Inhibitory Effects of Fatty Acids on Glucose-Regulated B-Cell Function: Association With Increased Islet Triglyceride Stores and Altered Effect of Fatty Acid Oxidation on Glucose Metabolism

Yun-Ping Zhou, Zong-Chao Ling, and Valdemar E. Grill

Long-term exposure to fatty acids (FA) inhibits B-cell function. We tested whether the inhibitory effects are associated with increased islet triglycerides (TG). Rat pancreatic islets were cultured for 48 hours in RPMI 1640 medium with 10% fetal calf serum (FCS) and 11 mmol/L glucose in the presence or absence of the long-chain FA, palmitate. Palmitate (0.125 mmol/L) exposure successively increased islet TG 70% after 6 hours and 200% after 48 hours of culture. The dose-response for palmitate was similar for the increase in TG and inhibition of glucose-induced insulin secretion. Reversal of elevated islet TG in RPMI medium (after 48 hours of palmitate) was 29% after 6 hours and 84% after 24 hours. A more rapid decline of TG was observed in Krebs-Ringer bicarbonate (KRB) medium in the absence of nutrients. This decline was totally prevented by 1 μmol/L of the carnitine palmitoyl transferase-I (CPT-I) inhibitor, etomoxir. Etomoxir enhanced glucose-induced insulin secretion from palmitate-cultured islets; however, this effect was lost when TG were normalized. Under conditions when oxidation of FA from islet TG stores was blocked with etomoxir, we tested the effects of octanoate, the oxidation of which is not blocked by etomoxir. Oxidation of [1-^{14}C]octanoate from islets precultured with palmitate (48 hours) did not differ from that in control islets. Conversely, after palmitate, octanoate inhibited glucose oxidation ($^{14}CO_2$ production from [U-^{14}C]glucose, 613 ± 41 pmol/10 islets/90 min *v* 1,129 ± 87 after control conditions, $P < .01$). In conclusion, (1) palmitate induces increases in islet TG that are associated with inhibition of B-cell function, and (2) long-term exposure to palmitate also induces an inhibitory effect of FA oxidation on glucose metabolism that is independent of TG.

THE INFLUENCES of fatty acids (FA) on pancreatic B cells are complex. Short-term administration of FA stimulates insulin secretion in vivo[1-3] and in vitro.[4-7] Accumulation of long-chain acyl-coenzyme A esters in the cytoplasm has been suggested to supply second messengers for a stimulatory effect.[8-10] Long-term exposure to FA leads to inhibition of glucose-induced insulin secretion.[11-15] Inhibition of B-cell glucose metabolism by FA oxidation likely causes the inhibitory effect.[13] Inhibition by FA oxidation could be due to a glucose–fatty acid cycle. This concept encompasses the notion of a reciprocal relationship between FA and glucose oxidation.[16,17]

It is not known what causes the changes with time in FA effects on B cells. One possibility is that prolonged exposure to FA facilitates FA oxidation. Such facilitation could be due to increased transport of FA from cytosol to mitochondria because of greater transport efficiency or greater availability of acyl-coenzyme A esters for transport. Another possibility is that the influence of a given rate of FA oxidation on glucose oxidation changes with time. Such influence on glucose metabolism could thus be stimulatory after short-term but inhibitory after long-term exposure to FA.

When assessing the mechanisms behind the time-dependent inhibitory effects of FA, the size and regulation of islet triglyceride (TG) stores must be considered. The inhibitory effects of long-term FA exposure could be coupled to increased islet TG stores. FA are freely taken up by many types of cells,[18] including pancreatic B cells.[19] If not oxidized, they are stored as TG in many cell types. Elevated free FA are thus likely to produce elevated TG. If islet TG stores were markedly enlarged by long-term elevated FA, they could, under in vitro conditions, release large amounts of FA available for oxidation. Increased FA oxidation due to islet lipolysis could therefore be important for the inhibitory effects of FA observed in vitro and for the reversal of these effects by mitochondrial carnitine palmitoyl transferase-I (CPT-I) inhibitors[20] such as etomoxir.

Herein, we have investigated the regulation of islet TG stores in relation to the inhibitory effects induced by previous exposure to palmitate. In this context, we have also tested the influence of exogenous FA independent from intraislet TG stores by testing the effects of octanoate, a FA that does not form a significant part of TG stores.

