RESEARCH PAPER

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Differences in insulin biosynthesis pathway between small and large islets do not correspond to insulin secretion

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In a variety of mammalian species, small islets secrete more insulin per volume than large islets. This difference may be due to diffusional limitations of large islets, or inherent differences in the insulin production pathways. The purpose of this study was to identify possible differences in the early phase of glucose-stimulated insulin biosynthesis between large and small islets. Isolated small and large rat islets were challenged with 30 minutes of high glucose. The expression of insulin gene transcription factors (MafA, NeuroD/ Beta2, and PDX-1), preproinsulin mRNA, proinsulin and insulin were compared between large and small islets. Under basal (low glucose) conditions, MafA and NeuroD had higher mRNA levels and greater protein amounts in large islets compared to small when normalized to GAPDH levels. 30 minutes of high glucose stimulation failed to alter the mRNA or subsequent protein levels of either gene. However, 30 minutes of high glucose suppressed activated PDX-1 protein levels in both small and large islets. High glucose stimulation did not statistically alter the preproinsulin mRNA (insulin 1 and insulin 2) levels. At the translational level, high glucose increased the proinsulin levels, and large islets showed a higher proinsulin content per cell than small islets. Insulin content per cell was not significantly different between small and large islets under basal or high glucose levels. The results fail to explain the higher level of insulin secretion noted in small versus large islets and may suggest that possible differences lie downstream in the secretory pathway rather than insulin biosynthesis.

Introduction

Within the mammalian pancreas lies a disperse set of spheroidal endocrine cell clusters, the islets, responsible for the regulated secretion of insulin, glucagon and a number of other hormones. Historically small islets have been shown to secrete more insulin than their large counterparts even from the same pancreas.[1-5] Further, transplantation of small islets into rodent models resulted in reversal of diabetes, while the same procedure with large islets failed to reverse the disease.[2,5] In human clinical trials, the patient outcomes were also found to be superior when more small islets comprised the transplanted tissue.[4] However, the underlying cause for the functional differences between large and small islets remains unclear.

Two obvious explanations for the higher level of insulin secretion from small islets are: 1) diffusion limitations prevent glucose from activating the pathway in the core of large islets, but not small ones, or 2) the cells within large and small islets are inherently different and the insulin production pathways are uniquely activated by glucose in small islets. To test the first theory, we eliminated the diffusion barrier in large islets by breaking them into smaller components. This improved the cellular survival, but failed to improve the inferior *in vitro* insulin secretion to the same rate as intact small islets.[6] This finding led to the conclusion that there might be cellular differences in insulin production biosynthesis or secretion capacity between small and large islets. Thus, the current study was designed to examine possible differences in the glucose-sensitive insulin biosynthesis pathway.

The insulin production pathway is regulated at the molecular level by increased intracellular glucose levels that activate insulin gene transcription factors via the: 1) homeodomain protein pancreas duodenum homeobox-1 (PDX-1);[7-11] 2) β-cell E-box trans-activator 2 (NeuroD);[12-14] 3) the basic region leucine zipper (MafA).[15-17] In cell culture, these effects peak within 30 minutes of the initial exposure to high glucose and decrease markedly after that time point. [18-19]

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Following activation of the insulin gene transcription factors, insulin is transcribed as preproinsulin mRNA, which exists in the cell for an extremely short period of time before being processed into proinsulin and stored in intracellular granules. Glucose stimulates the recruitment of preproinsulin mRNA from an inert cytosolic pool to the rough ER, the site of preproinsulin protein synthesis.[20-21] After one hour of incubation at high glucose, proinsulin levels in rat islets increased 6 – 25 fold.[22-24] The synthesis ends with the cleavage of proinsulin into c-peptide and mature insulin. The objective of present work was to determine whether the insulin synthesis steps (described above) were different between the β-cells from small (diameter $\leq$ 100μm) and large (diameter $\geq$ 200μm) rat islets.

Methods

Rat islet isolation and separation

Adult male Sprague Dawley rats (200∼250 g BW; Harlan Labs, Indianapolis, IN) were housed on a 12 hours light/dark cycle with free access to standard laboratory chow and water. All animals received care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Rat islet isolation methods followed our published procedures described in detail.[2,25] Briefly, rats were anesthetized by intraperitoneal injection of a mixture of ketamine and xylazine. After the peritoneal cavity was exposed, the pancreatic main duct to the intestine was clamped and the pancreas cannulated *in situ* via the common bile duct. The pancreas was distended with collagenase and removed. Islets were gently washed, and passed through a sterile stainless steel screen and centrifuged. The pellet was mixed with Histopaque (Sigmal-Aldrich, St. Louis, MO), centrifuged, and the islets floating on the gradient were collected and sedimented. Islets were passed through a sterile 40μm mesh cell strainer with Hanks' Balanced Salt Solution (HBSS; Sigma-Aldrich, St. Louis, MO). After this cleaning process, islets were placed into Connaught Medical Research Laboratories media containing 5.5 mM glucose (CMRL1066 medium; ThermoFisher, Waltham, MA) with the addition of 2 mM glutamine, 10% fetal bovine serum (FBS) and 1% antibiotic/ antimycotic solution and put into a 37°C culture chamber containing 5% CO_2.

