



Imaging of pancreatic β -cell mass by PET

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Practice Points

- The sensitivity and specificity of molecular imaging by PET makes the noninvasive measurement of pancreatic β -cell mass (BCM) possible.
- PET imaging provides an integrated measure of BCM within the pancreas, as opposed to the imaging of individual islets. For the purposes of clinical evaluation of β -cell survival, the integrated measurement of pancreatic BCM is a critical parameter.
- Approaches to correct for off-target binding leading to background binding have been developed to improve the accuracy and dynamic range of BCM measurements.
- Preclinical evaluation in rodents suggests that glucagon-like peptide-1 receptor can serve as a useful biomarker for the imaging of pancreatic BCM.
- Two independent Phase I clinical trials with PET imaging of vesicular monoamine transporter type 2 with [^{11}C]-dihydratetrabenazine and [^{18}F]-FP-(+)-dihydratetrabenazine have shown that the loss of pancreatic BCM in patients with Type 1 diabetes mellitus can be successfully measured.
- The longer radioactive half-life of ^{18}F (109.8 min) as compared with ^{11}C (20.4 min) makes it possible to distribute [^{18}F]-FP-(+)-dihydratetrabenazine for research trials, especially in longitudinal studies to evaluate the efficacy of therapies to preserve BCM.

SUMMARY Success in the noninvasive and quantitative imaging of pancreatic islet β -cell mass (BCM) has been made possible by the inherent sensitivity of PET and the development of radiotracers for β -cell enriched peptide targets. This article briefly discusses the methodological considerations that can impact upon the accuracy of measuring BCM, and approaches to optimize BCM measurements in the face of these potential limitations. The current status of two peptide receptors that show promise for imaging BCM will be discussed: glucagon-like peptide-1 receptor and vesicular monoamine transporter type 2.

Insulin secretion by the pancreatic islet β cells is regulated in response to changes in circulating glucose in order to maintain plasma glucose concentrations at optimal levels. Islet β cells sense

the postprandial rise in glucose and respond with an increased rate of insulin secretion, which acts to suppress hepatic glucose output and to stimulate the muscle and liver to take up

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and store glucose as glycogen [1]. The development of peripheral and hepatic insulin resistance compromises the ability of the β cell to secrete sufficient insulin to quickly restore and maintain euglycemia [2]. Initially, islet β cells compensate with increased rates of insulin secretion to overcome the deficient response of the muscle and liver towards insulin. With time, however, the vitality of the β cell suffers, leading to loss of both β -cell function and β -cell mass (BCM) [3], resulting in chronic hyperglycemia with its associated complications. Alternatively, the loss of BCM is the result of an autoimmune attack directed towards the β cell [4]. Therapeutic strategies directed towards the root causes of insufficient insulin converge on the benefits of restoring the ability of the pancreatic islet β cells to secrete adequate insulin. Although the debate is ongoing, a consensus of data supports that β -cell function, as measured by overall rates of glucose-stimulated insulin secretion, improves with increases in BCM [5].

Developing therapies to increase BCM is complicated, however, by the lack of any practical way, short of taking a pancreatic biopsy, to measure BCM. The pressing need to non-invasively measure BCM has prompted the concerted efforts of numerous laboratories to develop solutions to this challenge. Our primary objective is to noninvasively, quantitatively and reproducibly measure the mass number of endogenous β cells in the pancreas by PET. This is a daunting objective given that β cells, organized in the islets of Langerhans, are dispersed throughout the pancreas. Also, in healthy individuals, β cells constitute only 1–3% of the total cellular mass of the pancreas. In diabetic patients, both Type 1 and Type 2, pancreatic BCM is lost with the progression of the disease [6,7]. Our goal is simply to measure BCM; whether β cells are functionally competent for glucose-stimulated insulin secretion or not is secondary to our imaging objective. Of the various imaging modalities, significant progress has been made using PET imaging, and this is the subject of this report.

