

Contribution of Elevated Free Fatty Acid Levels to the Lack of Glucose Effectiveness in Type 2 Diabetes

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Increased circulating free fatty acids (FFAs) inhibit both hepatic and peripheral insulin action. Because the loss of effectiveness of glucose to suppress endogenous glucose production and stimulate glucose uptake contributes importantly to fasting hyperglycemia in type 2 diabetes, we examined whether the approximate two-fold elevations in FFA characteristic of poorly controlled type 2 diabetes contribute to this defect. Glucose levels were raised from 5 to 10 mmol/l while maintaining fixed hormonal conditions by infusing somatostatin with basal insulin, glucagon, and growth hormone. Each individual was studied at two FFA levels: with (NA+) and without (NA-) infusion of nicotinic acid in nine individuals with poorly controlled type 2 diabetes ($HbA_{1c} = 10.1 \pm 0.7\%$) and with (LIP+) and without (LIP-) infusion of lipid emulsion in nine nondiabetic individuals. Elevating FFA to $\sim 500 \mu\text{mol/l}$ blunted the ability of glucose to suppress endogenous glucose production (LIP- = -48% vs. LIP+ = -28%; $P < 0.01$) and increased glucose uptake (LIP- = 97% vs. LIP+ = 51%; $P < 0.01$) in nondiabetic individuals. Raising FFA also blunted the endogenous glucose production response in 10 individuals with type 2 diabetes in good control ($HbA_{1c} = 6.3 \pm 0.3\%$). Conversely, normalizing FFA nearly restored the endogenous glucose production (NA- = -7% vs. NA+ = -41%; $P < 0.001$) and glucose uptake (NA- = 26% vs. NA+ = 64%; $P < 0.001$) responses to hyperglycemia in individuals with poorly controlled type 2 diabetes. Thus, increased FFA levels contribute substantially to the loss of glucose effectiveness in poorly controlled type 2 diabetes. *Diabetes* 52:2748-2758, 2003

Type 2 diabetes is a multifaceted disorder characterized by hyperglycemia with insulin resistance and impaired insulin secretion, resulting in decreased peripheral glucose uptake and increased endogenous glucose production (1,2). The progression toward worsening glycemic control in type 2 diabetes is accompanied by moderate elevations in plasma free fatty

acid (FFA) levels (3,4). These chronically elevated FFA levels result from resistance to the effects of both insulin and glucose on adipose tissue lipolysis and triglyceride storage (5). Important consequences of increased circulating FFAs include hepatic and peripheral insulin resistance (6,7), hepatic steatosis (8), diminished hepatic insulin clearance (9,10), and impaired pancreatic β -cell function (11,12).

Increased endogenous glucose production is believed to be the major cause of postabsorptive hyperglycemia in type 2 diabetes (1,2). Hepatic insulin resistance probably contributes to these inappropriate elevations in endogenous glucose production, given the known suppressive effects of insulin on endogenous glucose production (13,14). In addition, the effectiveness of glucose per se to inhibit endogenous glucose production seems to be markedly blunted in individuals with less optimally controlled type 2 diabetes (4,15,16). Indeed, there are prominent inhibitory effects of hyperglycemia, independent of other hormonal or metabolic signals, on endogenous glucose production in nondiabetic individuals (15,17-19). Of note, glucose effectiveness was completely restored by 72 h of intensive insulinization in individuals with poorly controlled type 2 diabetes in concert with normalization of plasma FFAs (4). Although inhibitory effects of hyperglycemia on lipolysis result in reduced FFA levels in nondiabetic individuals (17) and thereby may facilitate glucose-mediated suppression of endogenous glucose production, adipocyte resistance to glucose and insulin in individuals with moderately controlled type 2 diabetes likely contributes to the elevated FFA levels and loss of hepatic glucose effectiveness in these individuals.

These studies directly addressed the hypothesis that moderate elevations in circulating FFAs contribute importantly to the lack of glucose-mediated modulation of glucose fluxes in human type 2 diabetes. We first examined the effect of raising FFAs to type 2 diabetes levels on glucose effectiveness in nondiabetic individuals. We then determined to what extent FFA levels predicted glucose effectiveness among individuals with type 2 diabetes. Specifically, we compared responses to an identical hyperglycemic challenge in individuals with type 2 diabetes and moderately elevated FFA levels versus demographically matched individuals with type 2 diabetes and near-normal FFA levels. Because glycemic control differed between the two groups, we defined the specific contribution of FFA elevation by raising FFA in the individuals with nearly normal glycemic control and FFA levels. Finally, we examined the reversibility of FFA-induced impairments in

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Additional information can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

FFA, free fatty acid; G6Pase, glucose-6-phosphatase; GK, glucokinase.

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TABLE 1
Patient characteristics

Subjects	<i>n</i>	Age (years)	BMI (kg/m ²)	HbA _{1c} (%)	Duration (years)	Treatments
Good control	10	53.0 ± 4.1	30.4 ± 1.5	6.3 ± 0.3	5.1 ± 1.0	1D, 9H
Poor control	9	49.0 ± 2.7	27.2 ± 1.1	10.0 ± 0.7	7.9 ± 1.4	5H, 2I, 1H/I, 1D
Nondiabetic	9	51.0 ± 4.3	29.3 ± 1.2	N/A	N/A	N/A

D, diet only; H, oral hypoglycemic agents; I, insulin.

glucose effectiveness by normalizing plasma FFA levels in individuals with poorly controlled type 2 diabetes. "Pancreatic clamp" techniques were used to examine specifically the effect of an acute increase in plasma glucose on regulation of hepatic glucose fluxes, independent of any other hormonal or metabolic signals. The results suggest that the moderate elevations in FFA levels characteristic of poorly controlled type 2 diabetes are an important component of the loss of glucose effectiveness.

RESEARCH DESIGN AND METHODS

Subject characteristics. The nondiabetic (*n* = 9) healthy volunteers were taking no medications and had no family history of type 2 diabetes (Table 1). A 2-h oral glucose tolerance test was performed to ensure normal glucose tolerance. Individuals with type 2 diabetes were stratified into two groups on the basis of habitual glycemic control, using a cutoff HbA_{1c} value of 8%. Rationale for this cutoff value is provided in online appendix 1. Except for diabetes, all individuals with type 2 diabetes were in general good health and not experiencing proliferative retinopathy, significant diabetic renal disease, or symptomatic neuropathy. Medication use and withdrawal are described in online appendix 2. In all individuals, informed written consent was obtained in accordance with the policy of the institutional Committee on Clinical Investigations.

Experimental design. To ensure initial plasma glucose levels below 140 mg/dl in all individuals, individuals with poor control were admitted to the hospital the evening before the study for low-dose overnight insulinization. An 18-G catheter was inserted in an antecubital vein for infusions. Beginning at 10:00 P.M. on the night before the study, a variable intravenous infusion of insulin was started and the insulin infusion rate was adjusted according to an algorithm based on hourly blood glucose measurement (20). Nondiabetic individuals and individuals with good control were admitted to the General Clinical Research Center on the morning of the study after an overnight fast. The decision not to hospitalize the other individuals overnight was based on our previous experience (4) and a recent report that overnight low-dose insulinization does not affect glucose effectiveness (21).

