

# Effects of Free Fatty Acids on Gluconeogenesis and Autoregulation of Glucose Production in Type 2 Diabetes

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**Effects of endogenously derived free fatty acids (FFAs) on rates of gluconeogenesis (GNG) (determined with  $^2\text{H}_2\text{O}$ ), glycogenolysis (GL), and endogenous glucose production (EGP) were studied in 18 type 2 diabetic patients and in 7 nondiabetic control subjects under three experimental conditions: 1) during an 8-h fast (from 16–24 h after the last meal), when plasma FFA levels increased slowly; 2) during 4 h (from 16–20 h) of nicotinic acid (NA) administration (fasting plus NA), when plasma FFAs decreased acutely; and 3) during 4 h (from 20–24 h) after discontinuation of NA (FFA rebound), when plasma FFAs increased acutely. During fasting, FFAs increased from 636 to 711  $\mu\text{mol/l}$  in type 2 diabetic patients and from 462 to 573  $\mu\text{mol/l}$  in control subjects ( $P < 0.04$ ), but GNG did not change in diabetic patients (6.9 vs. 6.5  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P > 0.05$ ) or in control subjects (5.1 vs. 5.4  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P > 0.05$ ). During fasting plus NA, FFAs decreased in diabetic patients and control subjects (from 593 to 193 and from 460 to 162  $\mu\text{mol/l}$ , respectively); GNG decreased (from 6.1 to 4.2 and from 4.7 to 3.5  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), whereas GL decreased in diabetic patients (from 5.3 to 4.4  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) but increased in control subjects (from 5.4 to 7.2  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). During the FFA rebound, FFAs increased in diabetic patients and control subjects (from 193 to 1,239 and from 162 to 1,491  $\mu\text{mol/l}$ , respectively); GNG increased (from 4.2 to 5.4 and from 3.4 to 5.3  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  respectively), and GL decreased (from 4.4 to 3.4 and from 7.3 to 4.3  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively). In summary, during an extended overnight fast, increasing plasma FFA levels stimulated GNG, whereas decreasing FFA levels inhibited GNG in both diabetic and control subjects; 20 h after the last meal, approximately one-third of GNG in both diabetic and control subjects was dependent on FFAs; and autoregulation of EGP by GL in response to decreasing GNG was impaired in diabetic patients. *Diabetes* 50:810–816, 2001**

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EGP, endogenous glucose production; FFA, free fatty acid; GL, glycogenolysis; GNG, gluconeogenesis; HMT, hexamethylenetetramine; ISR, insulin secretory rate; NA, nicotinic acid.

**E**levated plasma levels of free fatty acids (FFAs) cause acute as well as long-term peripheral and hepatic insulin resistance (1,2). Yet, increased plasma FFA levels are not associated with increased endogenous glucose production (EGP), at least not in normal subjects (3). The reason for this apparent paradox has recently become clear. In normal subjects, acute elevation of plasma FFA levels has been shown to stimulate gluconeogenesis (GNG) (4,5) and at the same time to inhibit glycogenolysis (GL) (5). The net effect of this reciprocal relationship between GNG and GL (called autoregulation [4,6]) was that EGP remained unchanged. There is currently little information on the effects of FFAs on GNG and on autoregulation of EGP in type 2 diabetes. However, there are reasons to suspect that either one or both of these processes may be abnormal. For instance, plasma FFA levels (7,8) and GNG (9) are frequently elevated in type 2 diabetes. Another source for potential problems is that autoregulation of EGP appears to be regulated by insulin. When plasma FFA levels were increased (by intravenous infusion of lipid plus heparin), EGP did not change, but insulin secretion increased. When the increase in insulin secretion was prevented (by pancreatic clamping), EGP and plasma glucose increased dramatically (3,9). This apparent dependence on insulin suggested that autoregulation of EGP might be disturbed in type 2 diabetic patients and that EGP in type 2 diabetic patients would increase in response to an increase in plasma FFAs, analogous to the increase in EGP that occurred in nondiabetic subjects during lipid infusion, when insulin secretion was clamped at basal levels (3,9).

Therefore, it was our objective to study effects of FFAs on GNG and on autoregulation of EGP in patients with type 2 diabetes and in nondiabetic control subjects. To this end, we have examined effects of changes in plasma FFA levels on rates of GNG, GL, and EGP under three conditions: 1) during an extended overnight fast (from 16–24 h after the last meal), when plasma levels increased slowly; 2) during 4 h of nicotinic acid (NA) administration (between 16–20 h after the last meal), when FFA levels decreased rapidly; and 3) during the 4 h after discontinuation of NA (20–24 h), when plasma levels increased rapidly as a result of the FFA rebound that predictably follows discontinuation of NA administration (5). GNG was measured with the  $^2\text{H}_2\text{O}$  technique, which was recently developed and validated by Landau et al. (10,11).

