# Effect of Physiological Hyperinsulinemia on Gluconeogenesis in Nondiabetic Subjects and in Type 2 Diabetic Patients

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**Gluconeogenesis (GNG) is enhanced in type 2 diabetes. In experimental animals, insulin at high doses decreases the incorporation of labeled GNG precursors into plasma glucose. Whether physiological hyperinsulinemia has any effect on total GNG in humans has not been determined. We combined the insulin clamp with the $^2H_2O$ technique to measure total GNG in 33 subjects with type 2 diabetes (BMI 29.0 ± 0.6 kg/m$^2$, fasting plasma glucose 8.1 ± 0.3 mmol/l) and in 9 nondiabetic BMI-matched subjects after 16 h of fasting and after euglycemic hyperinsulinemia. A primed-constant infusion of 6,6-$^2$H-glucose was used to monitor endogenous glucose output (EGO); insulin (40 mU · min$^{-1}$ · m$^{-2}$) was then infused while clamping plasma glucose for 2 h (at 5.8 ± 0.1 and 4.9 ± 0.2 mmol/l for diabetic and control subjects, respectively). In the fasting state, EGO averaged 15.2 ± 0.4 μmol · min$^{-1}$ · kg$_{ffm}^{-1}$ (62% from GNG) in diabetic subjects and 12.2 ± 0.7 μmol · min$^{-1}$ · kg$_{ffm}^{-1}$ (55% from GNG) in control subjects ($P$ < 0.05 or less for both fluxes). Glycogenolysis (EGO – GNG) was similar in the two groups ($P$ = NS). During the last 40 min of the clamp, both EGO and GNG were significantly ($P$ < 0.01 or less, compared with fasting) inhibited (EGO 7.1 ± 0.9 and 3.6 ± 0.5 and GNG 7.9 ± 0.5 and 4.5 ± 1.0 μmol · min$^{-1}$ · kg$_{ffm}^{-1}$ in diabetic and control subjects, respectively) but remained significantly ($P$ < 0.05) higher in diabetic subjects, whereas glycogenolysis was suppressed completely and equally in both groups. During hyperinsulinemia, GNG was reciprocally related to plasma glucose clearance. In conclusion, physiological hyperinsulinemia suppresses GNG by ~20%, while completely blocking glycogenolysis. Resistance of GNG (to insulin suppression) and resistance of glucose uptake (to insulin stimulation) are coupled phenomena. In type 2 diabetes, the excess GNG of the fasting state is carried over to the insulinized state, thereby contributing to glucose overproduction under both conditions.**
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EGO, endogenous glucose output; FFA, free fatty acid; GCMS, gas chromatography–mass spectrometry; GNG, gluconeogenesis; HPLC, high-performance liquid chromatography; Ra, rate of glucose appearance.

Insulin inhibition of endogenous glucose output (EGO) is a major mechanism controlling plasma glucose homeostasis (1). Insulin reduces EGO primarily by suppressing glycogenolysis but also by decreasing de novo glucose formation from three-carbon precursors, i.e., gluconeogenesis (GNG). The ability of insulin to inhibit GNG has been generally inferred from the pattern of incorporation of labeled precursors into plasma glucose (2). Using this approach in the dog, Cherrington et al. (2) have shown that a direct intrahepatic effect of insulin to suppress GNG from alanine is only observed at supraphysiological plasma insulin levels (3). We (4) and others (5) have provided evidence that in the overnight-fasted state, the gluconeogenic component of EGO is increased both in nondiabetic obese subjects and in patients with type 2 diabetes. In the latter, the excess GNG makes a quantitative contribution to the fasting hyperglycemia (4). Whether physiological hyperinsulinemia has any effect on total GNG, in nondiabetic subjects or in patients with diabetes, has not been determined. We, therefore, set forth to measure GNG in the insulinized state with the use of the deuterated water technique (6).

## RESEARCH DESIGN AND METHODS

**Subjects.** The study population consisted of 33 patients with type 2 diabetes and 9 healthy control subjects. The baseline metabolic measurements of 7 of the healthy control subjects and 24 of the patients with type 2 diabetes were included among the 18 healthy volunteers and 37 diabetic patients in our previous study (4). The clinical and metabolic characteristics of the study population are listed in Table 1. Except for diabetes, all subjects were free of major diseases, as determined by medical history, physical examination, and routine blood chemistry. Diabetic subjects were being treated with diet alone or in combination with oral hypoglycemic agents (5 patients on diet alone, 17 on sulfonylureas, 5 on metformin, and 7 on combined sulfonylureas and metformin); oral agents were withdrawn at least 4 weeks before the study. Nondiabetic subjects were not taking any medication known to affect glucose metabolism. The study protocol was approved by the Institutional Ethics Committee, and each subject gave his/her informed written consent to participate.

