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Measuring the Pancreatic β Cell Mass in Vivo with Exendin SPECT during Hyperglycemia and Severe Insulitis

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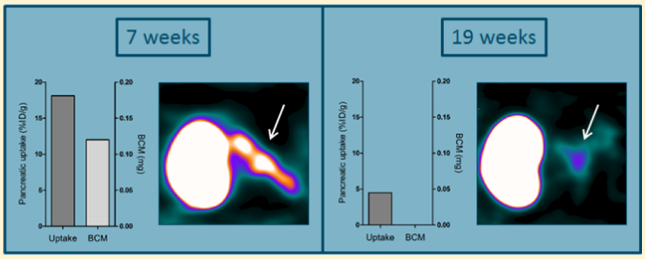
Supporting Information

ABSTRACT: Objective: Targeting the glucagon-like peptide-1 receptor with radiolabeled exendin is a very promising method to noninvasively determine the β cell mass in the pancreas, which is needed to unravel the pathophysiology of type 1 and type 2 diabetes. The present study aimed to explore the effects of both hyperglycemia and insulitis on the uptake of exendin in a spontaneous type 1 diabetes mouse model, nonobese diabetic (NOD) mice. Methods: NOD mice (n = 75, 7−21 weeks old) were injected intravenously with [^{111}In]In-DTPA-exendin-3, and single-photon emission computed tomography (SPECT) images were acquired 1 h pi. The pancreatic accumulation of [^{111}In]In-DTPA-exendin-3 was quantified in vivo using SPECT and by ex vivo counting and correlated to the β cell mass (BCM). The influence of insulitis and hyperglycemia on the exendin uptake was assessed. Results: The pancreas could be visualized longitudinally using SPECT. A linear correlation was found between the BCM (%) and pancreatic uptake (%ID/g) as measured by ex vivo counting (Pearson r = 0.64, p < 0.001), which was not affected by either insulitis (Pearson r = 0.66, p = 0.83) or hyperglycemia (Pearson r = 0.57, p = 0.51). Biodistribution and ex vivo autoradiography revealed remaining [^{111}In]In-DTPA-exendin-3 uptake in the pancreas despite total ablation of BCM. Conclusions: Despite hyperglycemia and severe insulitis, we have found a good correlation between BCM and pancreatic exendin uptake, even in a suboptimal model with relatively high background activity.



KEYWORDS: *NOD mice, GLP-1R, exendin, β cell mass, receptor imaging, SPECT*

INTRODUCTION

Type 1 diabetes (T1D) is characterized by islet-specific autoimmunity, followed by progressive depletion of the insulin-producing β cells leading to hyperglycemia.[1] Complete destruction of β cells in patients with long-standing type 1 diabetes has been believed to be the single cause of hyperglycemia for a long time; however, this theory has been challenged lately. In the last few years, several studies have found evidence of residual insulin-positive β cells in patients with long-standing T1D.[2,3] Furthermore, some patients with recent-onset T1D or patients positive for autoantibodies (ICA, GADA, and IA-2-Ab), but not (yet) diabetic showed lack of insulitis,[4−6] which has been considered to be the pathophysiological key feature required for the development of T1D. Insulitis is defined as the presence of lymphocytes infiltrating the islets of Langerhans,[4] leading to the destruction of β cells.

The evidence of remaining but functionally impaired β cells in long-standing T1D patients and the presence or absence of insulitis underline the population heterogeneity of T1D and with that the crucial need for a noninvasive method to determine the β cell mass. β cell function can be measured by clinical tests determining insulin production capacity following stimulation, but it appears that β cell mass and functional β cell mass are not identical,[7] emphasizing the relevance for individual monitoring of the β cell mass in relation to functional capacity. Such a method allows for monitoring of therapies targeting the autoimmune response to protect the β cells or therapies to increase β cell mass (BCM).

The β cells can be visualized noninvasively using radiolabeled exendin and SPECT or positron emission tomography (PET).[8,9] Exendin is a stable analogue of GLP-1 and targets the glucagon-like peptide-1 receptor (GLP-1R) with high affinity, which is highly expressed on β cells.

Previously, we have shown the feasibility of radiolabeled exendin to monitor the β cell mass in a rat model with chemically induced β cell destruction[8] and in a rat model for spontaneous type 1 diabetes.[10] In both studies, a strong linear correlation between exendin uptake and the β cell mass was found.

