Higher Insulin Concentrations Are Required to Suppress Gluconeogenesis Than Glycogenolysis in Nondiabetic Humans

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To determine the mechanism(s) by which insulin inhibits endogenous glucose production (EGP) in nondiabetic humans, insulin was infused at rates of 0.25, 0.375, or 0.5 mU · kg^{-1} · min^{-1} and glucose was clamped at ~5.5 mmol/l. EGP, gluconeogenesis, and uridine-diphosphoglucose (UDP)-glucose flux were measured using [3-^{3}H]glucose, deuterated water, and the acetaminophen glucuronide methods, respectively. An increase in insulin from ~75 to ~100 to ~150 pmol/l (~12.5 to ~17 to ~25 μU/ml) resulted in progressive (ANOVA; $P <$ 0.02) suppression of EGP (13.1 ± 1.3 vs. 11.7 ± 1.03 vs. 6.4 ± 2.15 μmol · kg^{-1} · min^{-1}) that was entirely due to a progressive decrease (ANOVA; $P < 0.05$) in the contribution of glycogenolysis to EGP (4.7 ± 1.7 vs. 3.4 ± 1.2 vs. –2.1 ± 1.3 μmol · kg^{-1} · min^{-1}). In contrast, both the contribution of gluconeogenesis to EGP (8.4 ± 1.0 vs. 8.3 ± 1.1 vs. 8.5 ± 1.3 μmol · kg^{-1} · min^{-1}) and UDP-glucose flux (5.0 ± 0.4 vs. 5.0 ± 0.3 vs. 4.0 ± 0.5 μmol · kg^{-1} · min^{-1}) remained unchanged. The contribution of the direct (extracellular) pathway to UDP-glucose flux was minimal and constant during all insulin infusions. We conclude that higher insulin concentrations are required to suppress the contribution of gluconeogenesis of EGP than are required to suppress the contribution of glycogenolysis to EGP in healthy nondiabetic humans. Since suppression of glycogenolysis occurred without a decrease in UDP-glucose flux, this implies that insulin inhibits EGP, at least in part, by directing glucose-6-phosphate into glycogen rather than through the glucose-6-phosphatase pathway. *Diabetes* **52:2213–2220, 2003**

Insulin is the primary regulator of endogenous glucose production (EGP). Insulin can inhibit hepatic glucose release via a variety of mechanisms. Insulin can decrease net hepatic release of glucose by increasing the uptake of glucose and by decreasing the conversion of glucose-6-phosphate to glucose (1–4). The former is regulated by the activity of glucokinase and the latter by glucose-6-phosphatase (5,6). Insulin can also stimulate glycogen synthesis, inhibit glycogen breakdown, and suppress gluconeogenesis (7–11). The relative contribution of these processes to insulin-induced inhibition of EGP in humans is presently not known.

Data in animals indicate that higher concentrations of insulin are required to suppress gluconeogenesis than are required to suppress glycogenolysis (12,13). In an elegant series of experiments, Chiasson et al. (13) reported that insulin concentrations of ~600 pmol/l suppressed EGP in dogs but did not alter the rate of incorporation of [^{14}C]glucose derived from [^{14}C]alanine (an index of gluconeogenesis) into hepatic glycogen. In contrast, insulin concentrations of ~6,000 pmol/l inhibited both EGP and [^{14}C]glucose incorporation into glycogen. These data implied that higher insulin concentrations were required to inhibit gluconeogenesis than were required to inhibit EGP. These conclusions are supported by the recent report of Edgerton et al. (12) that an increase in insulin to ~20 μU/ml suppressed glycogenolysis in dogs but did not alter gluconeogenesis measured using three different methods.

The extent to which these data apply to humans is not known. Chiasson et al. (14), using an experimental design similar to that previously used in their dog studies, reported that insulin levels of ~600 pmol/l suppressed EGP in three men who fasted 48 h but did not prevent the release of [^{14}C]glucose (derived from [^{14}C]alanine) during a subsequent glucagon infusion, implying ongoing gluconeogenesis and glycogen synthesis. In contrast, insulin concentrations of ~2,500 pmol/l both suppressed EGP and blunted glucagon-induced release of [^{14}C]glucose, suggesting an additional inhibitory effect on intrahepatic gluconeogenesis. Recently, Gastaldelli et al. (15), using the deuterated water method to measure gluconeogenesis, reported that insulin concentrations of ~700 pmol/l suppressed the contribution of gluconeogenesis and glycogenolysis to EGP in nondiabetic humans by ~33 and 100%, respectively. However, the subjects in those studies were obese and therefore presumably insulin resistant. While this manuscript was in preparation, Boden et al. (16), using a similar experimental design, reported that insulin concentrations of ~450 pmol/l resulted in ~45 and 100% suppression of the contribution of gluconeogenesis and glycogenolysis to EGP, again implying that glycogenolysis is more sensitive to insulin then gluconeogenesis. However, since the insulin concentrations in both of these studies resulted in nearly complete suppression of EGP

