Circulating Fatty Acids Are Essential for Efficient Glucose-Stimulated Insulin Secretion After Prolonged Fasting in Humans

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In the fasted rat, efficient glucose-stimulated insulin secretion (GSIS) is absolutely dependent on an elevated level of circulating free fatty acids (FFAs). To determine if this is also true in humans, nonobese volunteers were fasted for 24 h (n = 5) or 48 h (n = 5), after which they received an infusion of either saline or nicotinic acid (NA) to deplete their plasma FFA pool, followed by an intravenous bolus of glucose. NA treatment resulted in a fall in basal insulin concentrations of 35 and 45% and in the area under the insulin response curve (area under the curve [AUC]) to glucose of 47 and 42% in the 24- and 48-h fasted individuals, respectively. The 48-h fasted subjects underwent the same procedure with the addition of a coinfusion of Intralipid plus heparin (together with NA) to maintain a high concentration of plasma FFAs throughout the study. The basal level and AUC for insulin were now completely normalized (C-peptide profiles paralleled those for insulin). To assess the effect of an overnight fast, nonobese (n = 6) and obese (n = 6) subjects received an infusion of either saline or NA, followed by a hyperglycemic clamp (200 mg/dl). The insulin AUC in response to glucose was unaffected by lowering of the FFA level in nonobese subjects, but fell by 29% in the obese group. The data clearly demonstrate that in humans, the rise in circulating FFA levels after 24 and 48 h of food deprivation is critically important for pancreatic β-cell function both basally and during subsequent glucose loading. They also suggest that the enhancement of GSIS by FFAs in obese individuals is more prominent than that seen in their nonobese counterparts. *Diabetes* 47:1613–1618, 1998

We recently showed that the lowering of the circulating free fatty acid (FFA) level in 18- to 24-h fasted rats by infusion of nicotinic acid (NA) resulted in undetectable basal insulin concentrations and caused total ablation of insulin secretion in response to a glucose load (1,2). The latter effect was also true in the case of the nonglucose secretagogues, such as arginine, leucine, and glibenclamide (3). In all cases, the NA-induced suppression of insulin secretion was completely reversed if a high FFA concentration was maintained by coadministration of a lipid emulsion plus heparin. In addition, this insulinotropic action of FFAs was found to increase markedly with their chain length (over the range C_8–C_{18}) and degree of saturation (2).

Since elevated plasma FFA concentrations have been implicated in the etiology of diabetes and insulin-resistant states (4,5), it is important to determine whether efficient glucose-stimulated insulin secretion (GSIS) in humans is dependent on FFA availability. Previous studies have demonstrated that the insulin response to glucose is enhanced in individuals in whom FFA concentrations are elevated by combined administration of a fat meal and heparin (6,7) or by prolonged Liposyn/heparin infusion (8). Conversely, experimental reduction of the FFA level by oral dosing with the NA derivative, acipimox, has been shown to lower basal insulin concentrations by some investigators (9–11) but not by others (12,13). When intraveneous NA was infused to block endogenous lipolysis, the β-cell response to glucose and tolbutamide was impaired in obese subjects (7). To expand on and clarify the findings of these earlier studies, we have investigated whether FFAs are essential for efficient GSIS in lean human subjects and also if they contribute to the hyperinsulinemia associated with obesity. The results below provide an affirmative answer to both questions.

RESEARCH DESIGN AND METHODS

Subjects. Fourteen nonobese and six obese nondiabetic volunteers participated in the study after giving informed consent. They included men and women with a broad range of age and a racial composition representative of the general U.S. population. None of the subjects were receiving medication and all had maintained a stable weight for at least 2 months before the study. Baseline clinical characteristics are given in Table 1.

Materials. NA for infusion was purchased from U.S. Biochemical Corporation (Cleveland, OH) and dissolved in deionized water to a concentration of 250 mg/ml (pH adjusted to 7.0). This was passed through a 0.2-micron Nalgene filter and pyrogen-tested to ensure sterility. Five milliliters of the stock solution was diluted to a final volume of 50 ml with 0.9% sterile saline for infusion. Intralipid (Kabi Pharmacia, Clayton, NC) was infused as a 20% emulsion. Dextrose solutions (5, 20, and 50%) were from Baxter Healthcare Corporation (Deerfield, IL). Sodium heparin (1,000 U/ml) was obtained from Elkins-Sinn (Cherry Hill, NJ). Trisodium citrate (Tricitrasol) was from Cytosol Labs (Braintree, MA).

