Distinct Effects of Saturated and Monounsaturated Fatty Acids on β-Cell Turnover and Function

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Glucotoxicity and lipotoxicity contribute to the impaired β-cell function observed in type 2 diabetes. Here we examine the effect of saturated and unsaturated fatty acids at different glucose concentrations on β-cell proliferation and apoptosis. Adult rat pancreatic islets were cultured onto plates coated with extracellular matrix derived from bovine corneal endothelial cells. Exposure of islets to saturated fatty acid (0.5 mmol/l palmitic acid) in medium containing 5.5, 11.1, or 33.3 mmol/l glucose for 4 days resulted in a five- to ninefold increase of β-cell DNA fragmentation. In contrast, monounsaturated palmitoleic acid alone (0.5 mmol/l) or in combination with palmitic acid (0.25 or 0.5 mmol/l each) did not affect DNA fragmentation. Increasing concentrations of glucose promoted β-cell proliferation that was dramatically reduced by palmitic acid. Palmitoleic acid enhanced the proliferation activity in medium containing 5.5 mmol/l glucose but had no additional effect at higher glucose concentrations (11.1 and 33.3 mmol/l). The cell-permeable ceramide analog C2-ceramide mimicked both the palmitic acid–induced β-cell apoptosis and decrease in proliferation. Moreover, the ceramide synthetase inhibitor fumonisin B1 blocked the deleterious effects of palmitic acid on β-cell viability. Additionally, palmitic acid but not palmitoleic acid decreased the expression of the mitochondrial adenine nucleotide translocator and induced release of cytochrome c from the mitochondria into the cytosol. Finally, palmitoleic acid improved β-cell–secretory function that was reduced by saturated acid. Taken together, these results suggest that the lipotoxic effect of the saturated palmitic acid involves an increased apoptosis rate coupled with reduced proliferation capacity of β-cells and impaired insulin secretion. The deleterious effect of palmitate on β-cell turnover is mediated via formation of ceramide and activation of the apoptotic mitochondrial pathway. In contrast, the monounsaturated palmitoleic acid does not affect β-cell apoptosis, yet it promotes β-cell proliferation at low glucose concentrations, counteracting the negative effects of palmitic acid as well as improving β-cell function. Diabetes 50:69-76, 2001

At the initial stages of type 2 diabetes, individuals lose the ability to produce sufficient quantities of insulin to maintain normoglycemia in the face of insulin resistance (1). The capacity to produce insulin is determined by the total β-cell number and β-cell functional activity. The β-cell mass is capable of long-term adaptation by increasing the β-cell number through hyperplasia and neogenesis (2,3). However, β-cell expansion can be offset by concomitant apoptosis (4). The failure of these long-term feedback adaptations in genetically susceptible individuals with concurrent insulin resistance may result in type 2 diabetes (3,5). The etiology and mechanisms leading to apoptosis of β-cells together with an inadequately low proliferation rate has not been completely elucidated. Previously, we analyzed β-cell turnover in pancreata of Psammomys obesus, a rodent with a natural tendency to diet-induced type 2-like diabetes (6). Elevated glucose concentrations directly induced β-cell apoptosis in cultured islets from diabetes-prone P. obesus, but not in islets from diabetes-resistant rats (6,7). Glucose-induced β-cell proliferation was observed in both rat and P. obesus islets; however, P. obesus showed only a limited capacity of β-cell proliferation in response to elevated glucose concentrations. Based on these observations, we suggested the existence of a novel process of glucotoxicity, in which chronic hyperglycemia is linked to a progressive loss of β-cell mass, when genetic susceptibility to diabetes exists.

Apart from hyperglycemia, plasma long-chain free fatty acid levels are often increased in states of insulin resistance, further impairing β-cell–secretory function (8,9). In an adipogenic model of diabetes, the Zucker diabetic fatty rat, fatty acid–induced β-cell apoptosis was observed (5). However, it is not known whether islets of these diabetic models behave differently from those of normal rats. Furthermore, the effect of fatty acids on β-cell proliferation has been investigated only at high fatty acid concentrations (2 mmol/l) in rat islets maintained in suspension for 7 days (10).

