β-Cell Dysfunction in 48-Hour Glucose-Infused Rats Is Not a Consequence of Elevated Plasma Lipid or Islet Triglyceride Levels

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The abnormal insulin secretion found in human diabetics and animal models of diabetes has been attributed to the deleterious effects of chronic hyperglycemia and/or elevated circulating levels of nonesterified fatty acids (NEFAs). In this study, abnormal glucose-induced insulin secretion (GIIS) was generated by a 48-hour infusion of glucose and assessed by the isolated perfused pancreas technique. In these hyperglycemic animals, abnormal GIIS is accompanied by a decrease in plasma NEFAs, while plasma and, more importantly, islet triglycerides remain at levels comparable to those in the controls. It is concluded that the abnormal insulin secretion in this glucose infusion model was likely caused by 48 hours of hyperglycemia and not by changes in circulating or islet lipids.

ABNORMAL β-cell function with a specific loss of glucose-induced insulin secretion (GIIS) is a well-established characteristic of type 2 diabetes and is thought to be a consequence of the inability of the β cell to compensate adequately for the demands placed on them by overnutrition and/or an underlying insulin resistance.[1-3] When there is adequate compensation, insulin resistance is accompanied by hyperinsulinemia and an enhanced insulin response to a glucose challenge.[4] However, as glucose levels rise and subjects progress toward a state of impaired glucose tolerance (IGT) and frank type 2 diabetes, a marked loss in the acute GIIS occurs, although the ability of β cells to respond to non-glucose secretagogues such as arginine or isoproterenol is relatively well preserved.[1,2,5] Indeed, a reduction of the insulin response to either intravenous or oral glucose has been found to be a major predictor of the rate of progression of this disease.[6]

The cause(s) for the loss of GIIS is poorly understood, but is virtually never observed when fasting plasma glucose levels are truly normal, that is, less than 90 mg/dL. This loss in GIIS has been found to be closely related to rising fasting glucose levels, such that some reduction is found when fasting glucose exceeds 100 mg/dL, a level that usually would not even be associated with IGT. By the time fasting glucose levels reach and exceed 115 mg/dL, acute GIIS is virtually abolished.[5] One formulation for the progression is that the early failure might be caused by a β-cell mass that is inadequate for the degree of insulin resistance and/or by the modulation of processes that limit insulin secretion and thereby allow glucose levels to rise. The generation of this diabetic milieu, albeit mild, allows for the unmasking of a defect of GIIS in the β cell. One premise is that the abnormal β-cell function is caused by mild chronic hyperglycemia, a process termed glucotoxicity.[1,7] Another potential mechanism is that the high circulating NEFA levels found in the diabetic state could negatively influence β cells through lipotoxicity.[8-10] Perhaps the two processes are synergistic. The biochemical and/or molecular basis within the β cell for either glucotoxicity or lipotoxicity is yet unknown, although many hypotheses exist,[7] with recent interest focused on the possibility that an increased triglyceride content of β cells may somehow contribute to defective GIIS.[11]

In the present study, we used a model of β-cell dysfunction produced by 48-hour glucose infusion in normal rats,[12,13] which leads to insulin secretory changes similar to those observed in rodents with more chronic hyperglycemia.[14,15] These studies were undertaken to determine whether the β-cell dysfunction in this hyperglycemic model could be best linked to changes in plasma glucose or to plasma nonesterified fatty acid (NEFA) levels and/or islet triglyceride content.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250 to 280 g) were purchased from Taconic Laboratories (Germantown, NY) and maintained on a 12-hour light/dark cycle at 21° ± 1°C with free access to food (Purina Rodent Chow 5002, St Louis, MO) and water. All procedures in this study were in compliance with the Animal Welfare Act Regulations 9 CRF Parts 1, 2, and 3 and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.[16]

After a 4- to 7-day acclimation period, indwelling catheters were inserted in the left carotid artery and right jugular vein, tunneled subcutaneously, and externalized in the dorsal cervical region. The surgeries were performed under anesthesia using 68 mg/kg ketamine HCl (Aveco, Fort Dodge, IA), 3.6 mg/kg xylazine (Miles, Shawnee Mission, KS), and 0.7 mg/kg acepromazine (Aveco). The cannulas were filled with saline containing 20 IU/mL heparin and 25 KU penicillin G. Animals were allowed a 4- to 5-day postoperative recovery period before the infusions commenced.