Euglycemic/hyperglycemic pancreatic clamp studies. At 7 A.M. on the morning of the study, an 18-G catheter was inserted into an antecubital vein for infusions (in those individuals who had not been hospitalized overnight) and a contralateral hand vein was cannulated in a retrograde manner for arterialized venous blood sampling. For obtaining arterialized venous blood, this hand was kept in a warming plexiglas box maintained at 55°C. The experimental protocols lasted 6 h and consisted of an initial 4-h euglycemic period followed by a 2-h hyperglycemic period as previously described (4,15).

At *t* = 0 min, a primed-continuous infusion of high-performance liquid chromatography-purified [³-H]glucose (New England Nuclear, Boston, MA) was started (prime infusion 22 μCi), then continuously infused at 0.15 μCi/min for 6 h. Infusions containing somatostatin, growth hormone, glucagon, and insulin were also initiated at *t* = 0 min (4,16). Plasma glucose concentrations were measured at 10- to 15-min intervals during the initial 240 min of the study and maintained at basal levels (~90 mg/dl) by frequently adjusting the infusion rate of insulin during the first ~120 min and maintaining these optimal insulin infusion rates for the duration of the studies. At *t* = 240 min, plasma glucose concentrations were acutely increased to 180 mg/dl and then clamped at this level with variable 20% glucose infusions. [³-H]glucose (~0.1 μCi/ml) was added to the "cold" 20% glucose to maintain constant glucose specific activity (22). From *t* = 0 to *t* = 360 min, blood samples were obtained to measure plasma glucose, insulin, glucagon, C-peptide, FFAs, glycerol, lactate, and [³-H]glucose. All infusions were stopped at *t* = 360 min.

For determining the impact of FFAs on glucose effectiveness, glucose fluxes were compared between euglycemia and hyperglycemia in individuals with type 2 diabetes and nondiabetic individuals under the following conditions:

1. Nondiabetic individuals and individuals with type 2 diabetes and good control were studied under the following conditions: *a*) with infusion of lipid emulsion (LIP+; *n* = 10 in nondiabetic individuals and *n* = 9 in individuals with good control; Liposyn 20%, 0.42 ml/min) throughout the 6-h clamp studies to reproduce the moderately elevated FFA levels observed in poorly controlled type 2 diabetes, or *b*) with infusion of glycerol (GLY+; *n* = 6 in both groups; 12.25 mmol/h) to match the glycerol content of Liposyn (462 mmol/l); and *c*) LIP- (*n* = 10 in nondiabetic individuals and *n* = 9 in individuals with good control) and GLY- (*n* = 6 in both groups): 6 h saline control studies, paired with the above studies.
2. Individuals with poorly controlled type 2 diabetes were studied under the following conditions: *a*) with infusion of nicotinic acid (NA+; *n* = 9; USP grade; Sigma, St. Louis, MO) at 0.015 mg · kg⁻¹ · min⁻¹ throughout the 6-h clamp studies; this rate was selected to decrease FFA levels to those observed in nondiabetic individuals (23,24); the NA was pyrogen-free, pH adjusted to 7.0 with NaOH, and filter sterilized through a 0.2-μ filter before use; *b*) NA+/LIP+ (*n* = 5) with infusion of nicotinic acid (0.015 mg · kg⁻¹ · min⁻¹) while preventing a decline in FFAs by infusing Liposyn (0.42 ml/min), to rule out separate, unanticipated effects of NA on glucose metabolism; and *c*) NA- (*n* = 9) and NA-/LIP- (*n* = 5): saline control studies, paired with the above studies.

Thus, all individuals were studied under paired conditions with a control study and an infusion study, at least 1 month apart.

Analytical procedures. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) by use of the glucose oxidase method. Plasma [³-H]glucose and tritiated water specific activity were measured as previously described (4,20). Plasma insulin, C-peptide, glucagon, FFA, glycerol and lactate, and HbA_{1c} were measured as previously described (20).

Calculations. Rates of glucose appearance and glucose uptake (or glucose disappearance) were calculated using Steele's steady-state equation, as derived by Wolfe (25). Rates of endogenous glucose production, rates of glycolysis from plasma glucose, and glycogen synthetic rates were calculated as previously described (26). Data for glucose turnover, plasma hormones, and substrate concentrations represent the mean values during the final 60 min of the euglycemic period (*t* = 180–240 min) and the final 60 min of the hyperglycemic period (*t* = 300–360 min).

Statistical analysis of the data over time was performed using PROC MIXED in SAS System Version 6.12 (SAS Institute, Cary, NC). The random effect considered in this mixed model is the error measurement of individual subjects, and the within-individual fixed effect is the difference between groups. For averaged data, *t* test was used, with paired *t* tests for comparisons of euglycemic and hyperglycemic intervals and nonpaired *t* tests to compare the different groups.

RESULTS

Baseline (fasting) patient characteristics. After an overnight fast in all individuals and insulin infusion in the individuals with poor control, plasma insulin concentrations were similar in all groups. The rate of overnight insulin infusion required to maintain plasma glucose in the target range of 90–120 mg/dl in the individuals with poor control averaged 2.6 ± 0.25 units/h. Fasting plasma glucose levels averaged 127 ± 21 mg/dl in the individuals with poor control, 139 ± 10 mg/dl in the individuals with good control, and 101 ± 8 mg/dl in the nondiabetic individuals (averaged for all studies). Basal (*t* = 0) plasma FFA levels were as follows: nondiabetic = 355 ± 51, good control = 429 ± 87, and poor control = 285 ± 52 (*P* = 0.032 by ANOVA). This difference was attributed to a difference