Because ceramide is synthesized from long-chain fatty acids, it has been postulated that free fatty acid–induced cell death is mediated via formation of ceramide. Ceramide serves as a second messenger for cellular functions ranging from proliferation and differentiation to growth arrest and apoptosis (11). Shimabukuro et al. (5,12) showed that islets from Zucker diabetic fatty rats manifested elevated ceramide levels. In response to a challenge with fatty acids, these islets displayed increased incorporation of fatty acids into ceramide, accompanied by apoptosis. Fumonisin B1, a specific inhibitor of ceramide synthase activity, blocked both ceramide generation and apoptosis, indicating that ceramide

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ANT, adenine nucleotide translocator; BSA, bovine serum albumin; PBS, phosphate-buffered saline; TUNEL, terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling.
generation was necessary for the apoptotic response. However, the possible role of ceramide signaling on β-cell proliferation has not been addressed in these studies. A critical event leading to apoptosis involves changes in mitochondrial membrane, which culminates in the release of apoptogenic factors, such as cytochrome c, from the mitochondrial intermembrane space to the cytosol (13,14). The loss of barrier function of both mitochondrial membranes is controlled, at least in part, by the permeability transition pore complex, a polypeptide complex that includes the most abundant protein of the inner mitochondrial membranes, the adenine nucleotide translocator (ANT) (15). ANT catalyses the exchange of ADP and ATP across the inner mitochondrial membrane. Recent studies indicate that proapoptotic proteins, such as Bax or Bak, interact with ANT to facilitate apoptosis and induce release of cytochrome c from mitochondria of β-cells is unknown.

Therefore, we investigated the role of fatty acids at different ambient glucose concentrations on β-cell proliferation, apoptosis, and function in adult rat islets. Pancreatic islets were cultured onto plates coated with extracellular matrix derived from bovine corneal endothelial cells, allowing in situ identification of β-cell apoptosis and proliferation. In addition, the possible involvement of the ceramide and of the apoptotic mitochondrial pathway was studied. To elucidate whether possible effects could be ascribed to the degree of saturation at an identical carbon chain length, β-cells were exposed to palmitic (C16:0) and palmitoleic (16:1) acid alone and in combination.

**RESEARCH DESIGN AND METHODS**

**Islet isolation.** Male Sprague-Dawley rats (200–220 g) were anesthetized by ether, and the livers were perfused with the pancreas using an adaptation for rat islets of the method of Gototoh et al. (21). Briefly, after cannulation of the common bile duct and instillation of 10 ml cold Hank’s balanced salt solution (Gibco, Gaithersburg, MD) containing 0.17 mg/ml Liberase (Boehringer Mannheim, Mannheim, Germany), 0.1 mg/ml DNase I (Boehringer Mannheim), and 25 mM/L Hepes, each pancreas was removed and digested in 5 ml of the above-described Liberase solution for 35 min at 37°C in a shaking water bath. This was followed by dilution and washing with Hank’s balanced salt solution containing 0.1 mg/ml DNase I, 25 mM/L Hepes, and 10% fetal calf serum (Gibco). Islets from the crude pancreas digest were purified by centrifugation through a discontinuous Histopaque 1077 (Sigma Chemical, St. Louis, MO) gradient, washed with RPMI-1640 medium (Gibco), and dispersed into culture dishes.