**Protocol.** Subjects were asked to drink $^2H_2O$ (5 g per kg of total body water, which was taken to be 55 and 45% of body weight in men and women, respectively) the night before the study at 10:00 P.M. (~2 h after eating a light dinner consisting of 50% carbohydrate, 15% protein, and 35% fat). To minimize vertigo, subjects were instructed to sip the $^2H_2O$ over a period of 30 min and to rest in bed thereafter.

After the overnight fast, during which subjects were instructed not to drink, the study was initiated at 9:00 A.M. An indwelling catheter was placed into an antecubital vein for isotope infusion. A second catheter was inserted retrogradely into a wrist vein of the ipsilateral hand, and the hand was placed into a heated box (60°C) to achieve arterialization of venous blood. The study

TABLE 1
Clinical characteristics

| | Control subjects | Diabetic subjects | *P* |
|---|---|---|---|
| *n* | 9 | 33 | |
| Sex (men/women) | 4/5 | 25/8 | NS |
| Age (years) | 38 ± 4 | 59 ± 1 | 0.0001 |
| Body weight (kg) | 86 ± 3 | 82 ± 2 | NS |
| BMI (kg/m$^2$) | 29.4 ± 0.7 | 29.0 ± 0.6 | NS |
| Fat-free mass (kg) | 54 ± 2 | 54 ± 1 | NS |
| Fat mass (%) | 37 ± 1 | 34 ± 1 | NS |
| Waist circumference (cm) | 102 ± 4 | 99 ± 1 | NS* |
| Waist-to-hip ratio | 0.89 ± 0.04 | 0.97 ± 0.02 | NS* |
| Duration of diabetes (years) | — | 8 ± 1 | — |
| $HbA_{1c}$ | — | 7.3 ± 0.2† | — |
| Systolic blood pressure (mmHg) | 129 ± 7 | 143 ± 3 | NS* |
| Diastolic blood pressure (mmHg) | 79 ± 4 | 83 ± 2 | <0.03* |

Data are *n* or means ± SE. *Adjusted by sex; †at the time of study.

was divided into two parts. First, a primed-continuous infusion of 6,6-[$^2$H]glucose (4 mg · kg$^{-1}$ as the prime, followed by a continuous infusion at a rate of 0.04 mg · min$^{-1}$ · kg$^{-1}$) was given for 120 min in nondiabetic subjects. In diabetic subjects, the priming dose was increased in proportion to the increase (>5 mmol/l) in fasting plasma glucose concentration and was extended to 180 min to ensure tracer equilibration in the presence of reduced plasma glucose clearance (7). During the second part of the study, a primed-continuous infusion of exogenous insulin (40 mU · min$^{-1}$ · m$^{-2}$) was added to the 6,6-[$^2$H]glucose infusion, and plasma glucose concentrations were clamped for 120 min after they had been allowed to decrease to <6 mmol/l (euglycemic insulin clamp). The exogenous glucose solution used to maintain this level was not enriched with either 6,6-[$^2$H]glucose or $^2H_2O$. Blood samples for the determination of 6,6-[$^2$H]glucose enrichment, plasma insulin and glucagon concentrations, plasma free fatty acid (FFA), and blood β-hydroxybutyrate concentrations were obtained before starting the tracer infusion and every 10 min during the last 20 min of the first part of the study (equilibration period). During the insulin clamp, plasma samples were obtained every 20 min. For the determination of naturally enriched carbon 5 (C5) glucose, plasma samples were obtained the day before the study. On the day of the study, samples for C5 determination were taken at the end of the equilibration period and at the end of the insulin clamp. The mean duration of fasting for the whole study group was 16 h at the end of glucose tracer equilibration. In 16 of 42 subjects, one additional C5 enrichment determination was performed on a blood sample obtained at time −180 min, i.e., before the start of the 6,6-[$^2$H]glucose infusion.

**Validation study.** The $^2H_2O$ method for the measurement of total gluconeogenesis has not been used previously during the infusion of unlabeled glucose (e.g., insulin clamp). In this non–steady-state situation, the variable infusion of exogenous (unlabeled) glucose dilutes both the 6,6-$^2$H-glucose and the C5-enriched plasma glucose. To validate the calculation of total GNG under these conditions (see "Data analysis" for details), in one additional nondiabetic obese (BMI 41 kg/m$^2$) subject (not included in the control group), the protocol described above was extended to 150 min of insulin and tracer infusion, and samples for C5 enrichment were obtained every 30 min during the clamp to determine the time course of C5 enrichment.