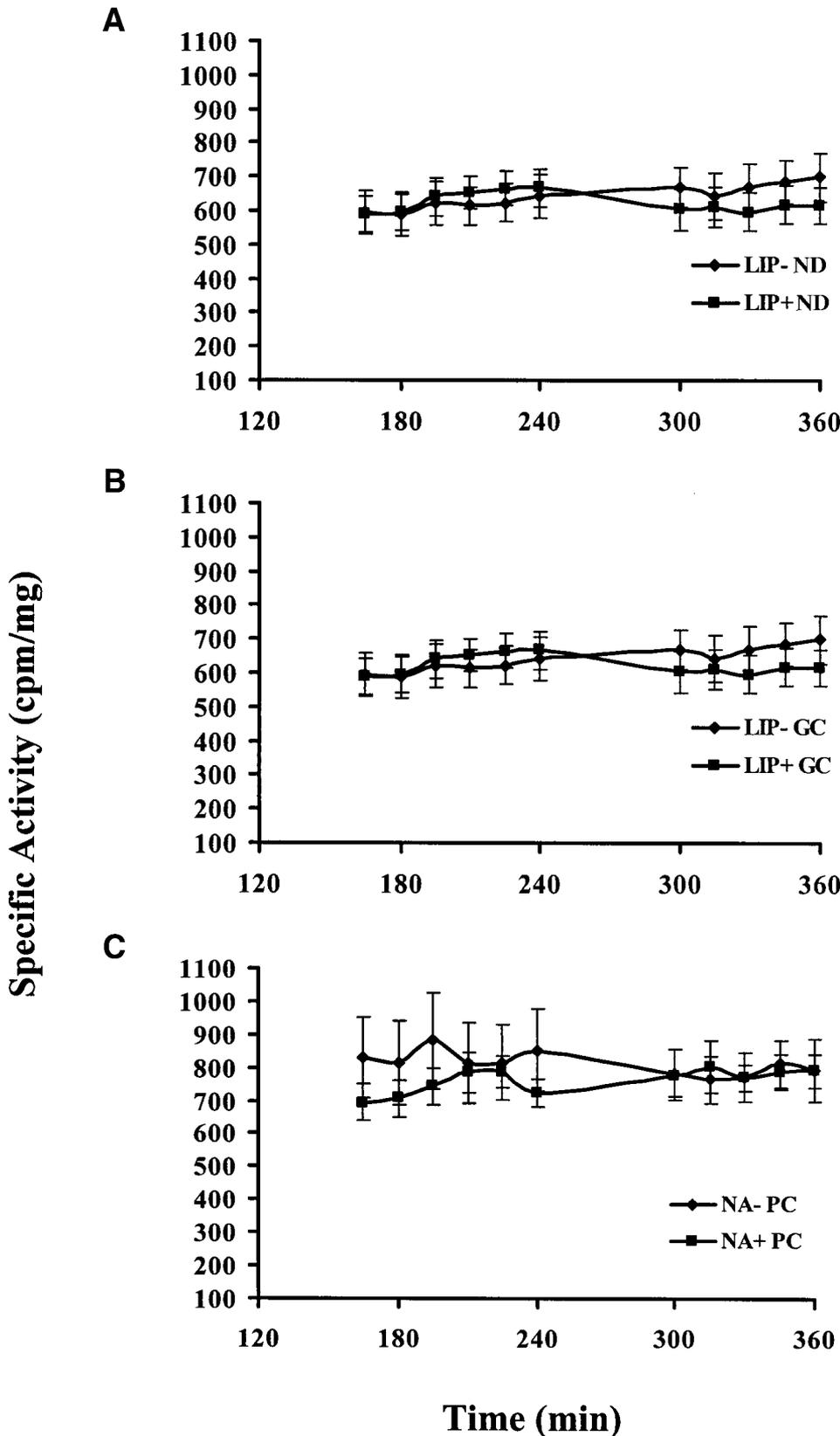


FIG. 1. Plasma specific activity (cpm/mg) in nondiabetic individuals during baseline (LIP-) versus lipid infusion (LIP+; A), individuals with good diabetic control during LIP versus LIP+ (B), and individuals with poor diabetic control (C) during baseline (NA-) versus nicotinic acid infusion (NA+).

between the two type 2 diabetes groups ($P = 0.04$ by t test), given the overnight insulin in the individuals with poor control. This also explains the difference in basal insulin values ($P = 0.04$ by ANOVA).

General clamp study conditions. Plasma glucose levels were similar in both groups of individuals with type 2

diabetes and in nondiabetic individuals during the euglycemic study period ($t = 180-240$; 95.3 ± 9.4 mg/dl for poor control, 96.8 ± 7.7 mg/dl for good control, and 95.2 ± 9.3 mg/dl for nondiabetic individuals) and the hyperglycemic study period ($t = 300-360$; 183.1 ± 3.3 mg/dl for poor control, 185.7 ± 5.9 mg/dl for good control, and 181.9 ± 4.0

mg/dl for nondiabetic individuals, respectively). Glucose specific activity was constant after tracer equilibration during both euglycemia and hyperglycemia in each group (Fig. 1).

The average insulin infusion rate needed to maintain euglycemia was similar in all study types and in all subject groups ($0.23 \pm 0.07 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for nondiabetic subjects, $0.30 \pm 0.24 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for subjects with good control, and $0.26 \pm 0.13 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for subjects with poor control; NS). Importantly, plasma insulin levels did not differ between the euglycemic and hyperglycemic study periods in any group. Plasma glucagon levels also remained stable in all groups (Tables 2 and 3). C-peptide levels were suppressed by overnight insulin infusion in the poorly controlled group. There was marked suppression of C-peptide levels by somatostatin infusion in all groups; although there was a trend toward increased C-peptide with the onset of hyperglycemia during LIP- studies in nondiabetic subjects, the levels remained suppressed at far lower than baseline levels. Plasma lactate levels were unchanged throughout the protocols in all study types, and the trend toward lower lactate levels in the individuals with poor control was not significant (Table 3).

Saline control studies. In the following text, results of all saline control studies are combined for each subject group. In the tables, all study results are compared with the paired saline control studies in the same individuals, e.g., LIP+ versus LIP-. The average rate of glucose infusion required to maintain the target hyperglycemic plateau during the last 60 min of the hyperglycemic period decreased by more than half in individuals with poor control compared with nondiabetic individuals ($0.69 \pm 0.21 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in individuals with poor control vs. $1.92 \pm 0.15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in individuals with good control and $2.59 \pm 0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in nondiabetic individuals; $P < 0.001$ for poor control vs. good control and $P < 0.001$ for poor control vs. nondiabetic; $P = 0.01$ for good control vs. nondiabetic). This suggests a marked decrease in "glucose effectiveness" in the individuals with poor control.

Endogenous glucose production was similar in all groups during euglycemia (nondiabetic = 1.83 ± 0.12 ; good control = 1.89 ± 0.11 ; and poor control = $1.94 \pm 0.09 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; NS), and there were significant and comparable decreases in endogenous glucose production in nondiabetic individuals and individuals with good control during hyperglycemia (nondiabetic = 0.92 ± 0.05 ; good control = $1.08 \pm 0.11 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.01$ vs. euglycemia). However, endogenous glucose production failed to suppress with hyperglycemia in individuals with poor control ($1.77 \pm 0.06 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Also, while the percentage increase in glucose uptake between the euglycemic and hyperglycemic study periods was $\sim 97\%$ and $\sim 79\%$ for nondiabetic individuals and individuals with good control, respectively (euglycemia: nondiabetic = 1.9 ± 0.1 , good control = $1.8 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; hyperglycemia: nondiabetic = 3.7 ± 0.1 , good control = $3.2 \pm 0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; NS for % change nondiabetic individuals vs. individuals with good control), there was a more modest increase of $\sim 25\%$ in poorly controlled (euglycemia = 1.9 ± 0.1 vs. hyperglycemia = $2.4 \pm 0.1 \text{ mg} \cdot$

TABLE 2
Plasma hormone and substrate values for nondiabetic (ND) and good control (GC) subjects

