Inhibition of Insulin Gene Expression by Long-Term Exposure of Pancreatic β Cells to Palmitate Is Dependent on the Presence of a Stimulatory Glucose Concentration

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Long-term exposure of pancreatic β cells to elevated levels of fatty acids (FAs) impairs glucose-induced insulin secretion. However, the effects of FAs on insulin gene expression are controversial. We hypothesized that FAs adversely affect insulin gene expression only in the presence of elevated glucose concentrations. To test this hypothesis, isolated rat islets were cultured for up to 1 week in the presence of 2.8 or 16.7 mmol/L glucose with or without 0.5 mmol/L palmitate. Insulin release, insulin content, and insulin mRNA levels were determined at the end of each culture period. Palmitate increased insulin release at each time point independently of the glucose concentration. In contrast, insulin content was unchanged in the presence of palmitate at 2.8 mmol/L glucose, but was markedly decreased in the presence of 0.5 mmol/L palmitate and 16.7 mmol/L glucose after 2, 3, and 7 days of culture. In the presence of a basal concentration of glucose, insulin mRNA levels were transiently increased by palmitate at 24 hours but were unchanged thereafter. In contrast, palmitate significantly inhibited the stimulatory effects of 16.7 mmol/L glucose on insulin mRNA levels after 2, 3, and 7 days. To determine whether the inhibitory effect of palmitate on glucose-stimulated insulin mRNA levels was associated with decreased insulin promoter activity, HIT-T15 cells were cultured for 24 hours in 11.1 mmol/L glucose in the presence or absence of palmitate, and insulin gene promoter activity was measured in transient transfection experiments using the insulin promoter-reporter construct INSLUC. INSLUC activity was decreased more than 2-fold after 24 hours of exposure to 0.5 mmol/L palmitate. We conclude that long-term exposure of pancreatic β cells to palmitate decreases insulin gene expression only in the presence of elevated glucose concentrations, in part through inhibition of insulin gene promoter activity.

PLASMA TRIGLYCERIDES and free fatty acids are increased in a significant proportion of patients with type 2 diabetes mellitus.[1,2] Excessive lipid levels within the islets have been suggested to contribute to β-cell dysfunction in type 2 diabetes, a hypothesis known as "lipotoxicity".[3,4] In vitro, the effects of exogenous fatty acids (FAs) on insulin secretion are time-dependent. With short-term exposure, FAs augment glucose-stimulated insulin secretion (GSIS), whereas prolonged exposure increases basal insulin release and decreases GSIS.[5-8] The secondary decrease in GSIS upon prolonged exposure to FAs may be due to overstimulation of insulin secretion by simultaneous glucose and palmitate, which eventually leads to β cell "exhaustion" if intracellular stores of insulin are not replenished at the same rate as insulin is released. Indeed, a 48-hour exposure of isolated rat islets to palmitate or oleate increases basal insulin secretion but does not increase the rate of proinsulin biosynthesis.[8-10] An alternative hypothesis is that FAs might also have direct inhibitory effects on insulin gene expression, thereby contributing to the decrease in insulin content. Gremlich et al[11] reported that a 48-hour exposure of islets to 0.6 mmol/L palmitate decreases insulin gene transcription in the presence of 30 mmol/L glucose through negative regulation of the transcription factor islet/duodenum homeobox 1 (IDX-1, STF-1, PDX-1, and IUF-1). In contrast, Bollheimer et al[9] found that a 24-hour exposure of islets to 0.125 mmol/L oleate at 5.6 mmol/L glucose increased insulin gene mRNA levels.

In view of this discrepancy, we hypothesized that FAs inhibit insulin gene expression only in the presence of elevated glucose levels. This study was designed to assess (1) whether the effects of long-term exposure (up to 1 week) of isolated rat islets to palmitic acid on insulin mRNA levels are dependent on the presence of a stimulatory concentration of glucose, and (2) whether these glucose-dependent effects are associated with a decrease of insulin gene transcription in HIT-T15 cells.

