

Electronic Supporting Information for

Microfluidic-enabled quantitative measurements of insulin release dynamics from single islets of Langerhans in response to 5-palmitic acid hydroxy stearic acid

*Basel Bandak, Lian Yi, and Michael G. Roper**

*Department of Chemistry and Biochemistry, Florida State University, 95 Chieftain Way,
Tallahassee, FL 32306*

Abstract. Additional details outlining the perfusion system, immunoassay, data analysis and processing, and human islet donor characteristics are presented. Results showing the characterization of the microfluidic system, finite element simulations of insulin release, PA exposure data, FFT analysis, and complete 48-h incubated human and murine islet GSIS data.

*Address Correspondence to:

Dr. Michael G. Roper
Department of Chemistry and Biochemistry
Florida State University
95 Chieftain Way
Dittmer Building
Tallahassee, FL 32306
Ph 850-644-1846
Fx 850-644-8281
E-mail: roper@chem.fsu.edu

Perfusion system

Perfusion solutions were held in two 60 mL plastic syringe tubes that were connected to the microfluidic device by Tygon tubing (0.03 in. o.d., 0.01 in. i.d., Cole-Parmer, Vernon Hills, IL). The perfusion solutions consisted of BSS and were supplemented with either glucose, PA, or 5-PAHFA as described in the text. The desired solution was delivered to the microfluidic device by changing the relative heights of the syringes, as described in detail elsewhere.¹⁻³ The volumetric flow rate of the perfusion solutions was a function of syringe height, and the mixing ratio of the two perfusion solutions as a function of relative syringe height was calibrated as previously described.^{2,3}

For the characterization of the perfusion system, BSS containing 200 nM insulin and 1 μ M fluorescein was placed in one syringe and BSS without insulin and fluorescein in the other. To calculate perfusion flow rate, a CCD camera (QImaging, Surrey, BC, Canada) was used to measure the time required for a fluorescent signal to be detected a specified distance in the perfusion channel after a change in solution from BSS to BSS with fluorescein and insulin.

Immunoassay system

A laser-induced fluorescence (LIF) detection system was used as previously described.^{2,3} Briefly, a 100 mW 635 nm laser (AixiZ, Houston, TX) was used as the excitation source. This light passed through a neutral density filter wheel and an iris prior to impinging on a dichroic filter cube that held a 405/488/561/635 nm dichroic mirror (Semrock). The light was then directed to the back of a 40X, 0.6 NA objective (Nikon Instruments, Inc.) and emission was collected with the same objective and reflected back through the dichroic cube, through a spatial filter, a 446/523/600/677 nm emission filter (Semrock), and a linear polarizer before reaching a PMT

(Hamamatsu Photonics, Middlesex, NJ). The spatial filter, emission filter, polarizer, and the PMT were all housed in a photometer (Photon Technology International, Inc., Birmingham, NJ). Data were collected using software written in LabView (National Instruments, Austin, TX).

Prior to experimentation, the microfluidic device was conditioned for 10-min with 1M NaOH, deionized water, and immunoassay reagents (Cy5-insulin (Ins*) and Ab). At the end of the day, the devices were conditioned with water for at least 10-min prior to storage in water. Reservoirs were capped with modified plastic caps that accommodated platinum electrodes for the application of voltages. For all experimentation, the immunoassay reagents used were 150 nM Ins* and 150 nM Ab and were prepared daily in a buffer containing 25 mM tricine, 1 mM EDTA, 40 mM NaCl, 1 mg mL⁻¹ BSA and 0.1% Tween-20, at a final pH of 7.4. For the remainder of the ESI and throughout the main text, the concentrations of the immunoassay reagents are referred to as the concentration in the fully mixed state (50 nM each), which assumes a 3-fold dilution in the microfluidic channels. The gate and waste reservoirs contained separation buffer that comprised 150 mM tricine and 20 mM NaCl at a final pH of 7.4. During experiments, -5000 V (UltraVolt, Inc., South Thief River Falls, MN) was applied to the waste reservoir, while the Ins*, Ab, and insulin sampling reservoirs were grounded. The sample was introduced into the separation channel using a flow-gated injection scheme⁴ that utilized a high-voltage relay (Gigavac, Carpinteria, CA). The EOF system was calibrated by introducing various concentrations of insulin (0 – 600 nM) in BSS into the insulin sampling reservoir and the ratio of bound (B) and free (F) Ins* (B/F) was measured.