	Insulin ($\mu\text{U/ml}$)		Glucagon (pg/ml)		C-peptide (nmol/ml)		Lactate (mmol/l)		Glycerol ($\mu\text{mol/l}$)	
	ND	GC	ND	GC	ND	GC	ND	GC	ND	GC
LIP-										
Basal ($t = 0 \text{ min}$)	11.0 ± 1.9	19.8 ± 5.8	68.9 ± 2.1	80.6 ± 1.1	0.60 ± 0.14	0.79 ± 0.04	1.60 ± 0.27	1.31 ± 0.20	117.1 ± 11.2	122.8 ± 6.6
Euglycemia ($t = 180\text{--}240 \text{ min}$)	20.8 ± 2.2	29.0 ± 8.1	64.7 ± 1.7	81.5 ± 0.4	0.07 ± 0.00	0.10 ± 0.01	1.04 ± 0.14	1.01 ± 0.24	70.7 ± 6.9	89.7 ± 3.8
Hyperglycemia ($t = 300\text{--}360 \text{ min}$)	20.7 ± 2.6	27.0 ± 9.6	61.8 ± 1.5	77.2 ± 1.3	0.11 ± 0.00	0.10 ± 0.01	0.96 ± 0.11	0.89 ± 0.21	78.3 ± 7.4	87.4 ± 5.1
LIP+										
Basal ($t = 0 \text{ min}$)	9.6 ± 1.3	29.8 ± 8.8	70.8 ± 2.5	73.2 ± 2.3	0.62 ± 0.02	0.76 ± 0.03	1.30 ± 0.31	1.40 ± 0.18	122.0 ± 6.5	137.1 ± 10.8
Euglycemia ($t = 180\text{--}240 \text{ min}$)	$27.1 \pm 3.5^{\dagger}$	39.0 ± 6.9	68.4 ± 2.2	69.9 ± 2.0	0.10 ± 0.01	0.11 ± 0.01	0.75 ± 0.16	1.12 ± 0.19	$241.3 \pm 10.4^{\dagger}$	$277.9 \pm 13.2^{\dagger}$
Hyperglycemia ($t = 300\text{--}360 \text{ min}$)	$25.0 \pm 3.1^*$	$37.0 \pm 8.6^*$	65.2 ± 2.1	66.7 ± 1.8	0.12 ± 0.01	0.14 ± 0.01	1.00 ± 0.14	0.92 ± 0.17	$201.9 \pm 9.5^{\dagger}$	$247.1 \pm 10.5^{\dagger}$
GLY-										
Basal ($t = 0 \text{ min}$)	12.0 ± 2.6	16.0 ± 2.9	60.2 ± 4.4	73.1 ± 2.0	0.63 ± 0.03	1.03 ± 0.05	1.75 ± 0.34	1.62 ± 0.33	135.5 ± 19.9	126.0 ± 10.9
Euglycemia ($t = 180\text{--}240 \text{ min}$)	21.6 ± 2.8	33.8 ± 11.2	58.9 ± 3.2	69.9 ± 2.0	0.06 ± 0.00	0.13 ± 0.01	1.10 ± 0.19	1.17 ± 0.43	85.0 ± 12.2	94.7 ± 6.6
Hyperglycemia ($t = 300\text{--}360 \text{ min}$)	21.9 ± 3.3	34.0 ± 13.3	58.5 ± 2.9	66.7 ± 1.8	0.10 ± 0.01	0.16 ± 0.02	1.00 ± 0.14	1.01 ± 0.32	85.4 ± 13.8	83.2 ± 9.1
GLY+										
Basal ($t = 0 \text{ min}$)	10.0 ± 1.7	23.8 ± 15.4	6.1 ± 1.8	63.9 ± 2.6	0.64 ± 0.04	1.18 ± 0.06	1.87 ± 0.24	1.64 ± 0.24	307.3 ± 52.6	244.2 ± 34.1
Euglycemia ($t = 180\text{--}240 \text{ min}$)	24.4 ± 5.5	29.1 ± 2.5	55.0 ± 3.5	73.7 ± 1.3	0.06 ± 0.01	0.15 ± 0.01	1.37 ± 0.19	1.29 ± 0.18	$328.5 \pm 41.4^*$	$231.6 \pm 22.8^*$
Hyperglycemia ($t = 300\text{--}360 \text{ min}$)	27.7 ± 4.4	26.1 ± 3.3	57.6 ± 3.0	73.0 ± 2.0	0.12 ± 0.01	0.19 ± 0.01	1.22 ± 0.11	1.26 ± 0.21	$283.6 \pm 34.2^*$	$171.1 \pm 16.3^*$

* $P < 0.05$, $^{\dagger}P < 0.001$ for comparison with saline controls.

TABLE 3
Plasma hormone and substrate values for individuals with poor diabetic control

	Insulin (μ U/ml)	Glucagon (pg/ml)	C-peptide (nmol/ml)	Lactate (mmol/l)	Glycerol (μ mol/l)
NA-					
Basal ($t = 0$ min)	33.5 \pm 5.1	61.3 \pm 2.9	0.28 \pm 0.02	1.18 \pm 0.28	91.7 \pm 6.4
Euglycemia ($t = 180$ -240 min)	25.2 \pm 5.8	63.1 \pm 2.1	0.05 \pm 0.00	0.68 \pm 0.10	82.0 \pm 5.8
Hyperglycemia ($t = 300$ -360 min)	20.0 \pm 4.2	63.0 \pm 2.1	0.05 \pm 0.00	0.68 \pm 0.11	76.6 \pm 4.0
NA+					
Basal ($t = 0$ min)	37.5 \pm 6.2	70.1 \pm 1.6	0.33 \pm 0.03	1.03 \pm 0.28	90.4 \pm 3.5
Euglycemia ($t = 180$ -240 min)	25.8 \pm 5.2	83.7 \pm 0.7	0.19 \pm 0.03	0.67 \pm 0.16	52.9 \pm 3.5
Hyperglycemia ($t = 300$ -360 min)	25.0 \pm 5.0	79.2 \pm 0.7	0.14 \pm 0.02	0.65 \pm 0.13	52.0 \pm 2.6
NA-/LIP-					
Basal ($t = 0$ min)	34.0 \pm 9.5	71.6 \pm 0.7	0.27 \pm 0.04	1.10 \pm 0.36	110.1 \pm 9.4
Euglycemia ($t = 180$ -240 min)	22.5 \pm 6.0	69.8 \pm 3.5	0.06 \pm 0.01	0.63 \pm 0.80	101.9 \pm 9.6
Hyperglycemia ($t = 300$ -360 min)	20.1 \pm 5.1	69.8 \pm 3.8	0.05 \pm 0.01	0.61 \pm 0.11	89.7 \pm 6.7
NA+/LIP+					
Basal ($t = 0$ min)	64.0 \pm 27.8	64.5 \pm 3.1	0.30 \pm 0.05	1.30 \pm 0.13	69.7 \pm 6.8
Euglycemia ($t = 180$ -240 min)	19.1 \pm 2.1	75.0 \pm 3.7	0.08 \pm 0.01	0.88 \pm 0.10	179.3 \pm 17.6
Hyperglycemia ($t = 300$ -360 min)	20.0 \pm 2.3	73.6 \pm 1.2	0.06 \pm 0.01	0.86 \pm 0.07	227.5 \pm 19.9*

* $P < 0.05$, for comparison with saline control.

$\text{kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.02$ for % change in glucose uptake between individuals with poor control and both nondiabetic individuals and individuals with good control). The glycolytic rates from plasma glucose were similar in the three groups during euglycemia (poor control = 0.80 ± 0.1 vs. good control = 1.13 ± 0.2 vs. nondiabetic = 1.28 ± 0.1 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; NS). However, rates of glycolysis during hyperglycemia trended lower in individuals with poor control than in nondiabetic (poor control = 1.21 ± 0.2 vs. good control = 1.68 ± 0.4 vs. nondiabetic = 1.71 ± 0.2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.09$).

