*Original Article*

# Time-Dependent Effects of Free Fatty Acids on Glucose Effectiveness in Type 2 Diabetes

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**Impaired effectiveness of glucose to suppress endogenous glucose production (EGP) is an important cause of worsening hyperglycemia in type 2 diabetes. Elevated free fatty acids (FFAs) may impair glucose effectiveness via several mechanisms, including rapid changes in metabolic fluxes and/or more gradual changes in gene expression of key enzymes or other proteins. Thus, we examined the magnitude and time course of effects of FFAs on glucose effectiveness in type 2 diabetes and whether glucose effectiveness can be restored by lowering FFAs. Glucose fluxes ([3-$^3$H]-glucose) were measured during 6-h pancreatic clamp studies, at euglycemia (5 mmol/l glucose, $t$ = 0–240 min), and hyperglycemia (10 mmol/l, $t$ = 240–360 min). We studied 19 poorly controlled subjects with type 2 diabetes ($HbA_{1c}$ 10.9 ± 0.4%, age 50 ± 3 years, BMI 30 ± 2 kg/m$^2$) on at least two occasions with saline (NA− group) or nicotinic acid (NA group) infusions for 3, 6, or 16 h (NA3h, NA6h, and NA16h groups, respectively) to lower FFAs to nondiabetic levels. As a reference group, glucose effectiveness was also assessed in 15 nondiabetic subjects. There was rapid improvement in hepatic glucose effectiveness following only 3 h of NA infusion (NA3h = 31 ± 6% suppression of EGP with hyperglycemia vs. NA− = 8 ± 7%; $P$ < 0.01) and complete restoration of glucose effectiveness after 6 h of NA (NA6h = 41 ± 8% suppression of EGP; $P$ = NS vs. nondiabetic subjects). Importantly, the loss of hepatic glucose effectiveness in type 2 diabetes is completely reversible upon correcting the increased FFA concentrations. A longer duration of FFA lowering may be required to overcome the chronic effects of increased FFAs on hepatic glucose effectiveness. *Diabetes* 55:1761–1768, 2006**

Increased endogenous glucose production (EGP) may be the principal cause of fasting hyperglycemia in type 2 diabetes (1–3). Several mechanisms have been proposed to explain this observation, including defective overnight insulin secretion and relative insulin deficiency, enhanced delivery of gluconeogenic substrates, hepatic insulin resistance, and impairment of the effectiveness of glucose per se to regulate its own production (1). Indeed, in addition to the suppressive effects of insulin on EGP (4,5), we and others (6–10) have reported direct inhibitory effects of hyperglycemia on EGP in nondiabetic individuals, independent of other hormonal or metabolic signals. These studies all demonstrated that hyperglycemia per se inhibits EGP in nondiabetic individuals, and this phenomenon is referred to as "glucose effectiveness." This glucose effectiveness is markedly blunted in people with type 2 diabetes (11–13) and contributes substantially to worsening hyperglycemia. Importantly, we have demonstrated that glucose effectiveness is completely restored in individuals with type 2 diabetes by normalizing the chronic metabolic defects (12).

In addition to chronic hyperglycemia, the metabolic milieu in less than optimally controlled type 2 diabetes is characterized by sustained elevations in free fatty acid (FFA) levels (14), which rise in proportion to worsening glycemic control (12). Of note, we have reported that 72 h of normoglycemia with intensive insulinization in subjects with poorly controlled type 2 diabetes restored glucose effectiveness together with normalization of plasma FFA levels (12). We recently demonstrated that elevating circulating FFA levels in nondiabetic subjects antagonized the regulation of EGP by hyperglycemia, while conversely, normalizing FFA levels for 6 h markedly improved this regulation in subjects with type 2 diabetes. Furthermore, in type 2 diabetic subjects with good glycemic control ($HbA_{1c}$ [A1C] <7%), both FFA levels and glucose effectiveness were comparable with nondiabetic subjects (15). Thus, the inability of hepatic glucose fluxes to respond to hyperglycemia in poorly controlled type 2 diabetic subjects may be, at least in part, due to sustained increases in plasma FFAs. Loss of insulin's inhibitory effects on lipolysis probably contributes to the FFA elevations in type 2 diabetes, thus ultimately impairing glucose effectiveness. Since long-term normalization of FFA levels remains an elusive goal, delineating the mechanism(s) whereby FFAs impact glucose effectiveness could be therapeutically advantageous.

An increase in de novo synthesis of glucose via gluconeogenesis is the major process responsible for increased EGP in type 2 diabetes. Increased FFAs are known to potently stimulate gluconeogenesis (16,17). Additionally, increased FFA availability has been shown to affect the relative flux through the key hepatic enzymes, glucokinase and glucose-6-phosphatase, by altering both gene expression and enzyme activity (18–23).

Thus, we studied the time-dependent effects of increased FFAs on glucose effectiveness and the extent to which these effects are reversible in type 2 diabetes. We thereby aimed to establish the magnitude, time course,

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Received for publication 2 August 2005 and accepted in revised form 16 March 2006.

EGP, endogenous glucose production, FFA, free fatty acid.