Experimental protocol. All experiments were conducted at the U.T. Southwestern General Clinical Research Center and were approved by the Institutional Review Board. Study subjects were separated into groups based on body habitus and length of fasting. In the overnight fasting studies, six nonobese and six obese subjects began fasting at 1800 the evening before each experiment. For the more prolonged fasts, volunteers began fasting at 0800 (after breakfast) either 24 h (n = 5) or 48 h (n = 5) before the start of each experiment. After an

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AUC, area under the curve; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; NA, nicotinic acid.

overnight stay in the center, intravenous catheters were placed in the left and right antecubital veins for infusion and sampling, respectively, at 0700. Catheters were flushed with 0.9% saline and the infusion line was connected to an automated glucose monitor (VIA Medical Corporation, San Diego, CA). The infusate for operation of the monitor was modified to include 1.2 ml of 46.7% trisodium citrate and 3.8 ml of 0.9% saline instead of the customary 5 ml of 100 U/ml heparin as the anticoagulant. The use of citrate in this setting was validated in separate studies (14).

Paired experiments were performed in random order spaced apart at least 1 week for overnight-fasted and 24-h fasted subjects and at 2 weeks for individuals who fasted for 48 h. Studies began at 0800 and lasted for 4 h. A baseline blood sample was drawn before subjects received ketorolac (Hoffman LaRoche, Nutley, NJ) at 0.6 mg/kg intravenously (maximum dose 60 mg) to minimize the prostaglandin-mediated flush associated with NA administration. After a 30-min equilibration period, a primed (50 mg) continuous infusion of NA at 0.045 mg · kg^{-1} · min^{-1} was begun, or an equal volume of 0.9% saline was administered during control studies. An intravenous glucose bolus (15 g/m^2) was given 2.5 h later as 50% dextrose over 2 min, and venous blood was collected into sodium citrate–coated syringes at 2, 5, 10, 15, 20, 30, 45, and 60 min after completion of the bolus. Five subjects underwent the above-mentioned procedures after a 24-h fast and five subjects (including one from the 24-h fasting study) underwent after a 48-h fast. The latter group completed a third protocol in which they received an infusion of Intralipid (0.9 ml · min^{-1} · m^{-2}) and heparin (1,000-U bolus followed by infusion at 1,000 U · h^{-1} · m^{-2}) beginning at –180 min to maintain normal plasma FFA levels when NA was started 30 min later.

Six overnight-fasted obese and six nonobese subjects completed slightly modified paired protocols with and without infusion of NA. These individuals received a bolus of 10 g/m^2 of intravenous glucose given as 20% dextrose over 3 min. Venous blood was collected at 2 min and then every 5 min after completion of the bolus. Plasma glucose concentrations were measured and clamped at ~200 mg/dl with a continuous variable infusion of 20% dextrose. The hyperglycemic clamp was used *1*) to assess both first and second phase insulin secretion, *2*) to augment the glucose stimulus and magnify any differences caused by NA treatment, and *3*) to minimize differences in plasma glucose concentration between nonobese and obese, insulin-resistant subjects.

When NA was infused, some individuals tended to show a decline in their plasma glucose concentration, as noted by Landau et al. (15). Therefore, glucose was monitored frequently, and the basal level was maintained by administering 5% dextrose until the glucose bolus was given. Despite our attempt to relieve symptoms by administering the anti-inflammatory drug, ketorolac, all subjects receiving NA experienced a pronounced flush reaction that was described variously as intense heat and/or pruritic in nature. This peaked within a few minutes and subsided over the next 20–30 min. Subjects also described a transient sour metallic taste. No clinically significant adverse reactions were noted, and blood pressure and heart rate were unaffected.

Treatment of blood samples. Immediately on collection, blood samples were centrifuged for 30 s in a microfuge to separate plasma. Glucose concentrations were measured with the automatic blood glucose monitor (see above) and subsequently verified using a Glucose Analyzer II from Beckman Instruments (Fullerton, CA). Other aliquots of plasma were frozen in liquid N_2. These were analyzed the same day for FFAs and total triglycerides, employing colorimetric assay kits from Boehringer Mannheim (Indianapolis, IN) and Sigma (St. Louis, MO) (Protocol 337). Basal plasma β-hydroxybutyrate concentrations were determined spectrophotometrically (16). Insulin was measured as previously described (1), employing a human insulin–specific antibody (SP 21–6) and human standards (lot 1112) from Linco (St. Charles, MO). For any given individual, all samples from experiments with and without NA were analyzed simultaneously to eliminate interassay variation. Fasting insulin levels were measured in a high sensitivity assay in which samples were preincubated for 24 h with antiserum alone, after which ^{125}I-insulin tracer was added for an additional 24 h, giving a minimal detection limit of 3 pmol/l, with IC-80 of 6 pmol/l and IC-20 of 130 pmol/l. All insulin samples were analyzed using the linear portion of the curve between the IC-20 and IC-80. The interassay coefficients of variation were 8.5 and 7.5% for the high and routine sensitivity assays, respectively. C-peptide was measured using an radioimmunoassay (RIA) kit, also from Linco, and had an interassay coefficient of variation of 5%.