MATERIALS AND METHODS

Palmitate Solution

Palmitic acid (sodium salt) was purchased from Sigma (St. Quentin Fallavier, France). A stock solution of 150 mmol/L palmitate was prepared by dissolving the powder in ethanol:H_2O (1:1 vol/vol) at 50°C. Aliquots of the stock solution were complexed with FA-free bovine serum albumin ([BSA] Sigma) by stirring for 1 hour at 37°C. The final molar ratio of palmitate to BSA was 5:1. All control conditions included a solution of ethanol:H_2O mixed with FA-free BSA at the same concentration as the palmitate solution.

Animals

Male Wistar rats 250 to 300 g were purchased from Charles River France (St Aubin les Elbeuf, France). Animals were housed on a 12-hour light/dark cycle with free access to water and standard laboratory chow (UAR, Epinay sur Orge, France).

Rat Islet Isolation and Culture

The pancreases were digested by intraductal injection of 5 mL collagenase type XI (Sigma) 0.5 mg/mL in Hanks buffered saline solution (HBSS) and stationary incubation as previously described.[12] Islets were purified on a bilayer HBSS/Histopaque 1077 (Sigma) gradient and cultured in a humidified atmosphere of 5% CO_2/95% air at 37°C. The islets were first cultured overnight in RPMI 1640 containing

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10% fetal calf serum (FCS) and 11.1 mmol/L glucose to ensure optimal recovery,[13] and then further cultured in 2.8 mmol/L glucose for 24 hours to reduce the insulin content and insulin mRNA to basal levels.[11] Batches of 100 islets were then cultured for 24 hours, 48 hours, 72 hours, or 7 days at a basal (2.8 mmol/L) or stimulatory (16.7 mmol/L) glucose concentration in the presence or absence of 0.5 mmol/L palmitic acid. For experiments longer than 72 hours, media were changed after 3 days.

Insulin Assay

Insulin was measured in the supernatant by radioimmunoassay. Insulin content was determined after acid-alcohol extraction as previously reported.[8]

Northern Blot Analysis of Insulin mRNA

Cultured islets were transferred to 15-mL conical tubes, rinsed twice with phosphate-buffered saline ([PBS] 137 mmol/L NaCl, 2.7 mmol/L KCl, and 4.3 mmol/L $NaH_2PO_4 \cdot 7H_2O$, pH 7.3), and resuspended in denaturing solution (4 mol/L guanidine thiocyanate, 25 mmol/L sodium citrate, pH 7, 0.5% sarcosyl, and 0.1 mol/L 2-mercaptoethanol). HIT-T15 cells were rinsed with PBS and scraped with denaturing solution. RNA was extracted according to the method of Chomczynski and Sacchi.[14] Total RNA was fractionated on a 1.5% agarose-formaldehyde gel and transferred to a nylon hybridization membrane (Hybond N^+; Amersham, Arlington Heights, IL) by electroblotting. The membrane was prehybridized in 50% formamide, 5× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate), 5× Denhardt solution, 50 mmol/L sodium phosphate, 0.1 mg/mL salmon sperm DNA, and 0.1% sodium dodecyl sulfate (SDS) at 42°C overnight, and then hybridized for 16 hours with ^{32}P-labeled Syrian hamster preproinsulin cDNA probe[15] in the same solution. The membrane was then washed 3 times at room temperature in 2× SSC and 0.1% SDS, twice at 60°C in 0.2× SSC and 0.1% SDS, and exposed to x-ray film (Kodak Biomax; Eastman Kodak, Rochester, NY) for 4 to 12 hours. Under these hybridization conditions, the probe hybridized with a single 0.5-kb band on agarose gel fractionation of total islet or HIT cell RNA. The membranes were stripped for 2 hours at 75°C in 1 mmol/L Tris hydrochloride, pH 8.0, 1 mmol/L EDTA, pH 8.0, and 0.1× Denhardt solution, and rehybridized with a ^{32}P-labeled oligonucleotide probe specific for 18S ribosomal RNA (islet experiments) or a ^{32}P-labeled β-actin cDNA probe (HIT-T15 cell experiments) to control for variations in the amount of total RNA. RNA levels were quantified by scanning densitometry of the autoradiographs (Imager; Appligène Oncor, Illkirch, France), and the data are expressed as the density ratio of insulin mRNA to 18S rRNA or β-actin mRNA.