Importantly, plasma FFA concentrations were higher in individuals with poor control than in individuals with good control and nondiabetic individuals during both euglycemia (poor control = 400 ± 95 $\mu\text{mol/l}$ vs. good control = 237 ± 28 $\mu\text{mol/l}$ and nondiabetic = 170 ± 28 $\mu\text{mol/l}$; $P < 0.01$ for poor control vs. nondiabetic and good control) and hyperglycemia (poor control = 381 ± 75 $\mu\text{mol/l}$ vs. 198 ± 19 $\mu\text{mol/l}$ for good control and 170 ± 28 $\mu\text{mol/l}$ for nondiabetic individuals; $P < 0.01$ for poor control vs. nondiabetic and good control; Fig. 2). Thus, although there were time-dependent decreases in FFA values with the onset of hyperglycemia in nondiabetic individuals ($P = 0.001$) and individuals with good control (NS), FFA levels remained elevated in the group with poor control throughout the entire 6 h. By contrast, there were no differences in glycerol levels among groups (Tables 2 and 3).

We previously reported that rates of endogenous glucose production and glucose uptake were stable during 6 h of euglycemia under pancreatic clamp conditions in nondiabetic individuals and individuals with type 2 diabetes (15). To facilitate intergroup comparisons of glucose fluxes over time, we compared the actual rates of endogenous glucose production and glucose uptake during all study conditions (Figs. 3 and 4). Glucose fluxes were comparable during euglycemia in all studies and during hyperglycemia in all control studies. These results were also presented as the percentage change in endogenous glucose production and glucose uptake with the onset of hyperglycemia (Fig. 5).

LIP+ studies. The infusion of Liposyn in nondiabetic individuals and individuals with good control raised FFAs to levels comparable to the saline control studies in individuals with poor control (Fig. 2). The average rate of glucose infusion required to maintain the target hyperglycemic plateau during the last 60 min of the hyperglycemic period was decreased significantly in nondiabetic individuals during LIP+ studies (LIP+ = 1.59 ± 0.29 vs. LIP- = 2.59 ± 0.20 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.01$), suggesting decreased "glucose effectiveness." The elevated FFA levels resulted in a blunting of the suppression of endogenous glucose production with hyperglycemia, in both nondiabetic individuals and individuals with good control (Fig. 3). The percentage increase in glucose uptake in nondiabetic individuals (Fig. 5) between euglycemia and hyperglycemia was reduced to ~51% in the LIP+ vs. ~97% in LIP- ($P = 0.01$).

The rate of glycolysis during hyperglycemia was markedly decreased by Liposyn infusion in the nondiabetic individuals (LIP+ = 1.19 ± 0.19 vs. LIP- = 1.71 ± 0.18 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.03$). However, rates of glycogen synthesis were not affected by Liposyn infusion (hyperglycemia: LIP+ = 1.71 ± 0.34 vs. LIP- = 1.65 ± 0.26 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; NS), indicating that these moderate FFA elevations did not affect the acute stimulation of glycogen synthesis by glucose. Of note, plasma insulin levels were higher in the LIP+ studies during both euglycemia and hyperglycemia in the nondiabetic group ($P = 0.01$ vs. LIP-) and during hyperglycemia in the good control group ($P = 0.02$ vs. LIP-) and trended higher during euglycemia in the good control group, despite comparable insulin infusion rates and suppression of C-peptide. This was likely due to FFA-induced decreases in hepatic insulin clearance (10,11). Thus, the Liposyn-induced blunting of glucose effectiveness is all the more striking considering that insulin levels were actually slightly higher in those studies.

GLY+ studies. Because Liposyn infusions (LIP+) raised plasma glycerol levels, we performed glycerol infusion studies (GLY+) to ensure that elevated glycerol levels per

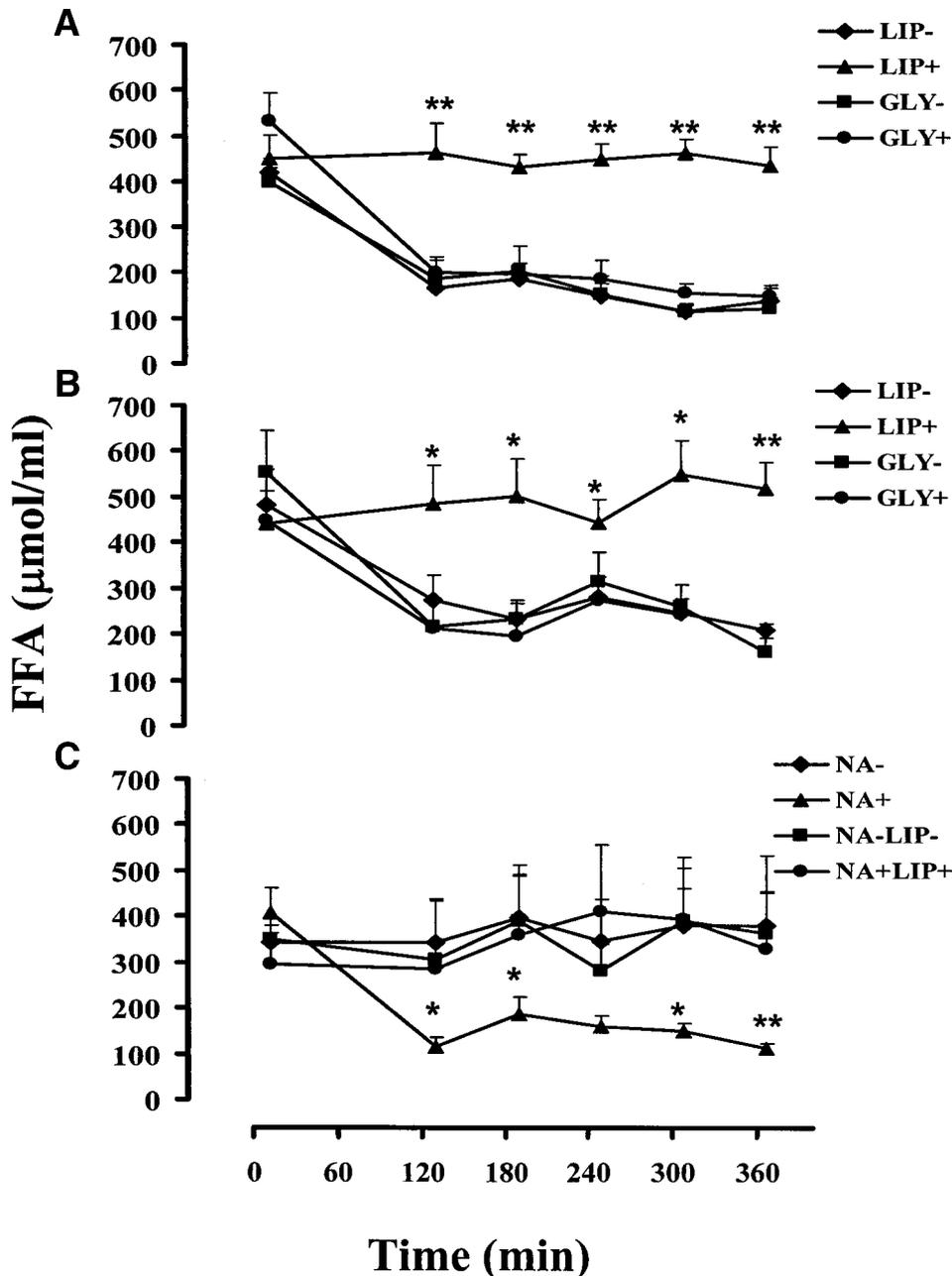


FIG. 2. Plasma FFA values ($\mu\text{mol/ml}$) in nondiabetic individuals with lipid infusion (LIP+) versus baseline (LIP-) and with glycerol (GLY+) infusion versus baseline (GLY-; A), individuals with good diabetic control with LIP+ versus LIP- and GLY+ versus GLY- (B), and individuals with poor diabetic control with baseline (NA-) versus nicotinic acid infusion (NA+) and with NA+/LIP+ versus NA-/LIP- (C). ** $P < 0.01$, * $P < 0.05$.

