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

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Abstract

Proper release of insulin from pancreatic islets of Langerhans is essential for maintaining glucose homeostasis. For full efficacy, both the pattern and the amount of hormone release are critical. It is therefore important to understand how insulin levels are secreted from single islets in both a quantitative fashion and in a manner that resolves temporal dynamics. In this study, we describe a microfluidic analytical system that can both quantitatively monitor insulin secretion from single islets while simultaneously maintaining high temporal sampling to resolve dynamics of release. We have applied this system to determine the acute and chronic effects of a recently-identified lipid, 5-palmitic acid hydroxy stearic acid (5-PAHSA), which is a member of the fatty acid hydroxy fatty acid class of lipids that are upregulated in healthy individuals. Chronic incubation (48-h) with 5-PAHSA significantly increased glucose-stimulated insulin secretion (GSIS) in murine islets compared to chronic incubation without the lipid or in the presence of palmitic acid (PA). The studies were continued in human islets from both healthy donors and donors diagnosed with type 2 diabetes mellitus (T2DM). Total amounts of GSIS were not only augmented in islets that were chronically incubated with 5-PAHSA, but the dynamic insulin release profiles also improved as noted by more pronounced insulin oscillations. With this quantitative microfluidic system, we have corroborated the anti-diabetic effects of 5-PAHSA by demonstrating improved islet function after chronic incubation with this lipid via improved oscillatory dynamics along with higher basal and peak release rates.

INTRODUCTION

A newly discovered class of endogenous mammalian lipids, branched fatty acid esters of hydroxy fatty acids (FAHFA), are directly correlated with insulin sensitivity. FAHFA are up-regulated in subcutaneous adipose tissue of healthy humans but reduced in insulin-resistant individuals.¹ This is in contrast to free fatty acids (FFA), which are found in higher circulating concentrations in insulin resistant individuals compared to healthy individuals.^{2, 3} Although the mechanisms of action are still being investigated, the beneficial effects of FAHFA appear to derive from their direct activation of the GPR40 (FFAR1) fatty acid receptor.⁴ Low levels of FAHFA were found in the breast milk of obese mothers,⁵ while they

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also appear to help protect against Colitis in humans.⁶ FAHFA have also been shown to improve insulin-stimulated glucose transport¹, as well as have anti-inflammatory effects demonstrated in their original report through their ability to decrease macrophage activation and the release of proinflammatory cytokines after LPS activation¹. They have also been shown to have beneficial effects on insulin secretion from pancreatic islets of Langerhans; incubation of human islets of Langerhans with FAHFA over a short time period (45 min) significantly increased glucose-stimulated insulin secretion (GSIS) compared to controls.¹

One aspect of FAHFA that has not been examined is their effect on the dynamic profiles of insulin secretion. GSIS occurs in a biphasic fashion with the first phase consisting of a burst of insulin secretion that lasts for 5–10 min, while the second phase consists of a slower and longer lasting level of release.^{7–9} In addition to these profiles, *in vivo* insulin levels oscillate with periods of 5–10 min,^{10, 11} and these oscillations are conserved at the single islet level.^{9, 12, 13} It has been demonstrated that these dynamic aspects of insulin release are important for maintaining proper glucose control.^{14–17} For example, oscillatory insulin secretion is more effective at promoting glucose uptake into peripheral tissues as compared to static levels of the hormone, has been linked to greater insulin sensitivity, and better regulates hepatic glucose output. Impairments in insulin release dynamics have been reported to be a hallmark of type 2 diabetes mellitus (T2DM).¹⁷ This loss of insulin dynamics is also observed in murine and human models of lipotoxicity where chronic (48-h) incubation with FFA impairs not only GSIS through lower secretion rates^{18–20}, but also via disruption of the dynamic patterns of release as observed by a loss of oscillations in murine islets²¹. Lipotoxicity is the collective term that describes the deleterious effects of elevated FFAs on the biological function of various tissues, such as pancreatic islets, where dysfunction and eventual cell death are observed.³ How incubation with FAHFA, which appears to be beneficial for insulin signaling, would impact the dynamic secretion profile from islets of Langerhans is unknown.

To examine the effects of FAHFA on dynamic insulin release profiles, techniques that allow for single islet measurements are necessary. One way to achieve this sensitivity and temporal resolution has been through the use of microfluidic systems. Various microfluidic platforms have been developed for the measurement of insulin secretion from islets.^{21–27} However, many of these approaches do not allow for single islet measurements, high temporal resolution monitoring, or they have not been applied to the investigation of FAHFA. In this work, we describe a microfluidic system which enables quantitative, inter-islet comparisons of insulin secretion rates, as well as the observation of dynamic patterns of release. The system was used to examine the acute and chronic effects of FFA and FAHFA on the dynamic profile of GSIS from single murine islets. We report augmented GSIS from islets that have been acutely exposed to both classes of these lipids. In contrast, chronic exposure (48-h) to these lipid classes indicated a large difference in their effects; palmitic acid (PA) impaired GSIS, yet incubation with FAHFA augmented both GSIS rates and the dynamic insulin release patterns. The chronic incubation studies were replicated on human islets from two healthy donors and showed similar improvements in GSIS rates and insulin patterns. Finally, chronic incubation of FAHFA was performed on islets from donors with T2DM resulting in elevated GSIS rates compared to control T2DM islets, although their patterns were less robust compared to islets from healthy donors. These experiments demonstrate the

anti-diabetic potential of this lipid and highlights the potential of this quantitative microfluidic platform for comparing inter-islet secretion rates and patterns at the single islet level.

