Acute Lowering of Plasma Fatty Acids Lowers Basal Insulin Secretion in Diabetic and Nondiabetic Subjects

Guenther Boden, Xinhua Chen, and Nayyar Iqbal

The objective of this study was to determine whether basal plasma free fatty acid (FFA) concentrations affect basal insulin secretion rates (ISRs). Effects of FFA levels on basal ISRs were evaluated by lowering basal plasma FFA levels with nicotinic acid (NA) (100–150 mg p.o., q 30 min × 4 h) in type 2 diabetic patients and in normal volunteers. Lowering of FFAs (from ~600 to ~100 μmol/l) lowered ISRs in type 2 diabetic patients during isoglycemic clamping (from 139 to 101 pmol/min; –23%; $P < 0.02$) and euglycemic clamping (from 99 to 63 pmol/min; –36%; $P < 0.03$) and in normal subjects during euglycemic clamping (from 127 to 96 pmol/min; –25%; $P < 0.03$). In addition, peripheral insulin concentrations decreased by ~30% in diabetic and nondiabetic subjects. NA had no direct effect on ISRs; that is, NA did not change ISRs when plasma FFAs were prevented from decreasing with a lipid/heparin infusion. We concluded that *1*) basal plasma FFAs exerted physiologically important, long-lasting effects supporting 25–33% of basal insulin secretion in nondiabetic and diabetic subjects; *2*) basal plasma FFAs were responsible for some of the hyperinsulinemia in normoglycemic obese subjects; and *3*) NA had no direct effect on insulin secretion. *Diabetes* 47:1609–1612, 1998

Obesity is commonly associated with insulin resistance, which has been recognized as an important cardiovascular risk factor (1,2). The fact that insulin resistance increases with weight gain and decreases with weight loss (3–6) suggests that this is a cause-and-effect relationship. Moreover, plasma free fatty acids (FFAs) have been shown to play a pivotal role in the development of insulin resistance in obesity; FFAs are elevated in obesity (7,8), and elevations of plasma FFA have been shown to produce peripheral insulin resistance in a dosage-dependent manner in normal and diabetic subjects (9–11). Based on these data, we recently proposed that whether or not the FFA-mediated insulin resistance results in hyperglycemia depends largely on the ability of the FFAs to potentiate glucose-stimulated insulin secretion (12). Thus information on the effects of FFAs on insulin secretion is critically important, but unfortunately the issue of whether or not FFAs are physiological potentiators of insulin secretion remains controversial. On one hand, elevating plasma FFA levels in normal subjects has been shown to potentiate glucose-stimulated insulin secretion, both acutely (13–17) and for as long as 48 h (18). On the other hand, exposure to FFAs for >24 h has been reported to inhibit insulin secretion from isolated perfused rat pancreases and isolated rat islet cells (19,20). Based on these and other animal study data, elevated plasma FFA levels have been postulated to have long-term adverse effects on insulin secretion (lipotoxicity) (21).

In the current study, we examined the effects of acute lowering of plasma FFAs with nicotinic acid (NA) on basal insulin secretion rates (ISRs) in normal subjects and in type 2 diabetic patients. To minimize the effect of changes in plasma glucose on ISRs, all subjects were studied at their prevailing blood glucose levels—diabetic patients at hyperglycemia (as well as at euglycemia) and nondiabetic control subjects at euglycemia. We reasoned that if lowering plasma FFA levels could be shown to decrease ISRs, this would support the notion that FFAs are physiologically important, long-acting potentiators of insulin secretion.

RESEARCH DESIGN AND METHODS

Subjects. Characteristics of type 2 diabetic patients and control subjects who participated in two different studies are shown in Table 1. All diabetic patients had been treated with oral hypoglycemic agents (sulfonylureas and/or biguanides) and some received small doses of NPH insulin (5–20 U) at bedtime in addition. These medications were withheld starting 1 day before the studies. Patients' weight was stable for at least 2 months, and their diet 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 these studies. 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. After an overnight fast, the studies began at ~8:00 A.M., 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 in a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood. Two different types of studies and two different types of glucose clamps were performed.

