Previous work in our lab has shown that low-frequency, low-intensity ultrasound can stimulate the release of insulin from pancreatic β-cells with no statistically significant effect on cell viability. The purpose of our current work is to study the mechanisms behind ultrasound-mediated insulin secretion, specifically its calcium dependence.

Type 2 diabetes mellitus is characterized by systemic insulin resistance, β-cell dysfunction and insufficient β-cell mass due to genetic and environmental factors.

Prevalence of Type 2 Diabetes
- As of 2015, 29 million Americans had type 2 diabetes and 1.5 million new cases of diabetes are diagnosed every year.
- Diabetes remains the 7th leading cause of death in the US.

Cost of Diabetes
In patients diagnosed with type 2 diabetes at between 25-44 years of age, the lifetime cost of medical treatment is $124,700 for women and $130,800 for men.

We are interested in low-frequency, low-intensity therapeutic ultrasound where the mechanical effects, not thermal effects, dominate. Ultrasound has been shown to induce calcium influx in various cells types including fibroblasts, neurons, and chondrocytes, among others. The primary mechanisms of this calcium influx are transient membrane poration from acoustic caviation and mechanotransduction.

Previous Studies
We have shown that ultrasound can be used to safely and effectively stimulate insulin release. No statistically significant difference in cell viability following ultrasound was found at 800 kHz and 1 MHz (Fig 1). A physiologically relevant increase in insulin was seen following five minutes of 800 MHz ultrasound.

All studies were conducted on INS-1 cells, a rat insulinoma pancreatic beta cell line. 800 kHz ultrasound was applied at 0.5 W/cm² at durations ranging from 5 to 5 min. Calcium dependence was tested through exposure to EGTA, an extracellular calcium chelator.

I. Carbon Fiber Amperometry
- Dopamine and L-DOPA loaded into the cells are co-released with insulin.
- Qualitative information about insulin release with high temporal resolution.

II. ELISA
- Samples of extracellular fluid taken before, during, and after ultrasound stimulation.
- Quantitative information about insulin release with lower temporal resolution.

III. Ca²⁺ Fluorescence Imaging
- Transducer placed into a KBS-filled wave coupler that positions it at the δx distance from the cells.
- Cells stained with Quest Rhod4AM
- Information about calcium dynamics with high temporal resolution.

Amperometry revealed an immediate response to ultrasound stimulation that lasted the duration of the stimulus (n=5). ELISA showed significant release of insulin in response to 800 kHz ultrasound as compared to sham and a glucose positive control (n=6, p<0.001). Ca²⁺ imaging studies indicated a calcium mobilization in response to ultrasound stimulation about 5 s after the start of the 10 s stimulus (n=3).

Future studies will focus on further probing the mechanism behind ultrasound-induced insulin release. Experimentally determine the impact of intracellular calcium and stretch-activated cation channels will be performed. In addition, we will also be moving towards more physiologically relevant models, including whole tissue and animal models.

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