METHODS

Reagents and Materials

Bovine serum albumin (BSA), fatty acid-free BSA (FA-free BSA), fatty acid-free human serum albumin (FA-free HSA), and ethylenediaminetetraacetic acid (EDTA) were from EMD Chemicals (San Diego, CA). 5-palmitic acid hydroxy stearic acid (5-PAHSA) was purchased from Cayman Chemical (Ann Arbor, MI). All other chemicals were obtained from Sigma-Aldrich (Saint Louis, MO), unless otherwise stated.

Reagents for microfluidic immunoassays and isolation and culture of murine islets were obtained as previously described.^{26, 27} All buffers and reagents were prepared using ultrapure deionized water (NANOpure Diamond™, Barnstead International, Dubuque, IA) and filtered using 0.2-μm nylon syringe filters (Pall Corporation, Port Washington, NY).

Stock solutions of 5-PAHSA were prepared in DMSO while PA was prepared in ethanol. For all acute exposure studies, fatty acid stocks were diluted into balanced salt solution (BSS), whereas for chronic exposure studies, stocks were diluted into RPMI 1640 culture media containing FA-free BSA (for murine islets) or FA-free HSA (for human islets). BSS contained 125 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl₂, 2.4 mM CaCl₂, 25 mM tricine, and 0.1% FA-free BSA (pH 7.4).

Microfluidic devices

Reagents for fabricating microfluidic devices and the fabrication procedures were performed as previously described.²⁷ A modification to the devices was performed as described in the text. Details on the characterization of the perfusion and immunoassay systems are given in the ESI. Information about the finite element simulations of the device are also given in the ESI.

Isolation and culture of islets of Langerhans

Murine islets of Langerhans were isolated from male CD-1 mice that weighed 20–40 g (Charles River Laboratories Internal, Inc., Wilmington, MA) according to the Florida State University Animal Use and Care Committee protocol #1813. Isolated islets were cultured as described elsewhere.²⁷ Human islets were obtained from Prodo Laboratories (Aliso Viejo, CA). Islets from two healthy and two donors with T2DM were utilized in this study. Human islet samples were obtained from deidentified cadaveric organ donors and, therefore, were exempt from Institutional Review Board approval. Donor characteristics are provided in the ESI.

Experimental Protocols

Islets, 150 μm in diameter, were held for 10-min in a dish of BSS that contained pre-warmed 3 mM glucose. Single islets were then transferred into the microfluidic islet chamber that

contained pre-warmed BSS with 3 mM glucose and were allowed to settle to the bottom of the chamber. A temperature of 37 °C was maintained in the islet chamber using a temperature control unit (CNi3233, Omega Engineering Inc., Stamford, CT) a thermofoil heater (KHLVA-0502, Omega Engineering, Inc.) affixed under the islet chamber, and a thermocouple sensor (SA1-J, Omega Engineering, Inc.) attached to the top of the device adjacent to the islet chamber.

Two experimental protocols were performed for examining the effect of 5-PAHSA: acute and chronic incubation studies. For the acute studies, single murine islets were perfused with BSS containing 11 mM glucose. After 20-min, either 5-PAHSA or PA in BSS containing 11 mM glucose was delivered for 20-min. Afterwards, the islets were perfused with BSS containing only 11 mM glucose. For the chronic exposure studies, murine islets were incubated for 48-h in one of three conditions. One condition utilized RPMI supplemented with 0.7% FA-free BSA and 40 μ M 5-PAHSA, another was RPMI supplemented with 0.7% FA-free BSA and 0.5 mM PA, and the last condition utilized RPMI supplemented with only 0.7% FA-free BSA for a lipid-free condition. Similar incubation conditions were used for human islets with 48-h incubations in media with and without 40 μ M 5-PAHSA, except FA-free HSA was used instead of FA-free BSA. After incubation, single islets were washed in BSS with 3 mM glucose at 37 °C for 10 min and loaded into the chamber of the microfluidic device. The islets were initially rinsed with 3 mM glucose for 2 min prior to data collection. The islets were then stimulated with 3 mM glucose for 3 min before raising the concentration to 11 mM glucose and holding for 45 min followed by a step-down to 3 mM glucose.

Data analysis and processing

Secretion rates were obtained by normalizing the measured concentration of insulin to the perfusion flow rate. The amount of insulin was quantified by measuring the area under the curve using Origin 9.0 (Origin Labs, Northampton, MA). Phase 1 was defined as the initial 9 min of elevated glucose (3 – 12 min experimental time), and phase 2 as the remaining time at elevated glucose (12 – 48 min experimental time). For the acute experiments, the insulin amount during exposure to either PA or 5-PAHSA (20 – 40 min experimental time) was normalized to the average insulin amount outside the exposure window (0 – 20 min and 40 – 60 min). All values are presented as the average $\pm$ one standard deviation unless otherwise stated. Significance was assessed by a two-tailed Student's t-test and differences were considered significant when $p < 0.05$. To present the integrated secretion data, box and whisker plots are used with the box hinges representing the 25th and 75th percentiles, and the median and mean represented by the bar and cross, respectively. To assess the oscillatory nature of insulin release in the chronically-incubated murine islets, phase 2 insulin levels were baseline subtracted and assessed using a fast Fourier transform (FFT) with a rectangular window consisting of 217 data points. Further details on data processing and analysis are provided in the ESI.