MATERIALS AND METHODS

Animals and Materials

Male Sprague-Dawley rats were obtained from B & K Universal (Stockholm, Sweden). At the time of experiments, they were 2 to 3 months old. All animals had free access to water and a standard pelleted diet (Lactamin R36 Brook Stock Feed for rats and mice; B&K Universal, Stockholm, Sweden) containing, on a weight basis, 55.7% carbohydrate, 18.5% protein, and 4% fat. They were exposed to a 12-hour light (6 AM to 6 PM)/dark cycle. The experimental protocols were approved by the Stockholm Ethics Committee for Research on Animals. Sodium 2-[b-(4-chlorophenoxy)-hexyl]oxirane-2-carboxylate (B-827-33, Etomoxir) was obtained from ASAT (Zug, Switzerland). D-[U-^{14}C]glucose and [1-^{14}C]octanoate were obtained from Dupont-NEN (Boston, MA). All other chemicals were from Sigma Chemical (St Louis, MO).

From the Department of Molecular Medicine, Endocrine and Diabetes Unit, Karolinska Institute and Hospital, Stockholm, Sweden; and the Department of Medicine, Section of Endocrinology, University of Trondheim, Trondheim, Norway.

Submitted September 2, 1995; accepted February 8, 1996.

Supported by the Swedish Medical Research Council (19X-04540), the Swedish Diabetes Association, the Magnus Bergvall Foundation, the Novo-Nordic Insulin Foundation, and the Karolinska Institute.

Address reprint requests to Valdemar E. Grill, MD, PhD, Department of Medicine, Section of Endocrinology, University of Trondheim, Trondheim 7003, Norway.

0026-0495/96/4508-0012$03.00/0

Islet Isolation and Culture

Pancreatic islets were isolated as previously described,[21] using digestion with collagenase obtained from *Clostridium histolyticum* (Boehringer, Mannheim, Germany). Digestion and sedimentation of islets was performed in Hanks solution containing 5.5 mmol/L glucose. Islets were then hand-picked under a stereo microscope and transferred to RPMI 1640 medium with 10% fetal calf serum (FCS), 2 mmol/L glutamine and 11 mmol/L glucose. Palmitate was added to the medium after being dissolved with 95% ethanol, as previously described.[13] The islets were cultured free-floating for 48 hours in an atmosphere of 95% air/5% CO_2. For control conditions, islets were cultured with the solvent (1% ethanol). It was previously ascertained that ethanol under these conditions does not affect insulin secretion, insulin biosynthesis, or glucose oxidation.[13]

Insulin release was determined in 60-minute batch-type incubations of islets in Krebs-Ringer bicarbonate buffer (KRB) as described previously,[13] following a 30-minute preincubation in the same medium with 3.3 mmol/L glucose. Electrolyte composition of the KRB medium was as follows (millimolars): Na^+ 143.5, K^+ 5.8, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 124.1, PO_4^{3-} 1.2, SO_4^{2+} 1.2, and CO_3^{2-} 25. The pH was adjusted to 7.4 immediately before use. The medium was supplemented with 10 mmol/L HEPES, 2 mg/mL bovine serum albumin (fraction V), and 3.3 mmol/L glucose. Insulin released to the incubation media was determined by radioimmunoassay.[22] In experiments with etomoxir, the CPT-I inhibitor was included in the KRB medium for both initial and final incubations, but not during the culture period with palmitate.

Islet Glucose and Octanoate Oxidation

Glucose oxidation was determined by measuring the formation of $^{14}CO_2$ from [U-^{14}C]glucose.[23] Duplicate batches of 10 islets each were incubated in 100 μL KRB containing a low (3.3 mmol/L) or high (27 mmol/L) glucose concentration together with 0.5 μCi D-[U-^{14}C]glucose. The incubations were performed in 1-mL glass vials inside 20-mL scintillation vials fitted with airtight rubber seals. Following 90 minutes of incubation at 37°C with continuous shaking, 0.1 mL 0.1-mol/L HCl was injected into the inner vials through the rubber seal (to stop islet metabolism), after which 0.25 mL Hyamine hydroxide (Packard, Meriden, CT) was injected. The sealed scintillation bottles were subsequently left overnight at room temperature to allow absorption of $^{14}CO_2$ into Hyamine.