**Islet culture.** Details of the procedure have been described previously (22,23). Islets pooled from four rats were suspended in 7 ml RPMI-1640 medium (11.1 mM/L glucose) containing 100 U/ml penicillin, 100 µg/ml streptomycin, 250 µg/ml amphotericin B, 40 µg/ml gentamicin, and 10% fetal calf serum (Gibco). Of the islet suspension, 200 µl (30–50 islets) were added to 2 ml of the above-described medium and cultured onto 35-mm plates coated with extracellular matrix derived from bovine corneal endothelial cells (23) (Novamed, Jerusalem, Israel). Two days after plating, when most islets were attached and began to flatten, the culture medium was changed to RPMI containing 5.5, 11.1, or 25.3 mM/L glucose supplemented with bovine serum albumin (BSA) alone or with fatty acids (0.5 mM/L palmitic acid, 0.5 mM/L palmitoleic acid, or a mixture of 0.25 mM/L or 0.5 mM/L each). Fatty acids (Sigma) were dissolved at 10 mM/L in RPMI-1640 medium containing 11% fatty acid-free BSA (Sigma) under an N2-atmosphere, shaken overnight at 37°C, sonicated 15 min, and filtrated under sterile conditions (stock solution). For control incubations, 11% BSA was prepared, as described above. Before use, the effective free fatty acids concentrations were controlled with a commercially available kit (Wako, Neuss, Germany). In some experiments, islets were cultured with 15 mM/L C2-derivative (Biomol, Plymouth Meeting, PA) or 15 mM/L fumonisin B1 (Sigma), both were first dissolved in prewarmed 37°C DMSO (Phka, Buchs, Switzerland) at 5 mM/L.

**β-cell replication.** For β-cell proliferation studies, a monoclonal antibody to Ki-67 was used (MIB-5; Dianova, Hamburg, Germany). Ki-67 is a nuclear antigen expressed by proliferating cells that is used as a marker for late G1, S, G2, and M phases of the cell cycle (24). After washing with phosphate-buffered saline (PBS), cultured islets were fixed in 4% paraformaldehyde (30 min at room temperature) followed by permeabilization with 0.5% Triton X-100 at room temperature. Afterwards, islets were incubated for 1 h at room temperature with monoclonal mouse anti-Ki-67 antibody diluted 1:10, followed by detection using a streptavidin-biotin-peroxidase complex (Histostain-Plus Kit, Zymed, San Francisco, CA). Subsequently, islets were incubated for 30 min at 37°C with guinea pig anti-insulin antibody diluted 1:50 (Dako, Carpenteria, CA), followed by a 10-min incubation with a 1:10 dilution of fluorescein-conjugated rabbit anti–guinea pig antibody (Dako, Glostrup, Denmark).

**Detection of apoptotic β-cells.** The free 3-OH strand breaks resulting from DNA degradation were detected by the terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) technique (25). After islet cultures were fixed and permeabilized as described above, the TUNEL assay was performed according to the manufacturer’s instructions (In Situ Cell Death Detection Kit, AP; Boehringer Mannheim). The preparations were then rinsed with Tris-buffered saline and incubated (10 min at room temperature) with a 5-bromo-4-chloro-indolyl phosphate/nitro blue tetrazolium liquid substrate system (Sigma). Thereafter, islets were incubated with a guinea pig anti-insulin antibody as described above and detection was performed using the streptavidin-biotin-peroxidase complex (Zymed).

The TUNEL assay detects DNA fragmentation associated with both apoptotic and necrotic cell death; therefore, islets were also treated with a fluorescent annexin V probe (Annexin-V-FLUOS staining kit; Boehringer Mannheim) according to the manufacturer’s instructions. Double staining of cells with propidium iodide and annexin V enables the differentiation of apoptotic cells from necrotic cells.

**For analysis of rat islet’s subcellular fractions,** islet cultures were fixed and permeabilized as described above, incubated for 2 h at room temperature with mouse anti–cytochrome c monoclonal antibody that was diluted 1:50 (PharMingen), and then incubated for 1 h with a 1:50 dilution of fluorescein-conjugated goat anti-mouse antibody (Jackson Immuno Research Lab, West Grove, PA) and embedded in Dako fluorescent mounting medium. Images were produced by a confocal laser scanning microscope (Zeiss Axiophot fluorescence microscope with a Zeiss Neofluar ×40/1.3 objective lens connected to a Bio-Rad MRC-600 confocal scanner (Bio-Rad, Lasership, Oxfordshire, U.K.) and a Silicon Graphics Personal Iris 4D/25 Workstation Graphics, Mountain View, CA).