RESULTS AND DISCUSSION

A quantitative examination of the effect of FAHFA on insulin dynamics has not been reported. We first describe a modification to our previous microfluidic system²⁷ whereby a sealed chamber in conjunction with a sampling chamber is used to capture all the secretions from a single islet of Langerhans, independent of its location within the chamber. The homogenized secretions are then sampled and analyzed via an online immunoassay. We show that with this modification, inter-islet comparisons of both insulin secretion rates and dynamic patterns of release are possible. After development of this platform, the acute and chronic effects of FAHFA on murine islets are reported, as well as the chronic effects of FAHFA on human islets from healthy and T2DM donors.

Development of quantitative sampling system

In our previous system²⁷, the outlet of the perfusion channel was directed to a 300 μm islet chamber where a single islet was located (**Fig. S1A**). The insulin chamber was open to the atmosphere allowing the perfusion solution to flow across the islet and out the device to waste. The islet chamber was electrically grounded causing a fraction of the perfusate to be continuously sampled into the analysis region by electroosmotic flow (EOF) where quantitative measurements of the sampled insulin were made using an electrophoretic immunoassay.^{22, 27–29} While this previous system enabled the patterns of release to be compared between islets, comparisons of absolute release rates between islets were difficult because the measured concentrations were dependent on the islet location within the chamber. If the islet was close to the sampling channel, higher insulin levels were observed compared to islets that were farther from the channel. To permit inter-islet comparisons of release rates, comprehensive capture of the secretions from the islet chamber is necessary. This has been achieved using a PDMS plug to close the islet chamber with a split channel after the islet chamber to direct the majority of perfusate out to waste while a fraction was analyzed.²¹ We describe a similar approach, albeit in a simpler fashion.

The new system was designed with two goals in mind: ensuring a constant fraction of the secretions were analyzed independent of the location of the islet within the chamber, and maintaining high temporal resolution monitoring. In **Fig. 1A**, the new design is shown which consisted of a closed islet chamber that allowed the perfusion solution to capture the entire secretions from the islet. The perfusate then traveled down a mixing channel where it homogenized prior to exiting the microfluidic device via a second chamber. This “sampling chamber” was electrically grounded, allowing a continuous sampling of the perfusate into the analysis region via EOF. Due to the complete capture of the insulin release, this configuration negated the dependence on islet position as a factor that would affect inter-islet comparisons assuming that the perfusate was well mixed before it entered the sampling chamber. One caveat to this new design is that the islet secretions would be more diluted compared to the previous system due to the extra volume of the chambers and the mixing channel; however, this dilution could be accounted for during calibrations and, if necessary, counteracted by modifying the concentrations of the immunoassay reagents.

As described in more detail in the ESI and Table S-1, finite element analysis simulations were used to optimize the time that the insulin spent in the mixing channel between the islet

chamber and insulin sampling chamber. Too short of a time would not allow for complete mixing of the secreted insulin, and irreproducible sampling; too long of a time would produce low temporal resolution and not resolve secretion dynamics. Three mixing channel lengths (3.0, 5.5, and 8.0 mm) were evaluated at three perfusion flow rates (0.20, 0.25, and 0.30 $\mu\text{L min}^{-1}$). To determine if there was a dependence of the measured insulin levels on the spatial location of the islet within the chamber, three different locations of the islet were simulated (A, B, and C) which are shown in the inset in Fig. 1B. The positions were simulated on the right, center, and left, respectively, of the chamber. The exact locations of the simulated islets are described in the ESI. Cross-sectional slices of the insulin profile within the mixing channel were then obtained for each of the islet positions at 5 points along the channel length (**Fig. S1B**) and the RSD of the concentrations were measured. Well-mixed solutions had high homogeneity which produced low RSD values. The insulin mixing RSDs for all simulations were then plotted against channel length and flow rate (**Fig. S1C**). The low RSD values, particularly at the longer channel lengths, demonstrate that the measured insulin sample was homogenous as it entered the sampling chamber, and that the levels of insulin measured were independent of spatial positioning of the islet within the chamber. While the lower flow rates of 0.2 $\mu\text{L min}^{-1}$ with the 8.0 mm channel length demonstrated the highest levels of mixing, due to the longest mixing time, this was at the expense of increased dilution and increased broadening of the insulin pulses. To minimize these effects while still achieving homogeneity of the insulin mixture, the 5.5 mm length was selected at a flow rate of 0.25 $\mu\text{L min}^{-1}$.

