

# Prolonged Elevation of Plasma Free Fatty Acids Impairs Pancreatic $\beta$ -Cell Function in Obese Nondiabetic Humans But Not in Individuals With Type 2 Diabetes

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Our recent *in vivo* observations in healthy nonobese humans have demonstrated that prolonged elevation of plasma free fatty acids (FFAs) results in diminished glucose-stimulated insulin secretion (GSIS) when the FFA-mediated decrease in insulin sensitivity is taken into account. In the present study, we investigated whether obese individuals and patients with type 2 diabetes are more sensitive than healthy control subjects to the inhibitory effect of prolonged elevation of plasma FFAs on GSIS. In seven patients with type 2 diabetes and seven healthy nondiabetic obese individuals, we assessed GSIS with a programmed graded intravenous glucose infusion on two occasions, 6–8 weeks apart, with and without a prior 48-h infusion of heparin and Intralipid, which was designed to raise plasma FFA concentration approximately twofold over basal. The nondiabetic obese subjects had a significant 21% decrease in GSIS ( $P = 0.0008$ ) with the heparin and Intralipid infusion, associated with a decrease in whole body insulin clearance. The impairment in GSIS was evident at low ( $<11$  mmol/l) but not at higher plasma glucose concentrations. In contrast, the patients with type 2 diabetes had a slight increase in GSIS ( $P = 0.027$ ) and no change in insulin clearance, although there was marked interindividual variability in response. Plasma proinsulin concentrations measured in a subset of subjects were not altered in either group by the infusion of heparin and Intralipid. In summary, 1) obese nondiabetic individuals are susceptible to a desensitization of GSIS with heparin and Intralipid infusion, and 2) patients with type 2 diabetes do not demonstrate such susceptibility when FFAs are elevated approximately twofold above basal with heparin and Intralipid. Our results suggest that FFAs could play an important role in the development of  $\beta$ -cell failure in obese individuals who are at risk for developing type 2 diabetes. They do not, however, seem to further deteriorate the  $\beta$ -cell function of patients who already

have established type 2 diabetes and may even result in a slight increase in GSIS in this latter group. *Diabetes* 49:399–408, 2000

It is well accepted that type 2 diabetes is characterized by defects in both insulin action and insulin secretion (1,2), with a specific defect in glucose-stimulated insulin secretion (GSIS) early in the evolution of this disease (3,4). Over the past decade, several investigators have focused their attention on the possible role of free fatty acids (FFAs), which are often elevated in states of insulin resistance, in selectively desensitizing the  $\beta$ -cell to glucose (5–9). The concept of FFA-induced  $\beta$ -cell desensitization has received support from prospective epidemiological studies, which show that elevated plasma FFA levels are a risk marker for the long-term development of glucose intolerance and progression toward type 2 diabetes both in Caucasians (10) and in Pima Indians (11).

The acute stimulating effect of FFAs on GSIS has been well described both *in vitro* (12,13) and *in vivo* (14–16), and it is also now recognized that FFAs play an important role in the maintenance of basal insulin secretion and GSIS in the fasting but not in the fed state (17–20). *In vitro* studies, however, have shown that prolonged ( $>24$  h) exposure of rat (21,22) and human islets (23) to fatty acids decreases GSIS. We have recently shown in a rat model that a prolonged *in vivo* elevation of FFAs, either with an intravenous infusion of heparin and Intralipid or with oleate, decreases insulin secretion in response to a two-step hyperglycemic clamp (24). The term “ $\beta$ -cell lipotoxicity” has been coined by Unger (8) to describe the glucose-induced secretory incompetence induced by FFAs.

Despite the evidence from *in vitro* studies, the effect of prolonged elevation of FFAs on GSIS in humans remains controversial. Studies by other investigators showed that prolonged (24–48 h) FFA elevation either increased (25) or decreased (14) GSIS in nondiabetic normal-weight humans. However, we have recently shown that prolonged (48 h) elevation of plasma FFAs in young healthy nonobese subjects results in a relative decrease in GSIS when the concomitant FFA-mediated decrease in insulin sensitivity is taken into account (16). Another very recent study by Paolisso et al. (26) reported that the acute insulin response was inversely correlated with plasma FFAs in nonobese nondiabetic relatives of patients with type 2 diabetes and that a 1-week treatment

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Received for publication 8 April 1999 and accepted in revised form 3 November 1999.

FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; ISR, insulin secretion rate;  $S_i$ , insulin sensitivity.

TABLE 1  
Characteristics of the subjects

Subject	Age (years)	Sex	BMI (kg/m <sup>2</sup> )	Fasting glucose (mmol/l)	HbA <sub>1c</sub> (%)	2-h Plasma glucose (mmol/l)	Fasting plasma FFA (mmol/l)	Fasting plasma insulin (pmol/l)	Fasting plasma triglycerides (mmo/l)	Duration of diabetes (years)
Diabetic subjects										
1	63	M	31.9	9.2	8.0	—	0.37	81.5	1.36	2
2	49	M	29.1	7.1	6.2	—	0.66	52.4	2.17	0.5
3	45	M	29.5	7.4	6.0	—	0.67	34.7	1.28	5
4	47	M	31.2	7.2	6.7	—	0.16	241.1	1.65	0.5
5	36	F	34.8	13.8	10.0	—	0.64	56.7	1.91	3
6	55	F	39.0	7.2	6.8	—	0.92	129.5	1.55	1
7	57	F	22.0	9.4	8.0	—	0.55	27.5	1.17	3
Mean ± SE	50.2 ± 3.4	—	31.5 ± 2.0	8.7 ± 0.9	7.4 ± 0.5	—	0.57 ± 0.09	89 ± 28	1.57 ± 0.13	2.1 ± 0.6
Obese nondiabetic subjects										
1	41	M	32.0	6.3	—	8.5	0.37	50.4	2.02	—
2	45	M	26.9	4.9	—	9.3	0.48	44.1	0.93	—
3	44	F	31.2	4.9	—	3.6	0.36	38.7	0.75	—
4	63	M	27.2	6.3	—	3.9	0.79	57.5	1.20	—
5	53	F	40.1	6.2	—	5.7	0.76	65.6	0.85	—
6	53	F	30.2	6.3	—	5.3	0.32	77.7	1.46	—
7	57	M	29.4	6.1	—	7.3	0.51	48.8	1.09	—
Mean ± SE	50.8 ± 3.0	—	31.4 ± 1.7	5.8 ± 0.3	—	6.2 ± 0.8	0.51 ± 0.07	55 ± 5	1.17 ± 0.16	—
P	0.91	—	0.97	0.01	—	—	0.65	0.26	0.09	—

For 2-h plasma glucose, measurements were made after an oral glucose tolerance test. P values were determined by unpaired two-tailed t test of patients with type 2 diabetes versus obese nondiabetic subjects.

with acipimox, with a resultant decrease in plasma FFAs, increased the acute insulin response in these individuals. This latter change was impaired when plasma FFAs were prevented from decreasing by the addition of an Intralipid infusion to the acipimox treatment. From these studies, it appears that the effect of FFAs on GSIS may be more evident if there is underlying susceptibility for  $\beta$ -cell failure. Furthermore, elevation of the intracellular triacylglycerol content of  $\beta$ -cells is associated with a decrease in insulin secretion and glucose intolerance in animal models of type 2 diabetes (27).