and since intra-hepatic glucose flux was not measured, it is not clear whether insulin suppressed EGP by inhibiting flux through glucose-6-phosphatase, intra-hepatic gluconeogenesis, or a combination of both.

The present experiments were undertaken in an effort to better define the mechanism(s) by which insulin inhibits EGP in humans. EGP, uridine-diphosphoglucose (UDP)-glucose flux, and the contribution of gluconeogenesis to EGP were measured using [3-³H]glucose, the acetaminophen glucuronide technique, and the deuterated water method (17–20). Responses in the presence of submaximal and maximal insulin concentrations were assessed. Glucose concentrations were maintained in the euglycemic range by means of a glucose infusion. Endogenous hormone secretion was inhibited with somatostatin, and glucagon replaced in order to ensure comparable portal glucagon concentrations on all occasions.

RESEARCH DESIGN AND METHODS

Subjects. After approval from the Mayo Institutional Review Board, 15 subjects (4 men and 11 women) participated in the study. All subjects were in good health and had a fasting glucose concentration <5.5 mmol/l. The subjects' age averaged 27 ± 2 years, BMI 22.4 ± 0.5 kg/m², and body fat 28 ± 2%. No subject regularly engaged in vigorous physical activity, and all were at a stable weight. All subjects were instructed to consume a weight-maintenance diet consisting of 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before study. Subjects were on no regular medications other than stable estrogen or thyroid hormone replacement. No subject had a first-degree relative with type 2 diabetes.

Experimental design. Subjects were admitted to the Mayo Clinic General Research Center at 1700 on the evening before study. A standard 10 cal/kg meal (55% carbohydrate, 30% fat, and 15% protein) was eaten between 1800 and 1830. The subjects then fasted for the remainder of the study. Each subject ingested 1.67 g/kg total body weight of deuterated water at 1900, 2100, and 2300. Each dose of deuterated water was consumed over 30 to 45 min to avoid dizziness. Any subsequent water drunk by the subject was enriched to 5% with deuterium.

Two intravenous catheters were placed at 0500 on the morning of the study. One catheter was inserted into an ante-cubital vein for tracer and hormone infusion. The second catheter was inserted in a retrograde fashion into a dorsal hand vein for blood sampling. The hand was then placed in a heated box and maintained at 49°C to permit sampling of arterialized blood. A primed (12 μCi), continuous (0.12 μCi/min) infusion of [3-³H]glucose was started at 0600. Infusions of [1-¹⁴C]galactose (15 μCi prime; 0.15 μCi/min constant), somatostatin (60 ng · kg⁻¹ · min⁻¹), human growth hormone (3 ng · kg⁻¹ · min⁻¹), and glucagon (0.65 ng · kg⁻¹ · min⁻¹) were started at 0830 (time 0) and 2 g acetaminophen given by mouth. Beginning at 0830, insulin was infused at rates of either 0.25, 0.375, or 0.5 mU · kg⁻¹ · min⁻¹ for 180 min. The order of study was random, and each study was separated by at least 2 weeks. Three subjects participated in three studies, nine subjects in two studies, and three subjects in one study. Glucose concentrations were maintained at ~5.5 mmol/l by means of an exogenous glucose infusion containing [3-³H]glucose. In addition, the basal [3-³H]glucose infusion was adjusted downward beginning at time 0 in a manner mimicking the anticipated pattern of fall of EGP in an effort to further minimize the change in plasma [3-³H]glucose specific activity as previously described (3). Data from three additional subjects were excluded from analysis since an error in tracer administration resulted in a marked change in plasma glucose specific activity, precluding accurate measurement of glucose turnover.

Blood was obtained for a baseline measurement of the enrichment of deuterium on the fifth carbon of plasma glucose (C5) and plasma deuterated water enrichment (²H₂O) before the administration of deuterated water at 1730 on the day before the study. Blood was also obtained for hormones, tracer, and glucose measurement at −30, −20, −10, 0, 150, 160, 170, 180, 330, 340, 350, and 360 min. Subjects voided at time 0, and urine was obtained for measurement of urinary [¹⁴C] and [³H] glucuronide enrichment from 0 to 180 min.