Octanoate oxidation in islets was determined in a similar way by recording the production of $^{14}CO_2$ during 120-minute incubations of islets with [1-^{14}C]octanoate (0.25 μCi for each sample) as reported previously.[24]

Islet Lipid Extraction

Islet lipids were extracted basically as previously described.[25] Briefly, 25 to 50 islets each were placed in 1.0 mL albumin-free KRB to which 3 mL $CHCl_3$:CH_3OH (2:1 vol/vol) was added. The samples were immediately mixed by vortexing for 30 seconds, followed by periodic mixing for 1 hour at room temperature. The phases were separated by centrifugation (3,000 rpm at 4°C for 15 minutes). The upper phase was removed and re-extracted with 2 mL $CHCl_3$. The two lower organic phases were then pooled and washed twice with 2 mL synthetic upper phase (prepared by processing KRB as above but without islets). The extracts were then dried by air-blow in a 50°C oven. The dried lipids were solubilized in 0.3 mL absolute ethanol containing 0.5 mol/L KOH and incubated for 30 minutes at 70°C. The hydrolyzed samples were dried as described above, mixed with 0.3 mL $MgSO_4$ (0.3 mol/L), and then centrifuged at 3,000 rpm for 15 minutes, after which the supernatants were saved.

Bioluminescent Assay of Glycerol

The method for determining islet TG contents used the following reactions, using previously described procedures[26,27] with minor modifications:

$$\text{glycerol} + \text{ATP} \xrightarrow{\text{glycerokinase}} \text{glycerol-3-phosphate} + \text{ADP} \quad \text{Eq 1}$$

and

$$\text{ATP} + \text{luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{light.} \quad \text{Eq 2}$$

An aliquot of extracted and hydrolyzed lipid (50 μl) was added to same volume of Tris hydrochloride buffer (pH 7.4) with (final concentration) 0.25 mmol/L ATP and 0.4 U/mL glycerokinase and then incubated at 37°C for 20 minutes. The reaction was stopped by transferring the tubes to an ice-cold water bath. Consumption of ATP during the first reaction (Eq 1) was then monitored by mixing 20 μL each of the incubated samples immediately with 80 μL ATP-monitoring reagent in a small glass tube inside a 6-mL scintillation vial. The ATP-monitoring reagent was composed of 1.0 mg/mL luciferin and 2 μL/mL luciferase in 25 mmol/L HEPES buffer. The luciferase preparation was purified from dessicated firefly lanterns essentially as previously described.[28] Chemiluminescence produced by the firefly reaction was measured in a Packard Tricarb Scintillation Analyzer (Packard Instrument, Meriden, CT) within 30 seconds after mixing ATP-containing samples and ATP-monitoring reagent. Pilot experiments showed that the linear range for ATP in this system was 10^{-7} to 10^{-12} mol/L. ATP consumption during the 20-minute incubation with glycerokinase was linear to the amount of glycerol added (results not shown).

TG contents of samples were located on a standard curve established in each assay with 0 to 250 pmol/0.1 mL glycerol. There was a good correlation between the number of islets included before extraction and the TG concentration determined in the range of five to 50 islets ($r = .98$, n = 6). The recovery rate for added triolein of the present method was 88% ± 3.1% with islets and 78% ± 3.6% without islets in the system. The basal glycerol content in freshly isolated islets, measured in the unhydrolyzed lipid extract, was 11% ± 2.2% of the amount of TG determined in the hydrolyzed lipids (4.4 ± 0.9 *v* 41 ± 2.3 pmol per islet, n = 6).

Statistical Analysis

All results are expressed as the mean ± SE. Significance testing was conducted with an unpaired Student's *t* test and ANOVA as appropriate.

RESULTS

Islet TG: Time Course of FA Effects

The effects of culturing islets with or without 0.125 mmol/L palmitate for 6 to 48 hours on islet TG content are shown in Fig 1. In the absence of added palmitate, islet TG increased moderately but significantly during the culture period. In the presence of palmitate, islet TG stores were significantly increased after 6 hours relative to levels in control islets. Prolonging the culture with palmitate for 24 and 48 hours successively increased the difference versus control islets. After 48 hours of palmitate exposure, islet TG content was increased twofold relative to levels in control islets.