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The expression of the GLP-1R can change during the development of diabetes. During insulitis, several cytokines (like Il-1ß and IFN-γ) are released by immune cells, which in turn alter the expression of many genes, among which the GLP-1R.[11] Furthermore, previous studies in mice showed that hyperglycemia reduced the expression of the GLP-1R and thereby the β cell function.[12,13]

To address the effects of long-standing hyperglycemia and long-standing inflammation of the islets on the GLP-1R expression and thus uptake of exendin in the pancreas, we performed a study using nonobese diabetic (NOD) mice. This mouse model is the only well-characterized spontaneous mouse model for studying T1D.[14] The development of diabetes in this model shows a variety of similarities to that in human patients, like the presence of particular immune cells and cytokines (like Il-1ß and IFN-γ) in the diabetic pancreas, and the presence of insulitis,[14−16] although the insulitis is much more severe in mice than in men. Furthermore, this model is suitable to study the effects of hyperglycemia since these mice do not necessarily require insulin treatment.

Here, we determined the correlation between the BCM and pancreatic uptake of radiolabeled exendin under conditions of severe insulitis and hyperglycemia.

MATERIALS AND METHODS

Animals. All animal experiments were approved by the Animal Ethical Committee of the Radboud University, Nijmegen, The Netherlands, and all experiments were performed according to the Institute of Laboratory Animal Research Guidelines.

Nonobese diabetic (NOD) female mice were purchased from Charles River Laboratories (L'Arbresle, France). Unilateral nephrectomy of the left kidney had been performed at the age of 5 weeks under isoflurane anesthesia using Buprenorphine as an analgesic and the mice arrived at our facility between 6 and 9 weeks of age. One group of mice (n = 6) did not undergo nephrectomy and was euthanized at an age of 12 weeks to determine the distribution of the tracer, without any further measurements. Mice were housed in a pathogen-free environment in ventilated filter-topped cages (5 mice per cage), required to maintain diabetes incidence and had ad libitum access to sterile water and chow. Mice were allowed to adapt to laboratory conditions for 1 week before the start of the experiments.

At least twice a week, the body weight of the mice was measured; blood was drawn by venous puncture, and blood glucose was measured using a blood glucose meter (Accu-Chek Sensor, Roche Diagnostics, Almere, The Netherlands). Every week, a group of 5 mice was studied using SPECT, starting at 7 weeks until 21 weeks of age. Mice were considered diabetic when glucose levels exceeded 11.1 mmol/L.[17]

Radiolabeling of diethylene triamine penta acetic acid (DTPA)-exendin-3 with ^{111}In. [Lys^{40}(DTPA)]exendin-3 was purchased from Peptide Specialty Laboratories (PSL, Heidelberg, Germany) and referred to as DTPA-exendin-3. Diethylene triamine penta acetic acid (DTPA) was conjugated to the ε-amino group of C-terminal lysine. $^{111}InCl_3$ was obtained from Mallinckrodt Medical (Petten, The Netherlands). DTPA-exendin-3 was labeled with ^{111}In, as described previously.[18] Briefly, DTPA-exendin-3 (1 μg, 200 pmol) was incubated for 20 min with 150 MBq $^{111}InCl_3$ and two volumes of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 5.5. Subsequently, 50 mM ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, St. Louis, MO) and 10% Tween-80 (Sigma-Aldrich, St. Louis, MO) to a final concentration of 5 mM and 0.1%, respectively, were added. The radiolabeled peptide was separated from the unbound ^{111}In using solid-phase extraction by a hydrophilic−lipophilic balance reversed-phase sorbent cartridge (Waters Oasis, Milford, MA), as described previously.[19] Pure ethanol was used for the activation of the cartridge, and 0.1 M MES buffer was used for conditioning and washing. Quality control was performed by instant thin-layer chromatography (ITLC) on silica gel ITLC strips (ITLC-SG Biodex, Shirley, NY). As a mobile phase, 0.1 M EDTA in 0.1 M NH_4Ac, pH 5.5, was used (R_f [^{111}In]In-DTPA-exendin-3 = 0, R_f ^{111}In-EDTA = 1).

