**Final progress report**

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**Project title:**

Structural-functional changes in primary cilia in T1D pancreas

**Project Progress:**

From March 2021 to March 2022, we used funds from Diabetes Research Connection to obtain key reagents and human donor pancreatic tissue from Prodo Labs to develop and optimize human islet staining protocols. We applied this knowledge to perform immunostaining of primary cilia in human islet tissues. Main accomplishments include:

* Immunofluorescence imaging:
* Established expression of canonical cilia markers including Arl13b, acetylated alpha tubulin, centrin, and polyglutamylated tubulin in human islets and exocrine tissue including acinar and ductal cells.
* Demonstrated human donor islet heterogeneity in the abundance and distribution of cilia among endocrine cells
* Live imaging:
* Following a novel hypothesis about islet cilia possessing dynein-driven motility, we made a breakthrough observation in our imaging experiments, demonstrating in fixed human islet tissue that primary cilia in fact contained motile cilia proteins that would confer the ability for cilia to move.
* Subsequently, we were able to use live-cell cilia sensors to capture for the first time movement of human islet cilia by confocal imaging. This is an extraordinary finding as it re-defines the classification of primary cilia as kinetic structures that possess both sensory and motile function.
* Cilia inhibition studies:
* As a clinical correlate to our imaging studies, we tested whether cilia motility is required for islet insulin secretion. We assayed for static insulin secretion where we observed blocking cilia motility using chemical inhibitors or by motility gene knockdown led to reduced ability of islets to secrete insulin.
* Secondly, in pursuit of a mechanistic explanation for reduced insulin secretion, we examined the intracellular calcium dynamics after cells were treated with cilia motility inhibitors. We found that, if cilia are prevented from moving, then the cilia are prevented from activating beta cells in response to glucose by altering their calcium entry.
* Ongoing studies using knockdown and knockout animal models are testing the hypothesis that the dynein genes DNAI1 and DNAH5 are responsible for islet cilia motility and maintenance of glucose homeostasis in the whole animal. These studies will be novel as it has never been demonstrated that cilia motility is linked to diabetes.

The above work using DRC funding contributed to the following manuscript which is in review at *Science Advances,* preprint at Biorxiv: https://www.biorxiv.org/content/10.1101/2021.12.14.472629v1.full.

Research support by the DRC was acknowledged in the manuscript.

Cho JH, Li ZA, Zhu L, Muegge BD, Roseman HF, Utterback T, Woodhams LG, Bayly PV, Hughes JW. Islet primary cilia motility controls insulin secretion. *BioRxiv* preprint published December 2021, manuscript in review.

Importantly, our work on the DRC project has fostered new collaborations between our group and other islet biologists at Indiana University and the University of Chicago, leading to a U01 grant proposal in spring 2022. New joint experiments are ongoing to apply our findings to human T1D and will likely result in more publications and extramural funding by the NIH.

Once again we are grateful for this unique funding opportunity through the Diabetes Research Connection. Your funds bridged a critical period in our lab’s work and enabled discovery-based, patient-oriented research in T1D. We would like to express our heartfelt thanks to the donors and the Diabetes Research Connection administrative team for your vision in supporting our work.