The time course of reversibility was tested in islets

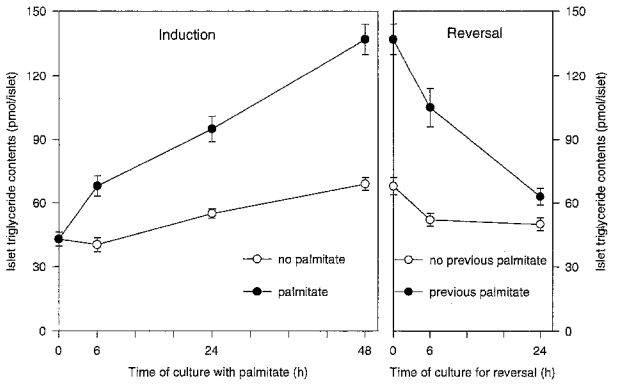

Fig 1. Time course of induction and reversibility for islet TG stores in islets preexposed to palmitate. Rat islets were exposed to 0.125 mmol/L palmitate for 6 to 48 hours in RPMI 1640 medium with 10% FCS and 11 mmol/L glucose. They were then transferred to this medium without added FA for recovery culture for 6 or 24 hours. Islet lipids were extracted with chloroform/methanol, and TG levels were measured by a bioluminescent assay. Data are the mean ± SE of 6 experiments.

cultured 48 hours in palmitate by continuing culture in RPMI 1640 medium with 10% FCS but without palmitate. The elevated TG after palmitate treatment declined in a linear fashion (Fig 1). The effect of previous FA was significant after 6 but not after 24 hours following omission of palmitate.

Islet TG: Dose-Response for FA Effects

The apparent threshold concentration of palmitate that (when added to RPMI 1640 medium with 10% FCS for 48 hours during culture) evoked an increase in TG was 100 μmol/L (Fig 2). A maximally effective concentration of palmitate could not be determined. Dose-response characteristics for the increase in TG were compared with those for inhibition of glucose-induced insulin release and found to be similar (Fig 2).

Islet TG: Effects of Glucose Concentration

To study a possible interaction between FA and glucose on TG accumulation, we cultured islets with 0.125 mmol/L palmitate and varying concentrations of glucose. Increasing the glucose concentration during culture from 5.5 to 27 mmol/L tended to increase islet TG ($P < .15$) during control conditions (ie, no palmitate added to the culture medium, Table 1). No such tendency was observed in palmitate-cultured islets.

Islet TG: Effects of Etomoxir

We next investigated to what extent inhibition of FA oxidation would affect the FA-induced elevation of TG. To induce a rapid decrease in TG stores, islets were cultured in KRB without glucose or other nutrients. During such conditions, islet TG contents declined significantly over 4 hours. This decline was wholly preventable by etomoxir, an inhibitor of CPT-I (Table 2). Taken together, these results demonstrate the potential for increased TG to supply FA for mitochondrial oxidation.

In previous studies, etomoxir partly restored the inhibition of glucose-induced insulin secretion caused by 48 hours of preexposure to palmitate.[13] To test whether such a restoring effect of etomoxir is associated with elevated TG, we measured, in five separate experiments, glucose-induced insulin secretion before and after 4-hour incubations in KRB without added nutrients. Following 48 hours of palmitate exposure, the insulin response to 27 mmol/L glucose was 110 ± 7 μU/islet/h. This response was increased to 144 ± 8 μU/islet/h ($P < .05$, n = 5) by eto-

