Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation

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Type 2 diabetes mellitus is characterized by impaired insulin secretion and action (16, 48). An early characteristic of the defect in insulin secretion is selective impairment of the insulin secretory response to glucose (34, 35). Recently, attention has been focused on the possible role of free fatty acids (FFA), which are often elevated in states of insulin resistance, in selectively desensitizing the β-cell to glucose (14, 32, 36, 49).

Both in vitro (15, 51) and in vivo (33) studies have shown that fatty acids acutely enhance glucose-induced and tolbutamide-stimulated (27) insulin secretion and play an important role in the maintenance of basal insulin secretion in the fasting but not in the fed state (18, 19, 42, 43). In contrast, in vitro studies in both rat and human islets have shown that prolonged exposure (≥24 h) to fatty acids decreases glucose-stimulated insulin secretion (GSIS; Refs. 11, 25, 28, 29, 40, 53–55). We have recently shown in a rat model that a prolonged elevation of FFA with an intravenous infusion of either heparin and Intralipid or oleate decreases insulin secretion in response to a two-step hyperglycemic clamp (30). It is known that fatty acids are actively taken up and metabolized by pancreatic islets (4, 5) and can regulate β-cell enzymes, ion channels, and genes (1, 24, 36), and there is initial evidence that FFA may chronically impair both potassium-ATP channel-dependent (55) and potassium-ATP channel-independent pathways of insulin secretion (28).

However, despite the evidence from in vitro studies, the effects of prolonged elevation of FFA on GSIS in humans remain controversial. Prolonged (24–48 h) FFA elevation either increased (8) or decreased (33) glucose-induced insulin secretion in nondiabetic normal-weight humans. The objective of the present study, therefore, was to determine the effect of acute and prolonged elevation of plasma FFA on insulin secretion in vivo in healthy nondiabetic human subjects under glucose stimulatory conditions of different intensity and duration.

MATERIALS AND METHODS

Subjects

Sixteen healthy male research volunteers participated in the study. Mean age was 25.4 ± 1.4 yr (means ± SE), and body mass index was 24.1 ± 0.5 kg/m². None of the subjects were taking any medication or had any known systemic illness. Informed written consent was obtained from all participants in accordance with the guidelines of the Human Subjects Review Committee of The Toronto Hospital, University of Toronto.

Experimental Protocols

Each subject was studied on at least two occasions, 6–8 wk apart (some subjects participated in a third study outlined in Acute heparin-Intralipid studies). Eight of the sixteen subjects underwent a graded glucose infusion with and without a prior 48-h infusion of heparin and Intralipid, and the other eight subjects underwent a two-step hyperglycemic clamp, with and without a prior 48-h infusion of heparin and Intralipid.

Graded glucose infusion studies. In the first study, subjects (n = 8) were admitted to the Metabolic Investigation Unit of The Toronto Hospital, where they received a control diet and a continuous infusion of Intralipid and heparin to raise plasma FFA for 48 h before and during the assessment of pancreatic insulin secretion. This intervention will be re-
Glucose (20% dextrose) was then started at a rate of 1 mg·kg\(^{-1}\)·min\(^{-1}\), followed by infusions of 2, 3, 4, 6, and 8 mg·kg\(^{-1}\)·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 as outpatients for 48 h before the assessment of pancreatic insulin secretion but did not receive an infusion of heparin and Intralipid. The diet was identical to that which had been provided during the 48-h heparin-Intralipid study described previously. Compliance with the diet was monitored by daily interviews with a dietician. This study will be referred to as the "control study." On the morning of day 3, subjects received a graded glucose infusion with blood sampling as described previously, with the exception that heparin and Intralipid were not administered.

Two-step hyperglycemic clamp. To test the glucose-induced maximal insulin secretion at two fixed levels of hyperglycemia, eight additional subjects underwent a two-step hyperglycemic clamp according to the modified DeFronzo et al. (17) method, with plasma glucose concentrations maintained at 10 mmol/l for the first 2 h and then at 20 mmol/l for the final 2 h of the clamp. One study was performed with a prior heparin and Intralipid infusion for 48 h that was continued during the hyperglycemic clamp studies as described in Graded glucose infusion studies (48-h heparin-Intralipid study), and a second study was performed on another occasion without the infusion of heparin and Intralipid (control study). In the hyperglycemic clamp studies, samples were drawn at 10-min intervals for insulin, FFA, and triglyceride during the 30-min basal period and the last 30 min of each step of the hyperglycemic clamp. Samples for glucose were drawn at 5-min intervals during the clamp and for plasma C-peptide were drawn at 5-min intervals during the last 30 min of each step of the clamp.