Statistical analysis. All statistical comparisons were calculated using SigmaStat computer software (Jandel Scientific, San Rafael, CA). Baseline characteristics of the nonobese subjects who fasted overnight for 24 or 48 h were compared by analysis of variance, and differences between the groups were determined by Newman-Keuls test for multiple comparisons. Baseline characteristics of the lean and obese subjects who fasted overnight were also analyzed by Student's t tests. The effects of treatments in individual subjects were determined using paired analyses.

RESULTS

Baseline clinical characteristics. As expected, plasma β-hydroxybutyrate concentrations progressively increased in the 24- and 48-h fasted nonobese volunteers (Table 1), verifying that all individuals were compliant with the study protocol. With prolonged fasting, plasma insulin and glucose concentrations tended to fall, while FFA concentrations increased. The only difference between the obese and nonobese subjects at baseline, was that the former group displayed basal hyperinsulinemia while maintaining normal basal plasma glucose levels.

Studies in 24- and 48-h fasted subjects. Figure 1 shows the results of studies carried out in five subjects after a 24-h fast. As seen from Fig. 1*A* and *C*, the intravenous bolus of glucose elicited an abrupt increase in the plasma insulin concentration that waned with time as the hyperglycemia subsided. As expected, this was accompanied by a sharp fall in the circulating FFA level because of the antilipolytic effect of insulin (Fig. 1*B*). However, when the FFA concentration was first lowered by infusion of NA, basal insulin concentrations fell from 14.4 ± 2.4 to 9.6 ± 2.4 pmol/l ($P < 0.001$), and GSIS was diminished by 47 ± 4% (Fig. 1*C* and *D*; $P < 0.05$).

Five subjects underwent the same procedure after a 48-h fast (Fig. 2). The effect of prior FFA depletion (using NA) on pancreatic β-cell function was again quite marked. Basal insulin concentrations fell from 19.2 ± 3.4 to 10.8 ± 1.0 pmol/l ($P < 0.05$), while the area under the curve (AUC) for insulin after the glucose challenge declined by 42 ± 5% (Fig. 2*C* and

TABLE 1
Baseline clinical characteristics of study subjects

	Nonobese 48-h fast	Nonobese 24-h fast	Nonobese ON fast	Obese ON fast
n	5	5	6	6
Sex (M/F)	1/4	2/3	2/4	3/3
Age (years)	39.4 ± 4.4	37.2 ± 4.3	36.2 ± 4.5	35.7 ± 3.6
Weight (kg)	62.9 ± 3.3	69.5 ± 5.4	58.1 ± 3.0	108.8 ± 8.8*
BMI (kg/m^2)	24.1 ± 0.8	24.6 ± 1.8	22.5 ± 1.5	35.7 ± 3.6*
Plasma βOHB (mmol/l)	1.93 ± 0.22†	0.83 ± 0.24*	0.08 ± 0.01	0.10 ± 0.02
Plasma insulin (pmol/l)	20 ± 2*	16 ± 2*	31 ± 3	74 ± 13*
Plasma glucose (mg/dl)	70 ± 3*	82 ± 4*	92 ± 2	96 ± 2
Plasma FFA (mmol/l)	1.08 ± 0.06†	0.83 ± 0.06*	0.56 ± 0.10	0.51 ± 0.07

Data are means ± SE, and plasma measurements include all studies completed by each subject. To convert insulin units from microunits per milliliter to picomoles per liter, divide by 6. *$P < 0.5$ vs. overnight-fasted nonobese subjects. †$P < 0.05$ vs. overnight-fasted and 24-h fasted nonobese subjects. βOHB, β-hydroxybutyrate; ON, overnight.

