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Cyclical and Alternating Infusions of Glucose and Intralipid in Rats Inhibit Insulin Gene Expression and Pdx-1 Binding in Islets

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Abstract

OBJECTIVE—Prolonged exposure of isolated islets of Langerhans to elevated levels of fatty acids, in the presence of high glucose, impairs insulin gene expression via a transcriptional mechanism involving nuclear exclusion of pancreas-duodenum homeobox-1 (Pdx-1) and loss of MafA expression. Whether such a phenomenon also occurs in vivo is unknown. Our objective was therefore to ascertain whether chronic nutrient oversupply inhibits insulin gene expression in vivo.

RESEARCH DESIGN AND METHODS—Wistar rats received alternating 4-h infusions of glucose and Intralipid for a total of 72 h. Control groups received alternating infusions of glucose and saline, saline and Intralipid, or saline only. Insulin and C-peptide secretion were measured under hyperglycemic clamps. Insulin secretion and gene expression were assessed in isolated islets, and β -cell mass was quantified by morphometric analysis.

RESULTS—Neither C-peptide secretion nor insulin sensitivity was different among infusion regimens. Insulin content and insulin mRNA levels were lower in islets isolated from rats infused with glucose plus Intralipid. This was associated with reduced Pdx-1 binding to the endogenous insulin promoter, and an increased proportion of Pdx-1 localized in the cytoplasm versus the nucleus. In contrast, MafA mRNA and protein levels and β -cell mass and proliferation were unchanged.

CONCLUSIONS—Cyclical and alternating infusions of glucose and Intralipid in normal rats inhibit insulin gene expression without affecting insulin secretion or β -cell mass. We conclude that fatty acid inhibition of insulin gene expression, in the presence of high glucose, is an early functional defect that may contribute to β -cell failure in type 2 diabetes.

Type 2 diabetes is due to the inability of pancreatic β -cells to compensate for insulin resistance induced by environmental factors such as obesity in genetically predisposed individuals (1). Chronic hyperglycemia (2), hyperlipidemia (3), or the combination of both (4) have been

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proposed to contribute to β -cell failure. However, the precise role of chronic metabolic alterations in β -cell dysfunction in vivo remains unclear.

The mechanisms by which elevated glucose and fatty acids affect β -cell function have mainly been studied in vitro. We (5–9) and others (10–13) have shown that prolonged exposure to elevated levels of glucose and fatty acids inhibits both glucose-stimulated insulin secretion (GSIS) and insulin gene expression in β -cells. Inhibition of GSIS occurs in the presence of either saturated or unsaturated fatty acids, whereas only the saturated fatty acid palmitate inhibits insulin gene expression (8), via de novo ceramide synthesis (7). Palmitate inhibition of insulin gene expression is transcriptional (7) and is associated with reduced binding activity of pancreas-duodenum homeobox-1 (Pdx-1) and MafA, two transcription factors essential for glucose regulation of the insulin gene, to the insulin promoter (9). Interestingly, palmitate affects Pdx-1 and MafA binding activities via distinct mechanisms: it reduces MafA mRNA expression while promoting nuclear exclusion of Pdx-1 (9).

While insightful, in vitro studies investigating the effects of fatty acids on β -cell function have limited pathophysiological relevance. First, addition of individual fatty acids does not mimic the mixture of saturated and unsaturated fatty acids present in the circulation. For example, Moffitt et al. (13) demonstrated that exposure of insulin-secreting cells to palmitate led to the formation of cytotoxic tripalmitin-rich triglycerides and subsequent morphological disruption of the endoplasmic reticulum (13). Second, during in vitro experiments, β -cells are exposed to stable, constantly elevated levels of fatty acids, a situation clearly different from the cyclical variations of fatty acid levels that occur in vivo during the prandial cycle. Third, the local concentration of biologically active fatty acids (unbound to albumin) in the vicinity of the β -cell is essentially unknown and determined by many factors, including the total fatty acid concentration, the molar ratio of fatty acids to albumin, and the activity of lipoprotein lipase (14).

