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Impact of biotin supplemented diet on mouse pancreatic islet β-cell mass expansion and glucose induced electrical activity

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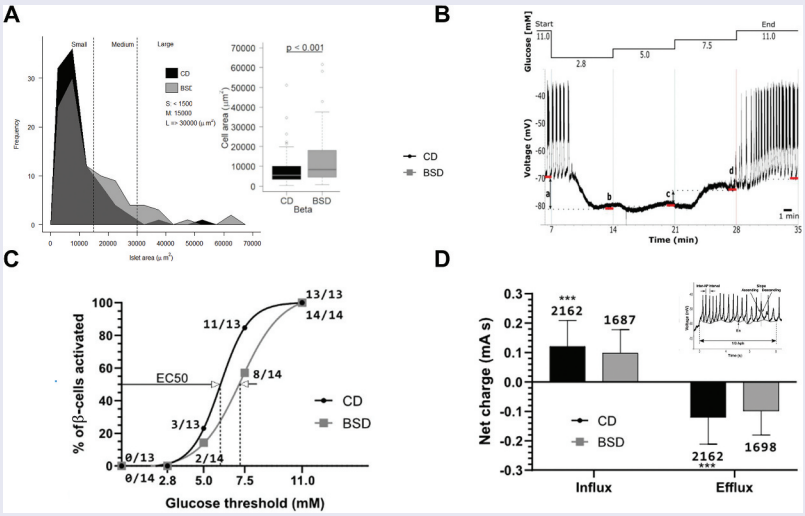
ABSTRACT

Biotin supplemented diet (BSD) is known to enhance β-cell replication and insulin secretion in mice. Here, we first describe BSD impact on the islet β-cell membrane potential (Vm) and glucose-induced electrical activity. BALB/c female mice (n ≥ 20) were fed for nine weeks after weaning with a control diet (CD) or a BSD (100X). In both groups, islet area was compared in pancreatic sections incubated with anti-insulin and anti-glucagon antibodies; Vm was recorded in micro dissected islet β-cells during perfusion with saline solutions containing 2.8, 5.0, 7.5-, or 11.0 mM glucose. BSD increased the islet and β-cell area compared with CD. In islet β-cells of the BSD group, a larger ΔVm/Δ[glucose] was found at sub-stimulatory glucose concentrations and the threshold glucose concentration for generation of action potentials (APs) was increased by 1.23 mM. Moreover, at 11.0 mM glucose, a significant decrease was found in AP amplitude, frequency, ascending and descending slopes as well as in the calculated net charge influx and efflux of islet β-cells from BSD compared to the CD group, without changes in slow Vm oscillation parameters. A pharmacological dose of biotin in mice increases islet insulin cell mass, shifts islet β-cell intracellular electrical activity dose response curve toward higher glucose concentrations, very likely by increasing K_{ATP} conductance, and decreases voltage gated Ca^{2+} and K^+ conductance at stimulatory glucose concentrations.



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Introduction

Diabetes includes various pathologies that have as a common sign hyperglycemia. Diabetes type 2 (DT2) represents 90% of the diabetes cases and constitutes a pandemic with an increasing global prevalence.[1] Insulin is the only hormone capable of reducing serum glucose levels. This hormone is exclusively produced by β-cells, which constitute the main central mass in a pancreatic islet (~80% in the mouse). Glucose is a key physiological

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stimulus for β-cell electrical activity, which is critical for adequate insulin secretion. In mouse islet β-cells, glucose between 0 and 7.5 mM depolarizes the membrane by inhibition of K_{ATP} ionic channels. Between 7.5 and 16.0 mM, glucose induces slow membrane potential (Vm) oscillations. Vm rhythmically alternates between a silent phase (Sph) (~ −55 mV) and a more depolarized or active phase (Aph) (~ −45 mV), where action potentials (APs) are generated. The APs result from the activation of voltage dependent Ca^{2+} and K^+ channels. The duration of the Aph and, the frequency of the APs, increases in a glucose concentration-dependent manner from 7.0 to 22.0 mM.[2–4] Due to the high incidence of electrical coupling, as demonstrated in freshly isolated pairs of double voltage clamped β-cells[5,6], as well as in perfused islets,[7,8] the slow Vm oscillations occur in synchrony and in phase in most islet β-cells.[9] The synchrony and phase in the electrical activity determines that the islet secretes insulin in a pulsatile manner at the same frequency.[10]

In patients with DT2, β-cell function is deteriorated,[11–13] and islet β-cell mass is decreased[14] owing to an enhanced apoptosis secondary to hyperglycemia, as well as elevated serum-free fatty acids and inflammatory adipokines.[15] Given the critical role of insulin for glucose homeostasis, it is essential to understand the regulation of β-cell expansion. There is evidence that DT2 patients that have used a biotin (vitamin H, B7) supplemented diet (BSD) improved their glycemic control (fasting and stimulated).[16,17] Also, male mice fed with a BSD for eight weeks after weaning, show an increase in β-cell mass by increasing the number of islets per pancreas (~1.15–1.3 times), islet size (~1.4–1.75), and β-cell fraction (~1.14), as well as islet insulin production.[18,19]

However, up to now, it is not known whether biotin supplementation in the diet changes the glucose induced β-cell electrical activity. In this study, we address this question by comparing the electrical activity induced by glucose recorded in islet β-cells in two groups of mice, one fed with a control diet (CD) and other fed with BSD (100X) for nine weeks. We found that BSD increased β-cell mass in female mice, as previously reported for male mice, and described for the first time that it also induced changes in β-cell Vm and electrical activity in response to sub-stimulatory and stimulatory concentrations of glucose, at both the non-excitable and excitable voltage range, suggesting biotin action in various ion channels.

Results

CD and BSD female mice exhibited similar metabolic parameters

There is evidence that glucose induces metabolic responses that vary between sexes[20] Figure 1 shows the food intake (A) and the body weight (B) changes measured for 9 weeks on a weekly basis for the CD (black dots) and BSD (gray squares) female fed mice. In addition, at the end of the 9-week period, the mean blood glucose (C) and triglyceride (D) values were measured after 4 and 16 h fasting in CD (black bars) and BSD (gray bars). No statistically significant differences were found between the two groups in these parameters, suggesting that BSD did not induce metabolic changes in the experimental group.

BSD expands the islet and β-cell mass

It is well known that the central islet mass is constituted mostly by β-cells, whereas other endocrine cells including α-cells are mainly distributed in the periphery.[21,22] To obtain information about the impact of BSD on the islet cell mass and topological distribution, islet β-cell and α-cell areas identified by immunofluorescent (IF) studies were compared between CD and BSD groups.