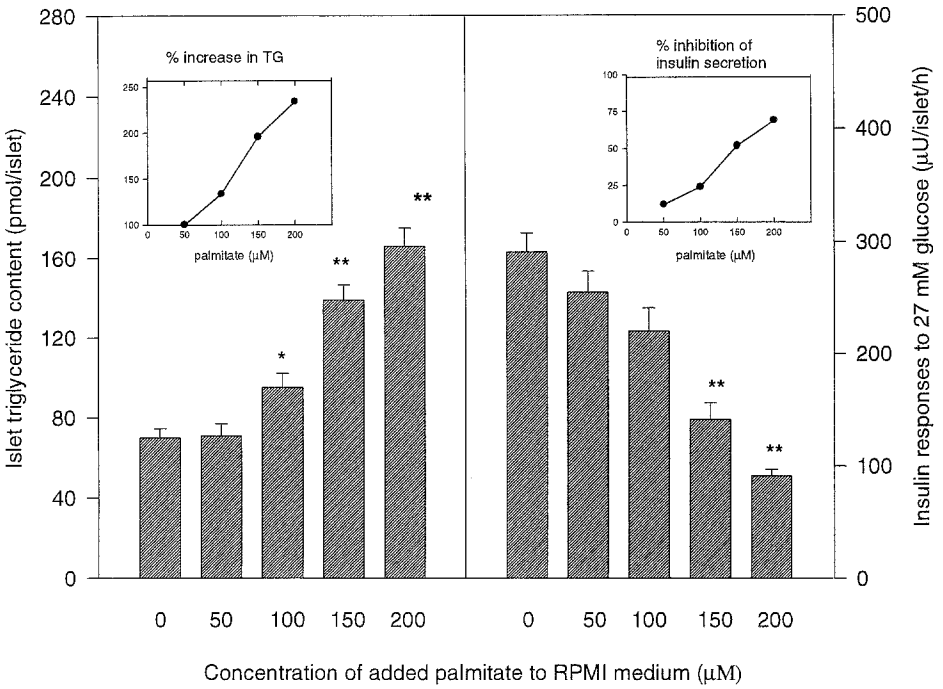

Fig 2. Dose-response (islet TG and glucose-induced insulin release) to 50 to 200 μmol/L palmitate for 48 hours. Islets were cultured in RPMI 1640 medium containing 10% FCA, 11 mmol/L glucose, and added concentrations of palmitate for 48 hours before extracting lipids and measuring TG content or—in separate experiments—measuring insulin release. Data are the mean ± SE of 6 (TG) or 3 (insulin release) experiments. *$P < .05$, **$P < .01$: *v* control (no added FA).

Table 1. Effects of Glucose Concentration During Culture on Islet TG Content

Glucose (mmol/L)	Islet TG (pmol per islet)	
	Control	Palmitate
5.5	63.6 ± 2.5	117 ± 7.1*
11	76.3 ± 3.5	133 ± 5.4*
27	87.5 ± 6.1	128 ± 5.6†

NOTE. Islets were cultured for 48 hours in RPMI 1640 medium containing 3 different concentrations of glucose in the presence or absence of 0.125 mmol/L palmitate. Data are the mean ± SE of 6 experiments.

*$P < .01$, †$P < .001$: *v* control.

moxir. Conversely, after the 4-hour incubation in KRB, etomoxir failed to affect glucose-induced insulin secretion (106 ± 7 in the absence and 109 ± 7 μU/islet/h in the presence of etomoxir).

Experiments with Octanoate

The rationale for the design of experiments with octanoate is summarized in Fig 3. It takes advantage of the fact that oxidation of FA derived from TG can be blocked by etomoxir, whereas oxidation of the medium-chain FA, octanoate, is not affected. Thus, etomoxir was always present when effects of octanoate were tested.

Effects of Octanoate on Insulin Secretion

In control islets (cultured without palmitate), 5 mmol/L octanoate enhanced insulin secretion both at low (3.3 mmol/L) and at high (27 mmol/L) glucose (Table 3). Conversely, in palmitate-cultured islets, octanoate failed to enhance insulin secretion at 3.3 or 27 mmol/L glucose.

Effects of Octanoate on Islet Glucose Oxidation

In control islets, octanoate moderately but significantly enhanced the production of $^{14}CO_2$ from [U-^{14}C]glucose (Table 4). At high glucose (27 mmol/L), such stimulation was not significant. In palmitate-exposed islets, the stimulatory effects of octanoate on oxidation were lost. In these islets, octanoate failed to increase glucose oxidation at 3.3 mmol/L glucose and inhibited (by 23%, $P < .01$) glucose oxidation at 27 mmol/L glucose.

Table 2. Effect of Etomoxir on the Decline of Islet TG Content Following Culture With Palmitate

Incubation Condition	Islet TG (pmol per islet)		
	Before Incubation	After Incubation	
		2 h	4 h
No additions	135 ± 7.9	98 ± 4.1	88 ± 4.2
1 μmol/L etomoxir	—	125 ± 4.8*	120 ± 7.2*

NOTE. Rat islets cultured with 0.125 mmol/L palmitate for 48 hours were incubated in bovine serum albumin and glucose-free KRB for 2 or 4 hours in the presence or absence of 1.0 μmol/L etomoxir. Data are the mean ± SE of 6 experiments.

*$P < .05$ *v* no etomoxir.

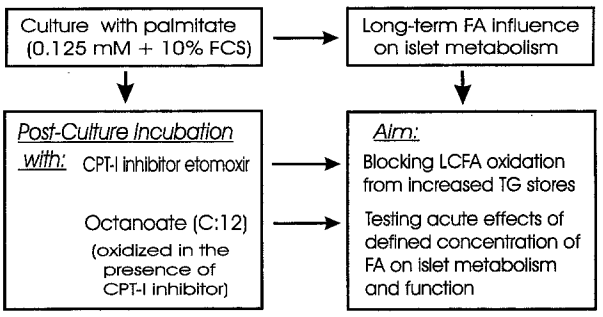

Fig 3. Experimental protocol for studies with etomoxir and octanoate in palmitate-exposed islets. LCFA, long-chain fatty acid.

