Effects of Free Fatty Acids on β-Cell Functions: A Possible Involvement of Peroxisome Proliferator-Activated Receptors α or Pancreatic/Duodenal Homeobox

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It is well known that acute administration of fatty acids enhances insulin release from β cells, although chronic exposure to fatty acids inhibits insulin release (lipotoxicity). The mechanism for these reciprocal effects of fatty acids on insulin release remains to be elucidated. The present study was performed to investigate the effects of fatty acids on gene expression related to glucose metabolism or insulin biosynthesis. In islets cultured with palmitate for 8 hours, glucose-induced insulin release was enhanced together with increment of pyruvate carboxylase (PC) mRNA or peroxisome proliferator-activated receptors (PPAR)α. In contrast, by extending the culture period up to 48 hours, glucose-induced insulin release or islet insulin content was significantly impaired by the coexistence of palmitate. Concomitantly, PC, PPARα, GLUT-2, glucokinase (GK), preproinsulin, or pancreatic/duodenal homeobox-1 (PDX-1) mRNA were significantly suppressed in those islets cultured for 48 hours with palmitate. These data may imply that during short-term culture period palmitate promotes PPARα gene expression, which enhances PC mRNA expression leading to the enhancement of insulin release, whereas during long-term culture period, palmitate rather inhibits PPARα mRNA, which reduces PC mRNA expression. Furthermore, palmitate reduces GLUT-2, GK, or preproinsulin mRNA expression probably through the inhibition of PDX-1 mRNA.

TYPE 2 DIABETES is often associated with obesity and increased levels of circulating free fatty acids.[1] In healthy individuals, levels of free fatty acids usually range between 0.2 to 0.7 mmol/L, whereas in diabetics, levels of those are higher and often reach 1.0 mmol/L.[2-4] It is well known that acute elevation of free fatty acids moderately stimulates insulin release both at normal and at elevated glucose concentration.[5,6] However, in most diabetic states, elevated levels of free fatty acids persist for years and even decades. This phenomena has led to the proposal that high free fatty acid levels might aggravate the diabetic condition by increasing the peripheral resistance to insulin and increasing hepatic gluconeogenesis.[1] Indeed, Zhou and Grill[7] first characterized long-term inhibitory effects of free fatty acids on β-cell functions, that is, free fatty acids inhibited glucose-induced insulin release and insulin biosynthesis in islets cultured for 48 hours. Our previous report showed that a 48 hour-lipid infusion to normal rats time-dependently inhibited glucose-induced insulin release.[8] Zhou and Grill[9] showed that free fatty acids decreased the activity and gene expression of enzymes involved in β-cell glucose utilization and oxidation. An alternative hypothesis is that free fatty acids might also have direct inhibitory effects on insulin gene expression, thereby contributing to the decrease in insulin content. Gremlich et al[10] reported that a 48-hour exposure of islets to palmitate decreases insulin gene transcription through negative regulation of the transcription factor pancreatic/duodenal homeobox-1 (PDX-1). To investigate the mechanisms for effects of free fatty acids on β-cell functions, we measured insulin release, insulin content, or the gene expressions related to glucose metabolism or insulin biosynthesis in rat pancreatic islets cultured with free fatty acids.

MATERIALS AND METHODS

Materials

Glucose, palmitate, Dulbeccos' Modified Eagle Medium (DMEM), histopaque, N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) were purchased from Sigma Chemical Co (St Louis, MO). Rat insulin was from Cosmo Bio Co (Tokyo, Japan). Collagenase was from Boehringer Mannheim (Mannheim, Germany). Penicillin, streptomycin, and fetal-calf serum (FCS) were from Life Technology (Grand Island, NY). Insulin RIA kit was from Kabi Pharmacia Co (Tokyo, Japan). Tissue culture flask was from Falcon Co (Plymouth, England).