These limitations prompted us to examine the effects of hyperglycemia and hyperlipidemia on pancreatic β -cell function in vivo using a time frame (72 h) similar to that of our previous in vitro studies. This study was designed to specifically examine whether cyclical and alternating 4-h infusions of glucose and Intralipid, over 72 h, alter 1) insulin secretion in vivo and in vitro in isolated islets; 2) insulin mRNA levels, Pdx-1 binding to the insulin promoter, and Pdx-1 and MafA expression; and 3) pancreatic β -cell mass.

RESEARCH DESIGN AND METHODS

Reagents

RPMT-1640 and fetal bovine serum were obtained from Invitrogen (Burlington, ON). Fatty acid-free bovine serum albumin was obtained from Equitech-Bio (Kerrville, TX), 50% Dextrose was from McKesson Canada (Montreal, QC, Canada), and sterile 0.9% saline was from Baxter (Mississauga, ON). All other reagents (analytical grade) were from Sigma unless otherwise noted.

Rat infusions

All procedures were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l'Université de Montréal. Male Wistar rats (Charles River, St.-Constant, QC, Canada) weighing 250–300 g were housed under controlled temperature (21°C) and 12-h light-dark cycle with unrestricted access to water and standard laboratory diet. Under general anesthesia, indwelling catheters were inserted into the left carotid artery and right jugular vein. The catheters were tunneled subcutaneously and exteriorized at the base of

the neck. The animals were recovered for 5 days after surgery. Catheter patency was maintained with 50 units/ml heparin in 0.9% saline.

One day before initiating the infusion, the animals were placed in cotton vests and connected by a flexible tether (Lomir Biomedical, Notre-Dame de l'Ile Perrot, QC, Canada) to a single channel swivel (Harvard Apparatus, Holliston, MA) suspended above the cage. The swivel was attached to a counter-balance mounted on the top of the cage (Instech, Plymouth Meeting, PA), affording the animal unrestricted motion. The animals were randomized into four groups, receiving either 0.9% saline (SAL), 50% glucose (GLU), 20% Intralipid (IL) with heparin (20 units/ml), or glucose plus Intralipid with heparin (GLU+IL). The infusion profile consisted of alternating cycles of glucose or saline for 4 h followed by 4 h of Intralipid + heparin or saline, repeatedly for 72 h (supplementary Fig. 1 [available in an online appendix at <http://dx.doi.org/10.2337/db07-1285>]). This infusion profile was controlled by a PC linked to Harvard infusion pumps (Pump 33; Harvard Apparatus) capable of independently operating two syringes simultaneously. Glucose was infused at 2 ml/h and Intralipid at 1 ml/h. Animals infused with saline alone received 4 h of saline at 2 ml/h followed by 4 h of saline at 1 ml/h. Where glucose or Intralipid were infused alone, the remaining volume was made up using saline at the appropriate infusion rate, such that all four groups received the same volume of fluid throughout the infusion. During the infusion, all animals had unrestricted access to food and water.

Samples for glucose and nonesterified fatty acid (NEFA) determinations were collected throughout the infusion period. At the end of the infusion, animals were either subjected to hyperglycemic clamps or killed for islet isolation or pancreas harvesting.

Intravenous glucose tolerance test

Intravenous glucose tolerance tests were performed on conscious, 1-h-fasted animals following the 72-h infusion by injecting 1 ml/kg of 50% glucose (0.5 g/kg). Blood glucose values were determined using a glucometer (Accu-Chek; Roche, Indianapolis, IN).

Hyperglycemic clamp studies

One-step hyperglycemic clamps were performed on conscious animals immediately following the 72-h infusion. A 50% dextrose solution was infused through the jugular vein to clamp plasma glucose levels at 13 mmol/l (upper physiological level for rats [15]) for 120 min and adjusted based on instantaneous assessments using a YSI sidekick glucose-analyzer (YSI, Yellow Springs, OH). Plasma samples were collected from the carotid artery for insulin and C-peptide measurements at 0, 2.5, 5, 10, 30, 60, 90, and 120 min.

Islet isolation and pancreas harvesting

In a second set of experiments, infused rats were anesthetized by intraperitoneal injection of a 100-mg/ml Ketamine Hydrochloride (Bimeda-MTC Animal Health, Cambridge, ON, Canada)/20 mg/ml Xylazine (Bayer, Toronto, ON, Canada) mixture, and islets were isolated by collagenase digestion and dextran density gradient centrifugation as described previously (7). Freshly excised whole pancreata were trimmed of fat, weighed, and fixed in 4% buffered paraformaldehyde and embedded in paraffin with 5- μ m sections mounted on glass slides for immunohistochemical and β -cell mass analyses.