For large and small islet separation, the islet culture media was changed to L15 containing 10% FBS and 5 mM HEPES, and islets were transferred into 37°C culture chamber without CO_2. Isolated islets were manually separated by size based on the criteria that small: diameter $\leq$ 100μm; large: diameter $\geq$ 200μm. After separation, a sample of islets from each group were manually measured using light microscopy with a 40X magnification. Diameters were measured in 2 directions, unless the islets were irregularly shaped, and then up to 4 measurements were taken for each islet. The cell number per islet was estimated based on the published equation $y = -0.0001\times3 + 0.0912\times2 - 6.2162x + 182.1125$, where y equals the total cell number and x equals the islet diameter (μm).[25]

Insulin and proinsulin content

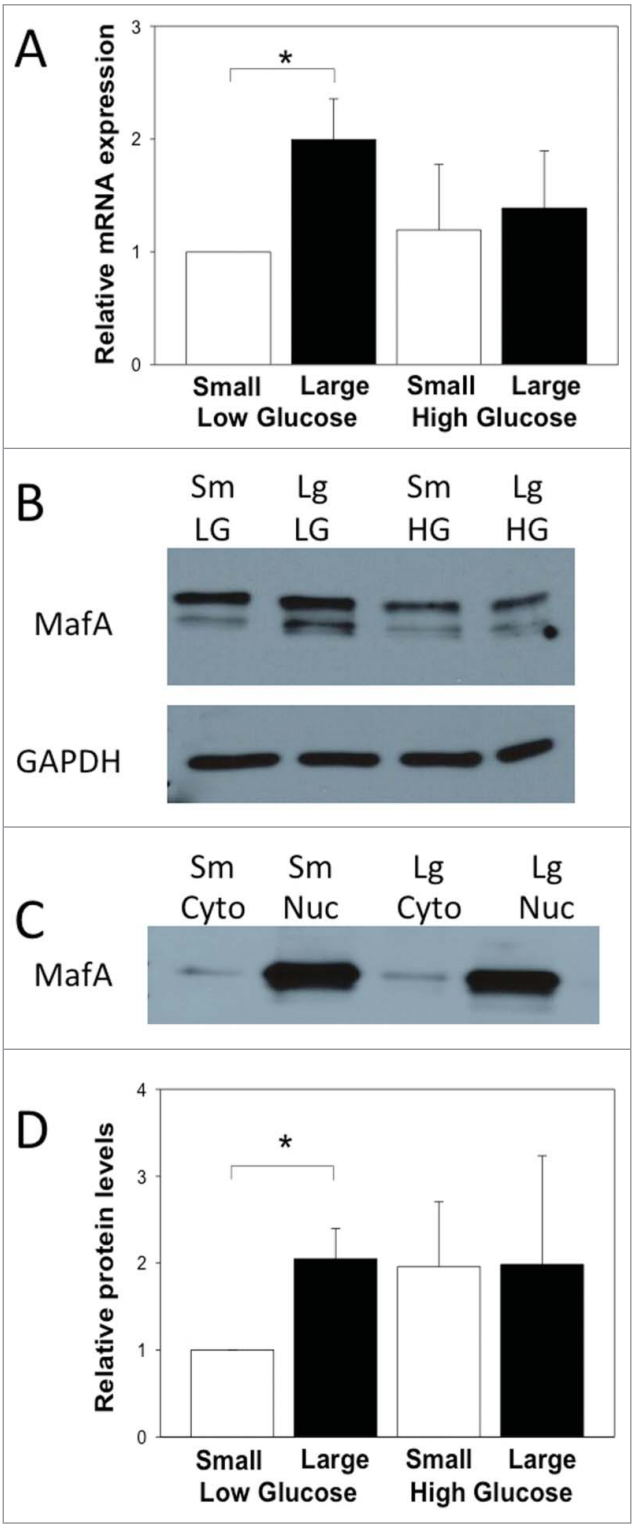
Islet static incubation assays followed our published procedures.[2,3,6] Briefly, islets were placed in 24-well plates with a minimum of 5 large or 15 small islets per well. All wells were preincubated for 2.5 hours in Roswell Park Memorial Institute medium with no glucose (RPMI 1640; Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum and 3 mM glucose in a 37°C containing 5% CO_2. After preincubation, media was removed from each well and discarded. Low (3 mM as basal condition) or high (16.7mM) glucose solutions were added, according to the design. After a 30 minutes static incubation at 37°C and 5% CO_2, media samples were removed and the islets were harvested and frozen at −80°C. The total protein in the islets was extracted by sonication in acid ethanol (0.18 M HCl in 95% ethanol) and incubated overnight at 4°C. The total intracellular proinsulin and insulin amounts in the extracts were determined separately by the ELISA (ALPCO, Salem, NH).