Acute heparin-Intralipid studies. Three subjects who participated in the graded glucose infusion protocol and five subjects who participated in the two-step hyperglycemic clamp underwent a third study to determine the effects of an acute elevation of plasma FFA on GSIS. After 2 days on the same control diet outlined in Graded glucose infusion studies for the control studies and after a 12-h overnight fast, the subjects were admitted to the Metabolic Test Center, and an intravenous catheter was inserted into a superficial vein in each forearm. One arm was maintained in a heating blanket to arterialize the venous blood samples. An infusion of heparin and Intralipid was started at the same rate as was administered in the 48-h heparin-Intralipid studies. One hour after the start of the infusion, four baseline blood samples were drawn at 10-min intervals, followed by either a graded glucose infusion or two-step hyperglycemic clamp as described in Graded glucose infusion studies and Two-step hyperglycemic clamp, depending on which protocol the subject had previously participated in.

Laboratory Methods

Glucose was assayed enzymatically at the bedside with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay with 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. 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. FFA were measured by a colorimetric method (kit supplied by Wako Industries, Osaka, Japan). Triglycerides were measured as esterified glycerol with an enzymatic colorimetric kit (Boehringer Mannheim). 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 insulin secretion rate was calculated from peripheral plasma C-peptide levels by deconvolution with a two-compartment mathematical model with standard parameters for C-peptide distribution and metabolism as previously described (Ref. 50; the software program for calculation of insulin secretion was kindly provided by Drs. K. Polonsky and J. Sturis, University of Chicago, Chicago, IL). 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 insulin secretion rates that differ in each subject by only 10–12% from those obtained with individual parameters, and there is no systematic over- or underestimation of insulin secretion (50). The same protocol was used without individual C-peptide boluses by Byrne et al. (12). Furthermore, 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. Other investigators have used and published studies in which they assessed ISR with and without an acute increase in plasma FFA with either a unique set of individually determined C-peptide kinetic parameters (21) or standard parameters (8).
Method of analysis of the relationship between glucose and ISI, insulin, and C-peptide. Baseline levels of glucose, insulin, ISI, FFA, and triglycerides were calculated as the mean of the four baseline samples in each study. For 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 ISI, 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 healthy subjects for each period differed between heparin-Intralipid and control studies and because glucose concentrations in each individual did not always include the 5.0–10.0 mmol/l range, the dose-response curves were compared in the following fashion: the average ISI, insulin, and C-peptide over each sequential 1 mmol/l glucose concentration interval between 6 and 9 mmol/l were calculated in each individual as the area under the curve with the trapezoidal rule; this area was then divided by 1 mmol/l to obtain the correct units (pmol/min for ISI, pmol/l for insulin, and nmol/l for C-peptide).

For the subjects who underwent the two-step hyperglycemic clamp, the statistical analysis was done on the mean of the four baseline samples and the mean of the last 30-min samples of each hyperglycemic step with untransformed data, because the glucose levels were matched for the three studies.

Insulin clearance. Clearance of endogenous insulin was calculated by dividing the mean ISI by the mean serum insulin in the last 20 min of each period of glucose infusion for the graded glucose infusion protocol and in the last 30 min of each hyperglycemic step for the two-step hyperglycemic clamp (13).

Insulin sensitivity index and DI. The insulin sensitivity index (SI) was calculated only for each step of the two-step hyperglycemic clamp studies according to the following formula

\[
SI_{clamp} = \frac{C_{Glc}}{(Ins_{period} - Ins_{baseline})}
\]

where \(C_{Glc}\) is the glucose clearance at each step of the hyperglycemic clamp, estimated as glucose infusion rate/glucose concentration during the last 30 min of each period; \(Ins_{period}\) is the insulin concentration during the last 30 min of the glucose step; and \(Ins_{baseline}\) is the mean insulin level during the baseline period. This equation is mathematically similar to that previously used to calculate the clamp-based insulin sensitivity index \((SI_{P(clamp)})\) (2) and assumes that the change in glucose uptake induced by a change in insulin concentration is proportional to the ambient glucose concentration. This makes the calculated SI independent of the glucose concentrations and therefore appropriate to use in the hyperglycemic state. SI is reported in units of deciliter per kilogram per minute per microunit per milliliter. No correction was made for the urine glucose loss, which was assumed to be equal between studies for the same individual because plasma glucose levels were similar. Further, because the plasma insulin levels were in the 150–250 and 450–650 pmol/l ranges during the 10 and 20 mmol/l clamp steps, respectively, the endogenous glucose production was assumed to be zero. The DI, which has been used as an index of insulin secretion corrected for the ambient degree of insulin resistance (3), was then calculated for each experimental period as an index of correction of ISR for the ambient degree of insulin sensitivity as the product of SI and ISR. DI was also calculated by fitting the equation \(SI = DI/ISR\), a hyperbolic function, to the SI and ISR data. These fitted parameters were not different from the average DI obtained from each protocol, and therefore only the average data are reported here. DI is given in arbitrary units (\(l^2 \cdot kg^{-1} \cdot min^{-2}\)).