Animals

Female Wistar rats (150 to 250 g of body weight) were bred under pathogen-free conditions at the Kyushu University Animal Center, Fukuoka, Japan. They had free access to tap water and standard pelleted chow (Clea Japan Inc, Tokyo, Japan). They were exposed to a 12-hour light (6 AM to 6 PM), 12-hour dark cycle. All experiments were approved by the ethics committee for animal experiments at the Faculty of Medicine, Kyushu University and performed according to the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, as well as Law No. 105 and Notification No. 6 of the Japanese Government.

Isolation of Islets

Pancreatic islets were isolated by collagenase digestion as previously described.[11] Collagenase was injected into the common bile duct at a concentration of 2 mg/mL in 10 mL Hanks solution. The pancreas was digested at 37°C for 20 minutes. Islets were partly separated from exocrine tissue using gradient centrifugation, (1,000×*g*, 20 minutes, 4°C) in histopaque. Islets were transferred to DMEM containing 5.5 mmol/L glucose, antibiotic (100 U/mL penicillin and 100 μg/mL streptomycin), and 10% FCS, then cultured free floating at 37°C in a humidified atmosphere of 5% CO_2/95% air for 12 hours (primary culture) to remove exocrine or other tissues.

Islet Culture

After the period of primary culture, islets were selected under stereomicroscope and transferred into tissue culture flasks. Cultures were

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performed in DMEM containing 5.5 mmol/L glucose with or without 250 μmol/L palmitate for up to 48 hours at 37°C in a humidified atmosphere of 5% CO_2/95% air. Palmitate was dissolved in 95% ethanol before being added to the cultured media. The final concentration of ethanol in medium was 0.1% at the concentration of palmitate used in this study. Control conditions with 0.1% ethanol during culture were included in each experiment.

Insulin Release

After each culture period, islets were preincubated at 37°C for 30 minutes in Krebs-Ringer bicarbonate (KRB) medium[12] with the following composition: 143 mmol/L Na^+, 5.8 mmol/L K^+, 2.5 mmol/L Ca^{2+}, 1.2 mmol/L Mg^{2+}, 124.1 mmol/L Cl^-, 1.2 mmol/L SO_4^{3-}, and 25 mmol/L CO_3^{2-}, pH 7.4, supplemented with 10 mmol/L HEPES, 0.2% bovine serum albumin (BSA), and 3.3 mmol/L glucose. After preincubation, islets were selected under microscope and batches of 3 islets were transferred into tubes containing 300 μL of KRB with 3.3 mmol/L or 27 mmol/L glucose. Final incubations were then performed at 37°C for 60 minutes in a water bath with continuous shaking and in an atmosphere of 5% CO_2/95% air. At the end of the incubation, aliquots of the incubation media were retrieved for assay of insulin concentrations. Islets that had been exposed to 3.3 mmol/L glucose in the final incubation were retrieved for later determination of islet insulin content.

Insulin Assay

Insulin was measured by RIA using rat insulin as a standard. For the determination of islet insulin content, 3 islets were each put into 200 μL of acid-ethanol (0.18 mol/L HCl in 95% ethanol). Insulin was extracted overnight at 4°C after sonication as previously described.[13]

Measurement of mRNA Levels

Total RNA was extracted by the TRIzol isolation method (Life Technologies, Gaithersburg, MD) from about 100 isolated islets of individual rats. The mRNA levels of each protein were measured by using semiquantitative polymerase chain reaction (PCR). Total RNA was reverse-transcribed by random priming using Avian Myeloblastosis Virus reverse transcriptase (RT) (first-strand DNA synthesis) according to the manufacturer's instructions. A total of 1 μL of RT reaction mix was amplified with primers specific for each protein described in Table 1 in a total volume of 50 μL.[14-19] The samples were amplified in 18 to 20 cycles for preproinsulin, in 30 to 32 cycles for GLUT-2, glucokinase (GK), hexisokinase-1 (HK), pyruvate carboxylase (PC), pyruvate dehydrogenase (PDHE1 α), PDX-1, or β-actin, in 32 to 34 cycles for peroxisome-activated rececptors (PPAR)α using the following parameters: 92°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minute. Aliquots (10 μL) of the PcR were tested on 1% agarose gels. Gels were stained with ethidium bromide. Signasl were quantified by scanning densitometry using NIH Image 1.56 software (NIH, Bethesda, MD).