The objective of the present study, therefore, was to determine the effect of prolonged elevation of plasma FFAs on GSIS in vivo in patients with type 2 diabetes and in healthy obese nondiabetic human subjects of similar age, sex, and weight. Glucose administered intravenously in a graded fashion allowed us to examine a dose-response insulin secretory effect at mild to moderate levels of hyperglycemia.

#### RESEARCH DESIGN AND METHODS

**Subjects.** Seven patients with type 2 diabetes (four men and three women) participated in the study. Table 1 shows their demographic and clinical characteristics. All the subjects were diet-treated, and no subject was treated with either oral hypoglycemic agents or insulin for their diabetes. Seven healthy overweight or obese subjects (Table 1) with similar age, BMI, and sex (four men and three women) also participated in the study. Diabetes was excluded in the obese subjects by assessing fasting plasma glucose levels at every visit and by performing a standard 2-h oral glucose tolerance test on a separate occasion. Five of the patients had impaired fasting glucose according to the American Diabetes Association criteria (28), and two of them had glucose intolerance as assessed by the 2-h oral glucose tolerance test. None of the patients or control subjects had systemic illness other than controlled high blood pressure and mild hyperlipidemia. The dose of antihypertensive medications remained the same throughout the study period, and no patients were taking any lipid-lowering agents for at least 1 month before and during the study. No patients or obese subjects were treated with  $\beta$ -blockers or any other agent known to affect pancreatic function. The pre-

menopausal female subjects were studied during the follicular phase of their menstrual cycle on both occasions. Informed written consent was obtained from all participants in accordance with the guidelines of the Human Subjects Review Committee of The Toronto General Hospital, University of Toronto.

**Experimental protocols.** Each subject was studied on two occasions, 6–8 weeks apart. In the first study, subjects were admitted to the Metabolic Investigation Unit of The Toronto General Hospital, where they received a control diet and a continuous infusion of Intralipid and heparin to raise plasma FFA levels for 48 h before and during the assessment of pancreatic insulin secretion. This intervention is referred to as the “48-h heparin-Intralipid study.” After a 12-h overnight fast, an intravenous catheter was placed in each forearm, one for infusion and one for blood sampling. The arm containing the sampling catheter was maintained in a heating blanket ( $-65^{\circ}\text{C}$ ) to arterialize venous blood. After a fasting baseline blood sample was drawn, Heparin sodium (Organon Teknika, Toronto, Canada) and Intralipid 20% solution (Baxter, Mississauga, Canada) were infused at 250 U/h and 40 ml/h, respectively, starting at 8:00 A.M., and continued for 48 h before and then during the subsequent intravenous glucose infusion. Intralipid is a sterile fat emulsion containing 20% soybean oil, 1.2% egg phospholipids, and 2.25% glycerin in water. During this time, subjects consumed an isocaloric diet consisting of 20% of calories derived from protein, 30% from fat, and 50% from carbohydrate. Blood samples were drawn at approximately 8:00 A.M. (after a 12-h overnight fast) and 4:00 P.M. on days 1 and 2 for measurement of glucose, insulin, FFA, and triglycerides. On the morning of day 3, after a 12-h overnight fast, the subjects underwent a graded glucose infusion, as has been previously described by Byrne et al. (29), to explore the dose-response relationship between glucose and insulin secretion rate (ISR). At approximately 8:00 A.M. on day 3, with the heparin-Intralipid infusion continuing at a steady rate until the end of the study, blood samples were drawn at 10-min intervals for glucose, insulin, C-peptide, FFA, and triglycerides during a 30-min basal period, before the start of the dextrose infusion. Samples for FFA and triglyceride analysis were collected into chilled EDTA tubes on ice containing 30 mg/ml blood of the lipase inhibitor tetrahydrolipstatin (Hoffman La Roche, Mississauga, Ontario, Canada) to prevent ongoing in vitro lipolysis of the samples. A stepped intravenous infusion of glucose (20% dextrose) was then started at a rate of  $1\text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , followed by infusions of 2, 3, 4, 6, and 8  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for a period of 40 min at each glucose infusion rate. Samples were drawn for measurement of the above parameters every 10 min throughout the experiment.

In the second study, subjects were provided with a control diet (prepackaged food was provided) as outpatients for 48 h before the assessment of pancreatic

TABLE 2  
Fasting plasma glucose, insulin, FFA, and triglyceride levels for 48 h before the graded intravenous glucose infusion (day 3)

	48-h Heparin-Intralipid study				Control study			
	Glucose (mmol/l)	Insulin (pmol/l)	FFA (mmol/l)	Triglyceride (mmol/l)	Glucose (mmol/l)	Insulin (pmol/l)	FFA (mmol/l)	Triglyceride (mmol/l)
Type 2 diabetic subjects								
Day 1								
8:00 A.M.	8.4 ± 1.1	101 ± 35	0.57 ± 0.11	1.64 ± 0.14	10.2 ± 2.4	141 ± 49	0.47 ± 0.16	2.07 ± 0.30
4:00 P.M.	8.4 ± 1.8	169 ± 37	2.14 ± 0.63*	5.41 ± 1.08*				
Day 2								
8:00 A.M.	7.9 ± 1.2	132 ± 47	2.13 ± 0.74*	5.50 ± 1.83*	11.7 ± 2.5	81 ± 41	0.26 ± 0.10	1.96 ± 0.25
4:00 P.M.	7.9 ± 1.5	205 ± 52*	2.01 ± 0.43*	6.01 ± 1.54*				
Day 3								
8:00 A.M.	9.0 ± 1.0	95 ± 25	1.09 ± 0.15*†	4.21 ± 0.54*†	8.7 ± 0.9	89 ± 28	0.57 ± 0.09	1.57 ± 0.13
Obese control subjects								
Day 1								
8:00 A.M.	4.3 ± 0.1‡	71 ± 15	0.67 ± 0.13	1.58 ± 0.46	4.3 ± 0.2‡	94 ± 23	0.49 ± 0.10	1.59 ± 0.39
4:00 P.M.	4.3 ± 0.1‡	218 ± 44*	1.13 ± 0.30*	2.62 ± 0.99*				
Day 2								
8:00 A.M.	4.6 ± 0.1‡	56 ± 11	0.98 ± 0.14	3.10 ± 1.03*	4.4 ± 0.3‡	91 ± 18	0.48 ± 0.12	1.77 ± 0.45
4:00 P.M.	4.4 ± 0.3‡	242 ± 67*	1.11 ± 0.20*	3.68 ± 1.09*				
Day 3								
8:00 A.M.	6.1 ± 0.2*‡	86 ± 17†	1.13 ± 0.15*†	2.84 ± 0.81*	5.8 ± 0.3*‡	55 ± 5	0.51 ± 0.07	1.17 ± 0.16