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 ANOVA for repeated measurements was performed to detect significant differences between the 48-h heparin-Intralipid and control studies. The results of the patients who underwent the acute heparin-Intralipid study were compared with the corresponding results obtained from those individuals in the control experiment and in the 48-h heparin-Intralipid study with two-way ANOVA for repeated measurements followed by a Tukey's t-test for multiple comparisons of means. For the comparison of DI between control and 48-h heparin-Intralipid studies in the two-step hyperglycemic clamp, a balanced ANOVA was used to isolate effects by subject, glucose concentration, heparin-Intralipid infusion, and interaction between glucose and heparin-Intralipid. Calculations were performed with SAS software (Statistical Analysis System, Cary, NC).

RESULTS

For clarity, results for the 48-h heparin-Intralipid infusion and control studies will be presented and illustrated graphically first, followed by the results for the acute heparin-Intralipid studies.

Plasma FFA, Triglyceride, Glucose, and Insulin Concentrations During the 48 h Before Both the Graded Glucose Infusion and the Two-Step Hyperglycemic Clamp (Data Pooled).

Table 1 shows the FFA and triglyceride levels measured at 0800 and 1600 on days 1 and 2 of the 48-h

<table>
<thead>
<tr>
<th>Glucose, mmol/l</th>
<th>Insulin, pmol/l</th>
<th>FFA, mmol/l</th>
<th>TG, mmol/l</th>
<th>Glucose, mmol/l</th>
<th>Insulin, pmol/l</th>
<th>FFA, mmol/l</th>
<th>TG, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800 fasting</td>
<td>4.3 ± 0.2</td>
<td>37 ± 7</td>
<td>0.38 ± 0.07</td>
<td>1.05 ± 0.21</td>
<td>4.3 ± 0.2</td>
<td>32 ± 6</td>
<td>0.35 ± 0.11</td>
</tr>
<tr>
<td>1600</td>
<td>4.6 ± 0.1</td>
<td>98 ± 15</td>
<td>0.98 ± 0.07</td>
<td>3.39 ± 0.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800 fasting</td>
<td>4.6 ± 0.1</td>
<td>43 ± 4</td>
<td>0.92 ± 0.12*</td>
<td>1.85 ± 0.27*</td>
<td>4.3 ± 0.1</td>
<td>38 ± 5</td>
<td>0.46 ± 0.11</td>
</tr>
<tr>
<td>1600</td>
<td>4.5 ± 0.2</td>
<td>113 ± 25</td>
<td>0.83 ± 0.05</td>
<td>1.86 ± 0.28</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. FFA, free fatty acids; TG, triglycerides. *P < 0.02 between studies.
heparin-Intralipid study. These levels were also measured at 0800 on days 1 and 2 of the control study in some of the subjects (n = 6). There was no difference between the preinfusion FFA and triglyceride levels of the 48-h heparin-Intralipid study vs. control study. After the heparin-Intralipid infusion was started, levels of FFA and triglycerides were elevated approximately two- to fourfold above fasting preinfusion levels and were maintained in that high physiological range for the entire study. Both FFA and triglycerides were significantly higher (P < 0.02) on day 2 of the heparin-Intralipid infusion vs. the control study.

On the day after the heparin-Intralipid infusion was started (day 2), there was no difference vs. control for fasting plasma glucose or insulin.

Graded Glucose Infusion Studies

Plasma glucose, FFA, triglycerides, insulin, and C-peptide. Glucose levels were significantly higher on day 3 in the 48-h heparin-Intralipid study vs. control during the baseline period (0–30 min) (5.6 ± 0.1 and 5.0 ± 0.1 mmol/l, respectively, P < 0.0001; Fig. 1A). Glucose levels rose from ~5 to 10 mmol/l during the subsequent glucose infusion. Plasma glucose concentrations were significantly higher in the heparin-Intralipid study during the glucose infusion (P < 0.0001), although the difference was accounted for entirely by the initial and not the latter part of the glucose infusion vs. time curve.

FFA levels (Fig. 1B) were significantly higher by design in the 48-h heparin-Intralipid study vs. control during the baseline period (0.91 ± 0.04 and 0.51 ± 0.03 mmol/l, respectively, P < 0.0001). Levels declined progressively during the subsequent glucose infusion in both studies (less so in the heparin-Intralipid study, when the interaction between experiment and time was significant at the P < 0.0005 level) but remained higher in the heparin-Intralipid than in the control study (P < 0.0001).

Plasma triglycerides were higher at baseline during the 48-h heparin-Intralipid vs. control study (Fig. 1C; 2.22 ± 0.09 vs. 1.10 ± 0.10 mmol/l, P < 0.0001) and during the graded glucose infusion (P < 0.0001).

Insulin levels (Fig. 1D) were significantly higher in the heparin-Intralipid vs. control study in the baseline period (51 ± 4 vs. 35 ± 2 pmol/l, P < 0.0001) and rose progressively in both studies in response to the graded glucose infusion. The area under the insulin vs. glucose curve (not shown) for the 6.0–9.0 mmol/l glucose range
was 22.4 ± 6.6% higher in the 48-h heparin-Intralipid study vs. controls (P < 0.004).