Western blot

Islets were homogenized using a $26G^1/_2$ syringe with extraction buffer containing 10 mM TRIS HCl pH7.4, 150 mM NaCl, 1 mM EDTA, 20 mM Na Molybdate, 50 mM Na Fluoride, 0.2 mM Na-Orthovanadate (pH 10), 1% Triton X-100, and 0.2 mM PMSF. The extracts were centrifuged for 15 minutes with 15,600 rcf at 4°C. Measurement of protein concentrations in supernatants was performed using Micro BCA Protein Assay Kit (Pierce, Rockford, IL). The protein samples from large and small islets extracts were prepared for electrophoresis by heating at 95°C for 3 min in SDS gel-loading buffer (0.125 M Tris, pH6.8, 5% glycerol, 2.5% mercaptoethanol, 2% SDS, and 0,001% bromophenol blue). For nuclear-enriched fractions, pellets from large and small islets were separated using the NE-PER Nuclear Cytoplasmic Extraction procedures (Thermo Fisher Scientific Inc.., Rockford, IL). A total of 10,000 small islets and 1500 large islets from 11 rats were used for the nuclear-enriched studies.

Proteins were separated on a 4–15% Tris-HCl Ready Gels (Bio-Rad Laboratories, Hercules, CA) with 0.025 M Tris, 0.192 M Glycine, 0.1% SDS running buffer. Equal amounts of total protein (10μg) were loaded in each lane. Molecular weight markers - SeeBlue Plus2 Pre-Stained Standard (Invitrogen, Carlsbad, CA) were used to determine the size of the antigen. After electrophoresis, the proteins were transferred from the gel to Bio Trace PVDF membranes 0.45μm (Pall Life Sciences, East Hills, NY) using 0.012 M Tris, 0.096 M Glycine transfer buffer. Blots were blocked with 5% nonfat dry milk diluted in 0.1 M PBS 0.1% Tween (PBST) for 1 hour, and primary antibodies were diluted in the 5% nonfat dry milk or 5% BSA in PBST according to the manufacturer's directions. Blots were probed with primary antibodies against MafA (Santa Cruz Biotechnology Inc., Santa Cruz, CA), PDX-1 (Cell Signaling Technology Inc. Danvers, MA), NeuroD (Cell Signaling Technology Inc. Danvers, MA)

and incubated at room temperature for 1 hour or at 4°C overnight according to the manufacturer's directions. After washing in 0.1 M PBS 0.1% Tween (10 minutes for 3 times), blots were incubated for 30 minutes with secondary antibody horseradish peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (Santa Cruz Biotechnology Inc., Santa Cruz, CA). After washing in 0.1 M PBS 0.1% Tween (10 minutes, 3 times), bound antibodies were detected using SuperSignal® West Pico Chemiluminescent Substrate (Thermo Fisher Scientific Inc., Rockford, IL). For a protein loading control, the membrane was reprobed with mouse anti GAPDH (**Sigma-Aldrich,** St. Louis, MO), for 1 hour at room temperature and densitometry was normalized to GAPDH levels for each sample.