**Analytical methods.** $^2H_2O$ and 6,6-[$^2$H]glucose were purchased from Mass Trace (Woburn, MA). Glucose concentration was determined by the glucose oxidase method (Beckman II Glucose Analyzer; Beckman, Fullerton, CA). Plasma insulin and glucagon concentrations were measured by radioimmunoassay (Linco Research, St. Louis, MO). Serum glycosylated $HbA_{1c}$ concentrations were measured by high-performance liquid chromatography (HPLC) (Menarini HA-8140; Menarini Diagnostics, Florence, Italy). Plasma FFA concentrations were measured by an enzymatic method (NEFA C; Wako Chemical, Neuss, Germany). For determination of β-hydroxybutyrate concentrations, blood was collected in chilled tubes containing 1N perchloric acid (1:1 wt/wt) and assayed in duplicate on the supernatant of perchloric acid precipitates by a spectrophotometric enzymatic method.

Plasma glucose enrichment due to 6,6-[$^2$H]glucose was determined by gas chromatography–mass spectrometry (GCMS) on a Hewlett Packard GC 5890/MS 5972 (Hewlett Packard, Palo Alto, CA) equipped with a 30-m capillary column, as described by Wolfe (8). Briefly, 100 μl of plasma were deproteinized using 2 ml cold methanol, and the supernatant was dried and derivatized using acetic anhydride:pyridine (1:1) to form penta-acetate glucose. The sample was then dried again and dissolved with ethylacetate for injection into the GCMS. The fragments 200, 201, and 202 were monitored, and enrichment was calculated as the ratio of 202:200. To correct for incorporation into glucose of $^2$H originating from the administered $^2H_2O$, the 202:200 ratio obtained at the end of the 6,6-[$^2$H]glucose infusion was corrected for the corresponding ratio measured in the plasma sample collected before the start of the 6,6-[$^2$H]glucose infusion. An additional correction was introduced to account for the possible incorporation of deuterium from $^2H_2O$ into glucose occurring between the beginning and the end of the 6,6-[$^2$H]glucose infusion (overlapping spectra correction, as per Eq. 5 in ref. no. 8).

The pattern of $^2$H incorporation into plasma glucose after $^2H_2O$ ingestion was determined according to the method developed by Landau et al. (6,9). Briefly, the fraction of glucose produced via GNG from all precursors can be quantified from the ratio of $^2$H enrichment of C5 to that of water. The precursor of the hydrogen bound to C5 of glucose is the hydrogen bound to C2 of glyceraldehyde-3-phosphate. That hydrogen equilibrates with the hydrogen of body water in the isomerization of glyceraldehyde-3-phosphate with dihydroxyacetone phosphate, an intermediate in the conversion of glycerol to glucose, and binds in the hydration of phosphoenolpyruvate (formed in the conversion of pyruvate into glucose). Because during glycogen breakdown there is no binding of hydrogen from body water to C5 of the glucose formed, enrichment at C5 in blood glucose versus water reflects the fractional contribution of total gluconeogenesis, i.e., from both phosphoenolpyruvate precursors and glycerol.

Plasma samples were first deproteinized by the Somogyi procedure. The supernatant was then passed through a mixed column of AG 1-X8 in the formate form and AG 50W-X8 in the $H^+$ form; the eluate was dried in a Speed-Vac (Savant, France). Samples were then reconstituted with 220 μl distilled water and injected into an HPLC device (Waters, Milford, MA) for further purification. Deuterium enrichment at C5 was obtained by converting glucose to xylose by the removal of carbon in position 6. The xylose was purified by HPLC; the C5 group was cleaved by oxidation with periodic acid, and the formaldehyde was collected by distillation. The formaldehyde was incubated with ammonia overnight: in the presence of ammonia, six molecules of formaldehyde react to form a molecule of hexamethylenetetramine. This step is used to increase the sensitivity of the method. Enrichment of hexamethylenetetramine obtained from C5 was determined by GCMS by monitoring peaks of mass 140 and 141. Precision and accuracy of C5 have been reported previously (4).

Water enrichment in the body water pool was monitored by reacting a sample of plasma or urine with calcium carbide ($CaC_2$), thereby obtaining acetylene ($C_2H_2$), the enrichment of which was then determined by GCMS by monitoring peaks of mass 26 and 27 (10). All samples were run through the GCMS processing in duplicate or triplicate.

**Data analysis.** Fat-free mass was estimated with the use of Hume's formula in its sex-specific version (11). All glucose fluxes were corrected for the fat-free mass because this normalization has been shown to correct for differences due to sex, obesity, and age (12). During the first part of the study (equilibration period), both plasma glucose concentrations and 6,6-[$^2$H]glucose enrichment were stable during the last 20 min of tracer infusion in all subjects. Therefore, total EGO was calculated as the ratio of the 6,6-[$^2$H]glucose infusion rate to the plasma 6,6-[$^2$H]glucose enrichment (mean of three determinations). The percent contribution of GNG to plasma glucose was calculated as the ratio of the enrichments C5:$^2H_2O$ (6). Gluconeogenic flux was calculated by multiplying percent GNG by EGO. The glycogenolytic flux was obtained as the difference between EGO and the gluconeogenic flux.