Data analysis and processing

An automated program was used to analyze the immunoassay electropherograms and to calculate the B/F using the peak heights.⁵ For the calibration curve, 15 successive electropherograms were used to calculate an average B/F which was plotted against insulin concentration. The plot was fitted with a weighted four-parameter logistic function. Error bars are presented as ± 1 standard deviation of each standard point unless otherwise noted. The limits of detection were calculated by using the calibration curve to calculate the concentration of insulin that would decrease the B/F of a blank solution by an amount greater than three times the standard deviation of the blank. The temporal resolution was calculated by averaging the rise and fall times (at 10% and 90% of the B/F signal change) during perfusions of standard insulin square waves.

Human islet donor characteristics

Healthy donor #1 was an 18 year old African American female, 5'3", 78 kg, BMI of 30.4, HbA1c of 5.1%.

Healthy donor #2 was a 40 year old Caucasian female, 64", 66 kg, BMI of 25, HbA1c was 5.7%.

T2DM donor #1 was a 35 year old Caucasian male, 69", 85 kg, BMI of 27.7, HbA1c was 6.3%. The donor had been diagnosed with T2DM for 3 years and had been on Metformin.

T2DM donor #2 was a 45 year old Hispanic male, 67", 79 kg, BMI of 27.3, HbA1c was 6.5%.

Finite element simulations

The proposed microfluidic system featuring the islet chamber, the perfusion channel, and the insulin sampling chamber was developed in a finite element simulation software (COMSOL

Multiphysics v4.3, COMSOL Inc., Stockholm, Sweden). Flow rates were solved using the incompressible Navier-Stokes equation with a no slip boundary condition on solid surfaces, while concentration profiles were solved using the convection-diffusion equation. The coordinates of the islets relative to the center point of the chamber (0,0) are (in μm): Position A: (75,10); Position B: (0,0); Position C: (-75,-10). All simulation parameters are given in **Table S1**.

Table S-1.**Parameters of the finite element simulation.**

Temperature (T)	310.15 K
Viscosity (η)	$1 \times 10^{-3} \text{ N s m}^{-2}$
Density (ρ)	$1 \times 10^3 \text{ kg m}^{-3}$
Volumetric Flow Rate (V)	$0.2 - 0.3 \text{ } \mu\text{L min}^{-1}$
Insulin Diffusion Coefficient (D)	$2.5 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$

