Lipotoxicity of the Pancreatic β-Cell Is Associated With Glucose-Dependent Esterification of Fatty Acids Into Neutral Lipids

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Prolonged exposure of isolated islets to supraphysiologic concentrations of palmitate decreases insulin gene expression in the presence of elevated glucose levels. This study was designed to determine whether or not this phenomenon is associated with a glucose-dependent increase in esterification of fatty acids into neutral lipids. Gene expression of *sn*-glycerol-3-phosphate acyltransferase (GPAT), diacylglycerol acyltransferase (DGAT), and hormone-sensitive lipase (HSL), three key enzymes of lipid metabolism, was detected in isolated rat islets. Their levels of expression were not affected after a 72-h exposure to elevated glucose and palmitate. To determine the effects of glucose on palmitate-induced neutral lipid synthesis, isolated rat islets were cultured for 72 h with trace amounts of [^{14}C]palmitate with or without 0.5 mmol/l unlabeled palmitate, at 2.8 or 16.7 mmol/l glucose. Glucose increased incorporation of [^{14}C]palmitate into complex lipids. Addition of exogenous palmitate directed lipid metabolism toward neutral lipid synthesis. As a result, neutral lipid mass was increased upon prolonged incubation with elevated palmitate only in the presence of high glucose. The ability of palmitate to increase neutral lipid synthesis in the presence of high glucose was concentration-dependent in HIT cells and was inversely correlated to insulin mRNA levels. 2-Bromopalmitate, an inhibitor of fatty acid mitochondrial β-oxidation, reproduced the inhibitory effect of palmitate on insulin mRNA levels. In contrast, palmitate methyl ester, which is not metabolized, and the medium-chain fatty acid octanoate, which is readily oxidized, did not affect insulin gene expression, suggesting that fatty-acid inhibition of insulin gene expression requires activation of the esterification pathway. These results demonstrate that inhibition of insulin gene expression upon prolonged exposure of islets to palmitate is associated with a glucose-dependent increase in esterification of fatty acids into neutral lipids. *Diabetes* 50:315–321, 2001

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BSA, bovine serum albumin; CPT-1, carnitine-palmitoyl-transferase 1; DAG, diacylglycerol; DGAT, AcylCoA:diacylglycerol acyltransferase; GPAT, *sn*-glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; PBS, phosphate-buffered saline; PL, phospholipid; RT-PCR, reverse transcriptase–polymerase chain reaction; TAG, triacylglycerol; TLC, thin-layer chromatography.

According to the lipotoxicity hypothesis, chronic exposure to elevated lipid levels impairs pancreatic β-cell function in type 2 diabetic patients (1,2). We (3) and others (4,5) have previously shown that prolonged (>1 day) culture of normal islets in the presence of supraphysiologic concentrations of palmitate decreases insulin content and impairs insulin gene expression only in the presence of elevated glucose levels. This occurs, at least in part, via decreased insulin gene promoter activity in HIT-T15 cells (3) and decreased binding of the transcription factor pancreas-duodenum homeobox-1 (PDX-1) to the insulin gene in islets (4). In Zucker diabetic fatty (ZDF) rats, it has been postulated that β-cell dysfunction is due to increased triacylglycerol (TAG) content in islets (6–8), which leads to increased production of nitric oxide (9) and ceramide synthesis (10). However, the ZDF rat is an extremely obese genetic model of type 2 diabetes bearing a mutation in the leptin receptor gene. It remains to be demonstrated that prolonged exposure of normal β-cells to fatty acids results in increased esterification into neutral lipids, eventually leading to intracellular TAG accumulation. In addition, whether increased neutral lipid synthesis occurs via modulation of expression of lipogenic and lipolytic enzymes or is simply driven by the amount of available substrates is unknown. Finally, the differential and possibly synergistic effects of glucose and palmitate on neutral lipid metabolism have not been studied in the context of prolonged exposure to both nutrients.

This study was based on the hypothesis that prolonged exposure to palmitate results in a glucose-dependent increase in neutral lipid synthesis. It was designed to *1*) determine whether *sn*-glycerol-3-phosphate acyltransferase (GPAT), AcylCoA:diacylglycerol acyltransferase (DGAT), and hormone-sensitive lipase (HSL)—three key enzymes of neutral lipid metabolism—are expressed in islets; *2*) ascertain whether prolonged exposure of islets to glucose and palmitate modulates the expression of these enzymes; and *3*) assess the effects of prolonged exposure to glucose and palmitate on neutral lipid metabolism in islets and insulin-secreting cells.

RESEARCH DESIGN AND METHODS

Reagents. Palmitic acid (sodium salt), palmitate methyl ester, octanoate, fatty-acid–free bovine serum albumin (BSA), triolein, and the GPO Trinder reagent (No. 339) were from Sigma (St. Louis, MO). 2-Bromopalmitate was from Aldrich (Milwaukee, WI). Reverse transcriptase–polymerase chain reaction (RT-PCR) primers and the Gold RT-PCR kit were from PerkinElmer

(Foster City, CA). [U-^{14}C]Palmitate, [1-^{14}C]cholesteryl oleate, and [carboxyl-^{14}C]triolein were from NEN (Boston, MA). Thesit was from Boehringer Mannheim (Mannheim, Germany).