Analytical measurements

Plasma glucose and NEFA levels were measured enzymatically using colorimetric kits (Wako Chemicals, Neuss, Germany). Plasma insulin was measured by radioimmunoassay (Linco

Research, St. Charles, MO) and C-peptide using a rat C-peptide ELISA kit (Mercodia, Uppsala, Sweden).

Real-time RT-PCR

Total RNA was extracted from aliquots of 100 islets as described previously (16). cDNA was synthesized from 1–2 µg islet RNA preincubated with 1 µg Pd(N)₆ (GE Santé Bio-Sciences, Baie D'Urfee, QC, Canada) at 73°C for 3 min followed by a 60-min incubation at 37°C with 100 units of Reverse Transcriptase M-MULV (Invitrogen) in RT-buffer containing 50 mmol/l Tris (pH 8.3), 8 mmol/l MgCl₂, 1 mmol/l dNTP mix, 10 mmol/l dithiothreitol, and 20 units RNase Inhibitor (Amersham). PCR was carried out using the Faststart DNA Master PLUS SYBR Green Kit (Roche). Primers, listed in supplementary Table 1, were designed using Primer3 (17). Results are expressed as the ratio of target mRNA to β-actin mRNA.

Insulin secretion and content in isolated islets

Insulin secretion was assessed in 1-h static incubations as described (18). Briefly, batches of 10 islets were washed twice in Krebs-Ringer buffer containing 0.1% bovine serum albumin and 2.8 mmol/l glucose for 20 min at 37°C, then incubated for 1 h at 37°C in either 2.8 or 16.7 mmol/l glucose. Each condition was run in triplicate. Intracellular insulin content was determined after acidified-ethanol extraction. Insulin was measured by radioimmunoassay (Linco).

β-Cell mass and Pdx-1 semiquantitative imaging

β-Cell mass and proliferation were measured as detailed previously (19). For Pdx-1 immunofluorescence, paraffin sections of rat pancreata were rehydrated, boiled in 10 mmol/l citrate (pH 6) for 30 min, blocked, and then immunostained with guinea pig anti-insulin IgG (Linco) and rabbit anti-Pdx-1 IgG (provided by C. Wright, Vanderbilt University, Nashville, TN) followed by donkey anti-guinea pig Cy2 (Jackson ImmunoResearch), donkey anti-rabbit Alexafluor 647 (Molecular Probes), and Hoechst (0.5 µg/ml).

Semiquantitative comparisons of Pdx-1 immunofluorescence intensity in islet β-cells were accomplished by batch staining and sampling under confocal microscopy (Zeiss LSM 510; UVM Microscope Imaging Core) for Pdx-1 (Alexa 647), the nuclear counterstain (Hoechst), and insulin (CY2). For each field, the microscope was focused to maximize the number of cells optically sectioned through the middle of the nucleus, and the Area-Measure tool (NIH Image J) was used to determine the mean pixel intensity (range 0–255 grayscale levels) of Pdx-1 immunofluorescence in a 20-pixel circle over the nuclear area (slightly smaller than the average nuclear surface area) and within the cytosol area of a β-cell. One hundred β-cells were counted for each rat.

Quantitative chromatin immunoprecipitation assay

Approximately 300 islets were fixed in 1% formaldehyde for 10 min, sonicated to shear DNA fragments in the range of 800–2000 bp, and subjected to chromatin immunoprecipitation (ChIP) as described previously (20). Each sample was quantitated in triplicate by SYBR Green I-based real-time PCR using the primers indicated in supplementary Table 1.

Expression of data and statistics

Data are expressed as means ± SE. Inter-group comparisons were performed by ANOVA followed by two-by-two comparisons using the Tukey-Kramer honestly significant difference test. $P < 0.05$ was considered significant.