Transient Transfections

The plasmid INSLUC containing the human insulin gene sequences −326 to +30 linked to the luciferase reporter gene was a gift from Dr Danielle Melloul (Hadassah Medical Center, Jerusalem, Israel). HIT-T15 cells (passage 70 to 76) were subcultured for 48 hours in RPMI 1640 containing 11.1 mmol/L glucose at a density of 1.2×10^6 cells per well in 6-well plates. Duplicate wells were cotransfected with 1 μg INSLUC and 20 ng pRL-CMV DNA by a liposome-mediated method (Lipofectin; GIBCO-BRL, Gaithersburg, MD). All transfections and subsequent incubations were performed in 11.1 mmol/L glucose. Cells were rinsed with FCS-free RPMI 1640, and then 1 mL FCS-free RPMI 1640 containing a 1:4 (wt/wt) ratio of plasmid DNA to lipofectin was added to each well. Cells were incubated for 4 hours at 37°C, after which the transfection medium was replaced by 4 mL RPMI 1640 containing 11.1 mmol/L glucose with or without 0.5 mmol/L palmitate. Cells were harvested 28 hours after transfection. Firefly luciferase (INSLUC) and Renilla luciferase (pRL-CMV) activities were measured using the Dual Luciferase Assay kit from Promega (Madison, WI). Data are expressed as relative INSLUC construct to pRL-CMV construct expression to control for the variability in transfection efficiency observed between different culture conditions.

Statistical Analysis

Data are expressed as the mean ± SE. Intergroup comparisons were performed by Student's paired *t* test or ANOVA, where appropriate. A P level less than .05 was considered significant.

RESULTS

Effects of Palmitate and Glucose on Insulin Release and Insulin Content in Isolated Islets

Isolated islets were cultured for 24, 48, or 72 hours in media containing either 2.8 or 16.7 mmol/L glucose in the absence or presence of 0.5 mmol/L palmitic acid. Palmitic acid was chosen because it is a predominant FA in human plasma. The concentration of 0.5 mmol/L is in the range of FA levels observed in moderately obese patients. The presence of 0.5 mmol/L palmitate in the culture media did not modify the amount of total protein recovered from the islets after 72 hours of culture (38 ± 5 *v* 41 ± 26 μg/mL in the absence of palmitate, n = 3, nonsignificant [NS]), ruling out a major effect of the FA on cell viability under these conditions. Figure 1A shows cumulative insulin release at the end of each culture period. As expected, insulin release was increased in the presence of high glucose (ANOVA, $P < .0001$). This effect was dependent on the length of time in culture (ANOVA, $P < .0001$) but independent of the presence of palmitate (ANOVA, NS). Insulin release was significantly increased in the presence of palmitate (ANOVA, $P < .0001$), an effect that was independent of the glucose concentration (ANOVA, NS). The stimulated fraction of insulin release, calculated as the difference in insulin release at 16.7 mmol/L glucose versus 2.8 mmol/L glucose, was not significantly affected by palmitate (358 ± 49 *v* 279 ± 44 μU/islet without palmitate after 24 hours, n = 15; 549 ± 89 *v* 490 ± 81 μU/islet without palmitate after 48 hours, n = 17; and 1,218 ± 150 *v* 831 ± 150 μU/islet without palmitate after 72 hours, n = 5; all P = NS), suggesting that palmitate increased basal, but not glucose-stimulated, insulin release in the media.