Biodistribution and SPECT. Every week five mice were injected intravenously with 19.7 ± 1.3 MBq (20 pmol). One hour pi mice were anesthesized and subjected to SPECT. Images were acquired with an acquisition time of 50 min using a dedicated small animal SPECT scanner (U-SPECT-II, MILabs, Utrecht, The Netherlands) and a 0.1 mm pinhole mouse collimator.

Mice were euthanized after SPECT using CO_2/O_2 suffocation. Blood, muscle, heart, lung, spleen, pancreas, stomach, intestine, kidney, and liver were dissected, weighed, and the radioactivity concentration was determined in a γ counter (Wallac 1480-Wizard, PerkinElmer, Boston, MA). Pancreata were fixed overnight in formaldehyde, dehydrated, and embedded in paraffin.

U-SPECT-Rec software (MILabs, Utrecht, The Netherlands) was used to reconstruct the SPECT images (OSEM with 16 subsets, 1 iteration, and a voxel size of 0.4 mm). The pancreatic uptake of [^{111}In]In-DTPA-exendin-3 was quantified using an Inveon Research Workplace (Preclinical Solutions, Siemens Medical Solutions, Inc., Knoxville, TN). A volume of interest over the pancreas was drawn manually and corrected for overlapping kidney uptake. For each mouse, a volume of 100 mm^3, including the voxels with the highest value, was measured by thresholding.

Macroautoradiography. Pancreatic sections of 5 μm were cut (one mouse per group). Two sections per mouse were exposed to a phosphor screen (Fuji Film BAS-SR 2025, Raytest, Straubenhardt, Germany) for 7 days; images were acquired with a radioluminography laser imager (Fuji Film BAS 1800 II System, Raytest) and analyzed using Aida Image Analyzer software (Raytest).

β Cell Mass Measurement and Insulitis. Four micrometer thin sections were cut, mounted on glass, and stained. Pancreatic sections were stained with guinea pig anti-insulin polyclonal antibody (1:3000; Van Schravendijk, Brussels) and species-matched Cy3-conjugated secondary antibody (Jackson ImmunoResearch, Cambridgeshire, UK) and counterstained with Hoechst (Sigma-Aldrich). Images were acquired with a Carl Zeiss LSM 710 multiphoton confocal laser scanning microscope and analyzed using IPLAB pathway 4.0 software. β cell mass was estimated morphometrically by quantifying the total insulin immunoreactive area within a pancreatic section area of at least 100 mm^2 for each mouse multiplied by the pancreatic weight as previously described.[20] Insulitis was examined histologically after hematoxylin and eosin staining using a bright-field microscope (Zeiss Axioskop, Oberkochen, Germany), and the degree of insulitis was scored as the percentage of islets that were infiltrated. The observer was blinded to the origin of the sections.

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Immunohistochemistry. Four micrometer thin sections were cut, mounted on glass, and stained. Pancreatic sections were treated with 10 mM citrate, pH 6.0, for 10 min at 96 °C to reverse epitope masking. Blocking was performed by incubating for 10 min at room temperature (RT) in 3% H_2O_2, followed by 30 min at RT in 20% normal goat serum. Subsequently, sections were stained with guinea pig anti-insulin (1:3000; A0564; DAKO, Agilent, CA) for 60 min at RT, followed by goat-anti-guinea pig-HRP (1:1000; A18775, Thermo Fisher, MA) for 30 min at RT. Adjacent sections underwent antigen retrieval by a 10 min incubation step at 37 °C in 0.1% pronase, followed by blocking in 3% H_2O_2 for 10 min at RT and 10 min at RT in 1% bovine serum albumin. Next, sections were stained with mouse-anti-GLP-1R (1:100; 7F382A, Novo Nordisk, Bagsvaerd, Denmark) overnight at 4 °C, followed by a 30 min incubation at RT with Opal Polymer HRP Ms + Rb (ARH1001EA; PerkinElmer). Sections were counterstained with hematoxylin, and images were acquired using a Leica DM3000 microscope, a Leica DMC2900 camera (Leica, Wetzlar, Germany), and Leica Application Suite software (v 4.11, Leica).

