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REVIEW ARTICLE

Beta-Cell Imaging: Call for Evidence-Based and Scientific Approach

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Abstract

Introduction: Advances in positron emission tomography (PET) imaging have provided opportunities to develop radiotracers specific for imaging insulin-producing pancreatic β-cells. However, a host of lingering questions should be addressed before these radiotracers are advocated for noninvasive quantification of β-cell mass (BCM) *in vivo* in the native pancreas.
Method: We provide an overview of tetrabenazine-based PET tracers developed to image and quantify BCM and discuss several theoretical, technical, and biological limitations of applying these tracers in clinical practice.
Discussion: VMAT2, a transporter protein expressed on pancreatic β-cells, has been advocated as a promising target for PET imaging tracers, such as dihydrotetrabenazine. However, the lack of radiotracer specificity for these proteins hampers their clinical application. Another important argument against their use is a striking discrepancy between radiotracer uptake and BCM in subjects with type I diabetes mellitus and healthy controls. Additionally, technical issues, such as the finite spatial resolution of PET, partial volume effects, and movement of the pancreas during respiration, impede PET imaging as a viable option for BCM quantification in the foreseeable future.
Conclusion: The assertion that BCM can be accurately quantified by tetrabenazine derived β-cell-specific radiotracers as density per unit volume of pancreatic tissue is not justifiable at this time. The fallacy of these claims can be explained by technical as well as biological facts that have been disregarded and ignored in the literature.

Key words: β-Cell mass, DTBZ, Pancreas, PET

Introduction

Despite afflicting an estimated 366 million people worldwide—a figure expected to increase to 522 million by 2030 [1]—diabetes mellitus (DM) is yet without a cure. Approximately 85 % of these patients suffer from type 2 diabetes mellitus (T2DM) and up to 15 % suffer from type 1 diabetes mellitus (T1DM). Current strategies to palliate DM and prevent its vascular complications [2] have laid tremendous constraints on healthcare systems worldwide [3] and demand endeavors to diminish its ever more intensifying burden [4].

As both T1DM and T2DM are associated with a functional loss of β-cell mass (BCM) [5], most efforts to cure DM have focused on preserving BCM and its function [6, 7]. Accordingly, an appropriate and important contribution to DM research would be the noninvasive, *in vivo* quantification of BCM for determining the longitudinal natural course of the disease and for quantitative measurements of the efficacy of novel antidiabetic therapies [8].

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Studies with positron emission tomography (PET) suggest that islets can be visualized using radioactive ligands that target insulin producing β-cells, and as such, this modality is claimed as a pivotal candidate for DM imaging and BCM quantification [9].

The requisite for noninvasive quantification of BCM has spurred the development of a large number of PET radiotracers [10–19]. Of these, radiolabeled dihydrotetrabenazine (DTBZ), a β-cell-specific imaging agent targeting the vesicular monoamine transporter 2 (VMAT2), has been claimed to hold particular promise [9]. Several preclinical studies with ^{11}C-DTBZ show lower pancreatic radiotracer activity in diabetic rodents compared to healthy controls [20, 21], and also results from subsequent clinical studies have shown similar findings [22, 23]. Encouraged by these provocative results, various groups are making major attempts to bring BCM imaging to the doorstep of clinical use for routine diagnostic purposes [24, 25].

However, the technical as well as biological issues associated with this approach and the ubiquitous patterns reported in the literature raise many enduring questions and should be addressed before these steps are taken to accomplish the goals proposed by both academia and the pharmaceutical industry. The bases for these concerns are enumerated in this scientific communication in great detail, and we hope these critical assessments will assist those who are or will be pursuing this research domain.

Biological Obstacles and Challenges for Imaging BCM with PET in the Native Pancreas

Among various tracers proposed for imaging BCM, many groups have extensively pursued radioligands targeting VMAT2 for this particular purpose [20, 22, 24–26]. Vesicular monoamine transporters (VMATs) mediate the uptake of monoamines from the cytoplasm into secretary granules. VMAT1 is found in neuroendocrine cells, while VMAT2 is expressed in the peripheral and central nervous system, as well as the hematopoietic system. Both are responsible for the storage and release of a variety of monoamines such as dopamine, norepinephrine, and serotonin in the synaptic terminals. In the pancreas, VMAT1 and VMAT2 are expressed on different types of pancreatic cells, mediating the pancreatic secretory function [27]. VMAT1 is expressed in the enterochromaffin cells within the pancreatic duct system, while VMAT2 is expressed in the insulin-secreting β-cells clustered in islets of Langerhans. In the neuroendocrine system outside the pancreas, VMAT2 is particularly detected in the chromaffin cells of the adrenal medulla as well as in the histamine-storing and enterochromaffin-like cells of the stomach.