Figures 2A and 2B show IF images of pancreatic sections from CD and BSD mice obtained after co-incubation with anti-insulin (FITC-green) and anti-glucagon (TRITC-red) antibodies. The sum of α and β-cell IF staining was considered to represent the total islet area. Figure 2C shows the distribution of the sizes of pancreatic islet area for CD (black) and BSD (gray) fed mouse groups. The vertical discontinuous lines indicate the cut points

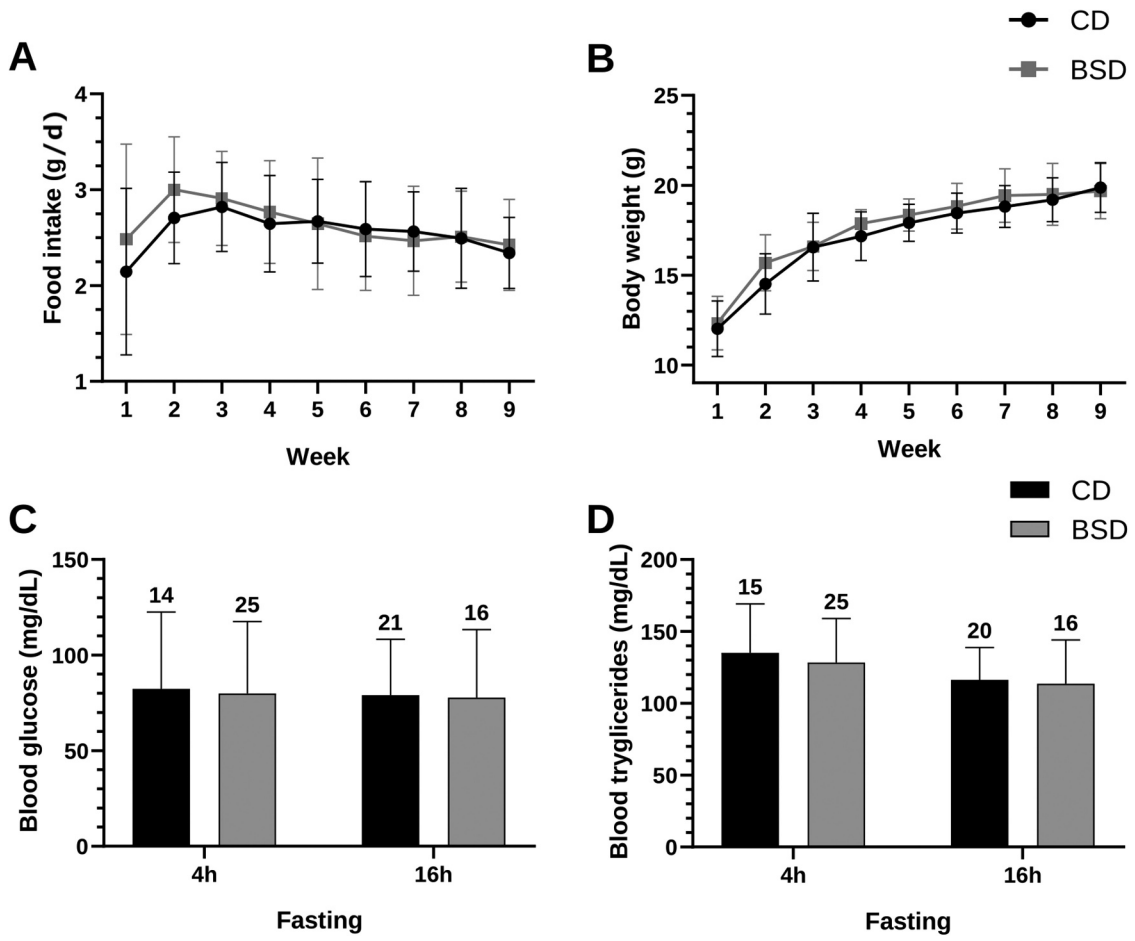

Figure 1. Food intake, body weight, blood glucose and triglycerides in female mice were similar in control diet (CD) and biotin supplemented diet (BSD) fed mice. After weaning, animals were fed with pellets Envigo 2018S (CD) or pellets supplemented with biotin (BSD) for nine weeks. Charts show the mean food intake **(A)** and body weight **(B)** measured at the end of each week in the CD (black dots) and the BSD (gray squares) groups (n = 20–25 mice per group). After 9 weeks, blood glucose **(C)** and triglyceride **(D)** levels were determined after 4 h and 16 h of fasting, in CD (black bars) and BSD (gray bars) groups; n = number of subjects analyzed. Significance was assessed by the two-way repeated measurements ANOVA (diet, time).

for further analyses of small ≤15000 μm^2, medium ≤30,000 or large >30,000 μm^2 islets. In both groups, the distribution shows peaks at about 5500 μm^2 for islet areas, but it was lower for the BSD group (Figure 2C). In addition, the proportion of medium and large islet size was larger in the BSD than for the CD fed mice (Figure 2D) (χ^2 test for trend p value = .004). Likewise, the median total islet area (Figure 2E) recorded was significantly larger in the BSD (median = 8964.5 μm^2) than in the CD (median = 6125.7 μm^2) group (*P* < *.001*, Mann–Whitney test). Also, the median of the total β-cell area was significantly larger (*P* < *.001*, Mann–Whitney test) in the BSD group (median = 8270.1, μm^2) than in CD group (median = 5448.3 μm^2) (Figure 2F), whereas it was similar for α-cells. Multiple comparison analysis showed that the β-cell/α-cell area increase is associated with the islet size (Figure 2G) (the median was 10.0, 10.4, 14.4 in small, medium, and large islets, respectively) (Dunn test p = .0032), as well as with the diet (a median of 10.0 and 6.5 in the small islets from BSD and CD, respectively (Dunn test p < .001); Bonferroni corrected significance <0.0033). No changes were found in the total median α-cell area in the BSD and CD group (Figure 2F), although when analyzed only in the islet center a decrease in α-cell was found in the BSD compared with the CD fed mice (significant in the small islets, Dunn test p value = .003, not shown). These results demonstrate that in our experimental protocol biotin expands the β-cell mass and changes islet size distribution.