C-peptide (Fig. 1E) was similar between studies at basal level (0.35 ± 0.02 vs. 0.34 ± 0.03 nmol/l, P = NS) but was slightly higher (P = 0.01) in the 48-h heparin-Intralipid vs. control study during the graded glucose infusion. However, the area under the C-peptide vs. glucose concentration curve (not shown) between 6.0 and 9.0 mmol/l of plasma glucose was similar [1.84 ± 0.17 nmol/l (6–9 mmol/l glucose interval) vs. 2.03 ± 0.24 nmol/l (6–9 mmol/l glucose interval)].

ISR and insulin clearance. There was no significant difference in basal ISR between studies (Fig. 1F). ISR increased to a similar extent with the graded glucose infusion in the heparin-Intralipid and the control studies. The glucose-ISR dose-response relationship (Fig. 2A) revealed no difference in mean total area under the curve of ISR between 6 and 9 mmol/l glucose in the 48-h heparin-Intralipid and the control study [579 ± 63 pmol/min (6–9 mmol/l glucose interval) vs. 597 ± 73 pmol/min (6–9 mmol/l glucose interval), respectively, P = NS], and the slope of the dose-response curves was similar.

Insulin clearance (Fig. 2B), calculated as the average insulin clearance at each incremental glucose infusion rate, was lower for the 48-h heparin-Intralipid vs. the control study (P = 0.0001).

Two-Step Hyperglycemic Clamp Studies

Plasma glucose, glucose infusion rate, FFA, triglycerides, insulin, and C-peptide. Basal glucose levels were slightly but significantly higher in the 48-h heparin-Intralipid vs. control study (5.9 ± 0.1 vs. 5.3 ± 0.1 mmol/l, respectively, P < 0.0001) but were comparable during the clamp (9.9 ± 0.1 vs. 9.7 ± 0.1 mmol/l, respectively, in the first step, P = NS; 20.0 ± 0.2 vs. 19.7 ± 0.2 mmol/l, respectively, in the second step, P = NS; Fig. 3A).

FFA (Fig. 3B) were significantly higher by design in the 48-h heparin-Intralipid study vs. control during the baseline period (0.95 ± 0.05 vs. 0.60 ± 0.04 mmol/l, P < 0.0001), the 10 mmol/l glucose step (0.68 ± 0.05 vs. 0.07 ± 0.01 mmol/l, P < 0.0001), and the 20 mmol/l glucose step (0.58 ± 0.03 vs. 0.02 ± 0.003 mmol/l, P < 0.0001).

Triglycerides were higher with the 48-h heparin-Intralipid infusion (Fig. 3C) at baseline (1.81 ± 0.11 vs. 0.86 ± 0.05 mmol/l, P < 0.0001), at 10 mmol/l glucose (1.96 ± 0.16 vs. 0.68 ± 0.04 mmol/l, P < 0.0001), and at 20 mmol/l glucose (1.60 ± 0.14 vs. 0.53 ± 0.03 mmol/l, P < 0.0001).

Insulin levels (Fig. 3D) were significantly higher in the heparin-Intralipid study vs. control at baseline (60 ± 4 vs. 37 ± 2 pmol/l, P < 0.0001), at 10 mmol/l plasma glucose (259 ± 17 vs. 168 ± 10 pmol/l, respectively, P < 0.0001), and at 20 mmol/l plasma glucose (666 ± 25 vs. 587 ± 20 pmol/l, P < 0.0001).

C-peptide levels (Fig. 3E) were higher in the heparin-Intralipid vs. control study at baseline (0.46 ± 0.03 vs. 0.36 ± 0.04 nmol/l, P < 0.0001) and slightly higher at 20 mmol/l glucose (2.13 ± 0.08 vs. 2.02 ± 0.07 nmol/l, P < 0.002) but were similar between studies at 10 mmol/l glucose (1.30 ± 0.04 vs. 1.27 ± 0.06 mmol/l, P = NS).

The amount of glucose infusion required to maintain the same glycemic level (Fig. 3F) was only slightly lower in the 48-h heparin-Intralipid vs. control study (33 ± 2 vs. 36 ± 3 µmol·kg⁻¹·min⁻¹ in the first step, P = 0.02) and was definitely lower (129 ± 9 vs. 201 ± 8 µmol·kg⁻¹·min⁻¹, P < 0.0001) in the second step.

ISR and insulin clearance. ISR (Fig. 4A) was the same in the 48-h heparin-Intralipid vs. control study at baseline (105 ± 11 vs. 96 ± 15 pmol/min, P = NS), at 10 mmol/l glucose (331 ± 26 vs. 340 ± 50 pmol/min, P = NS), and at 20 mmol/l glucose (540 ± 30 vs. 551 ± 34 pmol/min, P = NS).