Nicotinic acid administration. Nicotinic acid (NA) (Goldline Laboratories, Fort Lauderdale, FL) 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 type 2 diabetic patients and seven nondiabetic control subjects under euglycemic clamp conditions and to seven type 2 diabetic patients under isoglycemic clamp conditions. This caused facial and/or neck flushing during the first one to three doses in all subjects and mild and transient nausea in three subjects.

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 Dr. Guenther Boden, Temple University Hospital, 3401 North Broad St., Philadelphia, PA 19140.

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FFA, free fatty acid; ISR, insulin secretion rate; NA, nicotinic acid; β-OHB, β-hydroxybutyrate.

TABLE 1
Subjects and protocols (NA and NA + lipid/heparin (L/H) infusions)

	Euglycemic clamps			Isoglycemic clamp
	Type 2 diabetic patients	Control subjects	Control subjects	(type 2 diabetic patients)
Test agent	NA	NA	NA + L/H	NA
Sex (M/F)	1/5	3/4	7/0	3/4
Age (years)	70 ± 3	63 ± 3	29 ± 4	64 ± 2
Height (cm)	160.7 ± 4. 2	164.9 ± 3.1	170 ± 2.6	162 ± 6.0
Weight (kg)	77.7 ± 3.9	77.5 ± 4.8	84.6 ± 6.7	84.4 ± 5.0
Body fat (%)	35.4 ± 2.9	30.7 ± 2.8	24.7 ± 1.9	33.1 ± 2.9
BMI (kg/m^2)	30.2 ± 1.4	28.4 ± 1.2	29.4 ± 2.7	31.8 ± 0.9
Duration of type 2 diabetes (years)	14.0 ± 5.1	—	—	15.0 ± 3.7

Data are means ± SD.

NA plus lipid/heparin (FFA clamp). NA in the above-described dosages and lipid/heparin (Liposyn II [Abbott Laboratories, North Chicago, IL] 0.5–1.0 ml/min plus heparin 0.2 U/kg min) was given to seven nondiabetic control subjects under euglycemic clamp conditions. Liposyn II is a 20% triglyceride emulsion (10% safflower, 10% soybean oil) containing 272 mmol/l glycerol. These studies were performed to evaluate whether or not NA had effects on insulin secretion independent of its lowering action on plasma FFA levels.

Euglycemic clamps. In control subjects, blood glucose levels were maintained at ~5 mmol/l by a variable glucose infusion (9). To lower plasma glucose in type 2 diabetic patients from the prevailing hyperglycemia (~11 mmol/l) into the euglycemic range, small doses of regular human insulin were infused intravenously for several hours before the start of the experiments (10). Insulin infusions were discontinued at –90 min, and glucose concentrations were clamped at 5 mmol/l starting at 0 min for 4 h (by a feedback-controlled glucose infusion, if necessary).

Isoglycemic clamps. These studies were performed in type 2 diabetic patients. Glucose concentrations were clamped for 4 h at the patients' prevailing postabsorptive glucose concentration (9.4–11 mmol/l) by a feedback-controlled glucose infusion (10).

Measurements

C-peptide kinetics. Approximately 1 week before the studies, a 50 nmol/l i.v. bolus of biosynthetic human C-peptide (Lilly, Indianapolis, IN) was administered to each subject after an overnight fast. Plasma C-peptide concentrations were measured at frequent intervals for 3 h, as described by Polonsky et al. (22).

Insulin secretory rates. The C-peptide kinetic parameters were used to calculate the ISRs for each time interval between successive blood samples by deconvolution of peripheral C-peptide concentrations, according to Polonsky et al. (22) and Eaton et al. (23).

Analytical procedures. Plasma glucose was measured with a glucose analyzer with the glucose oxidase method. Serum free insulin was determined after deproteinization 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, St. Charles, MO). Total plasma fatty acids were determined enzymatically in chilled plasma containing EDTA and paroxypropione (Paroxon; Sigma, St. Louis, MO), a lipoprotein lipase inhibitor (0.275 mg/ml blood), with a kit from Wako (Richmond, VA). β-Hydroxybutyrate (β-OHB) was measured enzymatically.

Body composition. Body composition was determined by bioimpedance analysis (24).

