# Prolonged Elevation of Plasma Free Fatty Acids Desensitizes the Insulin Secretory Response to Glucose In Vivo in Rats

**Timothy M. Mason, Tracy Goh, Vaja Tchipashvili, Harmanjit Sandhu, Neehar Gupta, Gary F. Lewis, and Adria Giacca**

**Prolonged exposure of pancreatic islets to free fatty acids (FFAs) inhibits glucose-stimulated insulin secretion (GSIS) in vitro. However, FFA inhibition of GSIS has not been clearly demonstrated in vivo. We examined the in vivo effect of prolonged elevation of plasma FFAs on GSIS using a two-step hyperglycemic clamp in rats treated with a 48-h intravenous infusion of either 20% Intralipid plus heparin (INT) (5 µl/min plus heparin, 0.1 U/min; *n* = 8), oleate (OLE) (1.3 µEq/min; *n* = 6), saline (SAL) (*n* = 6), or bovine serum albumin (BSA) (vehicle for OLE; *n* = 5). Because there was no difference in any of the parameters between BSA and SAL rats, these groups were combined as control rats (CONT) (*n* = 11). At the end of the 48-h OLE/INT/CONT infusions, after an overnight fast, plasma glucose was clamped for 2 h at 13 mmol/l and for another 2 h at 22 mmol/l. Preclamp plasma FFAs were elevated twofold (*P* < 0.01) versus CONT with both INT and OLE (NS, INT vs. OLE). Preclamp glucose, insulin, and C-peptide levels were higher in INT than in CONT rats (*P* < 0.05), suggesting insulin resistance, but they were not different in OLE and CONT rats. The insulin and C-peptide responses to the rise in plasma glucose from basal to 13 mmol/l were lower in OLE (336 ± 72 pmol/l and 1.2 ± 0.1 nmol/l, *P* < 0.01 and *P* < 0.05, respectively) than in CONT (552 ± 54 and 1.9 ± 0.1) rats, but they were not different between CONT and INT rats (648 ± 150 and 2.0 ± 0.4). The insulin and C-peptide responses to the rise in plasma glucose from 13 to 22 mmol/l were lower in both INT (1,188 ± 204 pmol/l and 3.0 ± 0.3 nmol/l, *P* < 0.01 and *P* < 0.001) and OLE (432 ± 60 and 1.7 ± 0.2, *P* < 0.001 vs. CONT or INT) rats than in CONT rats (1,662 ± 174 and 5.0 ± 0.6). In summary, *1*) both INT and OLE decreased GSIS in vivo in rats, and *2*) the impairing effect of INT on GSIS was less than that of OLE, which might be due to the different type of fatty acid (mostly polyunsaturated in INT versus monounsaturated as OLE) and/or to differential effects of INT and OLE on insulin sensitivity. In conclusion, prolonged elevation of plasma FFAs can desensitize the insulin secretory response to glucose in vivo, thus inducing a β-cell defect that is similar to that found in type 2 diabetes.** ***Diabetes*** **48:524–530, 1999**

Type 2 diabetes is characterized by insulin resistance and by a defect in insulin secretion, the latter being characterized by a selective impairment of the insulin secretory response to glucose. More than 80% of type 2 diabetic individuals are obese. It is well-established that free fatty acids (FFAs), which are often elevated in obesity, can induce peripheral and hepatic insulin resistance (1). Recently, the effects of FFAs on insulin secretion have become the focus of extensive investigation (2–6). Acutely, FFAs stimulate glucose-induced insulin secretion both in vitro (7,8) and in vivo (9–11). However, in vitro and in situ studies in isolated rodent (12–14) and human islets (15) and in the perfused rat pancreas (16) have shown that FFAs chronically desensitize the β-cell secretory response to glucose. Therefore, elevated levels of FFAs could potentially induce defects in both insulin secretion and action as observed in type 2 diabetes.

The molecular mechanism of the impairment of glucose-stimulated insulin secretion (GSIS) induced by prolonged elevation of FFAs has only recently been addressed. It is known that fatty acids regulate β-cell enzymes, ion channels, and genes (4), and there is initial evidence that FFAs may chronically impair both $K_{ATP}$-dependent (17–19) and $K_{ATP}$-independent pathways of GSIS (19,20).

However, despite the fact that impairment in GSIS by prolonged elevation of FFAs has been well documented in in vitro and in situ studies, it is unclear whether a physiological elevation of FFAs desensitizes the insulin response to glucose in vivo. Prolonged elevation of plasma FFAs with Intralipid plus heparin (INT) either increased (21) or decreased (22) GSIS in healthy human subjects. These discordant results may be attributed to different experimental conditions, including a different intensity of the glucose stimulus.

To determine whether FFAs can inhibit GSIS in vivo at physiological and supraphysiological glucose concentrations and whether the postulated effect of FFAs depends on the type of fatty acid, we evaluated the insulin secretory response to glucose by means of a two-step hyperglycemic clamp after prolonged (48-h) intravenous infusion of a synthetic triglyceride emulsion (Intralipid) together with heparin (which stimulates its breakdown to fatty acids by the enzyme lipoprotein lipase) or oleate in normal Wistar rats. The rat

From the Departments of Physiology (T.M.M., T.G., V.T., H.S., N.G., A.G.) and Medicine (G.F.L., A.G.), University of Toronto, Toronto, Canada.

Address correspondence and reprint requests to Adria Giacca, MD, Department of Physiology, University of Toronto, Medical Sciences Building, Room 3363, Toronto, Ontario M5S 1A8, Canada. E-mail: adria.giacca@utoronto.ca.

Received for publication 17 July 1998 and accepted in revised form 9 November 1998.

T.M.M. and T.G. contributed equally to this work.

ANOVA, analysis of variance; BSA, bovine serum albumin; CONT, control; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; INT, Intralipid plus heparin; OLE, oleate; RIA, radioimmunoassay; SAL, saline.

model was chosen to provide a baseline for future tissue studies aimed at identifying the molecular mechanism of the FFA-induced β-cell desensitization to glucose.