Fatty-acid solutions. Stock solutions were prepared as follows: palmitic acid was dissolved in ethanol:H_2O (1:1, vol:vol) at 50°C at a final concentration of 150 mmol/l; 2-bromopalmitate and palmitate methyl ester were dissolved in ethanol at a final concentration of 300 mmol/l; and octanoate was dissolved in ethanol:H_2O (1:1, vol:vol) at a final concentration of 500 mmol/l. Aliquots of stock solutions were complexed with fatty-acid–free BSA (10% solution in H_2O) by stirring for 1 h at 37°C and then diluted in culture media. The final molar ratio of fatty acid:BSA was 5:1. The final ethanol concentration was ≤0.33% (vol:vol). All control conditions included a solution of vehicle (ethanol:H_2O) mixed with fatty-acid–free BSA at the same concentration as the fatty-acid solution, unless indicated otherwise.

Animals. Six-week-old male Wistar rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). Seven-week-old male Zucker diabetic fatty (ZDF/Gmi *fa/fa*) rats and Zucker lean control (ZLC/Gmi +/*fa* or +/+) rats were purchased from Genetic Models (Indianapolis, IN). Animals were housed on a 12-h light/dark cycle with free access to water and standard laboratory food. All procedures using animals were approved by the Institutional Animal Care and Use Committee.

HIT-T15 cells and isolated rat islets. HIT-T15 cells (passage 69–78) were routinely cultured as described (11). For Northern blot, labeling, and TAG content experiments, cells were detached and subcultured for 48 h by plating 1×10^6 cells per well in 6-well plates. Rat islets were isolated by collagenase digestion as described (3). After an overnight culture in RPMI 1640 containing 10% fetal bovine serum and 11.1 mmol/l glucose to ensure optimal recovery (12), batches of 100–200 islets were incubated in various experimental conditions as described in RESULTS.

Northern 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, 4.3 mmol/l $NaH_2PO_4.7H_2O$, pH 7.3), and resuspended in denaturing solution (4 mol/l guanidine thiocyanate, 25 mmol/l sodium citrate, pH 7, 0.5% sarcosyl, 0.1 mol/l 2-mercaptoethanol). HIT-T15 cells were rinsed with PBS and scraped with denaturing solution. RNA was extracted according to Chomczynski and Sacchi (13), and insulin mRNA was assessed by Northern blot as described (3). Membranes were stripped and rehybridized with a β-actin cDNA to control for variations in the amount of total RNA loaded on each lane.

TABLE 1
Primer and probe sequences for RT-PCR analysis

Gene (accession number)	Sequences (5′–3′)
Mouse DGAT (AF078752)	
Forward primer	TCCGCCTCTGGGCATTC
Reverse primer	GAATCGGCCCACAATCCA
Probe	6FAM-CCATGATGGCTCAGGT CCCACTGG-TAMRA
Rat GPAT (AF021348)	
Forward primer	CAGCTCTGCTGCCATCTTTG
Reverse primer	TGCAGCTTCTGCAGGTACTCA
Probe	6FAM-CACACCTTCCGCGGCC CAGTC-TAMRA
Rat HSL (X51415)	
Forward primer	GAGACGGGCCTCAGTGTGA
Reverse primer	CCACGCAACTCTGGGTCTATG
Probe	6FAM-TTCCCTCTTTACGGGTG GCCGATTC-TAMRA
Rat insulin II (J00748)	
Forward primer	GCCCAGGCTTTTGTCAAACA
Reverse primer	CTCCCCACACACCAGGTAGAG
Probe	6FAM-AGCTTCCACCAAGTGA GAACCACAAAGGT-TAMRA
Rat β-actin (J00691)	
Forward primer	ACGAGGCCCAGAGCAAGA
Reverse primer	TTGGTTACAATGCCGTGTTCA
Probe	6FAM-AGGCATCCTGACCCTG AAGTACCCCA-TAMRA

FAM, 6-carboxyfluorescein; TAMRA, 6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine.

Fluorescence-based RT-PCR of GPAT, DGAT, HSL, and insulin mRNAs. Comparative analysis of GPAT, DGAT, and HSL mRNA levels in isolated islets cultured with and without palmitate was performed using the Gold RT-PCR kit and an ABI Prism 7700 sequence detector equipped with a thermocycler (TaqMan technology), as described (14). β-Actin was used as an internal control, and insulin was used as a positive control for the effect of palmitate. Primer and probe sequences are given in Table 1.

Analysis of intracellular lipids by thin-layer chromatography. Fatty-acid solutions were prepared as described above with the addition of 5 µmol/l (4.25 mCi) [U-^{14}C]palmitate in all conditions. Islets or HIT-T15 cells were cultured in the various fatty-acid solutions, as described in RESULTS. At the end of the culture period, islets or cells were transferred to 12 × 75 mm prechilled glass tubes on ice, centrifuged at 1,200 rpm for 10 min at 4°C, washed with 2 ml ice-cold PBS, centrifuged as above, resuspended in 3 ml chloroform:methanol:HCl (200:100:1), and stored at 4°C overnight under N_2. After addition of 750 µl double-distilled H_2O, samples were vortexed and centrifuged for 10 min at 4°C. The upper (aqueous) phase was removed, and the lower (organic) phase was washed with 750 µl double-distilled H_2O and centrifuged as above. The upper phase was removed and the lower phase was dried under N_2. The lipid pellet was resuspended in 100 µl chloroform:methanol:HCl (200:100:1), and 40 µl was spotted in duplicate on silica gel thin-layer chromatography (TLC) plates (Whatman, Clifton, NJ). Lipids were resolved in petroleum ether:diethyl ether:glacial acetic acid (70:30:1). [1-^{14}C]Cholesteryl oleate, [U-^{14}C]palmitate, and [carboxyl-^{14}C]triolein were used as standards. Results obtained with this system were initially verified using *N*-hexane:diethyl ether:methanol:glacial acetic acid (90:20:2:3). TLC plates were exposed to X-OMAT AR autoradiography films (Eastman Kodak, Rochester, NY). Individual bands were scraped, transferred to scintillation vials with 5 ml Ecoscint scintillation fluid (National Diagnostics, Atlanta, GA), and counted.

