Glycemic Control Determines Hepatic and Peripheral Glucose Effectiveness in Type 2 Diabetic Subjects

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Glucose effectiveness is impaired in type 2 diabetes. We hypothesize that chronic hyperglycemia and hyperlipidemia contribute importantly to this defect. To test this hypothesis, we compared the effect of acute hyperglycemia on glucose turnover in type 2 diabetic subjects in good control (GC) (n = 14, age 51.7 ± 3.7 years, BMI 28.4 ± 1.0 kg/m^2, HbA_{1c} 5.9 ± 0.2%) and poor control (PC) (n = 10, age 50.0 ± 2.5 years, BMI 27.9 ± 1.5 kg/m^2, HbA_{1c} 9.9 ± 0.6%) with age- and weight-matched nondiabetic subjects (ND) (n = 11, age 47.0 ± 4.4 years, BMI 28.5 ± 1.0 kg/m^2, HbA_{1c} 5.1 ± 0.2%). Fixed hormonal conditions were attained by infusing somatostatin for 6 h with replacement of basal insulin, glucagon, and growth hormone. Glucose fluxes ([3-^{3}H]glucose) were compared during euglycemic (5 mmol/l, t = 180–240 min) and hyperglycemic (Hy) (10 mmol/l, t = 300–360 min, variable glucose infusion) clamp intervals. Acute hyperglycemia suppressed hepatic glucose production (GP) by 43% and increased peripheral glucose uptake (GU) by 86% in the ND subjects. Conversely, GP failed to suppress (−7%) and GU was suboptimally increased (+34%) in response to Hy in the PC group. However, optimal glycemic control was associated with normal glucose effectiveness in GC subjects (GP −38%, GU +72%; P > 0.05 for GC vs. ND). To determine whether short-term correction of hyperglycemia and/or hyperlipidemia is sufficient to reverse the impairment in glucose effectiveness, five PC subjects were restudied after 72 h of normoglycemia (~100 mg/dl; variable insulin infusions). These subjects regained normal effectiveness of glucose to suppress GP and stimulate GU and in response to Hy (GP −47%, GU +71%; P > 0.05 vs. baseline studies). Thus, chronic hyperglycemia and/or hyperlipidemia contribute to impaired effectiveness of glucose in regulating glucose fluxes in type 2 diabetes and hence to worsening of the overall metabolic condition. Short-term normalization of plasma glucose might break the vicious cycle of impaired glucose effectiveness in type 2 diabetes. *Diabetes* 51:2179–2189, 2002

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AMPK, cAMP-dependent protein kinase; FFA, free fatty acid; G-6-Pase, glucose-6-phosphatase; GC, good control; GCRC, General Clinical Research Center; GK, glucokinase; GP, glucose production; ND, nondiabetic; PC, poor control; PC3day, PC subjects participating in the 3-day treatment.

Increased glucose production (GP) is the major cause of fasting hyperglycemia in type 2 diabetes (1–3). Indeed, the gradual rise in fasting plasma glucose levels over time in type 2 diabetes is accompanied by progressive increases in basal GP (1). In addition to stimulating insulin secretion, acute elevations in plasma glucose levels exert direct effects on both GP and peripheral glucose uptake, which contribute to the maintenance of euglycemia in nondiabetic (ND) individuals (4). Indeed, elevations in plasma glucose suppress GP independently of hormonal signals, hence facilitating rapid modulation of glycemic levels.

However, despite the inhibitory effects of both insulin and glucose on GP in ND individuals, GP is increased in individuals with type 2 diabetes in the presence of both hyperinsulinemia and hyperglycemia. Indeed, "glucose effectiveness" refers to the ability of glucose per se to stimulate glucose uptake and suppress GP (5). Recently, we demonstrated the reduced effectiveness of glucose to both suppress GP and enhance peripheral glucose uptake in type 2 diabetic subjects relative to age- and weight-matched ND control subjects (6). This loss of regulation of GP in response to hyperglycemia per se could contribute to the increased rates of GP in individuals with type 2 diabetes, especially in the fasting state (7).

Hepatic autoregulation of GP by hyperglycemia probably resides at the level of the glucose-6-phosphate pool, which is partially regulated by the relative glucose flux through glucokinase (GK) and glucose-6-phosphatase (G-6-Pase). Flux through these enzymes represents the most proximal and distal steps of hepatic glucose metabolism, respectively (8). Type 2 diabetic subjects manifest dual abnormalities in this regulation, with decreased hepatic GK and increased G-6-Pase (9), both compatible with increased fasting GP and failure of GP to suppress with hyperglycemia. Because chronic hyperglycemia has inhibitory effects on hepatic GK expression (10,11) and both hyperglycemia and increased availability of free fatty acids (FFAs) enhance G-6-Pase expression (12–14), these dual characteristics of the chronic diabetic milieu are compatible with the abnormal activities of GK and G-6-Pase described above. Furthermore, correction of hyperglycemia in rodent models of diabetes normalized the impaired activity of GK and excess activity of G-6-Pase (10,11,13).

Thus, we proposed that the metabolic defects in human type 2 diabetes are responsible for the loss of normal regulation of GP and glucose uptake by hyperglycemia per

TABLE 1
Patient characteristics

Subjects	n	Age (years)	BMI (kg/m^2)	HbA_{1c} (%)	Diabetes duration (years)	Treatment
GC	14	51.7 ± 3.7	28.4 ± 1.0	5.9 ± 0.2	5.6 ± 1.3	2 D, 11 H, 1 H/I
PC	10	50.0 ± 2.5	27.9 ± 1.5	9.9 ± 0.6	10.2 ± 1.8	1 D, 5 H, 1 I, 3 H/I
ND	7	47.0 ± 4.4	28.5 ± 1.0	5.1 ± 0.2	NA	NA

Data are means ± SE. D, diet only; H, oral hypoglycemic agents; I, insulin.

se. We therefore hypothesized that hepatic and peripheral responses to hyperglycemia per se would differ among subjects with type 2 diabetes, such that individuals with relatively better glycemic control (reflecting lower chronic levels of both glucose and FFAs) would likely show more normal responses to hyperglycemia. Consequently, we studied the effect of an acute elevation in plasma glucose on hepatic and peripheral glucose fluxes under fixed hormonal conditions in type 2 diabetic subjects stratified by glycemic control and in ND individuals. Furthermore, because we anticipated that this regulation would be restored by correction of the metabolic profile, we restudied a subgroup of type 2 diabetic subjects in poor control after maintenance of euglycemia for 72 h.