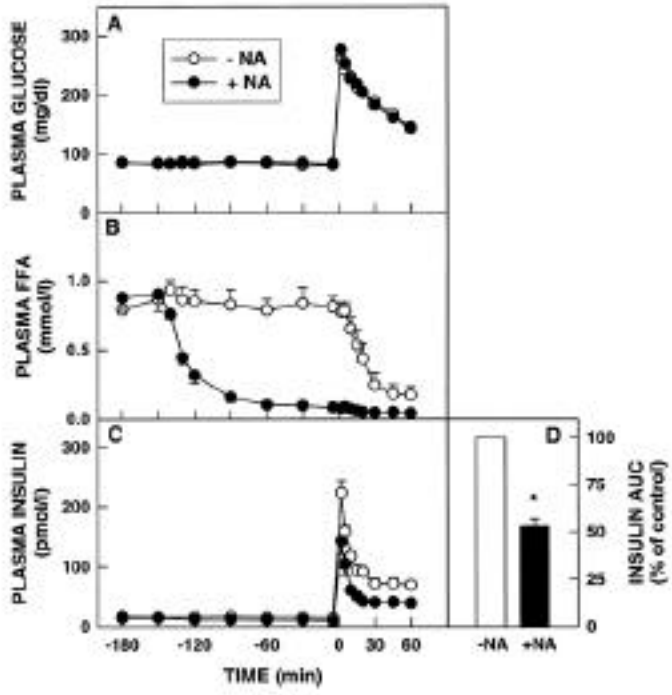

FIG. 1. Effect of NA on glucose-stimulated insulin secretion in 24-h fasted nonobese humans. When used, NA was infused from –150 min onward. A glucose bolus was given intravenously at 0 min. Plasma glucose concentrations before the bolus were 81 ± 5 and 83 ± 5 mg/dl in the NA^- and NA^+ groups, respectively. Mean peak plasma glucose (2 and 5 min values) were 256 ± 4 vs. 260 ± 11 in the NA^- and NA^+ groups, respectively. All plasma values are means ± SE for five subjects. AUC, area under curve above baseline. *P < 0.05 compared with NA^- control.

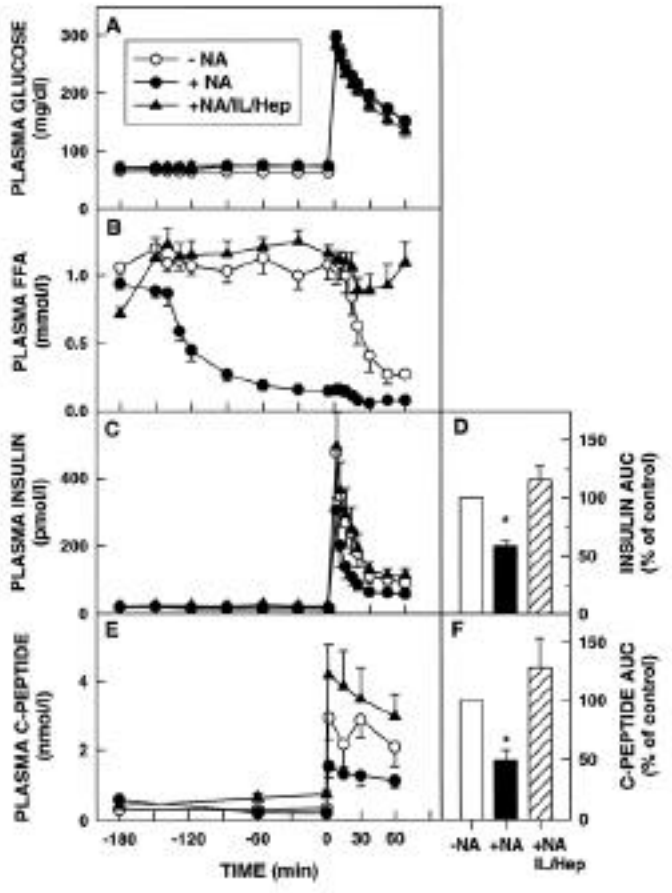

FIG. 2. Effect of NA in the absence and presence of lipid infusion on glucose-stimulated insulin secretion in 48-h fasted nonobese humans. When used, Intralipid (IL) and heparin (Hep) were administered from –180 min onward; NA infusion began at –150 min. A glucose bolus was given intravenously at 0 min. Basal glucose concentrations were 62 ± 2, 73 ± 4, and 75 ± 4; mean peak (2 and 5 min) glucose levels were 289 ± 10, 278 ± 8, and 282 ± 9 mg/dl in the NA^-, NA^+, and NA^+/IL/Hep groups, respectively. All plasma values are means ± SE for five subjects. AUC, area under curve above baseline. *P < 0.05 compared with NA^- control.

D). All values were normalized, however, when the plasma FFA concentration was maintained at its fasting level by coinfusion of the lipid emulsion plus heparin. Plasma triglyceride levels rose to ~350 mg/dl during the last hour of lipid/heparin infusion compared with ~60 mg/dl in the control and NA-only protocols (data not shown).