## RESEARCH DESIGN AND METHODS

**Animal models.** Female Wistar rats (Charles River, Quebec, Canada), weighing 250–300 g, were used for experiments. The female Wistar strain was chosen to allow for future comparison of the effect of FFAs on β-cell glucose competence with that of the Zucker diabetic fatty female rat, a prediabetic rat model that develops diabetes when fed a high-fat diet. The rats were housed in the University of Toronto's Department of Comparative Medicine. They were exposed to a 12-h light/dark cycle and were fed rat chow (Purina 5001, 4.5% fat; Ralston Purina, St. Louis, MO) and water ad libitum. All procedures were approved by the animal care committee of the University of Toronto.

**Surgical procedures.** After 3–5 days of adaptation to the facility, rats were anesthetized with ketamine:xylazine:acepromazine (100:0.1:0.5 mg/ml, 1 µl/g body wt), and indwelling catheters were inserted into the right internal jugular vein and the left carotid artery. Polyethylene catheters (PE-50; Cay Adams, Boston, MA), each extended with a segment of silastic tubing (length of 3 cm, internal diameter of 0.02 inches; Dow Corning, Midland, MI), were used for vascular catheterization. The venous catheter was extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch. Both catheters were tunneled subcutaneously, fed through a subcutaneous interscapular implant, and exteriorized. Catheters were filled with a mixture of 60% polyvinylpyrrolidone and heparin (1,000 U/ml) to maintain patency and were closed at the end with a metal pin. Catheters were flushed with saline (SAL) every 2–3 days.

**Preclamp (48-h infusion) period.** Rats were randomized to one of the following protocols: Intralipid plus heparin (INT) ($n$ = 8), oleate (OLE) ($n$ = 6), saline ($n$ = 6), or bovine serum albumin (BSA) (vehicle for oleate, $n$ = 5) infusion. At least 2–3 days after surgery, PE-50 infusion tubing was connected to each of the catheters. The infusion lines ran inside a tether that was fitted to the subcutaneous implant. Each rat was placed in a circular cage, and the infusion lines were run through a swivel, which was suspended on top of the cage. This procedure protected the infusion tubing and allowed the rat complete freedom of movement. Rats were allowed a 30-min adaptation period during which slow saline infusions (5 µl/min) kept the lines patent. Thereafter, a basal nonfasting (random) sample was taken for FFAs, glucose, and insulin. A slow infusion of heparinized saline (4 U/ml) was started at 5 µl/min to maintain the arterial line patent between samples in all rats. The Intralipid/oleate/saline/BSA infusions were given through the jugular catheter; 20% Intralipid was infused at 5 µl/min. Heparin had been added to the Intralipid to reach a final concentration of 20 U heparin/ml of infusate. Intralipid consists of a fat emulsion made from soybean oil and an emulsifier from egg phospholipids. According to the manufacturer, it typically contains 13% palmitic acid, 4.5% stearic acid, 22% oleic acid, 52% linoleic acid, 8% linolenic acid, and 1% other fatty acids. The composition may vary somewhat with different batches. Oleate was prepared in fatty acid–free BSA (Sigma, St. Louis, MO) according to the Bezman-Tarcher method (23) as modified by Miles et al. (24), diluted in saline to match the volume infused with Intralipid (the final BSA concentration was 2%), and given at a rate of 1.3 µEq/min. Oleate is, with palmitate, the most prevalent circulating fatty acid. Unfortunately, palmitate has a low solubility for intravenous infusion. Oleate, saline, or BSA were infused at a rate that matched the volume infused in Intralipid. Throughout the infusion period, rats had free access to water and to their standard pelleted food. Food intake did not significantly differ between the groups (INT = 11.7 ± 0.9, OLE = 13.3 ± 0.7, BSA = 13.3 ± 1.4, SAL = 13.0 ± 0.4 g/day), despite the fact that at least 1/3 of the daily caloric content was provided by the intravenous fat in the fat-infused groups. Because food intake was determined on day 1 of the fat infusion (on day 2, the rats started the overnight fast), this finding presumably indicates some delay between increased nutrient supply and adjustment of food intake.

Samples for FFAs, glucose, and insulin were taken at 18, 24, and 46 h after the onset of the Intralipid/oleate/saline/BSA infusion, i.e., at –30, –24, and –2 h before the onset of the hyperglycemic clamp (time = 0). Food was removed at 1900 the day before the two-step hyperglycemic clamp.

**Two-step hyperglycemic clamp.** Glucose-induced insulin secretion was determined by measuring the insulin and C-peptide responses to a two-step (~13 and ~22 mmol/l) hyperglycemic clamp. The two-step hyperglycemic clamp was performed in conscious rats fasted overnight. At –20 min, the continuous arterial infusion of heparinized saline was stopped in all rats, since the same total amount of heparinized saline was used to dilute the erythrocytes that were reinfused into the rats after plasma separation from blood samples. The venous infusion of oleate/saline/BSA was continued throughout the experiment. Intralipid was given at the same rate, however, without added heparin, since the same total amount of heparin (0.1 U/min) that was infused during the preclamp period was used to flush the arterial catheter when sampling during the experiment. Two basal samples were taken at –20 and 0 min, after which an infusion of 37.5% glucose was started (time = 0 min) to approximately double the plasma glucose levels (first step of the hyperglycemic clamp). The glucose infusion was giventhrough the jugular catheter. Both the glucose and the Intralipid/oleate/saline/BSA infusion lines were connected to the jugular line through a Y-shaped connector. We did not use a glucose prime to rapidly elevate plasma glucose levels because we wished to avoid FFA-induced heart arrhythmias caused by a bolus of oleate from the dead space of the venous line. The target plasma glucose level of 13 mmol/l was achieved and maintained by adjusting the rate of the glucose infusion according to frequent (every 5–10 min) plasma glucose determinations. At 120 min, the glucose infusion was again raised to achieve and maintain plasma glucose levels of ~22 mmol/l (second step of the hyperglycemic clamp) until the end of the experiment (time = 240 min). Samples for insulin, C-peptide, and FFAs were taken at regular intervals. The sample volume was minimized to avoid anemia. A total of 2.5 ml of blood was withdrawn from the rats. After removal of plasma from centrifuged whole blood samples, erythrocytes were suspended in heparinized saline (4 U/ml) and reinfused into the rats. Mean hematocrit at 0, 120, and 240 min was 44.7 ± 0.7, 41.9 ± 0.7, and 37.4 ± 0.8%, respectively.