After the length of this channel and optimum flow rate were determined, pulsatile insulin release was simulated from an islet at three varied spatial positions within the chamber while the insulin levels were monitored at the end of the mixing channel (**Fig. 1B**). As can be seen, the insulin levels measured had a sinusoidal pattern with the amplitudes and period of the waves independent of the spatial position of the islet. To highlight the difference in this new design versus the older design, a simulation of the previous system was also performed which showed a significant dependence of the measured insulin values on the islet location (**Fig. S1D**). A drawback to the new design is that higher levels of dilution were observed, as seen by the decreased amplitudes in **Fig. 1B** compared to **Fig. S1D**. Nevertheless, these results confirm that the new system enables quantitative inter-islet comparisons of secretion rates due to the comprehensive capture of the entire secretions from the islet. This ability to quantify inter-islet secretion rates are essential for understanding the effects of FAHFA on insulin release.

Upon completion of the simulations, the device was fabricated by drilling a 300 μm diameter sampling chamber 5.5 mm from the islet chamber. Upon loading an islet into the islet chamber, PCR film was used to seal the top of the chamber. PCR film was utilized for its practicality as an inert and adhesive material to effectively seal the chamber. Other methods to seal the chamber may be used but we found sealing to be simple and effective with the PCR film. The device was then characterized and calibrated as described in the ESI and the B/F ratios were plotted as a function of insulin concentration (**Fig. 1C**). The limit of detection was calculated to be 10 nM and the RSD of the B/F values was < 2% for each insulin concentration. These values are similar to what was achieved with the previous

system,²⁷ indicating that the quality of the assay was not affected by the new islet chamber design and that the device would be suitable for measurement of secretion dynamics.

Glucose stimulation of islets

The first application of this new system was to monitor GSIS from murine islets of Langerhans that had been incubated under conventional conditions (with serum) and compare secretion profiles with this system to what has been reported previously. The glucose level was raised from 3 to 11 mM and held for 45-min while the insulin response was monitored.

The basal release rate at 3 mM glucose for $n = 10$ islets tested was $28 \pm 6 \text{ pg min}^{-1}$. Upon exposure to 11 mM glucose, all islets displayed a biphasic profile in which the first phase consisted of an initial high rate of insulin secretion, followed by the second phase which showed a lower but sustained release. The phase 1 responses for the 10 islets peaked at $54 \pm 15 \text{ pg min}^{-1}$. The majority of the islets sampled ($n = 8$) showed regular pulsatility in their second phase, although there was variability in the amplitude as well as the frequency of the pulses. Representative secretory profiles for a “slow” and “fast” oscillating islet are shown in **Fig. 2A and 2B**. Spectral analysis was utilized to assess the pulsatility of the 10 islets. Although each islet had its own unique frequency, the islets generally oscillated between 2 and 5 min, with 3 islets possessing oscillations with 5 min periods, 4 islets showing periods of 4 min, and the remaining oscillating between 2–3 min. **Fig. 2C** shows the mean secretion dynamics from all 10 islets, with the error bars corresponding to $\pm 1 \text{ SD}$. Such reproducibility is consistent with the goal of this microfluidic system, which was designed to permit inter-islet comparisons. This average GSIS response is similar to what others have reported using different systems, validating the new system.^{9, 21, 24, 25} Finally, the patterns and levels of release are similar to what we and others have shown for single islet GSIS responses,^{9, 21, 26, 27} providing confidence that the system is capable of accurate monitoring of dynamic secretion profiles.

Acute stimulation of murine islets with 5-PAHSA or PA

After demonstrating that the new system enabled measurement of patterns and release rates of GSIS similar to literature values, the acute effects of 5-PAHSA and PA on insulin secretion from murine islets were evaluated. The FAHFA isomer used in the experiments, 5-PAHSA, was reported to be more upregulated in adipose tissue and have a greater efficacy on GSIS compared with other isomers in its class.¹ Concentrations of 20, 40, and 80 μM 5-PAHSA were delivered for 20-min to single islets that were being exposed to 11 mM glucose. The 20-min window was selected as it was deemed suitable to showcase the changes in insulin release dynamics. These results were compared with delivery of PA in a similar fashion.

As shown by the representative traces in **Fig. 3A** for delivery of 20, 40 and 80 μM 5-PAHSA, prior to delivery of the lipid, insulin oscillations were observed from most islets induced by the 11 mM glucose. As 5-PAHSA was applied, dose-dependent increases in insulin were observed with only modest increases in the amplitude, but large increases in the plateau fraction of the oscillations. This was exemplified by the 80 μM 5-PAHSA

stimulation where the insulin release was no longer oscillatory, but rather showed a sustained level of elevated secretion. These dynamics are similar to how islets respond when stimulated with extreme levels of glucose (~20 mM) when the glycolytic flux is so high that no oscillations are observed.³⁰ A similar trend was observed with delivery of 0.5 mM PA which produced a marked increase in GSIS ($52 \pm 33\%$ above baseline, $n = 3$) in all islets tested (**Fig. S3**). This similarity is not surprising given that both FAHFA and FFA act through GPR40,^{4, 31} and its subsequent IP₃-induced increase in intracellular Ca²⁺.³² Reversion to either a 5-PAHSA-free or PA-free glucose solution produced the return of normal pulsatility, demonstrating the transient effects of these agents. The percent secretion above pre-stimulatory levels for 5-PAHSA are shown in **Fig. 3B** which indicated a positive correlation of insulin release with concentration. The average ($\pm$ SD) increase for 20 μ M 5-PAHSA was $4 \pm 7\%$ ($n = 4$), while for 40 μ M was $20 \pm 9\%$ ($n = 5$), and for 80 μ M was $31 \pm 16\%$ ($n = 5$).