se did not affect glucose effectiveness. The infusion of glycerol in nondiabetic individuals and individuals with good control resulted in glycerol concentrations that were comparable to those observed with Liposyn (Table 2) but without any elevation in FFAs (GLY- vs. GLY+ in nondiabetic [NS] and GLY- vs. GLY+ in good control [$P = 0.05$]; Fig. 2). There was no difference in glucose infusion rate between GLY- and GLY+ in nondiabetic individuals or individuals with good control. There was also no significant difference in the endogenous glucose production or glucose uptake response to hyperglycemia between GLY+ and GLY- in nondiabetic individuals or individuals with good control.

NA+ studies. As shown in Fig. 2, FFA values in the NA+ studies were significantly lower than those in the baseline saline control (NA-) studies during both euglycemia and hyperglycemia. Indeed, plasma FFA levels in the NA+ studies in the group with poor control were comparable to

those in the saline control studies in the nondiabetic individuals during both euglycemia (NA+ in poorly controlled = 135 ± 21 vs. LIP- in nondiabetic = 145 ± 15 $\mu\text{mol/l}$; NS) and hyperglycemia (NA+ in poorly controlled = 133 ± 13 vs. LIP- in nondiabetic = 129 ± 16 $\mu\text{mol/l}$; NS). The average rate of glucose infusion required to maintain hyperglycemia during the last 60 min of the study in the NA+ studies in individuals with poor control was similar to the saline control studies in nondiabetic individuals (NS) and individuals with good control (NS). In fact, the glucose infusion rate in the NA+ studies was increased more than threefold over the rate required in the paired NA- studies. Thus, normalizing plasma FFA levels for 6 h significantly increased glucose effectiveness in the individuals with poor control.

The degree of suppression of endogenous glucose production in the NA+ studies ($-41 \pm 7.7\%$) was significantly lower than in the NA- studies in the same individuals

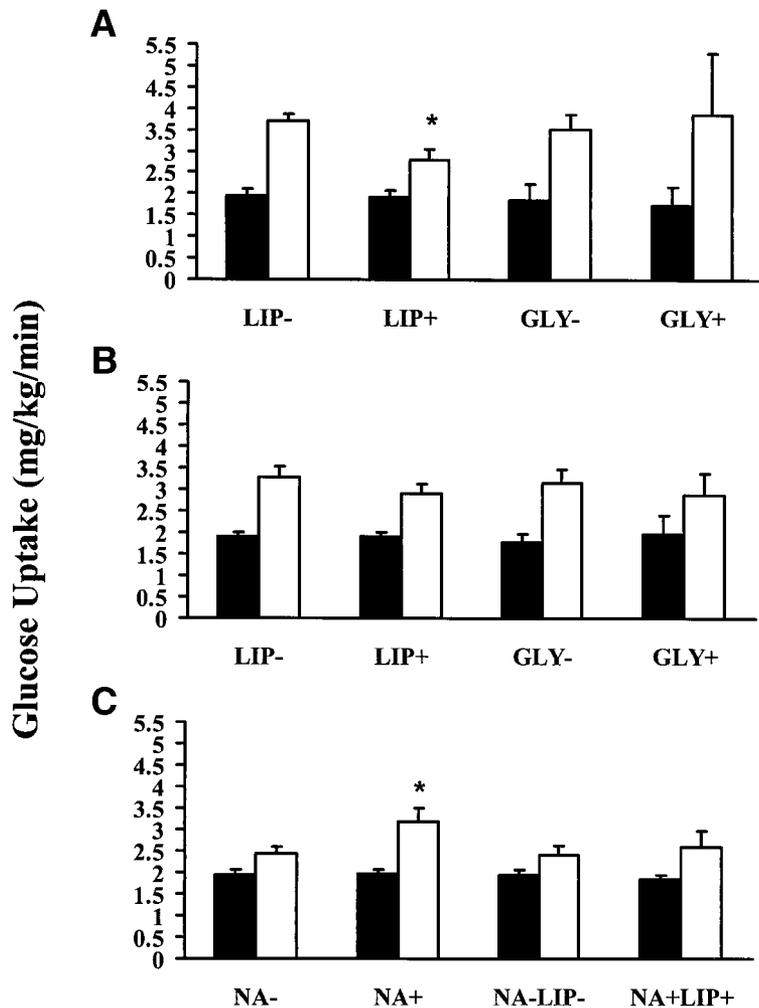


FIG. 3. Rates of glucose uptake ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during euglycemia versus hyperglycemia in nondiabetic individuals with lipid infusion (LIP+) versus baseline (LIP-) and with glycerol (GLY+) infusion versus baseline (GLY-; A), individuals with good diabetic control with LIP+ versus LIP- and GLY+ versus GLY- (B), and individuals with poor diabetic control with baseline (NA-) versus nicotinic acid infusion (NA+) and with NA+/LIP+ versus NA-/LIP- (C). * $P < 0.05$.

($-7 \pm 0.7\%$; $P = 0.001$) and comparable to the degree of suppression observed in the good control and nondiabetic groups (NS). Also, the percentage increase in glucose uptake between the euglycemic and hyperglycemic study periods was $64 \pm 10\%$ for the NA+ vs. $26.4 \pm 8\%$ change in the NA- ($P = 0.01$). Rates of glycolysis during euglycemia increased significantly with NA infusion (NA+ = 1.43 ± 0.26 vs. NA- = $0.80 \pm 0.15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.03$). **NA+/LIP+ studies.** Co-infusion of NA and Liposyn completely prevented the NA-induced decline in FFA levels, such that there were no significant differences in FFA measurements between the NA+/LIP+ during euglycemia ($537 \pm 147 \mu\text{mol/l}$) and hyperglycemia ($556 \pm 189 \mu\text{mol/l}$) and the saline control (NA-/LIP-) studies in the same individuals (NS). Endogenous glucose production and glucose uptake in the NA+/LIP+ studies were not different from NA-/LIP- (Figs. 3 and 4).

Correlation of FFA and glucose effectiveness. We examined the correlation between FFA levels and percentage suppression of endogenous glucose production by hyperglycemia in the group with poor control in all study types (saline control, NA+, and NA+/LIP+ studies) as an index of the importance of FFA in determining normal regulation of endogenous glucose production in these individuals. Examination of the data with a Spearman's rho test yielded a correlation coefficient of 0.642 ($P = 0.002$), suggesting a significant correlation.

DISCUSSION

Gradual increases in FFA levels may play an important role in the progression from normal glucose tolerance to fasting hyperglycemia and ultimately to frank type 2 diabetes in insulin-resistant individuals (5). Although the inhibitory effects of moderately elevated FFA levels on both hepatic and peripheral insulin action have been well documented (5), these are the first studies specifically designed to address whether increased FFA levels affect glucose effectiveness. Indeed, our results indicate that the moderately elevated FFA levels characteristic of type 2 diabetes markedly impair the ability of glucose to both stimulate glucose uptake and suppress glucose production. The magnitude of these findings suggests that the inhibitory effects of FFA on glucose effectiveness contribute importantly to worsening hyperglycemia in individuals with type 2 diabetes.