**Laboratory methods.** Plasma glucose concentrations were measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Fullerton, CA). Insulin and C-peptide levels in plasma were determined by radioimmunoassays (RIAs) using kits specific for rat insulin and C-peptide from Linco Research (St. Louis, MO). The coefficients of variation were less than 9 and 10.5% for insulin and C-peptide, respectively. Plasma FFA levels were measured using a colorimetric kit (Wako Industrials, Osaka, Japan). Plasma triglyceride levels were also measured using a colorimetric kit (Boehringer Mannheim, Mannheim, Germany). Plasma corticosterone levels were measured by RIA using a commercial kit (Diagnostic Products, Los Angeles, CA).

**Statistical analysis.** All data are presented as means ± SE. One-way analysis of variance (ANOVA) followed by Tukey's $t$ test for multiple comparisons was used to compare differences between experimental groups. The statistical calculations were performed using SAS software (SAS Institute, Cary, NC).

## RESULTS

Tables 1 and 2 show that there was no difference in any of the parameters under consideration in saline- or BSA-treated rats, during either the preclamp period or the two-step hyperglycemic clamp. Therefore, for clarity of presentation, the two groups were combined into a single control group (CONT) ($n$ = 11).

**Preclamp (48-h infusion) period.** The values reported in Fig. 1 refer to –48 (basal preinfusion levels), –30, –24, and –2 h (fasting sample) before the onset of the clamp.

There were no significant differences in the fed plasma FFA, glucose, and insulin levels at baseline before starting the infusions (Fig. 1). Intralipid and oleate infusions rapidly elevated plasma FFAs (Fig. 1*A*) to levels that were approximately twofold higher than those seen in control rats (INT vs. CONT, $P < 0.001$; OLE vs. CONT, $P < 0.01$) throughout the infusion period. There was no difference in FFA levels between Intralipid- and oleate-treated rats.

Plasma glucose levels (Fig. 1*B*) were slightly greater during the preclamp infusion period in Intralipid-treated than in

TABLE 1
FFA, glucose, and insulin levels during the preclamp (48-h infusion) period

| | SAL group | BSA group |
|---|---|---|
| FFA (µEq/l) | 467 ± 47 | 501 ± 44 |
| Glucose (mmol/l ) | 6.4 ± 0.2 | 6.0 ± 0.1 |
| Insulin (pmol/l ) | 204 ± 52 | 233 ± 79 |

Data are means ± SE. SAL, saline infusion ($n$ = 6). BSA, 2% BSA (vehicle for oleate) infusion ($n$ = 5). A two-step hyperglycemic clamp was initiated after 48 h of SAL/BSA infusion.

TABLE 2
Glucose, FFA, insulin, and C-peptide levels during the two-step hyperglycemic clamp

| | SAL group | BSA group |
|---|---|---|
| Glucose (mmol/l ) | | |
| Basal | 6.0 ± 0.3 | 5.9 ± 0.2 |
| First step | 13.3 ± 0.2 | 13.4 ± 0.2 |
| Second step | 22.8 ± 0.4 | 23.0 ± 0.9 |
| FFA (μEq/l ) | | |
| Basal | 536 ± 49 | 656 ± 85 |
| First step | 247 ± 47 | 245 ± 21 |
| Second step | 198 ± 37 | 159 ± 25 |
| Insulin (pmol/l ) | | |
| Basal | 93 ± 33 | 61 ± 19 |
| First step | 619 ± 83 | 631 ± 92 |
| Second step | 2,211 ± 243 | 2,382 ± 380 |
| C-peptide (nmol/l ) | | |
| Basal | 0.3 ± 0.07 | 0.2 ± 0.05 |
| First step | 2.2 ± 0.15 | 1.9 ± 0.19 |
| Second step | 6.8 ± 0.66 | 7.6 ± 1.13 |

Data are means ± SE. SAL, saline infusion ($n$ = 6). BSA, 2% BSA (vehicle for oleate) infusion ($n$ = 5). A two-step hyperglycemic clamp was initiated after 48 h of SAL/BSA infusion.

control rats (average values during the infusions: 6.9 ± 0.4 vs. 6.3 ± 0.1 mmol/l, $P < 0.05$). There was no difference in plasma glucose levels between oleate-treated (6.3 ± 0.4 mmol/l) and control rats. Preclamp plasma glucose levels tended to be higher in Intralipid- than oleate-treated rats; however, the difference failed to reach statistical significance ($P = 0.11$), presumably because the levels were superimposable after 18 h of fat infusion (–30 h point in Fig. 1*B*).

Plasma insulin levels (Fig. 1*C*) were greater throughout the preclamp period in Intralipid-infused rats than in control or oleate-treated rats ($P < 0.05$). There was no difference in plasma insulin levels between oleate-treated and control rats.

At the end of the infusion period (–2 h before the clamp), plasma triglyceride levels were higher ($P < 0.001$) in Intralipid-treated rats (17.9 ± 2.7 mmol/l) than in control rats (4.8 ± 0.5) or oleate-treated rats (4.7 ± 0.7). Plasma corticosterone levels were measured as a marker of stress and also because FFAs have been reported to stimulate the hypothalamus-pituitary-adrenal axis (4). There was a tendency for plasma corticosterone levels to be higher in both Intralipid- (646 ± 161 nmol/l) and oleate-treated rats (730 ± 389) than in control rats (331 ± 64), although the difference failed to reach statistical significance.

**Two-step hyperglycemic clamp.** Fasting plasma glucose levels in the basal period (–20 min to 0 min) were higher ($P < 0.001$) in Intralipid-infused rats (7.1 ± 0.3 mmol/l) than in control rats (6.0 ± 0.2) (Fig. 2*A*). Plasma glucose levels in oleate-treated rats (6.6 ± 0.4) were not significantly different from those observed in control or Intralipid-treated rats. During the first step of the hyperglycemic clamp, plasma glucose levels gradually rose to the target value of 13 mmol/l, which was maintained until 120 min. During the second step of the hyperglycemic clamp, plasma glucose levels gradually rose to the target level of 22 mmol/l, which was maintained until 240 min. The reason for not using glucose boluses to achieve a more rapid increase in glucose levels is described in METHODS. During both steps of the hyperglycemic clamp, the glucose levels were superimposable in all groups.