Methodological considerations & limitations of PET imaging

Before discussing examples of PET imaging of the pancreatic islet BCM, a discussion of methodological limitations common to any approach using PET to image BCM needs to be acknowledged. First and foremost, tracer binding density

per β cell must be constant irrespective of disease state. This concern will need to be evaluated on an individual basis with each intended β -cell protein target. A second methodological consideration is that the imaging resolution of PET is greater than the diameter of the islet, and may introduce errors termed partial volume effects [8–10]. The mismatch between image resolution and islet diameter, which differs by a factor of approximately 10, limits our ability to image and accurately determine the radio-tracer uptake within individual pancreatic islets, and has led to the objection that PET imaging cannot be used to measure pancreatic BCM [9]. Fortunately, for the purposes of quantitative imaging of BCM, it is not necessary to image individual islets [10]. Partial volume effects are minimized in the measurement of BCM, since the radioactivity is measured for all pancreatic voxels, and the spatial resolution of the PET scanner is greater than the pancreas diameter. An integrated measurement of the density of a β -cell-specific receptor within a defined volume of the pancreas meets our objective of measuring β -cell density. And, when β -cell-specific receptor density is summed over the entire pancreas, the result is a quantitative measurement of pancreatic BCM.

A third methodological consideration is the nonideality of the radioligand's specificity, which results in background binding to a greater degree than the specific binding to the β cell. As discussed below, BCM can be quantitatively measured in the presence of this off-target background binding, however, reducing or eliminating its contribution to the total binding is desirable for making diagnostically meaningful measurements. A primary objective of noninvasive imaging of BCM is to provide a means to evaluate the efficacy of treatments designed to preserve or restore BCM in longitudinal studies. This is an especially challenging objective, since the total BCM of healthy individuals is at most a few percent in the pancreas, decreasing to a few tenths of a percent with diabetes progression [6,7]. Ideally, the change in the signal intensity would have a one-to-one correspondence with BCM. The correspondence of the change in PET signal intensity to the change in BCM is a direct function of the specificity ratio of the radioligand for binding to the β cell versus anywhere else in the pancreas [11,12]. Rough calculations indicate that an exact correspondence of change in radioligand binding to BCM

would require a specificity of a ratio of more than 100,000:1, while a specificity ratio of 1000:1 would deviate from ideality by approximately 5%. Fortunately, for monitoring changes in BCM, an ideal correspondence is not necessary. As long as the radioligand binding is proportional to BCM, we have shown that meaningful results can be obtained with a specificity ratio less than 100. Our recent imaging study with [^{18}F]-FP-(+)-dihydrotrabenazine (DTBZ) found an approximately 50% reduction in [^{18}F]-FP-(+)-DTBZ binding potential in those patients with long-standing Type 1 diabetes mellitus (T1DM) and nondetectable functional BCM, corresponding to a specificity ratio on the order of approximately 100:1 [13]. The caveat to correlating any observed change in any radioligand binding with BCM is the assumption that the background binding either remains constant, or can be measured, for each imaging session.

Background, or off-target binding of the radioligand will contribute to the observed tissue uptake, and if not accounted for, will lead to errors in the interpretation of the measured amount or change of BCM [14]. The total observed signal in the target tissue is the sum of the radioligand: first, specifically bound to the target of interest; second, specifically bound to proteins other than the desired target; and third, nonspecifically bound to the tissue. Binding to anything other than the desired target can be considered off-target background binding. The relative contribution of the background signal to the total signal will be a factor of the tissue density of non- β -cell targets, and the radioligand's off-target specific and nonspecific binding affinities. In order to quantify BCM in the presence of background signal, the contribution of the background to the total signal must be established, and measurable in each subject. If a reference tissue can be identified that has the same degree of nonspecific binding and lacks the intended β -cell protein target, then this tissue can be used as a reference tissue to correct for the contribution of nonspecific binding to the total binding of the radiotracer in the pancreas, and arrive at a measure of BCM [14].

Techniques developed to account for background binding and accurately measure receptor density in the brain are well established and provide rational approaches for measuring the background signal in the pancreas. A proven strategy to measure the background signal of receptors and transporters in the brain exploits

the selectivity of the target receptor to specifically bind only one enantiomer of the two stereoisomers of the radioligand [15–17]. For example, to measure striatum dopamine D1 receptors, binding of the inactive (-) enantiomer was determined in the D1-rich striatum and the cerebellum, which lacks D1 receptors [15]. The cerebellum, with equivalent nonspecific binding of both the (+) and the (-) enantiomers, served as a reference tissue *in vivo* to correct for the contribution of nonspecific to the total binding of the (+) enantiomer for evaluating D1-receptor density in the striatum. A similar strategy has been used to account for nonspecific binding in the pancreas when targeting VMAT2 as a biomarker for pancreatic β -cells. VMAT2 binding has been shown to be selective and specific for the (+) enantiomer of [^{18}F]-FP-DTBZ, whereas the affinity for the (-) enantiomer is negligible [16,17]. Establishing a suitable reference tissue to correct for nonspecific binding of the (+) enantiomer was determined using the inactive (-) enantiomer, making it possible to then determine the specific binding of [^{18}F]-FP-DTBZ within the pancreas, and thereby calculate a truer value of BCM.