We used euglycemic/hyperglycemic pancreatic clamp techniques to study the ability of hyperglycemia per se to regulate glucose production and glucose uptake under fixed hormonal conditions. All individuals were studied under paired conditions, for intraindividual comparisons of glucose fluxes between euglycemia and hyperglycemia at different FFA levels. Infusion rates of Liposyn and NA were designed to ensure close matching of those FFA levels characteristic of individuals with poor control and

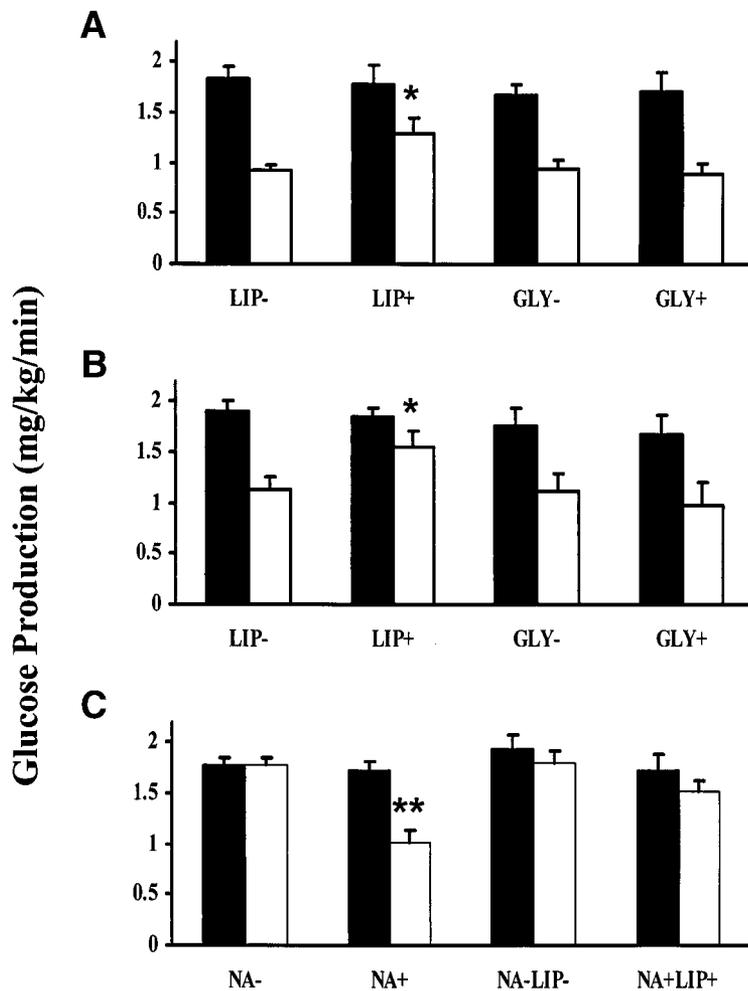


FIG. 4. Rates of endogenous glucose production ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during euglycemia versus hyperglycemia in nondiabetic individuals with lipid infusion (LIP+) versus baseline (LIP-) and with glycerol (GLY+) infusion versus baseline (GLY-; A), individuals with good diabetic control with LIP+ versus LIP- and GLY+ versus GLY- (B), and individuals with poor diabetic control with baseline (NA-) versus nicotinic acid infusion (NA+) and with NA+/LIP+ versus NA-/LIP- (C). * $P < 0.05$.

nondiabetic individuals, to study the impact of those moderately elevated FFA levels characteristic of type 2 diabetes on GE. Because individuals with poor control received overnight insulin infusions, plasma glucose levels were comparable in all groups during the euglycemic phase of the study and the absolute rise in plasma glucose with the onset of hyperglycemia was also comparable. In addition, because overnight insulin infusion has been shown to correct hepatic insulin resistance (27), basal insulin requirements did not differ between individuals with poor control and nondiabetic individuals. This was helpful in ensuring that differences in endogenous glucose production responses among groups were due to impaired glucose effectiveness and not to insulin resistance.

To establish further the importance of FFA levels in determining GE in type 2 diabetes, we made use of known heterogeneity in FFA levels among individuals with type 2 diabetes. Consistent with known suppressive effects of hyperglycemia per se on FFA levels (28), the onset of hyperglycemia was associated with FFA lowering in nondiabetic individuals. In contrast to individuals with type 2 diabetes in poor control, we have previously reported that those in good control retain a nearly normal ability to suppress FFA levels in response to hyperglycemia (4). In addition, these individuals respond to a doubling of plasma glucose concentration with a more robust increase in peripheral glucose uptake and a normal ability to suppress glucose production. Furthermore, attaining opti-

mal glycemic control for 72 h in individuals with poorly controlled type 2 diabetes both normalized the response of FFA to hyperglycemia and completely restored GE (4). These observations formed the rationale for using the three subject groups in the current studies and led us to hypothesize that the moderate elevations in FFA levels observed in poorly controlled type 2 diabetes could contribute importantly to the observed lack of glucose effectiveness.

These are the first studies to examine specifically whether elevated FFA levels contribute to the lack of GE in type 2 diabetes. However, some related observations are consistent with our conclusions. Elevated plasma FFA levels blunted the suppression of endogenous glucose production by a combination of both hyperinsulinemia and hyperglycemia in healthy women (6). When plasma FFA levels were elevated to >2 mmol/l for 9 h in healthy subjects under pancreatic clamp conditions, the resulting $\sim 50\%$ rise in glucose levels failed to suppress endogenous glucose production (29). Elevating FFA levels to ~ 3 mmol/l in nondiabetic men inhibited glucose-induced elevations in intramuscular glucose-6-phosphate levels and peripheral glucose uptake (30), although the hormonal conditions were not designed to examine endogenous glucose production. However, because elevations in FFA that are many-fold higher than the physiologic range can exert many effects on insulin signaling and other parameters, it is important to examine the effects of the moderate