Statistical Analysis

All results are presented as means ± SEM of more than 4 experiments. Data were analyzed by Students *t* test (paired). *P* values less than .05 were considered to indicate significant differences.

RESULTS

Effects of Palmitate on Glucose-Induced Insulin Release or Islet Insulin Content in Cultured Islets

In islets cultured with 250 μmol/L palmitate for 8 and 24 hours, 3.3 mmol/L glucose-induced insulin release significantly increased with no effect of palmitate after 48 hours (Fig 1A). A total of 27 mmol/L glucose-induced insulin release increased not significantly in palmitate-cultured islets after 8 hours. After a 24- or 48-hour culture period, however, 27 mmol/L glucose-induced insulin release was significantly impaired by the addition of palmitate (Fig 1B). Furthermore, islet insulin content was significantly impaired by coculture with palmitate for 48 hours (Fig 1C).

Effects of Palmitate on Gene Expression of Proteins Related to Glucose Metabolism or Insulin Biosynthesis in Cultured Islets

Coculture with palmitate for 8 hours significantly enhanced mRNA expression of PC or PPARα. After 48 hours of culture, however, mRNA expression of these proteins was significantly suppressed by the coexistence of palmitate (Fig 2). GLUT-2, GK, preproinsulin, or PDX-1 mRNA was not affected by coculture with palmitate for 8 hours, while similar inhibition of mRNA expression of these proteins was shown after a 48-hour culture with palmitate (Fig 3). Palmitate did not affect mRNA expression of HK, PFK, and PDHE1α mRNA during a 48-hour culture period (data not shown).

DISCUSSION

In the present study, we showed that in islets cultured with 250 μmol/L palmitate for 8 hours, glucose-induced insulin release was enhanced together with PPARα or PC gene expression. Free fatty acids are known to activate PPARα.[20] PPARα

Table 1. Sequences of PCR Primer

mRNA	Primer Sequences (5′-3′)	Reference No.	mRNA	Primer Sequences (5′-3′)	Reference No.
GLUT-2	TTA GCA ACT GGG TCT GCA AT	12	PC	ACT TGT ATG AGC GGG ACT GC	15
	GGT GTA GTC CTA CAC TCA TG			TGA CCT TGA CGG GGA TTG GA	
GK	AAG GGA ACT ACA TCG TAG GA	12	PDX-1	GAG CAG GAT TGT GCC GTA ACC	16
	CAT TGG CGG TCT TCA TAG TA			CTC AAA GTT TTC AGA AGC TCG	
HK	GGC TCA GAG GAG ACC CTT CG	12	preproinsulin	TGC CCG GGC TTT TGT CAA AC	16
	CCA GGT CGA ACT TGA ATC AT			CTC CAG TGC CAA GGT CTG AA	
PFK	TGA TGA GCT CTG CAT CCC	13	PPAR α	CCT TTT TGT GGC TGC TAT	17
	TGT CGA AGA TGC CCT TCC			TCC CTG CTC TCC TGT ATG	
PDH	TCA AGT ACT ACA GGA TGA TG	14	β-actin	CGT AAA GAC CTC TAT GCC AA	12
	GGC GTA CAT GTG CAT TGA TC			AGC CAT GCC AAA TGT GTC AT	

Abbreviations: GK, glucokinase; HK, hexokinase I; PFK, phosphofructokinase-A; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase E1 α.