Immunohistochemistry has revealed that anti-VMAT2 and insulin immunoreactivity were co-localized in islet β-cells, while VMAT2 was expressed to a lower degree in the other endocrine cells of the islets or the exocrine pancreas [20]. Recent studies in primates and humans confirmed these results and also showed that VMAT2 expression correlated with insulin levels in pancreatic tissue [25, 27]. The close association of VMAT2 and insulin has further suggested that VMAT2 might serve as a biomarker of BCM independent of the mechanics of insulin production [21]. Reduced BCM caused by partial pancreatectomy or streptozotocin induced β-cell necrosis indicated good correlations (approaching 70 %) between BCM and insulin secretion in animal models [28]. Because T1DM and T2DM are associated with a decrease in BCM, VMAT2 has become a potential molecular target for quantitative assessment of BCM.

In contrast to VMAT1, VMAT2 contains a binding site for DTBZ, a direct and active metabolite of tetrabenazine (TBZ). This enables DTBZ binding to VMAT2 with high specificity. Thus, DTBZ and its analogs developed for PET are reported to be 10,000 times more selective for VMAT2 than for VMAT1 and bind to VMAT2 with high affinity (0.5–1.0 nM dissociation constant) [21]. This finding spurred the hypothesis that DTBZ tracers could visualize and quantify BCM *in vivo*. However, this was not the first potential implication for clinical utility of DTBZ tracers. DTBZ, labeled with the positron emitter carbon-11, was already in clinical use for PET imaging of Parkinson's disease, with the first human studies being published in the 1990s [29–31]. It was not until much later that the first studies investigating the feasibility of [^{11}C]DTBZ PET evaluating BCM in rodents [20] and humans [22] were published.

The short half life of ^{11}C ($T_{1/2}$=20 min) used for labeling DTBZ, however, limits its application in clinical settings in wider scale. To overcome this drawback, ^{18}F-labeled analogs of DTBZ ($T_{1/2}$=110 min), such as [^{18}F] fluoropropyl [FP]-DTBZ, [^{18}F]fluoroethyl [FE]-DTBZ, and [^{18}F]FE-DTBZ-d4, have been explored [24–26, 32–34]. For example, a recently developed ^{18}F-labeled fluoropropyl derivative of DTBZ, [^{18}F]FP-(+)-DTBZ or ^{18}F-AV-133 (Avid Radiopharmaceuticals, Philadelphia, PA, USA), showed the advantage of an improved binding affinity to VMAT2 compared to [^{11}C]DTBZ (with K_i values for [^{18}F]FP-(+)-DTBZ and [^{11}C]DTBZ of 0.1 and 0.97 nM, respectively) [25, 26].

A potential confounding variable for radioligands targeting VMAT2 is the pancreatic polypeptide (PP) cells in the islets of Langerhans [35, 36]. PP cells are involved in regulating both endocrine and exocrine function of the pancreas, hepatic glycogen levels, and gastrointestinal secretions. In the body and tail of the pancreas, approximately 40 % of PP cells express VMAT2 [35]. It has been estimated that PP cells represent approximately 10 to 20 % of pancreatic islet cell mass, being predominantly located in the pancreatic head. These findings were confirmed in recent studies by Saisho *et al.*, who report that 39±7 % of islet PP positive cells expressed VMAT2, of which 70 % were located in the head of the pancreas [36]. The authors also pointed out that VMAT2 positive PP cells were more frequently observed in scattered groups in the exocrine

pancreas compared to PP cells clustered within islets. Triple staining for PP, insulin, and VMAT2 revealed that PP cells accounted for most of the VMAT2-positive insulin-negative cells. Only 1 % of VMAT2 positive cells were negative for both insulin and PP, most likely being mast cells or possibly nerve cells [36]. Interestingly, in their study based on autopsy findings, Saisho *et al.* [36] also point out that approximately 10 % of insulin producing β-cells in islets were actually VMAT2-negative, and this proportion increased up to 70 % in β-cells scattered in the exocrine tissue remote from pancreatic islets. The pattern was noted not only in healthy subjects but also in patients with T1DM and T2DM [36]. Furthermore, recent investigations in humans reported that a few somatostatin-positive cells (0–4 % of pancreatic islet cells) were also positive for VMAT2 [36]. These findings contradict the earlier suggestion that VMAT2 is a specific marker for pancreatic β-cells.