RESEARCH DESIGN AND METHODS

Subjects. We studied 27 patients with type 2 diabetes stratified into two groups by glycemic control (Table 1). In the type 2 diabetic subjects in good control (GC) (n = 14; 12 men, 2 women), HbA_{1c} was 5.8 ± 0.2%, age was 51.7 ± 3.7 years, BMI was 28.4 ± 1.0 kg/m^2, and duration of type 2 diabetes was 5.6 ± 1.3 years. Eleven subjects were treated with oral agents alone (seven with metformin, two with metformin and sulfonylureas, one with acarbose and sulfonylureas, and one with troglitazone and metformin), one was treated with a combination of insulin and oral agents, and two were controlled with diet alone. In the poor control (PC) group (n = 10; eight men, two women), HbA_{1c} was 9.9 ± 0.6%, age was 50.0 ± 2.5 years, BMI was 27.9 ± 1.8 kg/m^2, and duration of type 2 diabetes was 10.2 ± 1.8 years. Five subjects were treated with oral hypoglycemic agents alone (three with metformin, one with sulfonylureas, and one with troglitazone, metformin, and sulfonylureas), three were treated with a combination of insulin and oral agents, one was treated with insulin alone, and one was controlled with diet alone. Except for diabetes, all were in general good health. We also studied 11 ND healthy volunteers (eight men, three women) with average BMI of 28.5 ± 1.0 kg/m^2, average age of 47.0 ± 4.4 years, and average HbA_{1c} of 5.1 ± 0.2%. None of the ND subjects was taking any medications, and they had no family history of type 2 diabetes. Eligibility for study was determined by history, physical examination, and hematological and biochemical tests. Subjects with anemia, bleeding disorders, or recent weight changes were excluded. Informed written consent was obtained in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

A few subjects whose data were reported in a recent publication (6) were restudied in the current protocol. Specifically, four ND subjects, two GC type 2 diabetic subjects, and two PC type 2 diabetic subjects were restudied. Of note, the subjects were consistent with regard to both their glycemic control (type 2 diabetic subjects had no greater variations in HbA_{1c} than 0.2%) and glucose effectiveness (no more than 15% difference in GP or glucose uptake response to hyperglycemia) on separate study dates.

Experimental design. In the type 2 diabetic subjects on pharmacological therapy, sulfonylureas and metformin were discontinued at least 48 h before the study, troglitazone was withheld for 8 days, and long-acting insulin was withheld the evening before the study. The decision of how long to withhold the insulin-sensitizing agents metformin and troglitazone was based on the following information. At least 90% of ingested metformin undergoes renal excretion within 24 h, with an elimination half-life of about 6 h. Furthermore, a recent report indicates that metformin mediates its effects on hepatic GP via activation of cAMP-dependent protein kinase (AMPK) (15). Because enzymes of GP are rapidly regulated by AMPK (16), the effects of metformin on GP are likely to be rapid and transient. Troglitazone is eliminated fairly rapidly by hepatic metabolism, with a mean elimination half-life ranging from 7.6 to 24 h (17). Although circulating levels of three metabolites can be measurable in plasma for up to 3 days (18), only one has any pharmacological activity. Thus, a "1-week washout" is recommended as sufficient to ensure no further hepatic effects of troglitazone when switching to other thiazolidinediones (19).

To ensure that all subjects started the clamp studies with plasma glucose levels ≤140 mg/dl, PC subjects were admitted to the General Clinical Research Center (GCRC) the evening before the study for low-dose overnight insulinization. Patients were asked to begin fasting at 10:00 P.M. the night before the study, and a variable intravenous infusion of insulin was started. The insulin infusion rate was adjusted according to an algorithm based on hourly blood glucose measurement (20). The following morning, the experimental protocol was initiated. ND and GC subjects were admitted to the GCRC on the morning of the study after an overnight fast. The decision not to hospitalize the GC subjects overnight was based on our own unpublished observations and a recent report (21) that overnight low-dose insulinization does not affect glucose effectiveness in type 2 diabetic or ND subjects.

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, the 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 (Fig. 1). At 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 rate 22 μCi; continuous infusion rate 0.15 μCi/min) via an indwelling catheter. At 0 min, an infusion containing somatostatin (250 μg/h; Bachem, King of Prussia, PA), growth hormone (3.0 ng · kg^{-1} · min^{-1}; Genentech, San Francisco, CA), and glucagon (1.0 ng · kg^{-1} · min^{-1}; Eli Lilly, Indianapolis, IN) was initiated and maintained throughout the study. This infusion was prepared with 1 mg/ml albumin diluted in saline. At 0 min, an infusion of insulin (Novolin Regular [Novo-Nordisk, Princeton, NJ] prepared in albumin-containing saline) was also started. Plasma glucose concentrations were initially measured at 15-min intervals (for the period t = 0–240 min), and the rate of insulin infusion was adjusted to maintain euglycemia (90–100 mg/dl) without the need for exogenous glucose infusion. When a stable rate of insulin infusion was determined, it was kept constant for the remainder of the study. This occurred within the first 150 min of the euglycemic period (t = 0–150).

During the remaining 90 min of the euglycemic period, the individualized basal rate of insulin infusion was kept constant, and ambient euglycemia prevailed until 240 min. At 240 min, plasma glucose levels were rapidly increased by 90 mg/dl and clamped at 180 mg/dl for the following 2 h (until 360 min) using an exogenous infusion of 20% glucose. Tracer ([3-^{3}H]glucose) was added to the "cold" 20% glucose (~0.1 μCi/ml) to maintain glucose specific activity constant during hyperglycemia (22). All other infusions were kept at the rate used during the euglycemic period. Thus, a total of 35 pancreatic clamp studies were performed, 24 of which involved subjects with type 2 diabetes and 11 of which involved ND subjects.

Upon completion of the study, the tracer, glucose, and hormone infusions were terminated. Insulin infusion was continued in the diabetic patients for an additional 15 min. A meal was provided, and subjects were discharged, with diabetic individuals resuming their usual regimens.

Blood was sampled at 5-min intervals for measurement of plasma glucose, at 15-min intervals for specific activity of [3-^{3}H]glucose, and at 30-min intervals for plasma insulin, glucagon, C-peptide, FFA, lactate, and glycerol levels.

The 3-day intensive glycemic control studies. To prospectively determine the effects of glycemic control on glucose effectiveness, a representative subgroup of five PC subjects was admitted to the GCRC to acutely normalize plasma glucose levels for 3 days using variable low-dose insulin infusions (target plasma glucose ~100 mg/dl), followed by a euglycemic-hyperinsulinemic pancreatic clamp study. Among the PC subjects participating in the 3-day treatment (PC3day) (n = 5), HbA_{1c} was 10.7 ± 1.1%, age was 51.8 ± 5.1 years, BMI was 26.8 ± 1.6 kg/m^2, and duration of type 2 diabetes was 6.6 ± 2.9 years. Of the five subjects, four subjects were treated with oral agents (one with metformin and troglitazone, two with sulfonylureas, and one with metformin and sulfonylureas) and one with insulin alone. Except for diabetes, all subjects were in general good health. As in the glycemic control studies,

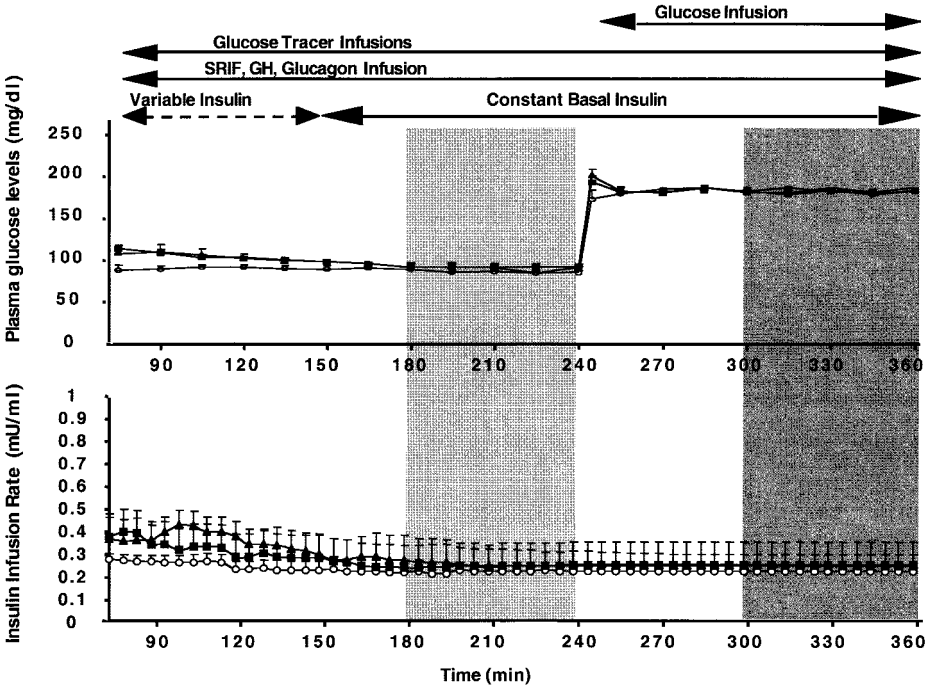

FIG. 1. Plasma glucose levels and insulin infusion rates during euglycemic-hyperglycemic clamp studies in ND (○) and type 2 diabetic (PC, ▲; GC, ■) subjects. The light and dark shaded areas represent the euglycemic (t = 180–240 min) and hyperglycemic (t = 300–360 min) study intervals, respectively. GH, growth hormone; SRIF, somatotropin release–inhibiting factor.