To determine whether the stimulatory effects of palmitate on insulin release are reversible, islets were first cultured in the presence of 16.7 mmol/L glucose for 48 hours with or without 0.5 mmol/L palmitate (period 1) and were then washed and further cultured for an additional 48 hours in the absence of palmitate (period 2). Insulin accumulation in the media containing palmitate was significantly increased at the end of period 1 (407 ± 53 *v* 150 ± 89 μU/islet, n = 4, $P < .05$), and this effect was fully reversed at the end of period 2, ie, after 48 hours in the absence of the FA (356 ± 27 *v* 350 ± 106 μU/islet, n = 4, NS).

Intracellular insulin content was measured at the end of each culture period (Fig 1B). The presence of palmitate did not affect insulin content in the presence of 2.8 mmol/L glucose. In contrast, palmitate significantly decreased the level of intracellular insulin in the presence of 16.7 mmol/L glucose after 48 and 72 hours. In the presence of palmitate, the values at 16.7 mmol/L glucose were similar to those at 2.8 mmol/L glucose, suggesting that palmitate blocked the stimulatory effect of glucose on insulin content.

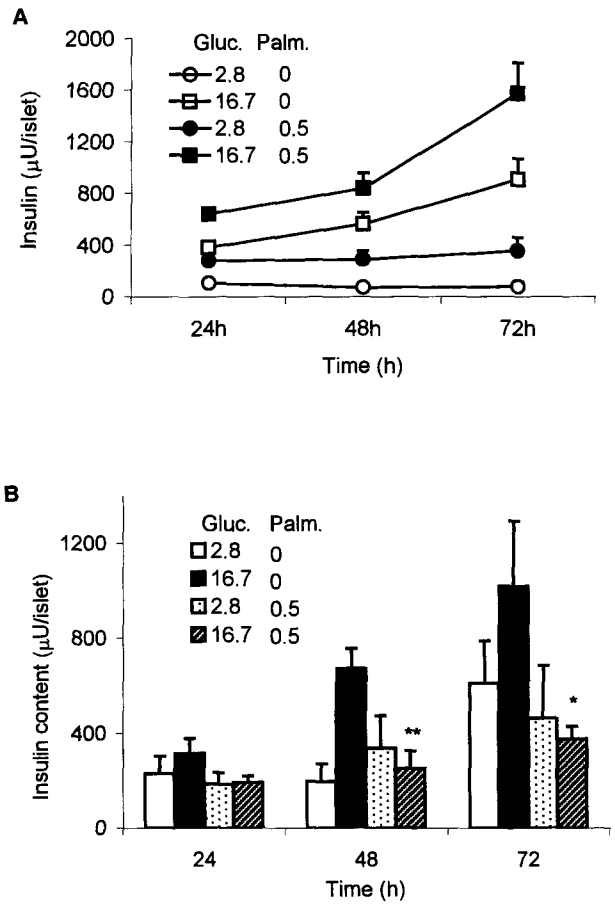

Fig 1. Isolated rat islets were cultured for 24, 48, or 72 hours in the presence of 2.8 or 16.7 mmol/L glucose (Gluc.) with or without 0.5 mmol/L palmitate (Palm.). (A) Mean ± SE insulin release for 15, 17, and 5 replicate experiments at 24, 48, and 72 hours, respectively. Insulin release was significantly increased in the presence of palmitate (ANOVA, $P < .0001$), but this effect was independent of the glucose concentration (NS). (B) Mean ± SE insulin content for 4 replicate experiments at each time point. *$P < .05$, **$P < .01$ *v* islets cultured in the presence of 16.7 mmol/L glucose and in the absence of palmitate.

To determine whether the glucose-dependent effects of palmitate on insulin content are transient or are also observed upon longer exposure, islets were cultured in the absence or presence of 0.5 mmol/L palmitate at 2.8 or 16.7 mmol/L glucose for 1 week. A similar pattern was observed after 1 week of culture compared with 48 and 72 hours, with no effect of palmitate on insulin content in the presence of 2.8 mmol/L glucose (179 ± 55 *v* 200 ± 24 μU/islet, n = 4, NS) and a complete blockade of the stimulatory effect of 16.7 mmol/L glucose (145 ± 22 *v* 582 ± 52 μU/islet, n = 4, $P < .05$).