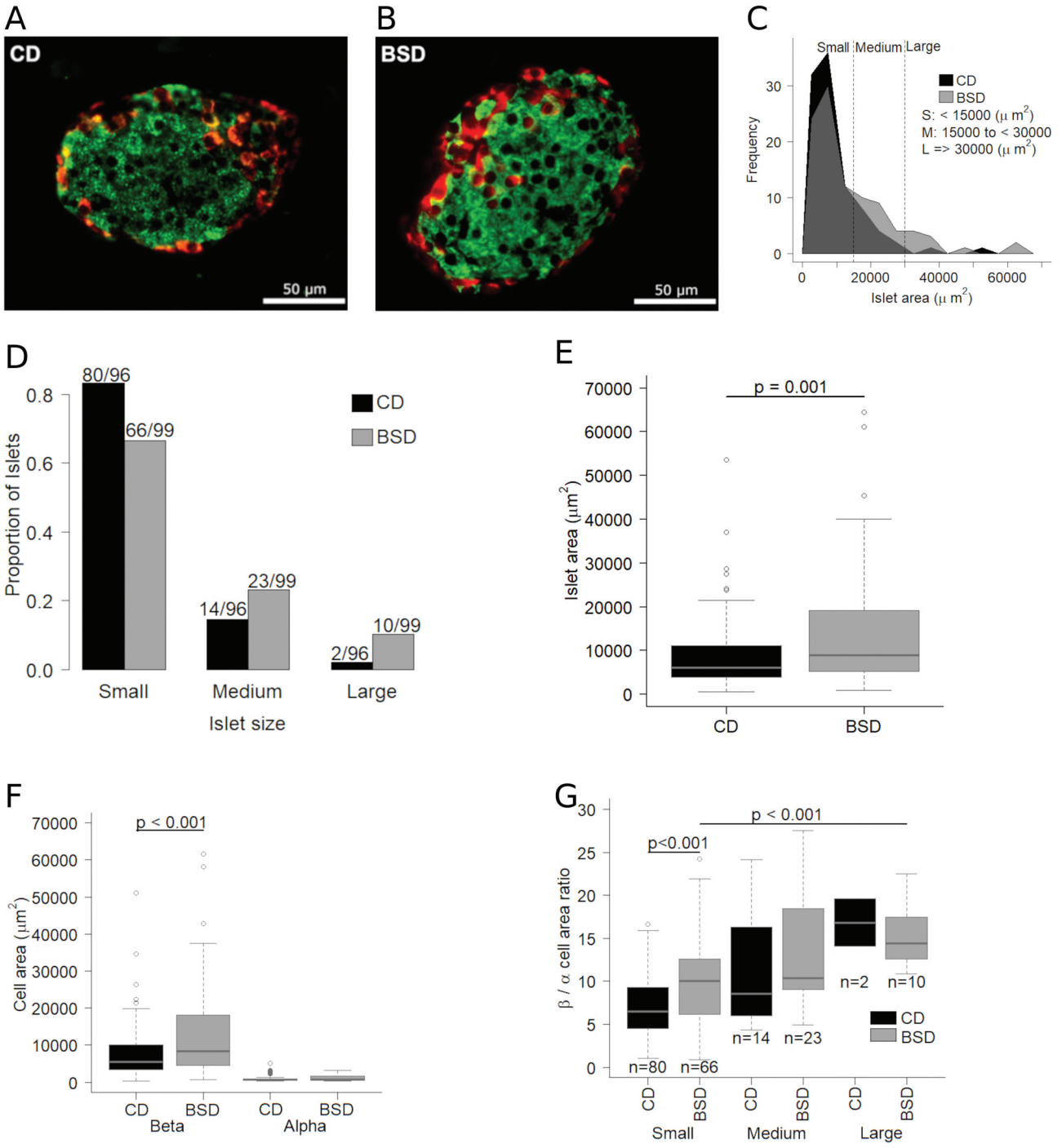

Figure 2. Biotin supplemented diet (BSD) increases the islet size and islet β-cell area. a-b) Immunofluorescence images of anti-insulin or β-cell (green) and anti-glucagon or α-cell (red) staining distribution in representative islets from **(A)** control diet (CD) and **(B)** BSD fed groups; scale bar 50 µm. **C)** Distribution of islet areas measured in both groups; black shaded area represents islet size distribution in the CD group and gray shaded area represents islet size distribution for the BSD group. Vertical dotted lines correspond to the cut points considered for the analysis of β-cell/α-cell area in small, medium and large islets β-cell/α-cell area. **D)** Proportions of small, medium, and large islets in CD and BSD feed groups (these proportions are different between groups, χ^2 test for trend p value = .004). **E)** Total islet area from CD (black) and BSD (gray) groups (Mann–Whitney test *P* = *.001*). **F)** Total β-cell and α-cell areas in CD group (black) compared with BSD (gray) group (Mann–Whitney test *P* < *.001*). **G)** Box plot of the β-cell/α-cell in small, medium and large islets. At least one islet size in a diet group was different from others in the β-cell/α-cell ratio (Kruskal–Wallis test p < .001). Multiple comparisons show that the median of the β-cell/α-cell was higher (at a Bonferroni corrected significance <0.0033) in small, medium and large islets of BSD groups as compared with small islets of CD group (Dunn test p values < .001). Also, large islets in BSD group had higher β/α ratio than small islets in BSD group (Dunn test p value = .0032). Data from five pancreases from each of the CD and BSD fed mice were used and a total of 96 and 99 islets, respectively, were analyzed.