Basal FFAs (Fig. 2*B*) were higher in Intralipid-treated ($P < 0.01$) and oleate-treated rats ($P < 0.05$) than in control rats. There was no significant difference in basal FFA levels between Intralipid- and oleate-treated rats. FFA levels declined with hyperglycemia and hyperinsulinemia in all groups. However, FFAs were still higher in the Intralipid- or oleate-infused rats than in control rats during the hyperglycemic clamp (during the first step of the clamp: OLE vs. CONT, $P < 0.05$; INT vs. CONT, $P = 0.051$, NS; during the second step of the clamp: OLE vs. CONT, $P = 0.08$, NS; INT vs. CONT, $P < 0.05$). There was no difference in the clamp FFA levels between Intralipid- and oleate-treated rats.

Basal fasting plasma insulin levels (Fig. 3*A*) were higher in Intralipid-infused rats than in oleate-treated or control rats (both $P < 0.05$) and nonsignificantly different between oleate-treated rats and control rats. During the first step of the hyperglycemic clamp, plasma insulin levels were lower in the oleate-treated rats ($P < 0.05$ vs. CONT) but higher in the Intralipid-treated rats ($P < 0.05$ vs. CONT) than in control rats. During the second step of the hyperglycemic clamp, insulin levels were lower in oleate-treated rats ($P < 0.001$) than in the Intralipid-treated group or in control rats. There was a trend for the insulin levels to be lower in the Intralipid-

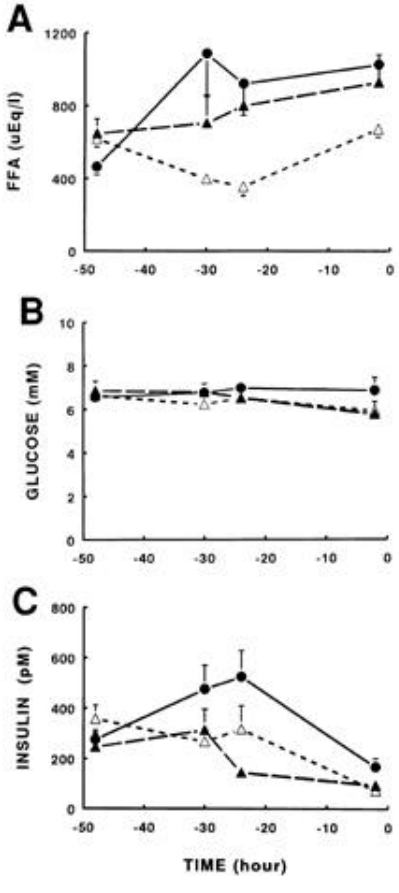


**FIG. 1. Plasma FFA (*A*), glucose (*B*), and insulin (*C*) levels during the 48-h infusion period before the two-step hyperglycemic clamp in control rats ( $n$ = 11) (△) and in rats infused with either Intralipid (5 μl/min plus heparin, 0.1 U/min, $n$ = 8) (●) or oleate (1.3 μEq/min, $n$ = 6) (▲). Data are means ± SE. Significances are described in RESULTS.**

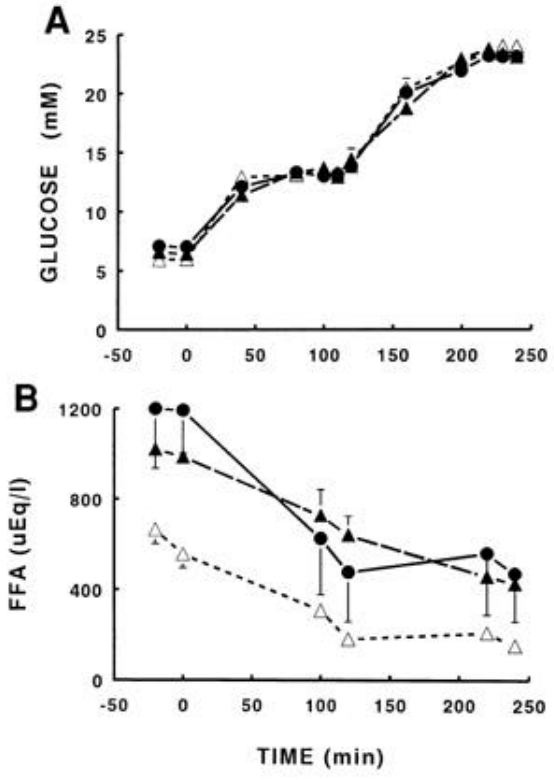


**FIG. 2. Plasma glucose (*A*) and FFA (*B*) levels during the two-step hyperglycemic clamp in control rats ($n$ = 11) (△) and in rats infused with either Intralipid (5 µl/min plus heparin, 0.1 U/min, $n$ = 8) (●) or oleate (1.3 µEq/min, $n$ = 6) (▲). Data are means ± SE. Significances are described in RESULTS.**

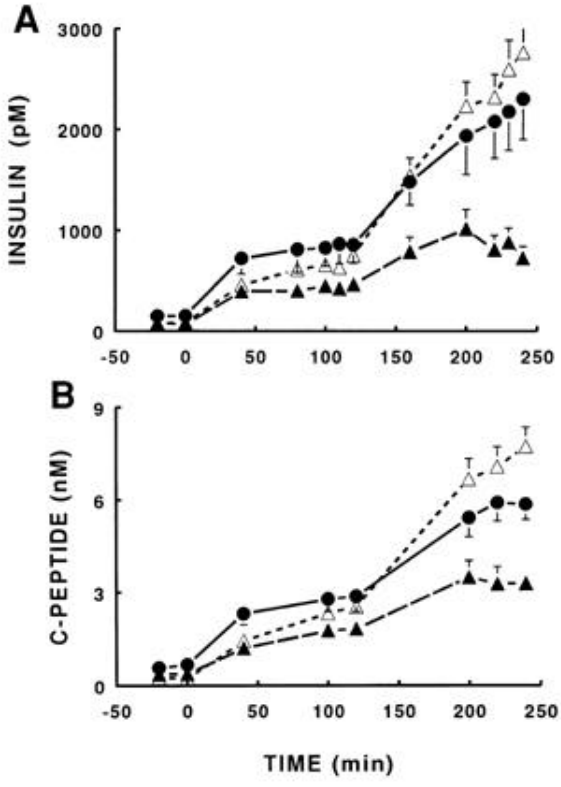


**FIG. 3. Plasma insulin (*A*) and C-peptide (*B*) levels during the two-step hyperglycemic clamp in control rats ($n$ = 11) (△) and in rats infused with either Intralipid (5 µl/min plus heparin, 0.1 U/min, $n$ = 8) (●) or oleate (1.3 µEq/min, $n$ = 6) (▲). Data are means ± SE. Significances are described in RESULTS.**

treated rats than in control rats, although the difference was not significant.