DOI: 10.2337/db05-0988

TABLE 1
Subject characteristics

| | Nondiabetic subjects | Type 2 diabetic subjects |
|---|---|---|
| *n* | 15 | 19 |
| Age (years) | 47 ± 2 | 50 ± 3 |
| Sex (men/women) | 11/4 | 14/5 |
| BMI (kg/m$^2$) | 28 ± 5 | 30 ± 2 |
| A1C (%) | NA | 10.9 ± 0.4 |
| Diabetes management | | |
| Diet alone | NA | 1 |
| Insulin alone | NA | 7 |
| Metformin or sulfonylurea alone | NA | 5 |
| Metformin and sulfonylurea | NA | 2 |
| Metformin and thiazolidinediones | NA | 2 |
| Metformin, sulfonylurea, and thiazolidinediones | NA | 1 |

Data are means ± SE, unless otherwise indicated. NA, not applicable.

and reversibility of the impact of FFAs on glucose effectiveness in type 2 diabetes. Indeed, we observed significant improvement in glucose effectiveness in poorly controlled type 2 diabetic individuals following only 3 h of FFA lowering and a maximal effect after 6 h. The complete restoration of glucose effectiveness with FFA lowering underscores the importance of FFA levels to this regulation and the potential therapeutic benefit of lowering FFAs in type 2 diabetes.

## RESEARCH DESIGN AND METHODS

Subjects aged 18–65 years were recruited, and informed written consent was obtained in accordance with the policies of the institutional committee on clinical investigations (Table 1). Subjects with type 2 diabetes ($n$ = 19) were in poor glycemic control (A1C ≥8%). Except for diabetes, all type 2 diabetic subjects were in general good health and did not have proliferative retinopathy, significant diabetic renal disease (urine microalbumin <100 mg/dl), or symptomatic neuropathy. Insulin was discontinued 24 h prior, sulfonylureas and metformin 3 days prior, and thiazolidinediones at least 8 days prior to the day of the clamp studies. The nondiabetic volunteers ($n$ = 15) were taking no medications and had no family history of type 2 diabetes. A 2-h oral glucose tolerance test was performed to ensure normal glucose tolerance.

Nondiabetic subjects were admitted to the General Clinical Research Center on the morning of the study. To ensure that initial plasma glucose levels were <140 mg/dl in all subjects on the morning of study, subjects with type 2 diabetes were admitted the evening before the study for low-dose overnight insulin infusion, and an 18-gauge 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 rate was adjusted according to an algorithm based on hourly blood glucose measurement. As previously described, overnight correction of glucose did not affect glucose effectiveness in these poorly controlled type 2 diabetic subjects (24).

**Euglycemic-hyperglycemic pancreatic clamp studies.** At 7 A.M. on the morning of the study, an 18-gauge catheter was inserted in an antecubital vein for infusions and a contralateral hand vein was cannulated in a retrograde fashion for arterialized venous blood sampling. To obtain 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 (12,15).

At $t$ = 0 min, a primed-continuous infusion of high-performance liquid chromatography–purified [3-$^3$H]-glucose (New England Nuclear, Boston, MA) was started (prime infusion 22 μCi) and then continued at 0.15 μCi/min for 6 h (Fig. 1). Infusions containing somatostatin, growth hormone, glucagon, and insulin were also initiated at $t$ = 0 min (12,15). Plasma glucose concentrations were measured at 10- to 15-min intervals during the initial 240 min of the study and maintained at normal fasting concentrations (~90 mg/dl) by frequently adjusting the infusion rate of insulin during the first 120 min and maintaining these individualized insulin infusion rates for the duration of the studies. At

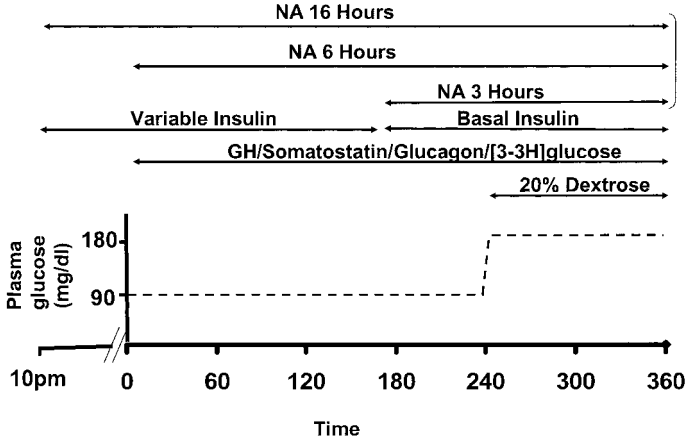


**FIG. 1. Schematic diagram of the clamp study. Type 2 diabetic subjects received either NA to lower FFA levels for 3, 6, or 16 h or saline. Variable insulin was infused overnight in type 2 diabetes. Basal insulin requirements were established for the first 3 h in both nondiabetic and type 2 diabetic subjects followed by constant insulin infusion. Blood glucose was maintained at ~90 mg/dl for the first 4 h and acutely raised to ~180 mg/dl for the next 2 h. Somatostatin was infused to inhibit endogenous insulin with replacement of growth hormone (GH) and glucagon at physiological levels.**

240 min, plasma glucose concentrations were acutely increased to 180 mg/dl and then clamped at this level with variable 20% glucose infusions. [3-$^3$H]-glucose (~0.1 μCi/ml) was added to the "cold" 20% glucose to maintain constant glucose specific activity (15). From 0 to 360 min, blood samples were obtained to measure plasma glucose, insulin, glucagon, C-peptide, FFAs, glycerol, lactate, and 3-$^3$H-glucose specific activity. All infusions were stopped at 360 min.