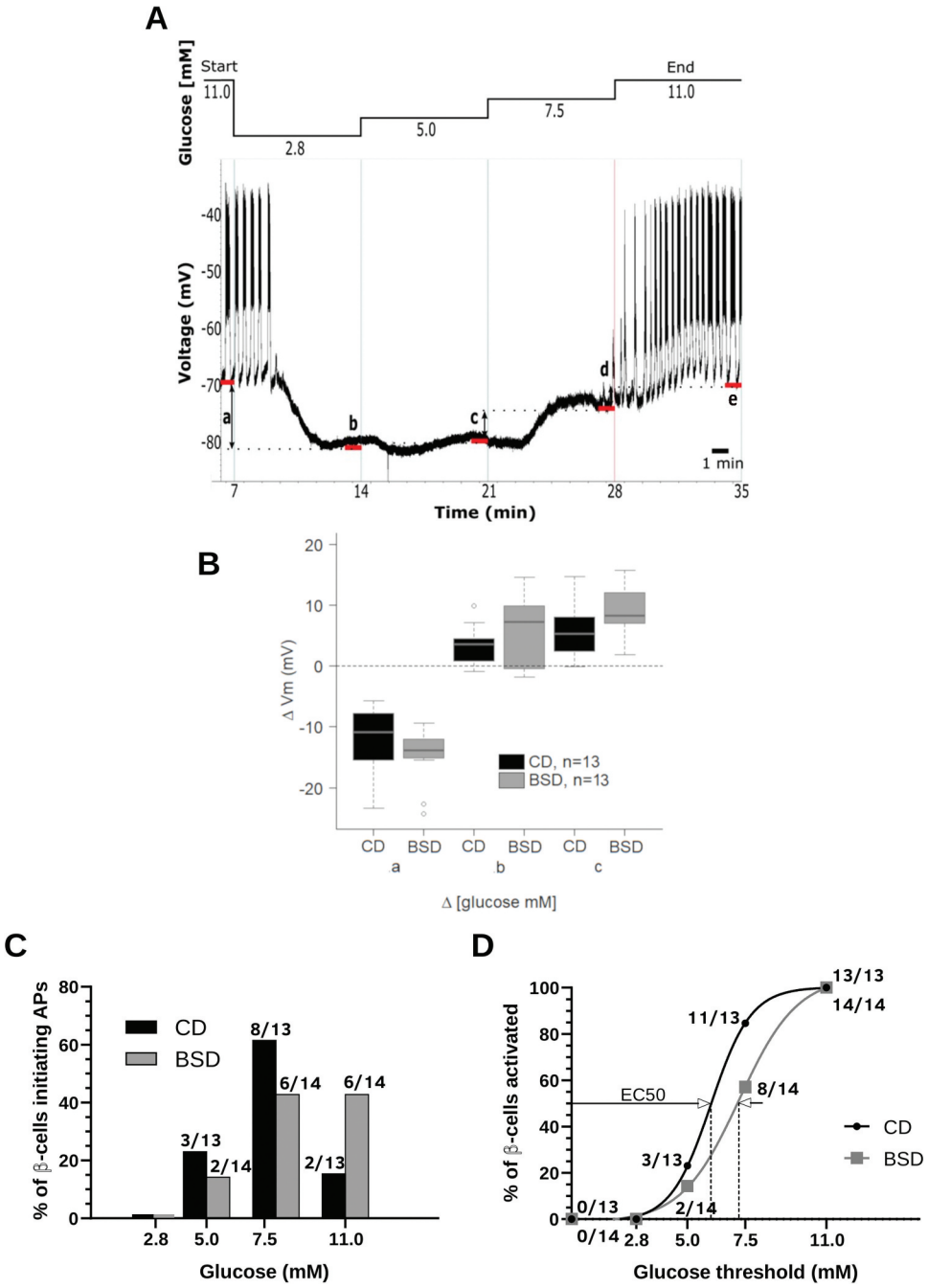

Figure 3. Electrophysiological parameters compared in islet β-cells in perfused micro dissected islets of mice fed with control diet (CD) and biotin supplemented diet 100X (BSD). A) Representative intracellular membrane potential (Vm) recording and ΔVm changes of a pancreatic islet β-cell from a BSD fed mouse obtained during a series of step changes in [glucose] (7 min each) in the perfusion solution as follows: 11.0, 2.8, 5.0, 7.5 and 11.0 mM. Letters a, b, c, d and e, indicate the ΔVm changes measured at the indicated pairs of [glucose] step changes (see Methods). **B)** Box plot of the median ΔVm and SD of islet β-cells from (CD) (black) and BSD (gray) groups measured at the specified a, b, c, and d [glucose] step changes. Parameters were measured in 13 CD and 14 BSD mice. **C)** Distribution of the percentage of islet β-cells initiating AP activity as a function of [glucose] for CD (black bars) and BSD (gray bars) groups; number of cells reaching threshold/cells recorded is shown over each bar. **D)** Cumulative percentage of active β-cells, exhibiting oscillations with APs, as a function of [glucose] from CD (black circles) and BSD (black squares) groups; number of active cells/total number of cells, as indicated next to each symbol. The [glucose] that induced APs in 50% of the islet β-cells, Ec50, was shifted toward a higher [glucose] by 1.23mM in the islets from BSD fed mice.