Data are means ± SE. The 8:00 A.M. measurements were performed after a 12-h overnight fast, whereas 4:00 P.M. samples were not performed in the fasting state. On day 1 and day 2, glucose values were measured on total blood in both studies. In the control study, for day 2 at 8:00 A.M., data were available for only three patients with type 2 diabetes and five nondiabetic obese control subjects. \*P < 0.05 vs. day 1 8:00 A.M. values; †P < 0.05 vs. control study; ‡P < 0.05 vs. patients with type 2 diabetes.

insulin secretion, but they did not receive an infusion of heparin and Intralipid. Subjects were instructed to eat only the food that was provided. The diet was identical in caloric content and composition to the one provided during the 48-h heparin-Intralipid study described above. Compliance with the diet was monitored by daily interviews with a dietician. Subjects were instructed to refrain from vigorous exercise during this 48-h period and were instructed to maintain a low level of physical activity, similar to that of an ambulatory inpatient. This study is referred to as the "control study." Plasma FFAs, triglycerides, glucose, and insulin were monitored daily in a subset of these subjects (Table 2). On the morning of day 3, subjects received a graded glucose infusion with blood sampling as described above, with the exception that heparin and Intralipid were not administered.

**Laboratory methods.** Glucose was assayed enzymatically at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay using a double antibody separation method (kit supplied by Pharmacia Diagnostic, Uppsala, Sweden) with intra- and interassay coefficients of variation of 5.8 and 11.2%, respectively. There is 41% cross-reactivity with proinsulin reported in this assay. C-peptide was measured by a double antibody C-peptide radioimmunoassay (kit supplied by Diagnostic Products, Los Angeles, CA). The intra- and interassay coefficients of variation for this assay are 4.1 and 15.1%, respectively. The samples for all studies in the same patient were assayed simultaneously with the same kit for both insulin and C-peptide. Proinsulin was measured in a subset of patients (n = 3 for patients with type 2 diabetes and n = 6 for obese control subjects) before glucose infusion (at times -20, -10, and 0 min) and during the last glucose infusion step (at times 220, 230, and 240 min) using a Linco human proinsulin RIA kit (coefficient of variation 3.2%). FFAs were measured by a colorimetric method (kit supplied by Wako, Osaka, Japan). Triglycerides were measured as esterified glycerol using an enzymatic colorimetric kit (Boehringer Mannheim, Mannheim, Germany). Free glycerol was eliminated from the sample in a preliminary reaction followed by enzymatic hydrolysis of triglyceride with subsequent determination of the liberated glycerol by colorimetry.

#### Calculations

**Estimation of ISR.** Pancreatic ISR was calculated from peripheral plasma C-peptide levels by deconvolution using a two-compartment mathematical model with standard parameters for C-peptide distribution and metabolism as previously described (30). (The software program for calculation of insulin secretion was kindly provided by Drs. K. Polonsky and J. Sturis, University of Chicago, Chicago). Although the determination of individual C-peptide kinetic parameters

would have been ideal, unfortunately C-peptide was no longer commercially available for in vivo use in humans at the time these studies were performed. The use of standard parameters for C-peptide clearance and distribution has been shown to result in ISRs that differ in each subject by only 10–12% from those obtained with individual parameters, and there is no systematic over- or under-estimation of insulin secretion (30). These parameters were validated for both obese nondiabetic and diabetic subjects in addition to normal control subjects. The same protocol was used without individual C-peptide boluses by Byrne et al. (31). Further, the present studies were performed in the same individuals, and the comparisons were within subjects. To our knowledge, there is no evidence that differences in plasma FFA levels or Intralipid and heparin infusion affect the kinetics of C-peptide in humans.

**Method of analysis of the relationship between glucose and ISR, insulin, and C-peptide.** Baseline levels of glucose, insulin, ISR, FFA, and triglycerides were calculated as the mean of the four baseline samples in each study. During the graded glucose infusion protocol, average levels of these parameters were also calculated for the last 20 min of the 40-min period for each infusion rate. Mean ISR, mean insulin, and mean C-peptide levels for each period were then plotted against the corresponding mean glucose level, thereby establishing a dose-response relationship between glucose and these variables. To statistically analyze the results—because the mean glucose level in subjects for each period differed between heparin-Intralipid and control studies and because glucose concentrations in each individual did not always include exactly the same range—the dose-response curves were compared in the following fashion. The average ISR, insulin, and C-peptide over each sequential 1 mmol/l glucose concentration interval between 13 and 17 mmol/l for patients with type 2 diabetes and between 6 and 11 mmol/l for the obese control subjects was calculated in each individual as the area under the curve using the trapezoidal rule. This area was then divided by 1 mmol/l to obtain the correct units (pmol/min for ISR, pmol/l for insulin, and nmol/l for C-peptide).

**Insulin clearance.** Clearance of endogenous insulin was calculated by dividing the mean ISR by the mean serum insulin in the last 20 min of each period of glucose infusion of the graded glucose infusion protocol (29).

**Statistical analysis.** The data were expressed as means ± SE. Basal levels of glucose, FFA, insulin, and ISR were compared by paired t test between the control and 48-h heparin-Intralipid study. Two-way analysis of variance for repeated measurements was performed to detect significant differences between the 48-h heparin-Intralipid and control studies. Calculations were performed with SAS software (SAS Statistical Analysis System, Cary, NC).

## RESULTS

Plasma FFA, triglyceride, glucose, and insulin concentrations during the 48 h before the graded glucose infusion. Table 2 shows the glucose, insulin, FFA, and triglyceride levels measured at 8:00 A.M. and 4:00 P.M. on days 1 and 2 and at 8:00 A.M. on day 3 of the 48-h heparin-Intralipid and control studies. In the control study, the parameters were measured only at 8:00 A.M. on each of the 3 days. There was no significant difference between the pre-infusion glucose, insulin, FFA, and triglyceride levels of the 48-h heparin-Intralipid study versus the control study in either patients with type 2 diabetes or obese subjects. After starting the heparin-Intralipid infusion, levels of FFA were elevated ~1.5 to 2-fold above fasting pre-infusion levels and were maintained in that high physiological range for the entire study in both diabetic patients and obese subjects. On days 1 and 2, the FFAs were even higher in the diabetic subjects and were higher than in the obese nondiabetic subjects, although the difference was not statistically significant. In both groups, FFAs were significantly higher ( $P < 0.05$ ) on day 3 of the heparin-Intralipid infusion than in the control study. Triglyceride levels also rose significantly with the heparin and Intralipid infusion ( $P < 0.05$ ), to a greater extent in the diabetic than in the obese subjects, although this difference was not significant. The glucose levels were significantly higher on day 3 than on day 1 in both studies in the obese subjects, although this was likely due to the fact that glucose was measured on total blood on day 1 and on plasma on day 3. As expected, the fasting glucose levels were significantly higher in the patients with type 2 diabetes than in the obese control subjects at day 1 and 3 during both the heparin-Intralipid and the control study ( $P < 0.05$ ). The insulin levels did not change significantly between day 1 and day 3 with heparin-Intralipid infusion in the patients with type 2 diabetes. In the obese control group, the insulin levels on day 3 were significantly higher in the heparin-Intralipid study than in the control study, although the elevation from day 1 during heparin-Intralipid infusion was not statistically significant.