Insulin clearance (Fig. 4B) tended to be lower at baseline in the 48-h heparin-Intralipid study (1.94 ± 0.34 vs. 2.58 ± 0.63 l/min, P = 0.21) and was significantly lower at the 10 mmol/l step (1.34 ± 0.13 vs. 2.15 ± 0.38 l/min, P = 0.04) but not at 20 mmol/l glucose (0.82 ± 0.08 vs. 1.00 ± 0.17 l/min; P = 0.50).

S₅ and DI. There was a significantly lower S₅ with prolonged elevation of plasma FFA (9.3 ± 1.1 × 10⁻⁴
Fig. 3. Mean profiles of glucose (A), FFA (B), TG (C), insulin (D), C-peptide (E), and glucose infusion rate (F) vs. time in response to a 2-step (10 and 20 mmol/l) hyperglycemic clamp started after a 30-min baseline-sampling period (time -30 min). Profiles are after 48 h of either a heparin-Intralipid infusion (●) or a control diet (○). In heparin-Intralipid study, infusion of heparin and Intralipid was continued throughout experiment. Glucose levels at baseline were significantly higher in 48-h heparin-Intralipid study (P < 0.0001), whereas they were similar in 1st and 2nd hyperglycemic steps. FFA, TG, and insulin were significantly higher for baseline, step 1, and step 2 in 48-h heparin-Intralipid study (P < 0.0001). C-peptide levels were higher in 48-h heparin-Intralipid vs. control study at baseline (P < 0.0001) and at 20 mmol/l of plasma glucose (P < 0.002) but were not different at 10 mmol/l. Glucose infusion rate was lower for 48-h heparin-Intralipid study for both 10 mmol/l step (P = 0.02) and 20 mmol/l step (P < 0.0001).

Acute Heparin-Intralipid Infusion Studies

Acute graded glucose infusion. Three subjects who underwent the graded glucose infusion studies, with and without 48-h infusion of heparin-Intralipid, underwent an acute (starting 1 h before baseline) heparin-Intralipid infusion study (Fig. 5). Comparative data for the control and 48-h heparin-Intralipid studies are reported here only for these three subjects. Baseline data are reported in Table 2. Baseline plasma glucose in the acute heparin-Intralipid study was similar to the control study but slightly lower than in the 48-h heparin-Intralipid study. During the graded glucose infusion, the glucose levels (Fig. 5A) rose to a higher level (P < 0.001) than in both the 48-h heparin-Intralipid and control studies, with a mean peak glucose level of 11.7 ± 0.4 mmol/l in the acute heparin-Intralipid study, 10.7 ± 0.8 mmol/l in the 48-h heparin-Intralipid study, and 10.0 ± 1.2 mmol/l in the control study.

The FFA (Fig. 5B) and triglyceride (Fig. 5C) levels were lower in the control study, intermediate in the acute heparin-Intralipid study, and higher in the 48-h heparin-Intralipid study both at baseline and during the glucose infusion (all differences significant, P < 0.001).

At baseline, the insulin levels (Fig. 5D) were similar between the acute heparin-Intralipid study and the control study. The DI was also significantly lower with the prolonged heparin-Intralipid infusion compared with control (6.1 ± 0.7 × 10⁻³ vs. 10.0 ± 1.2 × 10⁻³ [l²·kg⁻¹·min⁻２; P = 0.01]. This difference was significant when a balanced ANOVA was used to isolate the effects by subject, glucose level, treatment (control vs. heparin-Intralipid), and interaction between glucose and treatment. Figure 4C depicts the mean DI for both hyperglycemic clamp steps. At 10 mmol/l of glucose, DI tended to be lower with the Intralipid and heparin infusion vs. control (6.5 ± 0.9 × 10⁻³ vs. 9.8 ± 2.0 × 10⁻³ [l²·kg⁻¹·min⁻２, respectively), although the difference was not significant (P = 0.12). At 20 mmol/l, however, the difference of DI between the two studies was statistically significant (5.8 ± 0.8 × 10⁻³ vs. 10.2 ± 1.5 × 10⁻³ [l²·kg⁻¹·min⁻２ for Intralipid and heparin study vs. control, respectively, P < 0.02).
control study but were higher in the 48-h heparin-Intralipid study than in the two other studies. During the glucose infusion, the insulin levels were similar between the acute heparin-Intralipid study and the 48-h heparin-Intralipid study, and these were both higher than in the control study ($P < 0.001$). To normalize plasma insulin concentrations for corresponding glucose between studies, we calculated the area under the plasma insulin concentration vs. glucose concentration curves between 6.0 and 9.0 mmol/l glucose. The acute heparin-Intralipid study area under the curves for insulin ($354 \pm 73$ pmol/l (6–9 mmol/l glucose interval)) was intermediate between the control study ($289 \pm 13$ pmol/l (6–9 mmol/l glucose interval)) and the 48-h heparin-Intralipid study ($423 \pm 16$ pmol/l (6–9 mmol/l glucose interval)), but the difference was significant only between the control and the 48-h studies ($P < 0.05$).