Effects of Glucose and Palmitate on Insulin mRNA Levels in Isolated Islets

Isolated islets were cultured for 1, 2, 3, or 7 days in the presence of 2.8 or 16.7 mmol/L glucose with or without 0.5 mmol/L palmitate, and steady-state levels of insulin mRNA were determined by Northern blot analysis (Fig 2). A transient stimulatory effect of palmitate on insulin mRNA was observed after 24 hours of culture in the presence of 2.8 mmol/L glucose. This effect was no longer observed after 48 hours. Palmitate inhibited insulin mRNA levels after 2, 3, and 7 days of culture in the presence of 16.7 mmol/L glucose by 26% ± 14% (n = 5, $P < .05$), 27% ± 7% (n = 4, $P < .01$), and 23% ± 4% (n = 4, $P < .05$), respectively. The increment over basal in response to glucose, calculated as the value at 16.7 mmol/L glucose minus the value at 2.8 mmol/L glucose, was 89%, 62%, 52%, and 62% lower in the presence versus the absence of palmitate after 1, 2, 3, and 7 days of culture, respectively.

Effects of Palmitate on Insulin mRNA Levels and Insulin Promoter Activity in HIT-T15 Cells

To determine whether the HIT-T15 cell line represents an appropriate model to study glucose-dependent effects of palmitate on insulin gene transcription, HIT-T15 cells (passage 75) were cultured for 24 hours in the presence of 0.2, 2.8, or 11.1 mmol/L glucose (respectively, basal, submaximal, and maximal stimulatory concentrations of glucose for insulin gene transcription in HIT cells[16]) and in the absence or presence of 0.5 mmol/L palmitate. Insulin mRNA levels were assessed by Northern blot analysis (Fig 3). Consistent with the observations in isolated islets, palmitate had no effect at a low glucose

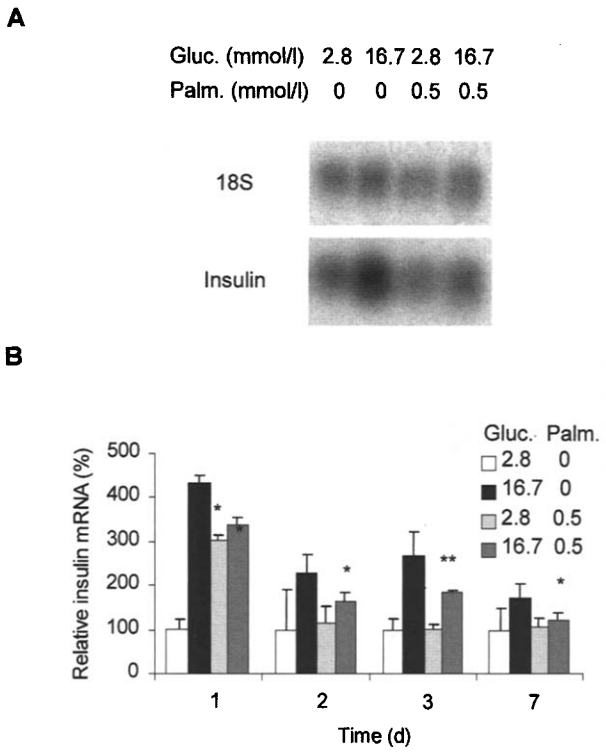

Fig 2. Isolated rat islets were cultured for 1, 2, 3, or 7 days in the presence of 2.8 or 16.7 mmol/L glucose (Gluc.) with or without 0.5 mmol/L palmitate (Palm.) and insulin mRNA levels were measured by Northern blot analysis. (A) Representative Northern blot after 1 week in culture. (B) Mean ± SE densitometric analysis of 3, 5, 6, and 4 replicate experiments at 1, 2, 3, and 7 days, respectively. Insulin mRNA levels are normalized to 18S rRNA levels and expressed relative to the level in islets cultured in 2.8 mmol/L glucose and in the absence of palmitate (control), set at 100%. *$P < .05$, **$P < .01$ *v* control islets in the absence of palmitate.