The data below will be presented first for the type 2 diabetic patients and then for the obese nondiabetic subjects. Graded glucose infusion studies in patients with type 2 diabetes

Plasma glucose, FFA, triglycerides, insulin, and C-peptide. Glucose levels were similar on day 3 in the 48-h heparin-Intralipid study and in the control study during the baseline period (0–30 min) ( $9.0 \pm 1.0$  and  $8.7 \pm 0.9$  mmol/l, respectively,  $P = 0.61$ ) (Fig. 1A). Glucose levels rose from ~9 to ~19 mmol/l during the subsequent glucose infusion and were slightly but significantly higher in the heparin-Intralipid study ( $P < 0.0019$ ).

FFA levels (Fig. 1B) were significantly higher by design in the 48-h heparin-Intralipid study versus the control study on day 3 during the baseline sampling period ( $1.09 \pm 0.15$  and  $0.57 \pm 0.09$  mmol/l, respectively,  $P < 0.0001$ ). Levels declined progressively during the subsequent glucose infusion in both studies but remained higher in the heparin-Intralipid than in the control study ( $P < 0.0001$ ).

Plasma triglycerides were higher on day 3 at baseline in the 48-h heparin-Intralipid than in the control study (Fig. 1C) ( $4.21 \pm 0.54$  vs.  $1.57 \pm 0.09$  mmol/l,  $P < 0.0001$ ) and remained higher during the graded glucose infusion ( $P < 0.0001$ ).

Insulin levels (Fig. 1D) were similar in the heparin-Intralipid and the control study on day 3 in the baseline

period ( $95 \pm 25$  vs.  $89 \pm 28$  pmol/l,  $P = 0.76$ ) and rose progressively in both studies in response to the graded glucose infusion. The area under the insulin versus glucose curve (not shown) for the 13.0–17.0 mmol/l glucose range was  $19.6 \pm 6.0\%$  higher in the 48-h heparin-Intralipid study versus the control study ( $P = 0.001$ ). Proinsulin levels were also similar between heparin-Intralipid and control studies at baseline ( $20 \pm 3$  vs.  $16 \pm 4$  pmol/l, NS) and rose proportionately in both studies during the last glucose infusion step, between 220 and 240 min of the glucose infusion ( $63 \pm 9$  vs.  $49 \pm 5$  pmol/l, NS) in a subset of the patients ( $n = 3$ ). C-peptide (Fig. 1E) was similar between studies on day 3 at baseline ( $0.87 \pm 0.08$  vs.  $0.86 \pm 0.11$  nmol/l, NS) but was significantly higher ( $P = 0.0009$ ) in the 48-h heparin-Intralipid than in the control study during the graded glucose infusion. Further, the area under the C-peptide versus glucose concentration curve (not shown) between 13.0 and 17.0 mmol/l of plasma glucose was  $11.3 \pm 3.7\%$  higher in the heparin-Intralipid study versus the control study ( $P < 0.0001$ ).

ISR and insulin clearance. There was no significant difference on day 3 in basal ISR between studies ( $219 \pm 28$  vs.  $236 \pm 42$  pmol/l for the heparin-Intralipid versus control study,  $P = 0.73$ ) (Fig. 1F). However, ISR increased to a greater extent with the graded glucose infusion in the heparin-Intralipid than in the control studies ( $P = 0.008$ ). The glucose-ISR dose-response relationship (Fig. 2A) revealed a  $8.0 \pm 5.5\%$  higher mean total area under curve of ISR between 13 and 17 mmol/l glucose in the 48-h heparin-Intralipid versus the control study ( $P = 0.027$ ). However, only two of the seven subjects had an obvious increase in their ISR when corrected for glucose in response to high FFAs, whereas the other five subjects demonstrated either no change or a slight (<5%) increase in ISR.

Insulin clearance (Fig. 2B), calculated as the average insulin clearance at each incremental glucose infusion rate, was similar for the 48-h heparin-Intralipid and the control study ( $P = 0.89$ ).

Graded glucose infusion studies in obese nondiabetic subjects

Plasma glucose, FFA, triglycerides, insulin, and C-peptide. Basal glucose levels on day 3 were not significantly different in the 48-h heparin-Intralipid versus control study and were significantly higher in the heparin-Intralipid versus the control study during the graded glucose infusion ( $P < 0.0001$ ) (Fig. 3A). FFAs (Fig. 3B) were significantly higher by design on day 3 in the 48-h heparin-Intralipid study versus the control study during the baseline period ( $1.13 \pm 0.15$  vs.  $0.51 \pm 0.07$  mmol/l,  $P < 0.0001$ ). FFAs declined but remained significantly higher during the graded glucose infusion in the 48-h heparin-Intralipid study ( $P < 0.0001$ ). Triglycerides were higher with the 48-h heparin-Intralipid infusion (Fig. 3C) on day 3 at baseline ( $2.84 \pm 0.81$  vs.  $1.17 \pm 0.16$  mmol/l,  $P < 0.0001$ ) and were also higher during the intravenous glucose infusion ( $P < 0.0001$ ).

Insulin levels (Fig. 3D) were significantly higher in the heparin-Intralipid study than in the control study on day 3 at baseline ( $86 \pm 17$  vs.  $55 \pm 5$  pmol/l,  $P = 0.0005$ ) and were significantly higher during the graded glucose infusion ( $P < 0.0001$ ). The mean area under the insulin versus glucose curve (not shown) between 6 and 11 mmol/l of plasma glucose was  $39.8 \pm 16.2\%$  higher during the 48-h heparin-Intralipid study versus the control study ( $P < 0.0001$ ). Proinsulin levels

