Effects of Fatty Acids and Ketone Bodies on Basal Insulin Secretion in Type 2 Diabetes

Guenther Boden and Xinhua Chen

The objective of this study was to assess the role of free fatty acids (FFAs) as insulin secretagogues in patients with type 2 diabetes. To this end, basal insulin secretion rates (ISR) in response to acute increases in plasma FFAs were evaluated in patients with type 2 diabetes and in age- and weight-matched nondiabetic control subjects during *1*) intravenous infusion of lipid plus heparin (L/H), which stimulated intravascular lipolysis, and *2*) the FFA rebound, which followed lowering of plasma FFAs with nicotinic acid (NA) and was a consequence of increased lipolysis from the subject's own adipose tissue. At comparable euglycemia, diabetic patients had similar ISR but higher plasma β-hydroxybutyrate (β-OHB) levels during L/H infusion and higher plasma FFA and β-OHB levels during the FFA rebound than nondiabetic control subjects. Correlating ISR with plasma FFA plus β-OHB levels showed that in response to the same changes in FFA plus β-OHB levels, diabetic patients secreted ~30% less insulin than nondiabetic control subjects. In addition, twice as much insulin was secreted during L/H infusion as during the FFA rebound in response to the same FFA/β-OHB stimulation by both diabetic patients and control subjects. Glycerol, which was present in the infused lipid (272 mmol/l) did not affect ISR. We concluded that *1*) assessment of FFA effects on ISR requires consideration of effects on ISR by ketone bodies; *2*) ISR responses to FFA/β-OHB were defective in patients with type 2 diabetes (partial β-cell lipid blindness), but this defect was compensated by elevated plasma levels of FFAs and ketone bodies; and *3*) approximately two times more insulin was released per unit change in plasma FFA plus β-OHB during L/H infusion than during the FFA rebound after NA. The reason for this remains to be explored.
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Free fatty acids (FFAs) have emerged as an important link between obesity, insulin resistance, and type 2 diabetes based on the observations that plasma FFA levels are commonly elevated in obesity (1,2) and that elevation of plasma FFAs produces peripheral insulin resistance in a dose-dependent manner in healthy nonpregnant (3,4), pregnant (5), and diabetic subjects (6). FFAs not only inhibit insulin action, however; they also stimulate insulin secretion. While it has been known for some time that FFAs can acutely stimulate insulin secretion (7–11), it has only recently been recognized that FFAs have also prolonged stimulatory action on insulin secretion. For instance, in normal subjects, increased plasma FFA concentrations have been demonstrated to potentiate glucose-stimulated insulin secretion for 48 h (12), and basal FFA concentrations have been shown to support approximately one-third of basal insulin secretion rates (13). In rats, elevated plasma FFA levels have been demonstrated to strongly increase insulin secretion (14) and to be absolutely essential for the increase in insulin secretion that occurs after refeeding of fasting animals (15). Based on these findings, we have proposed (16) that in normal obese subjects, increased plasma FFA concentrations stimulate insulin secretion in amounts sufficient or nearly sufficient to compensate for the FFA-induced insulin resistance. Hence, these individuals have either no deficit or only a small deficit between FFA-mediated insulin resistance and insulin secretion. In contrast, obese subjects who are genetically predisposed to develop type 2 diabetes will gradually fail to secrete appropriate amounts of insulin in response to FFAs and therefore will develop increasingly larger FFA-induced insulin resistance/secretion deficits. These deficits will have to be compensated by an increase in plasma glucose. Since their β-cells are at least partially glucose-blind, the rise in plasma glucose needed to compensate for the FFA-mediated insulin resistance/secretion deficit will grow increasingly larger and eventually result in diabetes (16). This hypothesis predicts that patients with fully developed type 2 diabetes will have defective insulin secretory responses to FFAs. Whether this is the case is not known because there is currently no published information on the effects of elevated plasma FFAs on insulin secretion in patients with type 2 diabetes. It was the goal of the present study, therefore, to evaluate insulin secretory responses to elevation of plasma FFA levels in patients with type 2 diabetes and in age- and weight-matched nondiabetic control subjects. Elevated FFA levels were produced *1*) by intravenous infusion of lipid/heparin (L/H), which results in rapid intravascular lipolysis of the infused lipid, and *2*) as a result of the FFA rebound, which occurs predictably after discontinuation of nicotinic acid (NA) administration. During the course of these studies, it became apparent that evaluating effects on insulin secretion rate (ISR) of FFAs alone was not sufficient and that ketone bodies, which are major FFA metabolites, needed to be considered as well.

RESEARCH DESIGN AND METHODS

Subjects. Some characteristics of the patients with type 2 diabetes, participating in five different studies, and of the nondiabetic control subjects, participating in two studies, are shown in Table 1. All diabetic patients had been treated with

From the Division of Endocrinology/Diabetes/Metabolism and the General Clinical Research Center, Temple University School of Medicine, Philadelphia, Pennsylvania.

Address correspondence and reprint requests to Guenther Boden, MD, Temple University Hospital, 3401 North Broad St., Philadelphia, PA 19140.