Statistical Analysis. GraphPad Prism software version 5.03 for Windows was used for data analysis; one-way ANOVA followed by a Tukey test was used to determine significance. A p value below 0.05 was considered significant. All correlation coefficients were calculated with a Pearson correlation coefficient using SPSS (IBM SPSS Statistics 22). To calculate the influence of insulitis and hyperglycemia on the correlation between the β cell mass and the uptake of radiolabeled exendin, a partial correlation analysis was performed, with hyperglycemia and insulitis as confounding factors. The statistical difference between these correlations was examined by a two-tailed Fisher r-to-z transformation.

RESULTS

Blood Glucose. The blood glucose level of the mice was measured at least twice a week. In Figure 1, all measured blood glucose values between 7−21 weeks of age of all mice are summarized. During the first 14 weeks, all mice were able to control their glucose levels, which ranged between 3.8 and 11.1 mmol/L (normoglycemic). From week 15 onward, elevated glucose levels were measured that increased rapidly, indicating hyperglycemia.

Biodistribution and SPECT. The highest pancreatic uptake was found in the group of mice of eight weeks (16.4 ± 3.3 %ID/g), whereas the lowest average uptake was observed in mice of 17 weeks (6.7 ± 2.0 %ID/g) (p = 0.0005). Overall, a clear decrease in exendin uptake was observed over time. The comparative biodistribution between mice that were nephrectomized and mice that were not is shown in Figure S1, Supporting Information.

SPECT with [^{111}In]In-DTPA-exendin-3 clearly visualized the pancreas. Figure 2 shows examples of three mice of different ages and with a different BCM. The decline in [^{111}In]In-DTPA-exendin-3 uptake in the pancreas is clearly visible in these images. Figure 2A was obtained from a healthy mouse of 7 weeks, with an [^{111}In]In-DTPA-exendin-3 uptake in the pancreas of 18.1 %ID/g and a BCM of 0.13%. Figure 2B shows a mouse of 13 weeks, with a pancreatic uptake of 12.2 %ID/g and a BCM of 0.06%. Both mice had normal blood glucose levels. Finally, Figure 2C is from a mouse of 19 weeks, with an uptake of 4.5 %ID/g, a BCM of 0.00%, and high blood glucose levels during several weeks. Notably, there was still uptake of exendin detected in the diabetic mouse. Next to pancreatic uptake, high uptake of exendin in the kidney was also observed.

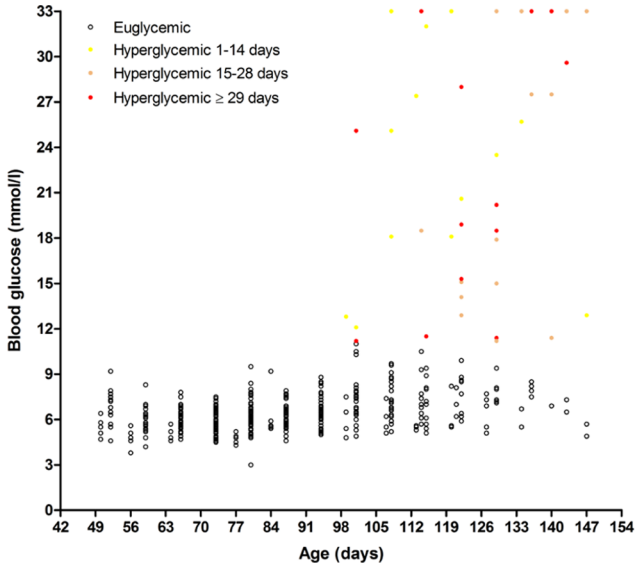

Figure 1. Blood glucose levels (mmol/L) in NOD mice up to 21 weeks of age. Blood glucose was measured twice a week. The duration of hyperglycemia was classified for every mouse: euglycemic (<11.1 mmol/L) (open circles) or hyperglycemic (≥11.1 mmol/L) for 1−14 days (yellow circles) (n = 8), 15−28 days (brown circles) (n = 4), and ≥29 days (red circles) (n = 3).

A linear correlation of 0.68 (Pearson r, p < 0.001) between the exendin uptake in the pancreas, measured by ex vivo counting and the SPECT signal, was found (Figure 3). When the SPECT signal declined, a decline in the pancreatic uptake measured by ex vivo counting was also found.