The procedure for this 48-hour glucose infusion study was similar to that previously described,[12] with some modifications. Briefly, the animals were generally acclimated to a rodent harness for the 24-hour period preceding the infusions. The indwelling cannulas were then extended out of the cage and connected to a swivel, which allowed the animal unrestricted mobility and free access to food and water. The study animals were infused with a 50% (wt/vol in sterile distilled water) glucose solution, while the control animals received a 0.45% (wt/vol) saline solution. All solutions were sterile and infused through the

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Submitted June 3, 1999; accepted November 4, 1999.

Supported by grants from the National Institutes of Health (DK-35449 to G.C.W. and DK-36836 to the Joslin Diabetes Endocrinology Research Center) and an important group of private donors, and in part by a Mentor-Based Fellowship from the American Diabetes Association (N.T.).

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0026-0495/00/4906-0005$10.00/0

doi:10.1053/meta.2000.6240

jugular cannula at a rate of 2 mL/h using a syringe pump (Harvard Apparatus, Boston, MA). All infusions began at about 9 AM, and blood samples were collected into heparinized tubes from the carotid cannula at 0, 6, 12, 24, 36, and 48 hours after commencement of the infusion. The plasma was then separated and stored at −20°C.

At the end of the 48-hour infusion period, the animals were anesthetized with sodium pentobarbital (65 mg/kg intraperitoneally [IP]) and the pancreas was distended and removed for isolation of islets using a collagenase digestion technique.[17] The retroperitoneal and epididymal white and interscapular brown fat pads from 9 saline- and 13 glucose-infused animals were excised and weighed for use as an indicator of body adiposity.

The isolated islets from each animal (900 to 1,200 islets per animal) were washed with the isolation buffer and centrifuged. The supernatant was discarded and the pellet containing the islets was stored at −20°C. The islets were sonicated for 2 minutes in 0.5 mL distilled water, and a 50-μL aliquot was stored for DNA and insulin assays. The remainder of the sonicate was dried in a Speedvac (Savant Instruments, Holbrook, NY) and used for extraction of islet triglycerides.[18] Briefly, the residue was reconstituted with 20 μL chloroform:methanol (2:1, vol/vol) and the entire volume was spotted onto a thin-layer chromatography (TLC) plate. The vial was washed with an additional 20 μL chloroform: methanol and added to the previous 20 μL spot on the TLC plate. A lipid lintrol standard (Sigma, St Louis, MO) was analyzed in parallel. The plate was allowed to run in ether:hexane:acetic acid (70:30:1) until the solvent front reached within 2 cm from the top of the plate. Triglyceride spots were visualized with iodine and scraped onto glass filter columns previously washed with methanol. Each glass filter was washed 3 times with 5 mL methanol wash, and the flow-through was collected into glass tubes and dried under N_2. The dried residue was then resuspended with 200 μL buffer (50 mmol/L sodium phosphate, 20 mmol/L EDTA, and 2 mmol/L NaCl at pH 7.4). This sample was then analyzed for islet triglycerides using a GPO-Trinder triglyceride kit (Sigma). The extraction coefficient was calculated from the lipid standard sample.