Intracellular TAG assay. We have experienced difficulties in trying to measure TAG content in islet extracts, as previously published (6,15–19). Therefore, we have modified this method by *1*) extracting the lipids before the assay, *2*) resuspending the extracted lipids in the detergent Thesit as recommended in other cells (20), and *3*) calculating the sample TAG concentrations from a triolein standard curve, as opposed to a single glycerol value. The assay is based on the colorimetric determination of glycerol produced by hydrolysis of neutral lipids in the presence of lipoprotein lipase. Islets or cells were transferred to 12 × 75 mm prechilled glass tubes on ice, centrifuged at 1,200 rpm for 10 min at 4°C, washed with 500 µl ice-cold PBS, and centrifuged as above. The pellet was resuspended in 3 ml of chloroform:methanol (2:1) and stored overnight at 4°C under N_2. After the addition of 1.5 ml double-distilled H_2O, the tubes were vortexed and centrifuged at 1,200 rpm for 10 min at 4°C. The upper phase was discarded, and 750 µl double-distilled H_2O was added to the lower phase. The samples were vortexed and centrifuged at 1,200 rpm for 10 min at 4°C. The upper phase was removed, and the lower phase was evaporated under N_2. The samples were resuspended in 50 µl chloroform; 10 µl was quickly transferred to glass tubes in duplicate and air dried. The dry pellet was resuspended in 10 µl Thesit. The triolein standard curve (1–50 µg) was prepared using triolein (Sigma) diluted in chloroform:methanol (2:1). Samples and standards resuspended in Thesit were air dried; 50 µl H_2O was added; and the tubes were vortexed and incubated in a 37°C shaking water bath for 10 min as described (20). One milliliter of GPO Trinder reagent was added to the tubes, which were then gently mixed and incubated at 37°C for 5 min. The absorbance was read at 540 nm in a Beckman (Fullerton, CA) DU-64 spectrophotometer. To test the efficiency of TAG extraction, samples consisting of 5, 10, or 50 µg total lipid, each containing 0.9 µg of [carboxyl-^{14}C]triolein, and the remainder unlabeled triolein underwent the extraction process. Aliquots were taken at each step during the extraction process and counted. The total loss of radioactivity during the extraction process was 3.0 ± 1.3% (n = 4).

Expression of data and statistics. Data are expressed as means ± SE. Intergroup comparisons were performed by paired Student's t test or analysis of variance with Dunnett's t test for multiple comparisons, where appropriate. $P < 0.05$ was considered significant.

RESULTS

Expression of GPAT, DGAT, and HSL in isolated rat islets. We first sought to determine whether critical enzymes of neutral lipid metabolism are expressed in islets and whether their level of expression is modulated upon prolonged exposure to palmitate and glucose. GPAT is a rate-lim-

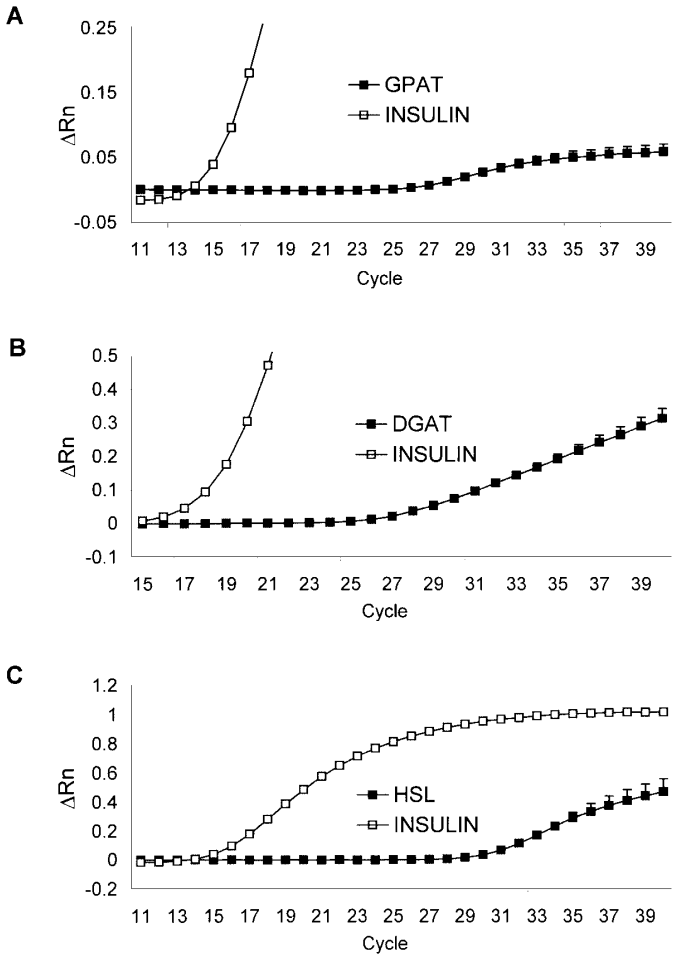

FIG. 1. Detection of GPAT (*A*), DGAT (*B*), and HSL (*C*) mRNAs by RT-PCR using Taqman technology in isolated rat islets. ΔRn designates changes in fluorescence emission. Representative amplification plots were from experiments performed in triplicate on two separate occasions.