To determine the impact of FFAs on glucose effectiveness, glucose fluxes were compared between euglycemia and hyperglycemia in the nondiabetic group and type 2 diabetic subjects under the various conditions outlined below:

1) Nondiabetic subjects underwent a control pancreatic clamp study as described above with saline infusion only. A subgroup of these subjects have been previously described (15).
2) All type 2 diabetic subjects were studied under paired conditions, with a control (saline infusion [NA−) study and an active infusion (nicotinic acid [NA]) study, at least 1 month apart under the following conditions: *a*) saline control studies (NA−) paired with one of the following study types: *b*) NA3h ($n$ = 9): with infusion of nicotinic acid (0.017 mg · kg$^{-1}$ · min$^{-1}$) for the final 3 h of the clamp study or *c*) NA6h ($n$ = 8): with infusion of nicotinic acid (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 subjects (33,34). The NA was pyrogen free, pH adjusted to 7.0 with NaOH, and filter sterilized through a 0.2-micron filter before use. *d*) NA16h ($n$ = 8): infusion of nicotinic acid (0.008 mg · kg$^{-1}$ · min$^{-1}$) starting at 10 P.M. and continuing for the duration of the clamp study at a rate of 0.015 mg · kg$^{-1}$ · min$^{-1}$.

These rates of NA infusion were chosen based on the literature and pilot studies to match FFA levels in the final hour in all groups.

**Analytical procedures.** Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) by use of the glucose oxidase method. Plasma [3-$^3$H]-glucose and tritiated water–specific activities were measured as previously described (4,11). Plasma insulin, C-peptide, glucagon FFAs, glycerol and lactate, and A1C were measured as previously described (15).

**Calculations.** Rates of glucose appearance ($R_a$) and glucose uptake ($R_d$) were calculated using Steele's steady-state equation (26). Rates of EGP 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.** Analysis of the data were performed using SPSS Version 11.5. For averaged data, Student's $t$ tests were utilized, using paired $t$ tests for comparisons of euglycemic and hyperglycemic intervals in each group and nonpaired $t$ tests to compare the nondiabetic with the type 2 diabetic group. ANOVA was used to compare the various study conditions within the type 2

TABLE 2
Hormone values during the clamp studies

| | Insulin (μU/ml) | C-Peptide (nmol/ml) | Glucagon (pg/ml) |
|---|---|---|---|
| Nondiabetic | | | |
| Basal* | 4.8 ± 0.7 | 0.50 ± 0.07 | 64.5 ± 2.8 |
| Euglycemia† | 13.2 ± 1.5 | 0.10 ± 0.01 | 73.3 ± 2.4 |
| Hyperglycemia‡ | 14.6 ± 2.0 | 0.16 ± 0.04 | 68.3 ± 2.7 |
| NA− | | | |
| Basal | 25.0 ± 6.3 | 0.21 ± 0.04 | 59.5 ± 1.0 |
| Euglycemia | 25.9 ± 3.1 | 0.08 ± 0.01 | 63.9 ± 0.6 |
| Hyperglycemia | 23.0 ± 3.5 | 0.10 ± 0.03 | 62.4 ± 0.7 |
| NA3h | | | |
| Basal | 26.3 ± 8.1 | 0.29 ± 0.10 | 64.9 ± 1.4 |
| Euglycemia | 25.1 ± 5.3 | 0.15 ± 0.04 | 68.6 ± 0.6 |
| Hyperglycemia | 23.9 ± 6.0 | 0.11 ± 0.02 | 64.9 ± 1.2 |
| NA− | | | |
| Basal | 33.6 ± 5.2 | 0.26 ± 0.06 | 69.3 ± 1.2 |
| Euglycemia | 19.9 ± 4.4 | 0.06 ± 0.01 | 81.1 ± 0.9 |
| Hyperglycemia | 16.7 ± 3.5 | 0.06 ± 0.01 | 79.3 ± 1.1 |
| NA6h | | | |
| Basal | 37.5 ± 6.2 | 0.25 ± 0.10 | 66.4 ± 1.7 |
| Euglycemia | 22.6 ± 4.8 | 0.09 ± 0.02 | 81.9 ± 0.7 |
| Hyperglycemia | 22.5 ± 5.0 | 0.07 ± 0.10 | 78.5 ± 0.7 |
| NA− | | | |
| Basal | 34.6 ± 12.7 | 0.37 ± 0.17 | 73.9 ± 1.3 |
| Euglycemia | 18.6 ± 2.7 | 0.08 ± 0.02 | 72.7 ± 1.2 |
| Hyperglycemia | 18.1 ± 3.0 | 0.11 ± 0.05 | 72.1 ± 1.4 |
| NA16h | | | |
| Basal | 31.6 ± 11.1 | 0.22 ± 0.02 | 62.4 ± 1.3 |
| Euglycemia | 20.4 ± 2.8 | 0.08 ± 0.02 | 77.3 ± 1.5 |
| Hyperglycemia | 15.5 ± 1.6 | 0.07 ± 0.02 | 70.7 ± 1.2 |

Data are means ± SE. *Basal: $t = 0$; †euglycemia: $t = 180$–$240$ min; ‡hyperglycemia: $t = 300$–$360$ min. See text for details.

diabetic group. All data are presented as means ± SE, unless otherwise specified. A $P$ value $<0.05$ was considered significant.

## RESULTS

**Baseline (fasting) patient characteristics.** The rate of overnight insulin infusion required to maintain plasma glucose in the target range of 90–120 mg/dl in the type 2 diabetic subjects averaged 2.67 ± 0.20 units/h. There was no difference in overnight insulin infusion rates for the different study conditions. In the nondiabetic subjects, fasting plasma glucose levels averaged 99 ± 1 mg/dl and plasma FFA levels were 443 ± 57 μU/ml. In type 2 diabetic subjects, basal ($t = 0$) plasma glucose was 124 ± 3 mg/dl and plasma FFA levels were 349 ± 44 μU/ml (averaged for all studies after overnight insulin infusion). There was no difference in fasting plasma glucose and fasting FFAs for the three NA groups (Tables 1 and 2).