β Cell Mass Measurement and Insulitis. Figure 4 shows that the β cell mass is linearly correlated to the exendin uptake in the pancreas (Pearson r = 0.64, p < 0.001). A decline in β cell mass corresponded to a decline in pancreatic uptake. Furthermore, all mice that were mild (11.1−22.2 mmol/L) or severe hyperglycemic (>22.2 mmol/L) at the end of the experiment had a low β cell mass. However, in mice that had complete absence of β cells, the uptake of exendin in the pancreas was still measured.

Already at an early age, the first leukocyte infiltrated islets became apparent, and in the few islets that were infiltrated, infiltration was already very severe. With increasing age, more islets were infiltrated by immune cells, and from 11 weeks of age onward, more than half of the islets were inflamed (Figure 5).

To investigate whether inflammation of the islets influenced the exendin uptake, the correlation between the exendin uptake determined ex vivo and the β cell mass was corrected for insulitis and was found to be 0.66 (Pearson r, covariant analysis) and was not statistically significant (p = 0.83). Insulitis did not significantly affect the uptake of exendin in the β cells. Furthermore, there was no correlation whatsoever (p = 0.40) between insulitis and exendin uptake (Supporting Information, Figure S2).

Correction for the blood glucose value at the time of SPECT imaging resulted in a correlation coefficient of 0.57 (Pearson r, covariant analysis), which was not significantly different than the uncorrected correlation coefficient (p = 0.51). There was,

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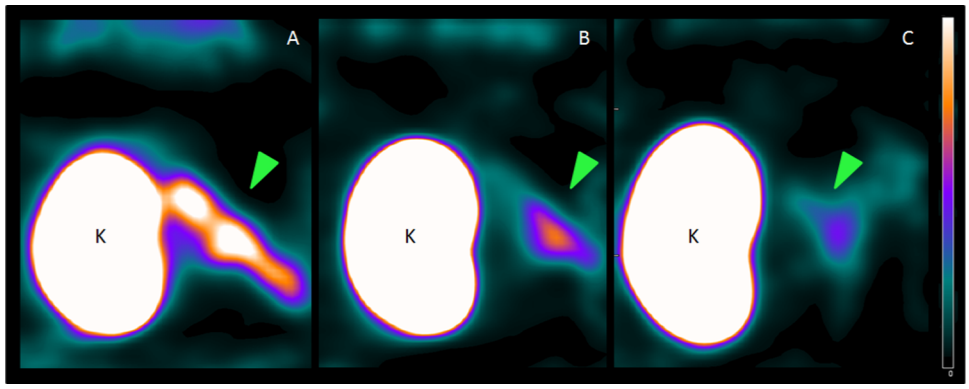

Figure 2. Coronal slices of SPECT images of NOD mice of 7, 13, and 19 weeks, respectively, with a pancreatic uptake and BCM of (A) 18.1 %ID/g and 0.13%, (B) 12.2 %ID/g and 0.06%, and (C) 4.5 %ID/g and 0%, respectively. The uptake in kidney is indicated with K and the pancreatic uptake with a green arrow.

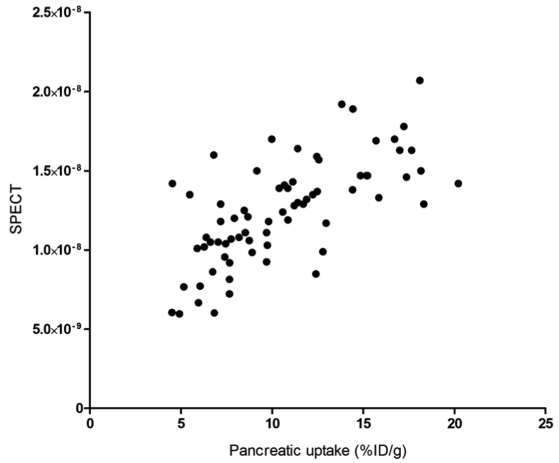

Figure 3. Correlation between the quantified SPECT signal in the pancreas and the uptake of exendin in the pancreas. The uptake is expressed as the percentage injected dose per gram of tissue (%ID/g) and determined by ex vivo counting of the pancreas.