Effects of Long-Term Exposure to Palmitate on the Oxidation of Octanoate

Oxidation of [1-^{14}C]octanoate was moderately (30%) inhibited by glucose (27 *v* 3.3 mmol/L) in control islets (Table 5). Such an inhibitory influence was also apparant in palmitate-precultured islets. Furthermore, previous palmitate tended to reduce octanoate oxidation at 3.3 mmol/L glucose (by 20%, $P = .08$). At 27 mmol/L glucose, octanoate oxidation was significantly reduced by previous palmitate (30%, $P < .05$).

DISCUSSION

Long-term exposure to FA in vivo[11,12] or in vitro[13-15] inhibits glucose-induced insulin secretion and islet glucose metabolism. The present results show that TG stores are increased in parallel with conditions that induce inhibition of B-cell function. The time-dependence of accumulation of TG stores is qualitatively in agreement with the time-dependence for induction by FA of inhibitory effects that was previously demonstrated.[13] Also, the time-dependence for reversal of enlarged TG stores in RPMI 1640 medium without added palmitate was similar to the time scale for

Table 3. Effect of Culture With Palmitate on Octanoate- and/or Glucose-Induced Insulin Release and Insulin Content

Incubation Condition	Insulin Release (μU/islet · h)		Insulin Content (ng per islet)	
	Control	Palmitate	Control	Palmitate
Glucose 3.3 mmol/L	6.2 ± 0.6	21 ± 1.9*	47 ± 2.9	25 ± 9.8*
Glucose 27 mmol/L	154 ± 9.6	138 ± 8.6		
Glucose 3.3 mmol/L + octanoate 5.0 mmol/L	22 ± 2.6	22 ± 2.4	49 ± 2.8	28 ± 2.4*
Glucose 27 mmol/L + octanoate 5.0 mmol/L	266 ± 15	134 ± 8.4*		

NOTE. Rat islets precultured in RPMI 1640 (11.0 mmol/L glucose) for 48 hours with or without 0.125 mmol/L palmitate were transfered to KRB with 1.0 μmol/L etomoxir for preincubation (30 minutes, with 3.3 mmol/L glucose) and final incubation (60 minutes, with the additions listed). Data are the mean ± SE of 5 experiments.

*$P \leq .01$ *v* control.

Table 4. Effects of 48 Hours' Exposure to Palmitate on D-[U-^{14}C]glucose Oxidation Induced by Glucose and Octanoate in Rat Islets

Incubation Condition	Glucose Oxidation (pmol per islet/90 min)	
	Control	Palmitate
Glucose 3.3 mmol/L	15 ± 1.8	27 ± 2.9‡
Glucose 3.3 mmol/L + octanoate 5 mmol/L	25 ± 4.0*	30 ± 2.2
Glucose 27 mmol/L	96 ± 11	79 ± 4.3
Glucose 27 mmol/L + octanoate 5 mmol/L	113 ± 8.7	63 ± 4.1†§

NOTE. Rat islets precultured with 0.125 mmol/L palmitate for 48 hours in RPMI 1640 medium were incubated with 1.0 μmol/L etomoxir, in addition to conditions listed. Data are the mean ± SE of 5 experiments.

*$P < .05$, †$P < .01$: glucose *v* glucose + octanoate.

‡$P < .01$, §$P < .001$: control *v* previous palmitate.

reversal of FA-induced inhibitory effects on insulin secretion.[13] Furthermore, the dose-response for different concentrations of palmitate on TG stores resembled the dose-response for inhibition of glucose-induced insulin secretion (Fig 2).

There was also a parallelism between etomoxir effects on glucose-induced insulin secretion and enhanced TG stores. Thus, etomoxir enhanced glucose-induced insulin secretion in palmitate-preexposed islets when TG stores were enhanced, but failed to do so after rapid (4-hour) normalization of TG stores in KRB. The fact that the absolute response to glucose was not enhanced by TG normalization could be due to unspecific effects of nutrient deprivation.

Taken together, our results indicate that lipolysis from enhanced TG stores with subsequent formation of acyl-coenzyme A esters plays an important role in the long-term inhibitory effects of FA on regulation of B-cell function by glucose observed in vitro. In particular, the use of FA oxidation inhibitors to demonstrate restoration of B-cell function seems to require lipolysis from enhanced TG stores.