**General clamp study conditions.** During the clamps, plasma glucose levels were similar in type 2 diabetic and in nondiabetic subjects (during the euglycemic study period, 98 ± 1 mg/dl for type 2 diabetic and 96 ± 1 mg/dl for nondiabetic subjects and during the hyperglycemic study period, 185 ± 1 mg/dl for type 2 diabetic and 183 ± 1 mg/dl for nondiabetic subjects, respectively). Plasma glucose levels during both euglycemia and hyperglycemia were similar between pairs of studies (NA versus NA−). Glucose specific activity was constant following tracer equilibration during both euglycemia and hyperglycemia in each group (Fig. 2).

Although there was a trend toward higher average insulin infusion rates required to maintain euglycemia in type 2 diabetic (0.23 ± 0.02 $\mu U \cdot kg^{-1} \cdot min^{-1}$) when compared with nondiabetic (0.18 ± 0.01 $\mu U \cdot kg^{-1} \cdot min^{-1}$) subjects, this did not reach statistical significance ($P = 0.056$). Importantly, plasma insulin levels did not differ between euglycemic and hyperglycemic study periods or between pairs of the NA studies (Table 2). The former comparison is particularly important since the principal comparison is between euglycemia and hyperglycemia in the same study. Plasma glucagon levels also remained stable in all groups. C-peptide levels were suppressed by somatostatin infusion in all studies and did not differ between pairs of studies in either basal, euglycemic, or hyperglycemic periods. Although there was a trend toward increased C-peptide with the onset of hyperglycemia in nondiabetic subjects, the levels remained suppressed at far-lower-than-baseline levels (Table 2). Plasma lactate levels were not different between paired studies.

**Saline control studies (NA− and nondiabetic subjects).** In the tables, all study results will be compared with the paired saline control studies in the same subjects, e.g., NA3h versus NA−. There was a marked decrease in glucose effectiveness in the type 2 diabetic subjects when compared with nondiabetic subjects. The average rate of glucose infusion required to maintain the target hyperglycemic plateau during the last 60 min of the hyperglycemic period was decreased by more than half in the type 2 diabetic compared with the nondiabetic subjects (1.00 ± 0.19 $mg \cdot kg^{-1} \cdot min^{-1}$ in type 2 diabetes vs. 3.10 ± 0.20 $mg \cdot kg^{-1} \cdot min^{-1}$ in nondiabetic subjects; $P < 0.001$). EGP was suppressed by 48 ± 3% with hyperglycemia in nondiabetic subjects but failed to suppress with hyperglycemia in type 2 diabetic subjects (9 ± 3%, $P < 0.001$ vs. nondiabetic

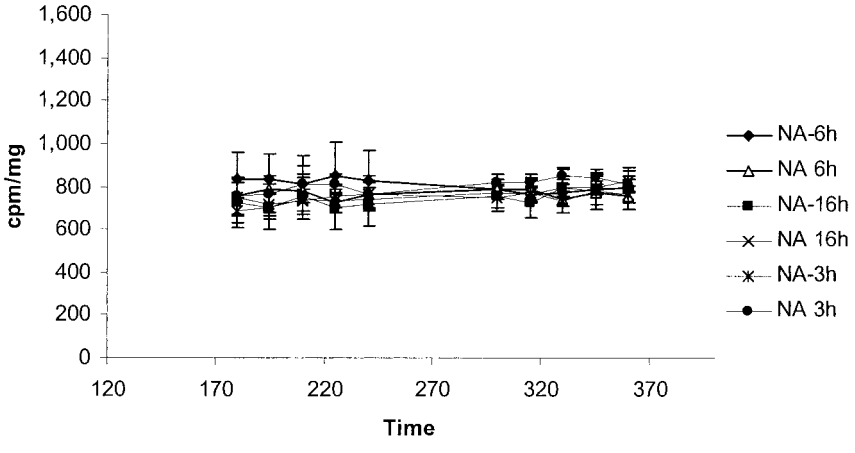


**FIG. 2. Plasma specific activity. Specific activity data were collected during the steady-state euglycemic and hyperglycemic phases and remained constant during those phases. NA, NA infusion for 3, 6, or 16 h; NA−, saline control.**