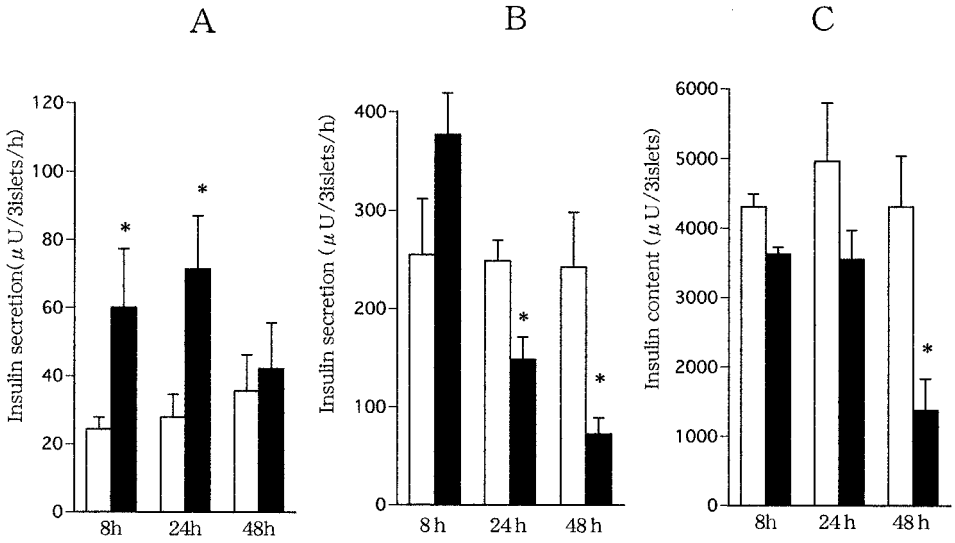

Fig 1. Glucose-induced insulin release and insulin content in islets cultured for 8, 24, and 48 hours. Open bar: islets cultured without palmitate. Closed bar: islets cultured with 250 μmol/L palmitate. (A) Represents 3.3 mmol/L glucose-induced insulin release, (B) 27 mmol/L glucose-induced insulin release, and (C) insulin content. Results are expressed as mean ± SEM of 4 experiments. *$P < .05$ *v* islets cultured without palmitate. Statistical significance was analyzed by Student's *t* test.

is primarily expressed in liver and regulates gene expression related to lipoprotein metabolism.[21] PPARα is also expressed in pancreatic islet,[22] while its role in β cells is unclear. Wang et al[23] reported that overexpression of leptin receptors in pancreatic islets of Zucker diabetic fatty (ZDF) rat augmented PPARα mRNA expression and restored glucose-stimulated insulin secretion. Their data are in line with our results except for the fact that GK or GLUT-2 mRNA was not affected by palmitate culture. PPARα is thought to be 1 of the transcription factors involved in direct upregulation of GLUT-2 and GK mRNA. This discrepancy may be due to the difference in the experimental model. PPARα mRNA increased by only 40% in the present study instead of the several times increment in their study. Unexpectedly, PC mRNA was increased in palmitate-cultured islets for 8 hours. PC is the important enzyme in pyruvate metabolism and it also plays a key role in regulating cytosolic nicotinamide adenine dinucleotide phosphate (NADPH) by participating in a pyruvate-malate shuttle.[24] Farfari et al[25] showed that phenylacetic acid, a PC inhibitor, reduced glucose-induced insulin release in both insulin-secreting (INS) cells and pancreatic islets. Although the relationship between PPARα and PC is unknown, it is possible that the increment of PC mRNA may play a part in the enhancement of glucose-induced insulin release in palmitate-cultured islets for 8 hours.