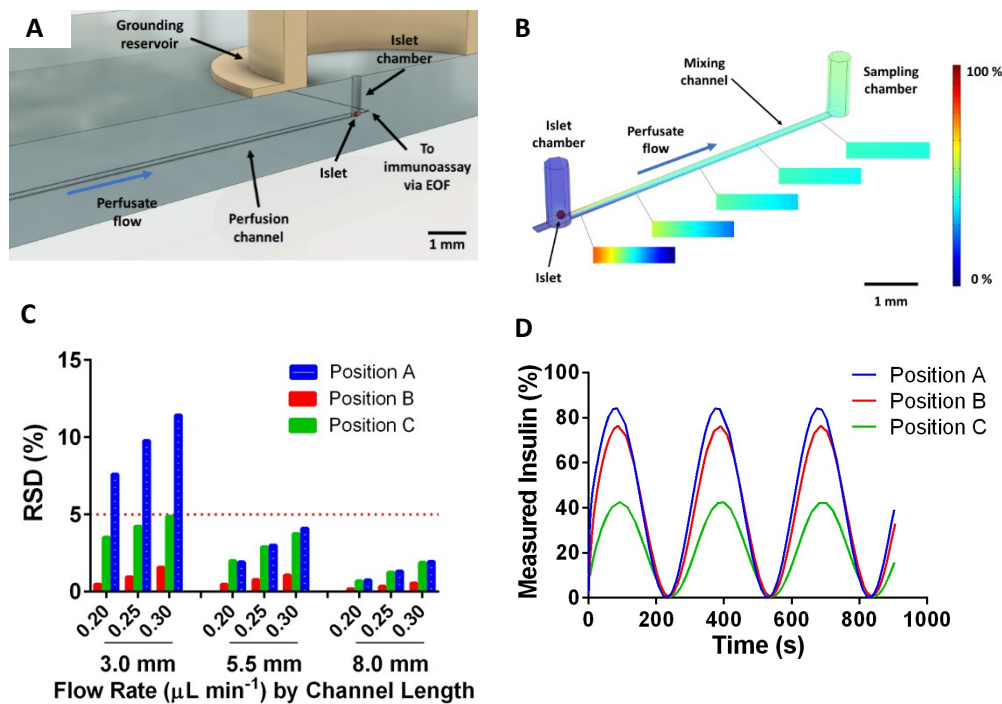


Fig. S1. Previous microfluidic system and finite element simulations. (A) 3D model of the previous islet chamber system^{2, 3}. Shown by the blue arrow, the flow from the perfusion system traveled through the perfusion channel and out the islet chamber and collected in the grounding reservoir. A Pt electrode (not shown) was inserted into the grounding reservoir and maintained a ground potential on the reservoir, which induced a continuous sampling of the perfusate into the EOF channel. Due to the size difference between the islet chamber and EOF channel, virtually no pressure-driven flow from the perfusion system entered the EOF channel. (B) Finite element analysis model of the new quantitative chamber system showing the islet chamber, the mixing channel, and the sampling chamber. As the perfusion solution flows through the islet chamber, it captures all of the releasate where it homogenizes in the mixing channel prior to reaching the sampling chamber. The release is being simulated from the islet and its concentration is shown as 0 – 100% by the color (scale bar on right). The concentration profile across the mixing channel is shown in 5 different positions. (C) The RSD at the final slice shown in (B), before the sampling chamber, is provided for 3 channel lengths at 3 different flow rates at 3 varying spatial islet positions within the chamber. The dashed red line indicates an RSD of 5% which was used to determine a minimum acceptable homogeneity. (D) The simulated insulin profile from an islet situated at 3 different positions within the previous microfluidic system (shown in Fig. 1B) demonstrate that the measured insulin values depend on the location of the islet within the chamber.

Characterization of microfluidic system

The integrated microfluidic system was characterized at a flow rate of $0.25 \mu\text{L min}^{-1}$ by perfusing insulin in 10 min square wave pulses over the course of an hour-long experiment (**Fig. S2**). The delay time was calculated by measuring the time it took to observe a signal rise upon a change in syringe position. That is, how long it takes for insulin to mobilize from the syringe to the LIF detection point in the EOF separation channel, and was calculated to be $2.9 \pm 0.2 \text{ min}$. The response time was calculated as the average of the rise and fall times of the signal from 10 - 90% and 90 - 10%, respectively, which was calculated to be $84 \pm 10 \text{ s}$.

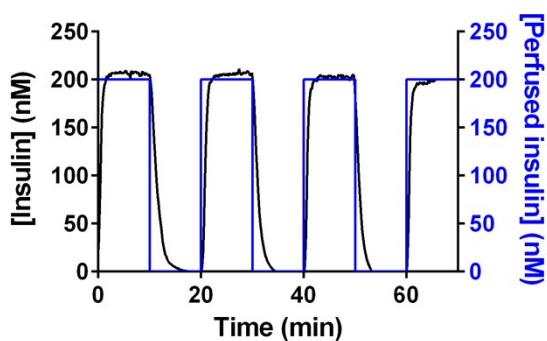


Fig. S2. Microfluidic system characterization. Insulin was perfused in a square wave (blue line, right y-axis) and quantified by the immunoassay (black line, left y-axis).

Acute stimulation of murine islets with PA

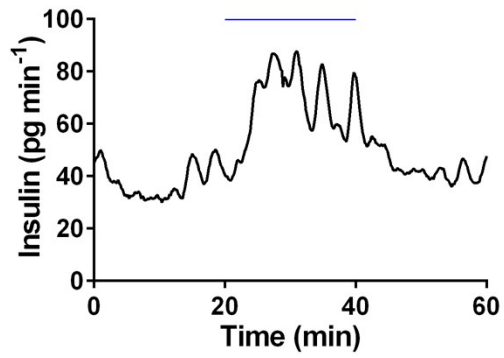


Fig. S3. Acute stimulation of murine islet with PA. A representative single islet response to 0.5 mM PA under 11 mM glucose conditions is shown. The delivery profile of PA is represented by the blue bar.