Plasma C-peptide levels (Fig. 5E) increased to a much greater extent during the glucose infusion in the acute heparin-Intralipid study vs. the two other studies ($P < 0.001$), even when normalized for plasma glucose concentration between studies. There was significantly higher area under the plasma C-peptide concentration vs. glucose concentration curves between 6.0 and 9.0 mmol/l of plasma glucose ($2.98 \pm 0.31$ nmol/l (6–9 mmol/l glucose interval) vs. $2.24 \pm 0.26$ nmol/l (6–9 mmol/l glucose interval)) for the 48-h heparin-Intralipid study, $P < 0.001$; vs. $2.11 \pm 0.28$ nmol/l (6–9 mmol/l glucose interval) for the control study, $P < 0.001$.

Consequently, the area under the ISR vs. glucose concentration curves (Fig. 5F) was higher during the glucose infusion in the acute study ($908 \pm 86$ pmol/min (6–9 mmol/l glucose interval)) vs. the 48-h study ($677 \pm 132$ pmol/min (6–9 mmol/l glucose interval), $P < 0.05$) and the control study ($599 \pm 104$ pmol/min (6–9 mmol/l glucose interval), $P < 0.01$).

Acute two-step hyperglycemic clamp studies. Five subjects who participated in the two-step hyperglycemic clamp studies, with and without 48-h heparin-Intralipid infusion, underwent an additional study in which heparin and Intralipid were infused acutely, starting 1 h before the beginning of the baseline-sampling period. Comparative data reported in Table 3 for the control and 48-h heparin-Intralipid studies are for these five subjects only.

Baseline plasma glucose was slightly higher in the 48-h study than in the acute study, which in turn was higher than the control study (all differences significant, $P < 0.001$). At baseline, plasma FFA levels were higher in the 48-h heparin-Intralipid study than in the two other studies ($P < 0.001$). For each step of the clamp, FFA levels in the 48-h study were higher than in the acute study, which in turn were higher than in the control study (all differences significant, $P < 0.001$). In contrast to FFA, triglyceride levels were significantly higher in the acute study than in the 48-h heparin-Intralipid study at baseline and for each step of the clamp. Basal insulin levels in the acute study were lower than in the 48-h study, but similar to control. At 10 mmol/l of glucose, insulin levels were similar in the acute and the 48-h heparin-Intralipid studies and both were higher than the control study. At 20 mmol/l of glucose, the insulin levels in the acute heparin-Intralipid study were higher than in the control study.
C-peptide levels were significantly higher in the acute than in the other two studies at 10 mmol/l glucose ($P < 0.001$) and at 20 mmol/l glucose ($P < 0.001$). Consequently, the ISR in the acute study was significantly higher than in the 48-h study at both 10 mmol/l glucose ($P < 0.05$) and 20 mmol/l glucose ($P < 0.01$; Fig. 6). ISR in the acute study was also higher than in the control study, but the difference was statistically significant only at 20 mmol/l of plasma glucose ($P < 0.001$).

The $S_I$ was decreased by the acute heparin-Intralipid infusion vs. control ($11.2 \pm 3.2$ vs. $13.1 \pm 2.5$ dl·kg$^{-1}$·min$^{-1}$ per mU/ml), similar to the effect of chronic heparin-Intralipid infusion ($9.8 \pm 1.4 \times 10^{-4}$ dl·kg$^{-1}$·min$^{-1}$ per mU/ml). However, due to the increase in insulin secretion seen in this group, DI was not different from the control group ($10.0 \pm 1.9 \times 10^{-3}$ vs. $8.9 \pm 1.4 \times 10^{-3}$ l$^2$·kg$^{-1}$·min$^{-2}$). This is in contrast to the decline in DI observed in the chronic heparin-Intralipid infusion group to $6.3 \pm 1.2 \times 10^{-3}$ l$^2$·kg$^{-1}$·min$^{-2}$.