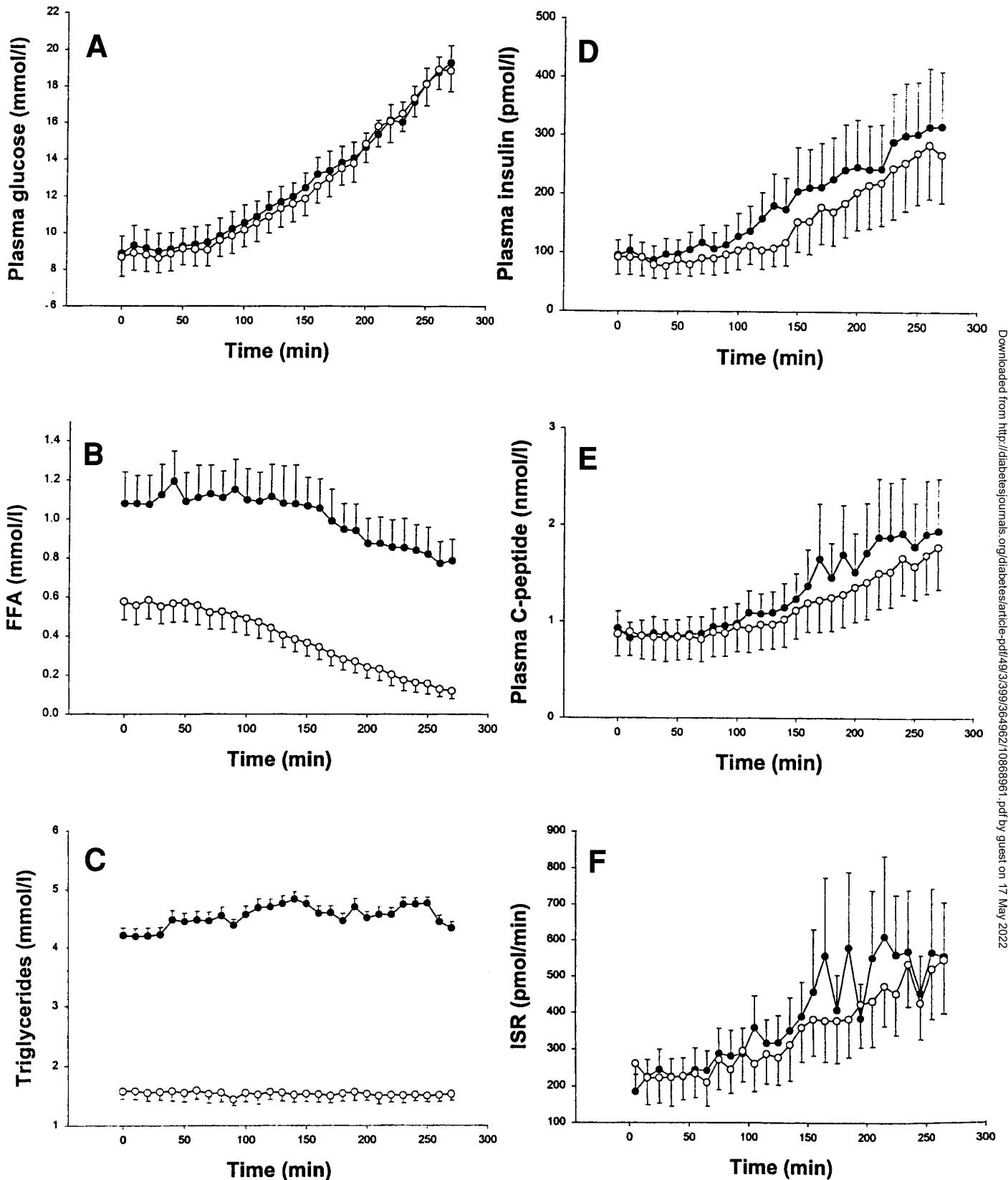


FIG. 1. Mean profiles of glucose (A), FFAs (B), triglycerides (C), insulin (D), C-peptide (E), and ISR (F) versus time in response to a programmed graded intravenous glucose infusion started after a 30-min baseline sampling period (time 0–30 min) in patients with type 2 diabetes ( $n = 7$ ). These figures illustrate profiles following 48 h of either a heparin-Intralipid infusion (●) or a control diet (○). In the heparin-Intralipid study, the infusion of heparin and Intralipid was continued throughout the experiment. The glucose, FFA, triglyceride, insulin, and C-peptide levels were higher in the heparin-Intralipid than in the control study ( $P < 0.0019$  for glucose;  $P < 0.0001$  for FFA, triglycerides, and insulin;  $P = 0.0009$  for C-peptide). ISR was significantly higher in the heparin-Intralipid than in the control study ( $P = 0.008$ ).

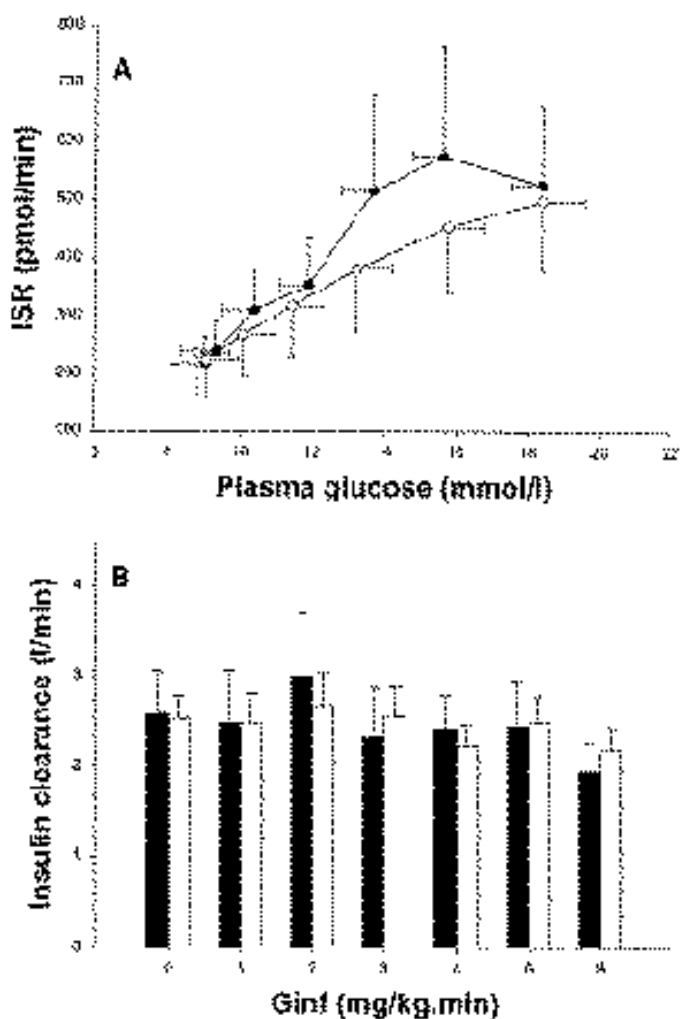


FIG. 2. Dose-response relationship between glucose and ISR (A) and mean insulin clearance at each glucose infusion rate (B) during the graded glucose infusion in patients with type 2 diabetes ( $n = 7$ ). There was a slight but significant increase in the average ISR for glucose levels between 13 and 17 mmol/l in the 48-h heparin-Intralipid study ( $\bullet$ ,  $\blacksquare$ ) versus the control study ( $\circ$ ,  $\square$ ) ( $P = 0.027$ ). The average insulin clearance was similar in the 48-h heparin-Intralipid study and the control study (NS).

were similar in both studies at baseline ( $11 \pm 2$  vs.  $14 \pm 3$  pmol/l for heparin-Intralipid study versus control study, NS) and rose proportionately in both studies in the last glucose infusion step between 220 and 240 min of the glucose infusion ( $45 \pm 6$  vs.  $54 \pm 8$  pmol/l, NS). The ratios of proinsulin to insulin were similar at baseline ( $28 \pm 7$  vs.  $32 \pm 7\%$ , NS) and lower at the end of the glucose infusion ( $19 \pm 3$  vs.  $33 \pm 6\%$ ,  $P < 0.05$ ) than in the diabetic subjects.