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FFA, free fatty acid; ISR, insulin secretion rate; L/H, lipid/heparin; NA, nicotinic acid; β-OHB, β-hydroxybutyrate.

oral hypoglycemic agents (sulfonylureas or biguanides or both), and some received in addition small doses of NPH insulin (5–20 U) at bedtime. These medications were withheld starting 1–3 days before the studies. The patients' body weights were stable for at least 2 months, and their diets contained a minimum of 250 g/day of carbohydrates for at least 2 days before the studies. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of the study. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Experimental design. All subjects were admitted to Temple University Hospital's General Clinical Research Center on the day before the studies. The studies began at ~8:00 A.M. after an overnight fast, with the subjects reclining in bed. A short polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood. Seven different types of studies using two different types of glucose clamps were performed (Table 1).

Study 1: L/H infusion during isoglycemia. Liposyn II (10% soybean and 10% safflower oil) plus heparin (0.4 U · kg^{-1} · min^{-1}) were infused at a rate of 1.5 ml/min for 4 h in 15 patients with type 2 diabetes. Their plasma glucose concentrations were maintained at their prevailing basal level (isoglycemia, ~11 mmol/l).

Study 2: saline infusions during isoglycemia. Saline instead of L/H was infused in 10 patients with type 2 diabetes. These studies served as controls for the L/H and glycerol studies.

Study 3: glycerol infusions during isoglycemia. Glycerol (24.5 mmol/h) was infused in six patients with type 2 diabetes. This dose, the same as that infused during L/H infusions, was needed as a control for the high content of glycerol in the Liposyn II emulsions (272 mmol/l).

Studies 4 and 5: L/H infusions during euglycemia. L/H was infused in six patients with type 2 diabetes (study 4) and in seven nondiabetic control subjects (study 5) under euglycemic conditions. To achieve the same plasma FFA levels in the patients with type 2 diabetes as in the nondiabetic control subjects, L/H was infused at reduced rates (0.75–1.0 ml/min). Those rates were adjusted as needed during each study.

Studies 6 and 7: NA administration during euglycemia. NA was given by mouth (100 mg at 0 and 30 min; 150 mg at 60, 90, 120, 150, and 180 min; and 100 mg at 210 and 240 min) to six patients with type 2 diabetes (study 6) and to five nondiabetic control subjects (study 7) under euglycemic clamp conditions. We have recently shown that NA given in these doses has no effect on ISR (13). NA caused facial and/or neck flushing during the first 1–3 doses in all subjects and mild, transient nausea in three subjects.

Euglycemic clamps. To lower plasma glucose in patients with type 2 diabetes from the prevailing hyperglycemia (~11 mmol/l) into the euglycemic range, small doses of regular human insulin (0.5–1.5 U/h) were infused intravenously for several hours before the start of the experiments. Insulin infusions were discontinued at –90 min, and glucose concentrations were clamped at ~5.0 mmol/l starting at 0 min for 4 h (by a feedback-controlled glucose infusion, if necessary).

Isoglycemic clamps. Isoglycemic clamp studies were performed in patients with type 2 diabetes. Glucose concentrations were clamped for 4 h at the patients' prevailing postabsorptive glucose concentrations (9.4–11.1 mmol/l) by a feedback-controlled glucose infusion.

C-peptide kinetics. Approximately 1 week before the studies, a 50-nmol IV bolus of biosynthetic human C-peptide (Eli Lilly, Indianapolis, IN) was administered to each subject after an overnight fast, plasma C-peptide concentrations were measured, and C-peptide kinetic parameters were calculated at frequent intervals for 3 h as described by Polonsky et al. (17).

Insulin secretory rates. The C-peptide kinetic parameters were used to calculate prehepatic ISRs for each time interval between successive blood samples by deconvolution of peripheral C-peptide concentrations according to Polonsky et al. (17) and Eaton et al. (18).

Analytical procedures. Plasma glucose was measured by the glucose oxidase method using a glucose analyzer. Serum free insulin was determined after deproteinization (19) by radioimmunoassay with a specific antibody that cross-reacts only minimally (<0.2%) with proinsulin (Linco, St. Charles, MO). C-peptide was determined by radioimmunoassay (Linco). Assay sensitivity was 0.05 ng/ml. The antiserum cross-reacted <4% with human proinsulin and did not cross-react with human insulin, glucagon, somatostatin, or pancreatic polypeptide. Intra- and interassay coefficients of variation were <4 and <7%, respectively. Total plasma fatty acids were determined enzymatically in chilled plasma containing EDTA and 0.275 mg/ml Paroxon (Sigma, St. Louis, MO), a lipoprotein lipase inhibitor, with a kit from Wako (Richmond, VA). Four individual fatty acids (palmitate C16, stearate C18, oleate C18:1, and linoleate C18:2) were measured by gas chromatography (Model 5730A; Hewlett-Packard, Palo Alto, CA) using heptadecanoic acid (C17) as internal standard and a Rtx-225 column (30 m long, ID 0.32 mm; Resteck, Bellfonte, PA). β-Hydroxybutyrate (β-OHB) was measured enzymatically.

Body composition. Body composition was determined by bioimpedance analysis (20).