sulfonylureas and metformin were discontinued at least 48 h before the study, and troglitazone was held for 8 days. A variable infusion of insulin was started on admission and adjusted according to an algorithm based on hourly blood glucose measurements for 72 h (20). Patients were asked to being fasting at 10:00 P.M. on the night preceding the clamp study. The following morning, the experimental protocol was initiated. The subjects underwent euglycemic-hyperinsulinemic pancreatic clamp studies that were identical to their original studies, described above.

Analytical procedures. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) using the glucose oxidase method. Plasma [3-^{3}H]glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide–zinc sulfate precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate tritiated water. Plasma tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness (23).

Plasma insulin, C-peptide, and glucagon were determined by radioimmunoassay (24). Plasma FFAs and glycerol were measured using colorimetric enzymatic methods (15) and plasma lactate by an enzymatic spectrophotometric assay (25). HbA_{1c} was measured by ion-exchange chromatography with an upper normal limit of 6.2% (26).

Calculations. Rates of glucose appearance and glucose disappearance (glucose uptake) were calculated using Steele's steady-state equation (27). Rates of endogenous glucose production were determined by subtracting the rates of glucose infusion from the tracer-determined glucose appearance. Rates of glycolysis from plasma glucose were estimated from the increment per unit time in tritiated water (disintegrations/minute per milliliter per minute) multiplied by body water mass (milliliters) per [3-^{3}H]glucose specific activity (disintegrations/minute per milligram), as previously validated (28,29). Glycogen synthetic rates were estimated as the difference between glucose disappearance and glycolysis from plasma glucose (23,24). 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. 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 comparison between the three subject groups (ND, GC, and PC), repeated measures ANOVA was applied. For comparison between the two test conditions, the paired Student's t test was used (i.e., for comparison of euglycemic and hyperglycemic intervals and for comparison between 3-day studies and the baseline studies).

RESULTS

Plasma glucose levels and insulin infusion rates. The rate of overnight insulin infusion required to maintain plasma glucose in the target range of 90–120 mg/dl in the PC group averaged 2.8 ± 0.6 units/h. Of note, insulin infusion rates were adjusted hourly and selected based on a sliding scale regimen, such that higher infusion rates of insulin were used in response to higher plasma glucose levels. Thus, since admission plasma glucose levels were generally >200 mg/dl, high infusion rates of insulin (>5 units/h) were required over the first 2–3 h overnight. However, plasma glucose levels averaged ~100–120 mg/dl during the majority of the night, such that most patients arrived in the study room with insulin infusion rates of ~1 unit/h (~0.2 mU · kg^{-1} · min^{-1}) (Fig. 1).

On the morning of the study, plasma glucose levels averaged 129 ± 9 mg/dl in the GC subjects, 113 ± 10 mg/dl in the PC subjects, and 104 ± 4 mg/dl in the ND subjects. Plasma glucose levels were similar in both groups with type 2 diabetes and in ND subjects during the euglycemic study period (t = 180–240 min; 91 ± 2 mg/dl for PC, 92 ± 3 mg/dl for GC, and 92 ± 3 mg/dl for ND subjects). Glucose levels were also similar in all groups during the hyperglycemic study period (t = 300–360; 182 ± 1 mg/dl for PC, 180 ± 1 mg/dl for GC, and 180 ± 1 mg/dl for ND subjects). Thus, the amplitude of the change in the ambient glucose concentrations generated with the hyperglycemic clamp was comparable in all three groups.

The average insulin infusion rate needed to maintain euglycemia during the final 30 min of the insulin adjustment period and thereafter was similar in all groups (0.23 ± 0.02 mU · kg^{-1} · min^{-1} for ND subjects, 0.25 ± 0.03 mU · kg^{-1} · min^{-1} for GC subjects, and 0.25 ± 0.05 mU · kg^{-1} · min^{-1} for PC subjects; $P > 0.05$). Importantly, the plasma insulin levels did not differ between the euglycemic

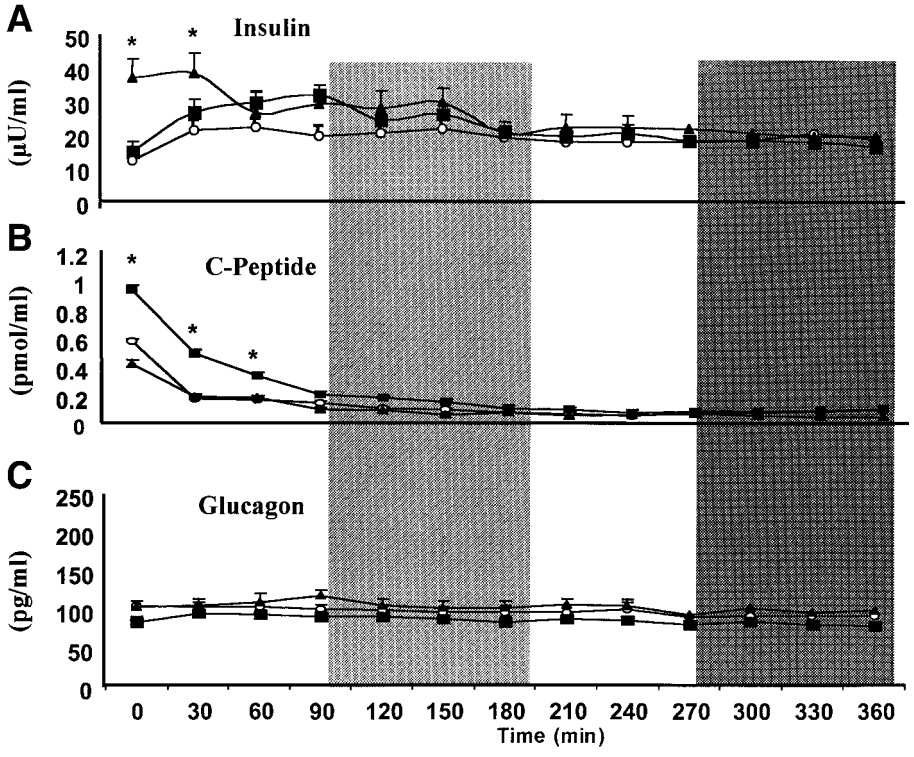

FIG. 2. Plasma insulin, C-peptide, and glucagon concentrations in ND (○) and type 2 diabetic (PC, ▲; GC, ■) subjects during euglycemic-hyperglycemic clamp studies. The light and dark shaded areas represent the euglycemic (t = 180–240 min) and hyperglycemic (t = 300–360 min) study intervals, respectively. *P < 0.01 for difference between groups.

and hyperglycemic study periods in any group (euglycemia vs. hyperglycemia: 23 ± 5 vs. 20 ± 3 μU/ml in PC, 21 ± 2 vs. 18 ± 2 μU/ml in GC, and 19 ± 2 vs. 19 ± 2 μU/m in ND subjects; $P > 0.05$ for euglycemic vs. hyperglycemic study periods) (Figs. 1 and 2).