Glucose stimulation of murine islets after 48-h incubation

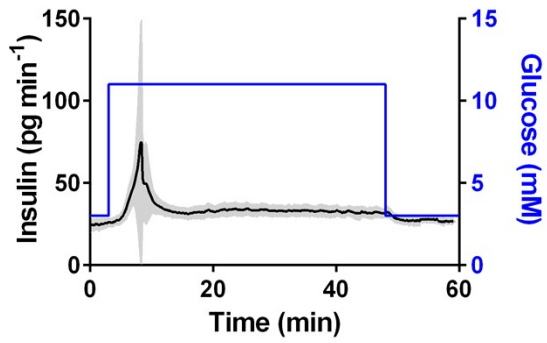
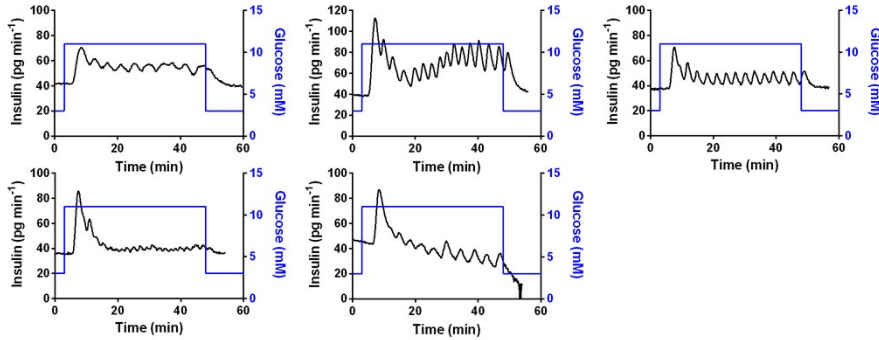


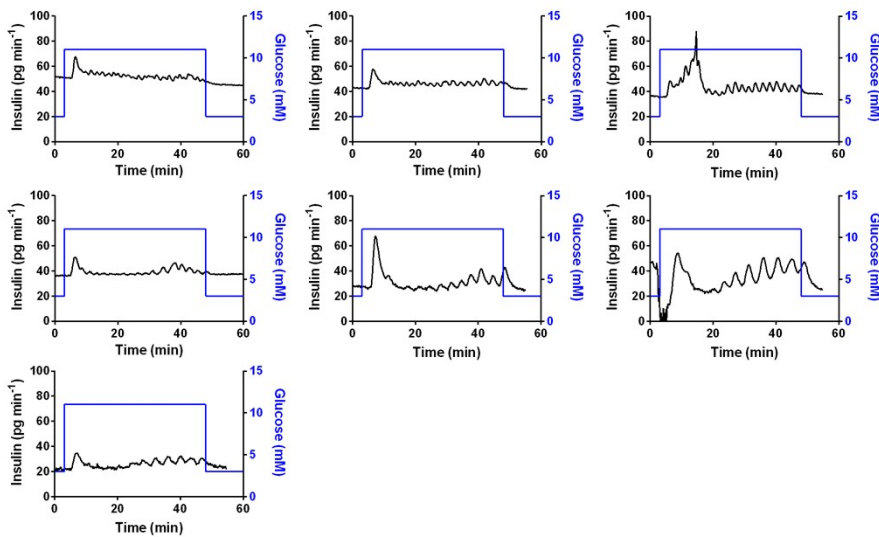
Fig. S4. Chronic incubations of murine islets with PA. The average (± 1 SD) GSIS from murine islets after 48-h incubation with 0.5 mM PA ($n = 5$ islets).