Similar to the insulin levels, basal fasting C-peptide levels (Fig. 3*B*) were higher in the Intralipid-infused rats than in control rats ($P < 0.001$) or oleate-treated rats ($P < 0.01$) and nonsignificantly different between oleate-treated rats and control rats. During the first step of the hyperglycemic clamp, there was a trend for the C-peptide levels to be lower in oleate-treated rats than in control rats, although the difference failed to reach statistical significance ($P = 0.054$). C-peptide levels were higher in the Intralipid-infused rats ($P < 0.05$) than in control rats. During the second step of the hyperglycemic clamp, C-peptide levels in the oleate-treated rats were lower than those observed in the Intralipid-treated rats or in control rats ($P < 0.001$). Unlike the insulin levels, C-peptide levels were lower in Intralipid-treated than in control rats ($P < 0.01$) during the second step of the hyperglycemic clamp.

Figure 4*A* shows that the rise in insulin (*left*) and C-peptide (*right*) levels from basal to the levels observed at 13 mmol/l glucose (first step of the hyperglycemic clamp) was not significantly different between Intralipid (649 ± 155 pmol/l and 2.0 ± 0.4 nmol/l) and control (562 ± 56 pmol/l and 1.9 ± 0.1 nmol/l) rats. The rise in both insulin and C-peptide levels was significantly lower in oleate-treated rats (343 ± 68 pmol/l and 1.2 ± 0.1 nmol/l).

Figure 4*B* shows that the rise in both insulin (*left*) and C-peptide levels (*right*) from the values observed at 13 mmol/l glucose (first step of the hyperglycemic clamp) to those found at 22 mmol/l glucose (second step of the hyperglycemic clamp) was significantly lower in both Intralipid-treated rats (1,192 ± 203 pmol/l and 3.0 ± 0.3 nmol/l) and oleate-treated rats (427 ± 68 pmol/l and 1.7 ± 0.2 nmol/l) than in control rats (1,665 ± 173 pmol/l and 5.0 ± 0.6 nmol/l). The rise in both insulin and C-peptide levels was significantly lower in the oleate-treated than in the Intralipid-treated rats.

The glucose infusion rates required to maintain the hyperglycemic clamp were lower ($P < 0.001$) in oleate-infused rats than in Intralipid-infused rats or in control rats during the first (126 ± 14, 179 ± 10, and 198 ± 11 µmol · kg$^{-1}$ · min$^{-1}$, respectively) as well as the second step of the clamp (274 ± 28, 359 ± 17, and 386 ± 17 µmol · kg$^{-1}$ · min$^{-1}$). There was a trend for the glucose infusion rates to be lower in Intralipid-treated rats than in control rats throughout the hyperglycemic clamp; however, the difference failed to reach statistical significance ($P = 0.08$ and $P = 0.053$ during the first and second steps of the clamp, respectively).

## DISCUSSION

In the present study, C-peptide responses to 22 mmol/l glucose were diminished after 48-h Intralipid or oleate infusion. This implies that prolonged fat infusion can desensitize the insulin secretory response to glucose in vivo in rats, as previously found in vitro. Because the inhibitory effect of oleate infusion was greater than that of Intralipid at 22 mmol/l glucose and could also be detected at 13 mmol/l, the present study further suggests that *1*) the type of fatty acid may be an important determinant of the decrease in GSIS induced by fat, and/or