Analytical methods. All blood samples were immediately placed on ice and centrifuged at 4°C, after which the plasma was removed. Plasma was then stored at −20°C until analysis. Plasma glucose was measured by a glucose oxidase method using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemilumines-

TABLE 1
Plasma C5 deuterium and plasma ²H₂O enrichments

	Basal	−30–0 min	150–180 min
Insulin infusion rates (mU · kg⁻¹ · min⁻¹)			
Plasma H₂O enrichment			
0.25	0.014 ± 0.009	0.41 ± 0.004	0.41 ± 0.004
0.375	0.022 ± 0.012	0.43 ± 0.004	0.41 ± 0.006
0.5	0.049 ± 0.014	0.44 ± 0.014	0.41 ± 0.002
C5 glucose enrichment			
0.25	0.00 ± 0.00	0.29 ± 0.004	0.16 ± 0.007
0.375	0.00 ± 0.00	0.28 ± 0.006	0.13 ± 0.004
0.5	0.00 ± 0.00	0.34 ± 0.001	0.08 ± 0.004
C2 glucose enrichment			
0.25	NA	NA	0.29 ± 0.04
0.375	NA	NA	0.19 ± 0.02
0.5	NA	NA	0.12 ± 0.02

Data are means ± SD. NA, not available.

cence method with the Access Ultrasensitive Immunoenzymatic Assay System (Beckman, Chaska, MN). C-peptide and glucagon concentrations were assayed by radioimmunoassay (Linco Research, St. Louis, MO). Growth hormone was measured with an hGH two-site assay (Beckman). Plasma [3-³H]glucose specific activity was measured after deproteinization using dual channel by liquid scintillation counting (17). Urinary [¹⁴C] and [3-³H]glucuronide specific activities were measured using high-performance liquid chromatography (HPLC) as previously described (21).

The C5 enrichment was measured using the original method of Landau et al. (18) in 3 of 27 studies and the shortened method of Schumann et al. (22) in 24 of 27 studies as described by Gastaldelli et al. (15). In brief, 50 μl of 0.197 mg/ml [6,6-²H₂]glucose was added to a 1-mg equivalent of plasma glucose as internal standard to achieve ~1% enrichment. The sample was then deproteinized using 0.3 mol/l Ba (OH)₂ and 0.33 mol/l ZnSO₄. The supernatant was then passed over columns containing AG 1-X8 and AG 50W X8 in the formate and H⁺ forms, respectively. The sample was converted to hexamethylenetetramine (HMT) using the method of Landau et al. (18) or Schumann et al. (22) both with and without the HPLC xylose purification step. The correlations between replicate standards and replicate plasma samples with and without the HPLC purification were r = 0.987 and 0.977, respectively. The analyses performed on the samples without the HPLC purification step were done in triplicate, and those data are presented in the figures and text. HMT was analyzed for isotope enrichment by gas chromatography–mass spectrometry (GC-MS; Hewlett-Packard, Avondale, PA) with selected ion monitoring under electron ionization conditions. The completeness of conversion of glucose to xylose was verified by monitoring ion 142 as well as ions 140 and 141 during the mass spectrometric analyses. In the presence of [6,6-²H₂]glucose, incomplete conversion of glucose to xylose results in a marked and easily detectable increase in the abundance of 142 m/z. This occurs due to the incorporation of carbons containing the two deuteriums derived from the sixth carbon of any residual [6,6-²H₂]glucose present during the formation of HMT from formaldehyde. As previously documented by Chandramouli et al. (23), the presence of [6,6-²H₂]glucose in plasma did not alter the 141:140 ratio when the enrichment [6,6-²H₂]glucose was <3%, as evident by the fact that the 141:140 ratio in HMT determined in three sets of plasma samples (n = 5) enriched with 0, 1, and 3% [6,6-²H₂]glucose averaged in 141:140 ratios 8.45 ± 0.12, 8.41 ± 0.10, and 8.47 ± 0.12, respectively. On each occasion, plasma C5 enrichment was measured on the evening before the study before ingestion of the ²H₂O to ensure that there was no residual labeling of C5 from a prior study, from −30 to 0 on the day of study to determine basal C5 enrichment, and from 150 to 180 min to determine enrichment during the insulin infusion. A plasma C5 enrichment <0.06 was considered undetectable and was set equal to zero. Plasma C5 enrichment was below the limits of detection in all subjects on all occasions on the evening before study (Table 1).