Statistical analysis. All data are expressed as means ± SE. Analysis of variance with repeated measures was used to determine differences in β-OHB and ISR during L/H infusion across all time points. Correlations of FFA and β-OHB concentrations and changes of ISR were assessed by least-square linear regression. Slopes of regression lines from different experimental groups were compared by comparing (with the Student t test) individual correlation coefficients (r). Paired comparison between baseline and study intervals were performed using the paired t test or the Wilcoxon signed rank test where appropriate.

RESULTS

Effects of L/H, saline, or glycerol on FFAs and ISR in isoglycemic patients with type 2 diabetes. To test whether pancreatic β-cells responded at all to rising plasma FFA levels, L/H was infused intravenously for 4 h in 15 patients with type 2 diabetes. As a control, saline was infused instead of L/H in 10 age- and weight-matched diabetic patients. Glycerol was infused in another six patients as a control for the large amount of glycerol present in Liposyn II (272 mmol/l).

Glucose was clamped at isoglycemic levels—the patient's postabsorptive blood glucose concentrations (~10–11 mmol/l)—in all three studies. Plasma FFAs increased during

TABLE 1
Subjects and protocols

	Isoglycemic clamps			Euglycemic clamps			
	Study 1 (type 2 diabetic patients)	Study 2 (type 2 diabetic patients)	Study 3 (type 2 diabetic patients)	Study 4 (type 2 diabetic patients)	Study 5 (control subjects)	Study 6 (type 2 diabetic patients)	Study 7 (control subjects)
Infusion	L/H	Saline	Glycerol	L/H	L/H	NA	NA
Sex (M/F)	3/12	2/8	3/3	2/4	5/2	1/5	2/3
Age (years)	62 ± 2	61 ± 3	63 ± 5	64 ± 3	60 ± 5	67 ± 4	61.3 ± 3
Height (cm)	163 ± 2.6	164.1 ± 3.4	159.8 ± 4.7	160.5 ± 4. 3	171.0 ± 3	159.2 ± 4.7	166.0 ± 4.1
Weight (kg)	87.7 ± 3.6	86.4 ± 4.4	78.1 ± 8.7	80.9 ± 7.6	86.9 ± 6.8	80.3 ± 8.3	82.8 ± 4.3
Body fat (%)	31.2 ± 2.1	36.2 ± 1.7	30.3 ± 3.7	36.2 ± 3.4	28.7 ± 3.9	34.5 ± 4.4	33.0 ± 3.4
BMI (kg/m^2)	31.8 ± 0.9	32 ± 1.2	30 ± 1.8	31.0 ± 1.5	29.5 ± 1.4	31.2 ± 1.6	30.0 ± 0.9
Duration of diabetes (years)	14.6 ± 2.5	18.4 ± 2.8	22.5 ± 5.2	13.5 ± 2.7	—	14.0 ± 5.1	—

Data are n or means ± SE.

L/H (from 504 ± 48 to 1,173 ± 205 µmol/l, $P < 0.001$) and did not change significantly in the saline (from 585 ± 68 to 498 ± 93 µmol/l, NS) and glycerol (from 605 ± 125 to 434 ± 102 µmol/l, NS) control subjects (Fig. 1). Plasma glycerol increased similarly during L/H (from 87 ± 10 to 597 ± 76 µmol/l, $P < 0.001$) and during glycerol infusion (from 67 ± 11 to 485 ± 61 µmol/l, $P < 0.001$). ISR increased during L/H (from 205 ± 46 to 322 ± 54 pmol/min, $P < 0.002$) and did not change during saline or glycerol infusions (Fig. 1). These results demonstrated that glycerol, infused in the same amounts as during L/H infusion, was not an insulin secretagogue. They also showed that diabetic patients could release insulin in response to FFA. To determine whether these FFA-induced ISR responses were normal or not, it became necessary to compare them with ISR responses in nondiabetic control subjects.

Effects of L/H on FFAs, β-OHB, and ISR in euglycemic patients with type 2 diabetes. We performed a second study in which L/H was infused under identical euglycemic conditions (~5 mmol/l) in six diabetic patients and seven nondiabetic control subjects matched for age and weight. In this study, plasma FFA concentrations rose comparably in response to L/H infusion in patients with type 2 diabetes (from 598 ± 66 to 1,301 ± 92 µmol/l, $P < 0.001$) and in control subjects (from 447 ± 46 to 1,115 ± 72 µmol/l, $P < 0.001$) (Fig. 2). β-OHB levels, however, rose higher in diabetic patients (from 240 ± 100 to 1,450 ± 290 µmol/l) than in nondiabetic control subjects (from 90 ± 30 to 690 ± 160 µmol/l). ISR rose equally in diabetic patients (from 104 ± 36 to 236 ± 51 pmol/min, $P < 0.01$) and control subjects (from 107 ± 6 to 257 ± 36 pmol/min, $P < 0.01$). Serum insulin concentrations rose from 54 ± 22 to 77 ± 27 pmol/l ($P < 0.05$) in diabetic patients and from 79 ± 11 to 130 ± 18 pmol/l ($P < 0.05$) in control sub-