The augmented insulin release upon acute exposure to 5-PAHSA is in agreement with the initial report of these lipids in which elevated levels of GSIS were measured from batches of human islets after a brief exposure to 5-PAHSA.¹ Similarly, our results reaffirm prior work that showed increased GSIS upon acute stimulation with PA and other FFAs.^{33–35} In addition to corroborating these general findings, using this newly developed system, we have also been able to elucidate the changes in the dynamic profile of insulin secretion that yields these augmented responses, the increased plateau fractions of the insulin oscillations.

Chronic incubation of murine islets with 5-PAHSA or PA

After examining the acute effects of 5-PAHSA on insulin dynamics, chronic effects were investigated by incubating islets in serum-free culture media in the presence and absence of 40 μ M 5-PAHSA for 48-h followed by initiation of GSIS in the absence of the lipid. In addition, these results were compared to a lipotoxic condition using 48-h incubations with 0.5 mM PA. The effects of chronic incubation with 5-PAHSA were of interest because FAHFAs are up-regulated in healthy individuals, yet chronic incubation with FFAs (like PA) are known to be detrimental to islets.^{3, 18–21} Therefore, we hypothesized that the GSIS response between the two lipids after a long-term culture would be different.

The average secretory profiles for each case are presented in **Fig. 4A-B** and **Fig. S4**. It is clear that 48-h incubation with 5-PAHSA ($n = 5$) maintained the dynamic profile of GSIS evidenced by the biphasic release profiles and strong oscillations observed during the second phase (**Fig. 4A**). Furthermore, the average basal insulin release rates in the presence of 5-PAHSA were 41 ± 12 pg min⁻¹ and the average peak release rate during phase 1 was 85 ± 17 pg min⁻¹. The lipid-free samples (**Fig. 4B**), which also showed a biphasic profile, had an average basal release rate of 36.5 ± 8.6 pg min⁻¹ ($p = 0.246$ vs 5-PAHSA), and peak secretions of 60.3 ± 16.7 pg min⁻¹, which were lower than the 5-PAHSA incubated islets ($p = 0.034$). The 5-PAHSA incubations also showed secretion rates that were increased over islets cultured with PA (**Fig. S4**) ($n = 5$), which showed a mean baseline of 27 ± 3 pg min⁻¹ and a peak phase 1 release rate of 79 ± 72 pg min⁻¹. Although these values were not significantly different at the 95% confidence level compared to the FAHFA-incubated islets,

one of the 5 islets tested with PA incubation had an extremely high level of peak first phase release at 207 pg min^{-1} .

To further characterize GSIS after chronic incubation, the amounts of insulin during phases 1 and 2 were calculated for each islet. During phase 1 (**Fig. 4C**), the amount of insulin released from the 5-PAHSA incubated samples ($524 \pm 76 \text{ pg}$) was significantly higher than the BSA-incubated samples ($366 \pm 93.4 \text{ pg}$, $p = 0.010$) as well as the PA-incubated samples ($364 \pm 101 \text{ pg}$, $p = 0.024$). For phase 2 release (**Fig. 4C**), the 5-PAHSA incubated islets secreted $1790 \pm 460 \text{ pg}$, which was significantly higher than their PA-incubated counterparts ($1184 \pm 125 \text{ pg}$, $p = 0.039$) and were increased over the lipid-free BSA incubated samples ($1405 \pm 333 \text{ pg}$, $p = 0.152$). The lower secretion levels for islets cultured with PA are consistent with its known lipotoxic effects. **Fig. S5** shows the responses from all chronically-incubated murine islets.

The dynamic responses observed during the 2nd phase were assessed by Fourier analysis, shown in **Fig. S6**, to determine the major periods of insulin oscillations. For the 5-PAHSA data, 80% of the islets showed oscillations, 2/5 having a major oscillation period of 3 min, and the remaining 2/5 with a period of 4 min. Islets incubated in lipid-free conditions showed a lower degree of pulsatility, with 4/7 having periods of 2.5, 3.5, and 5 min. The 5-PAHSA islets starkly contrasted with the PA-treated islets which showed almost no pulsatility. These FFT results are also evident in the average secretory profiles (**Fig. 4A-B** and **Fig. S4**).

While the chronic effects of FAHFAs have been recently investigated on human islets,⁴ the time-dependent insulin responses were not. We have observed that 48-h incubation in the presence of 5-PAHSA, even in the absence of serum, produced higher basal and GSIS, as well as greater pulsatility, than lipid-free or lipotoxic conditions.

Chronic incubation of human islets with 5-PAHSA

The effects of chronic exposure to 5-PAHSA were further investigated using islets from human donors. Four donors were utilized in this study: two healthy (non-diabetic) donors and two donors that had been diagnosed with T2DM. Incubations of islets were performed in serum-free culture media in the absence and presence of $40 \mu\text{M}$ 5-PAHSA for 48-h followed by initiation of GSIS in the absence of the lipid.