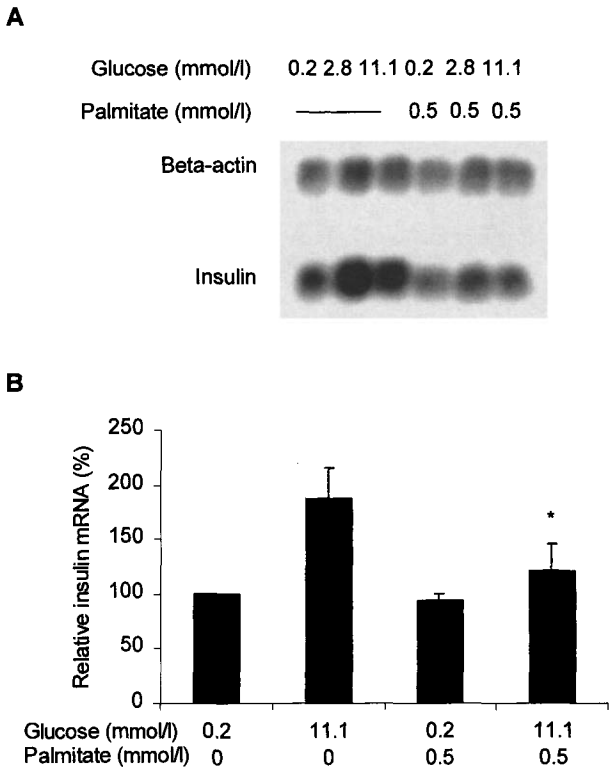

Fig 3. HIT-T15 cells (passage 72-75) were cultured for 24 hours in the presence of 0.2, 2.8, or 11.1 mmol/L glucose with or without 0.5 mmol/L palmitate. Insulin and β-actin mRNA levels were measured by Northern blot analysis. (A) Representative Northern blot. (B) Mean ± SE of 3 replicate experiments.

concentration (0.2 mmol/L) but significantly inhibited mRNA levels in the presence of 2.8 and 11.1 mmol/L glucose. HIT-T15 cells were then cotransfected with the insulin promoter-reporter INSLUC and the control construct pRL-CMV, cultured for 24 hours in the presence of 11.1 mmol/L glucose with increasing concentrations of palmitate, and analyzed for luciferase activity (Fig 4). Relative INSLUC activity, normalized to activity in the absence of palmitate (100 ± 24), was decreased to 43 ± 4 in the presence of 0.5 mmol/L palmitate ($n = 7$, $P < .01$), indicating that palmitate inhibits insulin gene promoter activity in HIT-T15 cells.

DISCUSSION

This study was designed to determine whether the inhibitory effects of palmitate on insulin gene expression are dependent on the presence of a stimulatory concentration of glucose. We found that culturing isolated rat islets in the presence of 0.5 mmol/L palmitate for 24 hours to 7 days had both glucose-independent and -dependent effects on β-cell function. Insulin release in the media was increased to the same extent regardless of whether a stimulatory glucose concentration was present. In contrast, neither the insulin content nor insulin mRNA level were affected by palmitate in the presence of a basal concentration of glucose (2.8 mmol/L), but both were significantly decreased in the presence of a stimulatory (16.7 mmol/L) concentration of glucose. This pattern of inhibition was observed after 48 hours of culture and persisted for at least 1 week. Inhibition of insulin mRNA levels by palmitate in the presence of elevated glucose was also observed in HIT-T15 cells after 24 hours of exposure, and was associated with decreased activity of the insulin gene promoter.