In chronic DM, the volume density of PP cells is in fact increased in proportion to that of β-cells because of the inherent effects of DM on BCM loss. An increased population of PP cells in association with ducts and periductal islet-like structures has also been reported in longstanding diabetes [35]. These findings suggest that an increased PP cell population diminishes the expected decrease in VMAT2 expression in DM. In turn, this reduces the quantification potential of PET imaging of BCM by targeting the VMAT2.

A multitude of additional BCM specific radioligands has been reported; however, except for VMAT2 imaging agents, none has been utilized to image diabetes in humans [24]. These imaging agents have been discussed elsewhere [10] and are beyond the scope of this review.

Loss of Pancreatic Volume in Type 1 and Type 2 Diabetes as well as Animal Models of This Disease

The effects of T1DM and T2DM on pancreatic structures on both the cellular as well as anatomic levels have been well described in the literature. These reports clearly state that the pancreas is substantially affected by this disorder [37]. Besides the pathognomonic endocrine dysfunction, DM also affects the acinar cells as indicated by the exocrine function affected in both the earlier stages as well as the clinically diagnosable state [37–39]. It is well established that impairment of cell function over an extended period of time will eventually lead to substantial loss of volume and detectable atrophy as has been shown in many organs. For example, in patients with early stage of Alzheimer's disease, the brain structures appear intact and similar to age-matched controls; however, as the disease progresses due to impaired metabolism and function of neuronal cells, a substantial degree of atrophy is noted by structural imaging such as MR imaging. A similar analogy exists in every organ in the body including the pancreas. In other words, a decrease in the metabolism and function of acinar tissues will eventually result in shrinkage of the pancreas, which has been well demonstrated by both *in vivo* imaging as well as postmortem examinations [40].

Pancreatic atrophy may result in underestimated values of radiotracer concentration per unit volume of tissue because of atrophy-related partial volume effects. These partial volume effects are the resultant of the finite spatial resolution of present day PET scanners as demonstrated by both phantom and *in vivo* setting studies. Although under ideal conditions (small phantoms imaged in a stable position) the spatial resolution of PET has been measured to be in the range of a few millimeters, in reality, this value is substantially larger when applied to the *in vivo* imaging settings. Therefore, the spatial resolution in human studies is estimated to be several millimeters at best. Furthermore, motion effects either due to respiration or cardiac cycles further degrade the spatial resolution of PET in assessing organ function or disease activity. This is primarily due to the fact that imaging with most PET preparations will require at least 2 min to provide statistically reliable data from the anatomic sites examined.

Regions of interest assigned to the center of structures that are larger than 3 cm in size will provide an accurate estimate of the true radioconcentration of the tracer accumulating at the site of interest. However, there is a substantial underestimation of values that are generated as the size of the structure decreases in diameter. This effect has been coined as the "partial volume effect (PVE)" and has been extensively discussed in the literature [41–46]. Therefore, the quantification of radiotracer concentration measured in structures that are small in size and are below 3 cm in diameter will be artificially below their real values inside the body. A great deal of effort has been made to correct for PVE, which have shown to substantially increase the values measured. Thus, partial volume correction is a must for accurate calculation of functional values of small organs or lesions.

We wish to point out that amino acids have been shown to concentrate in the acinar tissue by various groups over the past few decades [47–49]. In fact, selenomethionine, an amino acid labeled with selenium, was used in the 1970s to image the pancreas due to its concentration in the acinar tissue of this organ [47]. Since other tracers, such as 6-[^{18}F] fluoro-L-DOPA and DTBZ, are amino acids or similar compounds in nature [50], it is highly probable that these agents are heavily concentrated in the pancreas and, as such, will adversely interfere with visualizing the uptake in the islets that are imbedded in this organ.