To verify that the 48-hour glucose infusion resulted in abnormal insulin secretion similar to that previously described,[18] the pancreas of 2 animals from each treatment group was perfused immediately following termination of the glucose infusion. The rats were anesthetized with amobarbital sodium (100 mg/kg IP) and the pancreas was isolated and perfused in vitro as previously described.[19]

Plasma glucose concentrations were measured using a Beckman Glucose Analyzer II (Beckman, Brea, CA). Commercial diagnostic kits were used to measure plasma NEFA (Waco Pure Chemicals, Richmond, VA), plasma cholesterol and triglyceride, and islet triglyceride levels (Sigma). Islet DNA content was measured fluorometrically using the PicoGreen dsDNA Quantitation Kit (Molecular Probes, Eugene, OR). Insulin levels in plasma and islet extracts were determined with a double-antibody radioimmunoassay using a rat-specific insulin antibody and rat insulin as a standard (Linco Research, St Louis, MO).

Statistical Analysis

Data are presented as the mean ± SEM. Analyses were performed by Student's *t* test or repeated-measures 2-way ANOVA using the least-square means (LSM) for posteriori comparisons.

RESULTS

While the initial and final body weight differed between the saline (control) and glucose-infused groups, body weight changes during the 48-hour infusion period were comparable between the 2 groups (−14 ± 6 *v* −18 ± 2 g for control *v* glucose-infused, n = 10 and 13, respectively). Adiposity was increased in the glucose-infused group, as reflected by greater retroperitoneal (1.6 ± 0.2 *v* 1.0 ± 0.1 g, $P < .02$, Student's *t* test) and epididymal (2.7 ± 0.2 *v* 2.0 ± 0.1 g, $P < .02$, Student's *t* test) white fat, as well as interscapular brown adipose tissue (0.28 ± 0.02 *v* 0.12 ± 0.01 g, $P < .001$, Student's *t* test).

Pancreas perfusion data following the infusions indicate that the insulin secretory response to glucose was normal in the controls (Fig 1A) but dysfunctional in the glucose-infused group (Fig 1B), which concurs with the published literature for this model.[12,13] There was a paradoxical increase in insulin secretion from the pancreata of glucose-infused animals when the perfusate glucose level was reduced from 16.7 to 6.7 mmol/L. In contrast to the controls, there was a marked and persistent increase in the rate of insulin secretion from the pancreata of glucose-infused animals when perfusate glucose levels were decreased. With acute restimulation to 16.7 mmol/L glucose, insulin secretion increased in the glucose-infused group, but not as well as that observed in the control group, which is in keeping with the literature.[12,13]

In comparing plasma hormone and metabolite levels, preinfusion glucose and insulin levels were comparable between the 2 treatment groups. In the controls, plasma glucose levels remained unaltered over the 48-hour infusion period, while marked and sustained hyperglycemia approximately 10 mmol/L above basal was observed in the glucose-infused group (Table 1). Concomitant with the infusion-induced hyperglycemia, sustained hyperinsulinemia was observed in the glucose-infused group relative to the controls. With the start of the