iting enzyme for neutral lipid synthesis (21), and its expression is increased in islets from ZDF rats (6). DGAT is the enzyme responsible for the synthesis of TAG from diacylglycerols (DAGs), but its expression has never been documented in β-cells. HSL is responsible for the hydrolysis of TAG and is expressed and active in β-cells (22). Fluorescence-based RT-PCR demonstrated the expression of GPAT (Fig. 1*A*), DGAT (Fig. 1*B*), and HSL (Fig. 1*C*) in islets isolated from normal Wistar rats. Semiquantitative analysis of mRNA levels for each enzyme was then used to determine the effects of a 72-h culture in 2.8 or 16.7 mmol/l glucose with or without 0.5 mmol/l palmitate (Fig. 2). As expected, insulin mRNA levels were increased in the presence of 16.7 mmol/l glucose (P = 0.002, n = 8; Fig. 2*A*), and this effect was blocked by palmitate (P = 0.002, n = 8; Fig. 2*A*). However, neither GPAT (Fig. 2*B*), DGAT (Fig. 2*C*), nor HSL (Fig. 2*D*) mRNA was significantly affected by prolonged exposure to elevated glucose and/or palmitate. DGAT mRNA levels tended to be slightly lower in the presence of palmitate at 2.8 mmol/l glucose, but this difference was not statistically significant (NS, n = 7; Fig. 2*C*). The effect of the culture period itself was investigated by comparing insulin mRNA levels in freshly isolated islets to those in islets cultured for 72 h in either 2.8 or 16.7 mmol/l glucose. Insulin mRNA levels were lower in islets cultured in 2.8 mmol/l glucose than in freshly isolated islets (insulin:β-actin mRNA ratio 2.5 ± 0.9 vs. 5.7 ± 0.2, n = 3). Islets cultured for 3 days in 16.7 mmol/l glucose had insulin mRNA levels similar to those of freshly isolated islets (insulin/β-actin mRNA ratio 4.5 ± 1.5 vs. 5.7 ± 0.2, n = 3). Therefore, we cannot exclude the possibility that the lack of effect of palmitate on insulin mRNA levels at 2.8 mmol/l glucose is due to the fact that insulin mRNA is already downregulated at this glucose concentration and cannot be decreased further.

Effects of exogenous palmitate and glucose on incorporation of [^{14}C]palmitate into neutral lipids. Intracellular neutral lipid content results from the balance between lipogenic (GPAT and DGAT) and lipolytic (HSL) enzyme activities. To determine whether neutral lipids accumulate upon prolonged exposure to elevated palmitate and glucose, isolated islets were cultured for 72 h in the presence of 5 µmol/l [U-^{14}C]-labeled palmitate with and without addition of 0.5 mmol/l unlabeled palmitate, in 2.8 or 16.7 mmol/l glucose. Intracellular lipids were extracted and analyzed by TLC. In the absence of unlabeled palmitate, culturing islets with 16.7 mmol/l glucose increased the total number of counts incorporated from labeled palmitate into complex lipids (phospholipids [PLs] + DAG + TAG) 3.0 ± 0.9 fold compared with islets cultured in 2.8 mmol/l glucose (P < 0.05, n = 7; Fig. 3*A*). In islets cultured in 0.5 mmol/l unlabeled palmitate, the total number of counts incorporated into complex lipids was 2.4 ± 0.8–fold higher in the presence of 16.7 mmol/l glucose than in the presence of 2.8 mmol/l glucose (P < 0.05, n = 7; Fig. 3*A*). The ability of high glucose to increase incorporation of the counts into complex lipids was therefore similar in the absence or presence of unlabeled palmitate. The total number of counts incorporated into complex lipids was artificially lower in the presence of unlabeled palmitate because of the dilution of the specific activity of the tracer. The effect of palmitate on partitioning the counts is therefore expressed as the percentage of the counts incorporated into each lipid fraction (Fig. 3*B*). In the presence of 0.5 mmol/l unlabeled palmitate, the percentage of counts incorporated into DAG and TAG was increased at the expense of the counts incorporated into PLs. This effect was similar at 2.8 and 16.7 mmol/l glucose. These results indicate that glucose and palmitate have different effects on neutral lipid synthesis in islets upon prolonged exposure: glucose increases the total number of counts incorporated into complex lipids, whereas palmitate specifically directs partitioning of the counts toward neutral lipid synthesis.