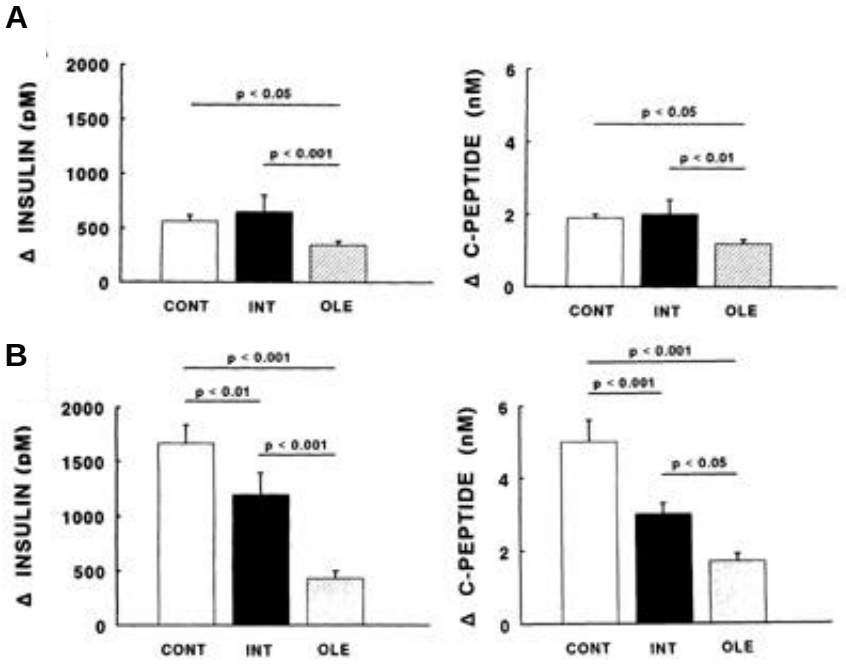


**FIG. 4.** ***A*****: Plasma insulin (*left*) and C-peptide (*right*) responses to the first step of the hyperglycemic clamp (rise in plasma glucose from basal to 13 mmol/l). Δ insulin and C-peptide levels correspond to the differences between the insulin and C-peptide levels observed at 13 mmol/l plasma glucose and those observed at baseline in control rats ($n$ = 11) (□) and in rats infused with either Intralipid (5 µl/min plus heparin, 0.1 U/min, $n$ = 8) (■) or oleate (1.3 µEq/min, $n$ = 6) (▨). Data are means ± SE of average individual change. The statistical analysis was performed using one-way ANOVA for repeated measures.** ***B*****: Plasma insulin (*left*) and C-peptide (*right*) responses to the second step of the hyperglycemic clamp (rise in plasma glucose from 13 to 22 mmol/l). Δ insulin and C-peptide levels correspond to the differences between the insulin and C-peptide levels observed at 22 mmol/l plasma glucose and those observed at 13 mmol/l plasma glucose in control rats ($n$ = 11) (□) and in rats infused with either Intralipid (5 µl/min plus heparin, 0.1 U/min, $n$ = 8) (■) or oleate (1.3 µEq/min, $n$ = 6) (▨). Data are means ± SE of average individual change. The statistical analysis was performed using one-way ANOVA for repeated measures.**

*2*) other factors that regulate GSIS in vivo, independent of FFAs, may be differentially affected by Intralipid or oleate.

The insulin responses appeared to be less affected by Intralipid than did the C-peptide responses. Because insulin levels are determined not only by insulin secretion but also by insulin clearance, this finding suggests that Intralipid might impair insulin clearance in accordance with our previous studies (25).

A comparable elevation of plasma FFAs was obtained with Intralipid or oleate. Interestingly, however, only the Intralipid-treated group showed greater C-peptide, insulin, and glucose levels after 48 h, which indicates insulin resistance. The impairment of both insulin sensitivity and insulin clearance by Intralipid suggests a link between the two effects (25). The apparent induction of insulin resistance with Intralipid but not oleate may indicate that hypertriglyceridemia is a major determinant of Intralipid-induced insulin resistance. The effect of hypertriglyceridemia may still be due to FFAs, since some of the FFAs released from triglycerides in peripheral tissues may be utilized locally, without first appearing in the circulating FFA pool (26,27). During the hyperglycemic clamp, glucose infusion rates were lower in oleate-treated rats than in control rats, in accordance with the markedly lower insulin response, but they were not significantly different in the Intralipid-treated and control groups. This is not surprising, since hyperglycemic clamps do not provide measures of insulin resistance as sensitive as those obtained by hyperinsulinemic-euglycemic clamps.

The results obtained with both Intralipid and oleate at 22 mmol/l plasma glucose are in accordance with previous in vitro studies that showed that prolonged islet exposure to fatty acids inhibited GSIS at glucose concentrations >16.7 mmol/l (12,13). At 11 mmol/l, no reduction or enhancement of insulin secretion was seen in vitro (12), whereas the range of 11–16.7 mmol/l has not been explored. In this range (13 mmol/l), we could detect an inhibitory effect of oleate but not of Intralipid. At glucose levels of ~3 mmol/l, studies in vitro show an enhancing effect of prolonged exposure of FFAs on GSIS in rat islets (12) and no effect in human (15) or mouse islets (14). At 5.5 mmol/l, which approximates our basal glucose levels of 6.5 mmol/l, the INS-1 rat cell line shows enhanced insulin secretion after prolonged exposure to FFAs (19). Our studies in vivo show no difference in insulin secretion under basal fasting conditions in the oleate-treated rats. In the Intralipid-treated rats, insulin secretion was stimulated at baseline, which may be due in part to the greater basal levels of plasma glucose.

The decrease in GSIS observed with oleate occurred in the absence of changes in glycemia or insulin levels throughout the preclamp period, which indicates that the FFA effect on GSIS is independent of hyperglycemia and may be, at least partially, independent of β-cell overstimulation (28,29). Notably, oleate increases GSIS acutely both in vitro and in vivo (10,30,31). The greater effect of oleate compared with Intralipid might depend on the molecular structure of the fatty acid, since the most prevalent fatty acids in the Intralipid emulsion are polyunsaturated, whereas oleate is monounsaturated. In vivo and in situ studies have shown that the acute enhancing effect of FFAs on GSIS is proportional to the length and degree of saturation of the fatty acids (32). Although no marked differences among fatty acids were seen in their chronic inhibitory effect on GSIS, the previous in vitro studies in rat islets (12) and INS-1 cells (19) suggest that oleate might be slightly more effective than palmitate or linoleate. Alternatively, the greater effect of oleate compared with Intralipid might be related to factors that regulate GSIS in vivo, independent of FFAs; for example, one cannot exclude that in the Intralipid group, insulin resistance might have stimulated insulin secretion independent of changes in plasma glucose and FFAs, and that this stimulatory effect might have counteracted in part the direct inhibitory effect of FFAs on GSIS. To differentiate between these two possibili-

ties, we are currently directly measuring insulin sensitivity and GSIS in isolated islets of Intralipid- or oleate-infused rats.

The similar elevation in plasma corticosterone with Intralipid or oleate suggests that the different insulin secretory response in the two fat-infused groups was not related to a different degree of stress. The trend toward an elevation in corticosterone levels in both groups is in accordance with previous studies showing stimulation of the hypothalamus-pituitary-adrenal axis by FFAs (33,34). It is unlikely that such a nonsignificant elevation in corticosterone would have affected GSIS. However, we have shown that 48-h dexamethasone treatment inducing insulin resistance increased insulin secretion during hyperglycemic clamps in healthy humans (35).