TABLE 1  
Study subjects

	Fasting plus NA		Fasting	
	Type 2 diabetic subjects	Control subjects	Type 2 diabetic subjects	Control subjects
<i>n</i>	9	6	14	7
Sex (M/F)	3/6	3/3	6/8	4/3
Age (years)	60.0 ± 4.1	58.3 ± 3.0	59.5 ± 3.4	58.3 ± 2.6
Weight (kg)	80.6 ± 6.4	76.2 ± 3.5	89.0 ± 7.1	80.7 ± 5.6
Height (cm)	161 ± 3	172 ± 2.6	166.5 ± 2.9	173 ± 2.5
Fat (%)	35.9 ± 3.3	28.2 ± 3.5	36.2 ± 3.0	28.6 ± 2.5
BMI (kg/m <sup>2</sup> )	30.6 ± 1.6	25.7 ± 0.6	31.8 ± 2.5	26.8 ± 1.3
FPG (mol/l)	9.6 ± 1.2	5.7 ± 0.3	11.0 ± 1.4	5.5 ± 0.2

Data are means ± SE unless otherwise indicated.

Major advantages of this method are that it determines GNG from all precursors (including glycerol) and that it avoids the problems related to unknown precursor-specific activity in the liver that has plagued all previous isotopic methods (12). One issue that has remained unresolved, however, is the possibility of glycogen cycling that could theoretically influence GNG estimates (13).

## RESEARCH DESIGN AND METHODS

A total of 18 patients with type 2 diabetes (5 patients participating in one study and 13 in both studies) and 7 nondiabetic control subjects (1 subject participating in one study and 6 in both studies) volunteered for these studies (Table 1). Weight, percent fat, and BMI measurements were higher in the diabetic patients than in the control subjects, but these differences were not statistically significant (weight  $P = 0.21$ , percent fat  $P = 0.14$ , and BMI  $P = 0.13$ ). All diabetic patients had been treated with oral hypoglycemic agents (sulfonylureas, biguanides, or both) and some had received, in addition, small doses of NPH insulin (5–20 U) at bedtime. These medications were withheld starting 2–3 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 the nature, purpose, and potential risks of the study were explained to them.

**Experimental design.** All subjects were admitted to the Temple University Hospital General Clinical Research Center the day before the studies. At 6:00 P.M., the subjects ingested a meal of 14 kcal/kg body wt; the meal was composed of 53% carbohydrate, 15% protein, and 32% fat. Then they fasted for 24 h, but were allowed water ad libitum. At 11:00 P.M., a baseline blood sample was obtained. The subjects drank 2.5 g of <sup>2</sup>H<sub>2</sub>O (99.9% hydrogen-2) (Isotec, Miamisburg, OH) per kilogram of body water at 11:00 P.M. and again 4 h later, at 3:00 A.M. Body water was assumed to be 50% of body weight in women and 60% of body weight in men. Additional water ingested during the fast was enriched to 0.5% with <sup>2</sup>H<sub>2</sub>O to prevent dilution of the isotopic steady state. The studies began at 8:00 A.M. the day after, with the subjects reclined in bed. A short polyethylene catheter was inserted into an antecubital vein for infusion of isotopes. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (–70°C) to arterialized venous blood. The following studies were performed.

**Study 1: fasting plus NA.** The objective of this study was to determine rates of GNG, GL, and EGP in response to acutely decreasing and acutely increasing plasma levels of FFA. A total of nine type 2 diabetic patients and six nondiabetic control subjects were studied. At 16 h after the last meal, NA (UDL Laboratories, Rockford, IL) was given orally every 30 min for 4 h (100 mg at 0, 30, 180, 210, and 240 min; and 150 mg at 60, 90, 120, and 150 min). Blood samples were drawn 16, 20, and 24 h after the last meal for determination of rates of GNG and glucose turnovers and for determination of plasma concentrations of substrates and hormones.

**Study 2: fasting.** This study served as a control to study 1. We studied 14 patients with type 2 diabetes and 7 control subjects in this protocol, which was identical to study 1, except that NA was not given.

### Analytical procedures

**C-peptide kinetics.** Approximately 1 week before the studies, a 50-nmol bolus of biosynthetic human C-peptide (Eli Lilly, Indianapolis, IN) was

administered intravenously 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. (14).

**Insulin secretory rates.** The C-peptide kinetic parameters were used to calculate prehepatic insulin secretory rates (ISRs) for each time interval between successive blood samples by deconvolution of peripheral C-peptide concentrations according to Polonsky et al. (14) and Eaton et al. (15).

**Rates of GNG and GL.** GNG was determined with the <sup>2</sup>H<sub>2</sub>O method of Landau and colleagues (10,11) using the C5-to-<sup>2</sup>H<sub>2</sub>O ratio. Enrichment in hexamethylenetetramine (HMT) from C5 of blood glucose was determined by gas chromatography–mass spectrometry (Hewlett-Packard 5,989 mass spectrometry, 5,890 gas chromatography; Palo Alto, CA) of mass plus 1 (mass 141) (5). Background enrichment was measured in blood samples obtained before <sup>2</sup>H<sub>2</sub>O ingestion. HMTs of 0.125, 0.25, 0.5, 0.75, 1.0, and 2.0% hydrogen-2 enrichment from 1-[<sup>3</sup>H]sorbitol served as standards to calculate the fraction of blood glucose produced from GNG.

Enrichment of hydrogen-2 in plasma water was determined 16, 20, and 24 h after the last meal with an isotope ratio mass spectrometer (PDZ Europa, London, U.K.) using a standard curve with known enrichment ranging from 0.25 to 1.0%.