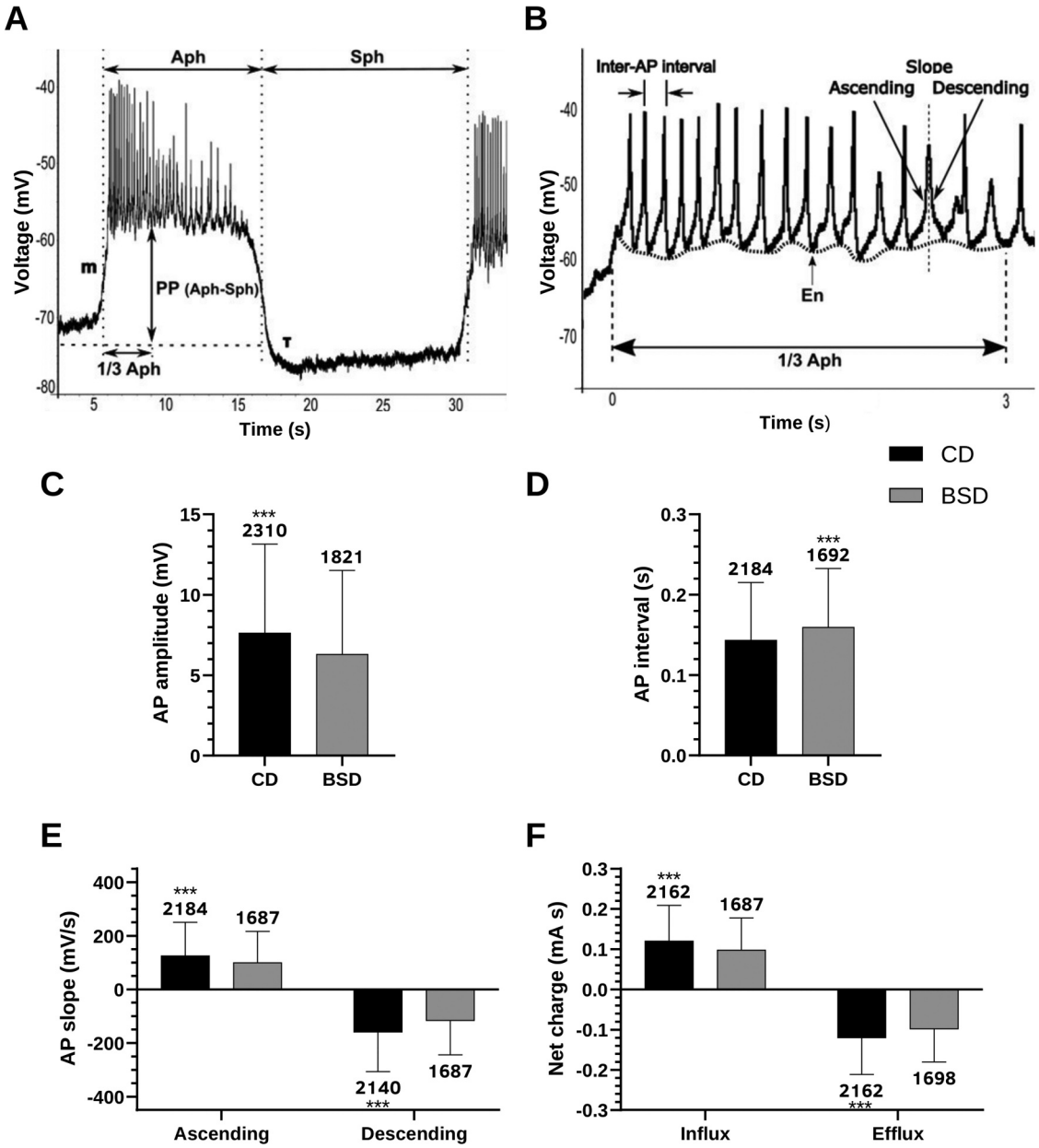

Figure 4. Biotin supplemented diet (BSD) decreases action potential amplitude and frequency during the active phase (Aph). Values were measured from recordings acquired during the last seven-minute period of perfusion of Krebs saline containing 11.0 mM glucose in islet β-cells from (CD) (black bars) and BSD (gray bar) groups. **A)** A slow Vm oscillation, showing a typical active (Aph) and silent (Sph) phase of electrical activity. The duration of the Aph and Sph was considered as the time delimited by the vertical dotted lines, drawn to intersect at 50% of the amplitude of the slow Vm oscillation. The plateau potential (PP) amplitude was calculated as the ΔVm measured from the mean voltage at the Sph, as indicated by the horizontal dotted line, to the mean voltage of the first third of the Aph, indicated by the horizontal solid line with arrow heads. The activation slope (m) corresponds to the maximum dV/dt change in Vm from the Sph to the Aph. The repolarization time constant (τ) was calculated as the time at which the Vm decayed to 36.8%, calculated by the best exponential fit to the voltage change from Aph to the Sph. **B)** Characteristic β-cell action potentials (APs) activated during the Aph of slow Vm oscillations induced by stimulating glucose concentrations. The wavy dotted line represents the "envelope" (En) or the best fit to the minimum membrane potential (Vm) recorded among APs obtained during the first third of the Aph and considered as the reference voltage for the AP parameters measured. **C)** AP amplitude, mean and SD, in islet β-cells from CD (black bar) and BSD (gray bar) groups. **D)** Inter AP interval, in CD and BSD groups, as indicated. **E)** AP ascending and descending slopes from CD and BSD groups, as indicated. **F)** Calculated net charge influx and efflux in islet β-cells from CD and BSD groups, as indicated. Differences between values from the CD compared with the BSD groups were significant using a Mann–Whitney test ***$P < .001$ in all cases; the number of events measured corresponding to each condition is indicated in the figure. All parameters were measured in 13 CD and 14 BSD fed mice.

BSD induces changes in electrical activity of islet β-cells

To identify whether biotin has an impact on the β-cell electrical activity and glucose sensitivity, Vm was recorded, as illustrated in Figure 3A, in islet β-cells from CD and BSD fed mice during the sequential perfusion of glucose from 11.0 to 2.8 (a) to 5.0 (b) to 7.5 (c) and again to 11.0 (d) mM. Only experiments where a stable membrane potential was recorded during the whole glucose perfusion protocol were included in the analyses. As expected, the change from 11.0 to 2.8 mM glucose induces hyperpolarization or negative ΔVm (Figure 3A, B). Thereafter, glucose concentration ([glucose]) step changes from 2.8 up to 11.0 mM induce positive ΔVm increments or depolarizations (Figure 3 A, C ,D, E).

BSD increases the ΔVm/Δ[glucose] relation in the subthreshold range

The ΔVm in islet β-cells recorded during the perfusion of different glucose concentrations was analyzed (Figure 3B). ΔVm changes were always larger in the BSD than in the CD group. Correspondingly, the median ΔVm measured at different glucose concentrations in the subthreshold range was larger for the islet β-cells from BSD (gray boxes) compared with those from CD (black boxes). These findings suggest that BSD increases K_{ATP} conductance in islet β-cells.

The histogram in Figure 3C shows the percentage of islet β-cells that reach the AP-[glucose] threshold or sustained Vm oscillations with bursts of APs as a function of the glucose concentration for CD (black bars) and BSD (gray bars) fed mice. At 5.0-, 7.5-, or 11.0-mM glucose the percentage of cells firing in the CD group was 23%, 61%, and 15%, whereas in the BSD group this was 14%, 42%, 42%, respectively.

The AP-[glucose] threshold was also analyzed by comparing the cumulative incidence curves calculated for islet β-cells for both groups. Figure 3D shows the percentage of β-cells activated at 5.0-, 7.5-, and 11.0-mM glucose for the CD (black dots) and BSD (gray squares) groups. The best fit with a sigmoidal adjustment for this relation indicates that the Ec50 (50% of the APs-[glucose] threshold relation) shifts toward higher glucose concentrations by 1.23 mM compared with the CD group. These findings give support to the hypothesis that BSD increases K_{ATP} conductance in islet β-cells.

BSD induces changes in islet β-cell electrical activity at supra-threshold glucose concentrations

The cell mechanisms underlying glucose-induced electrical activity can be divided into those controlling the slow Vm oscillations between silent and active phases and those controlling the action potentials during the active phase. No changes were found in the various parameters of the slow Vm oscillations recorded from islet β-cells from the CD group compared with the BSD group. Specifically, no changes were found in the range of cycles per minute (Aph + Sph)/min [CD (1 to 9) vs BSD (1 to 7)] (Figure 4A), nor in the mean burst frequency [CD (3.9) vs BSD (3.4)], nor in the duration of the Aph [CD (6.29 s) vs BSD (5.87 s)] and the Sph [CD (6.26 s) vs BSD (7.28 s)]. In addition, no statistically significant difference was found in the slow Vm oscillation amplitude, PP [CD (12.69 mV) vs BSD (11.46 mV)], nor in the mean slope of activation, m [CD (15.00 mV/s) vs BSD (14.57 mV/s)], nor in the mean slope of repolarization, τ [CD (0.60 ms) vs BSD (0.73 ms)] between groups.