It was possible that the observed alterations in insulin dynamics after manipulation of the plasma FFA concentration were more a reflection of changes in insulin clearance than in insulin secretion rates (17). That this was not the case is evident from the data on plasma C-peptide levels shown in Fig. 2*E* and *F*. It is seen that the C-peptide profiles essentially mirrored those for insulin, both basal and stimulated AUC falling with NA infusion (P < 0.05) but returning to normal when FFA concentrations were restored with Intralipid and heparin. Clearly, the prevailing FFA level at the time of glucose loading profoundly influenced the rate of insulin secretion.

Studies in overnight-fasted subjects. The importance of plasma FFA for efficient GSIS in 24- and 48-h fasted humans noted above raised the question of whether this also applies after the more commonly employed overnight fast. For reasons given in METHODS, we addressed this question using the hyperglycemic clamp instead of a simple intravenous bolus, as was done in Figs. 1 and 2. We also performed the experiments in both lean and obese subjects since a previous study had examined only obese subjects (7). As shown in Fig. 3, NA infusion in nonobese subjects again caused a brisk (90%) decline in plasma FFA concentrations (Fig. 3*B*). However, in contrast to the results obtained with the more prolonged fasts, this depletion of plasma FFAs had no effect on the plasma insulin profile when glucose concentrations were raised to 200 mg/dl. Both the initial peak insulin response and the total insulin AUC were equivalent in studies performed with and without NA infusion (Fig. 3*C* and *D*). However, the findings in obese subjects were noticeably different (Fig. 4). Here, administration of NA resulted in a somewhat delayed fall in plasma FFA concentrations (Fig. 4*B*), but this nevertheless resulted in a significant impairment of the insulin secretory response to hyperglycemia, the total stimulated insulin output falling by 29 ± 8% (Fig. 4*C* and *D*; P < 0.05). Interestingly, basal insulin levels in both lean and obese groups were significantly affected by lowering FFA with NA, falling from 34.8 ± 3.6 to 18.6 ± 4.2 pmol/l and 77.4 ± 21.0 to 57.6 ± 16.8 pmol/l in lean and obese subjects, respectively (P < 0.05).

DISCUSSION

The ability of FFAs to enhance both basal and glucose-stimulated insulin secretion in animals and humans has long been known (1–3,6–8,18). What has only recently come to

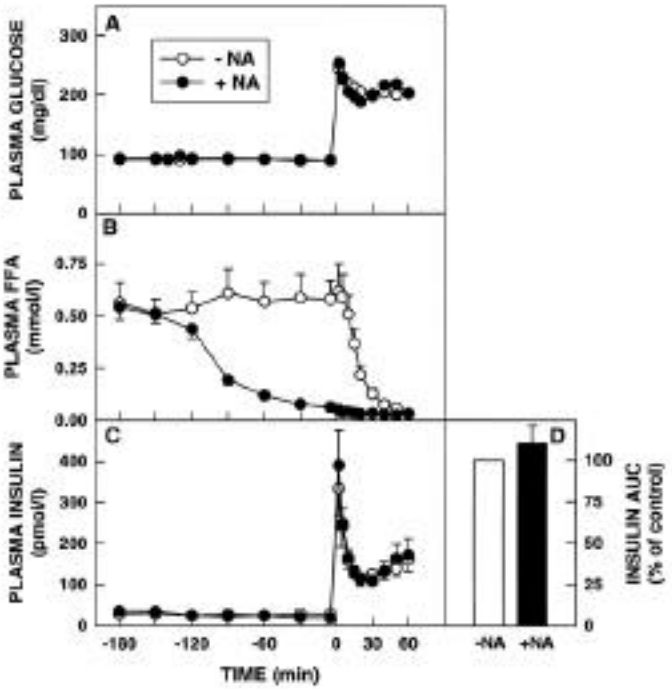

FIG. 3. Effect of NA on glucose-stimulated insulin secretion in overnight-fasted nonobese humans. When used, NA was infused from –150 min onward. A glucose bolus was given intravenously at 0 min when glucose concentrations were 89 ± 3 and 90 ± 2 mg/dl in the NA^- and NA^+ groups, respectively. Plasma glucose concentrations were clamped at 200 mg/dl using exogenous glucose infusion. Values are means ± SE for six subjects. AUC, area under curve above baseline.