GNG ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was calculated by multiplying the C5-to-<sup>2</sup>H<sub>2</sub>O ratio with EGP. GL was calculated as the difference between EGP and GNG.

**Glucose turnover.** Glucose turnover was determined with 3-[<sup>3</sup>H]glucose, which was infused intravenously for 9.5 h (14.5–24 h of fast), starting with a bolus adjusted proportionally to the degree of hyperglycemia (40  $\mu\text{Ci} \times \text{mmol/l glucose}/5.5$ ) followed by a continuous infusion of 0.4  $\mu\text{Ci}/\text{min}$ . This method has been shown to produce steady state tracer-specific activities within 60 min, even in severely hyperglycemic patients (16). In this study, specific activities were the same after 60 and 90 min of tracer infusions ( $0.47 \pm 0.05$  vs.  $0.47 \pm 0.04$   $\mu\text{Ci}/\text{mmol}$ ). Glucose was isolated from blood for determination of 3-[<sup>3</sup>H]glucose-specific activity, as described (17). Rates of total body glucose appearance ( $R_a$ ) and disappearance ( $R_d$ ) were calculated using Steele's equation for non-steady-state conditions (18). The rates of EGP were equal to glucose  $R_a$ , because no glucose was infused during these studies.

**Body composition.** Body composition was determined by bioelectrical impedance analysis (19).

**Substrate and hormone analyses.** Plasma glucose was measured with a glucose analyzer (YSI, Yellow Springs, OH). Insulin was determined by radioimmunoassay using an antiserum with minimal (0.2%) cross-reactivity with proinsulin (Linco, St. Charles, MO). Human growth hormone (Diagnostic Products, Los Angeles, CA) and glucagon (Linco) were determined with radioimmunoassay kits. Cortisol and epinephrine were measured with kits (Diagnostic Products and Amersham Life Sciences, Arlington Heights, IL, respectively). Plasma FFA concentration was determined with a kit from Wako Pure Chemical (Richmond, VA).

Plasma glycerol, lactate, alanine, glutamine, glutamate,  $\beta$ -hydroxybutyrate, and acetoacetate were determined enzymatically.

**Statistical analysis.** All data are expressed as the mean ± SE. Statistical analysis was performed using the SAS program (SAS Institute, Cary, NC). Analysis of variance with repeated measures was used to determine the differences in GNG, EGP, FFA, and GL across time points. Pairwise comparison for each time point was then performed if overall comparison was statistically significant.

## RESULTS

### FFA, glycerol, glucose, growth hormone, and insulin

**FFA.** Fasting (from 16–24 h) increased FFA levels gradually (from  $636 \pm 44$  to  $711 \pm 55$   $\mu\text{mol/l}$ ) in type 2 diabetic patients and from  $462 \pm 68$  to  $573 \pm 86$   $\mu\text{mol/l}$  in control subjects ( $P < 0.04$ ) (Fig. 1). Fasting plus NA (16–20 h) reduced plasma FFA levels from  $593 \pm 54$  to  $193 \pm 69$   $\mu\text{mol/l}$  in diabetic patients and from  $460 \pm 40$  to  $162 \pm 46$   $\mu\text{mol/l}$  in control subjects (both  $P < 0.01$ ). After discontinuation of NA (20–24 h), plasma FFA levels rebounded to  $1,239 \pm 200$   $\mu\text{mol/l}$  in diabetic patients and to  $1,491 \pm 252$   $\mu\text{mol/l}$  in control subjects (both  $P < 0.02$ ).

**Glycerol.** Fasting tended to increase glycerol levels slightly (from  $99 \pm 11$  to  $113 \pm 12$   $\mu\text{mol/l}$  in diabetic patients and from  $69 \pm 7$  to  $152 \pm 40$   $\mu\text{mol/l}$  in control subjects, both  $P > 0.05$ ). Fasting plus NA reduced plasma glycerol levels from  $81 \pm 10$  to  $35 \pm 6$   $\mu\text{mol/l}$  ( $P < 0.01$ ) in