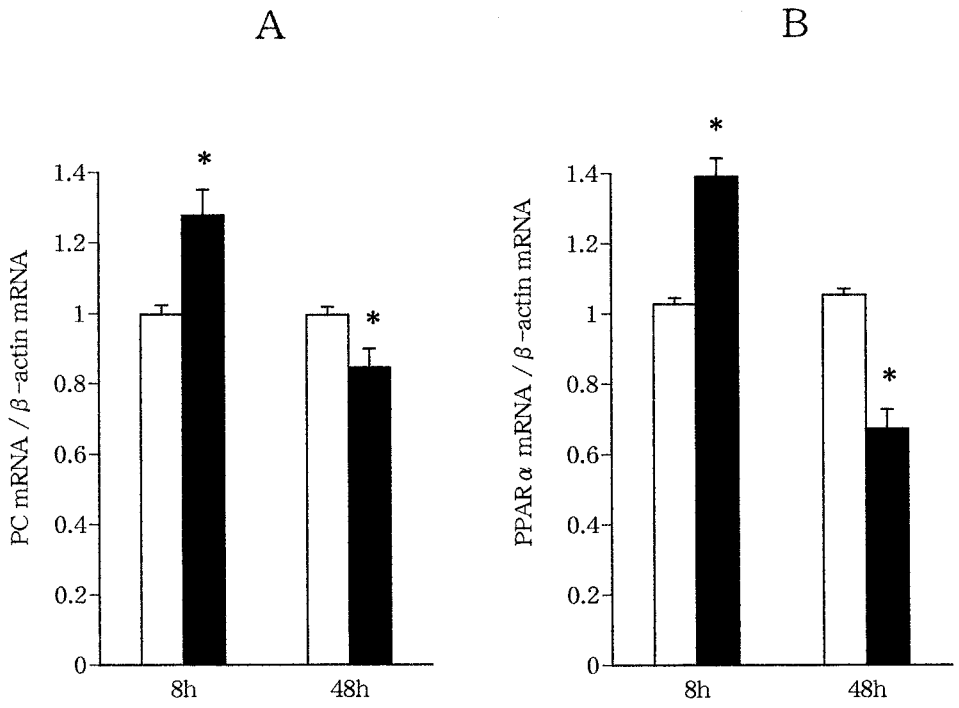

Fig 2. Quantification of PC or PPARα mRNA expression by semiquantitative RT-PCR in islets cultured for 8 or 48 hours. (A) Represents PC mRNA expression, (B) PPARα mRNA expression. Open bar: islets cultured without palmitate. Closed bar: islets cultured with 250 μmol/L palmitate. Mean ± SEM of 4 experiments. *$P < .05$ *v* islets cultured without palmitate. Statistical significance was analyzed by the Student's *t* test.