RT-qPCR

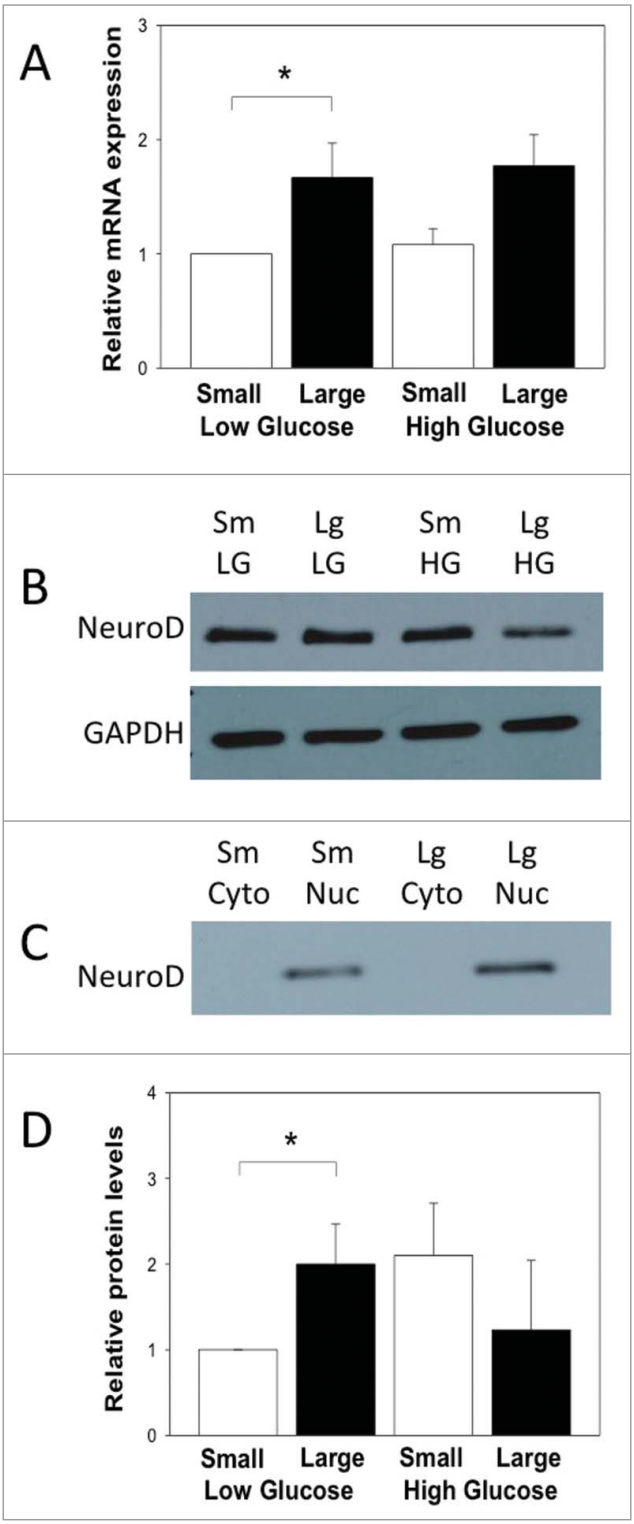
Total RNA was isolated from islets using RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and quantified by spectroscopy. Due to the fact that small islets have fewer cells,[25] large numbers of small islets had to be used to obtain sufficient RNA from small islets. Thus, 3000 small islets and 600 large islets from 18 rats were used for the mRNA studies. An equal quantity of total RNA (500ng) from each group was reverse transcribed using TaqMan Reverse Transcription Reagents (Applied Biosystems, Chicago, IL) according to the manufacturer's instructions. For real-time PCR, 150 ng cDNA products were mixed in TaqMan Gene Expression Master Mix (Applied Biosystems, Chicago, IL) together with gene specific primers: Insulin 1, Insulin 2, PDX1, MafA, NeourD (Applied Biosystems, Chicago, IL) and GAPDH (Applied Biosystems, Chicago, IL). Real-time PCR was carried out in an ABI Prism 7000® Sequence Detection System (Applied Biosystems, Chicago, IL). The amount of transcript was calculated using the comparative cycle threshold (C_T) method, which determines the amount of target genes against GAPDH as the normalization gene. Normalized C_T values were averaged to produce the mean C_T value for each gene analyzed.

Statistics

The experimental design included comparing islets from the same animals, thus each animal provided both large and small islets for the comparison studies (low and high glucose). The exact number of islets used in each experiment is shown in the figure legends. Results were expressed as mean of each group or islet population ± SEM. Where appropriate, differences between

Figure 1. MafA mRNA and protein levels in large and small islets. (**A**) Under basal conditions, MafA mRNA levels were greater in large islets compared to small. There was no difference in the high glucose condition. (**B**) Typical western blot for MafA shows 2 bands with a 49 kDa band as the most prominent. (Sm LG = small islets in low glucose, Lg LG = large islets in low glucose, Sm HG = small islets in high glucose, Lg HG = large islets in high glucose) (**C**) The 49 kDa band was predominantly found in the nuclear-enriched fraction of the islet cells. (**D**) Densitometry readings following normalization to GAPDH showed that, like the mRNA readings, there was more MafA protein in the large islets under basal glucose compared to small islets, with no differences measured in high glucose conditions. N = 3 experiments in each group. Each experiment contained at least 3,000 small islets or 600 large islets from 6 rats. The expressions in each group were presented as relative fold difference to small islets under low glucose. (*: $p < 0.05$ according to unparied t-test).

groups were examined for significance using one- or two-way ANOVA (factors: islet size and glucose concentration) followed by the Fisher's least significant difference (LSD) post hoc test. Unpaired Student's t-test was used for the comparisons between small and large islets under basal conditions. Significant differences were defined as $p < 0.05$.



Results

Transcriptional regulation of the insulin gene

Three transcription factors are involved in the upregulation of insulin gene expression; MafA, NeuroD/Beta2, and PDX-1. Each was measured in large vs. small islets at both the mRNA and protein levels.

MafA is a member of the Maf family of proteins, containing the basic leucine zipper.[16] The molecular weight of MafA ranges from 37–49 kDa, depending on post-translational modifications. In comparing the large and small islet groups, the gene expression of MafA was greater in the large islets compared to small islets under basal glucose conditions (**Fig. 1A**). All islets had more of the 49 kDa form of the protein, but smaller versions were found in each group (**Fig. 1B**). To ensure that the 49 kDa variation was the activated MafA, cellular fractions were enriched for cytoplasmic or nuclear components.[26] The 49 kDa form was predominantly found in the nuclear fraction (**Fig. 1C**). When the 49 kDa MafA protein was analyzed, there was statistically more protein in the quiescent large islets compared to small, but high glucose stimulation did not induce a further increase in levels (**Fig. 1D**).