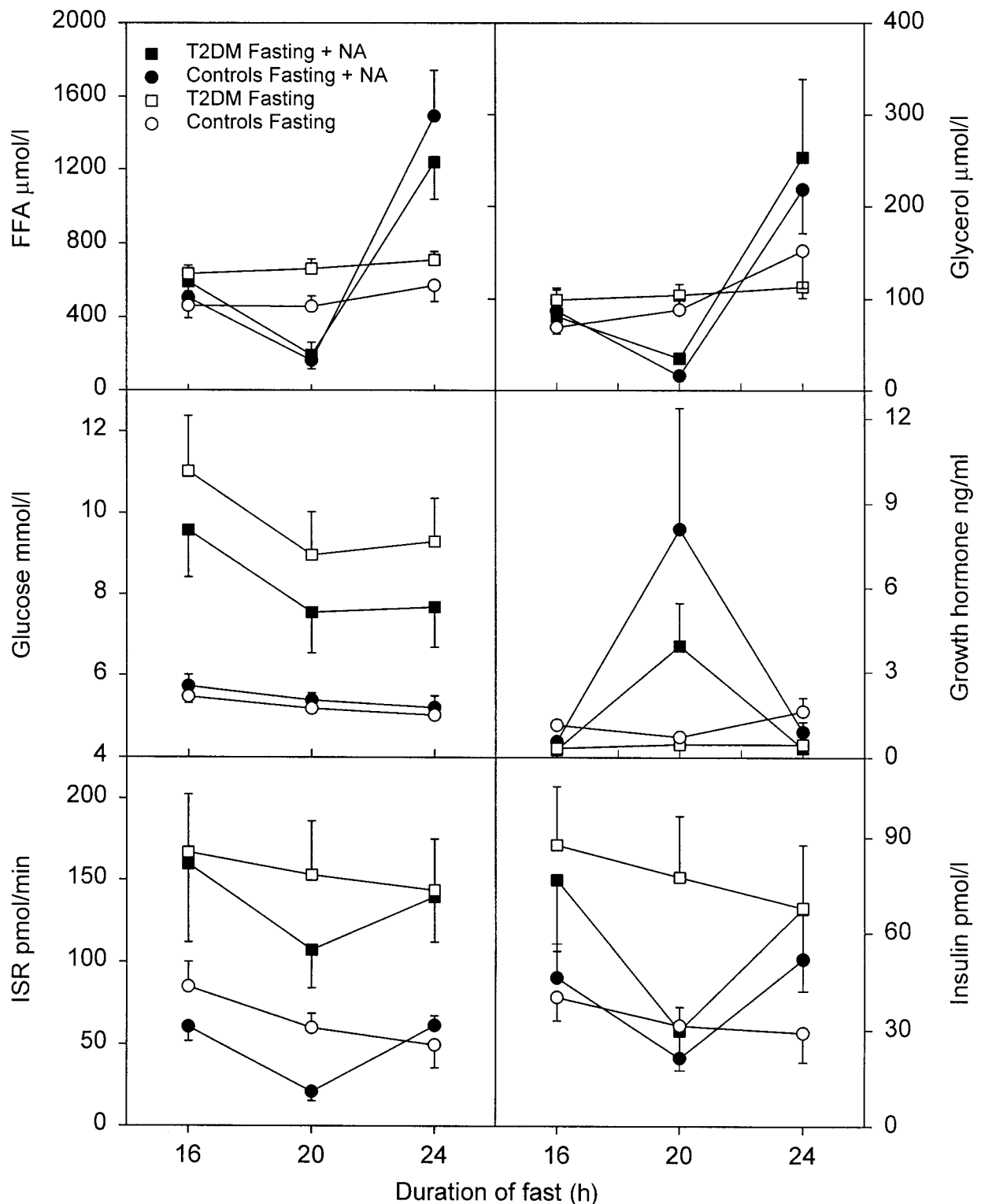


FIG. 1. Substrates and hormones in 14 subjects with type 2 diabetes and in 7 control subjects during an 8-h fast (from 16–24 h after the last meal) and in 9 subjects with type 2 diabetes and in 6 control subjects during (16–20 h) and after (20–24 h) NA administration. Data are means  $\pm$  SE.

diabetic patients and from  $87 \pm 25$  to  $16 \pm 5$   $\mu\text{mol/l}$  ( $P < 0.01$ ) in control subjects. After discontinuation of NA, glycerol levels rebounded to  $253 \pm 86$   $\mu\text{mol/l}$  in diabetic patients and to  $218 \pm 47$   $\mu\text{mol/l}$  in control subjects.

**Glucose.** During fasting, plasma glucose decreased from  $11.0 \pm 1.4$  mmol/l (at 16 h) to  $9.3 \pm 1.1$  mmol/l (at 24 h) in

diabetic patients ( $P < 0.05$ ) and from  $5.5 \pm 0.2$  to  $5.0 \pm 0.1$  mmol/l in control subjects ( $P < 0.01$ ). During fasting plus NA, glucose decreased in diabetic patients from  $9.6 \pm 1.1$  to  $7.5 \pm 1.0$  mmol/l ( $P < 0.001$ ) and did not change significantly in control subjects ( $5.7 \pm 0.3$  vs.  $5.2 \pm 0.3$