RESULTS

Cyclical and alternating infusions of glucose and Intralipid plus heparin do not alter insulin secretion in vivo

During the glucose cycles, glycemia increased to ~ 13 mmol/l in the GLU and GLU+IL groups, returning to basal levels during the nonglucose cycles (Fig. 1A). For the SAL and IL groups, blood glucose remained at basal levels (6 mmol/l) throughout the 72-h infusion period (Fig. 1A). In the IL and GLU+IL groups, circulating plasma NEFA levels exceeded 3 mmol/l during the Intralipid infusion cycles and returned to basal values during non-Intralipid cycles (Fig. 1B). In the SAL and GLU groups, plasma NEFA levels remained at or below 0.3 mmol/l (Fig. 1B).

Overall, postinfusion body weights averaged 265.1 ± 1.8 g, a gain of 13 g over average preinfusion weights of 253.5 ± 1.8 g. Saline-infused rats exhibited a 5% increase in body mass from 256.1 ± 3.5 to 270.1 ± 4.1 g, while glucose-infused rats gained slightly less (3%; 257.1 ± 4.3 to 266.8 ± 3.5). Interestingly, Intralipid-infused rats gained nearly 10% of their preinfusion weight of 243 ± 4.1 to 265.2 ± 3.9 , while the combined glucose and Intralipid animals gained significantly less (1%; 257 ± 2.8 to 260 ± 3.3 , $P < 0.05$).

Insulin secretion was initially assessed by intravenous glucose tolerance test and showed no difference in either glucose levels or secreted insulin among the four infusion groups (supplementary Fig. 2). To more accurately assess the effects of the infusion regimens on insulin secretion, we performed hyperglycemic clamps in a second group of animals. Glucose, insulin, and C-peptide curves during the hyperglycemic clamps are shown in supplementary Fig. 3. The area under the curve for insulin secretion was significantly elevated in the combined GLU+IL group, particularly during the second phase of insulin release (Fig. 2A). However, the M/I index, which serves as an index of insulin sensitivity (15) and is obtained by normalizing the glucose infusion rate with circulating insulin levels, was similar among all four groups (Fig. 2B). No significant difference among treatments was observed in C-peptide levels (Fig. 2C). Consistently, the disposition index, an index of insulin secretion corrected for insulin sensitivity (15), calculated as M/I index $\times$ C-peptide, was not significantly different across the four groups (Fig. 2D). Overall, these results indicate that cyclical, alternating infusions of glucose and Intralipid plus heparin over a 72-h period do not significantly affect insulin sensitivity or insulin secretion in vivo.

To further examine insulin secretion in vitro, islets isolated following the 72-h infusion were subjected to 1-h static incubations (Fig. 3). Islets from the GLU and GLU+IL groups tended to have higher insulin release in response to 16.7 mmol/l glucose, although these differences were not statistically significant whether expressed as secretion per islet (Fig. 3A) or as a percentage of intracellular content (data not shown). In contrast, insulin content was significantly decreased in islets from the GLU+IL group compared with the GLU group ($5,071 \pm 248$ pmol/islet, $n = 15$, vs. $6,297 \pm 363$ pmol/islet, $n = 16$; $P < 0.05$; Fig. 3B).

Cyclical and alternating infusions of glucose and Intralipid plus heparin inhibit insulin gene expression and Pdx-1 nuclear localization and binding

Following the 72-h infusion, we examined expression of insulin, Pdx-1, and MafA mRNA in islets from the four experimental groups (Fig. 4). Similar to the effects of glucose and fatty acids in vitro over a 72-h period (5,6), glucose infusion induced a marked increase in insulin mRNA levels ($P < 0.001$, $n = 17$; Fig. 4A), which was prevented by co-infusion of Intralipid plus heparin (NS vs. SAL group, $n = 21$; Fig. 4A). In contrast, we observed no differences in Pdx-1 (Fig. 4B) and MafA (Fig. 4C) mRNA expression between the groups. Similarly, no changes were observed in MafA and Pdx-1 protein levels (Fig. 4D). In isolated islets, we have