**Subcellular fractionation.** For analysis of rat islet’s subcellular fractions, islets were cultured in suspension in RPMI-1640 medium containing 11.1 mM/L glucose, as described above. One day after isolation, the medium was changed, and groups of 200 islets were incubated for 6 or 20 h in medium containing 11.1 mM/L glucose with 0.5 mM/L palmitate, 0.5 mM/L palmitoleic acid, or solvent. At the end of the incubations, islets were washed in PBS, and mitochondrial and cytosolic (S100) fractions were prepared from islets resuspended in 70 µl ice-cold containing 20 mM/L HEPES-KOH (pH 7.5), 10 mM/L KCl, 15 mM/L MgCl2, 1 mM/L Na-EDTA, 1 mM/L dithiothreitol, 0.1 mM/L phenylmethanesulfonyl fluoride, and 250 mM/L sucrose (26). Mechanical homogenization was achieved by repeated aspiration through a pipette. Unlysed cells and nuclei were pelleted by a 10-min centrifugation (750g for 4°C). The supernatant was centrifuged at 10 000g for 15 min at 4°C. This pellet representing the mitochondrial fraction was then resuspended in 2 ml of the above-described buffer. Finally, the supernatant was centrifuged at 100,000g for 1 h at 4°C. The supernatant from this final centrifugation represents the S-100 fraction (27). Both fractions were frozen at −80°C until use.

**Western blot analysis.** Mitochondrial and cytosolic fractions were diluted 1:3 in sample buffer containing 187.5 mM/L Tris-HCL, pH 6.8, 6% SDS, 30% glyceraldehyde, 1% β-mercaptoethanol, 1% sodium dodecyl sulfate, and 1% bromphenol blue, for 6 h at 100°C. Equivalent amounts of each treatment group at a ratio of 5:3 cytosolic to mitochondrial fraction were run on 15% SDS polyacrylamide gels. Proteins were electrophoretically transferred to nitrocellulose filters and incubated with a mouse anti-cytochrome c monoclonal antibody (PharMingen, San Diego, CA) (1 µg/ml for 1 h at room temperature), followed by incubation with horseradish peroxidase–linked anti-mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) (1 h at room temperature). After adding lumigen reagent (Phototope-HRP)
results

Modulation of β-cell proliferation and apoptosis after exposure to free fatty acids and glucose. Exposure of adult rat islets in long-term culture to palmitic acid for 4 days resulted in an increased number of β-cells with TUNEL-positive nuclei (Fig. 4A and B). The increase was 5.2- to 5.5-fold at 5.5, 11.1, and 33.3 mmol/l glucose, respectively, compared with islets exposed to identical glucose concentrations and solvent (albumin) (Fig. 3A). In contrast, the mono-unsaturated palmitoleic acid did not induce DNA fragmentation and, when present with palmitic acid (0.25 mmol/l and 0.5 mmol/l each), its effect on β-cell death was inhibited. Baseline β-cell death in the absence of fatty acids was minimal at 11.1 mmol/l glucose and increased 3.6- to 3.0-fold at 5.5 and 33.3 mmol/l glucose, respectively.

In parallel, the TUNEL assay, treated islets were incubated with annexin V and propidium iodide to discriminate apoptotic from necrotic cells (Fig. 1C–P). Exposure of cultured islets to palmitic acid markedly increased the number of cells exhibiting phosphatidylserine molecules translocated to the outer leaflet of the plasma membrane, as revealed by annexin V binding (1.96 ± 0.36 Annexin-V-FLUOS-positive cells/islet in control [11.1 mmol/l glucose] vs. 6.78 ± 1.05 in 0.5 mmol/l palmitic acid–treated islets, P < 0.01). Most of these cells had intact plasma membranes, impermeable to the DNA-binding dye propidium iodide (0.8 ± 0.01 propidium iodide–positive cells/islet in control vs. 2.14 ± 0.26 in 0.5 mmol/l palmitic acid–treated islets, P < 0.01). Therefore, the palmitic acid–induced DNA fragmentation, as determined by the TUNEL assay, mainly represents apoptotic cell death.