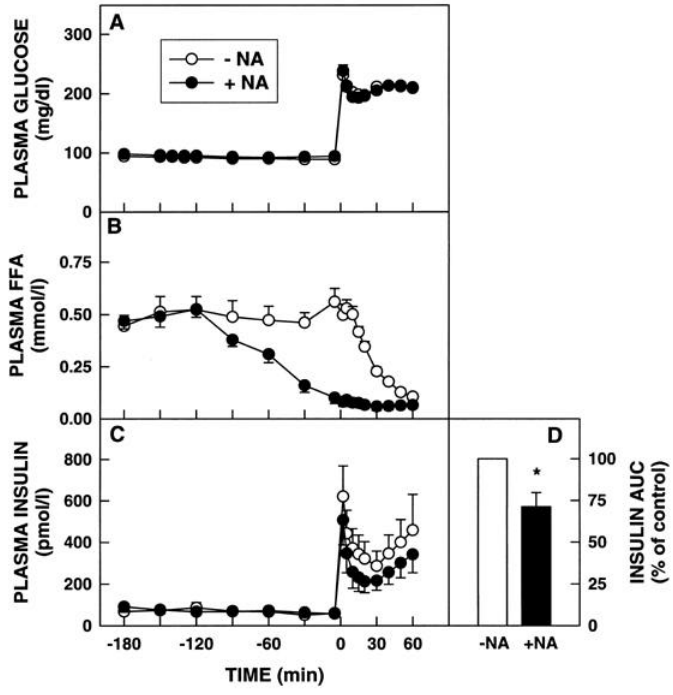

FIG. 4. Effect of NA on glucose-stimulated insulin secretion in overnight- fasted obese humans. When used, NA was infused from –150 min onward. A glucose bolus was given intravenously at 0 min when glucose concentrations were 89 ± 2 and 93 ± 2 mg/dl in the NA^- and NA^+ groups, respectively. Plasma glucose concentrations were clamped at 200 mg/dl using exogenous glucose infusion. Values are means ± SE for six subjects. AUC, area under curve above baseline. *P < 0.05 compared with NA^- control.

light is that under certain circumstances these substrates are actually essential for stimulus-secretion coupling in the pancreatic β-cell. The emerging picture, discussed in detail (1–3), can be summarized as follows. In the intact fed rat, the pancreatic β-cell responds well in terms of insulin secretion to a variety of secretagogues, such as glucose, arginine, leucine, and glibenclamide, regardless of whether the already low level of plasma FFAs is reduced still further by infusion of NA. By contrast, in the fasted state, which is characterized by a high plasma FFA concentration, all of these agents are completely ineffective if the FFA level is first lowered by treatment with NA. However, insulin secretion is fully restored if the NA-induced deficit of FFA is offset by simultaneous infusion of a lipid emulsion plus heparin. Indeed, maintenance of a high FFA level together with continuous administration of the secretagogue results in supranormal rates of insulin release (at least over a period of 1 h) in both the fed and fasted state. Importantly, over the range C_8–C_{18}, the longer the chain length and the greater the degree of saturation of the fatty acid, the greater is its insulinotropic potency (2).

To begin to explore the relevance of these new findings to pancreatic β-cell function in humans, we asked to what extent the ambient plasma FFA level influences GSIS in normal, lean volunteers after 24 or 48 h of food deprivation. Qualitatively, the results after a prolonged fast were similar to those we had obtained with the rat. Thus, in response to the same glucose challenge, the AUC for insulin in the 24-h and 48-h fasted subjects was reduced to 53 and 58%, respectively, of the control value simply by lowering their plasma FFA concentration with NA. A decrease of similar magnitude was seen in basal insulin concentrations in these two groups, consistent with prior reports of the effect of antilipolytic agents in fasted rats and humans (1–3,9–11). The impact of NA-induced FFA suppression on C-peptide dynamics, which is reflective of the insulin secretion rate, paralleled that seen for insulin in the 48-h fasted subjects. Moreover, maintenance of the fasting plasma FFA level by exogenous lipid infusion completely normalized both basal levels of insulin and C-peptide, as well as their responses to the glucose load. The effect of FFA withdrawal was uniform across patients studied under the same metabolic conditions.

The findings in overnight-fasted subjects produced something of a surprise. In the lean group, insulin responses after infusion of NA were unchanged despite the marked reduction in availability of circulating FFAs. It thus appears that in healthy, nonobese individuals, GSIS does not rely on an elevated plasma FFA level after only 14 h of fasting (just as in the nonfasted rat [1]), but does so to a very significant degree when the period of food deprivation is extended to 24–48 h. We did not explore the situation in fasts beyond 48 h, but we suspect that with longer times, say 4–5 days, GSIS will become even more dependent on a high plasma FFA concentration. This prediction is based on the fact that in the rat, with its higher basal metabolic rate, insulin secretion in response to glucose was completely ablated by NA infusion after just 18–24 h of fasting (1,2).