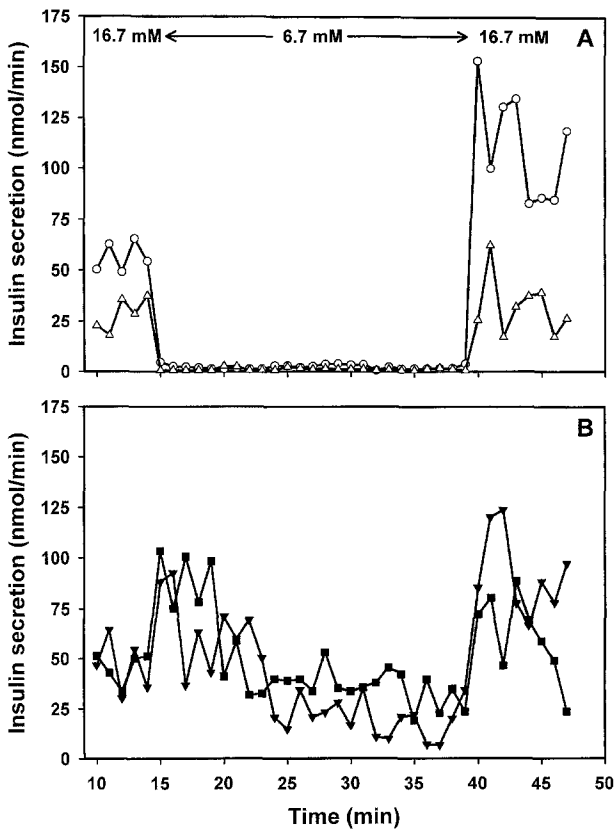

Fig 1. Effects of glucose on insulin secretion from the pancreata of 4 individual rats infused for 48 hours with saline (A) or 50% glucose (B). Insulin secretion was assessed using the ex vivo pancreas perfusion technique.

Table 1. Plasma Glucose and Insulin Levels in Normal Rats During a 48-Hour Infusion of 50% Glucose or 0.45% NaCl Solution

Parameter	Time After Commencement of Infusion (h)					
	0	6	12	24	36	48
Plasma glucose (mmol/L)						
Control	8.1 ± 0.5*	7.8 ± 0.3*	8.2 ± 0.4*	8.0 ± 0.3*	8.2 ± 0.5*	8.4 ± 0.4*
Glucose	7.7 ± 0.6*	18.3 ± 1.9†	16.9 ± 1.4†	19.6 ± 1.9†	19.0 ± 2.3†	17.7 ± 1.6†
Plasma insulin (μU/mL)						
Control	0.79 ± 0.16*	0.46 ± 0.12*	0.37 ± 0.05*	0.26 ± 0.03*	0.74 ± 0.19*	0.36 ± 0.07*
Glucose	0.67 ± 0.11*	5.22 ± 0.72†	5.48 ± 0.69†	5.84 ± 0.64†	5.50 ± 0.72†	5.33 ± 0.70†

NOTE. Data are the mean ± SEM. The number of animals in each group was 11 for the control and 14 for the glucose-infused group. Means with dissimilar superscripts differ at $P < .05$ by repeated-measures 2-way ANOVA and LSM.

infusion, plasma NEFA (Fig 2A) and triglycerides (Fig 2B) declined in both the control and glucose-infused groups. The decrease in plasma triglycerides was transient and comparable between the 2 groups, returning to preinfusion levels within 24 hours of commencement of the infusion. The decrease in NEFA was sustained, but the magnitude of the decrease relative to the controls was greater in the glucose-infused group during the first 24 hours of the 48-hour infusion period ($P < .05$, repeated-measures 2-way ANOVA and LSM; Fig 2A). Plasma cholesterol levels, while unchanged in the controls, were decreased by about 50% in the glucose-infused group for the duration of the 48-hour infusion period (Fig 2C).

Islet triglyceride levels were comparable between the 2 treatment groups, while islet insulin content per microgram DNA in the glucose-infused animals was only 26% of the level in the control group (Table 2).