Glucose stimulation of murine islets after 48-h incubation

A. (+) 5-PAHSA



B. (-) 5-PAHSA



C. (+) PA

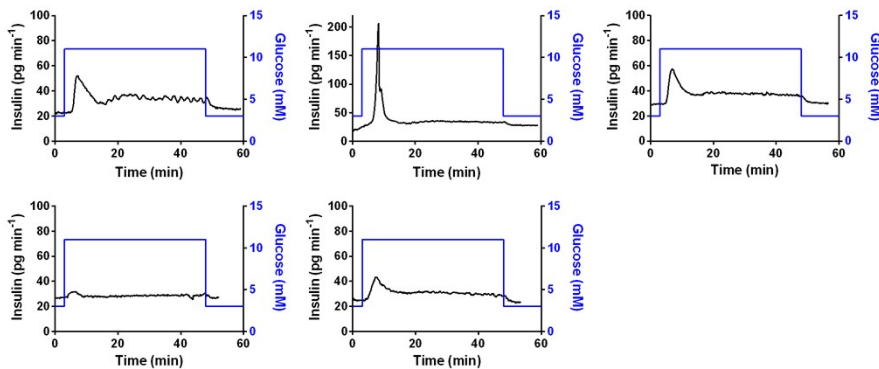


Fig. S5. Islet secretory traces of insulin from all chronically incubated murine islets. GSIS profiles for each islet sampled in (A) 5-PAHSA, (B) lipid-free, and (C) PA culture media conditions.