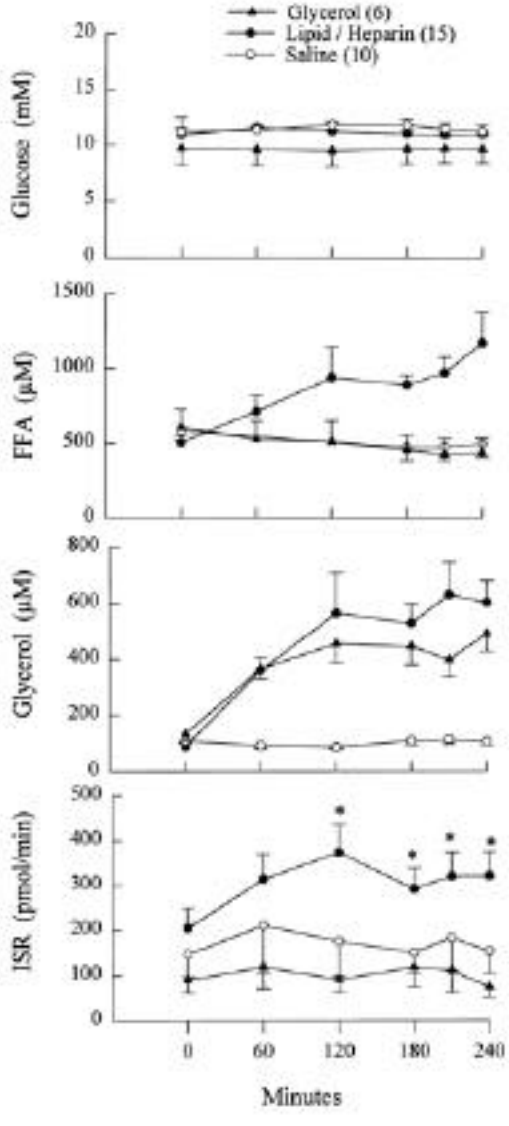

FIG. 1. Effects of 4 h (0–240 min) infusions of L/H (n = 15), saline (n = 10), or glycerol (n = 6) under isoglycemic clamp conditions on basal plasma FFA and glycerol concentrations and on prehepatic ISRs in patients with type 2 diabetes. Curves of FFA during L/H and of glycerol during L/H and glycerol infusions all rose significantly ($P < 0.01$ by analysis of variance) above baseline. Data shown are means ± SE. *$P < 0.002$ compared with basal values.

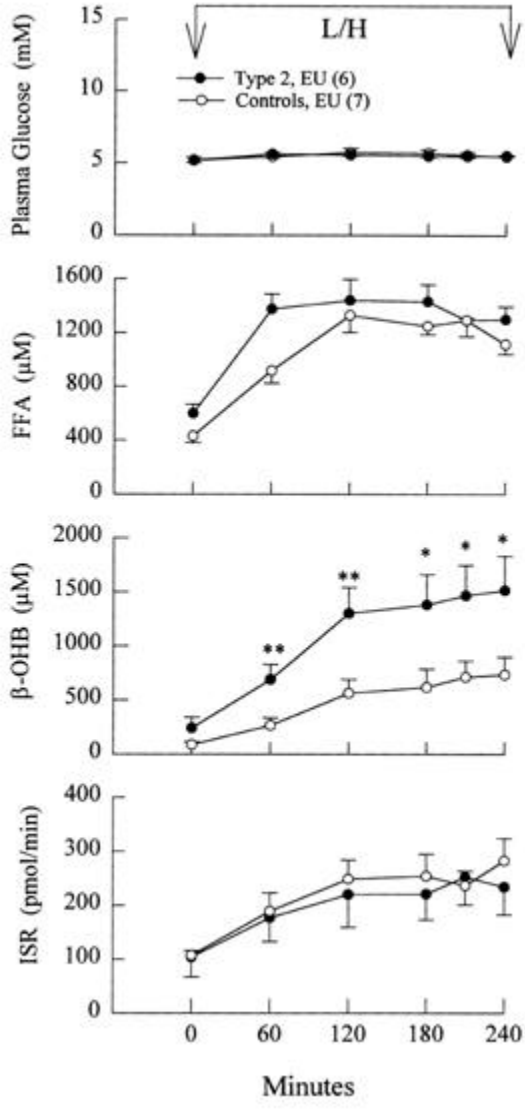

FIG. 2. Effects of L/H infusion under euglycemic clamp conditions on basal plasma FFA, β-OHB, and basal ISRs in patients with type 2 diabetic and nondiabetic control subjects. FFA and ISR curves in the two groups were not different from each other. *$P < 0.05$, **$P < 0.02$ for β-OHB in type 2 diabetes vs. control values.