In contrast, C-peptide levels (Fig. 3E) were significantly lower in the heparin-Intralipid than in the control study on day 3 at baseline ( $0.42 \pm 0.04$  vs.  $0.70 \pm 0.07$  nmol/l,  $P = 0.0013$ ). C-peptide levels also remained lower during the initial part of the following graded glucose infusion in the heparin-Intralipid study versus the control study ( $P = 0.006$  for the entire curve). This lowering of the plasma C-peptide levels in the 48-h heparin-Intralipid study was even more apparent when corrected for the higher glucose levels by calculating the area under the C-peptide curve between 6 and 11 mmol/l of plasma glucose ( $27.7 \pm 3.4\%$  lower,  $P < 0.0001$ ).

Estimated ISR and insulin clearance. ISR (Fig. 3F) was lower in the 48-h heparin-Intralipid than in the control study on day 3 at baseline ( $123 \pm 12$  vs.  $199 \pm 23$  pmol/min,  $P = 0.0073$ ) but was not different during the graded glucose infusion ( $P = 0.64$  for the entire infusion time) when not corrected for the plasma glucose. The mean area under the ISR curve between 6 and 11 mmol/l of plasma glucose (Fig. 4A) was  $21.0 \pm 5.5\%$  lower in the 48-h heparin-Intralipid study versus the control study ( $P = 0.0008$ ). However, it is obvious from Fig. 4A that the ISR versus glucose curves for both studies were converging at higher plasma glucose and that there were no differences in ISR versus glucose between the two studies at  $>11$  mmol/l of plasma glucose.

Insulin clearance (Fig. 4B) was much lower at all steps of the graded glucose infusion in the 48-h heparin-Intralipid study than in the control study ( $P < 0.0001$ ).

## DISCUSSION

In this study, we demonstrated that a prolonged infusion of heparin and Intralipid for 48 h, which resulted in an approximately twofold elevation of plasma FFA above fasting levels, was associated with a significant 21% reduction in GSIS in obese nondiabetic individuals, but not in patients with type 2 diabetes. In fact, our results suggest that patients with type 2 diabetes may have a small increase in GSIS after prolonged Intralipid and heparin infusion, although the response was highly variable between subjects (two had a frank increase, whereas five had either no change or only a very slight elevation [ $<5\%$ ] of GSIS). This is the first study to show an absolute suppression of GSIS, assessed by C-peptide deconvolution, with prolonged exposure to elevated plasma FFAs *in vivo* in humans.

Insulin secretion is chronically increased in obese insulin-resistant individuals, compensating for their decrease in insulin sensitivity (32). The increase in insulin, however, is unlikely to be due to elevated FFAs, because the 48-h elevation of plasma FFA levels was associated with a reduction in absolute GSIS. The patients with type 2 diabetes, on the other hand, experienced no reduction in GSIS with elevated FFA. In these latter patients, it could be hypothesized that an FFA-mediated decompensation of GSIS was not evident, because the  $\beta$ -cell was already dysfunctional. However, it is also possible that the "lipotoxic" effect of FFA on  $\beta$ -cell function was masked by the higher glucose range in patients with type 2 diabetes. Interestingly, the decrease in ISR mediated by FFAs in the obese subjects occurred at the lower range of glucose stimulus ( $<11$  mmol/l plasma glucose) and was no longer apparent at the higher range of glucose levels (Fig. 4A).

We have shown previously (16) in young nonobese healthy subjects that an increase in plasma FFA levels for 48 h did not result in an absolute reduction in GSIS, although it did result in a significant decrease in the disposition index (this index is constant for nondiabetic populations and is defined as the product of ISR and insulin sensitivity [ $S_{i1}$ ]). In other words, GSIS was inappropriately low when considering the reduction in  $S_{i1}$  produced by the plasma FFA elevation. Unfortunately, in the present study, the experimental protocol did not allow us to derive a quantitative measure of  $S_{i1}$ , although the increase in glucose levels in the presence of higher insulin concentrations suggests that heparin and Intralipid impaired  $S_{i1}$  in the obese subjects. Given the presumed reduction in  $S_{i1}$  in the obese nondiabetic subjects, the absolute decline in