Exposure to high concentrations of glucose for 4 days resulted in increased proliferation of β-cells by 1.9- and 1.8-fold at 11.1 and 33.3 mmol/l glucose, respectively, compared with islets at 5.5 mmol/l glucose (Figs. 2 and 3F). In contrast, addition of palmitic acid resulted in a similar decrease in β-cell proliferation at all glucose concentrations (45, 33, and 40% decreases at 5.5, 11.1, and 33.3 mmol/l glucose, respectively, compared with islets incubated in identical glucose concentrations and solvent) (Fig. 3B). On the other hand, palmitoleic acid augmented β-cell proliferation by 65% at 5.5 mmol/l glucose, whereas at higher glucose concentrations, when the maximal proliferative activity was achieved with glucose alone, no additional effect was observed. Treatment of the cells with a mixture of palmitoleic and palmitic acid had no influence on β-cell proliferation (Fig. 3B).

Ceramide signaling in palmitic acid induced changes in β-cell apoptosis and proliferation. In pancreatic islets cultured with 15 mmol/l C2-ceramide in the presence of 11.1 mmol/l glucose, β-cell DNA fragmentation was increased 2.6-fold, whereas β-cell proliferation was decreased by 71.1%, showing the same trend as that observed in palmitic acid–treated islets (Fig. 4). Addition of 15 mmol/l of the ceramide synthase inhibitor fumonisin B1 in cultured medium containing 11.1 mmol/l glucose and 0.5 mmol/l palmitic acid, reduced the effect of palmitoleic acid on β-cell apoptosis and had a tendency to reverse its antiproliferative effect (Fig. 4).

Palmitic acid–induced decrease in ANT expression and cytochrome c release. Exposure of pancreatic islets to palmitic acid, compared with palmitoleic acid and solvent-treated islets, induced a time-dependent decrease of mitochondrial ANT expression (Fig. 5). The decrease was already detectable after 6 h of treatment, and after 20 h, ANT in the mitochondrial fraction of palmitate–treated islets was almost undetectable. At the same time, cytochrome c of palmitate–treated islets was shifted from the mitochondrial to the cytosolic fraction, whereas most cytochrome c in palmitoleic acid or control islets was confined to the mitochondria (Figs. 5 and 6). No ANT was detectable in any cytosolic fraction, thereby confirming the integrity of the mitochondrial and cytosolic preparations.

Insulin release and content. Exposure of rat islets to 0.5 mmol/l palmitoleic acid for 4 days increased islet insulin content and chronic insulin secretion as compared to control and palmitic acid-treated islets (Fig. 7A and B). Incubation with 0.5 mmol/l palmitic acid decreased islet insulin content; addition of 0.5 mmol/l palmitoleic to 0.5 mmol/l palmitic acid prevented this effect. An acute glucose challenge of palmitoleic acid–treated cells induced a higher insulin release rate compared with control, whereas treatment with palmitic acid abolished the insulin response to glucose (Fig. 7C).

discussion

This study shows that the saturated palmitic acid reduces the proliferative capacity of β-cells and induces β-cell death mainly by apoptosis. Conversely, palmitoleic acid, a monounsaturated fatty acid with identical carbon chain length, exhibits the opposite effects: it does not affect apoptosis, but promotes β-cell proliferation and counteracts the toxic effects of palmitic acid. The cell-permeable ceramide analog C2-ceramide mimics the palmitic acid–induced changes in the cell cycle, which were blocked by the ceramide synthetase inhibitor fumonisin B1. Thus, formation of ceramide is required to mediate the palmitic acid effects on β-cell function. Palmitic acid increased islet insulin content as well as chronic and glucose-stimulated insulin secretion.
Furthermore, it prevented the palmitic acid-induced decrease in islet insulin content and impaired glucose-stimulated insulin secretion.