shown that a 72-h exposure to high glucose and palmitate leads to nuclear exclusion of Pdx-1 (9). To determine whether this phenomenon also occurs in vivo, we examined Pdx-1 subcellular localization in pancreatic sections of infused rats (Fig. 5). In the SAL, IL, and GLU groups, the representative pancreatic sections presented in Fig. 5A show predominantly cytoplasmic expression of insulin and nuclear localization of Pdx-1. In the combined GLU+IL group, Pdx-1 increasingly colocalized with insulin in the cytoplasm of β -cells. Semiquantitative assessment of Pdx-1 indicated that the GLU group exhibited a significant ($P < 0.001$) increase in the nuclear to cytoplasmic signal ratio relative to the SAL and IL control groups and that the combined effect of glucose plus Intralipid significantly reduced nuclear localization of Pdx-1 ($P < 0.001$; Fig. 5B). To examine the functional consequences of the change in Pdx-1 subcellular localization, we measured Pdx-1 binding to the endogenous insulin promoter in isolated islets by ChIP analyses. As shown in Fig. 5C, binding of Pdx-1 to the insulin promoter was reduced in rats co-infused with glucose plus Intralipid relative to the glucose-only infused controls ($P < 0.05$, $n = 5-10$). Thus, Intralipid infusion blocked the stimulatory effects of glucose on Pdx-1 nuclear localization and binding to the endogenous insulin gene promoter.

Neither glucose nor Intralipid infusions significantly alter β -cell mass

Morphometric measurements of β -cell surface area indicated a significant increase in the GLU+IL group ($P < 0.05$ vs. SAL group, $n = 8$; Fig. 6A). However, total pancreatic weight was decreased in this group ($P < 0.001$, $n = 8$; Fig. 6B), such that β -cell mass was unchanged across treatments (Fig. 6C). β -Cell proliferation frequency was not different among the four groups (Fig. 6D).

DISCUSSION

This study was designed to determine whether combined hyperlipidemia and hyperglycemia inhibit insulin gene expression in vivo as observed in vitro (5–9). To this aim, we infused normal Wistar rats with glucose, Intralipid plus heparin, or both, in a cyclical and alternating manner with a periodicity of 4 h for a total of 72 h. We reasoned that alternating infusions of glucose and lipids would more closely resemble the physiologic variations in plasma levels than continuous and simultaneous infusions of both fuels. Our results uniquely demonstrate that cyclical and alternating infusions of glucose and Intralipid plus heparin reduce insulin mRNA levels and insulin content without significant changes to β -cell mass or insulin secretion. This is associated with exclusion of Pdx-1 from the nucleus and marked inhibition of its binding to the endogenous insulin promoter. These results show that the transcriptional inhibition of the insulin gene by fatty acids that we observed in vitro (7,9) also operates in vivo, thereby demonstrating the significance of this phenomenon in vivo.

As assessed by hyperglycemic clamp, insulin secretion in vivo at 13 mmol/l (upper physiological level for rats [15]) was not significantly different among rats infused with glucose or Intralipid alone, or in combination. However, it is possible that differences might have been seen at maximal stimulatory glucose levels. Similarly, insulin sensitivity, as assessed by the M/I index, appeared unchanged, although we have not performed euglycemic-hyperinsulinemic clamps. The lack of effects of glucose and Intralipid on insulin secretion is in marked contrast with the results of previous studies using continuous infusion protocols (15,21–29). While Sako and Grill (22) reported that glucose infusion reduced GSIS, in other studies a marked enhancement was observed (27–29). Thibault et al. (23) observed that sustained hyperglycemia enhances insulin secretion in both normal and hyperglycemic rats. This effect, however, was lost in severely glucose-intolerant rats, a finding that was reproduced by Bernard et al. (24). Alternatively, while short-term lipid infusions enhanced insulin secretion (30), prolonged Intralipid infusion induced insulin resistance and inhibited insulin secretion in rodents (15). In the perfused pancreas model, Sako and Grill (21) observed that a 48-h lipid

infusion inhibited insulin secretion. Similarly, Mason et al. (25) reported impaired insulin secretion in Wistar rats following a continuous 48-h lipid infusion. More recently, Goh et al. (15) demonstrated that lipid infusions induced peripheral insulin resistance and impaired β -cell function in Wistar rats comparable with what had been previously observed in humans (15). In contrast, Steil et al. (27) reported no effect of a 96-h Intralipid infusion on insulin secretion. Although not directly tested, we suspect that the different effects of glucose or Intralipid on insulin secretion between our results and previous publications are due, in part, to the discontinuous nature of the infusion. This suggests that the β -cell functional response to a steady and continuous change in its metabolic environment is of much greater amplitude than that to more physiological fluctuations. While circulating insulin levels were found to be elevated in the GLU+IL group during the clamp, C-peptide levels were not significantly different, suggesting a difference in insulin clearance, although this parameter was not directly measured.