The general trend observed across all donors was that islets incubated with 5-PAHSA had higher basal insulin release rates and higher peak GSIS release rates compared to islets that had not been incubated with the lipid. **Fig. 5A and 5B** shows the average secretion dynamics of islets under the two incubation conditions. Basal secretion rates were higher ($p = 0.009$) in the 5-PAHSA pretreated islets compared to islets incubated without the lipid ($43 \pm 5 \text{ g min}^{-1}$ ($n = 9$ islets) vs $31 \pm 8 \text{ pg min}^{-1}$ ($n = 7$ islets), respectively). This effect on basal insulin release was not observed in murine islets. The 5-PAHSA pre-treated samples had a higher phase 1 release of $439 \pm 84 \text{ pg}$ compared to the untreated group $255 \pm 82 \text{ pg}$ ($p < 0.05$) (**Fig. 5C**). For phase 2, the insulin release amounts were also significantly higher ($1670 \pm 190 \text{ pg}$) in the 5-PAHSA group than the control group ($1158 \pm 229 \text{ pg}$, $p < 0.05$).

Similar trends were observed from T2DM islets. The samples exposed to FAHFA tended to exhibit more of a biphasic response and less variability in their secretion dynamics (**Fig. 6A**). In contrast, islets that were untreated showed a wide range of responses to glucose, and generally were not oscillatory (**Fig. 6B**). Basal release rates in the treated samples ($n = 7$ islets) were higher than their untreated counterparts ($n = 5$ islets) ($40 \pm 8 \text{ pg min}^{-1}$ vs $25 \pm 14 \text{ pg min}^{-1}$) although these values were not significantly different ($p = 0.513$). Across 1st phase (**Fig. 6C**), the 5-PAHSA group showed higher amounts of insulin released ($387 \pm 104 \text{ pg}$) compared to the untreated T2DM samples ($234 \pm 141 \text{ pg}$), although this was not significantly different at the 95% confidence level ($p = 0.0777$). During 2nd phase release, the 5-PAHSA group again showed higher release compared with their lipid-free counterparts ($1479 \pm 297 \text{ pg}$ vs $933 \pm 481 \text{ pg}$, $p = 0.064$).

In all cases, incubations with 5-PAHSA increased the amount of insulin released across all 4 donors examined (**Fig. 5C** and **Fig. 6C**). Islets from the two healthy donors that had been incubated with 5-PAHSA showed significantly larger increases in insulin release compared to islets from the same donors but untreated. Similarly, T2DM islets pre-treated with 5-PAHSA showed GSIS levels that were larger, but not at a statistically significant level. On average, the islets from healthy donors showed higher secretion than the T2DM samples in both phases of release, although the number of T2DM islets utilized was lower. Many of the T2DM islets that had not been exposed to 5-PAHSA exhibited weak responses, and at times, no response at all to glucose. **Fig. S7** shows the responses from all human islets sampled.

Spectral analysis was also utilized to examine the periodic nature of the insulin responses from the human samples (**Fig. S8**). While not as oscillatory as the murine islets, differences in pulsatility were observed between the two samples. In the healthy islets incubated with 5-PAHSA, 2 islets oscillated with a period of 3.0 min, one islet at 2.7, and one islet each at 4.5 and 5.0 min. For the untreated healthy islets, a weak oscillation at 3.0 and 3.5 min was observed in 2 islets, while the rest showed minimal pulsatility. The T2DM samples showed a smaller difference between the treated and untreated datasets, which were minimally pulsatile as evident in their secretion traces (**Fig. 6**).

In general, these trends show that long-term exposure to FAHFA results in not only higher GSIS in healthy and T2DM human islets, but also improved oscillatory dynamics, especially for islets from healthy donors. These results are consistent with the trends observed in murine islets that have been incubated in the presence of 5-PAHSA. However, some differences between murine and human islet responses were observed. First, 5-PAHSA incubation resulted in significantly higher baseline insulin release in healthy human islets, but not murine or T2DM human samples. In addition, while control murine islets showed minor GSIS, even the healthy human islet control group showed extreme reductions in GSIS and oscillatory dynamics (compare **Fig. S5** to **Fig. S7**). The different responses of these control groups may be attributed to the length of time from isolation; murine islets were collected immediately after isolation and underwent the 48-hr incubation, whereas human islets had a minimum of 5 days post-ischemic time before they underwent the 48-hr incubation. However, we believe this change in response between control and 5-PAHSA-incubated islets highlights the possible preventative properties of these lipids in maintaining

islet function. It would be interesting to observe how 5-PAHSA would impact islets taken from donors with varying degrees of T2DM.

CONCLUSIONS

A microfluidic platform has been developed for quantitative comparisons of inter-islet insulin secretion dynamics. Capturing the entire cellular secretions and ensuring they are homogenized prior to quantitation renders the measurements independent of islet placement within the chamber, and therefore quantitative. Using this system, we show that chronic incubation with PA impaired insulin release, as characterized by lower secretion rates and the loss of insulin pulsatility. While these trends are in good agreement with previously published reports that examined the long-term effects of fatty acids and GSIS, we report a departure in the long-term effects of 5-PAHSA. Across both murine and human islet samples, chronic exposure to 5-PAHSA demonstrated augmented GSIS at the single islet level. Recently published literature examined chronic effects of FAHFAs on human islets, and reported augmented responses of insulin to high glucose levels,⁴ which is consistent with our observations. However, we also report higher basal secretion rates and greater pulsatility as compared with untreated samples. GSIS was augmented in non-diabetic mouse and healthy human islets, and although it increased in islets from humans with T2DM, this increase was not statistically significant. Further studies are warranted on even longer-term incubations, as well as their possible protective effects, for example, by testing their efficacy at various stages in the progression to T2DM. Also, an examination of their actions on *in vivo* insulin pulsatility would help corroborate these *in vitro* studies. The quantitative microfluidic platform presented in this study could be used to examine time-dependent secretion changes from the same islet or group of islets by changing the perfusion solution to media and placing the device in an incubator, or by removing and placing the islet(s) back in an incubator after testing GSIS. The device could also be extended to study the effects of other pharmacological agents on islet biology and can be used to monitor cellular secretion dynamics other than insulin in a quantitative fashion in high temporal resolution by utilizing different affinity reagents.