**DISCUSSION**

In the present study we have demonstrated that the acute FFA-mediated potentiation of in vivo GSIS is diminished and is no longer evident when FFA are elevated for 48 h by infusion of heparin and Intralipid intravenously in healthy humans. We used two methods of determining GSIS: a programmed intravenous glucose infusion, which raises plasma glucose in a graded fashion to 10 mmol/l, and a two-step hyperglycemic clamp to determine GSIS in response to plasma glucose concentrations as high as 20 mmol/l. The advantage of the former method is that it provides a dose-response curve. The advantage of the latter method is that it allows us to determine the maximal insulin secretory response at the same steady-state concentrations of plasma glucose and to calculate an $S_I$.
Table 3. Glucose, FFA, TG, insulin, C-peptide, and ISR levels during 2-step hyperglycemic clamp in 48-h heparin-Intralipid, control, and acute heparin-Intralipid study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10 mmol/l</th>
<th>20 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.9 ± 0.1</td>
<td>5.1 ± 0.1c</td>
<td>5.3 ± 0.1c</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.90 ± 0.03</td>
<td>0.67 ± 0.06c</td>
<td>0.66 ± 0.06c</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>1.60 ± 0.06</td>
<td>0.86 ± 0.06c</td>
<td>1.80 ± 0.11c</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>49 ± 3</td>
<td>38 ± 3a</td>
<td>37 ± 5c</td>
</tr>
<tr>
<td>C-peptide, nmol/l</td>
<td>0.46 ± 0.04</td>
<td>0.41 ± 0.05c</td>
<td>0.35 ± 0.05c</td>
</tr>
<tr>
<td>ISR, pmol/min</td>
<td>114 ± 13</td>
<td>102 ± 21</td>
<td>91 ± 18</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 5 subjects who underwent all 3 studies. A, 48-h heparin-Intralipid; B, control; C, acute heparin-Intralipid; ISR, insulin secretion rate. *Significantly different from B, P < 0.001; †significantly different from B, P < 0.05; ‡significantly different from A, P < 0.001; ‡‡significantly different from A, P < 0.05.

The prolonged elevation of plasma FFA levels produced the expected decrease in whole body insulin sensitivity that has been previously described (9, 10, 39). An interesting observation is that although the ISR was not decreased by the chronic elevation of FFA levels, it was inappropriately low when corrected for the ambient degree of insulin sensitivity. The relationship between ISR and Si is hyperbolic, such that in a given population the product of Si and ISR (DI) is constant (3, 23). In individuals with normal β-cell function, a decline in Si should be followed by a compensatory increase in ISR, thus maintaining the ability of the body to dispose of glucose (3). This relationship suggests that insulin sensitivity and insulin secretion are linked through a negative feedback loop, and in situations such as type 2 diabetes or other cases in which β-cell function is defective and cannot fully compensate for the decline in Si, there is a concomitant decline in DI. The DI was significantly lower with prolonged elevation of plasma FFA levels in the two-step hyperglycemic clamp protocol, suggesting that there was failure of the normal compensatory pancreatic hypersecretion of insulin in the face of a reduction in Si induced by the elevation of FFA. Although Si also tended to decrease when FFA were elevated acutely, DI was not different from the control experiment because there was an appropriate elevation of ISR, which apparently compensated for the reduction in Si. We cannot exclude that ISR compensated for the mild reduction in Si induced by acute heparin-Intralipid but was unable to compensate for the greater reduction in Si induced by 48-h heparin-Intralipid infusion.

A critical issue is whether the decrease in DI occurs as a direct result of the effect of FFA on the β-cell or secondary to the concomitant decline in insulin clearance. Given that we observed a substantial decrease in insulin clearance with FFA elevation, which would have resulted in peripheral hyperinsulinemia if DI had not declined, the decline in DI may well be viewed as an adaptive response to the potential peripheral hyperinsulinemia. Likewise, the decline in insulin clearance may have occurred as a direct result of FFA on the hepatocyte and other tissues or may have been secondary to the decline in DI. We have no way of resolving the question whether the decline in DI was adaptive or maladaptive, either from our own data or by examination of the literature. However, in our studies in rats, prolonged intravenous oleate infusion diminished GSIS without apparently affecting insulin clearance (30). Also, we would argue that the slight, but significant, elevation of blood glucose that occurred with FFA elevation suggests that the decrease in Si was not perfectly countered by changes in insulin clearance or DI. In other words, FFA, either directly or indirectly, disable the β-cell compensation to the concomitant reduction in Si. One condition that is also associated with decreased Si, insulin clearance, and DI is type 2 diabetes. The hyperglycemia of type 2 diabetes represents a more extreme example of what we would consider to be failure of the β-cell to compensate for a decline in Si. Although this issue of adaptive vs. maladaptive response cannot readily be resolved, one should not lose sight of the very clear evidence from our studies that chronically elevated FFA in vivo are not stimulatory to the β-cell, as has been proposed by others to explain the hyperinsulinemia of insulin-resistant states (6–8).