NeuroD mRNA, along with the protein level, followed the same trends of MafA. NeuroD mRNA was greater in the large islets under basal glucose conditions compared to small islets (**Fig. 2A**). Exposure to high glucose made no impact on the NeuroD mRNA levels in either small or large islets. At the protein level, there was a trend toward more NeuroD in the small islets when stimulated with high glucose, but the difference was not significant (**Fig. 2B**). All of the NeuroD measured was found in the nuclear enriched fraction (**Fig. 2C**). The only statistically significant difference was the higher level of protein in the quiescent large islets compared to small (**Fig. 2D**). Exposure to high glucose did not cause a statistical increase in either group and there was no difference between the two groups under high glucose stimulation (**Fig. 2D**).

When assessing mRNA levels, there was no difference in PDX-1 between large and small islets or between low and high glucose (**Fig. 3A**). However, the activated PDX-1 protein levels

Figure 2. NeuroD mRNA and protein levels in large and small islets. (**A**) Like the results shown for MafA, NeuroD mRNA levels were greater in large islets compared to small under low glucose conditions. There was no difference in the high glucose condition. (**B**) Typical protein gel blot for NeuroD protein shows a single band with little difference between groups. (Sm LG = small islets in low glucose, Lg LG = large islets in low glucose, Sm HG = small islets in high glucose, Lg HG = large islets in high glucose) (**C**) The bands shown above were predominantly from protein in the nuclear-enriched fraction. (**D**) Densitometry readings following normalization to GAPDH showed that there was more NeuroD protein in the large islets under basal glucose compared to small islets, with no differences measured in high glucose conditions. N = 3 experiments in each group. Each experiment contained at least 3,000 small islets or 600 large islets from 6 rats. The expressions in each group were presented as relative fold difference to small islets under low glucose. (# $p < 0.05$ according to two-way ANOVA with Fisher's LSD test; *: $p < 0.05$ according to unparied t-test).

were significantly different between groups (**Fig. 3B**). Inactive PDX-1 is 31 kDa, while the activated form is a 46 kDa protein.[26] We confirmed that we were probing for active 46 kDa PDX-1 by demonstrating that our antibody only stained the nuclear-enriched fraction of islet proteins with no staining in the cytoplasmic fraction (**Fig. 3C**). The large islets had less activated PDX-1 protein in low glucose conditions compared to small islets. Stimulation with high glucose caused a further reduction in activated PDX-1 protein level that was statistically significant for both the large and small islet populations (**Fig. 3D**). Thus, PDX-1 was the only protein tested that demonstrated a statistical difference between low and high glucose conditions, and those differences were in the opposite direction of the anticipated result.