The distinct effects of the saturated palmitic acid and the monounsaturated palmitoleic acid on β-cell turnover and function are striking. The fact that fatty acids longer than 15 carbons may be harmful to various cell types has been previously reported (29–35). At the same concentrations, chronic exposure to saturated fatty acids of different length have similar effects. Likewise, monounsaturated fatty acids C16:1 and C18:1 induced similar effects (29–35). However, saturated fatty acids were associated with different effects than unsaturated fatty acids. A putative explanation for this difference is based on the relative high melting point of saturated fatty acids (63°C for palmitic acid) compared with unsaturated fatty acids (0.5°C for palmitoleic acid). Consequently, triacylglycerols synthesized from saturated fatty chains are insoluble at 37°C. This observation has led to the hypothesis that, immediately after their formation, saturated triacylglycerol molecules precipitate at the site of synthesis (i.e., the sarcoplasmatic reticulum) (36,37). These precipitates are thought to hamper sarcoplasmatic reticulum function. However, it remains to be established

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**FIG. 1.** Characterization of the effect of palmitic acid on β-cell death by double staining with the TUNEL assay (alkaline phosphatase) and anti-insulin antibody (peroxidase) (A and B) and by double fluorescent staining with Annexin-V-FLUOS (green) (C and D) and propidium iodide (red) (E and F). Islets were plated on extracellular matrix-coated dishes and exposed for 4 days to media containing 11.1 mmol/l glucose alone (A, C, and E) and with 0.5 mmol/l palmitic acid (B, D, and F). The arrows indicate nuclei stained positive for the TUNEL reaction (light and fluorescence microscopy ×400).
whether this process is linked to apoptosis. A second explanation may be related to changes in membrane fluidity. Indeed, enrichment of phospholipids by saturated fatty acids lowers membrane fluidity, which severely hinders membrane function (38,39). Moreover, it has been shown in a neuronal cell line that an increase of saturated fatty acids in the phospholipid pool forms an essential part of the apoptotic process (40). Furthermore, alterations in the composition of membrane phospholipids

FIG. 2. Double immunostaining for the Ki-67 nuclear antigen (peroxidase) (A and B) and insulin (fluorescein) (C and D). Islets plated on extracellular matrix-coated dishes were exposed for 4 days to media containing 11.1 mmol/l glucose alone (A and C) or including 0.5 mmol/l palmitic acid (B and D). The arrows indicate nuclei stained positive for Ki-67 (light and fluorescence microscopy ×400).

FIG. 3. Fatty acids and glucose-induced β-cell DNA fragmentation and proliferative activity. Islets were cultured for 4 days in the absence or presence of different fatty acids (0.5 mmol/l) or a mixture of palmitoleic and palmitic acid (0.25 mmol/l [mixture] or 0.5 mmol/l [mixture 0.5] each) in 5.5, 11.1, and 33.3 mmol/l glucose. Results are means ± SE of the relative number of TUNEL+ (A) and Ki-67+ β-cells (B) per islet, normalized to control incubations at 5.5 mmol/l glucose alone (100%) (in absolute value: 0.82 TUNEL+ cells per islet and 4.06 Ki-67+ cells per islet at 5.5 mmol/l glucose alone). The mean number of islets scored for DNA fragmentation was 40, 68, and 49 and the mean number for proliferative activity by anti–Ki-67 staining was 78, 69, and 63 in media containing 5.5, 11.1, and 33.3 mmol/l glucose, respectively. Islets were isolated from 40 rats. *P < 0.01 between control and palmitic or palmitoleic acid at the same glucose concentration; ‡P < 0.05 between palmitic acid and fatty acid mixture at the same glucose concentration; **P < 0.01 relative to islets at 5.5 mmol/l glucose.
may act as a trigger for apoptosis, as demonstrated in a promyelocytic cell line, by inhibition of the remodeling of long-chain unsaturated fatty acids between the phospholipids of cells (41). Finally, the specific toxic effects of saturated fatty acids may relate to ceramide formation. Recent studies indicate that signal transduction through the ceramide pathway activates apoptosis in various cell types (11), including islets from the Zucker diabetic fatty rats (5). Moreover, an increase in cellular levels of palmitic or stearic acid but not in levels of palmitoleic acid is correlated with de novo synthesis of ceramide (42). In line with these findings, the present study shows that the ceramide synthetase inhibitor fumonisin B1 blocked the deleterious effects of palmitic acid.