TABLE 2  
Substrates and hormones

	Type 2 diabetic subjects			Control subjects		
	16 h	20 h	24 h	16 h	20 h	24 h
Ketone bodies ( $\beta$ -hydroxybutyrate + acetoacetate) ( $\mu\text{mol/l}$ )						
Fasting	348 $\pm$ 73*	411 $\pm$ 82†	499 $\pm$ 101	140 $\pm$ 23‡	300 $\pm$ 40§	623 $\pm$ 139
Fasting and NA	210 $\pm$ 35¶	126 $\pm$ 36#	875 $\pm$ 131	328 $\pm$ 94¶	161 $\pm$ 11**	1,281 $\pm$ 353
Precursors ( $\mu\text{mol/l}$ )						
Fasting	2,269 $\pm$ 169	2,065 $\pm$ 142	2,052 $\pm$ 103	1,832 $\pm$ 114	1,804 $\pm$ 122	1,847 $\pm$ 84
Fasting and NA	2,112 $\pm$ 248	2,235 $\pm$ 241	2,344 $\pm$ 168	1,830 $\pm$ 93	1,797 $\pm$ 100	1,829 $\pm$ 96
Glucagon (pg/ml)						
Fasting	68 $\pm$ 5	66 $\pm$ 7	72 $\pm$ 8	60 $\pm$ 8	66 $\pm$ 7	63 $\pm$ 9
Fasting and NA	76 $\pm$ 10	70 $\pm$ 9	90 $\pm$ 9	57 $\pm$ 5	58 $\pm$ 4	72 $\pm$ 8
Epinephrine (pg/ml)						
Fasting	18 $\pm$ 5	23 $\pm$ 4	22 $\pm$ 3	17 $\pm$ 3	20 $\pm$ 4	25 $\pm$ 4
Fasting and NA	24 $\pm$ 4	37 $\pm$ 8	30 $\pm$ 4	28 $\pm$ 5	34 $\pm$ 8	77 $\pm$ 42
Cortisol (nmol/l)						
Fasting	320 $\pm$ 26††	275 $\pm$ 23	316 $\pm$ 58	225 $\pm$ 31¶	168 $\pm$ 16	131 $\pm$ 23
Fasting and NA	362 $\pm$ 54	369 $\pm$ 54	470 $\pm$ 165	203 $\pm$ 33	260 $\pm$ 55	304 $\pm$ 138

Data are means  $\pm$  SE. \* $P$  < 0.03 vs. control subjects; † $P$  < 0.01 vs. fasting and NA; ‡ $P$  < 0.01 vs. 24 h; § $P$  < 0.02 vs. fasting and NA; || $P$  < 0.03 vs. 20 h; ¶ $P$  < 0.05 vs. 24 h; # $P$  < 0.001 vs. 24 h; \*\* $P$  < 0.02 vs. 24 h; and †† $P$  < 0.03 s. control subjects.

mmol/l). During the FFA rebound, plasma glucose did not change in diabetic patients or control subjects.

**Growth hormone.** Plasma growth hormone levels did not change in either group during fasting. Growth hormone levels increased during fasting plus NA in seven of nine patients with type 2 diabetes (from  $0.26 \pm 0.03$  to  $3.93 \pm 1.53$  ng/ml,  $P$  < 0.05) and in six of six control subjects (from  $0.54 \pm 0.05$  to  $8.09 \pm 4.27$  ng/ml,  $P$  < 0.05). During the FFA rebound, growth hormone levels returned to basal values in all diabetic patients and control subjects.

**ISR.** During fasting, ISR decreased in diabetic patients (from  $167 \pm 36$  to  $143 \pm 32$  pmol/min) and in control subjects (from  $85 \pm 15$  to  $50 \pm 14$  pmol/min,  $P$  < 0.04). ISR decreased during fasting plus NA in diabetic patients and control subjects (from  $160 \pm 48$  to  $107 \pm 23$  pmol/min and from  $61 \pm 9$  to  $21 \pm 6$  pmol/min, respectively;  $P$  < 0.001) and increased during the FFA rebound (from  $107 \pm 23$  to  $139 \pm 27$ , pmol/min  $P$  < 0.04, and from  $21 \pm 6$  to  $61 \pm 6$  pmol/min,  $P$  < 0.002, respectively).

**Insulin.** During fasting, insulin levels tended to decrease in diabetic patients (from  $88 \pm 18$  to  $68 \pm 20$  pmol/l, NS) and in control subjects (from  $40 \pm 7$  to  $29 \pm 9$  pmol/l, NS). In diabetic patients and control subjects, insulin decreased during fasting plus NA (from  $77 \pm 22$  to  $30 \pm 8$ ,  $P$  < 0.05, and from  $46 \pm 10$  to  $21 \pm 4$  pmol/l,  $P$  < 0.04, respectively) and increased during the FFA rebound (from  $30 \pm 8$  to  $68 \pm 17$  pmol/l and from  $21 \pm 4$  to  $52 \pm 10$  pmol/l, both  $P$  < 0.02).

**Other substrates and hormones.** At 16 h, plasma concentrations of the sum of the five major GNG precursors (lactate, alanine, glutamine, glutamate, and glycerol) were not significantly different in type 2 diabetic patients compared with control subjects (Table 2). Precursor concentrations did not change in either group during fasting alone, during fasting plus NA, or during the FFA rebound.

There were no statistically significant differences in the plasma concentration of glucagon and epinephrine at any time between 16 and 24 h. Cortisol levels were higher at 16 h in diabetic patients than in control subjects ( $362 \pm 54$

vs.  $203 \pm 33$  nmol/l,  $P$  < 0.03), and cortisol levels in control subjects were lower at 24 than at 16 h ( $131 \pm 23$  vs.  $225 \pm 31$  nmol/l,  $P$  < 0.02).