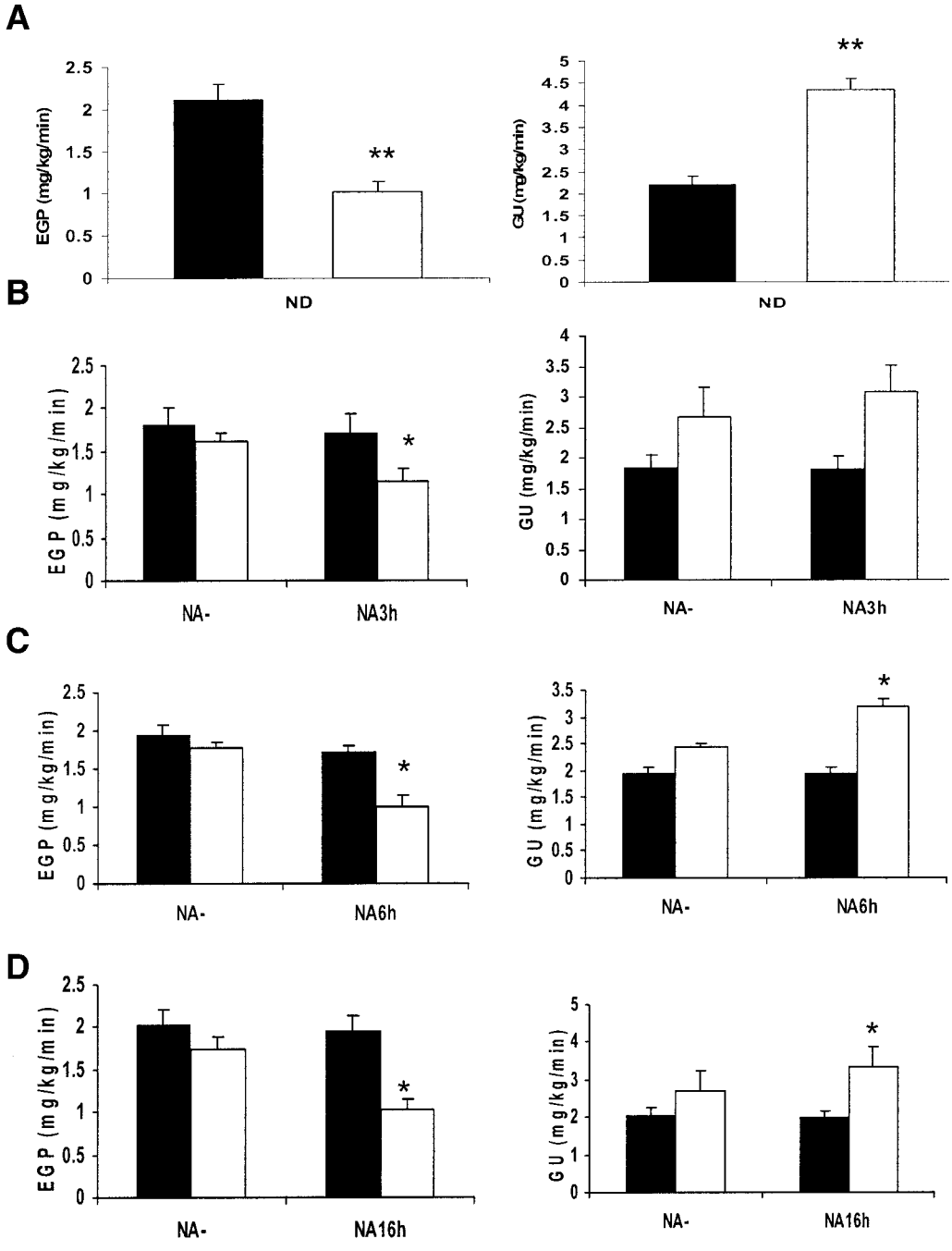


**FIG. 3. Rates of EGP and glucose uptake (GU). Rates of EGP and GU during euglycemia and hyperglycemia in nondiabetic subjects (*A*) is compared with the effect of lowering FFA for 3 h (*B*), 6 h (*C*), and 16 h (*D*) in type 2 diabetic subjects. Euglycemic (■) and hyperglycemic (□) phases are compared with the paired NA− studies in which FFA remained elevated. Data are means ± SE. *$P < 0.05$.**

subjects; Fig. 3). Also, while the percent increase in glucose uptake between the euglycemic and hyperglycemic study periods was 127 ± 72% for nondiabetic subjects, there was a more modest increase of 34 ± 8% in type 2 diabetic subjects ($P < 0.001$ vs. nondiabetic group). Importantly, plasma FFA concentrations were higher in type 2 diabetic than in nondiabetic subjects during both euglycemia (type 2 diabetes = 412 ± 99 μmol/l vs. no diabetes = 227 ± 31 μmol/l; $P < 0.001$) and hyperglycemia (type 2 diabetes = 435 ± 81 μmol/l vs. no diabetes = 190 ± 29 μmol/l; $P < 0.001$) (Fig. 4). Thus, while there were time-dependent decreases in FFA values with the onset of hyperglycemia in nondiabetic subjects ($P < 0.001$), FFA levels remained elevated in the type 2 diabetic group throughout the entire 6 h of NA− studies. This is consistent with our previous studies (15).

## NA studies

***FFA and glycerol levels.*** As shown in Fig. 4, FFA values during the final hour of all NA+ studies were comparable with the saline control studies in the nondiabetic subjects. Significant lowering in FFA levels was attained after ~1 h of NA infusion; hence, significant lowering of FFAs was observed for the final 2 h of the NA3h studies and during the final 5 h of the NA6h studies. In the NA16h studies, FFA levels were lower at the beginning of the clamp when compared with NA− ($P < 0.01$) and remained reduced throughout. Of note, the different NA infusion rates selected for each duration attained comparable FFA reduction in all three groups during the final hour ($P$ = NS by ANOVA).