Palmitic acid has a powerful acute insulinotropic effect (32). However, exposure to palmitic acid for 4 days did not increase total insulin secretion. Possibly, the acute insulinotropic effect gradually disappears in conjunction with increased β-cell apoptosis, leading to an overall unchanged insulin release.

The enhancement of TUNEL⁺ β-cells by ceramide is modest compared with the effect of palmitic acid. The difference is probably not due to reduced cell permeability of C₂-ceramide, because it has a dramatic effect on β-cell proliferation, stronger than the palmitic acid effect. The time course of the C₂-ceramide effect possibly differs from the palmitic acid effect, with an earlier increase in apoptosis that leads to a faster decrease in proliferation.

Glucose-induced apoptosis was observed in β-cells of ob/ob mice and of Wistar rats maintained in medium containing 10% fetal calf serum (43). In contrast, high glucose concentrations promoted survival of purified β-cells from adult Wistar rats cultured in serum-free conditions (7). Islets from the diabetes-prone P. obesus, which were cultured on extracellular matrix-coated plates in the presence of serum, responded to elevated glucose levels by increasing the rate of β-cell apoptosis, whereas no significant change was observed in similarly treated islets of adult Sprague-Dawley rats (6). In the present study, elevated glucose concentrations had only a marginal effect on β-cells apoptosis, whereas exposure to palmitic acid resulted in a substantial increase in the number of β-cells with TUNEL⁺ nuclei independent of the medium glucose levels.

The disruption of mitochondrial ATP/ADP exchange is among the earliest identified events that may initiate apoptosis (44). ANT catalyses the exchange of ADP and ATP across the inner mitochondrial membrane. Here we demonstrate that palmitic acid decreases the expression of ANT. The loss of ANT activity induces mitochondrial swelling (18), as well as rupture of the outer membrane leading to cytochrome c release and apoptosis (19), as observed in the present study. The mechanism leading to decreased ANT expression by palmitic acid is unknown. Yet, irrespective of the mechanism
involved, our results attest that the mitochondrion is an important target for palmitic acid–induced apoptosis in β-cells from adult rat islets.

Although various factors including glucose, fatty acids, and amino acids have been shown to govern β-cell proliferation (2,6,10,45), to our knowledge, this study is the first to demonstrate the distinct role of saturated and monounsaturated fatty acids in β-cell replication. Whereas palmitoleic acid was shown to stimulate β-cell proliferation at normoglycemic glucose concentrations, palmitic acid exhibited an inhibitory effect independent of a medium glucose level. With the induction of apoptosis, the deleterious effects of palmitic acid could lead to a reduction in β-cell mass, an important determinant of β-cell functional activity.

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FIG. 6. Confocal images showing cytochrome c diffusely localized to the cytoplasm but not significantly to the mitochondria in palmitic acid–treated islets. Islets were plated on extracellular matrix-coated dishes and were exposed for 4 days to media containing 11.1 mmol/l glucose alone (A) and with 0.5 mmol/l palmitic acid (B) and stained with anti-cytochrome c antibody.

FIG. 7. Effect of prolonged exposure of cultured islets to fatty acids on islet insulin content (A), on chronic insulin release into the culture medium (B), and on basal and glucose-stimulated insulin secretion (C). Islets were incubated in 11.1 mmol/l glucose in the absence or presence of 0.5 mmol/l palmitic or palmitoleic acid or a mixture of both (0.5 mmol/l each) for 4 days. Chronic insulin secretion represents the amount secreted into the culture medium during the experiment. Basal and stimulated insulin secretion denotes the amount secreted over 1 h incubation at 3.3 and 16.7 mmol/l glucose, respectively. Each bar represents the mean of 4 separate experiments ± SE. In each experiment, the data were collected from 4 plates per treatment. *P < 0.05 relative to solvent-treated controls; §P < 0.05 for the difference between palmitic acid and palmitoleic acid or fatty acid mixture.
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