In contrast, BSD alters several parameters of the action potential (AP). APs recorded during the first third of the burst Aph, in 11 mM glucose (see methods), were analyzed (Figure 4 A and B). The mean value of the AP amplitude decreased from 7.62 mV in the CD group to 6.30 mV in the BSD group (Figure 4C), a reduction of about 17%. In addition, an increase in inter-AP intervals was observed from 0.14 to 0.15 s (Figure 4D), and, thus, AP frequency decreased in islets from the BSD group by about 6%. Furthermore, ascending AP slopes (Figure 4E) were reduced from 125.8 in the CD group to 100.2 in the BSD group, and descending AP slopes were reduced from 158.9 in the CD group to 116 in the BSD group. Thus, net charge influx and efflux were also reduced by 0.02 and 0.02 mA·s, respectively, in the BSD group compared to the CD

group (Figure 4F). Differences were significant by Mann–Whitney test ***P < .001 in all cases. These measurements indicate that BSD decreased the ionic conductance of voltage dependent Ca^{2+} and K^+ channels involved in the generation of β-cell APs.

Discussion

Several in *vivo* and in *vitro* studies have shown that BSD up regulates the mRNA expression or the activity of different genes that participate in carbohydrate metabolism and islet β-cell differentiation.[23] However, to date, there are no reports of biotin impact on the electrical activity of pancreatic β-cell, which is well known to be critical for appropriate glucose sensing and insulin release. In the present study, we describe for the first time that a pharmacological dose of biotin supplied to mice for nine weeks in the diet, besides expanding the β-cell mass, induces changes in glucose-induced electrical activity in the β-cells of pancreatic islets compared with those from the control group. The possible functional implications of these changes are discussed.

BSD effects and gender

Here, we found that under fasting conditions female BSD mice were normoglycemic in agreement with another study using male mice.[18] In addition, here we did not find changes in fasting blood triglyceride levels. In contrast, another study in male mice found that BSD induced a decrease in triglycerides.[24,25] Future studies will be required to discern whether differences result from methods used to determine this variable or whether there are gender differences in BSD actions.

Biotin induced expansion of the islet β-cell mass

Using IF analyses of pancreatic sections co-incubated with anti-insulin and anti-glucagon antibodies, we describe that in-house prepared BSD induced an enhancement in the total β-cell and islet area. This expansion results from an increase in the proportion of medium and large area islets at the expense of small islets. In addition, it results from the islet size-dependent increase β/α cell area ratio. These observations are consistent with previous studies that showed an expansion of β-cell mass in male mice of the same strain fed with a commercial BSD 100X supplied for 8 weeks after weaning.[18] Since an enhancement of glucose-induced islet insulin release was previously found in BSD 100X compared with CD fed mice[18] and BSD induces β-cell proliferation,[19] it is likely that expansion of β-cell area may result from autocrine insulin trophic actions, which include β-cell proliferation.[26] In contrast with the first study, we did not detect that BSD induced an increase in the total α-area nor an increase in their cell number at the islet center. Overall, results from different laboratories, including ours, consistently showed that both commercially available and in-house prepared BSD diets expand the β-cell mass in mice of both sexes, strengthening the notion that biotin acts on the endocrine pancreas and is a regulator of β-cell growth.

Biotin induces changes in electrical activity in islet β-cells

BSD shifts the glucose threshold for the generation of action potentials (APs) in islet β-cells

So far, this is the first study to characterize a biotin effect on electrical activity. It is well known that the subthreshold Vm is mainly determined by K_{ATP} channels.[27] In our study, we observed that the individual and median ΔVm values were larger in the BSD than in the CD group for [glucose] changes in the non-stimulatory range. Also, the clousure of these K_{ATP} channels by glucose mainly determines the threshold for glucose-induced electrical activity. We also observed a shift of 1.23 mM in the Ec50 of the APs-[glucose] threshold, toward higher glucose concentrations in ß-cells from the BSD compared with the CD group. In addition, at 11 mM glucose , a greater percentage of β cells reached the APs-[glucose] threshold in the BSD compared with the CD group. Due to the relatively reduced number of islet β-cells recorded in both groups, the double experimental condition (diet and glucose) and the non-parametric distribution of ΔVm values recorded, the statistical significance of these changes was not proved. Nonetheless, the consistency among results shown in Figure 3 strongly

suggests that K_{ATP} conductance is increased in ß-cells from mice receiving a biotin supplement in the diet.

BSD does not alter slow Vm oscillations

From studies using islets perfused with stimulatory glucose concentrations it is well known that between 7.5 and 16.5 mM, bursts of APs are generated during the Aph of the slow Vm oscillations.[28–30] The ß-cell membrane Na^+-Ca^{2+} transporter has been implicated in the regulation of the slow oscillations in membrane potential during glucose stimulation. In contrast with APs, we did not find that BSD has an impact on the various parameters of the slow Vm oscillations, since no changes were found in the duration of the Aph and Sph, slow Vm amplitude or PP, the depolarization slope, m, from Sph to Aph, and in τ, the best exponential fit to the voltage decay from Aph to Sph. Thus, we conclude that biotin action does not affect the underlying mechanisms controlling slow Vm oscillation behavior during glucose stimulation.

BSD alters action potential parameters

In islet β-cells of mice, the APs result from the activation of voltage-gated Ca^{2+} channels (CaV), a key element for insulin release.[10,30,31] CaV channels are made up of four different subunits; the α1 subunit forms the channel pore, whereas the α2δ, β, and γ subunits regulate channel open time.[32,33] In mice islet β-cells, the expression of four variants of CaV channels has been described with a distinct proportion as follows: The CaV1.2 type or "L" (50%), the Cav2.3 or "R" type (20–25%), the Cav2.1 or P/Q (20%) and the Cav2.2 or "N" (10%).[34–36] The process of insulin granule docking and fusion with the cell membrane during secretion depends on local Ca^{2+}, presumably at the intracellular location of membrane Ca^{2+} channels, rather than on the overall intracellular Ca^{2+} levels.