Few studies have examined changes in β -cell gene expression in response to glucose or Intralipid infusions in vivo. No differences in the expression of islet-specific genes such as insulin, glucokinase, and Pdx-1 were observed in rats infused with glucose or Intralipid versus controls (27) or in cyclin genes involved in β -cell proliferation in glucose-infused mice (31). We observed an increase in insulin mRNA expression in glucose-infused rats, which was not observed in animals also receiving Intralipid. In isolated rat islets, glucose induction of insulin mRNA is blocked by the saturated fatty acid palmitate but not the unsaturated fatty acid oleate (5–7). The soybean oil emulsion Intralipid generates a mixture of ~80% unsaturated/20% saturated fatty acids when infused with heparin (32). Assuming that a parallel can be drawn between in vivo and in vitro situations, and considering that the circulating fatty acid levels achieved during the Intralipid infusions were ~3 mmol/l, the concentration of saturated fatty acids can be estimated to be ~0.6 mmol/l. Such concentration is within the range shown to affect the insulin gene in vitro (7,9). However, it is essential to bear in mind that the local concentrations of fatty acids in vivo in the vicinity of the β -cell are unknown and that, therefore, such parallels are likely inaccurate. Further, we show here that, as observed in vitro (9), the reduction in insulin mRNA expression is associated with a decrease in Pdx-1 binding activity and nuclear localization without changes in Pdx-1 mRNA and protein expression. These results confirm that fatty acids induce early posttranslational modifications of Pdx-1, which presumably contribute to the loss of insulin gene expression. Importantly, whereas in our previous in vitro study Pdx-1 binding activity was assessed by electromobility shift assays (9), in the present study we show a loss of Pdx-1 binding activity to the endogenous insulin promoter (by ChIP analysis) after infusions of glucose and Intralipid. In contrast to our previous study with isolated islets (9), in the present study the expression of MafA was not affected at either the mRNA or protein level by any of the infusion regimens. There are several potential explanations to this discrepancy between in vitro and in vivo results. First, it is possible that the duration of the isolation process could have blurred some differences in gene or protein expression between treatment groups. Second, it is conceivable that the decrease in MafA expression is secondary to the loss of Pdx-1 activity and would also be observed in vivo with longer infusions. Consistent with this possibility is the observation that Pdx-1 regulates MafA expression (33). Overall, although the contribution of a decrease in MafA expression was not indicated by the present results, our observations in this in vivo model of fuel oversupply confirm an early defect in insulin gene expression. The decrease in insulin gene expression while insulin secretion is unchanged is predicted to lead to depletion of intracellular insulin stores, which was indeed observed. Although not measured in this study, Bollheimer et al. (30) demonstrated that prolonged exposure to fatty acids does not enhance proinsulin biosynthesis. Therefore, it is likely that the loss of insulin gene expression in islets from the GLU+IL group results in decreased synthesis of insulin. Since intact regulation of insulin gene expression by glucose is required to maintain adequate intracellular insulin stores in the face of increased demand (34), the early loss of insulin gene expression upon infusion of glucose

and Intralipid will presumably lead to β -cell failure with longer infusions, although this hypothesis remains to be tested in our model.

Bonner-Weir et al. (35) reported a 50% increase in β -cell mass resulting from both hyperplasia and hypertrophy after 96 h of glucose infusion in rats. Similarly, Steil et al. (27) observed that 96 h of Intralipid or glucose infusion resulted in a 54 and 80% increase in β -cell mass, respectively, which was attributed to increased proliferation. Shorter, 48-h infusions of glucose in rats were also shown to increase β -cell mass by 60–65% (24,28). However, whereas Bernard et al. (24) associated these changes with cell hypertrophy, Paris et al. (28) observed no change in β -cell size and reported a decrease in β -cell proliferation. They concluded that the enhanced β -cell mass was due to neogenesis from ductal precursors (28). To assess metabolic adaptations to chronically elevated glucose, Topp et al. (29) carried out infusions for up to 6 days. They observed that prolonged glucose infusion increases β -cell mass twofold, stemming from both a 25% increase in β -cell size and enhanced replication (29). Collectively, these studies generally indicate that β -cell mass in rats is enhanced following continuous nutrient infusion. Interestingly, this was not the case in mice, where sustained glucose infusion, while enhancing β -cell replication, did not alter β -cell mass or cell size (31). In the present study, we observed no differences in β -cell mass in any of our infusion treatments, although rats in the GLU+IL group had significantly smaller pancreases. This was compensated for by an increase in β -cell surface area such that total β -cell mass was unchanged. The lack of increased β -cell proliferation in the GLU+IL group suggests that the increase in β -cell surface area may be due to β -cell hypertrophy rather than hyperplasia, although this was not measured directly. These modest changes in β -cell dynamics indicate first that discontinuous fuel oversupply has much more moderate effects than continuous infusions and second that the observed decrease in insulin gene expression is a bone fide functional change.