There were no significant changes in glycerol levels during the NA studies (NA3h = 85 ± 6 vs. NA− = 84 ± 7,

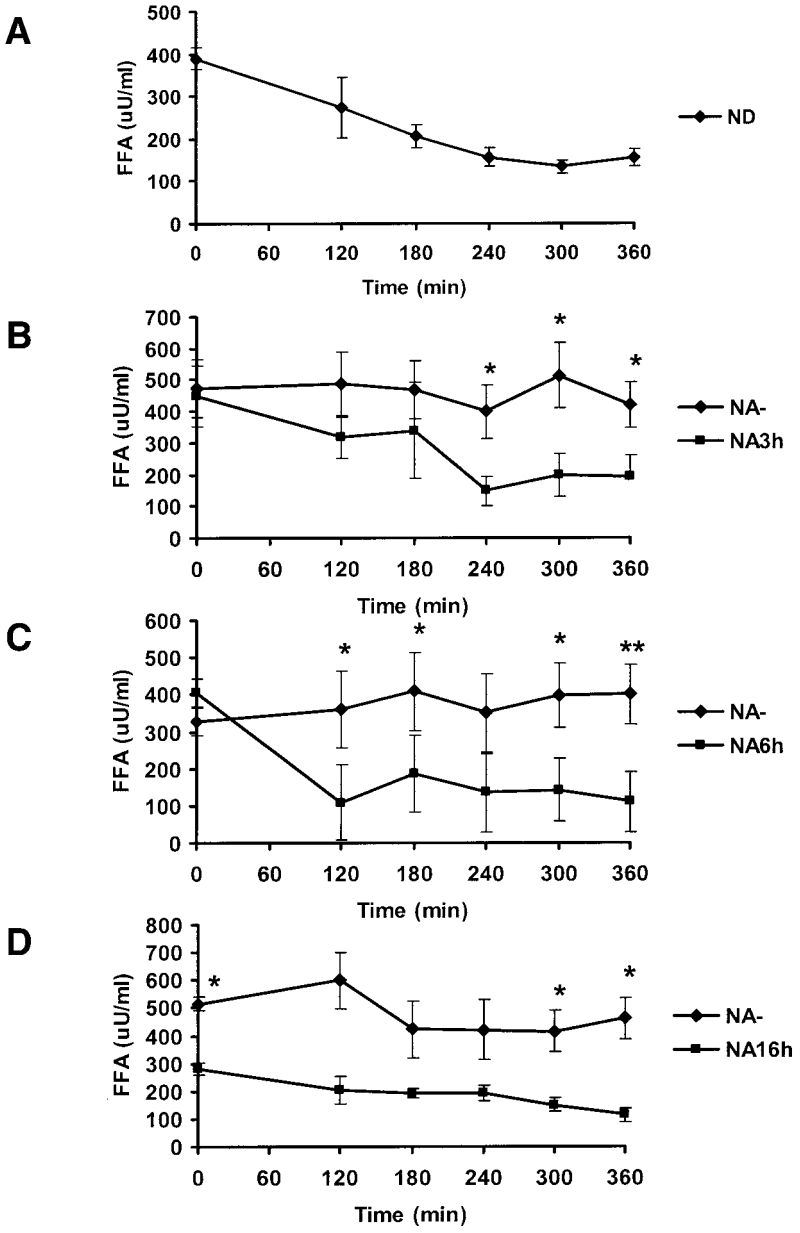


**FIG. 4. FFA levels in nondiabetic (ND) and type 2 diabetic subjects. *A*: FFA levels were decreased during the course of the clamp study in ND subjects. In type 2 diabetic subjects NA was infused to lower FFA values for 3 h (*B*), 6 h (*C*), or 16 h (*D*). Each NA study is compared with the saline control study (NA−) in a paired manner. Data are means ± SE. **P* < 0.05; ***P* < 0.01.**

NA6h = 52 ± 3 vs. NA− = 76 ± 4, and NA16h = 92 ± 15 vs. NA− = 108 ± 9 μmol/l; *P* = NS for all). These findings differ from two reports (27,28) in which nicotinic acid reduced glycerol levels in fasting individuals. However, a recent study (29) using nicotinic acid in the presence of high physiologic insulin was consistent with our findings, since nicotinic acid lowered FFAs but had no effect on plasma glycerol levels. Of note, the former pair of studies were performed under basal fasting conditions, while the latter pair of studies were in the presence of hyperinsulinemia and hyperglycemia, respectively. Both conditions inhibit gluconeogenesis, and hence there would be less consumption of glycerol via gluconeogenesis. This is likely to balance the reduced production of glycerol via lipolysis, particularly since lipolysis of a triacyl-glycerol molecule would yield only one-third as much glycerol as fatty acids when expressed on a molar basis.

**Glucose fluxes.** To raise blood glucose quickly to 180 mg/dl at 240 min, glucose infusion rates at the onset of hyperglycemia were calculated based on each subject's weight and were similar to the paired NA− studies. Glucose infusion rates became significantly different by 315 min in the NA6h group and 330 min in the NA3h and NA16h groups compared with the paired NA− studies. The average rate of glucose infusion required to maintain hyperglycemia during the last 60 min in the NA studies (NA3h = 2.22 ± 0.56, NA6h = 2.29 ± 0.33, and NA16h = 2.56 ± 0.46 mg · kg$^{-1}$ · min$^{-1}$) was increased more than twofold above the rates required in the paired NA− studies (1.11 ± 0.40, 0.66 ± 0.21, and 0.74 ± 0.18 mg · kg$^{-1}$ · min$^{-1}$, respectively). Thus, normalizing plasma FFA levels for ≥3 h significantly increased glucose effectiveness in the type 2 diabetic subjects. The degree of suppression of EGP with hyperglycemia in the NA studies (NA3h = 31 ± 6%, NA6h = 41 ± 8%, and NA16h = 47 ± 4%) was significantly greater than in the NA− studies in the same individuals (NA− 8 ± 7%, 7 ± 6%, and 14 ± 4%, respectively, *P* = 0.001; Figs. 3 and 5). The suppression of