Effects of palmitate and glucose on TAG content in islets. We next sought to determine whether prolonged and simultaneous exposure to palmitate and glucose leads to a net increase in TAG content. We first tested the modified assay against the previously published method using crude cellular extracts of islets (6,15–19) freshly isolated from normal Wistar, ZDF, and ZLC rats. The results are presented in Table 2 and indicate that the highest recovery of intracellular TAG is obtained using our modified method. TAG content was then measured in Wistar rat islets after a 72-h culture in the presence of 2.8 or 16.7 mmol/l glucose, with or without 0.5 mmol/l palmitate (Fig. 4). Palmitate did not affect TAG content in the presence of 2.8 mmol/l glucose (105 ± 5 ng/islet in the presence of palmitate vs. 76 ± 8 ng/islet in the absence of palmi-

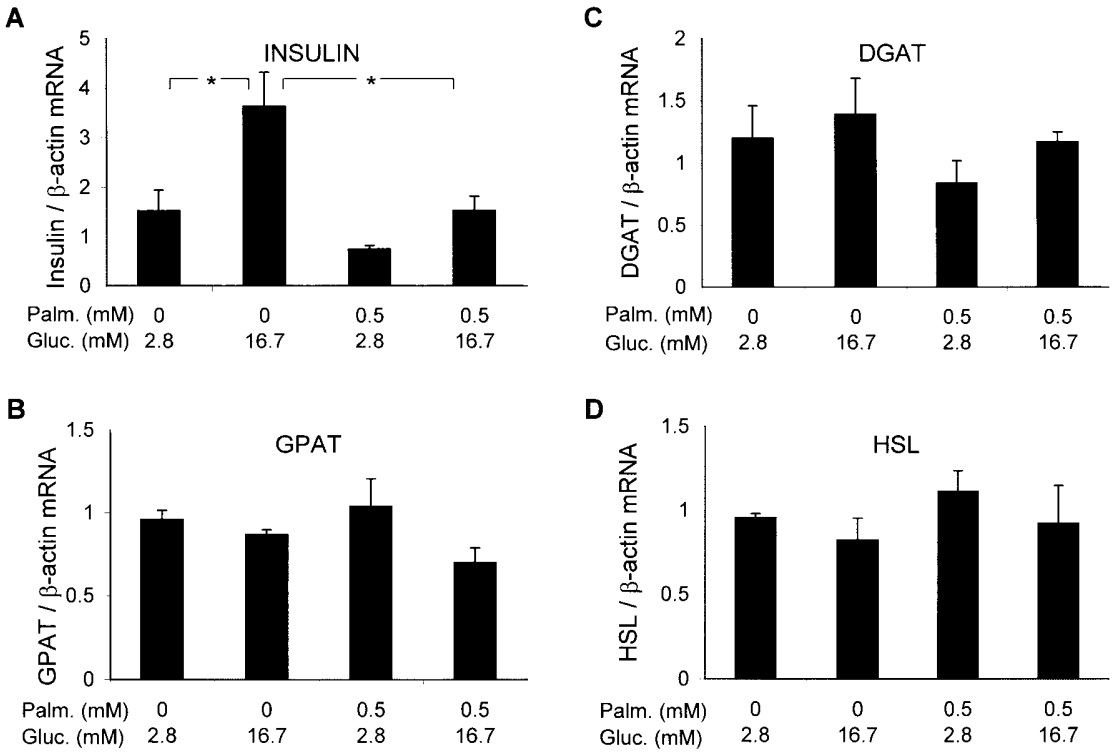

FIG. 2: Effects of prolonged exposure to glucose (Gluc.) and palmitate (Palm.) on insulin (*A*), GPAT (*B*), DGAT (*C*), and HSL (*D*) mRNA levels in isolated rat islets. Isolated islets were incubated in the absence or presence of 0.5 mmol/l palmitate and 2.8 or 16.7 mmol/l glucose for 72 h. Insulin (n = 8), GPAT (n = 3), DGAT (n = 7), and HSL (n = 3) mRNA levels were determined by fluorescence-based RT-PCR as described in RESEARCH DESIGN AND METHODS. Results (means ± SE) are expressed as the ratio between the signal corresponding to the gene of interest and the signal for β-actin mRNA. *P < 0.01.

tate, n = 3, NS) but significantly increased it in the presence of 16.7 mmol/l glucose (303 ± 58 ng/islet in the presence of palmitate vs. 171 ± 31 ng/islet in the absence of palmitate, P = 0.03, n = 6). These results indicate that the presence of high glucose is necessary for prolonged exposure to palmitate to result in a net increase in TAG content in islets.

Concentration dependency of the effects of palmitate on neutral lipid metabolism and insulin mRNA levels in HIT-T15 cells. The concentration dependency of the effects of palmitate on neutral lipid synthesis, TAG content, and insulin mRNA levels was assessed in HIT-T15 cells (Fig. 5). First, HIT-T15 cells were cultured for 72 h in the presence of 11.1 mmol/l glucose, 5 µmol/l [U-^{14}C]-labeled palmitate, and increasing concentrations of unlabeled palmitate at a constant palmitate:BSA molar ratio of 5:1. Analysis of incorporation of the counts into the various forms of complex lipids showed that palmitate dose-dependently shifted the partitioning of the counts into TAG and DAG, at the expense of PL, confirming the observations made in isolated islets (Fig. 5*A*). The effect of palmitate was statistically significant at 0.25 mmol/l and higher. Importantly, culturing HIT-T15 cells in the presence of increasing concentrations of BSA alone had no effect on the partitioning of the counts (data not shown). Next, HIT-T15 cells were cultured for 72 h in the presence of 11.1 mmol/l glucose with increasing concentrations of palmitate. TAG mass was measured as described above and was found to gradually augment as the concentration of palmitate was increased, with a significant and maximal effect observed at 0.5 mmol/l (P < 0.05, n = 5; Fig. 5*B*). This was associated with a dose-dependent decrease in insulin mRNA levels (Fig. 5*B* and *C*) that was statistically significant at and above 0.25 mmol/l. These results indicate that the effect of palmitate promoting neutral lipid synthesis in the presence of high glucose is concentration dependent and that there is an inverse relationship between TAG content and insulin gene expression.