Previous studies after an overnight fast have shown that when at steady state, deuterium enrichment on the second carbon of glucose equals plasma ²H₂O enrichment (23–25). To determine whether this was also true during the insulin infusions in the present experiment, plasma ²H₂O and C2 glucose enrichment were measured in each subject during the final 30 min of each insulin infusion. Plasma C2 enrichment was kindly measured by V. Chandramouli as previously described (18). Plasma ²H₂O enrichment was measured using isotope ratio mass spectrometry (26) following deproteinization and dilution 1:24.

Body composition and fat-free mass were measured using dual-energy

X-ray absorptiometry (DPX-IQ scanner, SmartScan Version 4.6; Hologic, Waltham, MA).

Data analyses. All turnover rates were expressed per fat-free mass. Total glucose appearance was calculated using the steady-state equations of Steele et al. (27). Endogenous glucose production was calculated by subtracting the exogenous glucose infusion rate from the total glucose appearance determined over the same interval. In the fasting state, plasma C5 glucose enrichment is diluted by unlabeled glucose derived from EGP, whereas during the clamps plasma C5 enrichment is diluted by unlabeled glucose derived from both EGP and the exogenously infused glucose. On the other hand, plasma H_2O is not diluted by the exogenous glucose. Therefore, as previously discussed (15), the contribution of gluconeogenesis to EGP is calculated by multiplying the plasma C5:^2H_2O ratio times total glucose appearance (12,16). On the other hand, both plasma C2 and C5 glucose enrichment are diluted by infusion of exogenous glucose. Therefore, the contribution of gluconeogenesis to EGP is calculated by multiplying the plasma C5:C2 glucose ratio by EGP. The contribution of glycogenolysis to EGP was calculated by subtracting the contribution of gluconeogenesis to EGP from EGP (12,15,16).

UDP-glucose flux was calculated by dividing the infusion rate of [^{14}C]galactose by the urinary [^{14}C]glucuronide specific activity (3,20). The contribution of the direct pathway to UDP-glucose flux was calculated by dividing the urinary [^{3}H]glucuronide specific activity by the plasma [^{3}H]glucose specific activity (3,20). Means of the plasma glucose and hormone concentrations present from −30 to 0 and 150 to 180 min were used to assess responses in the basal state and during the insulin infusions, respectively. Medians over the same intervals were used for assessment of glucose turnover. Repeated measures ANOVA followed by stepwise paired t tests, when appropriate, were used to assess the response to insulin. A P value <0.05 was considered statistically significant.

RESULTS

Plasma glucose, insulin, C-peptide, glucagon, and growth hormone concentrations (Fig. 1). Plasma glucose concentrations did not differ before (5.0 ± 0.1 vs. 4.9 ± 0.3 vs. 4.9 ± 0.1) or during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions (5.4 ± 0.3 vs. 5.1 ± 0.1 vs. 5.1 ± 0.2 mmol/l). Fasting plasma insulin concentrations also did not differ on the 3 study days (21 ± 1.4 vs. 23 ± 2.9 vs. 25.5 ± 2.4 pmol/l). Plasma insulin concentrations progressively increased ($P < 0.001$) during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions to 75 ± 4, 100 ± 6, and 157 ± 8 pmol/l, respectively.

Fasting C-peptide concentrations were slightly higher on the 0.375 mU · kg^{-1} · min^{-1} than the 0.25 and 0.50 mU · kg^{-1} · min^{-1} days (0.38 ± 0.02 vs. 0.31 ± 0.02 vs. 0.31 ± 0.02 nmol/l, respectively). Somatostatin, which was started at time 0, resulted in prompt and near complete suppression of C-peptide on all 3 study days (0.03 ± 0.002 vs. 0.02 ± 0.001 vs. 0.02 ± 0.001 nmol/l). The replacement infusions of glucagon and growth hormone (data not shown) resulted in constant and comparable concentrations on the 3 study days.

Glucose infusion rates, plasma [3-^{3}H]glucose specific activity, and plasma deuterated glucose and water enrichment. The glucose infusion rates required to maintain euglycemia progressively increased ($P < 0.003$) during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions to 10.4 ± 2.5 vs. 25.4 ± 5.2 vs. 40.5 ± 6.6 μmol · kg^{-1} · min^{-1}, respectively (Fig. 2, *upper panel*). Since all infused glucose contained [3-^{3}H]glucose, the plasma glucose specific activity remained essentially constant on all occasions (Fig. 2, *lower panel*).