In contrast to the lean subjects, obese individuals had already begun to show a partial dependence on circulating

FFAs for GSIS after an overnight fast, as their stimulated insulin AUC fell to 70% of the control value in response to NA infusion. However, the attenuated AUC still equaled that shown by lean subjects in the presence or absence of NA treatment. The behavior of the obese volunteers is reminiscent of the findings by Balasse and Ooms (7), and in light of newer developments in this area, could prove to be critically important for an understanding of the hormonal and metabolic abnormalities associated with the obese state. Two such derangements, both of which were clearly evident in the individuals studied here, are basal hyperinsulinemia and an exaggerated insulin response to a glucose challenge. The conventional view is that the hyperinsulinemia of obesity is a compensatory device to counteract the peripheral insulin resistance associated with the condition, the implication being that the former follows the latter. However, an alternative view is that both of these features of obesity arise simultaneously, and, at least in part, from a common signal, namely, increased availability of FFAs. As reviewed by Boden (5), numerous studies have demonstrated that elevated plasma FFA levels inhibit insulin-stimulated glucose utilization. In addition, the accumulation of triglyceride in muscle has been linked to impaired glucose disposal (19,20). Also, inefficient suppression of postprandial FFA levels has been noted in upper body obese individuals despite increased plasma insulin concentrations (21). Since insulin secretion in obese subjects appears to be particularly sensitive to circulating FFA levels, even after short-term fasting, it is attractive to suppose that increased availability of FFA directly stimulates the pancreatic β-cell while concomitantly contributing to insulin resistance in such individuals. If FFAs subserve this dual role (in addition to their other cellular functions), it would provide a simple mechanism whereby at any given time, the β-cell can "sense" how much insulin the muscle bed needs to maintain euglycemia in the early stages of obesity/type 2 diabetes syndromes. Consistent with this notion would be the correlation seen between pancreatic triglyceride content and GSIS in rat models with varying degrees of adiposity (22).

On the basis of the cited studies with rats (1–3), it seems likely that if we used the more saturated lard oil instead of Intralipid (mainly soybean oil) as the exogenous source of FFAs in the current studies with humans, the insulin response to glucose would have been greatly exaggerated, particularly under conditions of a hyperglycemic clamp where the glucose stimulus to the β-cell is maintained. It might also be expected that similar results would have been obtained using nonglucose secretagogues. However, both of these points will require validation in humans by direct experimental testing.

As noted elsewhere (1–3), still to be deciphered is in what form (carboxylic acid, CoA ester, or esterified product) FFAs exert this dramatic effect on β-cell function, precisely where in the insulin secretory pathway the effect is exerted, why saturated fatty acids are far more potent than their unsaturated counterparts, and why starvation renders insulin secretion so dependent on circulating FFAs. We have suggested (3) that the fatty acid, or one of is metabolites, acts at a late step in the insulin secretory process, in view of the fact that in the fasted rat FFAs are essential for stimulus-secretion coupling in response to nutrient stimuli (glucose or leucine), K^+ channel blockade (glibenclamide), or direct β-cell membrane depolarization (arginine or high extracellular K^+ concentration). Possible mechanisms underlying the starvation-induced dependence of insulin secretion on FFAs are outlined in Dobbins et al. (3).

Despite these uncertainties, the present findings clearly demonstrate that in humans, as in rodents, glucose-fatty acid crosstalk within the β-cell is critically important for short-term control of insulin secretion (1–3,23,24). In addition, obesity, a condition associated with more prolonged endogenous hyperlipacidemia, is shown to enhance β-cell dependence on circulating FFAs, resulting in basal hyperinsulinemia and increased responsiveness to a glucose load. These findings complement other studies revealing that chronic exposure to very high levels of exogenous FFAs increases basal insulin levels and impairs GSIS (25,26). The developing picture is that endogenous FFAs enhance insulin secretion in obesity/insulin resistance syndromes, but eventually accumulate to toxic levels and contribute to β-cell failure and the development of type 2 diabetes (22,27–29).