■ Heterovalent ligands

As discussed above, PET imaging can be used to obtain clinically useful measures of pancreatic BCM even in the presence of a significant background signal. The extent to which the uptake of the radiotracer by the pancreas is proportional to BCM will be determined by the specificity of its uptake by the β cell in relation to the rest of the pancreas. It follows, then, that improving the specificity of the radioligand for binding to the β cell will lead to a larger dynamic range in the correlation between the PET-determined binding parameters and actual pancreatic BCM. Rather than focusing on enhancing the binding affinity for a single receptor, an alternative approach for increasing β -cell specificity has been to design multivalent ligands targeted to two or more different β -cell receptors. The hypothesis spurring the use of the heteromultivalent ligand to increase selectivity is that, while many cell types, including β cells, may share expression of any one particular receptor, the expression of a particular combination of two or more receptors may be unique to β cells. The feasibility of this approach has been demonstrated in the design of a heterobivalent ligand engineered to bind two different G protein-coupled receptors

(GPCRs) [18]. *In vitro*, an approximately 24-fold increase in bivalent versus monovalent binding affinity was reported, resulting in a striking enhancement of the *in vivo* image intensity with a fluorescently tagged heterobivalent ligand. The use of heteromultivalent ligands for imaging BCM has the potential advantages of: first, lowering the concentration needed for imaging; and second, reducing the background binding, thereby improving the one-to-one correspondence of binding to BCM.

Specific methods/targets

Key to the success of molecular imaging is identifying a suitable β -cell-specific target. Two fundamentally different approaches have been used to identify imaging targets, the first targets peptides known to be highly associated with the β cell, with the ATP-sensitive K^+ channels being a prime example [19]. Early approaches to imaging BCM explored the potential of radioligands targeting the sulfonylurea receptor of the ATP-sensitive K^+ channels with fluorinated analogs of glyburide [20] and repaglinide [21]. While the low, and predominantly nonspecific pancreatic uptake suggested that the glyburide analogs were not suitable for imaging BCM [20], the repaglinide analog did show promise for further evaluation [21]. However, preclinical imaging trials confirming the ability to discern changes in BCM are still lacking.

The second approach seeks to identify novel β -cell-specific peptides with no *a priori* knowledge of the target. Alternatives to targeting protein receptors known to be enriched in the β cell are to generate monoclonal antibodies for islet β -cell surface antigens [22], or to use a phage display selection process for the discovery of novel β -cell binding peptides [23]. Phage particles that display peptides or single-chain antibodies (SCAs) are selected that trigger receptor-mediated endocytosis by islets or β cells. Infectious phage are recovered, and following subsequent rounds of selection, the specificity of the phage towards β -cell proteins can be substantially increased. Using this approach, Ueberberg *et al.* generated SCAs targeting rat and human islet β cells. The quicker vascular clearance of SCAs compared with monoclonal antibodies lends support to their use as imaging agents. And, in rat studies with [^{125}I]-labeled SCAs, their potential to measure BCM was favorably demonstrated [23].

The relative merits of these radioligands, potential β -cell receptors and peptide targets,

and strategies for the noninvasive imaging of BCM are discussed in several recent reviews [19,24–27]. For the purposes of this report, the focus of this contribution is on two molecular targets that continue to hold promise for moving towards clinical applications within the short term. Given the pace of recent research in this field however, it is highly likely that other imaging agents may soon move to the forefront of routine measurement of pancreatic BCM in the battle against diabetes.