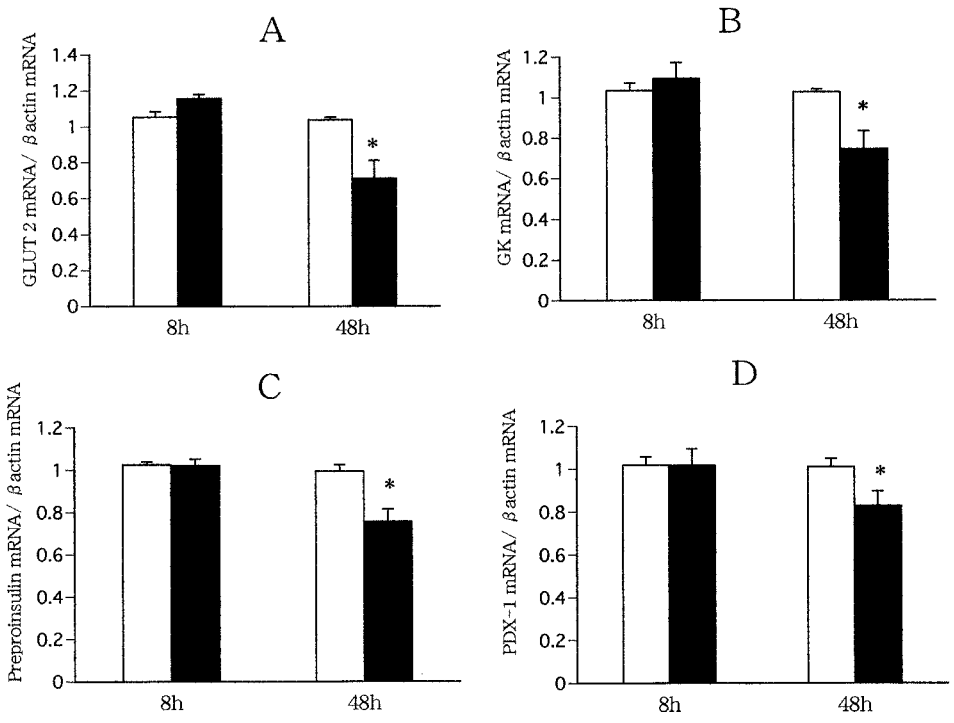

Fig 3. Quantification of GLUT-2, GK, preproinsulin, or PDX-1 mRNA expression by semiquantitative RT-PCR in islets cultured for 8 or 48 hours. (A) Represents GLUT-2 mRNA expression, (B) GK mRNA expression, (C) preproinsulin mRNA expression, and (D) PDX-1 mRNA expression. Open bar: islets cultured without palmitate. Closed bar: islets cultured with 250 μmol/L palmitate. Mean ± SEM of 4 experiments. *$P < .05$ *v* islets cultured without palmitate. Statistical significance was analyzed by the Student's *t* test.

In contrast to the 8-hour culture, 48-hour culture with palmitate rather suppressed glucose-induced insulin release or islet insulin content together with the suppression of gene expressions of PC or PPARα. Consistent with this result is the observation that pyruvate flux of PC was decreased in islets exposed to free fatty acids for the long term.[26] Furthermore, GLUT-2 or GK was suppressed in 48-hour culture with palmitate. As discussed above, PPARα could be 1 of the transcription factors involved in direct upregulation of GLUT-2 or GK mRNA.[23] We also showed that the gene expression of preproinsulin or PDX-1 was decreased in palmitate-cultured islets for 48 hours. These results imply that the impairment of insulin content in islets cultured with palmitate for the long term can be attributed to the inhibition of insulin biosynthesis. Gremlich et al[10] also reported that the exposure of isolated islets to palmitate for 48 hours decreased PDX-1 and insulin mRNA levels. Because PDX-1 transactivates GLUT-2, GK, or insulin genes,[27-30] PDX-1 may be 1 of the transcription factors in direct upregulation of GLUT-2 and GK mRNA, as well as PPARα in the palmitate culture period for 48 hours. However, the mechanism of the suppression of PPARα or PDX-1 by chronic exposure of free fatty acids needs to be clarified. It is possible that chronic exposure of free fatty acid may exert its inhibitory effects on PPARα or PDX-1 by some indirect mechanisms. Carlsson et al[31] reported that the generation of reactive oxygen species (ROS) was increased in palmitate-cultured islets for 24 hours. ROS-induced damage might influence β-cell dysfunction in palmitate-cultured isles for 48 hours.

In the present study, it is not clear whether those inhibitory effects by palmitate are reversible (desensitization) or irreversible (toxicity) ones. In this context, Bollheimer et al[32] reported that the effect of high circulating free fatty acid, which increased basal insulin release and decreased islet insulin content, could be blocked by somatostatin, a potent inhibitor of insulin release. Our preliminary results shown that coculture with diazoxide, K_{ATP} channel opener, restored the impaired insulin release or insulin content in islets cultured with 250 μmol/L palmitate for 48 hours. Furthermore, our preliminary results showed that wash-out of palmitate restored the impaired insulin release or content in palmitate-cultured islets. From these findings, desensitization is likely to occur in palmitate-cultured islets.

We showed that the expression of PDH, PFK, and HK mRNA did not change in palmitate-cultured islets for 8 or 48 hours. The glucose-fatty acid cycle (Randle cycle) is commonly known as the cause of the inhibition of glucose metabolism by free fatty acid (lipotoxicity). It entails 2 elements, the inhibition of PDH activity and PFK activity. The former inhibits glucose oxidation. The latter causes the accumulation of glucose-6-phosphate (G-6-P), which leads to the inhibition of HK activity and glucose utilization.[33-35] Indeed, it is reported that an exposure to free fatty acid for 48 hours led to the decrease of PDH activity in rat pancreatic islets.[10] In the present study, participation of PDH or PFK inhibition in lipotoxicity cannot be ruled out because we did not measure the activity of PDH or PFK. The concept of Randle cycle is now controversial. Liu et al[36] suggested that glucose-fatty acid cycle to inhibit glucose utilization and oxidation was not operative in fatty acid-cultured islets.