The amount of insulin released in the media was increased at all times in culture in the presence of palmitate. However, the stimulated fraction of insulin release was identical in the absence and presence of palmitate, suggesting that palmitate increases the basal release of insulin. This is in accordance with previous studies[8,9,11] and with the potential role of FAs as signaling molecules in stimulus-secretion coupling in the β cell.[17] It should be pointed out that measurements of cumulative insulin release in prolonged culture should be interpreted with caution, because they are influenced by the rate of insulin degradation of insulin in the media and a potential negative feedback of insulin on its own secretion. Nevertheless, our results support the hypothesis that the long-term effects of palmitate on insulin release are not dependent on the glucose concentration. The stimulatory effect of palmitate on insulin release was fully reversed upon removal of the FA, and the amount of total protein recovered from the cells after culture was unchanged in the presence of palmitate, ruling out a major toxic effect of the FA on cell function or survival.

In contrast to the results on insulin release, the effects of palmitate on insulin content were dependent on the presence of a stimulatory glucose concentration. In the presence of low glucose, palmitate did not affect insulin content; however, in the presence of high glucose, palmitate markedly decreased intracellular insulin to levels similar to those observed in islets cultured in low glucose. The amount of intracellular insulin is influenced by a number of factors, ie, the rate of insulin biosynthesis, the rate of insulin exocytosis, and the rate of intracellular degradation of insulin, none of which were directly measured in this study. Nonetheless, our data are consistent with the notion that in the presence of palmitate, islets are not able to compensate for the increase in secretion by a corresponding increase in insulin biosynthesis, as reported by Bollheimer et al.[9] The

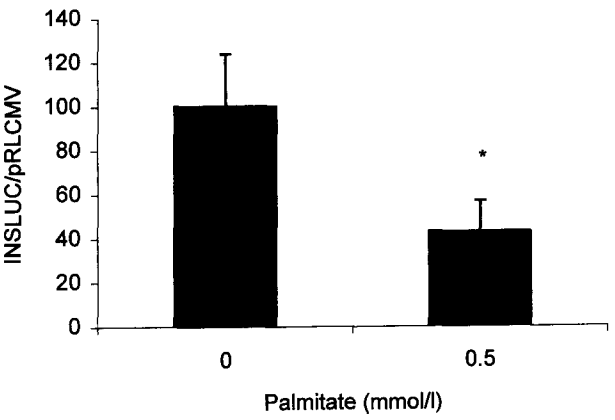

Fig 4. HIT-T15 cells (passage 70-76) were cotransfected with INSLUC and pRL-CMV. The cells were then cultured for 24 hours in media containing 11.1 mmol/L glucose in the absence or presence of palmitate. INSLUC activity was normalized to pRL-CMV activity and expressed relative to the activity in cells cultured in the absence of palmitate, set at 100%. Results are the mean ± SE of 7 replicate experiments. *$P < .01$ *v* control in the absence of palmitate.

decrease in insulin content in the face of increased secretion may be ascribed either to distinct effects of palmitate on insulin secretion and insulin biosynthesis, as suggested by other studies in clonal cells,[10] or to "overstimulation" of the cells, leading to depletion of intracellular stores.

Our findings that palmitate decreases insulin mRNA in the presence of high glucose levels reconcile the results of Bollheimer et al,[9] who reported that FAs do not affect insulin gene expression at low glucose concentration, and Gremlich et al,[11] who found that FAs decrease insulin mRNA in the presence of high glucose. Interestingly, Bollheimer et al[9] observed a transient increase in insulin mRNA levels after 24 hours of exposure to oleate at basal glucose levels, which was also observed in our study with palmitate.