■ GPCRs & glucagon-like peptide-1 receptor

A number of GPCRs have been identified that are enriched in the β cell relative to the exocrine pancreas, and serve to regulate β -cell function [25]. Recognizing the potential of these GPCRs as molecular targets, efforts have focused on identifying GPCR targets and developing GPCR ligands as a means to image islets and BCM [12]. Of the GPCRs identified as being sufficiently unique to the β cell for imaging, it may be argued that the most progress has been made in targeting glucagon-like peptide-1 receptor (GLP-1R). The majority of the GLP-1R imaging ligands have explored the efficacy of derivatives of the GLP-1 peptide mimetics, such as exendin-4 and exendin-3 [28–32]. The key consideration for introducing an imaging tag into these peptides is to avoid those amino acid residues that are involved in binding to the receptor. Specificity to islet β cells enabled the imaging of mouse islets *in situ* with exendin-4 modified with near-infrared fluorescent tag attached to the lysine-12 residue [28]. Analogs of exendin-4 for PET and SPECT imaging have relied on modifying the lysine-40 residue for the chelation of ^{111}In , ^{68}Ga or ^{99m}Tc [29–30], and their potential for imaging of human insulinomas has been shown in mice. The application of exendin analogs for imaging BCM has also been demonstrated in rats with [^{111}In]DTPA-exendin-3 [31]. However, quantitative imaging of BCM with GLP-1 analogs such as these may be compromised by the downregulation of GLP-1 receptor number in poorly controlled diabetes. Recent studies indicate that chronic hyperglycemia can lead to the downregulation of GLP-1 mRNA and protein in rats and humans, and lead to an underestimation of BCM [33,34]. Evaluating the potential impact of receptor downregulation will need to be resolved as work progresses towards moving GLP-1R imaging forward for the clinical imaging of BCM.