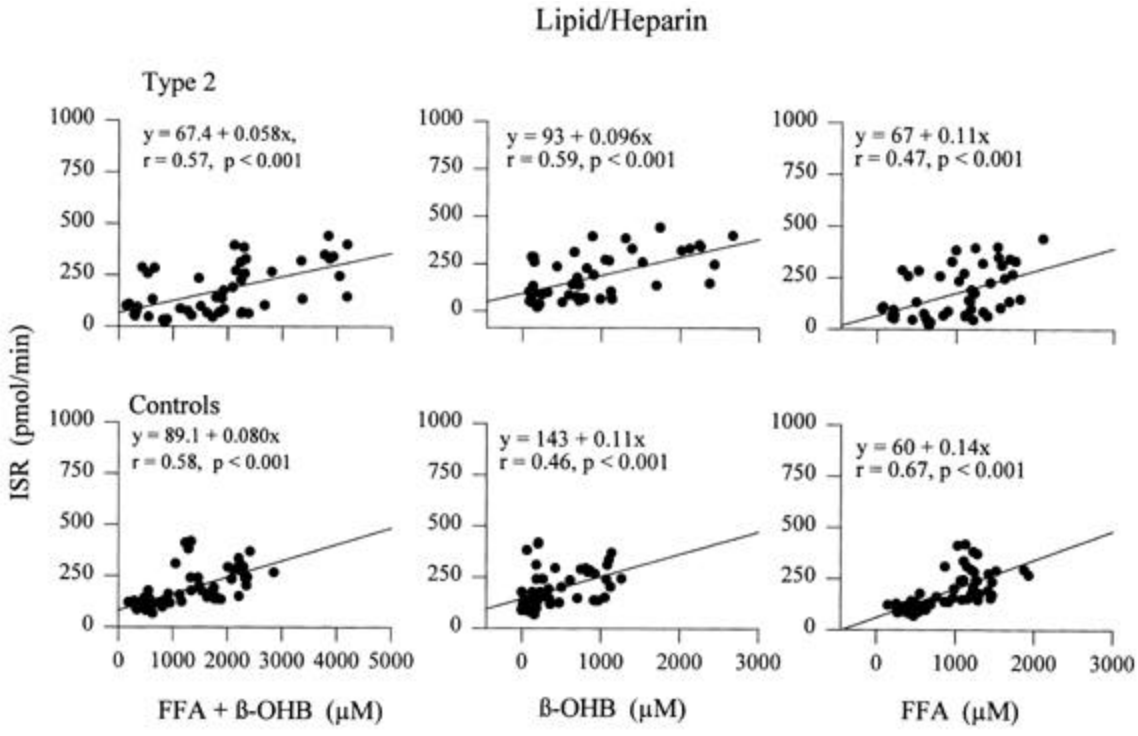

FIG. 3. Correlations between basal ISRs and plasma FFA plus β-OHB, β-OHB, and FFA concentrations in patients with type 2 diabetes (n = 6) and control subjects (n = 7) in response to L/H infusion (0–240 min). The slopes of the regression lines were significantly greater in control subjects than in diabetic patients only for ISR versus FFA plus β-OHB (0.090 ± 0.001 vs. 0.055 ± 0.001, P < 0.05) but not for ISR versus β-OHB or ISR versus FFA (P > 0.05). These results were obtained by comparison of individual correlation coefficients (r).

jects (Fig. 2). The differences in insulin levels and ISR between diabetic patients and control subjects were not significant. These results showed that diabetic patients had similar ISR responses to an FFA challenge as control subjects but at significantly higher β-OHB levels.

Since ketone bodies are known to stimulate insulin secretion in humans and animals (21–24), the data suggested that without the elevated β-OHB levels, ISR responses would have been lower in patients with type 2 diabetes than in control subjects. In fact, when ISRs were correlated with plasma FFA plus β-OHB levels, it was found that diabetic patients secreted ~28% less insulin than control subjects in response to the same stimuli (5.8 vs. 8.0 pmol/min per 100 µmol/l increase in plasma FFA plus β-OHB, P < 0.05) (Fig. 3). There were no significant differences in the slopes of the regression lines between type 2 diabetes and control subjects when ISRs were correlated with plasma β-OHB alone or with plasma FFA alone (Fig. 3).

Effect of FFAs released from adipose tissue on FFAs, β-OHB, and ISR. Raising plasma FFA levels by intravenous infusion of lipid and heparin, as was done in this and many other studies, is not an entirely physiologic approach because of the rather unusual composition of the lipid (see below). We have used another, and we believe novel, method, in which plasma FFA levels were increased as a result of increased lipolysis from the subject's own adipose tissue. To this end, plasma FFA levels were first lowered with NA (given every 30 min for 4 h) to <100 µmol/l in six patients with type 2 diabetes and in five control subjects under euglycemic clamp conditions. Within 2 h after discontinuation of NA, there was a sharp rise in plasma FFAs in all subjects (FFA rebound) accompanied by a smaller rise in β-OHB (Fig. 4). This provided an opportunity to assess effects on ISR of FFAs released from the study subjects' own adipose tissue, uncomplicated by changes in the pattern of individual plasma FFA concentrations.

The results showed that during the FFA rebound, patients with type 2 diabetes had similar increases in ISR to control subjects (46 ± 18 vs. 53 ± 21 pmol/min) but at considerably higher β-OHB (545 ± 78 vs. 260 ± 60 µmol/l, P < 0.05) and FFA (1,698 ± 98 vs. 891 ± 115 µmol/l, P < 0.05) levels (Fig. 4). These results were quantitatively similar to the results obtained during the L/H infusions, since ISR responses were ~39% lower in diabetic patients than in control subjects (increases in ISR of 2.8 vs. 4.6 pmol/min per 100 µmol/l increase in plasma FFA plus β-OHB), although the difference failed to reach statistical significance (P > 0.05) (Fig. 5). We interpreted these results to mean that patients with type 2 diabetes secreted less insulin than nondiabetic control subjects in response to comparable plasma FFA plus ketone body levels.