Other hormones and substrates. Plasma FFA levels for all groups were comparable at the beginning of the study (t = 0 min). Although FFA levels were expected to be higher in the PC subjects than in the other groups under comparable conditions, these subjects were studied after overnight insulinization. It is noteworthy that increased insulin availability overnight did not suppress plasma FFAs in these subjects below normal fasting levels. During both euglycemic and hyperglycemic study periods, the plasma FFA concentrations were higher in the PC group than in the GC and ND groups (euglycemia vs. hyperglycemia: 365.3 ± 64.2 vs. 377 ± 45.9 mmol/l for PC, 216.7 ± 35.3 vs. 224.9 ± 38.4 mmol/l for GC, and 215.9 ± 23.1 vs. 167.3 ± 12.2 mmol/l for ND subjects; $P < 0.01$ for PC vs. ND and GC, both euglycemia and hyperglycemia). There was no significant change in plasma FFA concentrations in any of the groups between euglycemia and hyperglycemia. However, the elevated FFA levels in the PC group throughout both intervals could affect the suppression of GP in response to hyperglycemia (see below) (Fig. 3).

The plasma concentrations of the remaining hormones and substrates are shown in Figs. 2 and 3. Initial (t = 0 min) plasma insulin levels were higher in the PC group, who had received overnight insulin infusions. However, plasma insulin levels did not differ significantly between groups throughout the euglycemic and hyperglycemic study periods. C-peptide levels were suppressed by overnight insulin infusion in the PC group and by somatostatin infusion in all groups throughout the length of the study. Although fasting plasma insulin levels were not significantly different in GC versus ND subjects, initial C-peptide levels were higher in GC subjects and took longer to fully suppress with somatostatin, potentially suggesting an increased secretory reserve in GC subjects versus ND subjects.

Plasma glucagon levels remained stable and at fasting levels throughout the euglycemic and hyperglycemic study periods in all groups. Plasma glycerol concentrations were slightly but significantly higher in the PC group than in the other groups during the euglycemic phase of the studies but were not different during the hyperglycemic period. Plasma lactate levels were slightly lower in the PC subjects throughout the studies.

Glucose infusion rates. Occasionally, subjects required infusion of small amounts of dextrose during the final minutes of the euglycemic interval to maintain target plasma glucose levels of ~90 mg/dl (because insulin infusion rates were fixed). Average glucose infusion rates during the euglycemic intervals were 0.27 ± 0.04 mg · kg^{-1} · min^{-1} in the ND subjects and 0.11 ± 0.03 mg · kg^{-1} · min^{-1} in the GC subjects, whereas none of the PC subjects required glucose infusion. Of note, these were low rates of glucose infusion in only a few subjects. Because rates of GP and glucose uptake were comparable in all groups during this euglycemic interval (see below), any discrepancy in glucose infusion rates was not likely to reflect any significant metabolic difference between groups.

The average rate of infusion of glucose required to maintain the target hyperglycemic plateau during the last 60 min of the hyperglycemic period was decreased by more than half in PC subjects compared with ND subjects (0.73 ± 0.11 mg · kg^{-1} · min^{-1} in PC vs. 2.13 ± 0.24 g · dg^{-1} · min^{-1} in GC and 2.82 ± 0.36 mg · kg^{-1} · min^{-1} in ND subjects; $P < 0.05$, with a significant difference between PC and both GC and ND groups). This difference suggests a marked decrease in glucose effectiveness with chronic hyperglycemia, which may underlie defects in the ability

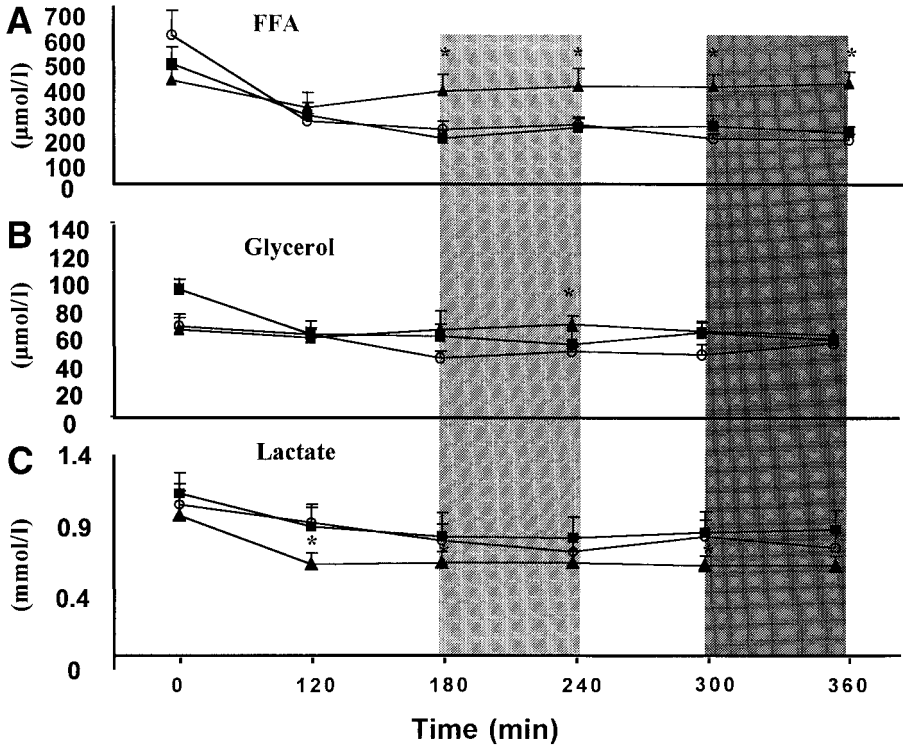

FIG. 3. Plasma FFA, glycerol, and lactate concentrations in ND (○) and type 2 diabetic (PC, ▲; GC, ■) subjects during euglycemic-hyperglycemic clamp studies. The light and dark shaded areas represent the euglycemic (t = 180–240 min) and hyperglycemic (t = 300–360 min) study intervals, respectively. *$P < 0.05$ vs. ND subjects, euglycemia vs. hyperglycemia.

of hyperglycemia to suppress GP and/or to promote glucose uptake and metabolism in peripheral tissues at basal insulin levels.

Specific activity. Glucose specific activity was constant after tracer equilibration during both euglycemia and hyperglycemia in each group: during euglycemia, specific activity averaged 722.4 ± 59.6 in ND subjects, 712.0 ± 44.1 in GC subjects, and 740.5 ± 69.8 in PC subjects. During hyperglycemia, specific activity averaged 721.3 ± 27.6 in ND subjects, 738.1 ± 35.4 in GC subjects, and 746.7 ± 54.7 in PC subjects (Fig. 4).