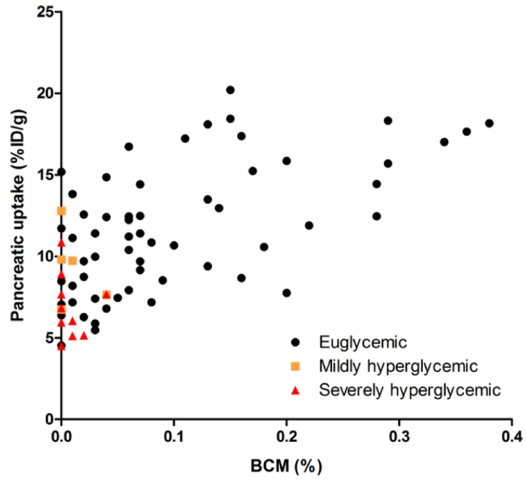

Figure 4. Correlation between the uptake of exendin in the pancreas and the BCM. The uptake is shown as percentage injected dose per gram of tissue (%ID/g) and determined by ex vivo counting of the pancreas; the BCM is presented as percentage of the total pancreas and determined by morphometric analysis after immunohistochemical staining with an anti-insulin antibody. The level of glycemia was determined at the endpoint and classified for every mouse: euglycemic (<11.1 mmol/L), mildly hyperglycemic (11.1−22.2 mmol/L), and severely hyperglycemic (>22.2 mmol/L).

however, a slight negative correlation between blood glucose (at the time of SPECT imaging) and the uptake of radiolabeled exendin (Pearson $r = -0.44$, $p < 0.01$) (Supporting Information, Figure S3).

Macroautoradiography and Immunohistochemistry. Figure 6 illustrates the high uptake of radiolabeled exendin in the islets of Langerhans and moderate uptake in the exocrine pancreas. The images depicted here show a decline of endocrine exendin uptake and thus β cell mass over time, as diabetes was progressing. When no insulin-positive cells were present in the section, there was still radioactive signal detected by macroautoradiography in the exocrine pancreas (Figure 6C).

To confirm the findings in the autoradiography, we have performed immunohistochemical analyses on a mouse with a high exendin uptake (17.7 %ID/g) and on a mouse with a low exendin uptake (5.1 %ID/g). Figure 7 demonstrates high insulin and GLP-1R expression in the mouse with high exendin uptake ((A) and (B), respectively), in contrast to the mouse with low exendin uptake, where the endocrine pancreas was nearly completely negative for both insulin and GLP-1R ((D) and (E), respectively). Furthermore, in both mice, there was no positive GLP-1R signal in the exocrine pancreas, which verifies that exendin binds to a different receptor than the GLP-1R.

DISCUSSION

In this study, we have validated [^{111}In]In-DTPA-exendin-3 SPECT for determination of the BCM in a type 1 diabetic mouse model. In NOD mice, we were able to visualize the pancreas with SPECT and to quantify the radioactive signal during the progression of the disease. Furthermore, we have found a linear correlation between the BCM and the exendin uptake as determined by ex vivo counting, which was not significantly altered by hyperglycemia or insulitis.

A study by Ortis et al. demonstrated that the GLP-1R was downregulated upon stimulation of primary rat β cells with pro-inflammatory cytokines.[11] Although this was an in vitro study, it is reasonable that in human T1D or in animal models for T1D insulitis could also influence the expression of the GLP-1R. We have previously demonstrated in biobreeding diabetes-prone (BBDP) rats that insulitis has no influence on

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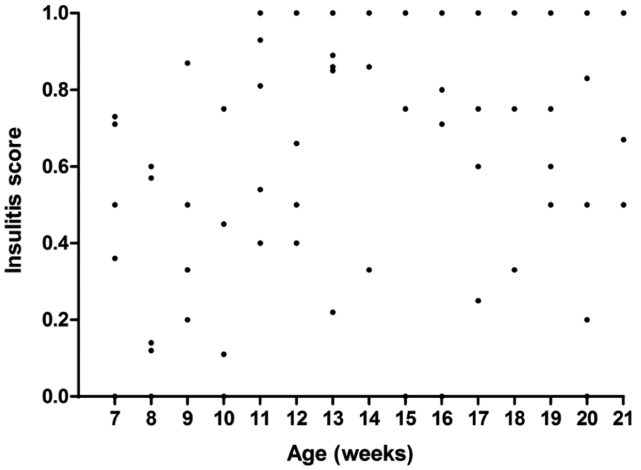