#### GNG, GL, and EGP

**GNG.** During fasting, GNG did not change significantly in diabetic patients (from  $6.9 \pm 1.0$  to  $6.5 \pm 0.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) or in control subjects (from  $5.1 \pm 0.3$  to  $5.4 \pm 0.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) (Fig. 2); although expressed as percent of EGP, GNG increased significantly in diabetic patients and control subjects (from  $58.1 \pm 4.0$  to  $72.3 \pm 2.2\%$  and from  $52.1 \pm 2.5$  to  $64.2 \pm 2.2\%$ , respectively,  $P$  < 0.04) (Table 2). During fasting plus NA, GNG decreased in diabetic patients (from  $6.1 \pm 0.7$  to  $4.2 \pm 0.3$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P$  < 0.03), whereas in control subjects the change (from  $4.7 \pm 0.4$  to  $3.5 \pm 0.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was not statistically significant. During the FFA rebound, GNG increased from  $4.2 \pm 0.3$  to  $5.4 \pm 0.4$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P$  < 0.03) in diabetic patients and from  $3.5 \pm 0.6$  to  $5.3 \pm 0.8$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in control subjects ( $P$  < 0.05).

GNG correlated positively with FFA in control subjects ( $r = 0.63$ ,  $P$  < 0.009) and in diabetic patients ( $r = 0.55$ ,  $P$  < 0.02) and correlated negatively with serum insulin ( $r = -0.51$ ,  $P = 0.04$ ) over the 8-h study period (16–24 h).

**GL.** Fasting was associated with a significant decrease of GL in diabetic patients (from  $4.7 \pm 0.6$  to  $2.4 \pm 0.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P$  < 0.001) and in control subjects (from  $4.8 \pm 0.5$  to  $3.0 \pm 0.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P$  < 0.01). During fasting plus NA, GL tended to increase in control subjects (from  $5.4 \pm 0.6$  to  $7.2 \pm 0.9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and tended to decrease in diabetic patients (from  $5.3 \pm 0.6$  to  $4.4 \pm 0.4$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ); so that at 20 h, GL was significantly higher in control subjects than in diabetic patients ( $7.3 \pm 0.9$  vs.  $4.4 \pm 0.4$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P$  < 0.02). During the FFA rebound, GL decreased in diabetic patients (from  $4.4 \pm 0.4$  to  $3.4 \pm 0.5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and in control subjects (from  $7.2 \pm 0.9$  to  $4.3 \pm 0.5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P$  < 0.02).