GP. GP was similar in all groups during the euglycemic study period (1.8 ± 0.1 mg · kg^{-1} · min^{-1} in ND, 1.9 ± 0.1 mg · kg^{-1} · min^{-1} in GC, and 1.9 ± 0.1 mg · kg^{-1} · min^{-1} in PC subjects; $P > 0.05$). However, during hyperglycemia, GP decreased significantly and to a similar extent in the ND and GC subjects, whereas it remained unchanged in the PC subjects (1.0 ± 0.1 mg · kg^{-1} · min^{-1} in ND, 1.2 ± 0.1 mg · kg^{-1} · min^{-1} in GC, and 1.7 ± 0.1 mg · kg^{-1} · min^{-1} in PC subjects; $P < 0.01$, with significant differences between PC and both GC and ND groups). This resulted in a 43.2 ± 3.5% suppression of GP in the ND subjects, a 38.3 ± 3.8% suppression in the GC subjects, and a 6.9 ± 5.5% suppression in the PC group ($P < 0.01$; significant difference PC vs. both GC and ND groups) (Fig. 5*A*).

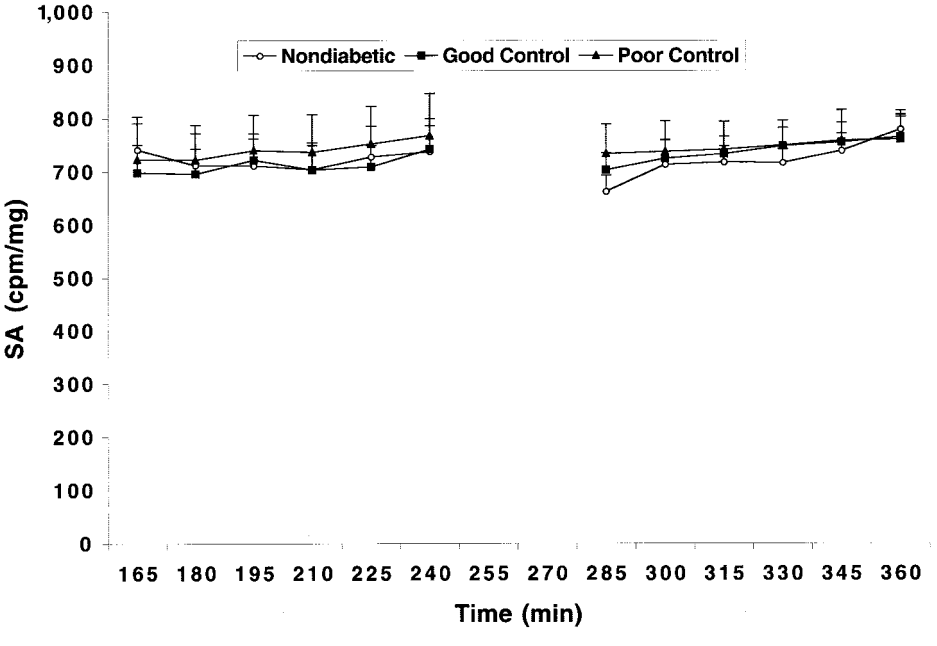

FIG. 4. Plasma levels of specific activity (SA) during euglycemic-hyperglycemic studies in the three study groups: ND, GC, and PC. Plasma samples for SA were obtained at 15-min intervals from 165 to 240 min and from 285 to 360 min in all subjects.

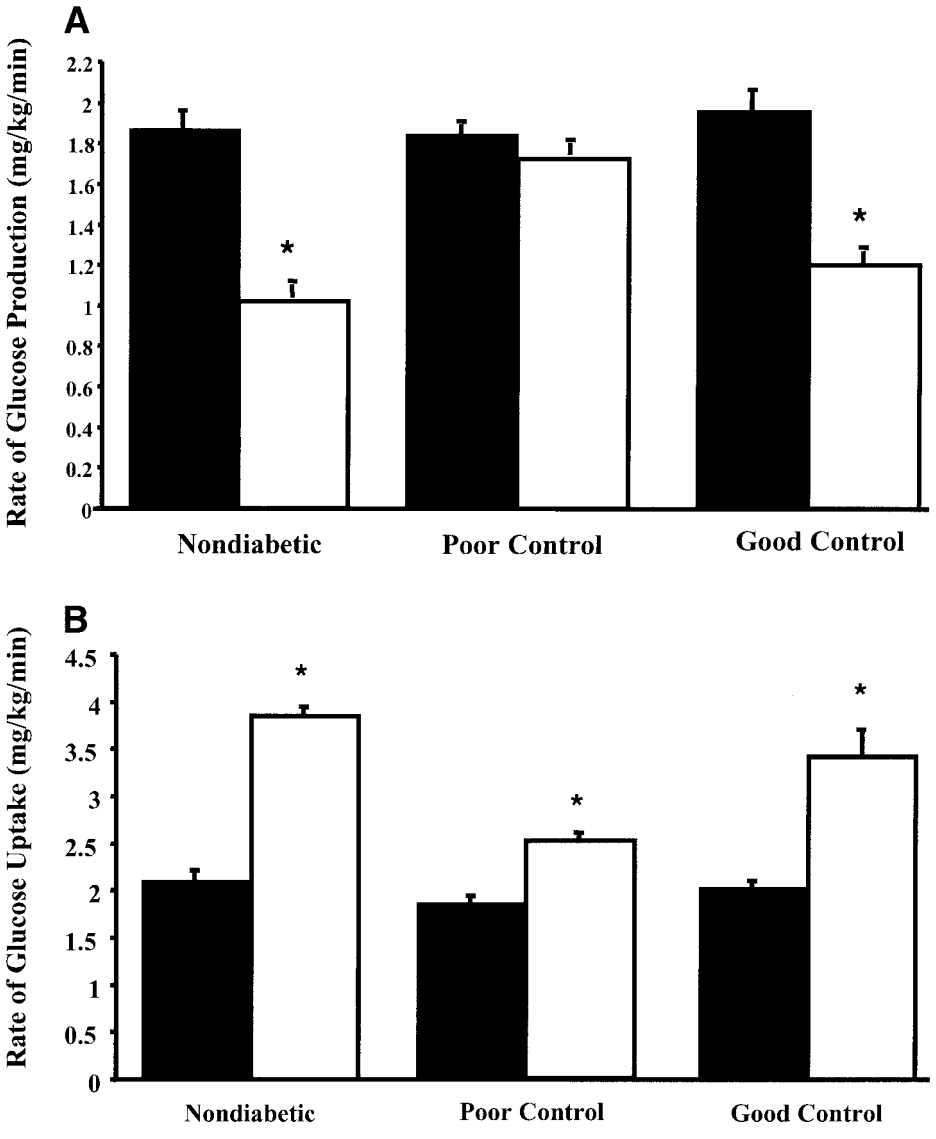

FIG. 5. *A*: **Rates of endogenous glucose production during the euglycemic study period (■) and the hyperglycemic study period (□) for the three study groups: ND, GC, and PC. *$P < 0.05$ in PC subjects, euglycemia vs. hyperglycemia.** *B*: **Rates of glucose uptake during the euglycemic study period (■) and the hyperglycemic study period (□) for the three study groups: ND, GC, and PC. *$P < 0.05$ for ND and GC subjects, euglycemia vs. hyperglycemia.**

To exclude any possible medication effects (because more GC than PC subjects were on insulin sensitizers), a subgroup analysis among the GC and PC groups was performed to compare results among subjects on metformin or troglitazone with subjects on other agents. Indeed, in both the GC and PC groups, there was no difference in the response of GP to hyperglycemia between those subjects previously on insulin sensitizers and those treated with other agents (results not shown).