Here, we observed that BSD induced a significant decrease in APs average amplitude, as well as a significant decrease in the rate of depolarization and repolarization of the APs and, thus, in the calculated net influx and efflux currents during the Aph. It is likely that the decrease in APs amplitude and net influx in islet β-cells from BSD, result from the reduced activity or levels of expression of one or more types of CaV channels. In contrast with our results, a previous study showed that BSD increased the levels of the Cacna1d, a gene transcript that encodes the α1D protein or pore subunit of the L type Ca^{2+} channel.[18] However, in that work, it was not explored whether there are changes in α1D protein or other subunits of the L type channel nor of other subunits from other types of CaV channels. Here, we also found that APs in islet β-cells from BSD mice showed a decrease in the repolarization rate (~0.73 times) as well as the calculated net efflux current (~0.81 times) compared to cells from CD fed mice, indicating that biotin decreases the conductance of voltage-gated K^+ channels (KV).[37–39] The changes in rate of rise and rate of fall contribute to the decrease in the frequency of APs here described. Then, changes in CaV and KV channels, shown here, suggest an overall reduced influx of Ca^{2+} during electrical activity in BSD ß-cells. These observations underline the need for further functional, pharmacological, and molecular studies to define the impact of BSD in islet β-cells.

Glucose-stimulated insulin secretion and electrical activity changes induced by BSD

Normoglycemia under fasting conditions was found here, consistent with another study in male mice fed for 8 weeks with a commercial BSD diet.[18] Nonetheless, changes in glucose-stimulated insulin secretion (GSIS) were found using a BSD protocol, similar to the one used here[18] . Specifically, during a glucose tolerance test (GTT), BSD fed mice showed an improved glucose tolerance and increased serum insulin release, without changes in insulin resistance, compared with mice fed with a control diet. . In addition, an increase in GSIS was also found in BSD cultured islets in the same study.[18] BSD effects on ionic conductance shown here will reduce maximum $[Ca^{2+}]_i$ levels in islet β-cells during glucose stimulation and may protect islet β-cell function. It is well known that cell death precedes the manifestation of DT2[13] and that glucotoxicity-induced cell death involves increased $[Ca^{2+}]_i$ levels.[40]Studies in cultured islets dissociated from transgenic mice with mutations in the K_{ATP} channel have shown that the continued exposure to high glucose concentrations (4 d) induced a high

state of β-cells excitability, with the consequently sustained increase in $[Ca^{2+}]_i$ which determines a decrease in the insulin content reserve and in GSIS.[41] Glucose-induced $[Ca^{2+}]_i$ alterations were also recorded in human islets under glucose induced-overstimulation.[42] Thus, the BSD effects on the electrical activity in islet ß-cells, as reported here, may have a protective effect on the insulin reserve content under glucotoxic conditions. This may contribute to the improvement of different metabolic parameters observed in BSD supplemented DT2 patients,[16,17] and DT2 animal models,[43,44] as well as may be participating in the improved GTT response, and enhanced *in vivo* and *in vitro* GSIS in BSD fed mice.[18] Moreover, enhanced DNA synthesis has been found to be associated with changes in $[Ca^{2+}]_i$.[45] Thus, the BSD reduced net Ca^{2+} influx shown here and the inferred reduction in maximum islet $[Ca^{2+}]_i$, may also have implications for gene regulation and islet β-cell proliferation. Future recordings of $[Ca^{2+}]_i$ changes will test this hypothesis.

Conclusions

A human pharmacological dose of BSD increases islet insulin cell mass and shifts the islet β-cell intracellular electrical activity dose response curve toward higher glucose concentrations, owing to a possible increase in K_{ATP} conductance. In addition, BSD induced a significant decrease in voltage gated Ca^{2+} and K^+ conductance during glucose stimulation. These changes may regulate optimum maximum $[Ca^{2+}]i$ levels in islet β-cells according to functional demands. Overall, the findings presented here have implications for the understanding of how biotin stimulates pancreatic β-cell expansion and function.

Materials and methods

Animal model

After weaning, 21-d old female BALB/c mice were used. Mice were kept in groups of five per box. Animal handling and experimental procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, Washington, DC, USA, 1996). All procedures were approved by the Ethics Committee for Experimentation, Faculty of Medicine, National Autonomous University of Mexico.

Biotin supplemented diet

Envigo 2018S food pellets (Envigo, Huntington UK) were ground and used as a base for the CD and the BSD pellet preparation. Envigo 2018S diet containing biotin levels of 0.90 mg/kg according to the specification sheet was used as a CD. For the BSD, 108 mg of biotin powder (B4501-10 G, Sigma-Aldrich, USA) per kg of food was added. Both diets were moistened with distilled water, and new pellets were prepared. Thereafter, pellets were dried by baking at 70°C (~4 h) and sterilized in an autoclave.

Diet supply

Mice were divided into two groups and fed ad libitum with CD or BSD for 9 weeks. Food pellets at 20 g/mouse were supplied three times per week. The remaining pellets per box were weighed to estimate the amount of food intake per mouse. Food consumption and body weight[46] changes were measured weekly.

Glucose and triglyceride blood levels

After 9 weeks of consumption of BSD or CD and after 4 h or 16 h of fasting, glucose and triglyceride levels were measured from a drop of blood from the mouse tail using test-strips (Accutrend kit Plus GCTL-mg/dL, Roche-cobas Mannheim Germany).

Immunofluorescence and morphometric analysis

Immunofluorescent (IF) studies were done using previously standardized methods.[46,47] Briefly, each pancreas was fixed in 4% buffered paraformaldehyde and embedded in paraffin. Permeabilized sections (5 µm) were co-incubated overnight with Guinea pig anti-insulin antibody (1:500; USA GeneTex, cat num. GTX27842) and mouse anti-glucagon antibody (1:1500; Sigma, USA, cat. Num. G2654) at 4°C. Then, the sections were

washed and stained with a goat anti-rabbit and a goat anti-mouse fluorescent FITC and TRITC antibodies, respectively (Invitrogen, USA).

Images were acquired at 20–40X with a C4742-95 Hamamatsu camera (Hamamatsu, Japan) attached to an Olympus 1 × 70 microscope (Olympus, Japan) adapted to a Metamorph imaging software (Universal imaging Corporation). Fluorescent images were analyzed with the Matlab software routine by means of edge (fluorescent contrast) detection and morphology tools. Five pancreases from each of the CD and BSD fed mice were used, and a total of 96 and 99 islets, respectively, were analyzed.