FFT analysis of chronically-incubated murine islet data

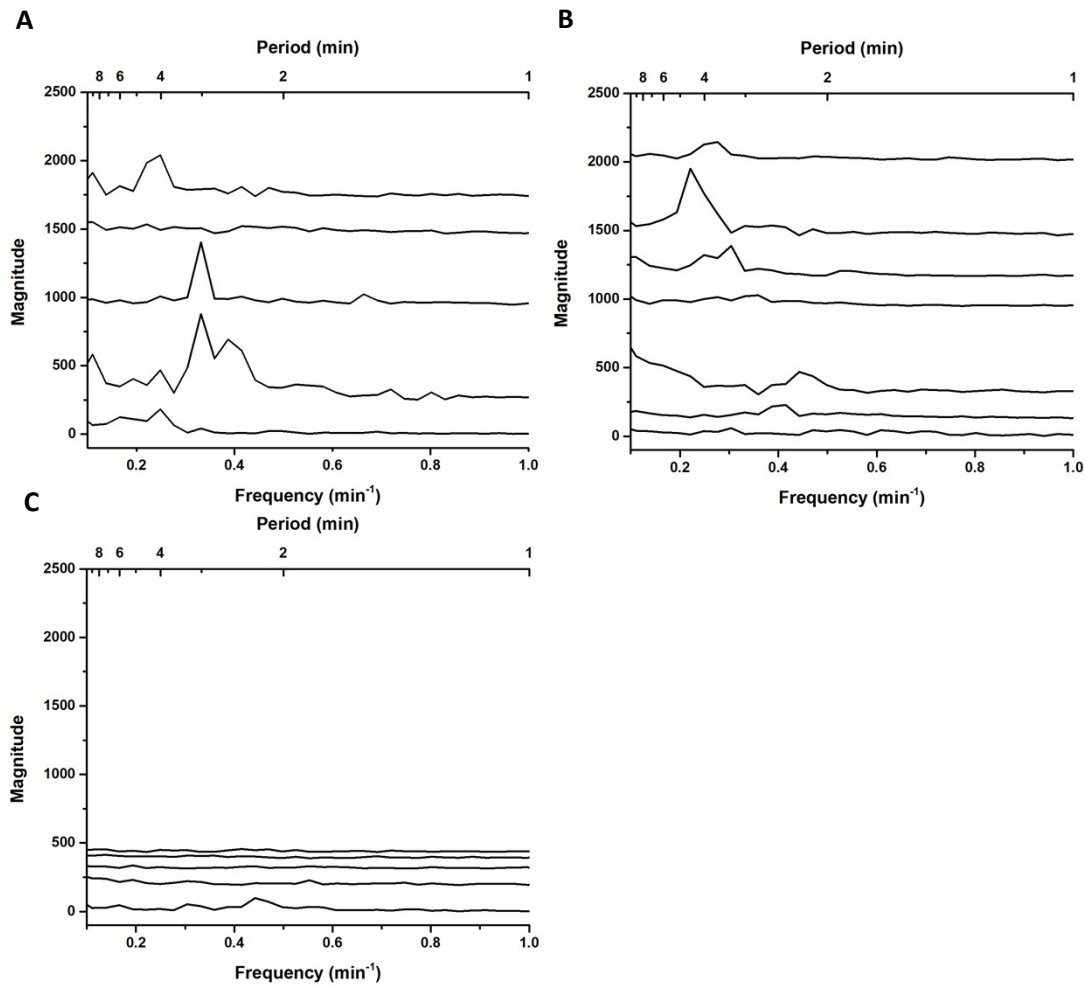


Fig. S6. Spectral analysis of chronically-incubated murine islet release during phase 2. (A) The FFT results for the 5-PAHSA pre-treated islets showed major oscillatory periods of 3.0 and 4.0 min for their 2nd phase GSIS. (B) Islets that were incubated without 5-PAHSA had lower pulsatility than their 5-PAHSA counterparts, with 4/7 islets oscillating with periods of 2.5, 3.5, and 5 min. (C) The PA-treated batch showed almost no pulsatility in their GSIS.