Statistical analysis. All data are expressed as means ± SE. Analysis of variance with repeated measures was used to determine differences in ISRs and serum insulin during NA administration across all time points.

RESULTS

Effects of lowering plasma FFAs on ISRs (Fig. 1). To assess the effects of decreasing plasma FFA concentrations on basal (postabsorptive) ISRs, NA was administered by mouth (100–150 mg, q 30 min × 4 h) to six type 2 diabetic patients and to seven control subjects under euglycemic conditions (mean 4-h plasma glucose 5.3 ± 0.2 and 5.1 ± 0.2 mmol/l, respectively) and to seven type 2 diabetic patients under isoglycemic conditions (mean 4-h plasma glucose 10.0 ± 0.9 mmol/l). The glucose infusion rates needed to maintain these glucose concentrations were 3.0 ± 1.1 and 3.4 ± 0.5 μmol/kg min for euglycemic type 2 diabetic patients and control subjects, respectively, and 6.0 ± 1.2 μmol/kg min for isoglycemic type 2 diabetic patients.

Plasma FFA concentrations decreased significantly ($P <$ 0.03) in all three groups, from 614 ± 109 (0 min) to 54 ± 6 μmol/l (240 min) in euglycemic type 2 diabetic patients, from 597 ± 84 to 110 ± 46 μmol/l in euglycemic control subjects, and from 643 ± 63 to 136 ± 42 μmol/l in isoglycemic type 2 diabetic patients.

Basal β-OHB concentrations were low in euglycemic type 2 diabetic patients and control subjects and decreased further to nearly undetectable levels during NA administration (from 0.14 ± 0.04 to 0.04 ± 0.01 mmol/l and from 0.11 ± 0.02 to 0.05 ± 0.01 mmol/l, respectively).

Associated with these decreases in plasma FFA and β-OHB levels were significant decreases in ISRs, from 99 ± 32 to 63 ± 16 pmol/min (–36%; $P < 0.03$) in euglycemic type 2 diabetic patients, from 127 ± 24 to 96 ± 25 pmol/min (–25%; $P <$ 0.03) in euglycemic control subjects, and from 139 ± 34 to 101 ± 23 pmol/min (–23%; $P < 0.02$) in isoglycemic type 2 diabetic patients. These results suggested that plasma FFA levels were responsible for ~25–33% of basal ISRs.

Peripheral insulin concentrations decreased in isoglycemic type 2 diabetic patients (from 82 ± 29 to 57 ± 17 pmol/l; –30%; $P < 0.03$), in control subjects (from 66 ± 18 to 46 ± 10 pmol/l; –30%; $P < 0.03$), and in euglycemic type 2 diabetic patients (from 64 ± 13 to 52 ± 10 pmol/l; –19%; $P < 0.05$).

To assess the possible direct effects of NA on ISRs unrelated to changes in FFA levels, NA (100–150 mg, q 30 min × 4 h) was given by mouth together with an infusion of lipid/heparin (to prevent plasma FFAs from decreasing) to seven nondiabetic subjects under euglycemic conditions (mean 4-h plasma glucose 5.0 ± 0.1 mmol/l). As seen in Fig. 2, NA did not affect ISRs when plasma FFA levels were prevented from falling.

DISCUSSION

FFAs support basal ISRs. Lowering FFA levels from ~600 to ~100 μmol/l was associated with a decrease of 25–35% in ISRs and ~30% in peripheral insulin concentrations in normal subjects and diabetic patients. Ketone bodies may have contributed to this effect (25); in the normal subjects, their contribution, if any, was probably small. β-OHB decreased by only ~60 μmol/l (from 110 ± 20 to 50 ± 10 μmol/l). Acetoacetate,