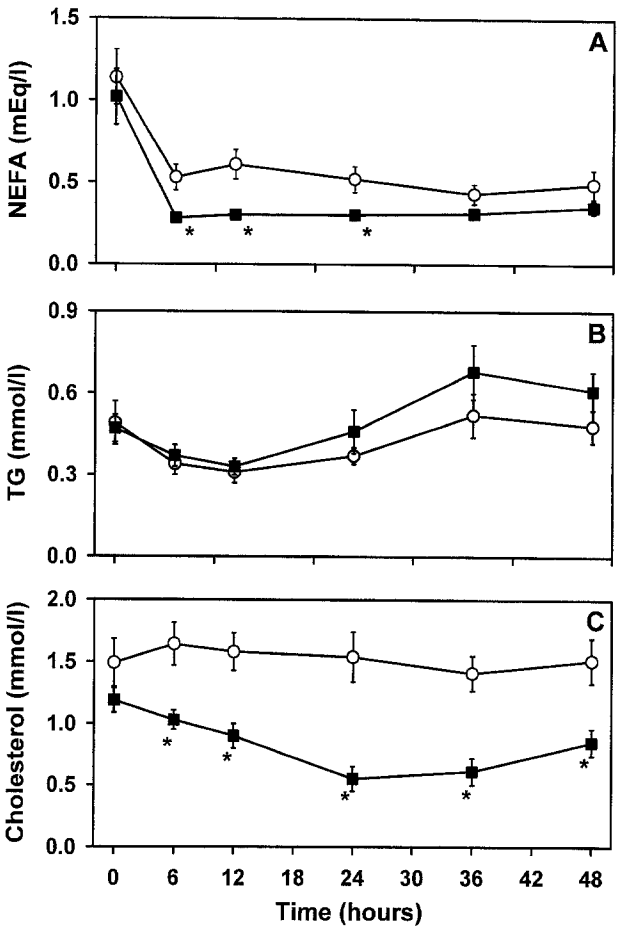

Fig 2. Changes in plasma NEFA (A), triglycerides (B), and cholesterol (C) in rats infused with saline (○) or 50% glucose (■) for 48 hours. Infusions were delivered via the jugular cannula and blood samples were obtained via the carotid cannula. Data are the mean ± SEM; n = 11 and 14 for saline- and glucose-infused animals, respectively. *$P < .05$ *v* respective control (repeated-measures ANOVA and LSM).

DISCUSSION

In this study, dysfunctional insulin secretion was clearly associated with the hyperglycemia produced by the 48-hour glucose infusion and therefore may be a direct effect of the elevated glucose level. Although various rodent models of diabetes have some differences in the characteristics of their insulin release patterns, they share a failure of the prompt suppression of insulin secretion when glucose concentrations are suddenly reduced, and there is a reduction or obliteration of the acute GIIS. It is of interest that with this protocol, there was some return of GIIS after 35 minutes of exposure to 6.7 mmol/L glucose, which shows the reversible nature of these defects. It is not clear whether we could obtain a similar rapid reversibility in rodents with more chronic hyperglycemia. In any event, it is difficult to attribute the β-cell dysfunction to an elevation in plasma lipid levels, since plasma NEFAs were lower in glucose-infused rats versus rats receiving saline, as should have been expected from the inhibition of adipose tissue lipolysis by the high insulin levels. Moreover, circulating triglyceride levels did not differ between the 2 groups, so they seem unlikely to account for the abnormal secretion. Nor can this dysfunction be attributed to increased lipid storage in β cells, since the triglyceride content of islets was similar in these glucose-infused rodents and the controls. This does not rule out the possibility that some lipid mediator in β cells may exert a negative influence, but it would be difficult to attribute such a mechanism to circulating lipid levels.

Table 2. Triglyceride and Insulin Content of Islets Isolated From Normal Rats Following a 48-Hour Infusion of 0.45% NaCl Solution (control) or 50% Glucose

Treatment	Triglyceride Content (nmol/μg DNA)	Islet Insulin Content (nmol/μg DNA)
Control (n = 4)	168 ± 14	1.32 ± 0.18
Glucose-infused (n = 4)	180 ± 16	0.35 ± 0.03*

NOTE. Data are the mean ± SEM.
*$P < .05$ *v* control, Student *t* test.

The results of this study raise questions about the influence of glucose and NEFAs on β-cell function in the normal and diabetic state. Although a dominant role for glucose in the normal control of insulin secretion has long been accepted, the concept of a glucotoxic influence on insulin secretion in the diabetic state has only been discussed more recently.[1,14] Likewise, an important role for lipids in β-cell function has only been appreciated in the past few years.[8,10] NEFAs have been found to play a crucial role in maintaining insulin secretion during fasting, which may provide an important brake to excessive ketogenesis.[10,20,21] Even during the fed state, NEFAs appear to work with glucose and other mediators such as amino acids, neurotransmitters, and gut hormones to provide optimal β-cell function.[10,22] Circulating NEFAs are presumably essential for providing acyl coenzyme A to β cells, which could serve, in a yet undefined manner, as mediators of secretion.[10,22]