In conclusion, using an in vivo model of combined fuel overload, which to our knowledge has never been reported before, this study uniquely demonstrates that cyclical, alternating infusions of glucose and Intralipid in normal rats over 72 h inhibit insulin gene expression via nuclear exclusion and reduced binding of Pdx-1, without altering insulin secretion and β -cell mass. These findings establish the in vivo relevance of previous in vitro observations and suggest that fatty acid inhibition of insulin gene expression is an early functional defect that may contribute to pancreatic β -cell failure, although the role of this phenomenon in human type 2 diabetes remains to be examined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Glossary

ChIP	chromatin immunoprecipitation
GSIS	glucose-stimulated insulin secretion
NEFA	nonesterified fatty acid

Pdx-1 pancreas-duodenum homeobox-1

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FIG. 1. Infusion protocol. Circulating blood glucose (A) and plasma NEFA (B) during the glucose (■) and lipid (□) infusion cycles. Data are time-averaged for the 72-h infusion period representing the mean $\pm$ SE from 15–50 samples per group.

FIG. 2.

One-step hyperglycemic clamps in 72-h infused rats. Circulating insulin (A) and C-peptide (C) levels were expressed as area under the curve (AUC) from 0 to 120 min. B: M/I index expressed as $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ glucose infusion per pmol/l insulin. D: Disposition index ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol/l}^{-1} \cdot \mu\text{mol/l}$). Data are the mean $\pm$ SE from 5–6 rats per experimental group. * $P < 0.05$.

FIG. 3.

Insulin secretion in islets. *A*: Insulin secretion as assessed ex vivo in 1-h static incubations of isolated islets at basal (2.8 mmol/l; □) and stimulatory (16.7 mmol/l; ■) glucose. *B*: Total islet insulin content. Data are the mean $\pm$ SE from 11–18 rats per group. * $P < 0.05$.

FIG. 4.

Gene expression in islets. Islet mRNA expression of mature insulin (A), Pdx-1 (B), and MafA (C) following 72 h of infusion. Data are the mean $\pm$ SE from 13–20 rats in each experimental group. * $P < 0.05$. D: MafA and Pdx-1 protein expression from infused rat islets. The blot is representative of four replicate experiments.

FIG. 5.

Pdx-1 localization and activity. *A*: Subcellular localization of Pdx-1 examined by immunofluorescence in pancreatic sections of infused rats. Insulin is shown in green and Pdx-1 in red. Cytoplasmic colocalization of Pdx-1 and insulin is indicated by the orange emission. *B*: Quantification of the nuclear to cytoplasmic ratio of Pdx-1. Data are the mean $\pm$ SE of five islets (100 β -cells) from three rats in each experimental group. $***P < 0.001$. *C*: Binding of Pdx-1 to the endogenous insulin promoter, as assessed by ChIP analysis. Data are the mean $\pm$ SE from 5–10 rats per experimental group. $*P < 0.05$. (Please see <http://dx.doi.org/10.2337/db07-1285> for a high-quality digital representation of this figure.)

FIG. 6.

β -Cell mass. *A*: β -Cell surface area measured by morphometric analysis. *B*: Total pancreatic weight. *C*: Pancreatic β -cell mass (β -cell surface area $\times$ pancreatic weight). *D*: β -Cell proliferation frequency assessed by nuclear Ki-67 staining. Data are the mean $\pm$ SE from 6–8 rats per experimental group. * $P < 0.05$.