Effects of palmitate, octanoate, palmitate methyl ester, and 2-bromopalmitate on insulin mRNA levels in HIT-T15 cells. We next investigated whether increased esterification into neutral lipids was necessary for the inhibitory effects of fatty acids on insulin mRNA levels (Fig. 6). After a 24-h exposure to 0.5 mmol/l palmitate, insulin mRNA was decreased by ~40% compared with control in the presence of BSA alone, confirming our previous findings (3; Fig. 5). 2-Bromopalmitate, which irreversibly binds to carnitine-palmitoyl-transferase 1 (CPT-1) and inhibits long-chain fatty acid β-oxidation (23), had a similar effect to palmitate. In contrast, the medium-chain fatty acid octanoate, which does not require CPT-1 to enter the mitochondria and is readily oxidized, and palmitate methyl ester, which is not activated into a fatty-acyl CoA in the cytosol, did not affect insulin mRNA levels. These results suggest that the fatty-acid–induced decrease in insulin mRNA levels is not related to β-oxidation but requires activation of the fatty acid into a fatty-acyl CoA. Considering that fatty-acyl CoAs undergo either β-oxidation or esterification, these results indirectly suggest that activation of the esterification pathway is necessary for fatty acids to inhibit insulin gene expression.

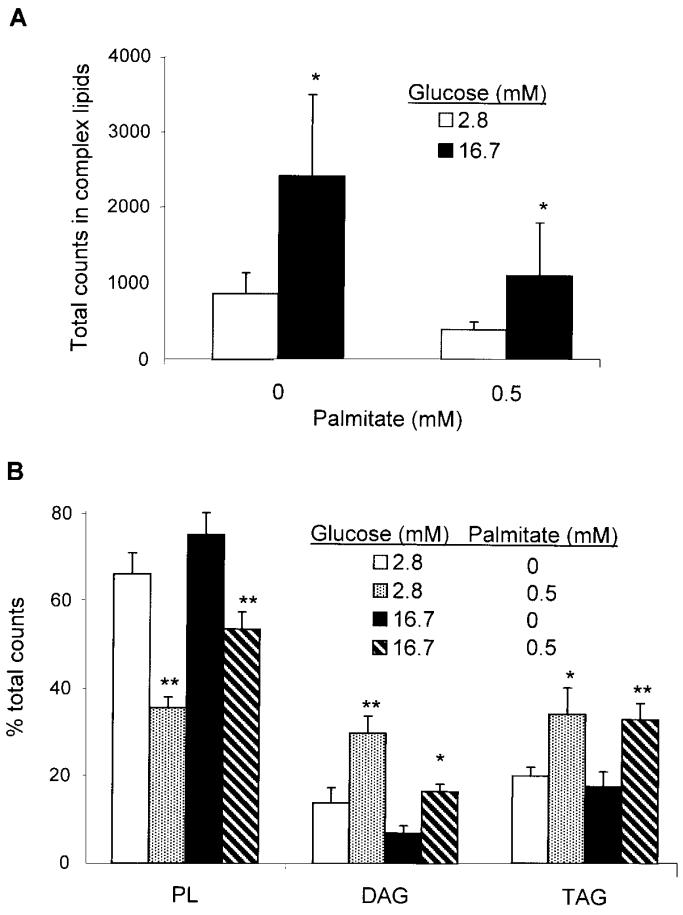

FIG. 3. Effects of palmitate and glucose on incorporation of [^{14}C] exogenous palmitate in isolated rat islets. Isolated rat islets were incubated with 5 µmol/l [U-^{14}C]palmitate in the absence or presence of 0.5 mmol/l unlabeled palmitate and 2.8 or 16.7 mmol/l glucose for 72 h. Extracted lipids were analyzed by TLC. For each lane, the lipid bands were scraped and counted. Results are means ± SE of seven to nine separate experiments. *A*: Effects of glucose on the total number of counts incorporated from labeled palmitate into complex lipids (PL + DAG + DAG). The effect of palmitate cannot be ascertained because of the dilution of the specific activity of the tracer in the presence of unlabeled palmitate. Indeed, the absolute number of counts is artificially lower in the presence of 0.5 mmol/l unlabeled palmitate. *P < 0.05. *B*: Effects of glucose and palmitate on the distribution of the counts into each complex lipid fraction. Results are expressed as percent of total counts recovered from each lane. *P < 0.05; **P < 0.01.

TABLE 2
Comparison of the modified TAG mass assay against the previously published method using crude cellular extracts in isolated islets

	TAG content (ng/islet)			
	Nonextraction		Extraction	
Rats	Solvent A	Thesit	Solvent A	Thesit
ZDF	3.2 ± 1.1	8.0 ± 1.4	7.1 ± 1.1	74.6 ± 8.8
ZLC	4.5 ± 0.8	10.1 ± 3.0	12.2 ± 2.3	39.2 ± 9.4
Wistar	13.3 ± 3.5	36.9 ± 13.3	9.1 ± 3.0	47.7 ± 13.4

TAG mass was measured in ZDF, ZLC, and normal Wistar rats, either on crude cellular extracts as published (6,15–19) or by extracting the lipids before the assay as described in RESEARCH DESIGN AND METHODS. Cell or lipid extracts were resuspended using either Solvent A (10 µl tert-butanol, 5 µl Triton X-100: methanol [1:1]) as described (6,15–19) or Thesit as recommended in other cells (20).