The latter work, by Boden et al. (8), showed that a ninefold elevation in plasma FFA concentration during

Fig. 6. Mean ISR in 5 patients who underwent acute heparin-Intralipid study with 2-step hyperglycemic clamp (open bars), 48-h heparin-Intralipid study (gray bars), and control study (filled bars). Mean ISR was similar at baseline in all 3 studies and was significantly higher at 20 mmol/l of glucose in acute heparin-Intralipid study vs. 2 other studies (P < 0.001). Mean ISR was significantly higher in acute than in 48-h heparin-Intralipid study (P < 0.05) at 10 mmol/l, but difference between acute heparin-Intralipid study and control study did not reach statistical significance at this level of hyperglycemia.
an Intralipid and heparin intravenous infusion compared with their control experiment over a 48-h period in healthy volunteers produced persistent hypersecretion of insulin under prolonged hyperglycemic clamp conditions (glucose ~ 8.6 mmol/l for 48 h). These investigators did not assess the insulin secretory response to acute changes in plasma glucose, as was done in the present study. It should also be noted that their subjects were fasting for the 48-h study period, although glucose was infused throughout. In view of the results in rats and humans showing the FFA dependency of GSIS in the prolonged fasting state (18, 19, 42, 43) plus the very low fasting FFA levels caused by glucose-induced hyperinsulinemia in the control group in Boden’s study, it is possible that this condition favored a maintenance of the difference between their heparin-Intralipid study vs. control, mainly by inducing a decrease in insulin secretion in the control group. Boden et al. also did not interpret the insulin secretory rate in their study in relation to changes in insulin sensitivity with elevated FFA, as we did.

Our findings agree more with those of Paolisso et al. (33), who reported that raising plasma FFA threefold with a short-term (6 h) Intralipid and heparin infusion, administered to healthy subjects, was associated with a significant increase in the acute insulin response to an intravenous glucose tolerance test. In contrast, prolongation of the infusion of heparin and Intralipid to 24 h was associated with an absolute inhibition of the acute insulin response to intravenous glucose. It is important to note that Paolisso et al. assessed insulin secretory response by calculating the incremental area of postglucose insulin levels. They did not measure plasma C-peptide levels nor did they calculate insulin secretory response as we did in the present study. We cannot fully explain why they observed an absolute decrease in GSIS after 24 h, whereas we found only a relative decrease in GSIS after 48-h elevation of plasma FFA, but it should also be noted that their FFA levels were higher than in our study and that they only studied the first phase acute insulin response to intravenous glucose.

The acute stimulatory effect of FFA on GSIS has been demonstrated both in vitro (15, 51) and in vivo (33), and its mechanism has been reviewed recently (37). The effect of prolonged FFA exposure on β-cell function is more controversial, although in vitro studies with cells from humans and rodents have shown an inhibitory effect on GSIS (11, 25, 28, 29, 40, 53, 54) at high physiological and pathological levels of glucose in the medium. These findings are in contrast to our results in vivo in humans, which showed only a relative FFA-mediated decrease in GSIS, in relation to the reduction in whole body insulin sensitivity. A direct comparison between in vivo and in vitro studies is limited for many reasons, not the least of which are the complex relationships that occur in vivo between insulin sensitivity, clearance, and secretion.

Although we did not measure the plasma free glycerol levels in our study, they were likely elevated with the heparin-Intralipid infusion, as we have previously shown (26). Previous in vitro studies have shown that glycerol fails to stimulate insulin secretion because of the low levels of glycerol kinase activity in islet cells (31). Therefore, it is unlikely that an increase in plasma glycerol could have mediated the increase in GSIS seen in the acute heparin-Intralipid study. In addition, glycerol infusion in fasting humans has been shown not to significantly increase endogenous glucose production (22) and fails to alter the ability of insulin to suppress hepatic glucose production (38, 41).

The elevated insulin levels in response to the glucose infusion in the present 48-h heparin-Intralipid infusion study were due to diminished insulin clearance and not to enhanced insulin secretion. Insulin clearance is mediated through the binding of the hormone to its receptor (20). Fatty acids have been shown to rapidly reduce insulin binding and degradation in isolated rat hepatocytes in a concentration-dependent manner, without changing the apparent receptor affinity (46). High concentrations of FFA in the portal blood are correlated with a decreased clearance of insulin in rat liver (44), and FFA added to the perfusate of in situ perfused rat liver reduces hepatic insulin clearance over the normal physiological range (47). This effect of FFA is probably mediated by fatty acid oxidation in the liver (45, 46). We have recently shown that heparin-Intralipid infusion in dogs reduces first-pass hepatic insulin extraction at both high and low insulin levels (52). Our data in humans are in accordance with those of Hennes et al. (21), who also demonstrated FFA suppression of endogenous insulin clearance in humans.

In conclusion, we have demonstrated that 1) both acute and chronic FFA elevations induce insulin resistance; 2) chronic but not acute elevation of FFA is associated with diminished insulin clearance, which results in peripheral hyperinsulinemia; 3) with acute FFA elevation, this insulin resistance is precisely countered by an FFA-induced increase in insulin secretion, such that DI does not change; and 4) chronic FFA elevation disables this β-cell compensation by an unknown mechanism. We are unable to determine whether the chronic effect of FFA on the β-cell is an adaptive or a maladaptive response, given the hyperinsulinemia that results from the concomitant reduction in insulin clearance, or whether this effect is likely to play a role in the β-cell dysfunction that is characteristic of type 2 diabetes. These important questions will have to be answered in future studies.

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