Based on what has been stated above, it is clear that without taking these important biological and technical factors into consideration, any attempt to visualize islets in the pancreas will result in erroneous and underestimated values. When these factors are taken into consideration, it is our view that the concentration of these agents per gram of tissue will not differ between patients with diabetes and age-matched controls. As such, the data in the literature that

claim "beta-cell imaging" agents truly reflect the loss of islets in the pancreas are likely to be incorrect and cannot be relied upon as definitive findings.

What has been described above is also applicable to animal models of diabetes (in particular, streptozotocin-induced diabetes in mice) [51]. In other words, these models also have been shown to be associated with significant atrophy of the pancreas which will have the same implications with regard to PET imaging. As such, the results reported in the literature about the loss of beta-cells being detectable by DTBZ are also likely to be incorrect [20, 24, 25].

Necessity of Achieving Optimal Contrast Between Target Tissue and Background

The principle requisite that allows for both structural and functional imaging techniques to succeed in medicine is to achieve a contrast between the structure of interest and the background (i.e., structures of non-interest). Functional imaging by positron emission tomography accomplishes this "requisite for contrast" by differential uptake of a radiopharmaceutical in the target tissue of interest compared to background activity. Accordingly, the first requirement for differential uptake and, ultimately, contrast is differential binding affinity—the binding affinity of the radiotracer for the target exceeding the affinity to bind to the background. However, differential uptake alone is not enough to establish a quantifiable contrast by positron emission tomography. Certain factors can adversely affect the contrast that is required for effective visualization of the target by differential uptake in the field of view, and all should be addressed extensively before successful and reliable utilization of this technique.

In short, avidity of β-cell-specific tracers must exceed the *in vivo* detection threshold of the instrumentation, be substantially related to β-cell activity, exceed that of neighboring cell types by at least 100 times, and exceed that of the extracellular space [52, 53]. The requisite that demands such high β-cell specificity for successful *in vivo* imaging originates from two factors: the finite spatial resolution of PET and the fact that pancreatic exocrine tissue exceeds the β-cells by roughly 100-fold in normal physiological states. In the typical clinical setting, it is estimated that a rise in blood sugar occurs when only about 10–20 % of the β-cells are still intact. As such, the demand for β-cell-specific radiotracer avidity increases exponentially for successful *in vivo* detection of BCM loss [52].

The poor spatial resolution of PET is related to the partial volume effect, which results in measured loss of radiotracer concentration in the target tissue, underestimation of the lesion's standardized uptake value (SUV), and thus, a decrease in contrast between the target structure and the surrounding background if not properly accounted for in the analysis of small structures [42, 44]. This effect is particularly evident in target structures that are less than 2.5 times the spatial resolution of PET, as measured by the full width at half-maximum in *x*-, *y*-, and *z*-dimensions [42, 54], and results in the blurring of the target-derived signal into the surrounding background.

Theoretically, the partial volume effect is especially strong when imaging β-cells with PET. β-cells cluster in organelles called islets of Langerhans, which are roughly 40 to 300 μm in diameter [55]. Such small structures fall far below the spatial resolution of PET, which is a few millimeters at best [56]. Furthermore, pancreatic movement artifacts induced by the respiratory cycle further degrade spatial resolution, thereby intensifying partial volume effects [42]. Correcting for partial volume effects can only be achieved when the target lesion (i.e., islets containing β-cells) is spatially resolved, a task currently unachievable by current noninvasive *in vivo* imaging techniques, such as MR and CT imaging. The resultant of these would be a prevalence of underestimated SUV data in the values generated.

Perhaps the most essential question about β-cell imaging concerns the ability of PET to visualize clusters of β-cells in the native pancreas. Assuming that β-cells are evenly dispersed in the pancreas and represent approximately 2 % of the pancreatic cellular volume, the uptake of β-cells specific tracers must exceed the uptake of tracer in exocrine tissue cells by roughly 440-fold [52].

When BCM decreases, which occurs in both T1DM and T2DM, the differential uptake ratio needed to image clusters of β-cells increases exponentially. For example, in patients with T1DM, in whom the BCM has declined below the symptomatic threshold of 0.2 % of the total pancreatic cellular volume, the differential uptake ratio would have to be at least 4,000 times that of the background. This is an achievement difficult for any radiotracer, but seems particularly improbable for β-cell-specific imaging agents considering the extent of their non-specific uptake in the pancreatic acinar tissue.