DISCUSSION

This study was designed to assess whether the decrease in insulin gene expression upon prolonged exposure to palmitate in the presence of high glucose was associated with modulation in the expression of lipogenic and lipolytic enzymes in β-cells and with increased esterification of fatty acids into neutral lipids.

GPAT, DGAT, and HSL mRNA were detected, demonstrating for the first time that DGAT is expressed in pancreatic islets. In ZDF rat islets, the dramatic increase in TAG content is associated with a marked elevation in GPAT mRNA (6). However, we have not found GPAT mRNA to be increased in normal islets after prolonged exposure to palmitate. In other cell types, it has been suggested that DGAT, together with GPAT, is an important enzyme in TAG synthesis because it catalyzes the final and only committed step in this pathway (24). DGAT activity is increased in adipose tissue from obese Zucker rats (25). Its expression was not found to be modified in islets cultured in palmitate and glucose for 3 days. HSL, the rate-limiting enzyme for intracellular TG hydrolysis, is expressed and active in rat islets and several β-cell lines (22). Its expression, however, does not seem to be modified by prolonged exposure to either glucose or palmitate.

Pancreatic β-cells have the capacity to store energy in the form of TAG from both glucose and palmitate upon short-term incubation (26). Glucose is mainly incorporated into the glycerol-3-phosphate backbone of TAG (27). In the fasting state, the β-cell preferentially utilizes fatty acids as metabolic fuels, and therefore palmitate oxidation is high compared with esterification (28,29). At high glucose concentrations, lipid metabolism is switched to preferential incorporation into TAG and PL (28), at least in the short term. Our results show that these observations also apply to long-term situations and demonstrate that exposing islets to elevated concentrations of palmitate results in a sustained increase in intracellular TAG stores only if the concentration of glucose is elevated. The metabolic signals responsible for the switch from fatty-acid oxidation to esterification in the presence of high glucose in the β-cell are thought to be malonyl-CoA and cytosolic long-chain acyl-CoA (30). Acceleration of glucose metabolism following a rise in intracellular glucose concentration leads to increased cytosolic levels of malonyl-CoA (31,32), which potently inhibits CPT-1, thereby decreasing fatty-acid oxidation and resulting in accumulation of fatty acyl-CoA esters in the cytosol (33). Our results suggest that this hypothesis, originally proposed to explain the role of fatty acids in stimulus-secretion coupling in the β-cell, also applies to situations in which β-cells are chronically exposed to elevated levels of glucose and fatty acids. Furthermore, our results directly demonstrate that esterification of fatty acids into neutral lipids is increased upon prolonged exposure to glucose and palmitate, and that both nutrients have synergistic effects on neutral lipid metabolism. Glucose promotes incorporation of the fatty acid into complex lipids, whereas palmitate specifically directs partitioning toward neutral lipid synthesis. As expected, this results in a net increase in neutral

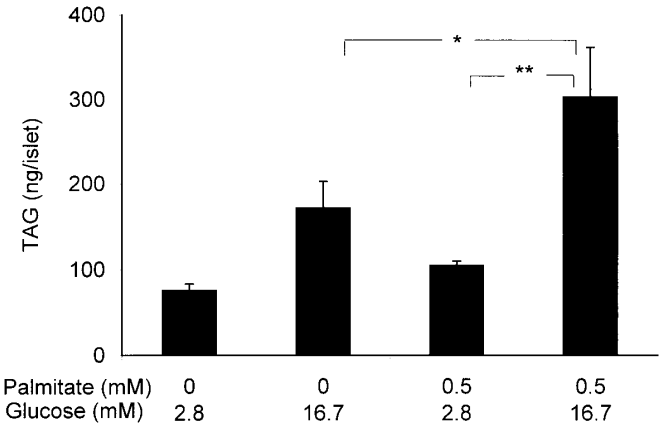

FIG. 4. Effects of palmitate and glucose on TAG content in isolated rat islets. Isolated rat islets were incubated in the absence or presence of 0.5 mmol/l palmitate and 2.8 or 16.7 mmol/l glucose for 72 h. TAG content was measured as described in RESEARCH DESIGN AND METHODS. Results are means ± SE of three to six separate experiments. *$P < 0.05$; **$P < 0.01$.

lipid mass only when both fuels are present simultaneously. The fact that neutral lipid accumulation is not associated with changes in the level of expression of lipogenic and lipolytic enzymes suggests that activation of the esterification pathway is driven by the amount of substrates, i.e., fatty acids and glucose. Intracellular accumulation of neutral lipids results mainly from the balance between the activities of lipogenic and lipolytic enzymes. Our results indicate that overall lipogenic activity is augmented upon prolonged exposure to palmitate in the presence of high glucose, resulting in increased DAG and TAG content.