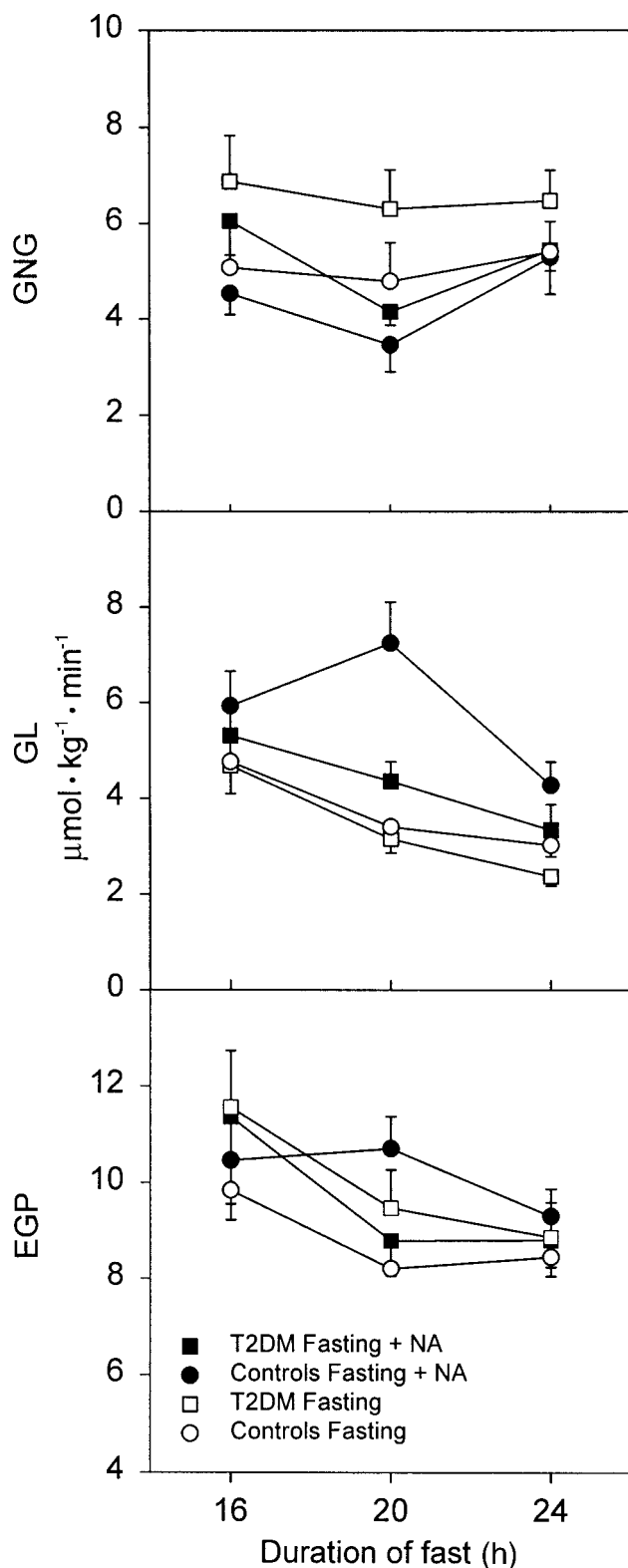


FIG. 2. GNG, GL, and EGP in 14 subjects with type 2 diabetes and in 7 control subjects during an 8-h fast (16–24 h after the last meal) and in 9 subjects with type 2 diabetes and 6 control subjects during (16–24 h) and after NA administration (20–24 h). GNG: type 2 diabetic patients (T2DM) 16 vs. 20 h,  $P < 0.03$ , and 20 vs. 24 h,  $P < 0.03$ ; and T2DM NA 20 h vs. T2DM fasting 20 h,  $P < 0.02$ . Control subjects NA 20 h vs. control subjects fasting 20 h,  $P < 0.05$ . GL: T2DM fasting 16 vs. 24 h,  $P < 0.001$ ; control subjects fasting 16 h vs. 24 h,  $P < 0.01$ ; and T2DM NA 20 h vs. control subjects NA 20 h,  $P < 0.02$ . EGP: T2DM fasting 16–24 h,  $P < 0.05$ ; control subject fasting 16 h vs. 24 h,  $P < 0.03$ ; and T2DM NA 16 vs. 20 h,  $P < 0.02$ .

**EGP.** EGP decreased during fasting in both groups (from  $11.4 \pm 0.7$  to  $8.9 \pm 0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in diabetic patients,  $P < 0.05$ , and from  $9.8 \pm 0.6$  to  $8.5 \pm 0.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in control subjects,  $P < 0.03$ ). During fasting plus NA, EGP declined in diabetic patients (from  $11.4 \pm 1.0$  to  $8.8 \pm 0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.02$ ), whereas it did not change in control subjects ( $10.5 \pm 0.4$  vs.  $10.7 \pm 0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , NS). During the FFA rebound, EGP did not change in diabetic patients ( $8.8 \pm 0.5$  vs.  $8.8 \pm 0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , NS) or in control subjects (from  $10.7 \pm 0.7$  to  $9.4 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , NS).

## DISCUSSION

**FFA and GNG.** One important result of this study was that GNG responses to changes in plasma FFA concentrations were similar in diabetic patients and control subjects, i.e., we found no evidence that GNG in patients with type 2 diabetes responded abnormally to changing plasma FFA levels. The reason for this may have been that most of our diabetic patients were reasonably well controlled and were able to compensate for their insulin resistance with augmented insulin secretion. Therefore, it may well be that patients with more severe type 2 diabetes and more compromised  $\beta$ -cell function may have more exaggerated GNG responses to changes in plasma FFA concentrations. In fact, recent findings that postabsorptive rates of GNG were higher than normal in subjects who had fasting plasma glucose concentrations of  $>10 \text{ mmol/l}$  and had reduced insulin secretion rates are compatible with this notion (20).

Our diabetic patients had, although not statistically significant, slightly higher weight, percent fat, and BMI than control subjects. To assess possible effects of obesity on GNG and GL, we compared BMI with GNG and found no significant correlation. Very similar findings have been reported by Magnusson et al. (21). Gastaldelli et al. (22), on the other hand, recently reported that GNG was higher in obese than in lean nondiabetic subjects ( $8.3 \pm 1.0$  vs.  $5.6 \pm 0.6 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{FFM}$ ,  $P < 0.01$ ), whereas there was no difference between obese and lean patients with type 2 diabetes ( $10.8$  vs.  $10.3 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{FFM}$ ).

In addition, the results demonstrated that fasting plasma FFA levels were needed to maintain fasting rates of GNG in both diabetic patients and control subjects. Compared with GNG during fasting, decreasing plasma FFAs with NA decreased GNG by 35% (from  $6.5$  to  $4.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in diabetic patients and control subjects (from  $5.4$  to  $3.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). This decrease in GNG was not caused by a direct effect of NA; as we and others have shown, NA per se does not affect GNG (5,23). On the other hand, NA administration was associated with significant decreases in insulin secretion and increases in plasma growth hormone concentrations in diabetic patients and control subjects. Insulin inhibits GNG (24), and growth hormone has been shown to inhibit insulin action on the liver and the periphery (25). Therefore, the inhibitory effect on GNG of lowering of FFA levels was probably underestimated to the extent that lower insulin and higher growth hormone levels stimulated GNG.

GNG continued at a rate of between  $3$  and  $4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in some subjects, even when plasma FFA concentrations had decreased to very low levels ( $<100 \mu\text{mol/l}$ ).

TABLE 3  
GNG and GL during and after NA

	Duration of fast		
	16 h	20 h	24 h
GNG (%)			
Fast	58.1 ± 4.0*	64.9 ± 3.5*	72.3 ± 2.2*
Fast and NA	53.1 ± 3.4	49.2 ± 3.0†	63.2 ± 3.8
Type 2 diabetic subjects (%)			
GL			
Fast	41.9 ± 4.0*	35.1 ± 3.5*	27.7 ± 2.2*
Fast and NA	46.9 ± 3.4	50.8 ± 3.0†	36.8 ± 3.8
GNG			
Fast	52.1 ± 2.5‡§	58.4 ± 1.2‡	64.2 ± 2.2‡
Fast and NA	47.0 ± 3.3	33.2 ± 6.1	54.6 ± 6.2
Control subjects (%)			
GL			
Fast	47.9 ± 2.5‡§	41.6 ± 1.2‡	35.8 ± 2.2‡
Fast and NA	53.0 ± 3.3	66.8 ± 6.1	45.4 ± 6.2

Data are means ± SE. \* $P < 0.06$ ; † $P < 0.012$  vs. 24 h; ‡ $P < 0.0001$ ; § $P < 0.05$  vs. 20 h; || $P < 0.04$  vs. 24 h.