Glucose uptake. The percent increase in glucose uptake between the euglycemic and hyperglycemic study periods was 85.9 ± 10.8% and 72.5 ± 12.1% for ND and GC subjects, respectively (euglycemia: 2.1 ± 0.1 mg · kg^{-1} · min^{-1} in ND subjects vs. 2.0 ± 0.1 mg · kg^{-1} · min^{-1} in GC subjects; hyperglycemia: 3.9 ± 0.1 mg · kg^{-1} · min^{-1} in ND subjects vs. 2.0 ± 0.1 mg · kg^{-1} · min^{-1} in GC subjects; $P >$ 0.05 between ND and GC subjects in both euglycemia and hyperglycemia), versus a more modest increase of 33.7 ± 5.1% in PC subjects (euglycemia vs. hyperglycemia: 1.8 ± 0.1 mg vs. 2.4 ± 0.1 mg · kg^{-1} · min^{-1}; $P < 0.02$ for comparison of percent change in glucose uptake between PC and both ND and GC subjects) (Fig. 5*B*).

Glycolysis and glycogen synthesis. Glycolysis from plasma glucose was calculated in the ND and type 2 diabetic subjects from the generation of [3-^{3}H]-H_2O during [3-^{3}H]glucose infusions. The glycolytic rates from plasma glucose were similar in the three groups during euglycemia and hyperglycemia (euglycemia vs. hyperglycemia: 1.5 ± 0.1 vs. 1.4 ± 0.2 mg · kg^{-1} · min^{-1} in PC, 1.9 ± 0.3 vs. 1.8 ± 0.2 mg · kg^{-1} · min^{-1} in GC, and 1.5 ± 0.1 vs. 1.7 ± 0.2 mg · kg^{-1} · min^{-1} in ND subjects; $P > 0.05$). Glycogen synthesis accounted for a small proportion of all glucose uptake during euglycemia. Although glycogen synthesis increased significantly during hyperglycemia in both type 2 diabetic and ND subjects, this response was markedly blunted in the PC group relative to the other groups (euglycemia vs. hyperglycemia: 0.3 ± 0.2 to 1.1 ± 0.3 mg · kg^{-1} · min^{-1} for PC, 0.1 ± 0.2 to 1.5 ± 0.2 mg · kg^{-1} · min^{-1}

TABLE 2
Rates of glycolysis and glycogen synthesis in glycemic control studies

	Glycolysis			Glycogen synthesis		
	PC	GC	ND	PC	GC	ND
Euglycemia ($mg \cdot kg^{-1} \cdot min^{-1}$)	1.5 ± 0.1	1.9 ± 0.3	1.5 ± 0.3	0.3 ± 0.2	0.1 ± 0.2	0.6 ± 0.2
Hyperglycemia ($mg \cdot kg^{-1} \cdot min^{-1}$)	1.4 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.1 ± 0.3	1.5 ± 0.2	2.2 ± 0.4
Δ (euglycemia vs. hyperglycemia)	−0.1	−0.1	+0.2	+0.8	+1.4	+1.6

Data are means ± SE. *$P < 0.05$ for PC vs. GC and ND.

for GC, and 0.6 ± 0.2 to 2.2 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$ for ND [$P < 0.01$ for all groups between euglycemia and hyperglycemia; $P < 0.05$ for PC subjects vs. both ND and GC subjects]). This increase accounted for virtually all of the increase in glucose uptake in all groups (Table 2).

The 3-day intensive glycemic control studies. Five PC subjects were restudied after 3 days of intensive glycemic control, using variable insulin infusions to achieve and maintain normal plasma glucose levels during the 72-h hospitalizations (PC3day). Average plasma glucose levels were 118 + 9 mg/dl throughout the 72-h admissions. During the euglycemic and hyperglycemic study periods, plasma glucose levels for the PC3day group were similar to the baseline studies for ND, GC, and PC patients, averaging 91 ± 6 mg/dl during euglycemia (t = 180–240 min) and 185 ± 6 mg/dl during hyperglycemia (t = 300–360 min). Hence, the amplitude of change in glucose concentrations for the PC3day group was comparable to the baseline studies for the ND, GC, and PC groups.

The average insulin infusion rate required to maintain euglycemia during the final 30 min of the insulin adjustment period and thereafter in the PC3day group was similar to the baseline studies for ND, GC, and PC groups and averaged 0.28 ± 0.05 $mU \cdot kg^{-1} \cdot min^{-1}$. Similarly, plasma levels did not differ between baseline and PC3day studies during either the euglycemic interval (baseline 26.4 ± 7.7 μU/ml vs. PC3day 26.1 ± 7.7 μU/ml) or the hyperglycemic interval (baseline 24.2 ± 4.8 μU/ml vs. PC3day 23.2 ± 4.5 μU/ml). Plasma glucagon levels also did not differ between baseline and PC3day studies during either the euglycemic interval (baseline 88.4 ± 5.2 pg/ml vs. PC3day 97.3 ± 11.3 pg/ml) or the hyperglycemic interval (baseline 82.2 pg/ml ± 5.3 vs. PC3day 94.6 ± 13.2 pg/ml).

As shown in Fig. 6*A*, FFA levels were comparable between the PC and PC3day groups under basal conditions (t = 0 min; PC = 349 ± 101 mmol/l, PC3day = 462 ± 147 mmol/l, $P > 0.05$). However, PC3day values were significantly lower than those in the baseline PC studies during both the euglycemic and hyperglycemic periods (PC3day: 221 ± 30 mmol/l during euglycemia and 189 ± 36 mmol/l during hyperglycemia; $P < 0.01$ for PC vs. PC3day, during both euglycemia and hyperglycemia) and were now comparable to the GC and ND subjects.

The average rate of glucose infusion required to maintain the hyperglycemic plateau during the last 60 min of the hyperglycemic period in PC3day subjects was similar to that of baseline studies for ND and GC subjects. This level of glucose infusion was increased more than twofold over the baseline PC glucose infusion rate. Thus, normalizing plasma glucose levels over 72 h increased glucose effectiveness. Indeed, the degree of suppression of GP by hyperglycemia in the PC3day studies (47.3 ± 3.9% suppression) was significantly greater than that in the baseline studies in the same individuals (17.9 ± 4.7% suppression; $P < 0.01$) and comparable to that observed in the GC and ND groups ($P > 0.05$) (Fig. 6*B*). The percent increase in glucose uptake between the euglycemic and hyperglycemic study periods was 71.2 ± 3.3% for the PC3day group versus the 39.9 ± 1.2% increase in baseline studies in the same subjects ($P < 0.05$) (Fig. 6*C*). These results demonstrate a complete recovery of the ability of hyperglycemia per se to suppress GP and stimulate glucose uptake after intensive normalization of glycemic control in PC subjects.

DISCUSSION

We examined whether the degree of chronic hyperglycemia in individuals with type 2 diabetes would determine the liver's ability to regulate hepatic glucose fluxes in response to an elevation in plasma glucose. We used a euglycemic-hyperglycemic pancreatic clamp technique to study the ability of hyperglycemia per se to regulate GP and glucose uptake in two groups of type 2 diabetic subjects stratified by degree of glycemic control (GC [n = 14, HbA_{1c} 5.9 ± 0.3%] vs. PC [n = 10, HbA_{1c} 9.9 ± 0.6%], relative to 11 age- and weight-matched ND subjects. In contrast to PC type 2 diabetic subjects, GC subjects retained a normal ability to stimulate peripheral glucose uptake and to suppress GP in response to a doubling of the plasma glucose concentration. Indeed, we have previously demonstrated a lack of glucose effectiveness to suppress GP and stimulate glucose uptake in type 2 diabetic subjects (6). Of note, the nine type 2 diabetic subjects studied had an average HbA_{1c} of 8.5 ± 0.4% and thus also could be considered to be in poor control.