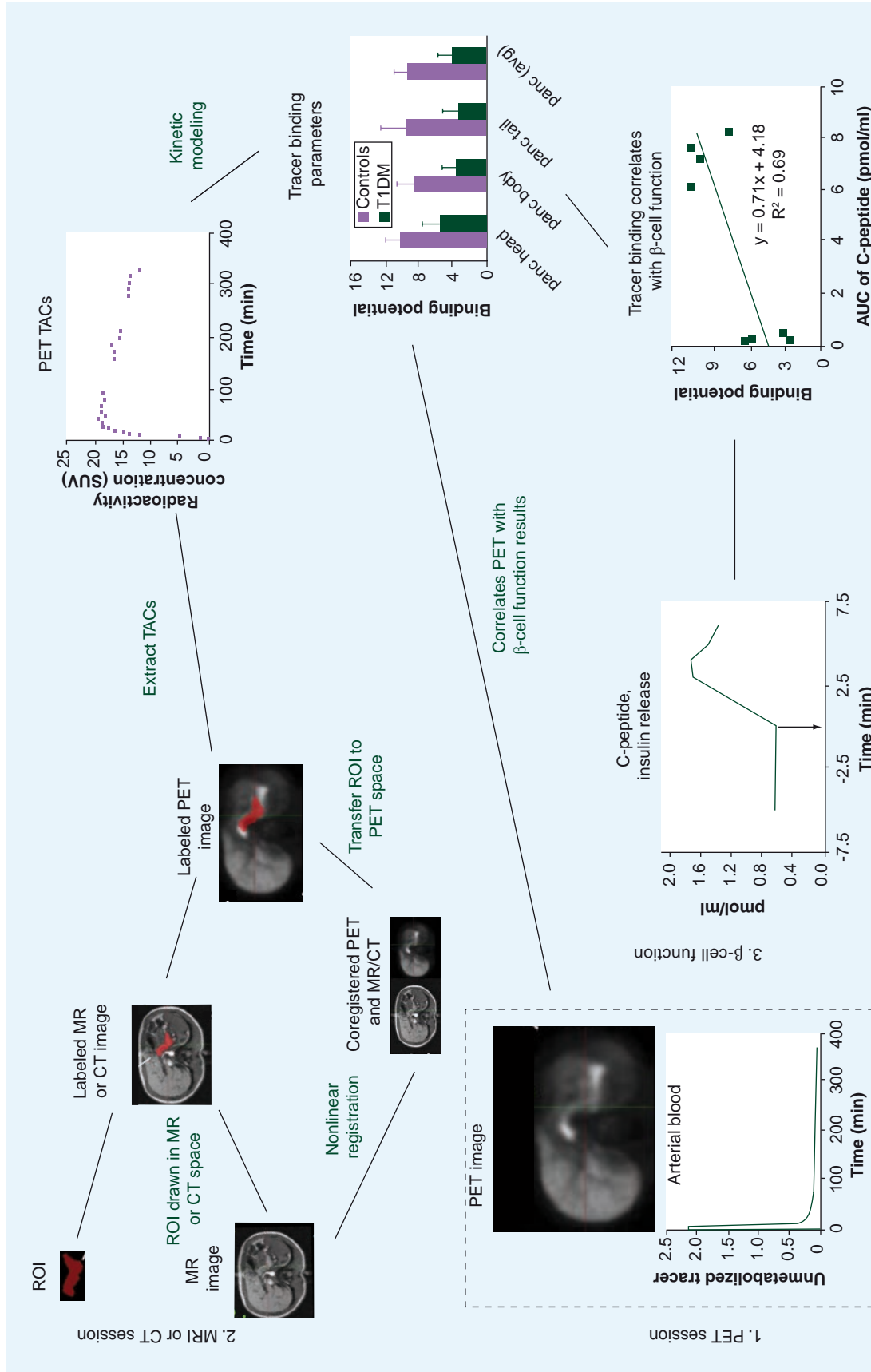


Figure 1. Imaging study protocol to noninvasively determine β -cell mass. Sessions include: first, PET imaging session with β -cell-specific radiotracer; second, MR or CT imaging for anatomical localization; and third, stimulus test to determine β -cell function. The pancreatic ROI in the PET image is derived from coregistration of the MR/CT image with the PET image. TACs of tissue radiotracer uptake are determined for the pancreatic ROI. Radiotracer activity in arterial blood during the PET session and the pancreatic TACs are used as inputs for kinetic modeling to calculate tracer binding parameters, as a biomarker of β -cell mass (BCM). The correlation of PET-determined BCM with β -cell function provides a measure of confidence in the ability of the radiotracer to quantify BCM.

AUC: Area under curve; CT: Computed tomography; MR: Magnetic resonance; Panc: Pancreas; ROI: Region of interest; SUV: Standardized uptake value; T1DM: Type 1 diabetes; TAC: Time-activity curves.

■ Vesicular monoamine transporter type 2

Of the various approaches for imaging of BCM *in vivo*, to date the clearest examples of quantitatively imaging pancreatic BCM in human clinical trials have been with ligands directed towards vesicular monoamine transporter type 2 (VMAT2). VMAT2 is responsible for the storage and release of monoamines such as dopamine, norepinephrine and serotonin in the transport vesicles of synaptic terminals of monoaminergic neurons, and is coexpressed in pancreatic β cells [26,27,35,36]. A strong correlation between the expressions of VMAT2 with insulin was found in the human pancreas. And, most importantly, the coexpression of VMAT2 and insulin was no different in patients with T1DM or Type 2 diabetes mellitus (T2DM) compared with those without diabetes, thereby lending credence to its use as a biomarker of BCM [35]. Radiolabeled analogs of DTBZ have been shown to be specific ligands for VMAT2 [16,37,38], and [^{11}C]DTBZ has been successfully employed to evaluate changes in VMAT2 levels in the brain associated with Parkinson's disease by PET imaging [39,40]. Developing imaging techniques to monitor the loss of BCM in relation to β -cell function is driven largely by the need to be able to evaluate the efficacy of therapies to restore or prevent loss of BCM with progression of T1DM or T2DM. Substantial progress towards this goal has been made by Harris and colleagues in imaging studies of pancreatic VMAT2 as a biomarker of BCM in rodent models of diabetes and humans [41–43]. Longitudinal PET-imaging studies of diabetes progression in biobreeding diabetic prone rats, and in streptozotocin-treated Lewis rats, showed positive correlations of [^{11}C]DTBZ binding with BCM [41,42]. Recently, Goland *et al.* provided the critical proof that PET imaging with [^{11}C]DTBZ could also measure the loss of BCM in patients with T1DM [43]. However, the relatively high background signal remaining in these long-standing T1DM patients may limit the overall utility of [^{11}C]DTBZ for detecting relatively small, but physiologically-relevant, changes in BCM.

A significant improvement in imaging VMAT2 for clinical applications to monitor BCM was achieved with the development of a fluoropropyl-derivative of DTBZ, [^{18}F]FP-(+)-DTBZ, with approximately eightfold higher binding affinity for VMAT2 [16,38]. Additionally, the longer radioactive half-life of ^{18}F (109.8 min) as compared with ^{11}C (20.4 min) makes it possible to distribute [^{18}F]FP-(+)-DTBZ for research