The specificity of TG measurements should be considered. Theoretically, the method used will assay glycerol-containing phospholipids in islets in addition to TG. However, in the study by Malaisse et al,[25] from which the present method was taken, removal of islet phospholipids did not measurably affect measured TG values.[25] Nevertheless, it has not been excluded that our TG measurements could, to a minor extent, include a phospholipid component.

Table 5. Effect of 48 Hours' Exposure to Palmitate on [1-^{14}C]octanoate Oxidation in Cultured Rat Islets

Incubation Condition	Octanoate Oxidation (pmol per islet/2 h)	
	Control	Palmitate
Glucose 3.3 mmol/L + octanoate 5.0 mmol/L	29 ± 3.0	25 ± 2.4
Glucose 27 mmol/L + octanoate 5.0 mmol/L	22 ± 1.0†	16 ± 1.9*‡

NOTE. Islets exposed to 0.125 mmol/L palmitate for 48 hours in RPMI 1640 medium (11 mmol/L glucose) were incubated in KRB with additions listed, plus 1.0 μmol/L etomoxir and 0.1 μCi [1-^{14}C]octanoate for 2 hours at 37°C. Data are the mean ± SE of 5 experiments.

*$P < .05$ *v* control.

†$P < .05$, ‡$P < .01$: *v* stimulation with 3.3 mmol/L glucose.

Two additional aspects of the TG results are relevant to the validity of our data. First, TG stores in freshly isolated islets were similar to those previously reported.[25] Second, a high glucose concentration during control conditions tended to increase TG stores. The latter findings are consistent with a slight inhibitory effect of glucose on FA oxidation, which was previously reported,[29,30] and an enhancing effect of the hexose on de novo synthesis of islet TG and phospholipids.[30-32]

The increase in TG after 48 hours of palmitate exposure (200%) was fivefold less than that recently reported in diabetic Zucker rats with chronically elevated free FA.[15,33] This difference cannot be ascribed to differences in assay methodology, since the methods of assay were similar. The differences indicate that prolongation of culture with elevated FA would further elevate TG stores and/or that a diabetic state enhances the TG-elevating effect of elevated FA. The larger size of TG stores than that present in our experiments could imply a more pronounced influence on glucose-induced insulin secretion and glucose oxidation in in vivo conditions than in our experiments. Further studies are needed to elucidate this point. The potential cellular toxicity of enlarged TG stores in islets has recently been proposed by others.[33]

Our results on TG thus indicate a permissive role of elevated TG stores for in vitro manifestation of long-term FA influence. Our results with octanoate indicate a major role for a time-dependent shift in the influence of a given rate of FA oxidation on glucose metabolism. Hence, long-term culture with palmitate led to a negative effect of octanoate on glucose oxidation, as well as a loss of the stimulating effect on insulin secretion that was seen in the absence of previous exposure to palmitate. This negative effect did not appear secondary to enhanced oxidation of octanoate, since the oxidation of [1-^{14}C]octanoate was decreased rather than enhanced after previous culture with palmitate. However, our results do not exclude the possibility of upregulation of the CPT-I enzyme in response to long-term FA exposure, since such a putative effect would not affect the oxidation of octanoate but could enhance the oxidation of long-chain FA.

The molecular mechanisms behind a shift from stimulation to inhibition by FA oxidation are not clear. However, we have shown that long-term exposure to FA leads to inhibition of pyruvate dehydrogenase (PDH) activity.[34] PDH activity is multifactorially regulated by nutrients and cofactors. It is also regulated by dephosphorylation (activating PDH) and phosphorylation (inactivating PDH). We have shown that PDH kinase activity is enhanced by long-term FA exposure.[34] It seems possible that the balance of activating and inactivating factors on PDH activity is shifted by long-term FA in such a way that acute exposure to FA inhibits PDH activity.

In summary, FA-induced enlargement of islet TG stores plays a permissive role in the time-dependent inhibition by FA of B-cell function observed in vitro. The results also

indicate that a switch from stimulatory to inhibitory effects by FA oxidation on glucose metabolism is important for the inhibition of B-cell functions seen after previous exposure to FA.

ACKNOWLEDGMENT

We are indebted to Annika Lindgren and Professor Hans Löw for supplying the luciferase preparation.