In rodents, both in vivo and in vitro studies indicate that NEFAs can have a short-term stimulatory effect on insulin secretion,[8] but with chronic exposure as occurs with in vivo NEFA infusions for 48 hours or with the addition of NEFAs to cultured islets, an inhibitory effect is found.[10] In addition to a chronic inhibitory effect of NEFAs on secretion, inhibition of proinsulin biosynthesis has also been reported.[23-25] It is postulated that the insulin deficiency of diabetes (either absolute or relative) is responsible for circulating FFA levels that are even higher than those found in obesity, thus producing an inhibitory rather than a stimulatory effect on β cells.[8,10] Unfortunately, the relative contributions of hyperglycemia and elevated NEFAs to the β-cell dysfunction of diabetes have not been elucidated. One complexity is that glucose and lipids may have a synergistic or complementary inhibitory influence.[8] In studies with the rat partial pancreatectomy model wherein diabetes is caused by surgical reduction of the β-cell mass, the characteristic selective loss of GIIS can be found even with relatively modest elevations of plasma glucose.[15,26,27] These secretory changes, which are associated with the loss of β-cell differentiation as determined by altered gene expression, are tightly associated with increasing glucose, but not at all with plasma NEFA levels.[28]

The potential contribution of NEFAs to the abnormal insulin secretion of diabetes in humans is even more complex. It is difficult to convincingly correlate the loss of GIIS in IGT and early diabetes to increased NEFA levels. For example, normoglycemic obese subjects have elevated NEFAs and increased GIIS, with evidence that NEFAs in this situation contribute to both hyperinsulinemia and the increased GIIS.[28,29] However, a complete loss of GIIS is found with only modest increases in plasma glucose, levels that do not even meet the criteria for IGT.[5] No studies have yet shown that under conditions of mild hyperglycemia and an abolished GIIS, NEFAs are higher than the levels observed in normoglycemic obesity with its increased GIIS. When diabetes is more severe, it is easier to see elevated levels of NEFA, which is an expected consequence of the relative adipose resistance to the antilipolytic effects of insulin and/or the insulin deficiency of the diabetic state. Thus, until more detailed studies are performed, it must be concluded that the early loss of GIIS in humans is well correlated with increasing plasma glucose, but poorly correlated with NEFA levels. These findings implicate glucotoxicity rather than lipotoxicity in the early loss of GIIS in humans, but do not rule out an important role for NEFAs as a contributing variable or permissive factor, or exclude an important role for NEFAs when the diabetic state is more severe.

In summary, the current study of glucose infusion in rats produced a marked loss of GIIS that cannot be attributed to an increase in the circulating level of NEFAs or triglycerides or an increase in islet stores of triglycerides, indicating that the abnormal secretion in this model is not caused by lipotoxicity as the phenomenon is generally understood. We recognize the limitations of this model, as even the rats receiving saline infusion have reduced food intake and reduced NEFA levels, so extrapolation to human diabetes must be made with great caution. Nonetheless, experiments with such model systems allow one to probe the mechanisms responsible for the influence of changes in circulating glucose and lipid levels on β-cell function and could provide clues about the pathophysiology of diabetes.

REFERENCES

1. Weir GC, Bonner-Weir S: Insulin secretion in NIDDM, in LeRoith D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus: A Fundamental and Clinical Text. Philadelphia, PA, Lippincott-Raven, 1996, pp 503-508

2. Porte D Jr: β-Cells in type II diabetes mellitus. Diabetes 40:166-180, 1991

3. Leahy JL, Bonner-Weir S, Weir GC: Beta cell dysfunction induced by chronic hyperglycemia: Current ideas on mechanism of impaired glucose-induced insulin secretion. Diabetes Care 15:442-455, 1992