Supplementary Material

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REFERENCES

1. Yore MM, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, Patel RT, Lee J, Chen S, Peroni OD, Dhaneshwar AS, Hammarstedt A, Smith U, McGraw TE, Saghatelian A and Kahn BB, Cell, 2014, 159, 318–332. [PubMed: 25303528]
2. Boden G, Diabetes, 1997, 46, 3–10. [PubMed: 8971073]
3. McGarry JD and Dobbins RL, Diabetologia, 1999, 42, 128–138. [PubMed: 10064091]

4. Syed I, Lee J, Moraes-Vieira PM, Donaldson CJ, Sontheimer A, Aryal P, Wellenstein K, Kolar MJ, Nelson AT, Siegel D, Mokrosinski J, Farooqi IS, Zhao JJ, Yore MM, Peroni OD, Saghatelian A and Kahn BB, *Cell Metab*, 2018, 27, 419–427.e414. [PubMed: 29414687]
5. Brezinova M, Kuda O, Hansikova J, Rombaldova M, Balas L, Bardova K, Durand T, Rossmeisl M, Cerna M, Stranak Z and Kopecky J, *Biochim Biophys Acta*, 2018, 1863, 126–131. [PubMed: 29154942]
6. Lee J, Moraes-Vieira PM, Castoldi A, Aryal P, Yee EU, Vickers C, Parnas O, Donaldson CJ, Saghatelian A and Kahn BB, *J Biol Chem*, 2016, 291, 22207–22217. [PubMed: 27573241]
7. Lacy PE, Walker MM and Fink CJ, *Diabetes*, 1972, 21, 987–998. [PubMed: 4561331]
8. Lenzen S, *Am J Physiol*, 1979, 236, E391–400. [PubMed: 373466]
9. Nunemaker CS, Wasserman DH, McGuinness OP, Sweet IR, Teague JC and Satin LS, *Am J Physiol Endocrinol Metab*, 2006, 290, E523–529. [PubMed: 16249252]
10. Pørksen N, Nyholm B, Veldhuis JD, Butler PC and Schmitz O, *Am J Physiol*, 1997, 273, E908–914. [PubMed: 9374676]
11. Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC and Butler PC, *J Clin Endocrinol Metab*, 2000, 85, 4491–4499. [PubMed: 11134098]
12. Gilon P, Shepherd RM and Henquin JC, *J Biol Chem*, 1993, 268, 22265–22268. [PubMed: 8226733]
13. Song SH, Kjems L, Ritzel R, McIntyre SM, Johnson ML, Veldhuis JD and Butler PC, *J Clin Endocrinol Metab*, 2002, 87, 213–221. [PubMed: 11788649]
14. Bratusch-Marrain PR, Komjati M and Waldhäusl WK, *Diabetes*, 1986, 35, 922. [PubMed: 3525288]
15. Komjati M, Bratusch-Marrain PR and Waldhäusl WK, *Endocrinology*, 1986, 118, 312–319. [PubMed: 3000741]
16. Matveyenko AV, Liuwantara D, Gurlo T, Kirakossian D, Dalla Man C, Cobelli C, White MF, Copps KD, Volpi E, Fujita S and Butler PC, *Diabetes*, 2012, 61, 2269–2279. [PubMed: 22688333]
17. Satin LS, Butler PC, Ha J and Sherman AS, *Mol Aspects Med*, 2015, 42, 61–77. [PubMed: 25637831]
18. Zhou YP and Grill VE, *J Clin Invest*, 1994, 93, 870–876. [PubMed: 8113418]
19. Tian G, Sol ERM, Xu YJ, Shuai HY and Tengholm A, *Diabetes*, 2015, 64, 904–915. [PubMed: 25281428]
20. Barlow J, Jensen VH, Jastroch M and Affourtit C, *Biochem J*, 2016, 473, 487–496. [PubMed: 26621874]
21. Dishinger JF, Reid KR and Kennedy RT, *Anal Chem*, 2009, 81, 3119–3127. [PubMed: 19364142]
22. Roper MG, Shackman JG, Dahlgren GM and Kennedy RT, *Anal Chem*, 2003, 75, 4711–4717. [PubMed: 14674445]
23. Hu J, Yu Y, Brooks JC, Godwin LA, Somasundaram S, Torabinejad F, Kim J, Shannon C and Easley CJ, *J Am Chem Soc*, 2014, 136, 8467–8474. [PubMed: 24827871]
24. Lenguito G, Chaimov D, Weitz JR, Rodriguez-Diaz R, Rawal SAK, Tamayo-Garcia A, Caicedo A, Stabler CL, Buchwald P and Agarwal A, *Lab Chip*, 2017, 17, 772–781. [PubMed: 28157238]
25. Hu J and Easley CJ, *Anal Chem*, 2017, 89, 8517–8523. [PubMed: 28696682]
26. Yi L, Bandak B, Wang X, Bertram R and Roper MG, *Anal Chem*, 2016, 88, 10368–10373. [PubMed: 27712062]
27. Yi L, Wang X, Dhumpa R, Schrell AM, Mukhitov N and Roper MG, *Lab Chip*, 2015, 15, 823–832. [PubMed: 25474044]
28. Schultz NM and Kennedy RT, *Anal Chem*, 1993, 65, 3161–3165.
29. Tao L and Kennedy RT, *Anal Chem*, 1996, 68, 3899–3906. [PubMed: 8916449]
30. Bertram R, Satin LS and Sherman AS, *Diabetes*, 2018, 67, 351–359. [PubMed: 29463575]
31. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y and Fujino M, *Nature*, 2003, 422, 173–176. [PubMed: 12629551]