Furthermore, intensively restoring euglycemia for 72 h in a representative subgroup of five poorly controlled type 2 diabetic individuals completely restored the responses of peripheral and hepatic glucose fluxes and plasma FFA levels to hyperglycemia per se. This result suggests that the impaired effectiveness of glucose to regulate glucose fluxes in type 2 diabetes is due to the chronic metabolic milieu and thus is a reversible defect.

Whereas decreased peripheral glucose clearance appears to be a major component of fasting hyperglycemia in mild type 2 diabetes (i.e., fasting plasma glucose of 126–140 mg/dl) (30), the contribution of an elevated basal GP becomes more prominent as plasma glucose levels increase above ~140 mg/dl. Inappropriately elevated GP is thus a major factor responsible for the worsening of the metabolic state in type 2 diabetes (1–4). Moreover, basal

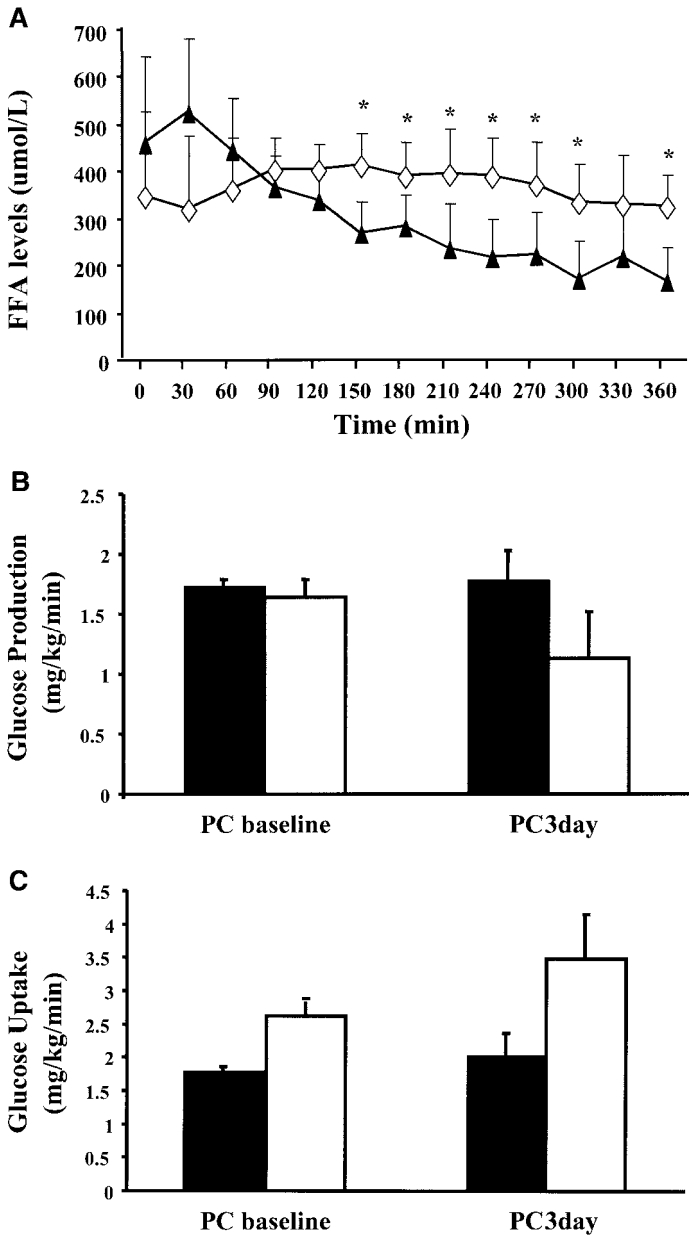

FIG. 6. Comparison of plasma FFA levels and rates of GP during euglycemic and hyperglycemic study periods in the PC3day protocol versus the same individuals studied under baseline conditions (PC). *A*: Comparison of average plasma FFA levels in PC versus PC3day subjects throughout euglycemic-hyperglycemic studies. ◇, PC; ▲, PC3day. **P* < 0.05 for PC vs. PC3day. *B*: Comparison of rates of GP during euglycemic and hyperglycemic study periods in the same PC subjects in the PC3day protocol versus baseline conditions. ■, Euglycemia; □, hyperglycemia. *C*: Comparison of rates of glucose uptake during euglycemic and hyperglycemic study periods in the same PC individuals in the PC3day protocol versus baseline conditions. ■, Euglycemia; □, hyperglycemia.

rates of GP are strongly correlated with fasting plasma glucose concentrations in type 2 diabetes (1). This finding has led us to predict that an individual's response to acute rises in plasma glucose might be at least in part determined by the habitual level of glycemia and that the specific glycemic threshold required for GP to be suppressed by hyperglycemia might vary along with glycemic control.

This rationale underlies the levels of glycemia we chose for the current stratification by glycemic control. Based on known relationships between HbA_{1c} and mean plasma blood glucose levels (31), individuals in the GC group (HbA_{1c} ~6%) are expected to have mean daily plasma glucose levels of ~120 mg/dl. Individuals in the PC group (HbA_{1c} ~8.8%), who demonstrated a failure to regulate GP in response to plasma glucose levels of 180 mg/dl, likely have mean daily plasma glucose levels of >180 mg/dl. It is therefore possible that these individuals have a higher threshold glucose level required for suppression of GP.

Indeed, a similar relationship between chronic levels of glycemia and basal rates of GP was observed in a large group of type 2 diabetic subjects with a wide range of plasma glucose levels (1). Subjects with fasting plasma glucose levels <140 mg/dl had rates of GP that were comparable to age- and weight-matched normal control subjects. However, progressive increases in fasting plasma glucose levels were associated with increasing rates of basal GP. Based on these data, a further increase in an individual's fasting plasma glucose level from 150 to 250 mg/dl would be expected to result in an additional 1.0 mg · kg^{-1} · min^{-1} of basal GP or ~60 g glucose produced overnight in a 100-kg person. These inappropriately elevated rates of GP could contribute substantially to fasting hyperglycemia in these subjects. Our studies suggest that an inability to respond to hyperglycemia per se with a reduction in GP could contribute to the progressive increases in GP in the type 2 diabetic subjects described above.

Whereas regulation of GP by hyperglycemia may occur in both ND and type 2 diabetic individuals, it may be activated at a higher set-point in type 2 diabetes. This is suggested both by the differential response among our subgroups of type 2 diabetic subjects to an identical level of hyperglycemia (180 mg/dl) and by the known association between progression of type 2 diabetes and inappropriate elevations in GP (3). A careful assessment of the literature reveals further support for an association between degree of hyperglycemia and hepatic glucose regulation. A group of 11 type 2 diabetic individuals with variable levels of glycemic control were heterogeneous in their responses to a stable-labeled frequently sampled insulin-modified intravenous glucose tolerance test, such that there was blunted suppression of GP by hyperglycemia in those individuals with higher levels of HbA_{1c} (32).

Conversely, Nielsen et al. (33) recently reported normal glucose-induced suppression of GP in a group of nine type 2 diabetic subjects. These subjects differed from our PC group in that they were leaner and possibly in better glycemic control (although only glycohemoglobin levels were reported) and retained partial ability of hyperglycemia to suppress FFA levels. Clearly, these differences in metabolic profiles could have all contributed to the preserved effectiveness of glucose to suppress GP in this group.