Figure 5. Insulitis score in NOD mice (n = 75). The degree of insulitis was scored as the percentage of islets that were infiltrated and was measured at the endpoint (n = 5 mice per week).

the strong correlation between BCM and exendin uptake.[10] In the present study, we confirm these findings in a mouse model with even stronger insulitis.[4] In nearly all mice, insulitis was found, while in diabetic patients, the incidence of insulitis is much less. Furthermore, the amount of islets that is infiltrated is hugely different between mice and human.[4,21] Based on these results, we presume that the weaker insulitis in humans will not have a significant influence on the uptake of exendin. Clinical evaluation is needed to address the influence of insulitis on the uptake of exendin in human since the cytokines involved in insulitis are not completely identical between mice and human.

The present study demonstrates that despite long-term hyperglycemia, there is a good correlation between the β cell mass and the uptake of exendin. Xu et al. and more recently Rajan et al. reported the downregulation of the GLP-1R both in vitro and in vivo after hyperglycemia.[12,13] We did not observe a significant difference in the correlation between β cell mass and exendin accumulation when corrected for the blood glucose levels, indicating that the observation of Rajan and co-workers may not be relevant for β cell quantification by GLP-1R imaging. However, as the duration of hyperglycemia varied in our study, and the blood glucose values at the time of [^{111}In]In-DTPA-exendin-3 and BCM measurement were mainly concentrated at the high and low blood glucose levels, one might consider to perform in vivo experiments specifically designed to determine the influence that the duration and severity of hyperglycemia have on GLP-1R expression and radiolabeled exendin accumulation in the pancreas.

With immunohistochemical staining of insulin, we have confirmed a near-complete ablation of the β cells in diabetic mice. However, uptake of the tracer was still found by ex vivo counting of the total pancreas in these mice, as well as the presence of a signal on SPECT. Furthermore, autoradiographic images of the pancreas of these mice confirmed the presence of a radioactive signal also in the exocrine pancreas. On the contrary, the validation of this tracer in BBDP rats demonstrated that when the amount of β cells dropped to zero, also no radioactive signal could be detected with either SPECT or autoradiography.[10]

Since there is exendin accumulation in the pancreas of diabetic mice, while immunohistochemistry shows a total depletion of the β cells, it is plausible that exendin is binding to the exocrine tissue of the pancreas. These data are in line with a previous study in which we compared GLP-1R targeting using exendin in various mouse and rat strains.[22] We report that in mice the pancreatic uptake of exendin after chemical β cell destruction is higher than after coinjection of an excess of unlabeled exendin. This stands in contrast to rats, where the accumulation of exendin is similar after β cell ablation and blocking of uptake by an excess of unlabeled exendin. Because the endocrine-to-exocrine mRNA ratio of GLP-1R was similar in mice and rats, it is likely that in mice exendin has cross-reactivity in the exocrine tissue with another protein. In accordance, the GLP-1R staining of the murine exocrine tissue was negative in the alloxan study as well as in this study. Similar findings are described by Eriksson et al., who report that the exendin uptake in islets has a larger contribution to the total pancreatic uptake in rats than in mice.[23] Furthermore, we have shown before that the uptake of exendin in patients with T1D drops to background levels, indicating that this cross-reactivity most probably does not play a role in human exocrine pancreas.[8]

Diabetes imaging in the NOD mouse model has been described using two different approaches, namely, immune imaging[24] or imaging the pancreatic β cells,[25] but thus far none of these approaches were sufficient for proper in vivo use. To the best of our knowledge, to date, no study reported successful noninvasive in vivo imaging of the β cells using a radioactive tracer in the NOD mouse model. Thus, the NOD mouse model can be used for studying the β cell mass with radiolabeled exendin, but the uptake of exendin in the exocrine pancreas should be taken into account. In the present study,