This suggested that a part of GNG was independent of FFA levels and may have been driven by energy generated through protein oxidation, as has been suggested by Jungas et al. (26).

After discontinuation of NA, endogenous plasma FFA levels rebounded sharply in diabetic patients and control subjects (from  $<200$  to  $>1,200$   $\mu\text{mol/l}$ ). The increase in FFAs was associated with an increase in GNG, which was similar in both groups. Interestingly, GNG increased only to levels that were reached during fasting alone ( $8\text{--}9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) when plasma FFA concentrations were much lower than during the FFA rebound ( $600\text{--}700$  vs.  $1,200\text{--}1,500$   $\mu\text{mol/l}$ ). There are several possible reasons why GNG did not further increase: insulin levels also increased during this period (from  $30$  to  $68$   $\text{pmol/l}$  in diabetic patients and from  $21$  to  $52$   $\text{pmol/l}$  in control subjects) and may have prevented a greater increase in GNG; and a GNG rate of  $8\text{--}9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  may have been the maximal rate that could be achieved under these conditions. This interpretation is supported by data from Rothmann et al. (27), who found that GNG (determined with  $^{13}\text{C}$ -nuclear magnetic resonance spectroscopy) in healthy volunteers was  $8.3 \pm 0.5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (range  $6.4\text{--}10.4$ ) after  $46\text{--}64$  h of fasting when GNG accounted for nearly all ( $96 \pm 1\%$ ) of the EGP.

The mechanisms by which FFAs have been postulated to modify GNG include changes in acetyl-CoA (to change pyruvate carboxylase activity), changes in NADH (needed for the glyceraldehyde 3-phosphate reaction), and changes in ATP (to provide energy for GNG) (28). In addition, we cannot exclude that glycerol, which is released with FFAs during lipolysis, may have contributed to the observed changes in GNG (29).

**FFAs, GL, and autoregulation of EGP.** When GNG increased (during the FFAs rebound), GL decreased proportionally, and EGP remained unchanged in type 2 diabetic patients; this suggested that autoregulation of EGP functioned normally. In contrast, when GNG decreased (during fasting plus NA) in diabetic patients, GL also decreased, and EGP decreased precipitously. This indicated that autoregulation of EGP in response to decreasing GNG was impaired in diabetic patients. However, one

may question why these patients had normal autoregulation in response to increasing GNG, but abnormal autoregulation in response to decreasing GNG. An explanation for this peculiarity may be that patients with type 2 diabetes did not truly autoregulate EGP in a feedback-like manner when GNG increased, i.e., the decrease in GL was not caused by the increase in GNG, but by something else. This interpretation is supported by the observation that the GL decrease in diabetic patients and control subjects was apparently unrelated to changes in GNG, as GL decreased at similar rates, regardless of whether GNG remained unchanged (during fasting) or increased (during the FFA rebound). Moreover, because rates of GL are controlled largely by the hepatic glycogen content (30,31), it seems likely that the decreasing hepatic glycogen content during the fast, rather than the increasing GNG, was responsible for the decreasing GL. On the other hand, our data provided evidence for a feedback-like autoregulation of EGP under conditions of decreasing rates of GNG. In fact, the reciprocal increase in GL when GNG decreased occurred not only in the elderly and mildly overweight control subjects (this study), but also occurred in a group of younger and leaner nondiabetic control subjects (5).

The mechanism responsible for autoregulation of EGP in response to changing FFA levels and the reason why autoregulation was impaired in type 2 diabetic patients were not investigated and remain uncertain. It may be more difficult for patients with type 2 diabetes to increase GL during the extended fast because their glycogen stores were lower than those of the control subjects; Magnusson et al. (21) have recently reported lower glycogen stores in patients with type 2 diabetes than in nondiabetic control subjects.

**Summary and significance.** Our results showed that the regulation of GNG by FFA appeared to be normal in type 2 diabetic patients. However, this may not be true for subjects who are more severely insulin deficient than those studied here. In fact, a recent study showed decreased insulin secretion to be a main cause for elevated GNG in patients with type 2 diabetes (20). In addition, the data demonstrated that after a 20-h fast at least 35% of GNG in type 2 diabetic patients and nondiabetic subjects

depended on FFAs. Moreover, the current study showed that autoregulation of EGP by GL in response to decreasing GNG was impaired in type 2 diabetic patients. This defect of autoregulation can be used therapeutically. If plasma FFAs are lowered in patients with type 2 diabetes, both GNG and GL will decrease together, resulting in a marked decrease in EGP and fasting plasma glucose. This has, in fact, been recently demonstrated in patients with type 2 diabetes in whom elevated plasma FFA levels were normalized overnight with the long-acting NA analog acipimox (2). It also suggests that lowering plasma FFA levels may be one of the mechanisms by which thiazolidinediones, a new class of insulin-sensitizing drugs that lower plasma FFAs, improve fasting plasma glucose (32).

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