4. Karam JH, Grodsky GM, Forsham PH: Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. Diabetes 12:197-204, 1963

5. Brunzell JD, Robertson RP, Lerner RL, et al: Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. J Clin Endocrinol Metab 42:222-229, 1976

6. Lillioja S, Mott DM, Spraul M, et al: Insulin resistance and beta cell function in the development of non–insulin-dependent diabetes mellitus: Prospective studies of Pima Indians. N Engl J Med 329:1988-1992, 1993

7. Weir GC, Sharma A, Zangen DH, et al: Transcription factor abnormalities as a cause of beta cell dysfunction in diabetes: A hypothesis. Acta Diabetol 34:177-184, 1997

8. Sako Y, Grill VE: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B cell oxidation through a process likely coupled to fatty acid oxidation. Endocrinology 127:1580-1589, 1990

9. Lee Y, Hirose H, Ohneda M, et al: β-Cell lipotoxicity in the pathogenesis of non–insulin-dependent diabetes mellitus of obese rats: Impairment in adipocyte-cell relationships. Proc Natl Acad Sci USA 91:10878-10882, 1994

10. McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. Diabetologia 42:128-138, 1999

11. Shimabukuro M, Zhou Y-T, Lee Y, et al: Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. J Biol Chem 273:3547-3550, 1998

12. Leahy JL, Cooper HE, Deal DA, et al: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion. J Clin Invest 77:908-915, 1986

13. Leahy JL, Weir GC: Evolution of abnormal insulin secretory responses during 48 hours of in vivo hyperglycemia. Diabetes 37:217-222, 1988

14. Bonner-Weir S, Trent DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. J Clin Invest 71:1544-1553, 1983

15. Leahy JL, Bumbalo LM, Chen C: Beta-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. Diabetologia 36:1238-1244, 1993

16. National Institutes of Health: Guide for the Care and Use of Laboratory Animals. Washington, DC, US Government Printing Office, Department of Health, Education, and Welfare Publication (NIH) 85-23, 1995

17. Gotoh M, Maki T, Kiyoizumi T, et al: An improved method for isolation of mouse pancreatic islets. Transplantation 40:437-438, 1985

18. Malaisse WJ, Best L, Kawazu S, et al: The stimulus-secretion coupling of glucose-induced insulin release: Fuel metabolism in islets deprived of exogenous nutrient. Arch Biochem Biophys 224:102-110, 1983

19. Weir GC, Knowlton SD, Martin DB: Glucagon secretion from the perfused rat pancreas. J Clin Invest 54:1403-1412, 1974

20. Stein DT, Esser V, Stevenson BE, et al: Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. J Clin Invest 97:2728-2735, 1996

21. Dobbins RL, Chester MW, Daniels MB, et al: Circulating fatty acids are essential for efficient glucose-stimulated insulin secretion after prolonged fasting in humans. Diabetes 47:1613-1618, 1998

22. Prentki M, Corkey BE: Are the β-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? Diabetes 45:273-283, 1996

23. Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-stimulated insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93:870-876, 1994

24. Gremlich S, Bonny C, Waeber G, et al: Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin and somatostatin levels. J Biol Chem 272:30261-30269, 1997

25. Bollheimer LC, Skelly RH, Chester MW, et al: Chronic exposure to free fatty acid reduces pancreatic b cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. J Clin Invest 101:1094-1101, 1998

26. Zangen DH, Bonner-Weir S, Lee CH, et al: Reduced insulin, GLUT2 and IDX-1 in beta cells after partial pancreatectomy. Diabetes 46:258-264, 1997

27. Jonas J-C, Sharma A, Hasenkamp W, et al: Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. J Biol Chem 274:1414-1421, 1999

28. Boden G, Chen X: Effects of fatty acids and ketone bodies on basal insulin secretion in type 2 diabetes. Diabetes 48:577-583, 1999

29. Boden G, Chen X, Iqbal N: Acute lowering of plasma free fatty acids lowers basal insulin secretion in diabetic and non-diabetic subjects. Diabetes 47:1609-1612, 1998