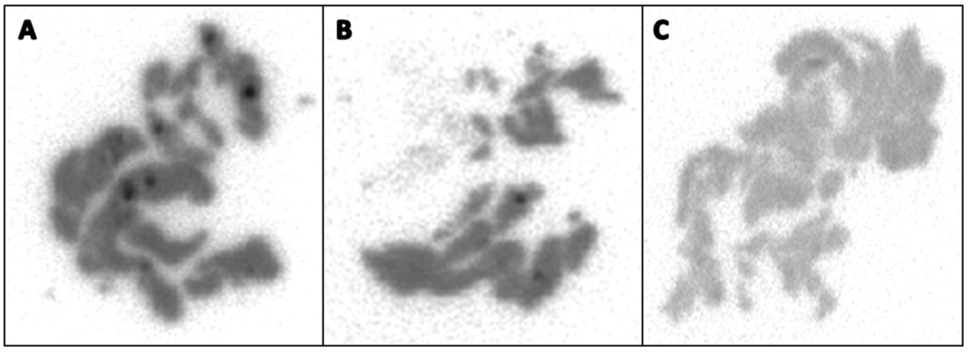

Figure 6. Macroautoradiography of pancreas of NOD mice of 11, 14, and 19 weeks, respectively, with a pancreatic uptake and BCM of (A) 15.7 % ID/g and 0.29%, (B) 8.5 %ID/g and 0.09%, and (C) 5.1 %ID/g and 0.01%, respectively.

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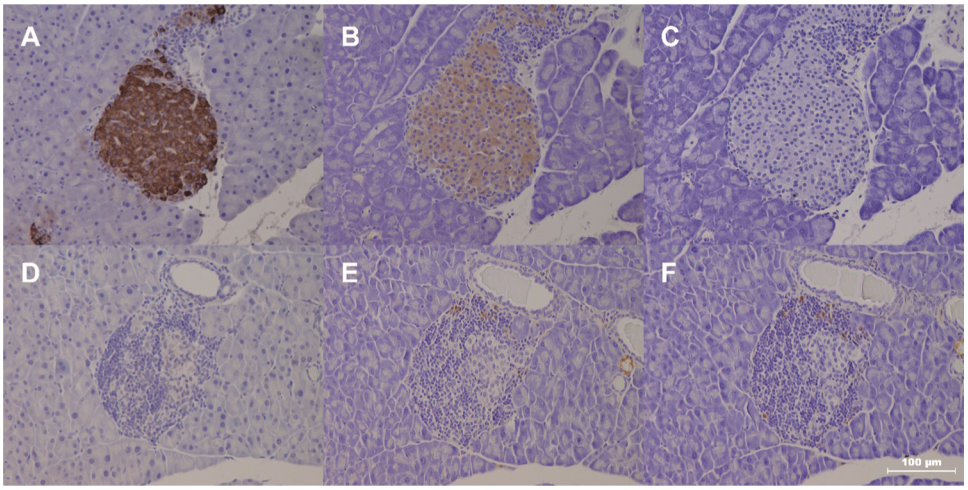

Figure 7. Immunohistochemistry of pancreatic sections of an NOD mouse with high (A−C) and an NOD mouse with low (D−F) exendin uptake. Anti-insulin staining (A + D), anti-GLP-1R staining (B + E), and blank controls (C + F).

the mice underwent unilateral nephrectomy to visualize the pancreas in vivo, otherwise the left kidney would mask the pancreas. For clinical SPECT or PET imaging, this is not an issue because there is a larger distance between the pancreas and the kidneys.

Population heterogeneity in T1D and new insights about β cells underline the necessity to determine the BCM non-invasively,[2,3] as currently all knowledge about β cells is obtained via autopsies. Detection of remaining β cells opens possibilities for therapy in which these β cells could be preserved, regenerated, or even activated to proliferate. Immune therapy in diabetic NOD mice revealed the recovery of β cells, while at time of diagnosis, no β cells were detected immunohistochemically.[26] In men, immunotherapy thus far cannot replace standard insulin treatment due to safety reasons of the therapy or lack of (long-term) effects, but shows great potential.[27,28] Based upon imaging and other (phenotypic) markers, patients could be categorized to provide patients with optimal therapy. Furthermore, monitoring the efficacy of novel immune-based therapeutic strategies would be of crucial value, especially in an individual-dependent manner.[27]

Despite hyperglycemia and severe insulitis, we have found a good correlation between BCM and pancreatic exendin uptake, even in a suboptimal model with relatively low differences in uptake due to high background activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.9b00728.

Biodistribution of mice with and without nephrectomy; correlation graph between insulitis and exendin uptake; and correlation graph between blood glucose and exendin uptake (PDF)

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Notes

The authors declare no competing financial interest.

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