REFERENCES

1. Crespin SR, Greenough WB, Steinbergh D: Stimulation of insulin secretion by long-chain free fatty acids, a direct pancreatic effect. J Clin Invest 52:1979-1984, 1973

2. Balasse EO, Ooms HA: Role of plasma free fatty acids in the control of insulin secretion in man. Diabetologia 9:145-151, 1973

3. Hicks EO, Taylor CI, Vij SK, et al: Effects of changes in plasma levels of free fatty acids on plasma glucagon, insulin, and growth hormone in man. Metabolism 26:1011-1023, 1977

4. Malaisse WJ, Malaisse-Lagae F: Stimulation of insulin secretion by noncarbohydrate metabolites. J Lab Clin Med 72:438-448, 1968

5. Opera EC, Hubbard VS, Burch WM, et al: Characterization of the insulinotropic potency of polyunsaturated fatty acids. Endocrinology 130:657-662, 1992

6. Conget I, Rasschaert J, Sener A, et al: Secretory, biosynthetic, respiratory, cationic, and metabolic responses of pancreatic islets to palmitate and oleate. Biochem Med Metab Biol 51:175-184, 1994

7. Warnotte C, Gilon P, Nenquin M, et al: Mechanisms of the stimulation of insulin release by saturated fatty acids: A study of palmitate effects in mouse β-cells. Diabetes 43:703-711, 1994

8. Prentki M, Matschinsky FM: Ca^{2+}, cAMP and phosphoinositide derived messengers in the coupling mechanisms of insulin secretion. Physiol Rev 67:1185-1248, 1987

9. Corkey BE, Glennon MC, Chen KS, et al: A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic B-cells. J Biol Chem 264:21608-21612, 1989

10. Prentki M, Vischer S, Glennon MC, et al: Malonyl-CoA and long chain acyl CoA esters as metabolic coupling factors in nutrient induced insulin secretion. J Biol Chem 267:5802-5810, 1992

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

12. Capito K, Hansen SE, Hedeskov CJ, et al: Fat-induced changes in mouse pancreatic islet insulin secretion, insulin biosynthesis and glucose metabolism. Acta Diabetol 28:193-198, 1992

13. Zhou YP, Grill V: 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

14. Zhou YP, Grill VE: 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

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

16. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

17. Randle PJ, Priestman DA, Mistry SC, et al: Glucose fatty acid interactions and the regulation of glucose disposal. J Cell Biochem 55S:1-11, 1994

18. Sherratt HSA, Spurway TD: Regulation of fatty acid oxidation in cells. Biochem Soc Trans 22:423-427, 1994

19. Hamilton JA, Civelek VN, Kamp F, et al: Changes in internal pH caused by movement of fatty acids into and out of clonal pancreatic β-cells (HIT). J Biol Chem 269:20852-20856, 1994

20. Wolf HPO: Aryl-substituted 2-oxirane carboxylic acids: A new group of antidiabetic drugs, in Bailey CJ, Flatt PR (eds): New Antidiabetic Drugs. Nishimura, Japan, Smith-Gordon, 1990, pp 217-229

21. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35-39, 1967

22. Herbert V, Lau KS, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1385, 1965

23. Keen H, Field JB, Pastan IH: A simple method for in vitro metabolic studies using small volumes of tissue and medium. Metabolism 12:143-147, 1963

24. Berne C: The metabolism of lipids in mouse pancreatic islets: The oxidation of fatty acids and ketone bodies. Biochem J 152:661-666, 1975

25. 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

26. Hercules DM, Sheehan TL: Chemiluminescent determination of serum glycerol and triglycerides. Anal Chem 50:22-25, 1978

27. Björkhem I, Arner P, Thore A, et al: Sensitive kinetic bioluminescent assay of glycerol release from human fat cells. J Lipid Res 22:1142-1147, 1981

28. Beny M, Dolivo M: Separation of firefly luciferase using an anion exchanger. FEBS Lett 70:167-170, 1976

29. Tamarit-Rodriguez J, Vara E, Tamarit JA: Starvation-induced changes of palmitate metabolism and insulin secretion in isolated rat islets stimulated by glucose. Biochem J 221:317-324, 1984

30. Vara E, Tamarit-Rodriguez J: Glucose stimulation of insulin secretion in islets of fed and starved rats and its dependence on lipid metabolism. Metabolism 35:266-271, 1986

31. Berne C: The metabolism of lipids in mouse pancreatic islets: The biosynthesis of triacylglycerols and phospholipids. Biochem J 152:667-673, 1975

32. Best L, Malaisse WJ: Effects of nutrient secretagogues upon phospholipid metabolism in rat pancreatic islets. Mol Cell Endocrinol 32:205-214, 1983

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

34. Zhou YP, Grill VE: 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