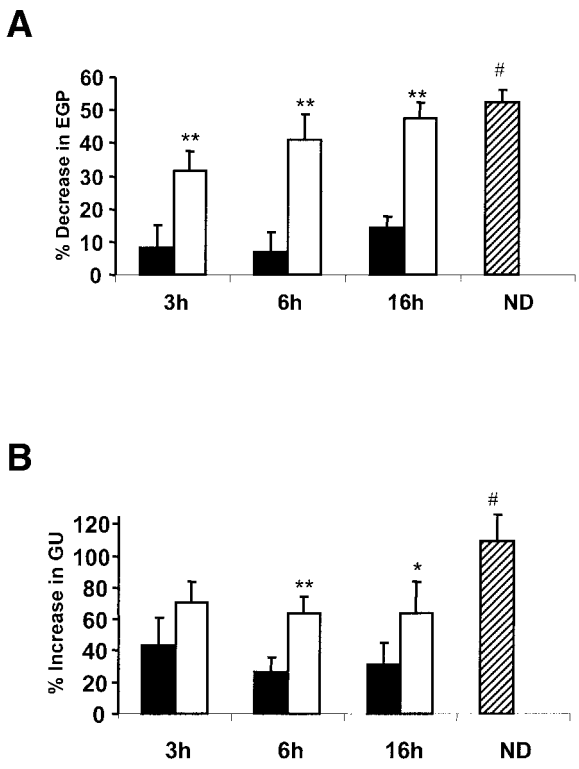


**FIG. 5. Change in EGP (*A*) and glucose uptake (GU) (*B*) with changes in FFA levels. Percent change in EGP and GU is shown in type 2 diabetic subjects (euglycemic versus hyperglycemic phases) after infusion of NA (□) to lower FFA for 3, 6, or 16 h compared with paired saline infusion studies (NA−; ■). In nondiabetic subjects (▨), EGP was suppressed to ~52% and GU was increased ~109% with hyperglycemia. Data are means ± SE. *$P < 0.05$; **$P < 0.01$.**

EGP with NA for 6 and 16 h was comparable with the degree of suppression observed in the nondiabetic subjects, while there was less suppression of EGP in the NA3h studies ($P = 0.02$). This indicates that significant improvements were seen at 3 h and complete normalization observed after 6 h of FFA lowering.

Also, the percent increase in glucose uptake (Figs. 3 and 5) between the euglycemic and hyperglycemic study periods was 63 ± 10% for the NA6h vs. 26 ± 10% in the NA− ($P = 0.01$) and 64 ± 20% for the NA16h vs. 30 ± 14% change in the NA− studies ($P = 0.04$). However, this stimulation of glucose uptake by hyperglycemia was substantially less than the 127% increase observed in nondiabetic subjects. While there was a trend toward increased glucose uptake after only 3 h of FFA lowering in type 2 diabetic subjects (NA3h 70 ± 14% vs. NA− 43 ± 17%), it did not reach statistical significance ($P = 0.09$). Thus, there were improvements in peripheral glucose effectiveness following 6 h of FFA lowering, but normalization was not achieved even after 16 h.

Of note, we previously performed studies with coinfusion of both nicotinic acid and liposyn in type 2 diabetic subjects ($n = 5$) to prevent the nicotinic acid–induced decline in FFA levels (15). The percent change in EGP and glucose uptake in the coinfusion studies were −9 ± 10 and 37 ± 17%, respectively, and thus were not significantly different from the current NA− studies. Thus, the effects of nicotinic acid on glucose effectiveness are likely due to FFA lowering and not to nonspecific effects of nicotinic acid.

## DISCUSSION

Although the effects of increased FFA levels on hepatic insulin action are well established (30), the effects of FFAs on hepatic glucose effectiveness are underrecognized. Indeed, a careful review of the literature suggests that this is an important phenomenon. Previous studies (31) have suggested that glucose-induced reductions in FFA levels account for the majority of suppression of EGP by glucose in nondiabetic individuals. In support of this idea, we previously reported that in type 2 diabetic individuals with optimal glycemic control, EGP displays normal suppression of FFA levels by hyperglycemia and thus normal glucose effectiveness (12). Moreover, 72 h of intensive glycemic control with insulin in poorly controlled type 2 diabetic subjects restored glucose effectiveness in concert with normalization of plasma FFA levels (12). Shah et al. (33) reported that elevation of plasma FFA levels in healthy women blunted the suppression of EGP by both hyperinsulinemia and hyperglycemia (35), although glucose effectiveness was not specifically examined. When plasma FFA levels were increased to >2 mmol/l for 9 h under pancreatic clamp conditions in healthy subjects, the resulting 50% rise in plasma glucose concentrations failed to suppress EGP (33).

The present experiments examined the impact of altering FFA levels for variable durations on glucose effectiveness in type 2 diabetic individuals. Euglycemic-hyperglycemic pancreatic clamp techniques were used to study the ability of hyperglycemia per se to regulate EGP and glucose uptake under fixed hormonal conditions. In these subjects with poorly controlled type 2 diabetes, there was a rapid improvement in hepatic glucose effectiveness following only 3 h of nicotinic acid infusion (~2 h of FFA lowering) and a complete restoration of glucose effectiveness with 6-h infusion (~5 h of FFA normalization). This time course of FFA-induced changes in glucose effectiveness is consistent with rapid changes in flux through gluconeogenesis and/or rapid changes in the activity of key hepatic enzymes. The magnitude of the effect we report suggests that the inhibitory effects of FFAs on glucose effectiveness contribute importantly to worsening hyperglycemia in individuals with type 2 diabetes.