Only a few studies have evaluated the effect of prolonged infusion of Intralipid on GSIS in vivo, and none have evaluated the effect of prolonged infusion of oleate. In preliminary studies in rats, Magnan et al. (36) found that 48-h Intralipid infusion increased GSIS during an intragastric glucose tolerance test. The enhancing effect of FFAs was attributed to an increased β-cell mass, as well as increased parasympathetic versus sympathetic activity in Intralipid-infused rats. These factors have been previously shown to counteract the inhibition of GSIS induced by prolonged hyperglycemia (37). The study by Magnan et al. is not necessarily in contrast with our data, because the intragastric glucose loading dose used should have resulted in plasma glucose levels lower than those obtained during the first step of our hyperglycemic clamp (13 mmol/l). The same can be said with regard to the difference in our results compared with those of Boden et al. (21), who found that a 48-h Intralipid infusion led to persistent enhancement in GSIS during a 48-h hyperglycemic clamp at 8.8 mmol/l in humans. In contrast, 24 h of Intralipid infusion, resulting in a threefold elevation of FFA levels, reduced the plasma insulin response in humans when insulin secretion was stimulated with an intravenous glucose tolerance test by Paolisso et al. (22) (the glucose levels were not reported; however, the glucose-loading dose used should have resulted in peak glucose levels >16.7 mmol/l).

It might be argued that if the desensitization of the insulin secretory response to glucose occurs only at supraphysiological glucose levels in vivo, it might not be a relevant factor in the pathogenesis of type 2 diabetes. Our results suggest that with oleate, which is a more prevalent circulating fatty acid than is linoleate (the main fatty acid released from Intralipid), the fat-induced desensitization occurs at physiological glucose levels in rats. In addition, the effects of chronic elevation of FFAs, as observed in obesity and in insulin-resistant states, are presumably greater than those of a 48-h fat infusion. Furthermore, the prediabetic and diabetic β-cell might be genetically more susceptible to the FFA-induced inhibition of GSIS, as suggested by studies in isolated islets of homozygotes and heterozygotes in prediabetic Zucker diabetic fatty rats (38).

Finally, with the present study, we have established an in vivo model of FFA-induced inhibition of GSIS. Further tissue studies in this rat model are needed to investigate the molecular mechanism of the FFA-induced impairment in β-cell function. Previous studies in isolated islets and in β-cell lines have shown that prolonged exposure to FFAs *1*) reduces glucose oxidation through upregulation of the FFA-glucose cycle (17–19), which may be due in part to increased gene expression of enzymes involved in FFA oxidation (18,19); *2*) decreases the activity and gene expression of enzymes involved in β-cell glucose utilization and oxidation (17) and in the formation of malonyl-CoA from glucose (39); *3*) opens the $K^+$-ATP channels (which inhibits insulin secretion) in proportion to intracellular long-chain fatty acyl-CoA accumulation (40); *4*) might activate uncoupling protein 2, which would result in reduction of glucose-derived ATP (2); and *5*) decreases preproinsulin mRNA translation (30) and content (39,41). Protein kinase C downregulation, and other mechanisms that have been implicated in the β-cell impairment induced by hyperglycemia (42,43), might also contribute to the FFA-induced inhibition of GSIS. In addition, it is likely that the FFA-induced inhibition of GSIS is only one reversible aspect of the FFA-induced impairment in β-cell function. Greater intracellular triglyceride might have a negative effect on the β-cell life span, ultimately decreasing β-cell mass (3,44–46).

In conclusion, the present study suggests that a prolonged elevation of plasma FFAs can desensitize the insulin secretory response to glucose in vivo, independent of hyperglycemia and of β-cell overstimulation. Future studies will determine whether the β-cells of prediabetic and diabetic animals are genetically more susceptible to the FFA-induced impairment in glucose recognition in vivo.

**ACKNOWLEDGMENTS**

This study was funded by the Eli Lilly/Banting and Best Diabetes Program at the University of Toronto.

We are grateful to L. Lam and M.A. Honig for excellent technical assistance and to Dr. M. Prentki for helpful criticism of the manuscript.

Parts of this study were presented at the 58th Annual Meeting and Scientific Sessions of the American Diabetes Association, Chicago, Illinois, 13–16 June 1998.

**REFERENCES**

1. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997
2. Bjorklund A, Yaney G, McGarry JD, Weir G: Fatty acids and β-cell function. *Diabetologia* 40 (Suppl. 3):B21–B26, 1997
3. Unger R: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. *Diabetes* 44:863–870, 1995
4. Prentki M, Corkey BE: Are the β-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 45:273–283, 1996
5. Milburn JL, Hirose H, Lee YH, Nagasawa Y, Ogawa A, Ohneda M, Beltrandel-Rio H, Newgard CB, Johnson JH, Unger RH: Pancreatic β-cells in obesity. *J Biol Chem* 270:1295–1299, 1994
6. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH: 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 U S A* 91:10878–10882, 1994
7. Warnotte C, Gilon P, Nenquin M, Henquin JC: Mechanisms of the stimulation of insulin release by saturated fatty acids. *Diabetes* 43:703–711, 1994
8. Chen S, Ogawa A, Ohneda M, Unger RH, Foster DW, McGarry JD: More direct evidence for a malonyl-CoA-carnitine palmitoyltransferase I interaction as a key event in pancreatic β-cell signaling. *Diabetes* 43:878–883, 1994
9. Crespin SR, Greenough WB, Steinberg D: Stimulation of insulin secretion by long-chain free fatty acids. *J Clin Invest* 53:1979–1984, 1973
10. Stein DT, Esser V, Stevenson BE, Lane KE, Whiteside JH, Daniels MB, Chen S, McGarry JD: Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *J Clin Invest* 97:2728–2735, 1996
11. Hennes MMI, Dua A, Kissebah AH: Effect of free fatty acids and glucose on splanchnic insulin dynamics. *Diabetes* 46:57–62, 1997
12. Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J Clin Invest* 93:870–876, 1994