It has been argued that the partial volume effect and the requisite radiotracer specificity in a ratio of 440:1 only apply when imaging individual clusters of β-cells in the pancreas. However, as islets are dispersed in the pancreas and the signal from an individual islet gets lost in the background noise due to the partial volume effect and lack of radiotracer specificity, the signal derived from every islet combined suffers from similar issues [57, 58].

Anomalous Patterns of Uptake of β-Cell Radiotracers in Health and Disease Following Signal Blockade Studies and Residual Uptake in Diabetic Patients

The percentage of radioactive signal emitted by the radioligand bound to VMAT2 was estimated in various blocking studies via either pre-treatment or co-administration of (+)-DTBZ or pre-treatment with FP-(+)-DTBZ or FP-(−)-DTBZ. Kung *et al.* [25] reported 30 % of radioligand displacement for pre-treatment with (+)-DTBZ and 36 % displacement for co-administration of (+)-DTBZ. In the same experiments, pre-

treatment with FP-(+)-DTBZ increased the radioligand displacement by 78 %. The likely mechanism for this higher displacement was attributed to different *in vivo* kinetics of (+)-DTBZ and FP-(+)-DTBZ, pointing out that the higher pancreas blockage of [^{18}F]FP-(+)-DTBZ uptake by FP-(+)-DTBZ (78 %) suggests matching kinetics between the cold and hot ligand binding to the same VMAT2 binding sites in the pancreas [25]. Interestingly, in the same experiments, the striatal region showed a complete blocking of [^{18}F]FP-(+)-DTBZ uptake by the active isomer [25]. Although this appeared in contrast to the radiotracer displacement in the pancreas, the findings are consistent with the existing reports of ^{18}F-DTBZ (AV-133) suitability for imaging of brain striatum and diagnosing Parkinson's disease.

In separate experiments, Singhal *et al.* [26] report the displacement of approximately 60 % of pancreatic [18F]FP-(+)-DTBZ with either pre-treatment or co-injection of unlabeled FP-(+)-DTBZ [26]. This displacement pool of radioligand has been interpreted to represent FP-(+)-DTBZ binding sites on VMAT2. In addition, the authors showed that the fraction of displaceable [^{18}F]FP-(+)-DTBZ was dependent upon BCM, supporting the hypothesis that this represents VMAT2 binding sites within the pancreatic β-cells [26]. The unlabeled FP-(+)-DTBZ also displaced approximately 80 % of the stomach [^{18}F]FP-(+)-DTBZ, consistent with known VMAT2 mediated histamine-storing and enterochromaffin-like cells of the stomach, but had no discernable effect on liver or kidney activity.

The results of residual uptake after PET signal blockade studies show that the pancreatic PET signal represents a combination of specific VMAT2-bound radioligand and nondisplaceable background signal. The nonspecific background signal may originate from both endocrine and exocrine pancreas and represented 22 to 65 % of the total PET signal in animal models. In addition, it has been estimated that a β-cell specificity of at least 440 (β-cells *versus* other tissues, including exocrine cells) would be necessary to produce a signal with enough β-cell contribution to be clinically useful [12, 52]. Reports involving [^{11}C]DTBZ, however, showed a β-cell specificity of approximately 70 [12], far below the necessary threshold. This may also contribute to the encountered difficulties in differentiating healthy controls from patients with T1DM in prior studies [12].

An important argument against the use of PET in BCM imaging is the striking discrepancy between the degree of pancreatic radiotracer uptake in patients with T1DM compared to that of healthy controls. Since patients with T1DM are virtually depleted of all β-cells, quantification of BCM with β-cell-specific radiotracers should theoretically result in zero radiotracer activity.

In preclinical research, streptozotocin (STZ), a glucosamine-nitrosourea compound that is particularly toxic to the insulin-producing β-cells of the pancreas, can induce T1DM in murine diabetes models. Quantitative studies showed that STZ-induced DM caused a significant reduction in total β-cell volume (84 %), reduction in volume density of β-cells/islets (64 %), reduction in islets mass (61 %), reduction in total islet volume (55 %), reduction in volume density of islet/pancreas (54 %), reduction of volume weighted mean islets volume (52 %), reduction of BCM (38 %), and reduction of pancreatic mass (13 %) compared to controls [59].