trials and clinical applications. We recently evaluated [^{18}F]FP-(+)-DTBZ for PET imaging and quantification of BCM in T1DM and healthy controls matched for age and BMI using the protocol outlined in Figure 1 [13]. All measures of pancreatic binding, calculated from the dynamic PET data acquired after administration of [^{18}F]FP-(+)-DTBZ, were strikingly reduced in the T1DM subjects. Moreover, all binding parameters correlated positively with the insulin secretory capacity of β cells as measured by an arginine challenge. Notably, the reduction in the BCM was not dependent upon pancreas volume, but reflected a loss of islets throughout all regions in the pancreas of the T1DM patients. However, when the decrease in pancreas volume in the T1DM subsets was accounted for, the relationship between PET-determined BCM and function was even more evident. In comparison to the previous studies using [^{11}C]DTBZ, [^{18}F]FP-DTBZ substantially improves, both qualitatively and quantitatively, the ability to noninvasively image pancreatic BCM in humans. The high tracer uptake in the pancreas and the quality of the time–activity curves give confidence that the methodological errors would be no higher than previous results in brain, with a test–retest reliability of approximately 10% [44,45], and that clinically relevant changes in BCM can be monitored during the course of longitudinal studies. Using metabolic measures, the rate of decline of endocrine pancreas function in new onset disease was shown to decay by 40–50% in the first year following diagnosis of disease [4]. Thus, PET studies spaced approximately 9 months apart and initiated within 10 weeks of diagnosis are likely to be properly spaced to evaluate the efficacy of therapies to prevent the decline in BCM during this critical phase.

Conclusion & future perspective

The sensitivity of PET imaging and the ability to quantify β -cell receptor number is of significant benefit for longitudinal noninvasive, direct evaluations of BCM in health and diabetes. The clinical utility of PET imaging of VMAT2 as a biomarker of BCM has been proven with [^{11}C]DTBZ, and independently with [^{18}F]FP-(+)-DTBZ. Ongoing studies to determine the reliability of PET scans with [^{18}F]FP-(+)-DTBZ, and to resolve and correct for off-target binding, will advance the wider application for its use in clinical studies. The experience gained with the clinical VMAT2 imaging studies serve as a prototype

and benchmark for alternative β -cell-specific radioligands as they come online. Peptide receptors such as GLP-1R and other β -cell GPCRs are probable candidates, with heteromultivalent ligands showing promise to improve β -cell specificity. Novel β -cell binding peptides will likely be identified by phage-display selection, stimulating the development of new imaging agents and opening up new areas of research of β -cell biology. Existing and future PET imaging probes to noninvasively image BCM will be of significant value in: first, studying the natural history of T1DM and T2DM; second, measuring the efficacy of pharmaceutical and cell-based interventions; and third, making clinical diagnoses related to incipient disease.

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References

Papers of special note have been highlighted as:

■ of interest

■ ■ of considerable interest

- Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in Type 2 diabetes: implications for therapy. *Diabetes* 59, 2697–2707 (2010).
- Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unraveling the mechanism. *Lancet* 375, 2267–2277 (2010).
- DeFronzo RA. From the triumvirate to the ominous octet: a new paradigm for the treatment of Type 2 diabetes mellitus. *Diabetes* 58, 773–795 (2009).
- Steele C, Hagopian WA, Gitelman S *et al.* Insulin secretion in Type 1 diabetes. *Diabetes* 53, 426–433 (2004).
- Provides the data needed to guide longitudinal studies of interventional studies to preserve β -cell mass (BCM) and function in early-onset Type 1 diabetes mellitus.**
- Wajchenberg BL. β -cell failure in diabetes and preservation by clinical treatment. *Endocr. Rev.* 28, 187–218 (2007).
- Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β -cell deficit and increased β -cell apoptosis in humans with Type 2 diabetes. *Diabetes* 52, 102–110 (2003).
- Löhr M, Klöppel G. Residual insulin positivity and pancreatic atrophy in relation to duration of chronic Type 1 (insulin-dependent) diabetes mellitus and microangiopathy. *Diabetologia* 30, 757–762 (1987).
- Hickeson M, Yun M, Matthies A *et al.* Use of a corrected standardized uptake value based on the lesion size on CT permits accurate characterization of lung nodules on FDG-PET. *Eur. J. Nucl. Med.* 29, 1639–1647 (2002).
- Kwee, TC, Basu S, Saboury B, Torigian DA, Naji A, Alavi A. Beta-cell imaging: opportunities and limitations. *J. Nucl. Med.* 52, 493–495 (2011).
- Ichise M, Harris PE. Reply: Beta-cell imaging: opportunities and limitations. *J. Nucl. Med.* 52, 493–495 (2011).
- Resolves the objections that measuring BCM by PET imaging is not possible due to partial volume effects. Clearly describes the analogy between PET imaging of pancreatic islets and the PET imaging of brain neuroreceptors.**
- Sweet IR, Cook DL, Lernmark A *et al.* Systematic screening of potential β -cell imaging agents. *Biochem. Biophys. Res. Commun.* 314, 976–983 (2004).
- Cline GW, Zhao X, Jakowski AB, Soeller WC, Treadway JL. Islet-selectivity of G-protein coupled receptor ligands evaluated for PET imaging of pancreatic β -cell mass. *Biochem. Biophys. Res. Commun.* 412, 413–418 (2011).
- Normandin MD, Petersen KF, Ding Y-S *et al.* *In vivo* imaging of endogenous pancreatic beta cell mass in healthy and type 1 diabetic subjects using [¹⁸F]FP-(+)-DTBZ and positron emission tomography. *J. Nucl. Med.* (2012) (In Press).
- Phase I clinical trial with improved dynamic range of PET-determined BCM necessary for measuring clinically relevant changes in BCM in longitudinal studies.**
- Innis RB, Cunningham VJ, Delforge J. Consensus nomenclature for *in vivo* imaging of reversibly binding radioligands. *J. Cereb. Blood Flow Metab.* 27, 1533–1539 (2007).
- Kassiou M, Loc'h C, Bottlaender M *et al.* (+)-[⁷⁶Br]A-69024: a non-benzazepine radioligand for studies of dopamine D1 receptors using PET. *Nucl. Med. Biol.* 29, 295–302 (2002).
- Kung MP, Hou C, Goswamia R *et al.* Characterization of optically resolved 9-fluoropropyl-dihydrotrabenazine as a potential PET imaging agent targeting vesicular monoamine transporters. *Nucl. Med. Biol.* 34, 239–246 (2007).
- Ichise M, Easwaramoorthy B, Bae SA *et al.* VMAT2 imaging of baboons with two ¹⁸F-FP-DTBZ enantiomers. *J. Nucl. Med.* 50, 227 (2009).
- Josan JS, Handl HL, Sankaranarayanan R *et al.* Cell-specific targeting by heterobivalent ligands. *Bioconjugate Chem.* 22, 1270–1278 (2011).
- Shiue C-Y, Schmitz A, Schirrmacher R, Shiue GG, Alavi A. Potential approaches for beta cell imaging with PET and SPECT. *Curr. Med. Chem. Immun. Endoc. Metab. Agents* 4, 271–280 (2004).
- Schmitz A, Shiue CY, Feng Q *et al.* Synthesis and evaluation of fluorine-18 labeled glyburide analogs as beta-cell imaging agents. *Nucl. Med. Biol.* 31, 483–491 (2004).
- Wangler B, Schneider S, Thews O *et al.* Synthesis and evaluation of (S)-2-(2-[F-18] fluoroethoxy)-4-([3-methyl-1-(2-piperidin-1-yl-phenyl)-butylcarbamoyl]-methyl)-benzoic acid ([F-18]repaglinide): a promising radioligand for quantification of pancreatic beta-cell mass with positron emission tomography (PET). *Nucl. Med. Biol.* 31, 639–647 (2004).
- Moore A, Bonner-Weir S, Weissleder R. Noninvasive *in vivo* measurement of β -cell