A similar effect of palmitate on insulin mRNA was observed in HIT-T15 cells and isolated islets, indicating that the HIT cell is a useful model for studying the transcriptional mechanisms by which palmitate affects insulin gene expression. The decrease in insulin gene promoter activity observed in HIT cells in the presence of palmitate suggests that this effect is not the mere consequence of β-cell exhaustion, but also results, at least in part, from inhibition of insulin gene transcription. Indeed, Gremlich et al[11] have shown that FAs decrease the activity of IDX-1, a transcription factor involved in glucose regulation of the insulin gene. Transcriptional effects of FAs have also been observed on other genes in the insulin-secreting cell line INS-1.[18,19] Furthermore, FAs can bind to and modulate the expression of the transcription factor hepatic nuclear factor-4,[20] which is the maturity-onset diabetes of the young (MODY 1) gene[21] and is thought to play a role in normal β-cell function.[22]

In conclusion, this study uniquely demonstrates that long-term exposure of isolated islets and HIT-T15 cells to palmitic acid decreases insulin gene expression in vitro only when a stimulatory concentration of glucose is present concomitantly, therefore providing insight into the mechanisms whereby simultaneous elevations of blood glucose and islet lipid levels adversely affect pancreatic β-cell function in type 2 diabetes.

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REFERENCES

1. Lewis B, Mancini M, Mattock M, et al: Plasma triglyceride and fatty acid metabolism in diabetes mellitus. Eur J Clin Invest 2:445-453, 1972

2. Reaven GM: The fourth musketeer—From Alexandre Dumas to Claude Bernard. Diabetologia 38:3-13, 1995

3. Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. Diabetes 44:863-870, 1995

4. Unger GH: How obesity causes diabetes in Zucker diabetic fatty rats. Trends Endocrinol Metab 8:276-282, 1997

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

6. Elks ML: Chronic perifusion of rat islets with palmitate suppresses glucose-stimulated insulin release. Endocrinology 133:208-214, 1993

7. Zhou Y-P, Grill V: Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. J Clin Endocrinol Metab 80:1584-1590, 1995

8. 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

9. Bollheimer LC, Skelly RH, Chester MW, et al: Chronic exposure to free fatty-acid reduces 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

10. Skelly RH, Bollheimer C, Wicksteed BL, et al: A distinct difference in the metabolic stimulus-response coupling pathways for regulating proinsulin biosynthesis and insulin secretion that lies at the level of a requirement for fatty acyl moieties. Biochem J 331:553-561, 1998

11. 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

12. Gotoh M, Maki T, Satomi S, et al: Reproducible high yield of rat islets by stationary in vitro digestion following pancreatic ductal or portal venous collagenase injection. Transplantation 43:725-730, 1987

13. Ling Z, Hannaert JC, Pipeleers D: Effects of nutrients, hormones and serum on survival of rat islet beta cells in culture. Diabetologia 37:15-21, 1994

14. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159, 1987

15. Bell GI, Sanchez-Pescador R: Sequence of a cDNA encoding Syrian hamster preproinsulin. Diabetes 33:297-300, 1984

16. Redmon JB, Towle HC, Robertson RP: Regulation of human insulin gene transcription by glucose, epinephrine, and somatostatin. Diabetes 43:546-551, 1994

17. 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

18. Assimacopoulos-Jeannet F, Thumelin S, Roche E, et al: Fatty acids rapidly induce the carnitine palmitoyltransferase I gene in the pancreatic β-cell line INS-1. J Biol Chem 272:1659-1664, 1997

19. Brun T, Assimacopoulos-Jeannet F, Corkey BE, et al: Long-chain fatty acids inhibit acetyl-CoA carboxylase gene expression in the pancreatic β-cell line INS-1. Diabetes 46:393-400, 1997

20. Hertz R, Magenhein J, Berman I, et al: Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4α. Nature 392:512-516, 1998

21. Yamagata K, Furuta H, Oda N, et al: Mutations in the hepatocyte nuclear factor-4 alpha gene in maturity-onset diabetes of the young. Nature 384:458-460, 1996

22. Stoffel M, Duncan SA: The maturity-onset diabetes of the young (MODY1) transcription factor HNF-4 alpha regulates expression of genes required for glucose metabolism. Proc Natl Acad Sci USA 94:13209-13214, 1997