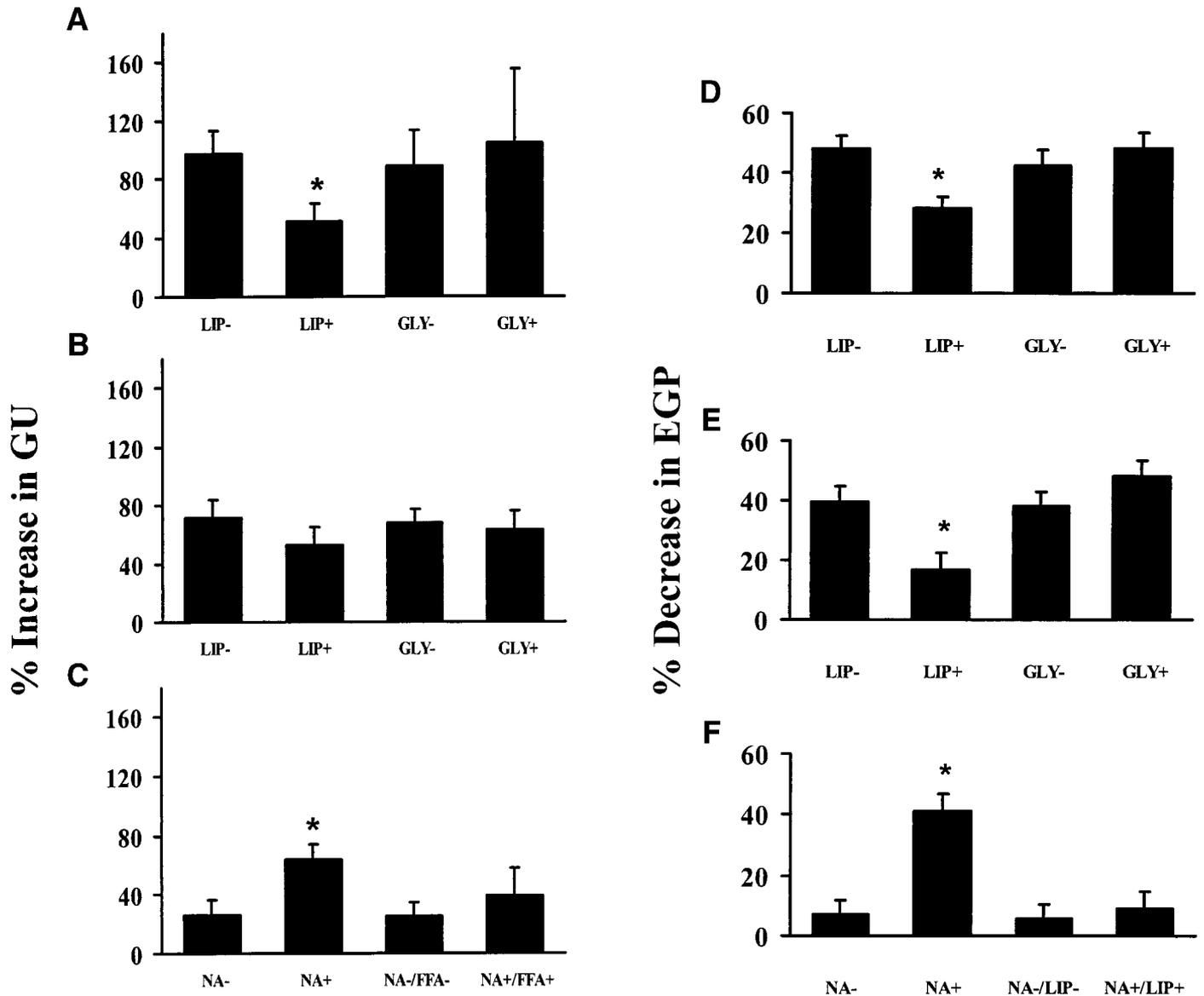


FIG. 5. Percentage change in glucose uptake and glucose production in nondiabetic individuals (A and D) with lipid infusion (LIP+) versus baseline (LIP-) and with glycerol (GLY+) infusion versus baseline (GLY-), percentage change in glucose uptake and endogenous glucose production in individuals with good diabetic control, LIP+ versus LIP- and GLY+ versus GLY- (B and E), and percentage change in glucose uptake and endogenous glucose production in individuals with poor diabetic control (C and F) with nicotinic acid infusion (NA+) versus baseline (NA-) and with NA+/LIP+ versus NA-/LIP-. * $P < 0.05$, ** $P < 0.001$.

elevations in FFA levels characteristic of type 2 diabetes. Under the controlled experimental conditions in the current studies, moderate changes in FFA levels had a major impact on GE in all groups.

It has been demonstrated that FFA levels declined with increasing blood glucose levels in nondiabetic individuals in parallel with suppression of endogenous glucose production, suggesting that glucose-induced decreases in FFA might make up a substantial component of the effectiveness of glucose to suppress endogenous glucose production (17). Our previous work has furthered this observation with the finding that inter-group differences in the ability of hyperglycemia to suppress FFAs predicted the degree of loss of glucose effectiveness (4). If, however, alterations in FFA levels completely explained the suppressive effects of glucose on endogenous glucose production, then one would expect that lowering FFAs would

also affect rates of endogenous glucose production during euglycemia. Instead, it is important to note that changes in FFAs do not alter rates of endogenous glucose production during euglycemia at basal insulin levels in these studies or in the literature (31,32). The contrasting observation that elevating FFA levels impaired the ability of increased portal insulin levels to suppress glucose production during euglycemia (33) suggests that portal insulin levels are likely to be important in determining this relationship.

The current observations suggest instead that circulating FFA levels, probably by affecting the relative activities of the enzymes glucokinase (GK) and glucose-6-phosphatase (G6Pase), play an important inhibitory role in the regulation of hepatic glucose fluxes by hyperglycemia. Indeed, the regulation of hepatic glucose fluxes by glucose is apparently determined by the relative flux through these key hepatic enzymes (34). It has been postulated that the

decreased hepatic glucose effectiveness in individuals with poorly controlled type 2 diabetes may be due to both decreased GK and increased G6Pase activity (15,35,36). Increased circulating FFA levels can contribute importantly to both defects (37–40). The current findings are consistent with other experimental models examining the impact of similar changes in GK or G6Pase activity. Neither acutely inhibiting GK in normal rats (41) nor disrupting the GK allele in transgenic mice (42) affected rates of endogenous glucose production during euglycemia, but both approaches markedly blunted the suppression of endogenous glucose production by hyperglycemia. In addition, G6Pase overexpression in the liver of normal rats caused further increases in plasma glucose levels with oral glucose loading but had no effect on blood glucose levels under fasting conditions (43).

FFA levels also affected the ability of hyperglycemia per se to affect whole-body glucose uptake. It is important to note that these studies were performed in the presence of low physiologic insulin levels, not in the absence of insulin. Indeed, insulin has marked potentiating effects on the ability of glucose to stimulate its own uptake (17). It has previously been shown that FFA levels did not affect glucose-mediated glucose uptake in the absence of insulin, yet they exerted marked inhibitory effects when insulin was infused at rates comparable to the present studies (44,45). The defect in glucose's effectiveness to stimulate glucose uptake in poorly controlled type 2 diabetes may be attributed in large part to a decreased availability of glucose transporters at the plasma membrane (46), such that glucose would be less able to enhance its own disposal (47). Of note, there seem to be direct effects of FFA on both the expression (48) and the translocation (49) of glucose transporters in skeletal muscle.

Together, these studies suggest that chronic increases in FFA availability impair the effectiveness of glucose to regulate glucose fluxes in type 2 diabetes and hence lead to a worsening of the overall metabolic condition. While regulation of glucose fluxes by hyperglycemia may occur in both nondiabetic individuals and individuals with type 2 diabetes (50), it may be activated at a higher set point in poorly controlled type 2 diabetes, and increased FFA levels might contribute to this altered set point. Indeed, the differential responses among the two type 2 diabetes groups and the marked improvement in regulation with normalizing FFA levels in the individuals with poor control are compatible with reversible metabolic effects on both enzymes. Given the vital importance of glucose effectiveness in determining glucose disposal in individuals with poorly controlled type 2 diabetes, lowering of plasma FFA levels might substantially restore the impaired glucose effectiveness in type 2 diabetes.

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