Intracellular electrophysiological recordings

Intracellular β-cell Vm recordings were performed using previously standardized methods.[28,29,48] Briefly, medium-to-large islets (>150 μm for the largest oval diameter) were dissected from the pancreas using iridectomy scissors, transferred and fixed through adherent exocrine tissue with pins to a micro chamber perfused with Krebs ringer solution. Once in the chamber, the microelectrode potential connected to a current clamp amplifier was adjusted to zero using the reference electrode and lowered into the islet. Thereafter, a step decrease in membrane potential (m. p.), was indicative of successful impalement. After recording cyclic APs induced by 11 mM glucose, the islet β-cell was allowed for 7 min to recover from the impalement and stabilize. Thereafter, islet β-cell Vm was recorded while perfusing with Krebs saline solution containing glucose concentrations of 11.0, 2.8, 5.0, 7.5, and 11.0 mM, each one for 7 min (as illustrated in Figure 3A). Cells were considered as β-cells, only when they were silent at low glucose concentrations[35] and responded to [glucose] increments with oscillations in Vm and bursts of spikes on the Aph.[48] Likewise, our study only includes islet β-cell whose m. p. was stable and continuously recorded through the whole glucose concentration perfusion protocol and whose ΔVm changes induced by glucose were reversible.

The recording chamber (66 μl) was perfused (1 ml/min) with a modified Krebs solution containing 11.0 mM glucose, 120.0 mM NaCl, 5.0 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 24.0 mM $NaHCO_3$, equilibrated to a pH 7.4 by using a mixture of 95% O_2 and 5% CO_2 (5% carbogen) and maintained at 37°C using an electronic water bath and an electronically controlled Peltier device at the entrance of the perfusion chamber. Solution changes were performed by electronic valves controlled with a Digidata 1440a acquisition card (Axon Instruments, USA) and a Clampex 10.7 software.

Electrophysiological parameters

An amplifier with an input impedance of about 1×10^{13} Ω and zero-input adjustable bias current (typically 0.075 pA) was used. Data acquisition was obtained at a sampling frequency of 10 kHz, without filtering, using Clampex 10.7 software and the Digidata 1440a acquisition card (Axon Instruments, USA). The intracellular recording microelectrodes were filled with a filtered solution of 3 M potassium citrate ($C_6H_5K_3O_7$) and 50 mM KCl.[49] Microelectrodes with a resistance between 100 and 260 MΩ were used. Cell input resistance was between 320 and 520 MΩ. The number of β-cells recorded for each measurement is specified in each figure. Data was acquired using a Clampfit 10.7 (Axon Instruments, USA).

Evaluation of Vm parameters at sub-stimulatory and stimulatory glucose concentrations

The change in membrane potential (ΔVm) as a function of glucose concentration (ΔVm/Δ [glucose mM]) was considered as the difference between the mean Vm values recorded during the last 60 s of a 7 min period of perfusion at each glucose concentration. Vertical lines labeled with the letters a, b, c, and d indicate the ΔVm measured for each respective step increment in glucose concentration (Figure 3A).

For glucose concentrations that generate Vm cyclic oscillations in the β-cell, ΔVm changes were measured during the Sph. The AP-[glucose] threshold for electrical activity was defined as the minimum glucose concentration at which the islet β-cells began to generate sustained oscillations of Vm with bursts of APs.

Calculation of slow Vm oscillation parameters

Slow Vm oscillation parameters were analyzed during the last 60 s of perfusion with 11.0 mM glucose, both at the beginning and end of each experiment (Figure 4A). The analyzed parameters were: a) The frequency of the slow Vm oscillations, calculated as the number of cycles (Aph + Sph) per minute (arrows on top of trace), b) The duration of the Aph and the Sph, measured as the time between the mean Vm 50% amplitude at the beginning and end or between the end and the beginning of the slow Vm oscillation, respectively (Figure 4A, vertical dashed lines), c) The amplitude of the Aph or plateau potential (PP), considered as the ΔVm between the average Vm recorded during the Sph (Figure 4A, horizontal dotted lines) and the mean voltage increment recorded during the first third of the Aph (Figure 4A, horizontal and vertical bars with arrow heads), d) the activation slope, m, considered as the steepest slope (dV/dt) that corresponds to the fast change in voltage from the Sph to the Aph, and e) the decay time constant, tau (τ), which represents the time at which Vm decayed to 36.8% from the Aph to the Sph, calculated from the best fit exponential function (Figure 4A).

Calculation of AP biophysical parameters

AP analysis was performed during the first third of the Aph duration of slow Vm oscillations. The following AP parameters were compared between CD and BSD: time to generation interval (inter-AP interval), amplitude, ascending slope, descending slope, net influx charge, net efflux charge. As a reference to the minimum AP Vm, an envelope (En) (as illustrated in Figure 4B) was fitted to the minimum voltage recorded at the end of each AP using a Matlab software routine (The MathWorks Inc., USA). Using the same routine, the inter-AP interval was calculated as the time duration between AP peak amplitudes (as indicated in Figure 4 B, D). The maximum amplitude of each AP was separated into an ascending and a descending phase (as indicated in Figure 4C) and the fastest respective rates of change or slopes calculated. Then, the voltage signal was derived (dV/dt) and the integral, or area under the curve, of the resulting capacitance scaled current signal was used to calculate the charge (Figure 4 E and F).

Statistical analysis

As data of CD and BSD groups did not show a normal distribution in morphological and electrophysiological variables, inferences about differences were carried out with non-parametric tests as cited in the corresponding text and figure legends for each figure. For action potential (AP) analysis an algorithm developed in Matlab by Dr Morales Reyes was used. For comparisons of more than two groups such as in cell areas per cell type and set or in β-cell/α-cell area ratio per islet size and diet, after getting a significant Kruskal–Wallis test, and adjusting significance for multiple comparison with Bonferroni methods, a Dunn test was carried out. In comparisons of delta Vm per diet and delta [glucose] (related samples) differences were evaluated by aligned rank transformation (non-parametric) two-way repeated measurements ANOVA. Statistical analysis was performed using Prism (GraphPad Software, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Figures of the electrophysiological parameters analyzed were created using Prism and R (R Core Team, 2022).

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Author's contributions

Dr Israel Morales Reyes contributes with investigation, methodology, algorithm generation for data analyses, validation, formal analysis, and data curation, writing original draft, review and editing. Dr Illani Atwater, contributes with funding acquisition, equipment, resources, critical reading and writing, review, and editing. Dr Marcelino Esparza contributes with data validation and curation, algorithm generation for statistical analysis, writing, reviewing, and edition of final draft. E. Martha Pérez Armendariz contributes with conceptualization, supervision, validation, funding acquisition and administration,

project resources, writing original draft, review, and editing of final draft.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data accessibility

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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