Glucose stimulation of human donor islets after 48-h incubation

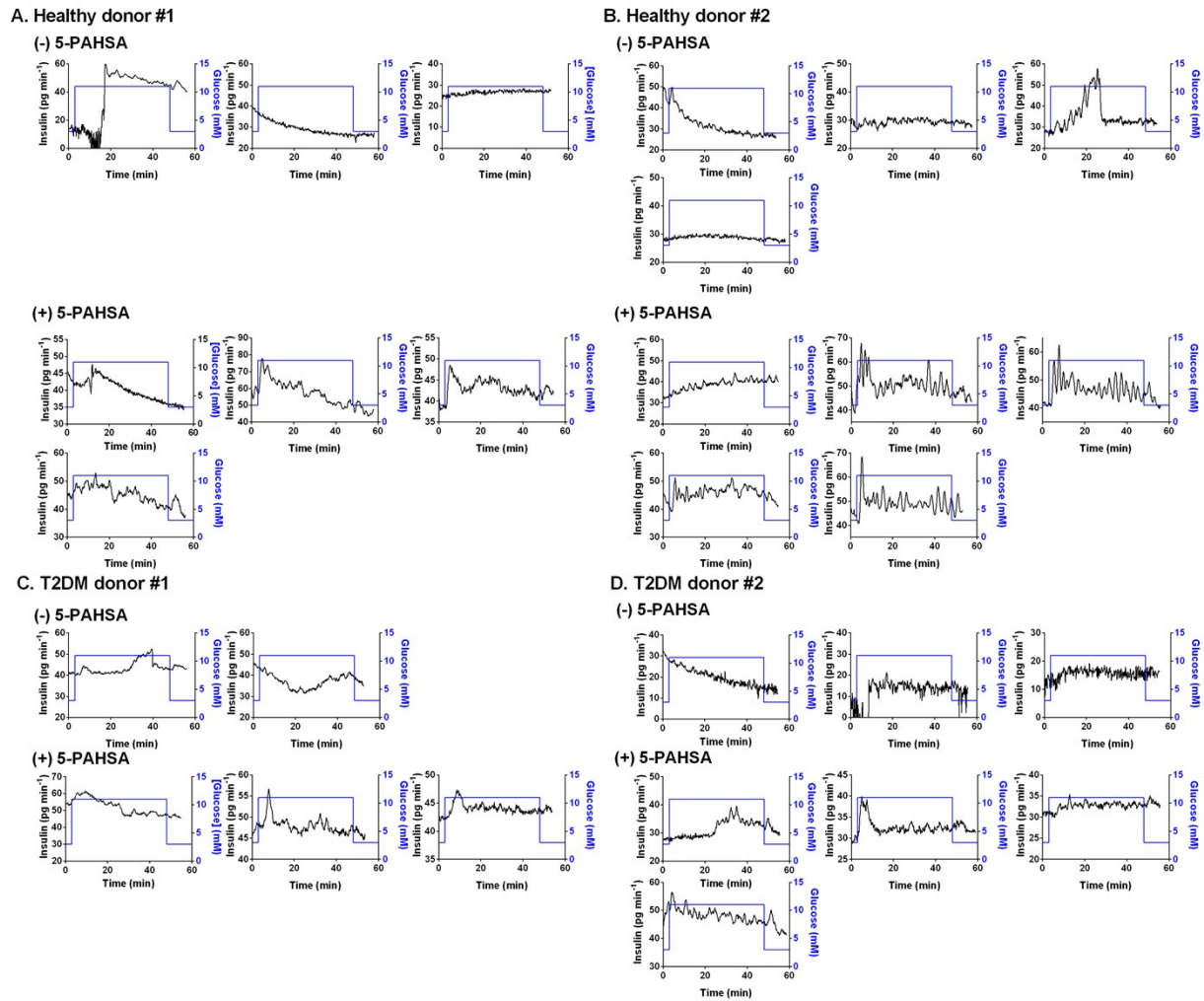


Fig. S7. Islet secretory traces of insulin from all human islets. GSIS profiles for each islet sampled in the lipid-free ((-) 5-PAHSA) and 5-PAHSA ((+) 5-PAHSA) culture media conditions. The islets came from (A) Healthy donor #1, (B) Healthy donor #2, (C) T2DM donor #1, (D) T2DM donor #2.

FFT analysis of chronically-incubated human islet data

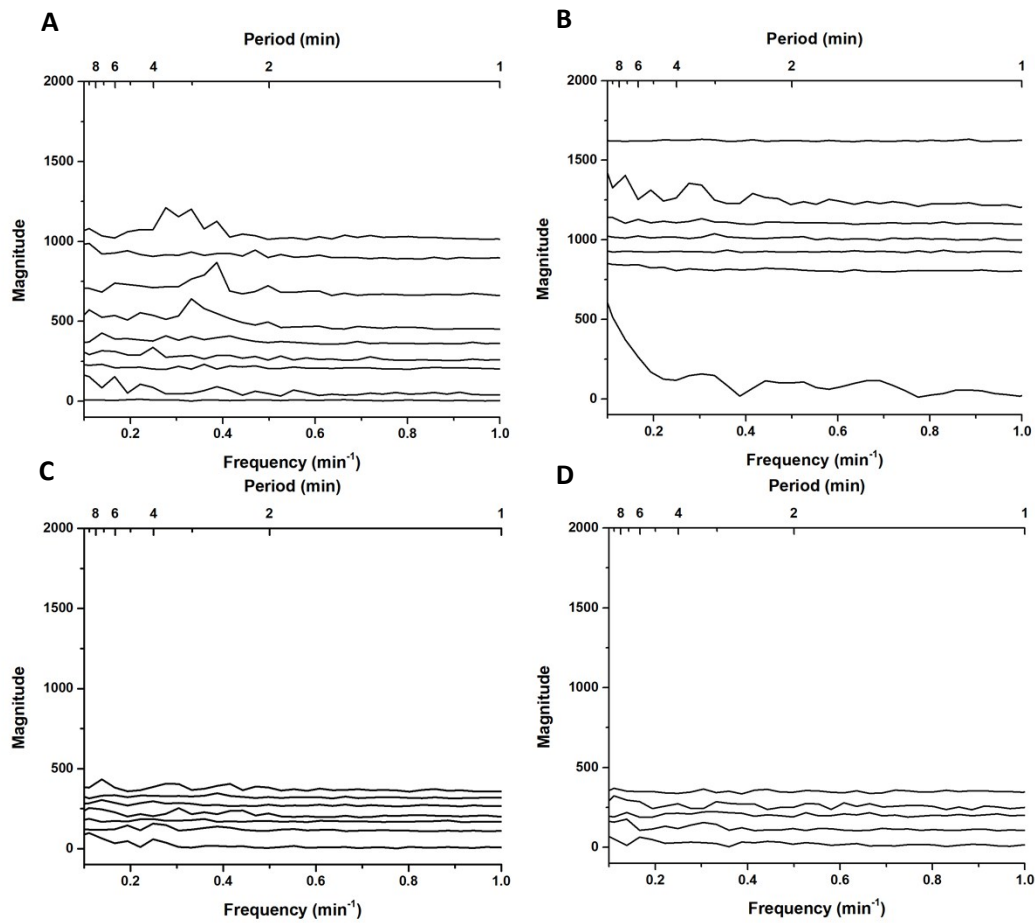


Fig. S8. Spectral analysis of chronically incubated human islet data. To assess the oscillatory nature of human islet responses, an FFT was performed on the 2nd phase of each human islet trace for the healthy human donor islets incubated (A) with 5-PAHSA (n = 9) and (B) without 5-PAHSA (n = 7), as well as T2DM human islets samples incubated (C) with 5-PAHSA (n = 7) and (D) without 5-PAHSA (n = 5).

References

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