Individual plasma FFAs during L/H and during the FFA rebound. Liposyn has a high content of linoleic acid (65.8%), and its infusion was likely to produce an atypical pattern of individual plasma FFAs, which may have influenced ISR. Therefore, concentrations of the four major FFAs (palmitic, stearic, oleic, and linoleic acids) were measured before and after L/H infusion and the FFA rebound. Basal concentrations of these four FFAs, expressed as percentage of total FFAs, did not change significantly during the FFA rebound (Fig. 6*B*). L/H infusion, on the other hand, more than doubled plasma linoleic acid (from 11.8 ± 1.4 to 25.7 ± 2.2%, P < 0.001) and correspondingly decreased the other three fatty acids (oleic acid from 25.9 ± 1.4 to 20.1 ± 1.5%, P < 0.01; stearic acid from 18.1

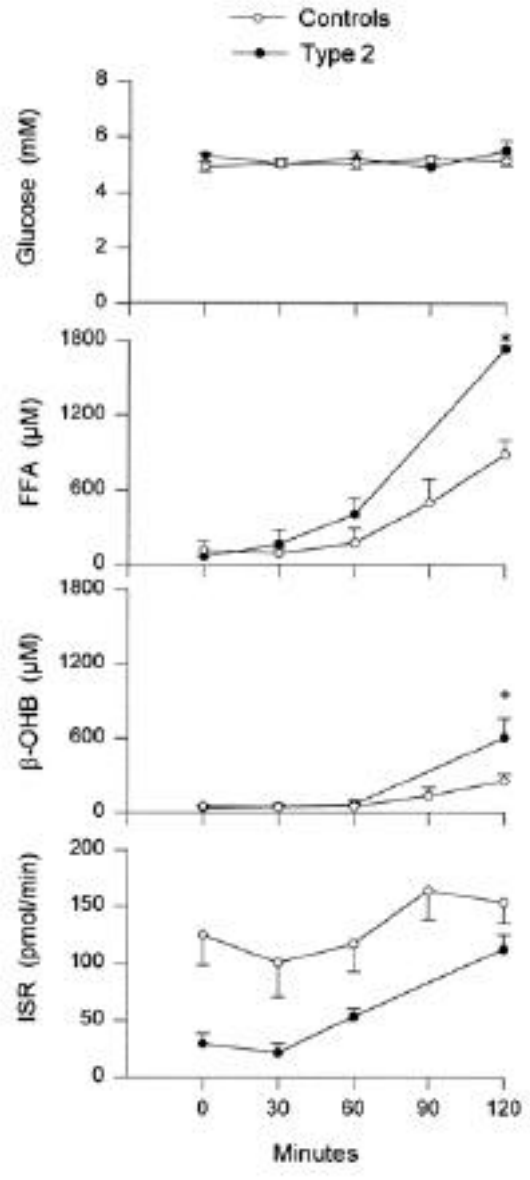

FIG. 4. ISR, plasma FFAs, and β-OHB in euglycemic patients with type 2 diabetes (n = 6) and control subjects (n = 5) during the FFA rebound after discontinuation of NA (at 0 min). In diabetic patients, blood was collected at 0, 30, 60, and 120 min only. *P < 0.05 for diabetic patients vs. control subjects.

± 1.5 to 15.3 ± 0.5%, P < 0.05; palmitic acid from 26.6 ± 1.0 to 21.0 ± 1.2%, P < 0.05) (Fig. 6*A*). The reason for these changes was the composition of the infused Liposyn II, which was very rich in linoleic acid and correspondingly lower in the other three fatty acids (C18:2, 65.8%; C18:1, 17.7%; C18:0, 3.4%; and C16:0, 8.8%). Thus, after 2 h of L/H infusion, the percentage of contribution to total plasma FFA of linoleic acid was 2.5-fold higher, that of oleic acid was 33% lower, that of stearic acid was about equal, and that of palmitic acid was 24% lower compared with the FFA rebound (Fig. 6*C*).

DISCUSSION

The main objective of this study was to examine the relationship between plasma FFAs and insulin secretion rates in patients with type 2 diabetes. Because there was no published information on this issue, it was necessary to test first

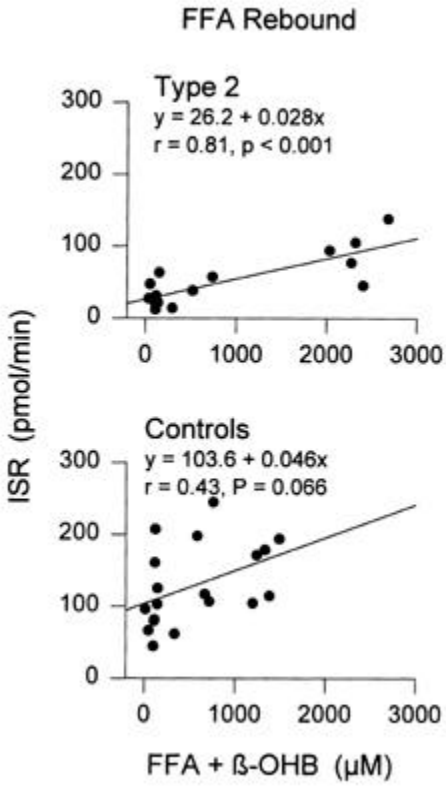

FIG. 5. Correlations between plasma FFA plus β-OHB concentrations and ISRs in patients with type 2 diabetes (n = 6) and control subjects (n = 5) during the FFA rebound after discontinuation of NA. The difference in the slopes of the regression lines was not statistically significant (0.054 ± 0.018 vs. 0.028 ± 0.006, P > 0.05).