In this study, the concentration of palmitate was dissolved in 95% ethanol before being added to the culture media, and final concentration of ethanol in medium was 0.1%. The use of ethanol-solubilized low concentration of free fatty acid is a commonly used experimental modality. In vivo, free fatty acid

levels in the blood stream are much higher, but much is in association with blood albumin. Free fatty acid and ethanol can have physiochemical and membrane permeability interactions that are concentration-dependent. A previous study showed that in islets cultured for 48 hours with or without 1% ethanol, glucose-induced insulin release and islet insulin content were not changed.[7]

In conclusion, free fatty acids enhance insulin release during a short-term culture period by promoting PPARα or PC. On the other hand, free fatty acids suppress insulin release or content after a long-term culture period probably due to the inhibition of PPARα or PDX-1. Although, these reciprocal effects on insulin release are quite in line with previous reports, a distinct mechanism could be operative in each effect of free fatty acids.

REFERENCES

1. Elks ML: Fat oxidation and diabetes of obesity: The Randle hypothesis revisited. Med Hypotheses 33:257-260, 1990
2. Chen YD, Golay A, Swislocki ALM, et al: Resistance to insulin suppression of plasma free fatty acid concentrations and insulin stimulation of glucose uptake in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 64:17-21, 1987
3. Coon PJ, Rogus EM, Goldberg AP: Time course of plasma free fatty acid concentration in responses to insulin: Effect of obesity and physical fitness. Metabolism 41:711-716, 1992
4. Swislocki ALM, Chen YDI, Golay M, et al: Insulin suppression of plasma-free fatty acid concentration in normal individuals and patients with type 2 (non-insulin-dependent) diabetes. Diabetologia 30:622-626, 1987
5. Crespin S, Greenough W, Steinberg D, et al: Stimulating of insulin secretion of fatty acids. J Clin Invest 48:1934-1943, 1969
6. Malaisse WJ, Malaisse LF: Stimulation of insulin secretion by non-carbohydrate metabolites. J Lab Clin Med 72:438-448, 1968
7. Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93:870-876, 1994
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 couple to fatty acid oxidation. Endocrinology 127:1580-1589, 1990
9. Zhou YP, Grill V: Palmitate-induced B-cell insensitivity to glucose is coupled to decreased pyruvate dehydrogenase activity and enhanced kinase activity in rat pancreatic islets. Diabetes 44:394-399, 1995
10. Gremlich S, Bonny C, Waeber G, et al: Fatty acids decreased IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. J Biol Chem 272:30261-30269, 1997
11. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35-39, 1967
12. Umbreit WW, Burris RH, Stauffer JF: Manometric Techniques. Minneapolis, MN, Burgess, 1957, pp 149-150
13. Grill V, Rundfeldt M, Efendic S: Previous exposure to glucose enhances somatostatin secretion from the isolated perfused rat pancreas. Diabetologia 30:495-500, 1981
14. Tokuyama Y, Sturis J, Depaoli AM, et al: Evolution of β-cell dysfunction in the male Zucker diabetic fatty rat. Diabetes 44:1447-1457, 1995
15. Ma Z, Ramanadham S, Kempe K: Characterization of expression of phosphofructokinase isoforms in isolated rat pancreatic islets and purified cells and cloning and expression of the rat phosphofructokinase—A isoform. Biochem Biophys Acta 1308: 151-163, 1996
16. Amessou M, Fouqeu F, Soussi N, et al: Longitudinal study of tissue- and subunit-specific obesity-induced regulation of the pyruvate dehydrogenase complex. Mol Cell Endocrinol 144:139-147, 1998
17. Melendez RR, Perez-Andrade ME, Diaz A, et al: Differential effects of biotin deficiency and replenishment on rat liver pyruvate and propionyl-CoA carboxylase and on these mRNAs. Mol Genet Metab 66:13-16, 1999