13. Elks ML: Chronic perifusion of rat islets with palmitate suppresses glucose-stimulated insulin release. *Endocrinology* 133:208–214, 1993
14. Zhou Y, Berggren P, Grill V: A fatty acid–induced decrease in pyruvate dehydrogenase activity is an important determinant of β-cell dysfunction in the obese diabetic *db/db* mouse. *Diabetes* 45:580–586, 1996
15. Zhou YP, Grill V: Long-term exposure to fatty acids and ketones inhibits β-cell function in human pancreatic islets of Langerhans. *J Clin Endocrinol Metab* 80:1575–1580, 1995
16. Sako Y, Grill V: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and β-cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology* 127:1580–1589, 1990
17. Zhou Y, Grill VE: Palmitate-induced β-cell insensitivity to glucose is coupled to decreased pyruvate dehydrogenase activity and enhanced kinase activity in rat pancreatic islets. *Diabetes* 44:394–399, 1995
18. Assimacopoulos-Jeannet F, Thumelin S, Roche E, Esser V, McGarry JD, Prentki M: Fatty acids rapidly induce the carnitine palmitoyltransferase I gene in the pancreatic β-cell line INS-1. *J Biol Chem* 272:1659–1664, 1997
19. Brun T, Assimacopoulos-Jeannet F, Corkey BE, Prentki M: Long-chain fatty acids inhibit acetyl-CoA carboxylase gene expression in the pancreatic β-cell line INS-1. *Diabetes* 46:393–400, 1997
20. Liang YL, Buettger C, Berner DK, Matchinsky FM: Chronic effect of fatty acids on insulin release is not through the alteration of glucose metabolism in a pancreatic β-cell line (βHC9). *Diabetologia* 40:1018–1027, 1997
21. Boden G, Chen X, Rosner J, Barton M: Effects of 48-h fat infusion on insulin secretion and glucose utilization. *Diabetes* 44:1239–1242, 1995
22. Paolisso G, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varricchio M, D'Onofrio F: Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* 38:1295–1299, 1995
23. Bezman-Tarcher A: Method for continuous intravenous infusion of large amounts of oleic acid into rats. *J Lipid Res* 10:197–206, 1969
24. Miles JM, Ellman MG, McClean KL, Jensen MD: Validation of a new method for determination of free fatty acid turnover. *Am J Physiol* 262:E431–E438, 1987
25. Wiesenthal SR, Sandhu H, McCall RH, Tchipachvili V, Yoshii H, Polonsky K, Shi ZQ, Lewis GF, Mari A, Giacca A: Free fatty acids impair hepatic insulin extraction in vivo. *Diabetes*. In press
26. Piatti PM, Monti LD, Baruffaldi L, Magni F, Paroni R, Fermo I, Costa S, Santambrogio R, Nasser M, March M, Kienle-Galli M, Pontiroli AE, Pozza G: Effects of an acute increase in plasma triglyceride levels on glucose metabolism in man. *Metabolism* 44:883–889, 1995
27. Wolfe RR, Durkot MJ: Role of very-low-density lipoproteins in the energy metabolism of the rat. *J Lipid Res* 26:210–217, 1985
28. Sako Y, Grill VE: Coupling of β-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. *Diabetes* 39:1580–1583, 1990
29. Hosokawa YA, Leahy JL: Parallel reduction of pancreas insulin content and insulin secretion in 48-h tolbutamide-infused normoglycemic rats. *Diabetes* 46:808–813, 1997
30. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ: Chronic exposure to free fatty acid reduces pancreatic β-cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. *J Clin Invest* 101:1094–1101, 1998
31. Crespin SR, Greenough WB, Steinberg D: Stimulation of insulin secretion by infusion of free fatty acids. *J Clin Invest* 48:1934–1943, 1969
32. Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD, McGarry JD: The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. *J Clin Invest* 100:398–403, 1997
33. Widmaier EP, Rosen K, Abbott B: Free fatty acids activate the hypothalamic-pituitary-adrenocortical axis in rats. *Endocrinology* 131:2313–2318, 1992
34. Widmaier EP, Margenthaler J, Sarel I: Regulation of pituitary-adrenocortical activity by free fatty acids in vivo and in vitro. *Prostaglandins Leukot Essent Fatty Acids* 52:179–183, 1995
35. Wajngot A, Giacca A, Grill V, Vranic M, Efendic S: The diabetogenic effects of glucocorticoids are more pronounced in low than in high insulin responders. *Proc Natl Acad Sci U S A* 89:6035–6038, 1992
36. Magnan C, Berthault MF, Laury MC, Leveteau J, Penicaud L, Ktorza A, Assimacopoulos J: Prolonged exposure of pancreatic β-cells to free fatty acids in vivo increases glucose-induced insulin secretion and β-cell mass in rats (Abstract). *Diabetologia* 40 (Suppl. 1):A26, 1997
37. N'Guyen J-M, Magnan C, Laury M-C, Thibault C, Leveteau J, Gilbert M, Penicaud L, Ktorza A: Involvement of the autonomic nervous system in the vivo memory to glucose of pancreatic β-cell in rats. *J Clin Invest* 94:1456–1462, 1994
38. Hirose H, Lee YH, Inman LR, Nagasawa Y, Johnson JH, Unger RH: Defective fatty acid–mediated β-cell compensation in Zucker diabetic fatty rats: pathogenic implications for obesity-dependent diabetes. *J Biol Chem* 271:5633–5637, 1996
39. Gremlich S, Bonny C, Waeber G, Thorens B: Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. *J Biol Chem* 272:30261–30269, 1997
40. Larsson O, Deeney JT, Branstrom R, Berggren P-O, Corkey BE: Activation of the ATP-sensitive $K^+$ channel by long-chain acyl-CoA. *J Biol Chem* 271:10623–10626, 1996
41. Laser B, Meda P, Philippe J: Palmitate inhibits insulin biosynthesis in islet cells (Abstract). *Diabetes* 46 (Suppl. 1):210A, 1997
42. Zawalich WS, Zawalich KC, Shulman GI, Rossetti L: Chronic in vivo hyperglycemia impairs phosphoinositide hydrolysis and insulin release in isolated perifused rat islets. *Endocrinology* 126:253–260, 1990
43. Leahy JL, Bonner-Weir S, Weir GC: β-cell dysfunction induced by chronic hyperglycemia. *Diabetes Care* 15:442–455, 1992
44. Man Z-W, Zhu M, Noma Y, Toide K, Sato T, Asahi Y, Hirashima T, Mori S, Kawano K, Mizuno A, Sano T, Shima K: Impaired β-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. *Diabetes* 46:1718–1724, 1997
45. Shimabukuro M, Zhou Y-T, Levi M, Unger RH: Fatty acid–induced β-cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 95:2498–2502, 1998
46. Shimabukuro M, Ohneda M, Lee Y, Unger RH: Role of nitric oxide in obesity-induced β-cell disease. *J Clin Invest* 100:290–295, 1997