During the second part of the study (euglycemic clamp), total rates of glucose appearance (Ra) were calculated using a two-compartment model, as previously described (13). EGO was then obtained as the difference between Ra and the exogenous glucose infusion rate. The percent contribution of GNG to plasma glucose was calculated as the ratio of the enrichments C5:$^2H_2O$ at the end of the clamp in each study subject. During the clamp, the infusion of the 20% glucose solution involved the administration of 2.2 ± 0.1 ml (range 0.6–4.8) of water per kg body wt, which corresponds to 4.0 ± 0.3% (range 1–10%) of the total body water pool. If urine output (~100 ml) and water loss through blood sampling (~100 ml) are considered, the water pool can be expected not to have changed significantly during the clamp period. As a consequence, $^2H_2O$ enrichment is essentially unaltered by the infused fluid (see RESULTS). In contrast, C5 enrichment is diluted by the exogenous glucose. Therefore, the C5:$^2H_2O$ ratio gives the contribution of GNG to the total (endogenous plus exogenous) concentration of glucose in the plasma. By applying the standard precursor-product relationship, gluconeogenic flux was therefore calculated by multiplying the C5:$^2H_2O$ ratio by the total glucose Ra at any time point during the clamp.

Data are given as mean ± SE. Comparison of mean group values was performed by the unpaired Student's *t* test; when appropriate, these compar-

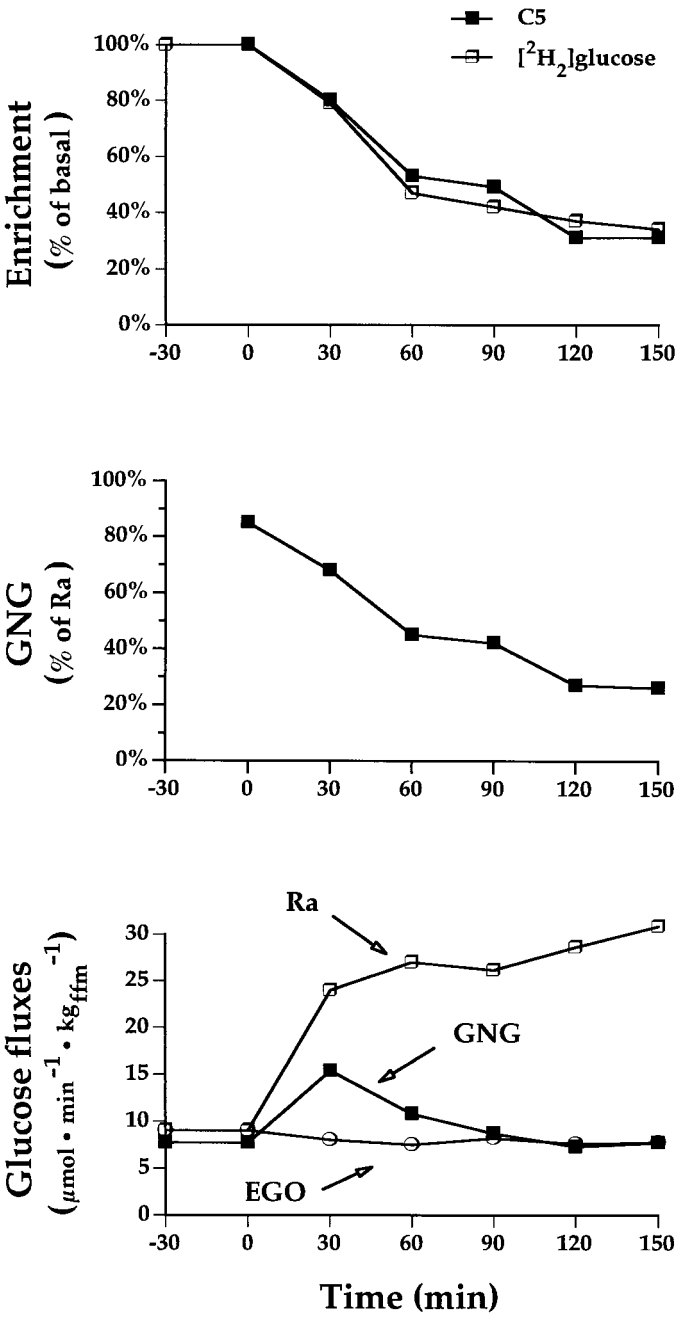


**FIG. 1. C5 and $^2H_2$ enrichment in plasma glucose (both expressed as percent of baseline), GNG (as a fraction of total rate of appearance), and whole-body glucose fluxes in an obese nondiabetic subject during 150 min of euglycemic hyperinsulinemia.**

isons were adjusted for continuous variables by regression analysis. The effect of insulin was tested by two-way analysis of variance for repeated measures. Simple and partial correlation analyses were used to estimate associations among continuous variables in the whole data set.