whether patients with type 2 diabetes were able to increase ISR in response to FFAs. When plasma FFAs were raised (from 504 ± 48 to 1,173 ± 205 μmol/l) and glucose was clamped at isoglycemic levels (~11 mmol/l), ISR increased from 205 ± 46 to 322 ± 54 pmol/min (P < 0.002). This increase suggested that diabetic patients could release insulin in response to FFAs. In fact, the increase in ISR in these older diabetic patients was comparable to that reported previously by us in younger (25 ± 4.1 years), nonobese (BMI 23.4 ± 0.4) men in whom an increase in plasma FFAs from ~600 to ~1,200 μmol/l produced an increase in ISR from ~240 to ~350 pmol/min (12). Because these control subjects were younger, leaner, and had slightly lower blood glucose levels than the diabetic patients of the current study, the data did not prove that patients with type 2 diabetes had normal insulin responses to an increase in plasma FFAs. It was therefore necessary to perform a second study, in which ISR responses could be compared under comparable euglycemic conditions in diabetic patients and age- and weight-matched nondiabetic control subjects. In that study, plasma FFA levels were raised to ~1,100–1,300 μmol/l in diabetic and nondiabetic subjects by intravenous infusion of L/H (which acutely increased intravascular lipolysis), whereas plasma glucose was maintained at euglycemic levels. Under those conditions, ISR rose similarly by 130–150 pmol/min in both groups. β-OHB levels, however, rose higher in diabetic than in nondiabetic subjects. Since ketone bodies stimulate insulin secretion (21–24), the data suggested that diabetic patients would have secreted less insulin than nondiabetic control subjects had they been exposed to comparable plasma

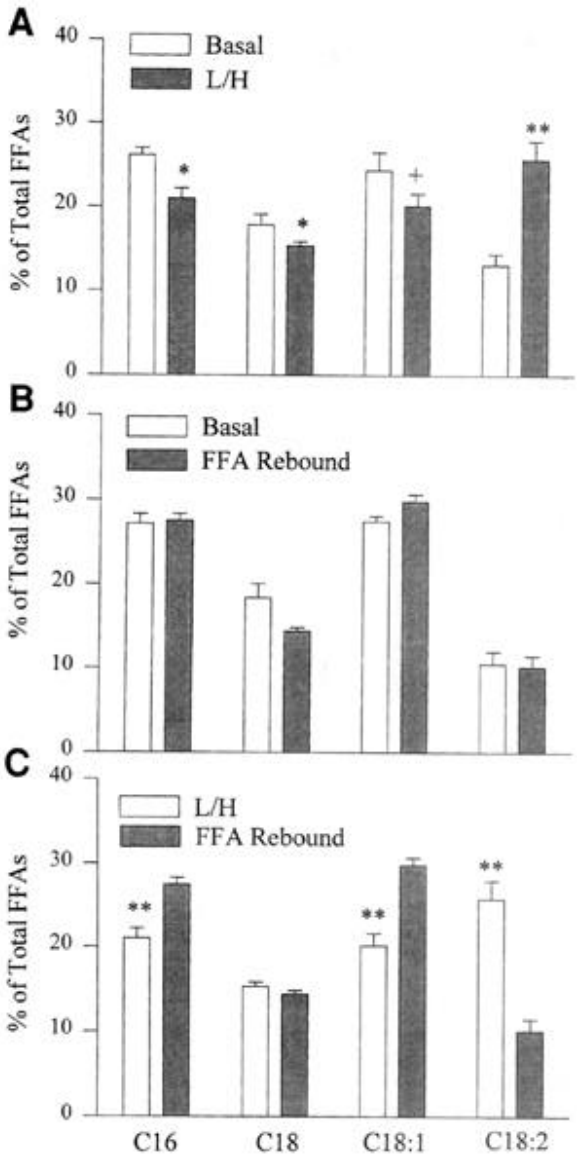

FIG. 6. Four individual FFAs, shown as percentage of total plasma FFAs before and during L/H (*A*), before and during the FFA rebound (*B*), and during the last 2 h of L/H and FFA rebound (*C*). C:16, palmitic acid; C:18, stearic acid; C:18:1, oleic acid; C:18:2, linoleic acid. **P* < 0.05; +*P* < 0.01; ***P* < 0.001.