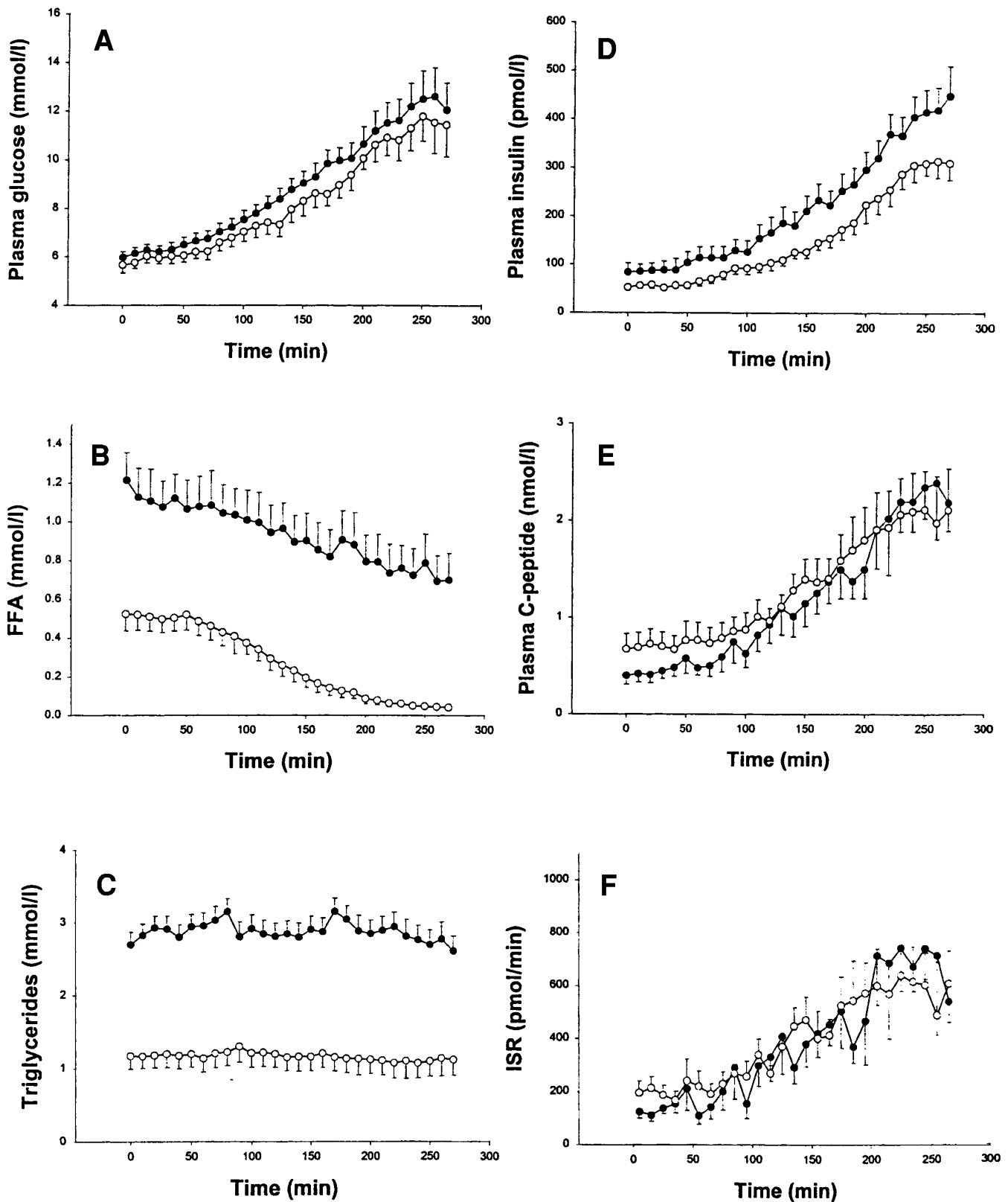


FIG. 3. Mean profiles of glucose (A), FFA (B), triglycerides (C), insulin (D), C-peptide (E), and ISR (F) versus time in response to a programmed graded intravenous glucose infusion started after a 30-min baseline sampling period (time 0–30 min) in obese nondiabetic subjects ( $n = 7$ ). These figures illustrate profiles following 48 h of either a heparin-Intralipid infusion (●) or a control diet (○). In the heparin-Intralipid study, the infusion of heparin and Intralipid was continued throughout the experiment. The glucose, FFA, triglyceride, and insulin levels were significantly higher in the 48-h heparin-Intralipid study ( $P < 0.0001$ ). In contrast, C-peptide levels were lower in the 48-h heparin-Intralipid study than in the control study both at baseline ( $P = 0.0013$ ) and during the intravenous glucose infusion ( $P = 0.006$ ). In consequence, ISR was lower at baseline ( $P = 0.0073$ ) in the 48-h heparin-Intralipid study than in the control study.

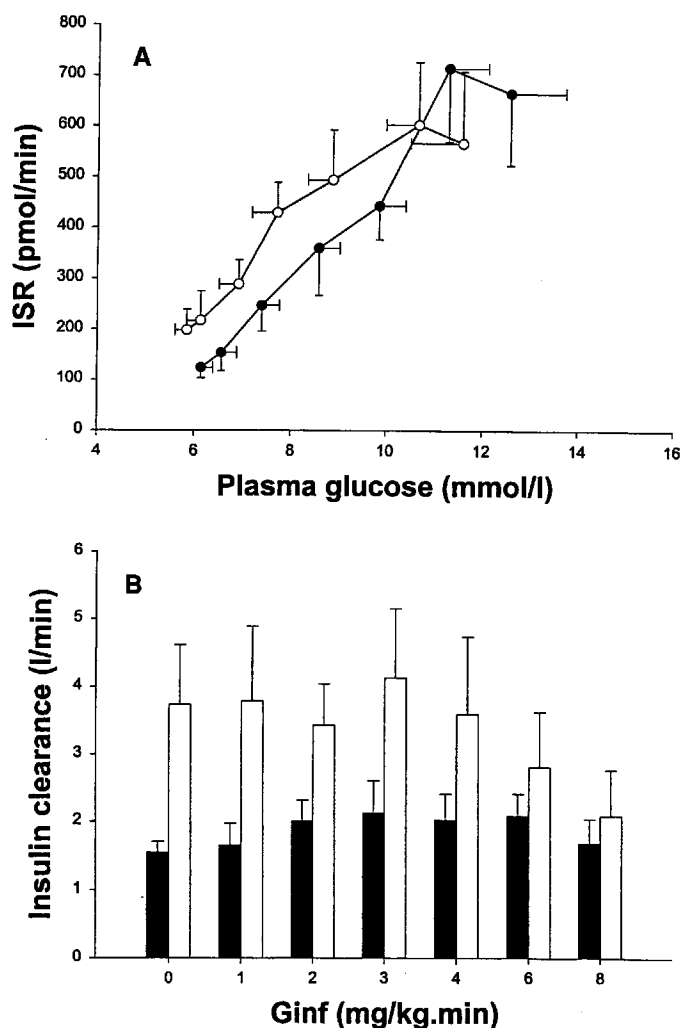


FIG. 4. Mean ISR (A) at baseline and during the last 20 min of each glucose infusion step in obese nondiabetic subjects ( $n = 7$ ) during the 48-h heparin-Intralipid study (●) and the control study (○). There was a significant decrease in the area under the ISR curve between 6 and 11 mmol/l of plasma glucose ( $P = 0.0008$ ). Mean insulin clearance rate (B) was much lower throughout the intravenous glucose infusion in the 48-h heparin-Intralipid study (■) versus the control study (□) ( $P < 0.0001$ ).

insulin secretion was all the more remarkable, because insulin secretion would be expected to increase to compensate for the decline in  $S_I$ , thus maintaining a constant disposition index. The situation in the diabetic patients, however, cannot be predicted. There was great interindividual variability in the response of the diabetic subjects, with at least two individuals demonstrating an absolute increase in GSIS after 48-h exposure to heparin and Intralipid. This finding may represent an absolute stimulation of GSIS with elevated FFAs in the diabetic subjects, although a true evaluation of this would require knowledge of the change in  $S_I$ , to calculate the disposition index.

Our results are not in accordance with the hypothesis proposed by others that chronically elevated FFAs are stimulatory to the  $\beta$ -cell in the fed state and thereby could explain the chronic hyperinsulinemia of insulin-resistant states (25,33–35). The first study of this group (25) found that an elevation of plasma FFAs in nondiabetic nonobese subjects using a

heparin-Intralipid infusion increases the ISR during a prolonged hyperglycemic clamp (glucose  $\sim 8.6$  mmol/l for 48 h). The subjects in their study were fasting for the entire 48-h period (although they did receive over 8,000 kcal/day intravenously with the infusion of glucose and Intralipid), which differed from our study, in which subjects only fasted overnight before the glucose infusion study. Results in rats and humans have shown that GSIS is dependent on FFAs in the prolonged fasting state but not in the fed state (17–20). It is also possible that the prolonged hyperglycemia induced in these individuals masked an FFA-mediated decrease in ISR. Finally, the authors did not calculate insulin secretion in relation to the expected FFA-induced reduction in insulin sensitivity, and there may indeed have been a reduction in the disposition index.