- mass in mouse models of diabetes. *Diabetes* 50, 2231–2236 (2001).
- 23 Ueberberg S, Meier JJ, Waengler C *et al.* Generation of novel single-chain antibodies by phage-display technology to direct imaging agents highly selective to pancreatic β - or α -cells *in vivo*. *Diabetes* 58, 2324–2334 (2009).
- 24 Brom M, Andraojc K, Oyen WJG, Boerman OC, Gotthardt M. Development of radiotracers for the determination of beta-cell mass *in vivo*. *Curr. Pharm. Design* 16, 1561–1567 (2010).
- 25 Ahren B. Islet G-protein-coupled receptors as potential targets for treatment of Type 2 diabetes. *Nat. Rev. Drug Discovery* 8, 369–385 (2009).
- 26 Harris PE, Ferrara C, Barba P, Polito T, Freeby M, Maffei A. *VMAT2* gene expression and function as it applies to imaging beta-cell mass. *J. Mol. Med.* 86, 5–16 (2008).
- 27 Ichise M, Harris PE. Imaging of beta-cell mass and function. *J. Nucl. Med.* 51(7), 1001–1004 (2010).
- 28 Reiner T, Kohler RH, Wee Liew CW *et al.* Near-infrared fluorescent probe for imaging of pancreatic β -cells. *Bioconjugate Chem.* 21, 1362–1368 (2010).
- 29 Wild D, Wicki A, Mansi R *et al.* Exendin-4-based radiopharmaceuticals for glucagon-like peptide-1 receptor PET/CT and SPECT/CT. *J. Nucl. Med.* 51, 1059–1067 (2010).
- 30 Brom M, Oyen WJ, Joosten L, Gotthardt M, Boerman OC. ⁶⁸Ga-labelled exendin-3, a new agent for the detection of insulinomas with PET. *Eur. J. Nucl. Med. Mol. Imaging* 37, 1345–1355 (2010).
- 31 Brom M, Baumeister P, Melis M *et al.* Determination of the beta-cell mass by SPECT imaging with In-111-DTPA-Exendin-3 in rats. *J. Nucl. Med.* 50(Suppl. 2), 147 (2009).
- 32 Mukai E, Toyoda K, Kimura H *et al.* GLP-1 receptor antagonist as a potential probe for pancreatic beta-cell imaging. *Biochem. Biophys. Res. Commun.* 389, 523–526 (2009).
- 33 Xu G, Kaneto H, Laybutt DR *et al.* Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. *Diabetes* 56, 1551–1558 (2007).
- 34 Cheong YH, Kim MK, Son MH, Kaang BK. Glucose exposure pattern determines glucagon-like peptide-1 receptor expression and signaling through endoplasmic reticulum stress in rat insulinoma cells. *Biochem. Biophys. Res. Commun.* 414, 220–225 (2011).
- 35 Saisho Y, Harris PE, Butler AE *et al.* Relationship between pancreatic vesicular monoamine transporter 2 (*VMAT2*) and insulin expression in human pancreas. *J. Mol. Histol.* 39, 543–551 (2008).
- Establishes the constancy of vesicular monoamine transporter type 2 (*VMAT2*) in β -cells in health and diabetes. Justifies the use of *VMAT2* as a biomarker of BCM.
- 36 Maffei A, Liu Z, Witkowski P *et al.* Identification of tissue-restricted transcripts in human islets. *Endocrinology* 145, 4513–4521 (2004).
- 37 DaSilva JN, Kilbourn MR. *In vivo* binding of [¹¹C]tetrabenazine to vesicular monoamine transporters in mouse brain. *Life Sci.* 51, 593–600 (1992).
- 38 Kung MP, Hou C, Lieberman BP *et al.* *In vivo* imaging of beta-cell mass in rats using ¹⁸F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus. *J. Nucl. Med.* 49, 1171–1176 (2008).
- 39 Bohnen NI, Albin RL, Koeppe RA *et al.* Positron emission tomography of monoaminergic vesicular binding in aging and Parkinson disease. *J. Cereb. Blood Flow Metab.* 26, 1198–1212 (2006).
- 40 Martin WR, Wieler M, Stoessl AJ, Schulzer M. Dihydrotetrabenazine positron emission tomography imaging in early, untreated Parkinson's disease. *Ann. Neurol.* 63(3), 388–394 (2008).
- 41 Sousa F, Simpson N, Raffo A *et al.* Longitudinal noninvasive PET-based β -cell mass estimates in a spontaneous diabetes rat model. *J. Clin. Invest.* 116, 1506–1513 (2006).
- 42 Simpson NR, Souza F, Witkowski P *et al.* Visualizing pancreatic β -cell mass with [¹¹C]DTBZ. *Nuc. Med. Biol.* 33, 855–864 (2006).
- 43 Goland R, Freeby M, Parsey R *et al.* ¹¹C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing Type 1 diabetes and in healthy controls. *J. Nucl. Med.* 50, 382–389 (2009).
- Phase I clinical trial that establishes the proof-of-principle that *VMAT2* PET imaging can be used to measure changes in BCM associated with Type 1 diabetes mellitus.
- 44 Kim JS, Ichise M, Sangare J, Innis RB. PET imaging of serotonin transporters with [¹¹C]DASB: test–retest reproducibility using a multilinear reference tissue parametric imaging method. *J. Nucl. Med.* 47(2), 208–214 (2006).
- 45 Hirvonen J, Aalto S, Hagelberg N *et al.* Measurement of central mu-opioid receptor binding *in vivo* with PET and [¹¹C]carfentanil: a test–retest study in healthy subjects. *Eur. J. Nucl. Med. Mol. Imaging* 36(2), 275–286 (2009).