FFA and β-OHB levels. Indeed, correlating ISR with plasma FFA plus β-OHB levels indicated that patients with type 2 diabetes secreted ~28% less insulin than nondiabetic control subjects in response to the same FFA/β-OHB stimulation (5.8 vs. 8.0 pmol/min per 100 μmol/l change in plasma FFA/β-OHB, $P < 0.05$) (Fig. 3). Moreover, loss of responsiveness to FFA plus β-OHB correlated with the duration of diabetes such that maximally FFA plus β-OHB–stimulated ISRs decreased by ~6 pmol/min for every year of known diabetes (ΔISR = 275 – 6.34 years, $r = 0.43$, $P < 0.05$).

The L/H infusions, however, were associated with several potential problems. First, the commercial lipid emulsion (Liposyn II) used in these and many other studies contained a large amount of free glycerol, raising the possibility that this infused glycerol was at least partially responsible for the observed increase in ISR. This possibility could be excluded by showing that glycerol infused in the same doses as L/H did not affect ISR (Fig. 1). Another concern was the high content of linoleic acid in Liposyn II (65.8%). As expected, infusion of Liposyn II produced a doubling in plasma linoleic acid and a decrease in the other FFAs (Fig. 6). This was of concern because of a recent report demonstrating that different FFAs have vastly different effects on ISRs in rats (25), raising the possibility that the observed ISR changes during L/H infusion may have been skewed by the abnormal FFA composition of the infused Liposyn II. We therefore felt it necessary to validate the results with a different technique. This was done taking advantage of the FFA rebound that predictably follows the lowering of plasma FFAs with nicotinic acid. Using this technique, we found that during the FFA rebound, plasma FFAs and β-OHB rose to higher levels in diabetic than in nondiabetic subjects, whereas ISR increased similarly in both groups (Fig. 4). Hence, the results obtained during the FFA rebound were qualitatively very similar to those obtained during L/H infusions. Both methods showed that ISR responses to the same FFA plus β-OHB stimulation were on average ~30% lower in diabetic patients than in nondiabetic control subjects. Thus, it appeared that our patients with type 2 diabetes were partially "lipid blind" in addition to being partially "glucose blind."

Were these results compatible with the hypothesis (outlined in the introduction) that in subjects genetically predisposed to develop type 2 diabetes, FFAs will eventually fail to adequately stimulate insulin secretion? First, this hypothesis will now have to be amended to include ketone bodies. (Only β-OHB was determined in this study. In an earlier study, however, we reported that under identical experimental conditions, β-OHB accounted for 70–80% of total ketone bodies [26].) Second, whether FFAs/ketone bodies produce an increase in the insulin resistance/secretion ratio will depend primarily on whether ketone bodies produce insulin resistance. If they do, the insulin resistance/secretion ratio would rise and probably lead to hyperglycemia. If, on the other hand, ketone bodies stimulate ISR but don't affect insulin resistance, the result would likely be compensated by insulin resistance, i.e., hyperinsulinemia. Thus, further validation of this hypothesis will require examination of effects of ketone bodies on insulin resistance and relationships between FFA/ketone bodies and ISRs in "prediabetic" subjects at various stages of development of the disease and the demonstration of an increasing FFA/ketone body–mediated insulin resistance/insulin secretion deficit in such individuals.

We are not aware of previously published data on the effects of FFAs or ketone bodies on basal ISR in humans. Evidence is accumulating, however, that the role of FFAs as insulin secretagogues has not been fully appreciated in the past. For instance, we have recently found that basal plasma FFA levels were responsible for maintaining about one-third of basal ISR in normal volunteers (13). Stein et al. (15) have shown that FFAs were absolutely essential for ISR to increase on refeeding of fasted rats, and we and others have demonstrated that FFAs are potent long-term insulin secretagogues in human subjects and rats (12,14).

Whereas FFAs produced qualitatively similar insulin responses regardless of whether they were released from infused fat or from endogenous adipose tissue, there were nevertheless large quantitative differences. About two times more insulin was released per unit change in plasma FFA plus β-OHB during L/H than during the FFA rebound (Figs. 2 and

3 versus Figs. 4 and 5). The reason for the difference remains uncertain but may be related to differences in individual plasma FFA levels, specifically the linoleic acid levels, which were ~2.5-fold higher during L/H than during the FFA rebound (Fig. 6*C*). Stein et al. (25) have shown that at least in the isolated perfused rat pancreas, linoleic acid was by far the weakest insulin secretagogue of the four major fatty acids. That finding does not exclude the possibility, however, that linoleic acid may be a stronger insulin secretagogue in humans than in rats.

Last, it was noteworthy that regardless of whether plasma FFAs were raised by L/H infusion or as result of the FFA rebound, patients with type 2 diabetes compensated for their defective ISR responses by increasing plasma concentrations of FFAs or ketone bodies or both. While this could have been coincidental, it is more likely that it indicated operation of a feedback, such that β-cell lipid blindness in longstanding type 2 diabetes caused a compensatory increase in plasma FFA/ketone body concentrations in much the same way as glucose blindness causes a rise in blood glucose. The existence of such a feedback and its regulation need to be validated with more studies and in greater detail.

In summary, we found that ISR responses to FFA/β-OHB were partially defective in patients with type 2 diabetes (partial β-cell lipid blindness). This defect appeared to become more severe with longer duration of the disease but was compensated by elevated plasma levels of FFAs or ketone bodies or both.

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