A preclinical study of [^{11}C]DTBZ by Souza *et al.* reported a statistically significant ($p<0.005$) decline in radiotracer uptake in a rodent model of progressive autoimmune DM (i.e., the BB-DP rat) [20]. More so, the reduction in pancreatic maximum SUVs was significantly ($p=0.024$) associated with the presence of abnormal glucose tolerance and persistent hyperglycemia. However, the reported residual radiotracer activity of 50 % at 8–9 weeks cannot stem from β-cells, as their destruction is almost 100 % in this animal model. A similar pattern emerges in a recent clinical study on [^{18}F]FP-(+)-DTBZ uptake in pancreas of long standing T1DM compared to healthy control subjects [23]. Here, the authors reported a significant decrease (approximately 40 %) in radiotracer uptake in the pancreas of patients with T1DM compared to control subjects. However, the average pancreatic uptake of the compound in T1DM subjects was still in excess of SUV 10, implying substantial accumulation of the radiotracer.

These contradictory findings can be explained by either (a) substantial non-specific binding of the tracer in pancreatic acini or (b) specific receptor binding to non-beta-cell tissues such as neuronal or acinar cells. Both mechanisms have been previously reported [32, 60, 61], and their variability within and between subjects has not been thoroughly investigated despite their significant influence on pancreatic uptake of ^{11}C/^{18}F-DTBZ analogs.

In a study comparing pancreatic [^{11}C]DTBZ PET scans of nine normal subjects with six patients with long-standing type 1 diabetes, Goland *et al.* showed an average decrease of 86 % of pancreatic binding potential (BP_{ND}) and a 40 % reduction of functional binding capacity in patients with T1DM compared to healthy controls [22]. The ROI-based quantification analysis suggested the presence of a significant amount of VMAT2 binding signal in the pancreas of T1DM patients, a rather unexpected finding in view of near complete depletion of β-cell mass in long-standing T1DM. The recognition of these findings prompted speculation on the possibility that the low-to-moderate BP_{ND} signal in T1DM patients might be either due to the higher [^{11}C]DTBZ nonspecific binding in the pancreas than in the renal cortex or due to the presence of non-β-cell VMAT2 binding in the pancreas in contrast to renal cortex rather than representing only β-cell mass. The authors conclude that [^{11}C]DTBZ allows quantification of VMAT2 binding in the human pancreas; however, BP_{ND} and functional binding capacity appear to overestimate the β-cell mass and that the high nonspecific binding in the exocrine pancreas may appear to overestimate BCM with pancreatic [^{11}C]DTBZ PET scans [22].

In a STZ-induced diabetic model comparing the utility of two PET imaging ligands [^{11}C]DTBZ and [^{18}F]FP-(+)-DTBZ, Singhal *et al.* reported that both tracers correlated

positively with BCM, although only about 25 % of uptake could be attributed to β-cell uptake [26]. In particular, from a comparison of the healthy and STZ-treated rats, the authors estimated that approximately 65 % of the specifically bound [^{18}F]FP-(+)-DTBZ was binding to VMAT2 (or another saturable site) that was not associated with insulin-positive β-cells. When also including the nonspecifically bound tracer, only about 25 % of the total uptake could be attributed to β-cell uptake [26]. It has also been noted that even in those rats with minimal residual pancreatic insulin or BCM, a substantial pool of displaceable and, hence, specifically bound radioligand remained in the pancreas, confirming that a substantial fraction of specific binding was not to pancreatic islet β-cells. Measured SUVs showed that the mean nonspecific pancreatic SUV was the same in both control and STZ diabetic rats, whereas there was a 35 % reduction in the specific uptake in the STZ diabetic rats compared to the controls [26]. Using an alternative rat model of type 1 diabetes (BB-PD), Souza *et al.* reported that loss of more than 65 % of the original SUV correlated significantly with the development of persistent hyperglycemia [20].

Recent human autopsy findings, however, showed that VMAT2 expression was not changed by the presence of diabetes and the overall pattern of pancreatic VMAT2 expression was comparable in T1DM and T2DM subjects [36]. As in non-diabetic subjects, most, but not all, β-cells were positive for VMAT2 (72±12 and 81±4 % of β-cells in subjects with T1DM and T2DM, respectively). In both T2DM and T1DM, β-cells scattered in exocrine tissue were less often VMAT2-positive than β-cells in islets, similar to non-diabetic subjects. Interestingly, the authors report a close linear correlation between VMAT2 and insulin expression in the tail of the pancreas in humans with and without diabetes, however, recommend caution in interpreting these findings because of VMAT2 expression in PP cells as well as heterogeneous VMAT2 expression among β-cells [36].