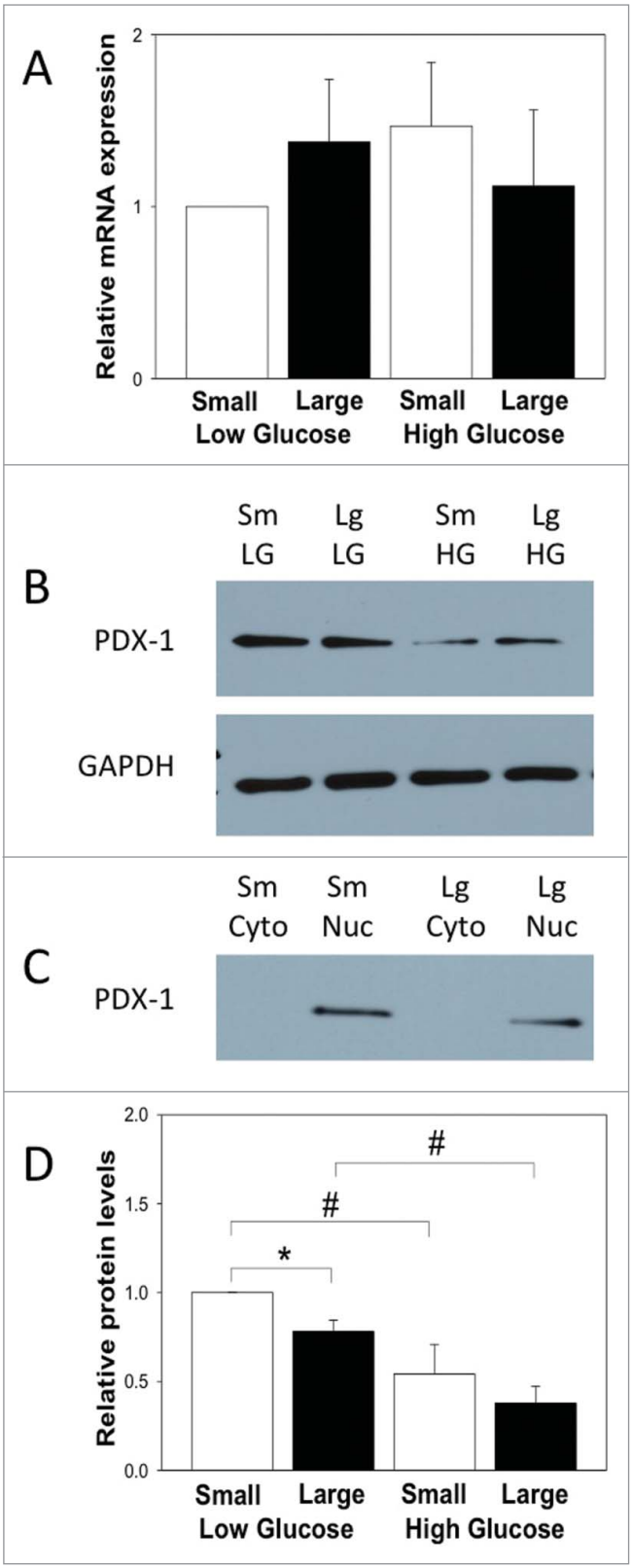


Figure 3. PDX-1 mRNA and protein levels in large and small islets. (**A**) There was no difference in PDX-1 mRNA levels between all four groups. (**B**) A typical western blot for PDX-1 shows a band at 46 kDa. (Sm LG = small islets in low glucose, Lg LG = large islets in low glucose, Sm HG = small islets in high glucose, Lg HG = large islets in high glucose) (**C**) The 46 kDa band was predominantly found in the nuclear-enriched fraction of the islet cells. (**D**) Densitometry readings following normalization to GAPDH showed that there was a decline in active PDX-1 protein in the large islets under basal glucose compared to small islets, and further declines in high glucose. N = 3 experiments in each group. Each experiment contained at least 3,000 small islets or 600 large islets from 6 rats. The expressions in each group were presented as relative fold difference to small islets under low glucose. (# $p < 0.05$ according to two-way ANOVA with Fisher's LSD test; *: $p < 0.05$ according to unparied t-test).

Insulin gene transcription

Downstream of MafA, NeuroD and PDX-1 are two separate insulin genes in rats that are transcribed into two preproinsulin mRNAs, insulin 1 and insulin 2.[27] Under basal conditions, insulin 1 mRNA in small or large islets was not statistically different (**Fig. 4A**). The high glucose challenge failed to induce an increase in mRNA levels of insulin 1 in either the small or large islets, yet there was a statistically significant difference between the two groups after stimulation. The mRNA levels of insulin 2 in small or large islets were not statistically different under basal or stimulated conditions. While not statistically significant, at least there was a trend toward increased production with high glucose, which had not been seen with insulin 1 (**Fig. 4B**).

Insulin gene translation

While there were no clues in the gene transcription factors suggesting a glucose-stimulated difference in islets of different sizes, the proinsulin levels did demonstrate stark differences in the basal and glucose-stimulated levels between large and small islets. Proinsulin levels were higher in the large compared to the small islets in the low glucose concentration (**Fig. 5A**). When stimulated with high glucose for 30 minutes, the proinsulin levels rose statistically for both groups. Still the large islets had more proinsulin/cell than the small islets after stimulation.

The final downstream product of this pathway ends in the production of insulin. Total insulin protein content was measured and no differences were found either between groups (large and small islets) or within groups under varying glucose concentrations (low and high glucose) (**Fig. 5B**).