## RESULTS

**Validation study.** To validate the calculation of total GNG by the $^2H_2O$ method during the insulin clamp, we obtained multiple measurements of both 6,6-$^2$H-glucose and C5 enrichment in a nondiabetic obese subject. As shown in Fig. 1, the variable exogenous (unlabeled) glucose infusion led to a time-varying dilution of both 6,6-$^2$H-glucose and C5 enrichment, whereas $^2H_2O$ enrichment was constant throughout (data not plotted). As a consequence, the fractional contribution of GNG to total Ra (i.e., the C5:$^2H_2O$ ratio) decreased in a time course parallel to that of C5 enrichment. The corresponding glucose fluxes calculated by non–steady-state analysis are also shown in Fig. 1. After the start of insulin infusion, EGO flux was hardly changed, indicating the presence of severe hepatic insulin resistance (presumably due to the severe obesity). At baseline, GNG flux (given by the product C5:$^2H_2O$ · Ra) was 85% of EGO, consistent with our previous finding of an increased contribution of GNG to EGO in obesity (4). Following insulin, GNG flux slightly exceeded EGO at 30 and 60 min (probably due to incomplete label mixing and modeling error). From 90 min onward, GNG represented ~100% of EGO and glycogenolysis was completely suppressed.

**Metabolic results.** The control subjects were younger than the diabetic subjects but well matched for total adiposity and fat distribution (Table 1). In nondiabetic subjects, fasting plasma glucose did not change significantly during the 2-h tracer glucose equilibration (from 5.1 ± 0.2 to 5.0 ± 0.2 mmol/l, $P$ = NS), whereas in diabetic subjects, it declined from 9.5 ± 0.4 to 8.1 ± 0.3 mmol/l ($P < 0.0001$) during the 3-h tracer glucose infusion. In the 16 subjects in whom both measurements were available (10 diabetic and 6 control subjects), the plasma C5:$^2H_2O$ ratio averaged 0.56 ± 0.03 at time −180 min (i.e., before initiation of 6,6-$^2$H-glucose infusion) and 0.62 ± 0.03 at time 0, a significant ($P < 0.004$) increase of 0.02 ± 0.01 per hour. By linear forward extrapolation, the plasma C5:$^2H_2O$ ratio would have been 0.66 ± 0.03 at time +120 min if the fasting condition had been extended. The value actually observed in these subjects (i.e., with insulin infusion between 0 and 120 min) was 0.22 ± 0.02 ($P < 0.0001$).

During insulin administration, $^2H_2O$ enrichment did not change from baseline in either group of subjects. Plasma glucose and insulin concentrations were clamped at similar levels in diabetic and control subjects; plasma glucagon and FFA as well as blood β-hydroxybutyrate concentrations were suppressed to similar extents in the two groups (Table 2).

The plasma C5:$^2H_2O$ ratio was significantly higher in diabetic subjects than in control subjects and was markedly reduced by insulin administration in both groups (Table 3). In the fasting state, both EGO and GNG flux were significantly elevated in diabetic patients (by 25 and 40%, respectively) as compared with the control group, whereas glycogenolytic flux was similar (5.8 ± 0.3 vs. 5.5 ± 0.5 μmol · min$^{-1}$ · kg$_{ffm}^{-1}$, $P$ = NS).

During euglycemic insulin administration (Fig. 2), both EGO and GNG flux were significantly suppressed (by 57 ± 4 and 17 ± 6%, respectively). However, both EGO and GNG flux remained significantly higher in diabetic subjects than in control subjects. Glycogenolysis was suppressed completely and equally in diabetic and control subjects.

As expected, insulin administration caused a significant stimulation of plasma glucose clearance (Table 3); this insulin effect tended to be smaller in the diabetic subjects (the difference falling just short of statistical significance, $P$ = 0.11). In the whole data set, insulin-stimulated plasma glucose clearance was significantly related to insulin-mediated GNG in an inverse fashion (Fig. 3).