Unfavorable Observations on Autoradiographic Imaging Results

Autoradiography studies with DTBZ analogs on tissue sections, thin-layer chromatography samples, and freshly isolated islets performed in rat and human pancreatic tissue yielded conflicting results [32, 33, 60, 62]. Using autoradiography for localizing DTBZ radioactivity in rat and human pancreatic tissue sections, Fagerholm *et al.* [62] reported that at 10 min after the injection of [^{11}C]DTBZ, radioactivity was heterogeneously distributed with higher levels toward the head of the pancreas (head-to-tail ratio of 1.7). This gradient, however, was no longer seen at 60 min post-injection. Measurements of [^{3}H]DTBZ binding in sections of the rat pancreas showed that radiotracer distribution was homogenous and pancreatic islets could not be visualized. At either time point, [^{11}C]DTBZ radioactivity did not accumulate specifically in pancreatic islets, and [^{11}C]DTBZ bindings assays suggested different kinetics for [^{11}C]DTBZ binding in the pancreas and the VMAT2-rich striatum. Saturable [^{3}H]DTBZ binding was observed in the rat brain striatum and the bovine adrenal medulla, whereas in the rat pancreas, [^{3}H]DTBZ binding was nonsaturable. These *in vitro* binding results suggest that [^{3}H]DTBZ binding in the rat pancreas, in contrast to binding in the rat striatum and bovine adrenal medulla, is nonspecific [62].

In a rodent model of diabetes, [^{11}C]DTBZ binding in the pancreas was lower in diabetic rats than in control rats, supporting previous findings obtained by *in vivo* PET [62]. However, [^{11}C]DTBZ binding was not directly associated with a decrease in β-cell mass. The observed binding competition between antagonists for [^{3}H]DTBZ and VMAT2 suggested that DTBZ binding in rat pancreas was nonspecific and, therefore, did not demonstrate binding to VMAT2 [62]. In human pancreas, [^{11}C]DTBZ and [^{3}H]DTBZ binding in most pancreatic islets did not exceed binding in the exocrine pancreas. Some intermediate to large islets were, however, discernable against the exocrine background labeling. Upon comparison, the levels of nonspecific binding were similar in rat exocrine pancreas and human exocrine pancreas [62]. While most studies show a substantial fraction of binding to VMAT2 in β-cells, there is also evidence of significant nonspecific radioligand binding that may limit the quantification of relevant changes in BCM in patients with diabetes.

Conclusions

In conclusion, the assertion that BCM can be accurately detected and quantified by tetrabenazine-derived β-cell-specific radiotracers, as density per unit volume of pancreatic tissue, is not justifiable at this time [57, 63–65]. This review sheds light on several theoretical, technical, and biological limitations of applying VMAT2 specific tracers in the field of BCM imaging. Most theoretical and technical aspects discussed in the review are also applicable to other BCM specific tracers currently investigated for the purpose of noninvasive *in vivo* BCM imaging. The reasons for this statement are the following: First, the volume of the target tissue (BCM) to be imaged is far beyond the capabilities of current PET imaging techniques. Secondly, the levels of incorporation of the proposed agents in the islets are very low and come nowhere close to reaching the minimum values needed to be detectable by the existing methods in the pancreas. Thirdly, the most difficult obstacle is significant uptake of these agents in the non-islet acinar tissue in the pancreas. This results in substantial loss of contrast between the uptake in the supposedly targeted cells compared to the background. Finally, the issue of pancreatic atrophy due to diabetes (either type 1 or type 2) poses a significant challenge for accurate measurement of tracer uptake in this organ. By now, it is well established that diabetes results in significant exocrine dysfunction in the pancreas and eventually leads to atrophy of this organ. Therefore, partial volume

correction will demonstrate that the difference between controls and patients are not as striking as the superficial quantification methods currently reported in the literature imply. Thus, we believe efforts to visualize islets in the native pancreas may not provide desired results, and therefore, resources should be diverted to more fruitful domains such as pancreatic islet cell transplantation in favorable sites. Intramuscular or subcutaneous islet transplants in particular appear reasonable and quantifiable targets for PET [65]. The presence of a large number of β-cells at these known locations will provide an opportunity to perform partial volume correction and, as such, quantitate the exact concentration of these agents in the transplanted tissue.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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