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REFERENCES

1. Stein DT, Esser V, Stevenson B, Lane KE, Whiteside JH, Daniels MB, Chen S, McGarry JD: Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *J Clin Invest* 97:2728–2735, 1996
2. Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD, McGarry JD: The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. *J Clin Invest* 100:398–403, 1997
3. Dobbins RL, Chester MW, Stevenson BE, Daniels MB, Stein DT, McGarry JD: A fatty acid-dependent step is critically important for both glucose and non-glucose stimulated insulin secretion. *J Clin Invest* 101:2370–2376, 1998
4. McGarry JD: What if Minkowski had been ageusic? An alternative angle on diabetes. *Science* 258:766–774, 1992
5. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 45:3–10, 1996
6. Schalch DS, Kipnis DM: Abnormalities in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. *J Clin Invest* 44:2010–2020, 1965
7. Balasse EO, Ooms HA: Role of plasma free fatty acids in the control of insulin secretion in man. *Diabetologia* 9:145–151, 1973
8. Boden G, Chen X, Rosner J, Barton M: Effects of 48-h fat infusion on insulin secretion and glucose utilization. *Diabetes* 44:1239–1242, 1995
9. Fulcher GR, Walker M, Catalano C, Farrar M, Alberti KGMM: Acute metabolic and hormonal responses to the inhibition of lipolysis in non-obese patients with non-insulin dependent (type 2) diabetes mellitus: effects of acipimox. *Clin Sci* 82:565–571, 1992
10. Fulcher GR, Walker M, Catalano C, Aguis L, Alberti KGMM: Metabolic effects of suppression of nonesterified fatty acid levels with acipimox in obese NIDDM subjects. *Diabetes* 41:1400–1408, 1992
11. Fery F, Plat L, Melot C, Balasse EO: Role of fat derived substrates in the regulation of gluconeogenesis during fasting. *Am J Physiol* 270:E822–E830, 1996
12. Nuutila P, Knuuti MJ, Raitakari M, Ruotsalainen U, Teras M, Voipio-Pulkki LM, Haaparanta M, Solin O, Wegelius U, Yki-Jarvinen H: Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans. *Am J Physiol* 267:E941–E946, 1994
13. Puhakainen I, Yki-Jarvinen H: Inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate but not fasting hyperglycemia or total hepatic glucose production in NIDDM. *Diabetes* 42:1694–1699, 1993
14. Dobbins RL, Stein DT: Compatibility of sodium citrate anticoagulation with short- and long-term glucose monitoring (Abstract). *Diabetes* 46

(Suppl. 1):367A, 1997
15. Landau C, Chen YD, Skowronski R, Hollenbeck CB, Jaspan JB, Reaven GM: Effect of nicotinic acid on plasma glucose concentrations in normal individuals. *Horm Metab Res* 24:424–428, 1992
16. McGarry JD, Guest MJ, Foster DW: Ketone body metabolism in the ketosis of starvation and alloxan diabetes. *J Biol Chem* 245:4382–4390, 1970
17. Peiris AN, Mueller RA, Smith GA, Struve MF, Kissebah AH: Splanchnic insulin metabolism in obesity: influence of body fat distribution. *J Clin Invest* 78:1648–1657, 1986
18. Crespin SR, Greenough WB, Steinberg D: Stimulation of insulin secretion by long-chain free fatty acids: a direct pancreatic effect. *J Clin Invest* 52:1979–1984, 1973
19. Phillips DIW, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, Taylor R: Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* 45:947–950, 1996
20. Stein DS, Dobbins R, Szczepaniak L, Malloy C, McGarry JD: Skeletal muscle triglyceride stores are increased in insulin resistance (Abstract). *Diabetes* 46 (Suppl. 1):23A, 1997
21. Roust LR, Jensen MD: Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes* 42:1567–1573, 1993
22. Koyama K, Chen G, Lee Y, Unger RH: Tissue triglycerides, insulin resistance, and insulin production: implications for hyperinsulinemia of obesity. *Am J Physiol* 273:E708–E713, 1997
23. Prentki M, Corkey BE: Are the β-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 45:273–283, 1996
24. Kruszynska YT, Mulford MI, Yu JG, Armstrong DA, Olefsky JM: Effects of non-esterified fatty acids on glucose metabolism after glucose ingestion. *Diabetes* 46:1586–1593, 1997
25. Paolisso G, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varricchio M, D'Onofrio F: Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* 38:1295–1299, 1995
26. Lee Y, Hiroshi H, Ohneda M, Johnson JH, McGarry JD, Unger RH: β-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-β-cell relationships. *Proc Natl Acad Sci U S A* 91:1078–1082, 1994
27. Zhou Y-P, Grill VE: Long term exposure to fatty acids and ketones inhibits β-cell function in human pancreatic islets of Langerhans. *Clin Endocrinol Metab* 80:1584–1590, 1995
28. Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. *Diabetes* 44:863–870, 1995
29. Man Z-W, Zhu M, Noma Y, Toide K, Sato T, Asahi Y, Hirashima T, Mori S, Kawano K, Mizuno A, Sano T, Shima K: Impaired β-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. *Diabetes* 46:1718–1724, 1997

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

Q1: “area under the curve (AUC) for insulin” ok?

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