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Reply:

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I want to thank Gotthardt and colleagues for bringing this important clinical question to the forefront of discussion – the independent measurement of BCM and beta cell function. Indeed, this distinction has major implications for both type 1 and type 2 diabetes research. Their group and others have pioneered GLP-1 receptor (GLP-1R) imaging in the clinic, moving this research beyond preclinical animal models. Despite great progress, significant challenges remain.

Challenges

Beta cells present an extremely difficult imaging target in the clinic in part due to their low fraction (~1%) in the pancreas and small size of islets resulting in significant volume averaging with surrounding exocrine tissue. In addition, as Willeken's et al. have shown[1], the expression of GLP-1R has significant inter-species variability. The important question for the clinic is if and how much GLP-1R protein expression exists in off-target cells in humans. Specifically, expression of GLP-1R on exocrine cells is critical, since even expression levels 100-fold lower than beta cells can still be significant given their abundance relative to beta cells, and estimates have indicated 1000-fold lower is needed for BCM quantification[2].

Unfortunately, many studies cited previously[3] indicate measurable expression of GLP1-R protein in human exocrine cells[4]. The importance of quantifying target expression cannot be overstated, and this has to be done at the protein level. In this case, the transcriptional data is not representative of protein levels, which are the relevant metric for molecular imaging. For example, the mRNA expression data from Willekens et al. show similar mouse and rat mRNA endocrine to exocrine ratios despite very different protein levels[1]. Absolute quantification (i.e. number of receptors per cell) is critically important. The 'small differences in exocrine pancreatic uptake between wild type and GLP-1R knock-out mice' on a per-cell basis translate into over half of the total signal in the pancreas due to their 100-fold higher prevalence. For mice, we quantified 54,000 GLP-1R per beta cell and ~50-fold lower levels for exocrine cells (~40-fold lower based on single-cell flow cytometry measurements[3] and 60-fold lower based on bulk %ID/g[5]). Indeed, 1,000 receptors per cell is below the limit of detection for most single-cell and tissue fluorescent methods and can be easily overlooked compared to the intense beta cell staining. Despite this difficulty in detection, a robust signal unfortunately remains, and this level in mice is well above the limits necessary to cause problems in whole body imaging.

Relevance

To clarify, we do not claim "the lack of clinical distinction between healthy volunteers and subjects with long-term diabetes" was caused by exocrine uptake, but rather the full sentence indicates this is a *possible* result consistent with the evidence. To rigorously and unambiguously identify residual BCM in patients, uptake from the exocrine pancreas has to be discounted.

Even more problematic, Waser and Reubi demonstrated only about half of human samples (3 of 5) appear to express detectable exocrine signal[6]. If it were uniform among patients, we would

agree that some long-standing T1D patients showing background levels would support a lack of exocrine uptake. However, since some patients appear to lack exocrine expression, the background levels could simply be the patients lacking exocrine expression. Now in contrast, the preliminary results reported by Gotthardt et al. indicate less variability in the 7 patients they studied. Here, the endocrine to exocrine ratio was 3.9 +/- 0.5 with little variation. However, this number is very similar to the ratios they reported in mice (4.11 +/- 0.9 and 4.56 +/- 0.9) rather than rats (44 to 106). This clinical data appears consistent with histology reports of significant human exocrine GLP-1R expression[7, 8].

Although these clinical data are similar to mice, it does not mean the absolute expression levels are the same in mice and humans. At the tracer doses used, it is more likely a reflection of delivery, i.e. higher vascularization in endocrine versus exocrine tissue. This is why the mouse autoradiography ratio is only ~4 at tracer doses while the absolute expression differences we measured are close to 50 at saturating doses. It is currently unclear what this ratio is in humans.

Where do we go from here?

The most recent data reported by Gotthardt and colleagues is exactly the type of data that needs to be collected if we want to determine whether the exocrine uptake can be selectively blocked. I commend them for their significant efforts to collect this important data, since pancreatic samples are difficult to obtain yet exactly the type of data the scientific community needs. Similar to our collaboration using intraoperative imaging agents to look at antibody distribution in tumors and associated healthy tissue[9], *ex vivo* analysis can enable absolute probe uptake following *in vivo* administration.

If their preliminary results continue to mimic the mouse pancreas, selective blocking may be needed to suppress exocrine uptake and reliably detect beta cells as we propose. The method we utilized in mice is not perfect, and the absolute endocrine/exocrine expression ratio in humans likely needs to be greater than in mice to practically work in humans. However, potential blocking agents are available that could be used in a similar study. While we used a lipophilic fluorescent dye to simultaneously bind albumin and facilitate imaging, a lipophilic conjugate like liraglutide would be a potential FDA-approved blocking agent.

The path is difficult, but it's an exciting time to investigate beta cell biology. Importantly, these results do not discount the potential presence of beta cells within the diabetic pancreas. Rather, they indicate that the exocrine expression level has to be addressed to definitively image residual BCM within the human pancreas. The method outlined in Khera et al. is one approach that can be pursued in humans to address this issue. The work by the Gotthardt lab and others staining for beta cell markers such as GLP-1R in patients with long-standing diabetes raises the specter of reversing this disease. To interpret these imaging results, we need to definitively know the endocrine versus exocrine uptake of these molecular probes. Exendin has many attributes of an ideal imaging agent – tight binding, high retention from metabolic trapping, low non-specific sticking, and rapid clearance. We just need to ensure the cellular specificity to be able to utilize this agent in the clinic.

References

1. Willekens, S.M. et al. Strain Differences Determine the Suitability of Animal Models for Noninvasive In Vivo Beta Cell Mass Determination with Radiolabeled Exendin. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging* **18**, 705-714 (2016).
2. Sweet, I.R., Cook, D.L., Lernmark, A., Greenbaum, C.J. & Krohn, K.A. Non-invasive imaging of beta cell mass: a quantitative analysis. *Diabetes technology & therapeutics* **6**, 652-659 (2004).
3. Khera, E. et al. Blocking Glucagon Like Peptide-1 Receptors in the Exocrine Pancreas Improves Specificity for Beta Cells in a Mouse Model of Type 1 Diabetes. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* (2019).
4. Pyke, C. et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* **155**, 1280-1290 (2014).
5. Zhang, L. & Thurber, G.M. Quantitative Impact of Plasma Clearance and Down-regulation on GLP-1 Receptor Molecular Imaging. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging* (2015).
6. Waser, B. & Reubi, J.C. Radiolabelled GLP-1 receptor antagonist binds to GLP-1 receptor-expressing human tissues. *Eur J Nucl Med Mol Imaging* **41**, 1166-1171 (2014).
7. Korner, M., Stockli, M., Waser, B. & Reubi, J.C. GLP-1 receptor expression in human tumors and human normal tissues: Potential for in vivo targeting. *Journal of Nuclear Medicine* **48**, 736-743 (2007).
8. Kirk, R.K. et al. Immunohistochemical assessment of glucagon-like peptide 1 receptor (GLP-1R) expression in the pancreas of patients with type 2 diabetes. *Diabetes, obesity & metabolism* **19**, 705-712 (2017).
9. Lu, G. et al. Co-administered antibody improves penetration of antibody-dye conjugate into human cancers with implications for antibody-drug conjugates. *Nat Commun* **11**, 5667 (2020).