TABLE 2
Metabolic data

| | Control subjects | | | Diabetic subjects | | |
|---|---|---|---|---|---|---|
| | Basal | *P** | Insulin | Basal | *P** | Insulin |
| $^2H_2O$ enrichment (molar percent excess) | 0.51 ± 0.03 | NS | 0.49 ± 0.03 | 0.45 ± 0.01† | NS | 0.44 ± 0.01 |
| Plasma glucose (mmol/l) | 5.0 ± 0.2 | NS | 4.9 ± 0.2 | 8.1 ± 0.3† | 0.0001 | 5.8 ± 0.1 |
| Plasma insulin (μU/ml) | 11.5 ± 1.4 | 0.0001 | 114 ± 9 | 12.2 ± 1.2 | 0.0001 | 113 ± 6 |
| Plasma glucagon (ng/ml) | 82 ± 10 | 0.004 | 64 ± 6 | 76 ± 4 | 0.004 | 68 ± 4 |
| FFAs (μmol/l) | 693 ± 32 | 0.0001 | 106 ± 28 | 537 ± 40 | 0.0001 | 93 ± 11 |
| β-OH-butyrate (μmol/l) | 170 ± 52 | 0.0001 | 15 ± 3 | 160 ± 24 | 0.0001 | 26 ± 3 |

Data are means ± SE or *n*. *Difference between basal and insulin; †$P < 0.001$ for the difference between diabetic and control subjects.

## DISCUSSION

**Methodology.** The $^2H_2O$ technique has been used in various laboratories (6,14–18) and has gained wide acceptance as a robust method to measure total GNG in humans. The physiological circumstances so far investigated have included the overnight and prolonged fast and the response to stimuli (glucagon, FFA, acipimox) not involving the administration of exogenous glucose. In general, when attempting to determine the effect of a metabolic perturbation on total GNG, two problems arise. One relates to the time kinetics of GNG, i.e., the time required for any change in the GNG pathway to be reflected in measurable changes in the enrichment pattern of circulating glucose (which is what the $^2H_2O$ technique measures). The second problem is how to compute gluconeogenic glucose fluxes in the nonsteady state, particularly when exogenous glucose (infused or ingested) dilutes the plasma tracer enrichment established during the equilibration phase. The validation study we performed showed that, after initiation of the insulin/glucose infusion, plasma glucose C5 enrichment declined in parallel with the decrease in 6,6-$^2$H-glucose enrichment as a result of dilution. Because $^2H_2O$ was stable as expected, the ratio C5:$^2H_2O$ continues to represent the fractional contribution of gluconeogenic glucose to total plasma glucose; however, unlike the basal state, total plasma glucose is now the sum of endogenous and exogenous glucose. Because glucose of any origin (exogenous, gluconeogenic, or glycogenolytic) is handled in the same way by body tissues, one can use a simple precursor-product relationship to calculate gluconeogenic glucose flux (i.e., C5:$^2H_2O$ times total glucose rate of appearance, which is the sum of EGO and the exogenous glucose infusion rate). Theoretically, by measuring C2 enrichment during the clamp, GNG flux can also be calculated as the product of C5:C2 ratio and residual EGO. The two approaches would give the same result (within the experimental errors involved in $^2H_2O$ and C2 enrichment) if glucose cycling (through the hexosemonophosphate pool) was nil during insulin infusion. In fact, in the presence of glucose cycling, some exogenous glucose also becomes labeled in C2; C2 enrichment in plasma glucose would consequently be overestimated, and GNG would be underestimated. The persistence of glucose cycling during insulin infusion has been previously shown in diabetic as well as nondiabetic subjects (19).

In the very obese subject of the validation study, GNG accounted for 85% of EGO at baseline and became 100% of EGO during the last hour of the clamp (Fig. 1). Thus, the only effect of insulin in this very insulin-resistant subject was to completely turn off the small glycogenolytic flux that prevailed in the fasting condition. The time course also shows that during the first hour of the clamp, GNG actually exceeded EGO, a result that can be explained by incomplete label mixing and/or modeling error. It should be recalled that GNG flux is obtained as the product of fractional GNG and Ra and is therefore sensitive to experimental and modeling errors. In summary, the validation study indicated that true changes in GNG flux during metabolic perturbation probably take 60–90 min to be reliably reflected in the labeling pattern of circulating glucose and that the precursor-product relationship can be applied to GNG when the precursor (i.e., $^2H_2O$) is in steady state.

An important methodological consideration and a potential source of error is glycogen cycling. Previous work using [$^{13}$C] nuclear magnetic resonance spectroscopy has provided evidence that in nondiabetic subjects, euglycemic hyperinsulinemia (similar to that used in the present work) stimulates glycogen synthase activity without completely blocking glycogen phosphorylase activity (both activities being inferred from in vivo glucose flux measurements) (20). Under these circumstances, significant cy-