Of note, subjects in the GC group had a shorter duration of type 2 diabetes than the PC group. However, within each group of type 2 diabetic subjects, duration of diabetes did not predict glucose effectiveness ($P > 0.05$ for percent change in glucose uptake or GP with type 2 diabetes duration; results not shown). Furthermore, the five subjects who underwent 72 h of intensive normaliza-

tion of their plasma glucose were representative of the entire PC group, yet their responses to increased plasma glucose were completely restored by the intensive insulinization. Thus, the tendency of the degree of hyperglycemia to progress with time most likely explains the difference in diabetes duration between the two groups.

The PC subjects are likely to lack the ability to increase the flux through GK and/or to inhibit the flux through G-6-Pase in response to an elevation in plasma glucose at basal levels. Given the important roles of hepatic GK and G-6-Pase in determining the response of GP to hyperglycemia and the effect of the chronic metabolic milieu on their gene expression, we predicted that subjects with milder degrees of antecedent hyperglycemia would have less severe abnormalities of these enzyme activities. Partial deficiencies in hepatic glucose phosphorylation capacity occur in humans with type 2 diabetes and may contribute to its pathophysiology (34). Although defects in hepatic GK characterize individuals with maturity-onset diabetes of the young (MODY)-2 (35,36), these genetic defects account for a minority of the type 2 diabetic population. Similar defects in GK enzymatic activity may also be acquired secondarily to associated hormonal and metabolic alterations in some patients with type 2 diabetes (35). Indeed, liver biopsies from type 2 diabetic subjects have revealed increased G-6-Pase and decreased GK activities (9).

Defects in either hepatic enzyme can disrupt the normal regulation of hepatic glucose fluxes by hyperglycemia. Reduction in hepatic GK activity, either chronically in a transgenic mouse model (37) or acutely by glucosamine infusion in normal rats (38), markedly blunted the ability of the liver to sense hyperglycemia and thus to suppress GP. In addition, G-6-Pase gene expression is stimulated by both high glucose levels (12,13) and increased availability of FFAs (14). Because increased expression of G-6-Pase results in elevated rates of GP, even in the absence of hyperglycemia (39), this could clearly contribute to the abnormally elevated rates of GP in type 2 diabetes and the lack of suppression with hyperglycemia. Correction of chronic hyperglycemia in the above experimental models completely and rapidly reversed the abnormal gene expression of both enzymes (11,13). Together, these data suggest that the combined abnormalities of GK and G-6-Pase activities contribute substantially to the inappropriately elevated rates of GP in type 2 diabetes. Furthermore, the differential responses among the two type 2 diabetic groups and the marked improvement in regulation with chronic restoration of euglycemia are compatible with reversible metabolic effects on both enzymes.

In addition to beneficial effects on the regulation of both GP and peripheral glucose uptake, improved glycemic control was also associated with normal control of lipolysis. Thus, the type 2 diabetic subjects in good control demonstrated FFA levels that approximated those in the ND subjects throughout the study period. Although plasma FFA levels in the PC group were persistently more than twofold higher than those in the other groups, 72 h of intensive therapy restored normal suppression of FFA levels in these subjects. Elevations in circulating levels of FFAs and/or in rates of lipid oxidation can antagonize the actions of insulin on both GP and uptake (40–43). Furthermore, increased FFA availability results in both increased gluconeogenesis (44,45) and enhanced glucose-6-Pase gene expression (14). In addition, high intake of dietary fatty acids and increased intracellular content of long-chain fatty acyl-CoA esters are both associated with decreased expression of hepatic GK (46), suggesting that increased FFA levels may also play a role in the decreased GK expression observed in type 2 diabetes.

Given the increase in hepatic G-6-Pase gene expression with increased FFA availability, chronic elevations in circulating FFA levels could increase flux through G-6-Pase in the PC subjects and thus hinder the liver's ability to suppress GP in the presence of hyperglycemia. Indeed, whereas the inhibitory effects of hyperglycemia on lipolysis result in reduced FFA levels in ND individuals (6,47) and thereby likely facilitate glucose-mediated suppression of GP, adipocyte resistance to hyperglycemia in poorly controlled type 2 diabetic individuals might contribute to the loss of hepatic glucose effectiveness in these individuals.

In contrast to the differential response of circulating FFA levels between groups, plasma glycerol levels were only significantly higher in the PC group during the euglycemic period. Because the PC group did not demonstrate a decrease in GP with hyperglycemia, there would have been no decrease in use of glycerol via gluconeogenesis during the hyperglycemic interval. Indeed, it has been estimated that ~60% of total body glycerol utilization is accounted for by the liver (L. Rossetti, unpublished data). Decreased hepatic utilization of glycerol during hyperglycemia in the other two groups, together with increased generation of glycerol by lipolysis in the PC group, might explain why the plasma glycerol levels were comparable in all groups during the final phase of the studies.

Furthermore, the similar levels of plasma glycerol in all groups during the hyperglycemic phase indicate that the failure to suppress GP in the PC group was not due to increased availability of glycerol for gluconeogenesis. In addition, plasma lactate levels were actually slightly lower in the PC group throughout the study and thus would not have provided a stimulatory effect on gluconeogenesis (48).

Because these studies were performed in the presence of basal insulin levels, which were prevented from rising in response to the elevations in plasma glucose, the stimulation of glucose uptake in response to hyperglycemia was presumably due to the phenomenon on non–insulin-mediated glucose uptake (49,50). Indeed, non–insulin-mediated glucose uptake is believed to account for the majority of glucose uptake under such basal conditions. The inability of hyperglycemia to normally stimulate glucose uptake in type 2 diabetes has been documented by other investigators who used similar pancreatic clamp techniques (33,51,52). It is interesting to note, however, that a group of type 2 diabetic individuals in good control (mean HbA_{1c} 6.6%) manifested preserved stimulation of glucose uptake by glucose (32). This supports our observations in the type 2 diabetic subjects in good control and suggests that the ability of hyperglycemia to stimulate glucose uptake may also be impaired by the chronic diabetic milieu.

One probable explanation for the defect in non–insulin-mediated uptake with chronic hyperglycemia is decreased availability of glucose transporters at the plasma membrane, such that glucose would be less able to enhance its

own disposal by mass action (53). Indeed, it has recently been proposed that glucose stimulates its own disposal in skeletal muscle cells by direct activation of the insulin receptor kinase, probably because of cytosolic translocation of protein kinase C-α (54). This postulated direct effect of glucose on proximal insulin signaling might be downregulated in the presence of chronic hyperglycemia. Although overnight restoration of euglycemia was unable to improve peripheral glucose effectiveness in our PC group or in a similar group of type 2 diabetic subjects (21), normalizing both glucose levels and FFA response with 72-h insulinization was able to nearly correct the underlying defect.

Another important feature of the experimental design was to ensure that plasma specific activity was maintained at constant levels between the euglycemic and hyperglycemic study periods in all subject groups. Most importantly, the specific activity was held constant by adding tracer to the glucose infusate (55), to minimize the fluctuation of specific activity due to the introduction of exogenous "cold" glucose at variable infusion rates. Failure to add tracer to the glucose infusate in the presence of high glucose infusion rates has been shown to result in inappropriately negative values for GP (56).

Together, these studies demonstrate the effect of the chronic metabolic milieu on peripheral and hepatic glucose effectiveness in type 2 diabetes. Chronic hyperglycemia and/or hyperlipidemia contribute to impaired effectiveness of glucose to regulate glucose fluxes in type 2 diabetes and hence to the worsening of the overall metabolic condition. Acute normalization of plasma glucose might break the vicious cycle leading to impaired glucose effectiveness in type 2 diabetes.

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