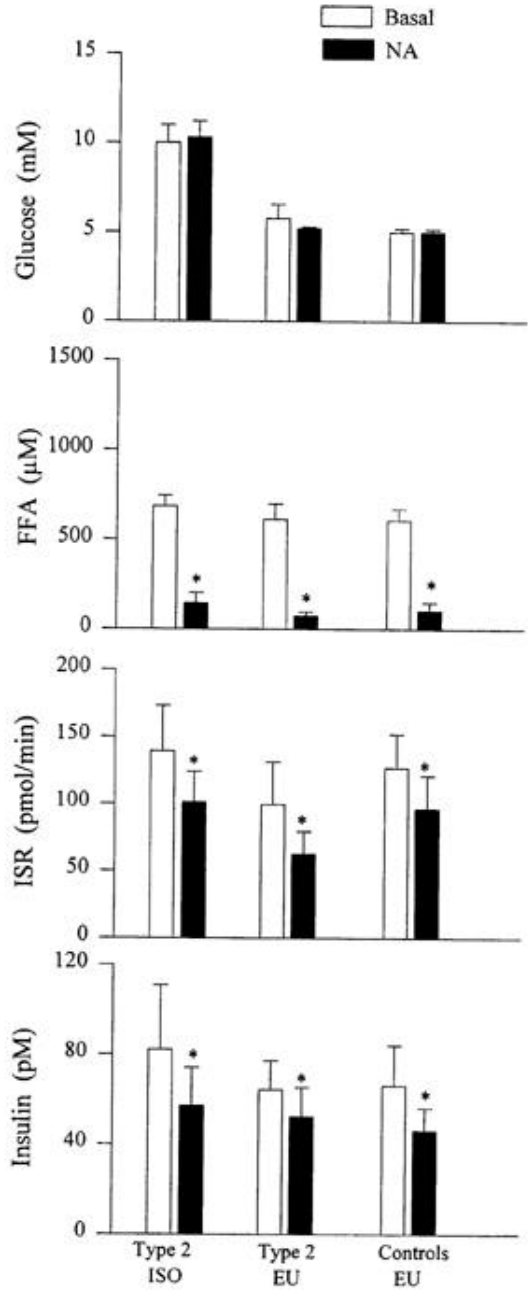

FIG. 1. Effects of NA (100–150 mg, q 30 min × 4 h) on plasma FFA levels, insulin, and ISRs in type 2 diabetic patients during euglycemic (EU) and isoglycemic (ISO) clamping and in nondiabetic control subjects during euglycemic clamping. Data are means ± SE. *$P < 0.03$, basal values (mean of values from –30 to 0 min) vs. values during NA (mean of values from 180 to 240 min).

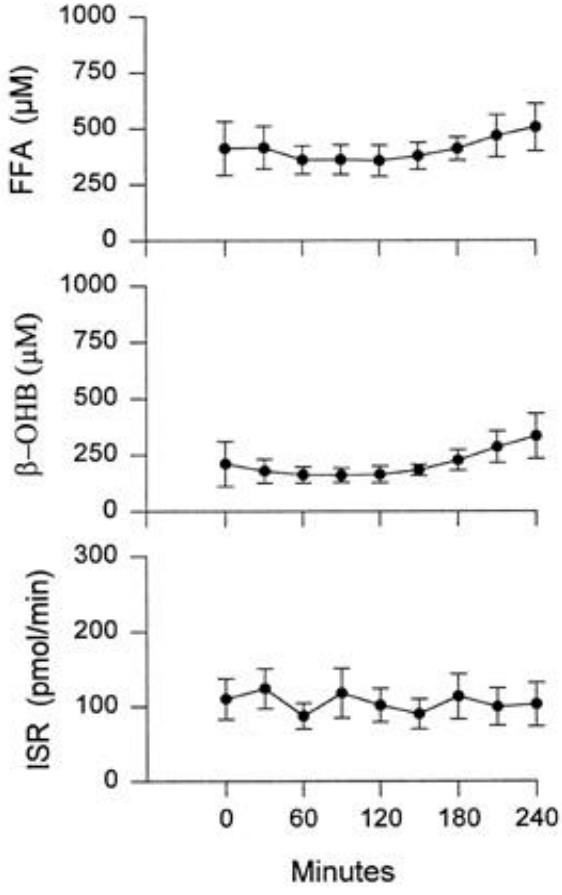

FIG. 2. Effects of NA (100–150 mg, q 30 min × 4 h) plus lipid/heparin (Liposyn II, 0.5–1.0 ml/min; heparin, 0.2 U/kg min) during euglycemic clamping in seven normal subjects on ISRs and plasma FFA and β-OHB concentrations.

which was measured under identical conditions during a previous study (26), may have added another 20 μmol/l, for a total decrease in ketone bodies of ~80 μmol/l compared with a fall in plasma FFAs of ~500 μmol/l. In type 2 diabetic patients (during isoglycemic clamping), however, the fall in β-OHB was larger (from 252 ± 88 to 38 ± 8 μmol/l). Hence, it is likely that ketone bodies supported a larger part of basal ISRs in type 2 diabetic patients than in normal subjects.