Our findings are in agreement with those of Paolisso et al. (14), who reported that raising plasma FFA threefold in healthy subjects with a 12-h Intralipid and heparin infusion was associated with an absolute inhibition of the acute insulin response to an intravenous glucose tolerance test, as assessed by the incremental area under the curve of the acute plasma insulin versus time response curve. However, they did not measure plasma C-peptide levels, nor did they calculate insulin secretory response, as we did in the present study. In line with our results, Paolisso et al. (26) have also recently published findings that showed that the acute insulin response to an intravenous glucose tolerance test was increased when plasma FFAs were lowered for 1 week by treatment with acipimox in healthy nonobese relatives of patients with type 2 diabetes, and that this effect of acipimox was prevented by the concomitant infusion of Intralipid to keep plasma FFA levels from decreasing with acipimox treatment.

The mechanisms leading to a decrease in ISR with prolonged exposure of  $\beta$ -cells to elevated FFAs are still debated. An attractive explanation would be that a “Randle cycle” is operating at the level of the  $\beta$ -cell. In line with this, Zhou and colleagues (23,36) have found that oxidation of FFA in  $\beta$ -cell in vitro leads to a decrease in pyruvate dehydrogenase activity, which causes a decrease in glucose oxidation. However, this was refuted by others who showed that the FFA-mediated decrease in GSIS in the  $\beta$ HC9  $\beta$ -cell line was not through an impairment of glucose oxidation (37). Therefore, alterations of GSIS by prolonged elevation of FFAs at the  $\beta$ -cell are likely to occur via other mechanisms, such as induction of uncoupling (38). IDX-1 mRNA, a transcription factor necessary for GLUT2, glucokinase, and insulin gene expression in rat  $\beta$ -cells, has been shown to be decreased after 48 h in vitro exposure to palmitate (39). Prolonged elevation of plasma FFAs may also have direct effects on insulin synthesis (39). Very recently, it has been shown that prolonged exposure of both rat and human islets to FFAs in vitro leads to an increase in proinsulin secretion due to an FFA-mediated impairment of conversion of proinsulin to insulin (40,41). However, in the current study, we were unable to demonstrate any significant FFA-mediated increase in proinsulin levels in vivo at baseline and during intravenous glucose stimulation in either patients with type 2 diabetes or obese control subjects. It is therefore unlikely that the FFA-mediated reduction in GSIS seen in the obese subjects in the current study was caused by an impairment in proinsulin processing.

The elevated insulin levels in response to the glucose infusion—despite a decrease in ISR in the obese subjects in the



present 48-h heparin-Intralipid infusion study—were due to a marked reduction in insulin clearance. This is in contrast to our findings of unchanged insulin clearance with elevation of plasma FFAs in patients with type 2 diabetes but in accordance with our earlier findings in young nonobese healthy subjects (16). Insulin clearance is mediated through the binding of the hormone to its receptor (42). Studies in isolated rat hepatocytes showed a concentration-dependent FFA-mediated decrease in binding and degradation of insulin (43). This effect was also shown *ex vivo* in rat liver (43–46). We have recently shown that heparin-Intralipid infusion in dogs reduces hepatic insulin extraction *in vivo* (47). Our data in humans are in contrast to those of Boden *et al.* (25) but in accordance with those of Hennes *et al.* (48), who also demonstrated FFA suppression of endogenous insulin clearance in nondiabetic humans. Insulin secretion was calculated in the present study by deconvolution of peripheral C-peptide concentrations, using standard kinetic parameters for C-peptide distribution and metabolism in diabetes and obesity, as previously described (30). The issue arises as to whether these parameters remain valid in the presence of elevated FFAs induced by the infusion of heparin and Intralipid. Unfortunately, biosynthetic C-peptide is no longer available for administration to humans, so we were unable to determine whether there is an effect on the kinetic parameters. We are not aware of any studies in which this has been tested, but could find no plausible biological reason to suggest that C-peptide's kinetic parameters should be affected by heparin and Intralipid infusion.

Heparin and Intralipid infusion results not only in an elevation of plasma FFAs, but in an elevation of triglycerides, heparin, glycerol, and calories, as well. It is not possible to absolutely exclude an effect of factors other than FFAs on GSIS, and a possible effect of these other factors needs to be considered. Heparin is not known to have a direct effect on  $\beta$ -cell function, and glycerol does not stimulate insulin secretion, since human islets are deficient in glycerol kinase (49). Plasma triglyceride elevation, on the other hand, has been shown by others to affect GSIS (50), although in this latter study plasma FFA level was not reported. Intra-islet triglyceride content has been shown to correlate with impairment of  $\beta$ -cell function (27). Our previous studies in rats, however (24), demonstrated an impairing effect of oleate infusion on GSIS, showing that FFAs *per se* can affect  $\beta$ -cell function *in vivo*. We cannot exclude an effect of the calories derived from Intralipid in the present study. To test this, future studies would have to be performed in which the subjects receive a hypocaloric diet during the heparin-Intralipid infusion. In addition to the above, the full effects of the heparin-Intralipid infusion on GSIS *in vivo* could have been modified by other hormonal or neural mediators. The autonomic system has recently been shown by others to play a role on GSIS in a rat model (51).

In conclusion, we have demonstrated that 1) prolonged *in vivo* elevation of plasma FFAs is associated with a decrease in GSIS and a marked reduction in insulin clearance in obese nondiabetic individuals, and 2) such an elevation of plasma FFAs is not associated with a decrease in GSIS in patients with type 2 diabetes. These results suggest that prolonged exposure of the  $\beta$ -cells to elevated FFAs in individuals at risk of developing type 2 diabetes could play a role in the progression toward pancreatic  $\beta$ -cell failure. In patients who have already developed overt  $\beta$ -cell failure

with consequent hyperglycemia, prolonged exposure to elevated FFAs does not seem to induce a further deterioration in  $\beta$ -cell function and may even result in some degree of stimulation of insulin secretion.

#### ACKNOWLEDGMENTS

This work was funded by a Research Grant from the Eli Lilly Banting and Best Diabetes Research Program at the University of Toronto. A.C. is the recipient of a Juvenile Diabetes Foundation International fellowship. S.D.M. is supported by the National Institutes of Aging (T32 AG 00093). R.N.B. is supported by funding from the National Institutes of Health (DK 27619 and 29867). G.F.L. is a Career Investigator of the Heart and Stroke Foundation of Canada.

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