32. Mancini AD and Poitout V, Trends Endocrinol Metab, 2013, 24, 398–407. [PubMed: 23631851]
33. Gravena C, Mathias PC and Ashcroft SJ, J Endocrinol, 2002, 173, 73–80. [PubMed: 11927386]
34. Sun MY, Yoo E, Green BJ, Altamentova SM, Kilkenny DM and Rocheleau JV, Biophys J, 2012, 103, 2379–2388. [PubMed: 23283237]
35. Fujiwara K, Maekawa F and Yada T, Am J Physiol Endocrinol Metab, 2005, 289, E670–E677. [PubMed: 15914509]

Fig. 1. Development and calibration of the quantitative microfluidic system.

(A) 3D rendition of the quantitative islet chamber system where the perfusion solution (blue arrows) captures all of the islet releasate. The released compounds homogenize as they travel through the perfusion mixing channel. As the perfusate leaves the device through the sampling chamber, a small fraction of the solution enters the EOF channel for quantitation by the immunoassay system. The inset shows a picture of a single murine islet situated in the islet chamber with the perfusion mixing channel situated below the islet. (B) A simulation of a periodic insulin signal emanating from an islet at 3 positions within the newly developed design. Positions A, B, and C refer to the 3 spatial positions of the islet within the chamber as depicted by the inset. The measurements show reproducible detection of insulin that are independent of islet position. (C) Calibration curve of the immunoassay system shows the measured B/F plotted against the insulin concentration. The red line is a best-fit curve using a 4-parameter logistic function. The LOD was calculated to be 10 nM and RSD for each standard was < 2%.

Fig. 2. GSIS profiles of murine islets incubated with serum.

Representative GSIS profiles (black lines, left y-axis) from single mouse islets in response to a glucose challenge (blue line, right y-axis). These traces were chosen to show biphasic pulsatile responses with (A) slower and (B) faster periods. (C) The mean response of insulin release stimulated with 11 mM glucose is shown by the black line with the error bars (grey) corresponding to ± 1 SD.

Fig. 3. Acute delivery of 5-PAHSA to mouse islets.

Representative single islet insulin release profiles in response to the acute exposure of (A) 20, 40, and 80 μM 5-PAHSA. The timing of the lipid delivery is denoted by the blue bar on the graph from 20 – 40 min. (B) The percent increase in secretion is plotted as a function of the 5-PAHSA concentration applied.

Fig. 4. Chronic incubations of murine islets with 5-PAHSA or PA.

The average (± 1 SD) GSIS from murine islets after 48-h incubation with (A) 40 μ M 5-PAHSA ($n = 5$ islets) or (B) FA-free BSA ($n=7$ islets). (C) The amount of insulin released (in pg) during phase 1 and phase 2 incubated with (+) and without (-) 5-PAHSA, or in a lipotoxic condition ((+) PA). * $p < 0.05$.

Fig. 5. Chronic incubation of healthy human islets.

The average insulin release profiles (black lines, left y-axis) from healthy human islets exposed to 11 mM glucose levels (blue lines, right y-axis) after 48-h incubation of healthy islets in culture media containing (A) 40 μM 5-PAHSA ($n = 9$ islets) and (B) FA-free HSA ($n = 7$ islets) are shown. The error bars (grey) correspond to $\pm 1\text{SD}$. (C) The amounts of insulin (in pg) for phase 1 and phase 2 from the healthy human islets that have been incubated with (+) and without (-) 5-PAHSA. * $p < 0.05$.

Fig. 6. Chronic incubation of T2DM human islets.

The average insulin release profiles (black lines, left y-axis) from T2DM human islets exposed to 11 mM glucose levels (blue lines, right y-axis) after 48-h incubation of T2DM islets in culture media containing (A) 40 μM 5-PAHSA (n = 7 islets) and (B) FA-free HSA (n = 5 islets) are shown. The error bars (grey) correspond to ± 1SD. (C) The amounts of insulin (in pg) for phase 1 and phase 2 from the T2DM human islets that have been incubated with (+) and without (-) 5-PAHSA.