TABLE 3
Glucose fluxes

| | Control subjects | | | Diabetic subjects | | |
|---|---|---|---|---|---|---|
| | Basal | *P** | Insulin | Basal | *P** | Insulin |
| Plasma C5 enrichment (molar percent excess) | 0.28 ± 0.01 | 0.0001 | 0.07 ± 0.02 | 0.28 ± 0.01 | 0.0001 | 0.11 ± 0.01† |
| Plasma C5/$^2H_2O$ ratio | 0.55 ± 0.03 | 0.0001 | 0.14 ± 0.03 | 0.62 ± 0.02† | 0.0001 | 0.25 ± 0.02† |
| EGO ($\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$) | 12.2 ± 0.7 | 0.0001 | 3.6 ± 0.5 | 15.2 ± 0.4† | 0.0001 | 7.1 ± 0.9† |
| Gluconeogenic flux ($\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$) | 6.7 ± 0.5 | 0.003 | 4.5 ± 1.0 | 9.4 ± 0.4† | 0.003 | 7.9 ± 0.5† |
| Exogenous glucose infusion rate ($\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$) | — | — | 28.9 ± 3.4 | — | — | 26.9 ± 1.9 |
| Glucose Ra ($\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$) | 12.2 ± 0.7 | 0.0001 | 32.3 ± 3.5 | 15.2 ± 0.4† | 0.0001 | 33.9 ± 2.2 |
| Glucose clearance rate ($ml \cdot min^{-1} \cdot kg_{ffm}^{-1}$) | 2.5 ± 0.2 | 0.0001 | 6.7 ± 0.7 | 1.8 ± 0.1 | 0.0001 | 6.0 ± 0.4 |

*Difference between basal and insulin; †$P \leq 0.05$ difference between control and diabetic subjects.

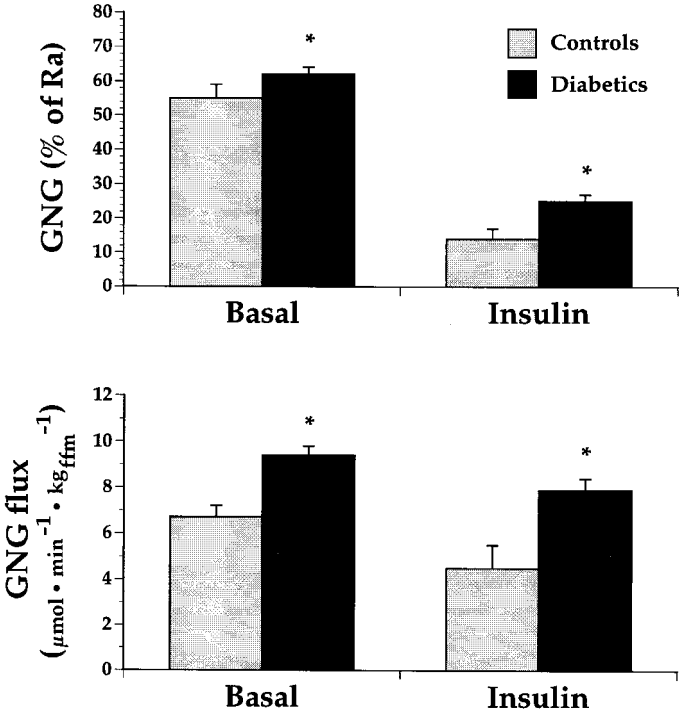


**FIG. 2. Gluconeogenesis (as a fraction of total Ra and as a flux rate) in patients with type 2 diabetes and obese nondiabetic control subjects in the fasting state and after 120 min of euglycemic hyperinsulinemia. *Mean values that are significantly different between the two groups (see also Table 3).**

cling of plasma glucose in and out of glycogen would occur. With our approach, to the extent that circulating, 6,6-[$^2$H]glucose-enriched glucose should be taken up into glycogen to be released at later times, the 6,6-[$^2$H]glucose tracer/tracee ratio would be artificially elevated, and consequently, rates of glucose appearance would be underestimated. This phenomenon could account for the negative rates of EGO that have been reported previously and that were also calculated in some of the present studies (e.g., GNG flux exceeding EGO in the validation study of Fig. 1). This bias is equivalent to overestimating the ability of insulin to suppress glycogenolysis. However, it is important to stress that in our studies, virtually all the 'endogenous' plasma glucose analyzed during insulin infusion was enriched in C5. This indicates that, whether directly from gluconeogenesis or following some detour through glycogen, the endogenous glucose released during insulin stimulation was entirely of gluconeogenic origin.

An additional problem with glycogen cycling relates to the possibility that some glycogen may become C5-enriched during the hours after the ingestion of $^2H_2O$; if such glycogen is then broken down during the EGO measurement (6,6-[$^2$H]glucose infusion) period, it would artificially increase the C5 labeling of plasma glucose and the attendant GNG estimate. Here, however, it can be argued that C5 enrichment of plasma glucose at early times after $^2H_2O$ administration is low; furthermore, in the overnight-fasted state, glycogen cycling is nil (20). Therefore, the quantitative impact of such a cycle should be minimal. In summary, the $^2H_2O$ technique makes it possible to quantitate the flux of gluconeogenic glucose through plasma under all circumstances; the extent to which such glucose derives directly from glucose-6-phosphate or indirectly (via some passage through glycogen) remains somewhat indeterminate, likely dependent on the experimental conditions and presumably small.