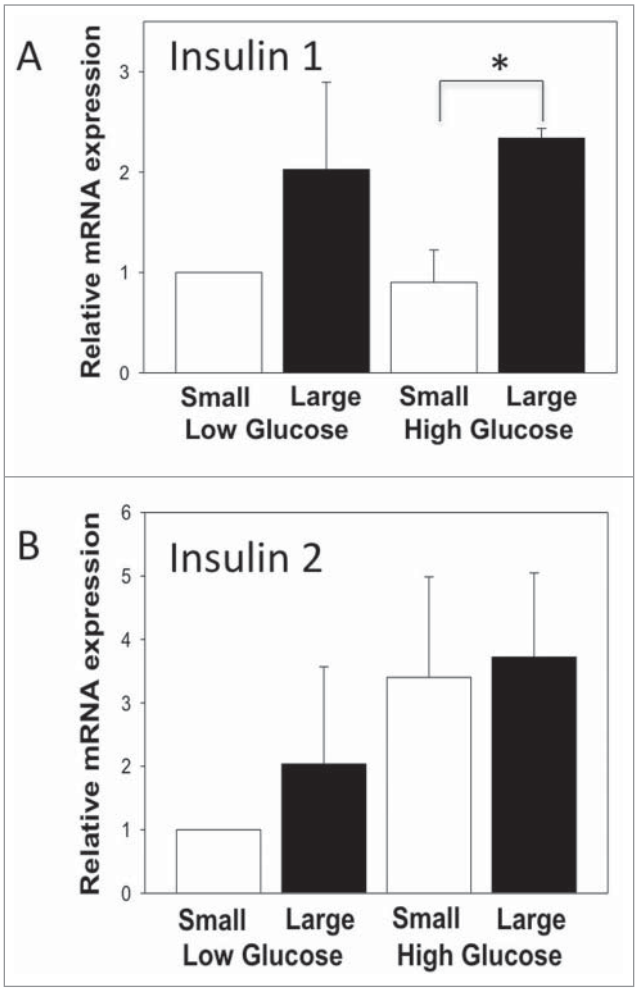


Figure 4. Preproinsulin mRNA levels. (**A**) After 30 minutes of exposure to high glucose, only the rat insulin1 gene was greater in the large islets compared to small. (**B**) There was no difference between large and small islets or between the 2 glucose conditions in rat insulin2. N = 3 experiments in each group. Each experiment contained at least 3,000 small islets or 600 large islets from 6 rats. The expressions in each group were presented as relative fold difference to small islets under low glucose. (* $p < 0.05$).

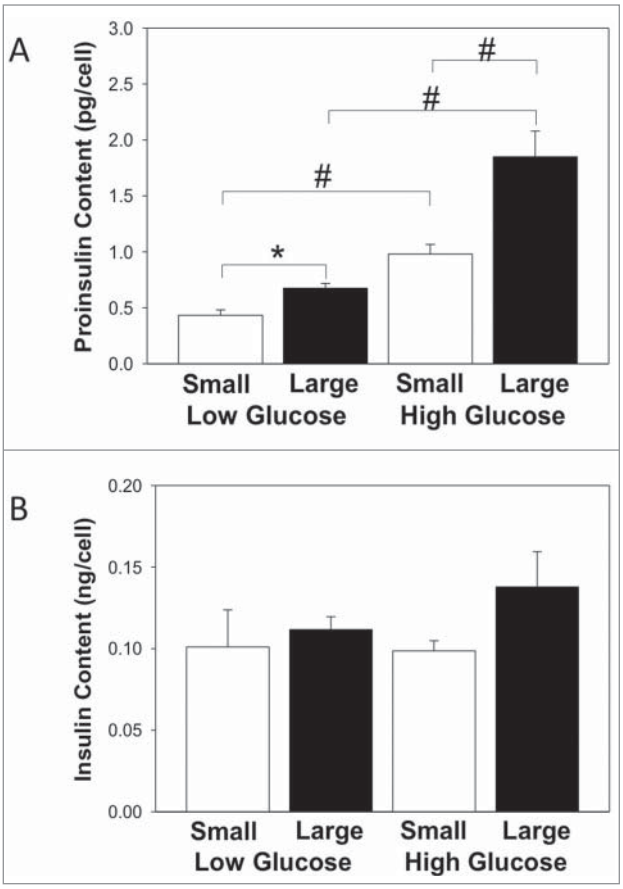


Figure 5. Proinsulin and insulin content per cell. (**A**) When normalized to cell, total proinsulin content from small and large isolated islets showed that large islets contained more proinsulin per cell in low and high glucose. Both small and large islets contained higher proisnulin levels in high glucose when compared to basal conditions. (**B**) When normalized to cell, total insulin content showed no significant difference in both small and large islet under basal conditions or high glucose. N = 6 experiments in each group. Each experiment contained at least 40 small islets or 10 large islets from 2 rats. (#: $p < 0.05$ according to two-way ANOVA with Fisher's LSD test; *: $p < 0.05$ according to unpaired t-test).

Discussion

Our group has been engaged in identifying the mechanism to explain the consistently higher insulin secretion/tissue volume from small islets compared to large islets in rodents and humans. First, we hypothesized that the diffusion barrier in large islets may play a role. Elimination of the diffusion barrier, however, in the large islets improved the cellular viability, but did not significantly improve the insulin secretion or content.[6] Those results suggested that the diffusion barrier alone could not account for the inferior insulin secretion per volume in large islets. Based on those findings, we hypothesized that there could be inherent differences between small and large islets in the insulin production pathway, starting with the insulin biosynthesis.

In the present study, we determined that large islets had more proinsulin per cell than the small islets in both low glucose and high glucose concentrations. This finding implies that large islets either have a superior capability of translating preproinsulin mRNA into proinsulin or an inferior capability of converting proinsulin into insulin. In addition, both small and large islets had a significant increase in proinsulin content after the high glucose challenge. Yet, there was no increase in insulin content in either group, which may have been due to the short incubation time (30 minutes). One explanation may be that the relative amounts of insulin and proinsulin are vastly different in individual β-cells. Although not reported, we found on average, the proinsulin content was approximately 0.43∼1.85 pg per cell, while the insulin content was approximately 99∼138 pg per cell, which is about 110 times higher than proinsulin. Therefore, it is reasonable that the changes the relatively small amount of proinsulin might not confer measurable differences in the intracellular insulin level.