Various methods have been used for measuring TAG content in islets, generating quantitatively variable results. The differences between studies are in the mode of preparation of the lipid extract utilized for the assay. Most studies in ZDF animals have used crude cellular extracts and found intracellular levels of TAG to increase from <50 ng/islet in control rats to up to 1,000 ng/islet in 12-week-old ZDF animals (6,15–19). Two studies using normal islets in culture have extracted the lipids before the GPO Trinder assay (34,35). Our results

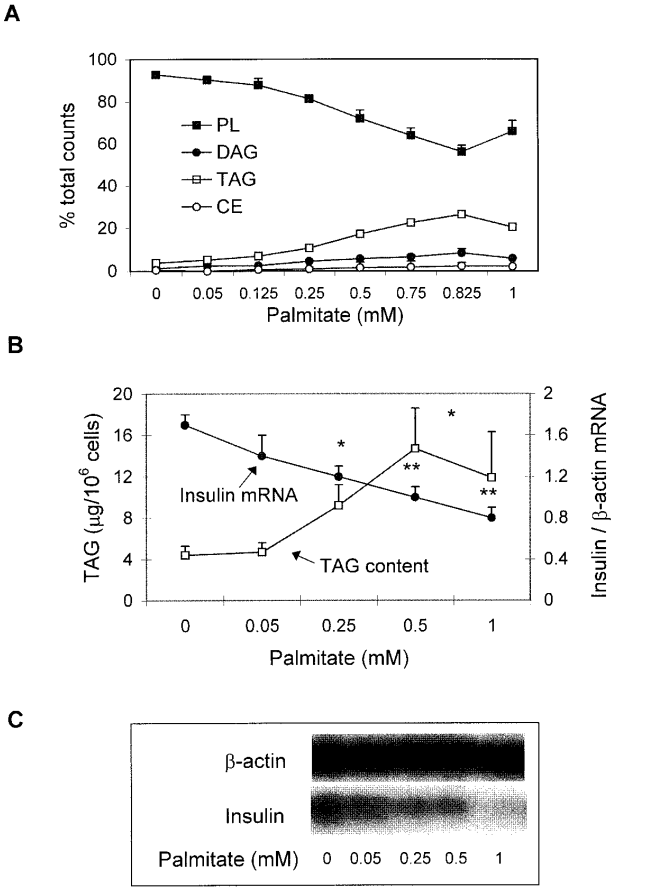

FIG. 5. Dose-dependent effects of palmitate on lipid partitioning, TAG content, and insulin mRNA levels in HIT-T15 cells. HIT-T15 cells were incubated for 72 h in RPMI 1640 containing 11.1 mmol/l glucose with increasing concentrations of palmitate. *A*: The culture medium contained 5 µmol/l [U-^{14}C]palmitate. Lipids were extracted and analyzed by TLC. For each lane, the lipid bands were scraped and counted. Results are means ± SE of three separate experiments and are expressed as percent of total counts recovered from each lane. All points at and above 0.25 mmol/l are significantly different from the control (0 palmitate) for PL, TAG, and DAG. CE, cholesterol ester. *B*: Inverse correlation between TAG content and insulin mRNA levels. Results are expressed as means ± SE of five or six separate experiments. *C*: Representative Northern blot of insulin mRNA.

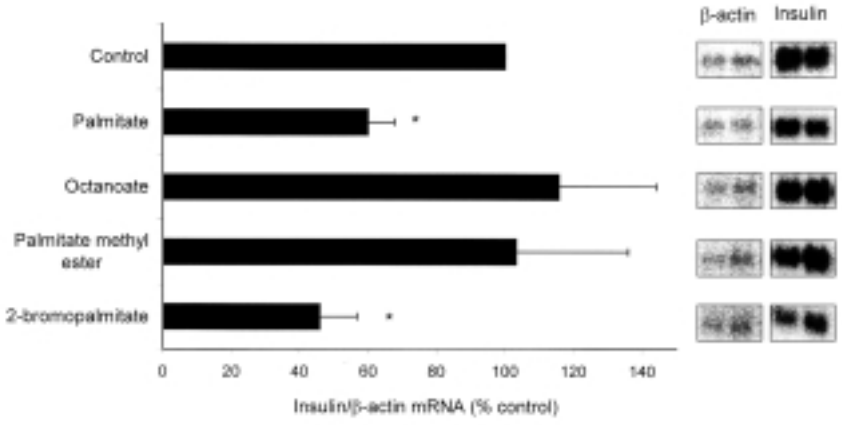

FIG. 6. Effects of fatty acids on insulin mRNA level in HIT-T15 cells. HIT-T15 cells were cultured for 24 h in the presence of 0.5 mmol/l palmitate, 2.5 mmol/l octanoate, 0.5 mmol/l palmitate methyl ester, or 0.5 mmol/l 2-bromopalmitate. The control condition contained an equivalent amount of BSA. Insulin mRNA was measured by Northern analysis and was normalized to β-actin mRNA. Results are means ± SE of three separate experiments and are expressed as percent control. A representative Northern blot is shown on the right.

are quantitatively similar to those of Zhou et al. (34) and uniquely demonstrate that the presence of glucose is necessary for TAG content to increase significantly upon prolonged exposure to exogenous palmitate.

In conclusion, the results presented herein demonstrate for the first time that neutral lipid synthesis is increased in normal β-cells exposed to elevated palmitate and glucose for prolonged periods of time. Glucose is required for palmitate to increase intracellular TAG mass, and both fuels have synergistic effects on neutral lipid metabolism. The glucose-dependent activation of the esterification pathway upon prolonged exposure to palmitate does not seem to be mediated by modulation of expression of key enzymes involved in neutral lipid metabolism, and it is therefore likely to be driven by the amount of available substrates.

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