18. Wang MY, Shimabukuro YL, Trinh KY, et al: Adenovirus-mediated overexpression of uncoupling protein-2 in pancreatic islets of Zucker diabetic rats increases oxidative activity and improves β cell function. Diabetes 48:1020-1024, 1999
19. Inoue I, Noji S, Shen MZ, et al: The peroxisome proliferator-activated receptor regulates the plasma thiobarbituric acid-reactive substance (TBARS) level. Biochem Biophys Res Commun 237:606-610, 1997
20. Herz R, Magenhiem J, Berman I, et al: Fatty acyl-CoA thioesters are ligand of hepatic nuclear factor-4α. Nature 392:512-513, 1998
21. Staels B, Dallongeville J, Auwerx J, et al: Mechanism of action of fibrate on lipid and lipoprotein metabolism. Circulation 98:2088-2093, 1998
22. Braissant O, Foufelle F, Scotto C, et al: Differential expression of peroxisome proliferator-activated receptor (PPARs): Tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology 137:354-366, 1996
23. Wang MY, Koyama K, Shimabururo M, et al: Overexpression of leptin receptors in pancreatic islets of Zucker diabetic fatty rats restores GLUT-2, glucokinase, and glucose-stimulated insulin secretion. Proc Natl Acad Sci USA 95:11921-11926, 1998
24. MacDonald MJ: Feasibility of a mitochondria pyruvate malate shuttle in pancreatic islets: Further implication of cytosolic NADPH in insulin secretion. J Biol Chem 270:20051-20058, 1995
25. Farfari S, Schulz V, Corkey B, et al: Glucose-regulated anaplerosis and cataplerosis in pancreatic β-cells: Possible implication of a pyruvate/citrate shuttle in insulin secretion. Diabetes 49:718-726, 2000
26. Iizuka K, Nakajima H, Nanba M, et al: Long-term exposure of pancreatic beta cells to fatty acid attenuates glucose-stimulated insulin secretion via the alternation of glucose and lipid metabolism. Diabetes 49:1095, 2000 (suppl 1, abstr A263)
27. Watada H, Kajimoto Y, Miyagawa J, et al: PDX-1 induced insulin and glucokinase gene expression in alpha TC1 clone 6 cells in the presence of betacellulin. Diabetes 45:1478-1488, 1996
28. Watada H, Kajimoto Y, Umayahara U, et al: The human glucokinase gene beta-cell-type promoter: An essential role of insulin promoter factor 1/PDX-1 in its activation in HIT-T15 cells. Diabetes 45:1478-1488, 1996
29. Lu M, Seufert J, Habener J: Pancreatic beta-cell-specific repression of insulin gene transcription by CCAAT/enhance-binding protein beta inhibitory interactions with basic helix-loop-helix transcription factor E47. J Biol Chem 272:28349-28359, 1997
30. Bonny C, Roduit R, Gremlich S, et al: The loss of GLUT2 expression in the pancreatic beta-cells of diabetic db/db mice is associated with an impaired DNA-binding activity of islet-specific transacting factors. Mol Cell Endocrinol 135:59-65, 1997
31. Carlsson C, Hakan Borg LA, Welsh N: Sodium palmitate induced partial mitochondrial uncoupling and reactive oxygen species in rat pancreatic islets in vitro. Endocrinology 140:3422-3428, 1999
32. Bollheimer LC, Skelly RH, Chester MW, et al: Chronic expo-

sure to fatty acid reduced pancreatic β-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

33. Randle PJ, Priestman DA, Mistry S, et al: Mechanism modifying glucose oxidation in diabetes mellitus. Diabetologia 37:S155-161, 1994 (suppl 2)

34. Randle PJ, Kerbey AL, Espinal J: Mechanism decreasing glucose oxidation in diabetes and starvation: Role of lipid fuels and hormones. Diabetes Metab Rev 4:623-638, 1988

35. Randle PJ: Regulatory interactions between lipid and carbohydrates: The glucose fatty acid cycle after 35 years. Diabetes Metab Rev 14:263-283, 1998

36. Liu YQ, Tronheim K, Leahy JL, et al: Glucose-fatty acid cycle to inhibit glucose utilization and oxidation is not operative in fatty acid-cultured islets. Diabetes 48:1747-1753, 1999