One of the most interesting findings of this study was the lack of changes in gene expression in response to high glucose stimulation. Glucose is the main regulator of insulin biosynthesis including insulin gene transcription and translation in β-cell. From the normal physiological perspective, β-cells in the pancreatic islets are exposed to the elevated glucose for minutes after food-uptake. In the present study, we chose a 30 minute high glucose stimulation to investigate the mechanisms of early phase of glucose-stimulated insulin gene transcription and translation. This study confirmed the commonly held understanding that over a short period, glucose regulates the insulin biosynthesis mainly by increasing the translation of preproinsulin mRNA rather than insulin gene transcription. We measured a significant increase of proinsulin proteins in both small and large islets but not on the preproinsulin mRNA levels after 30 minutes high glucose stimulation. While most studies suggest that the effect of glucose on insulin gene transcription is long-term (over several hours or even days), two groups previously reported that a short time period of glucose stimulation elevated the preproinsulin mRNA levels in β-cells. Efrat et al. suggested that 10 minutes high glucose (16.7 mM) stimulates insulin gene transcription about 3-fold, and the transcriptional activity is maximal at 30 minutes.[18] Leibiger et al. reported that incubating isolated rat islets in high glucose (16.7 mM) for only 15 minutes, resulted in a 5-fold elevation in preproinsulin mRNA levels within 60 minutes after stimulation.[19] However, the former group used β tumor cell lines rather than native islets. The latter group extracted the RNA from isolated rat islets 60 minutes after the start of 15 minutes high glucose stimulation. Contrastingly, islets from fasted rats failed to respond to a 1 hour high glucose challenge, showing no changes in insulin gene expression.[28] Here, we analyzed the RNA levels from native rat islets immediately after a 30-minute glucose stimulation, and we found no significant increase of preproinsulin mRNA levels in both small and large islets. In addition, 30 minutes of high glucose stimulation also failed to alter the mRNA or subsequent protein levels of two insulin gene transcription factors, MafA and NeuroD.

In contrast, 30 minutes of high glucose stimulation significantly decreased the active form of PDX-1 protein level in both groups of islets. This is in contrast to a study using MIN6 cells (β-cell line) showing that PDX-1 protein levels were increased after 30 minutes high glucose (16mM) stimulation.[29] Another study showed an increased PDX-1 gene expression in mRNA levels in rat islets after one hour of high glucose (16mM) stimulation.[27] While others have shown decreases similar to ours with longer high-glucose incubation times,[30] this report appears to be the first to describe such a decrease with a short (30 min) exposure to high glucose. It is important to note that our antibody only recognized the 46 kDa form of the protein, so we can only discuss a decrease in the active form of PDX-1.

In addition, a challenge when working with native islets is that PDX-1 is expressed in α-cells, although at a lower level than β-cells.[31] Thus, a difference in the percentages of β- to α-cells in large and small islets could potentially skew the results. However, we have previously published extensive analysis of the percentage of α-, β-, and δ-cells in large and small rat and human islets.[3,32] In rats, analysis of pancreatic sections indicated that there was no difference in the distribution of α-, β-, or δ-cells between the large and small islets. Only when the islets were isolated from the pancreas was there a slight increase in the α-cells in the large islets.[3] The small difference (from 19 to 24%) would not appear to explain the differences in PDX-1 levels noted here.

Previously, we published work showing that small and large islets have an equivalent amount of intracellular total protein when normalized by either cell number or total DNA.[25] Surprisingly, four of the five proteins tested here demonstrated statistically different levels between small and large islets. Under the basal condition, higher levels of MafA, NeuroD and proinsulin were measured in large islets rather than small islets. However, PDX-1 protein levels were lower in large islets. Thus, only active PDX-1 levels followed the anticipated trend of more protein in the small islets. It is unclear whether the modest changes in PDX-1 levels identified in this study were sufficient to change insulin production. For example, in one study PDX-1 heterozygous mice did not have major defects in insulin production,[33] but in a similar study PDX-1 heterozygous mice demonstrated impaired insulin secretion.[34] Likewise, in patients with chronic pancreatitis [35] or type 2 diabetes[36] there is an association between reduced PDX-1 gene levels and insulin production. These findings suggest that small and large islets may possess some cellular heterogeneity in gene expression, but the interpretation of those differences are more complex than originally hypothesized as none of these differences correlated with total insulin content and insulin secretion, which is downstream of the insulin production pathway.

In summary, potential differences in insulin gene transcription between small and large islets were not identified during the early phase of high glucose stimulation. The findings do not correspond with the often-measured enhanced insulin secretion from small islets compared to large ones. Later steps in the insulin secretion pathway, which are also glucose-sensitive, may be a place to search for mechanistic differences that explain the superior insulin secretion from small islets.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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