We lowered plasma FFA levels with NA, a potent but short-acting inhibitor of lipolysis (27). Short-term administration of NA has been reported to have highly variable effects on glucose tolerance—increasing, decreasing, or not changing it. In contrast, prolonged administration has been found to decrease glucose tolerance consistently (28). Our observation that ISRs did not change during NA administration when plasma FFA levels were prevented from falling (Fig. 2) appeared to rule out direct as well as indirect effects of NA on insulin secretion. We also believe that the results of the NA + lipid/heparin studies (Fig. 2) were applicable to all study subjects, even though they were obtained in normal volunteers who were younger and slightly leaner than the diabetic patients and the other control group.

Several diabetic patients had their sulfonylurea and/or biguanide medications discontinued only 24 h before the studies, which may have been too short for a complete washout of these drugs. Therefore we cannot completely rule out that the remaining sulfonylureas may have influenced our results. We consider this possibility remote, however, because chronic administration of sulfonylureas has little or no effect on basal insulin levels (29), and also because the sulfonylurea-treated patients' basal ISRs and ISR responses to FFAs did not seem to differ from those of our patients who received biguanides, which are known not to affect insulin secretion. Our observations thus demonstrated that under these study conditions, basal plasma FFA levels were responsible for 25–33% of basal ISRs—that is, they exerted long-term poten-

tiating effects on glucose-stimulated ISRs. Our results are in accord with a previous report by Balasse and Ooms (30), who found that lowering plasma FFA levels produced a decrease in serum insulin responses to intravenous glucose, tolbutamide, or glucagon in normal subjects and a recent report by Stein et al. (31), who found that the ability to secrete insulin in response to a glucose load in 18-h– to 24-h–fasted rats was dependent on elevated plasma FFA levels (31).

FFAs as a link between obesity and hyperinsulinemia. Lowering plasma FFA levels in our mildly obese nondiabetic and diabetic subjects normalized those subjects' elevated basal insulin levels. In control subjects, for example, serum insulin decreased from 66 ± 16 to 46 ± 10 pmol/l ($P < 0.03$) (normal for our laboratory, 45 ± 11 pmol/l). A smaller decrease in plasma FFAs than the one produced by NA would likely have resulted in a smaller decrease in serum insulin (unpublished data from our laboratory suggest that for every 100 µmol/l change in plasma FFAs, peripheral insulin levels rise or fall by ~4–6 pmol/l). Hence elevated plasma FFA levels may be responsible for at least some of the hyperinsulinemia in normoglycemic obese subjects. They may not always account for all of it, as insulin levels in some cases of normoglycemic obesity are higher than can be accounted for from the degree of FFA elevation.

In summary, the results of this study demonstrated that basal plasma FFA levels supported 25–33% of postabsorptive insulin secretion in obese nondiabetic and diabetic subjects and, hence, may be physiologically important, long-acting potentiators of glucose-stimulated insulin secretion. Moreover, FFAs were probably responsible for some of the hyperinsulinemia in normoglycemic obese subjects.

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Author Queries (please see Q in margin and underlined text)

Q1: Were both the diabetic and control subjects mildly obese? It would be useful to mention that here given that you discuss FFAs and obesity, below.>
Q2: Are these the subjects later referred to as normal subjects? See under DISCUSSION in which you make a distinction between normal volunteers and control subjects.>
Q3: Please check that the correct subheadings have been included under this main heading.>
Q4: Does this refer to euglycemic and isoglycemic diabetic patients?>
Q5: Can you make the distinction between the normal volunteers and control subjects clearer in the text?>
Q6: As meant for “their”?>
Q7: Is "year" the correct unit of measure for duration of type 2 diabetes?>

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Table 1: Okay as set?