Increased circulating FFAs have been shown to alter both the gene expression and the activity of hepatic enzymes responsible for regulating EGP. Specifically, FFAs promote hepatic glucose-6-phosphatase gene expression (18,19) and decrease hepatic glucokinase expression (20). Studies of the roles of these enzymes in regulating glucose fluxes are consistent with their importance in determining glucose effectiveness. Dual defects in these key regulatory enzymes could impair glucose effectiveness, since defective glucokinase activity would impair the liver's ability to "sense" rising glucose levels (34,35), while increased glucose-6-phosphatase would stimulate EGP by both glycogenolysis and gluconeogenesis. The fact that 6 h of FFA lowering was required to fully normalize glucose effectiveness in the current studies suggests that adequate time was required to reverse chronic effects of FFAs on hepatic gene expression.

Increased FFAs have also been shown to increase gluconeogenesis both in vivo and in vitro (18,36). Indeed, type 2 diabetes is characterized by augmented rates of gluconeogenesis, which contribute to the inappropriate elevations in EGP in these individuals (37). The current studies were designed to explore potential mechanisms

whereby increased FFA levels impact glucose effectiveness in humans. The rapid effects of lowering FFAs observed in these studies could be consistent with rapid changes in gluconeogenesis. This will be the focus of further investigation.

It is important to note that our studies were performed under basal insulin conditions, since they were designed to study glucose effectiveness and not insulin sensitivity. Of note, there was no effect of lowering FFA levels on rates of EGP during euglycemia (i.e., NA16h or NA6h versus their respective controls). This demonstrates that sensitivity to basal insulin was not affected by the modest lowering in FFA levels. This is consistent with the observation of Whiteall et al. (38) that lowering triglycerides and FFAs with gemfibrozil did not alter insulin sensitivity during low-dose insulin infusion. We also found that there was no change in insulin sensitivity (as assessed by the insulin infusion rates required to lower blood glucose) overnight despite lower FFA levels in the NA16h studies. This confirms that the improved suppression of EGP observed during hyperglycemia with FFA lowering was due to improved glucose effectiveness rather than a change in insulin sensitivity.

It is intriguing that nicotinic acid acutely improved glucose effectiveness in these poorly controlled type 2 diabetic subjects, despite concerns about its chronic use in type 2 diabetes (39). Use of niaspan in 125 subjects with type 2 diabetes for up to 65 weeks had very modest increases in plasma glucose (<10 mg/dl) and no change in A1C (40), although FFA levels were not assessed systematically. Of note, rebound elevations in FFA levels occur following administration of nicotinic acid, generally rising well above the pretreatment FFA levels. Indeed, 7-day treatment with nicotinic acid in nondiabetic subjects resulted in insulin resistance that was proportional to the increases in fasting FFAs (41). It is therefore likely that repeated rebound rises in FFA levels may have mild detrimental effects on glucose control with chronic administration of nicotinic acid. Use of extended-release nicotinic acid was recently shown to reduce the degree of FFA rebound. Despite a nonsignificant trend of more type 2 diabetic patients needing adjustment of diabetes medication, there was no change in A1C (42).

Lowering FFA levels also improved peripheral glucose effectiveness, i.e., the ability of hyperglycemia per se to stimulate whole-body glucose uptake. Previous studies have demonstrated that elevated FFAs inhibited glucose-mediated glucose uptake under conditions of physiologic insulin levels, similar to the present studies (43). Of note, FFAs have been shown to directly impact both the expression (44) and translocation (45) of glucose transporters in skeletal muscle. In contrast to the complete restoration of hepatic glucose effectiveness, FFA lowering for up to 16 h only partially restored peripheral glucose effectiveness. However, we previously reported that correction of glucose and FFA levels for 72 h improved peripheral glucose effectiveness to the level of nondiabetic subjects (12). Therefore it is possible that a longer duration of FFA lowering would have completely restored glucose effectiveness. Additionally, since peripheral insulin resistance is not rapidly restored by correcting metabolic defects (46), the permissive effects of insulin on peripheral glucose effectiveness (47) are still likely to be impaired in these poorly controlled type 2 diabetic subjects.

To conclude, our data suggest that elevated FFA levels contribute to the lack of hepatic and peripheral glucose effectiveness in individuals with type 2 diabetes. Of note, FFA levels are chronically elevated in type 2 diabetes, and attaining long-term FFA lowering in these individuals is currently not a specific therapeutic goal in type 2 diabetes. Since elevated FFA levels appear to significantly contribute to the loss of glucose effectiveness in type 2 diabetes and thus overall glycemic control, understanding the pathogenesis of this important defect could have considerable therapeutic benefit.

## ACKNOWLEDGMENTS

This work was supported in part by grants from the American Diabetes Association (to M.H. and J.T.) and the National Insitutes of Health (Einstein General Clinical Research Center [GCRC] RR12248, K12 RR17672 [to P.K.], P01-AG021654, and DK20541 [Diabetes Research and Training Center {DRTC}]). Dr. Hawkins was a Clinical Associate Physician of the GCRC (3 M01 RR 12248–03S1) and is a recipient of a Beeson Award from the American Federation of Aging Research.

The authors thank Angela Stangarone for coordinating studies, Robin Sgueglia for determinations performed in the DRTC Hormone Assay Core, Dr. Hillel Cohen for biostatistical assistance, and the staff of the Albert Einstein College of Medicine GCRC for their outstanding patient care. We also acknowledge Drs. Luciano Rossetti, Daniel Stein; and Harry Shamoon for many helpful discussions.

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