**Pathophysiology.** The present series included ambulatory patients studied under ordinary living conditions (diet and sleep pattern) and in fair glycemic control (no patients had a $HbA_{1c}$ level >12% after 4 weeks of pharmacological washout). Circulating insulin and glucagon concentrations as well as FFA and β-OH-butyrate concentrations were similar in diabetic and control subjects after 16 h of fasting and after 2 h of euglycemic hyperinsulinemia. In the fasting state, EGO was increased in the diabetic group as compared with the control group; this excess glucose release was entirely due to an increase of the gluconeogenic component. As previously shown (4), within the age range of this series, there was no effect of age on percent GNG. It should also be noted that GNG flux was higher than normal also in the control group; this is explained by the fact that these subjects were obese (mean BMI 29 kg/m$^2$), and obesity is associated with enhanced GNG, independent of diabetes (4).

During physiological hyperinsulinemia, EGO was incompletely suppressed in both the diabetic and control subjects: this was expected because all study subjects were insulin-resistant. The main finding is that in both groups, total GNG flux was suppressed by only ~20%, whereas glycogenolysis was almost completely inhibited. This result is in line with previous studies showing that the primary effect of insulin is the direct inhibition of net glycogenolysis (2,3,20,21). The effect of insulin on GNG in humans has not been previously quantitated. In dogs, a direct intrahepatic effect of insulin to suppress GNG from alanine was only observed at plasma insulin levels approximately fivefold higher than those applied in the current experiments (3). By catheterization and net balance analysis, Cersosimo et al. (22) could not detect inhibition of hepatic GNG-precursor uptake during 3 h of low-dose insulin infusion. Our clamp studies establish what is to be expected when physiological hyperinsulinemia is applied for a relatively short period of time in the face of euglycemia.

Any effect of insulin on GNG may be direct, i.e., on

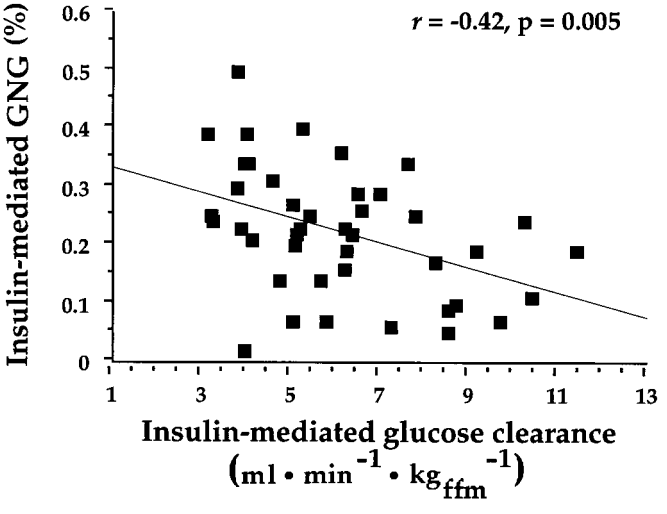


**FIG. 3. Reciprocal relationship between insulin-mediated gluconeogenesis (as a fraction of total Ra) and insulin-mediated glucose clearance in 42 study subjects (diabetic and nondiabetic subjects pooled).**

enzyme activities in the pathway, or indirect, i.e., mediated by insulin-induced decrements in the amount of glycerol and amino acids reaching the liver (and the kidney) and a reduction in FFA availability (due to inhibition of lipolysis and increased FFA re-esterification) (3,23). In our studies, insulin suppression of GNG was similar in diabetic and nondiabetic subjects and paralleled their respective decrements in circulating FFA and β-OH-butyrate concentrations. However, despite the fact that fatty acid availability and oxidation were almost completely suppressed during insulin infusion, GNG flux was reduced by only 20%. Therefore, although high plasma FFA concentrations have been shown to increase the contribution of GNG to EGO in normal subjects (18), this substrate-driven mechanism cannot be solely responsible for the increased GNG flux that obese and diabetic patients show both in the fasting state and after short-term physiological insulinization.

Another result of pathophysiological relevance is the reciprocal association between GNG and plasma glucose clearance during the clamp. Because the two measures were obtained by fully independent methods, this result clearly indicates that resistance of GNG to insulin suppression and resistance of peripheral glucose uptake to insulin stimulation are coupled phenomena. Whether this is due to a common defect in intracellular insulin signaling (2) or whether a causal connection exists between peripheral insulin resistance and enhanced gluconeogenic activity (4) remains to be determined. What has been established is that the excess GNG in the diabetic patient is carried over from the fasting to the insulinized state, thereby substantially contributing to glucose overproduction under both conditions.

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