Although studies were separated by at least 2 weeks, as anticipated, basal (i.e., before ^2H_2O ingestion on the evening before study) plasma enrichment of ^2H_2O was slightly elevated in some subjects who had been studied previously (Table 1). However, basal plasma C5 glucose enrichment was below the limits of detection in all sub-
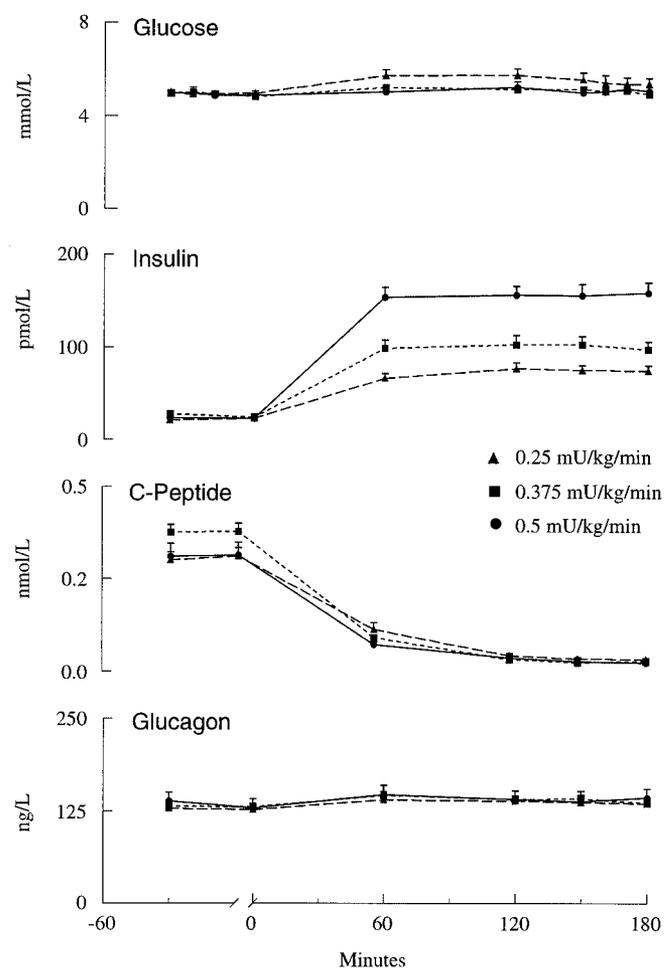
FIG. 1. Plasma glucose, insulin, C-peptide, and glucagon concentrations observed when insulin was infused at rates of 0.25 or 0.375 or 0.5 mU · kg^{-1} · min^{-1}.

jects on all occasions, enabling accurate measurement of gluconeogenesis on the day of study. Plasma deuterated water enrichment before the insulin infusions (i.e., from −30 to 0 min) did not differ from that during the insulin infusions (i.e., 150–180 min).

On the other hand, due to dilution with the exogenously infused glucose, plasma C2 enrichment progressively decreased during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions (Table 1, second column). Whereas plasma C2 enrichment was above the limits of detection in all subjects during the 0.25 and 0.375 mU · kg^{-1} · min^{-1} infusions, it was below the limits of detection in two subjects during the 0.5 mU · kg^{-1} · min^{-1} insulin infusion. The plasma C5 glucose enrichment also decreased as the insulin infusion rate increased with it being above the limits of detection in 11 of 12 subjects during the 0.25 mU · kg^{-1} · min^{-1} insulin infusion, 8 of 9 subjects during the 0.375 mU · kg^{-1} · min^{-1} insulin infusion, and 7 of 7 subjects during the 0.5 mU · kg^{-1} · min^{-1} insulin infusion.

Effect of insulin on EGP, and the contribution of gluconeogenesis and glycogenolysis to EGP. Basal rates of EGP and the contribution of gluconeogenesis or glycogenolysis to EGP did not differ on the 3 study days, averaging 18.4 ± 0.8, 12.2 ± 1.3, and 5.5 ± 1.1 μmol · kg^{-1} · min^{-1}, respectively. EGP was progressively ($P < 0.02$) suppressed during the 0.25, 0.375, and 0.5 mU · kg^{-1} ·
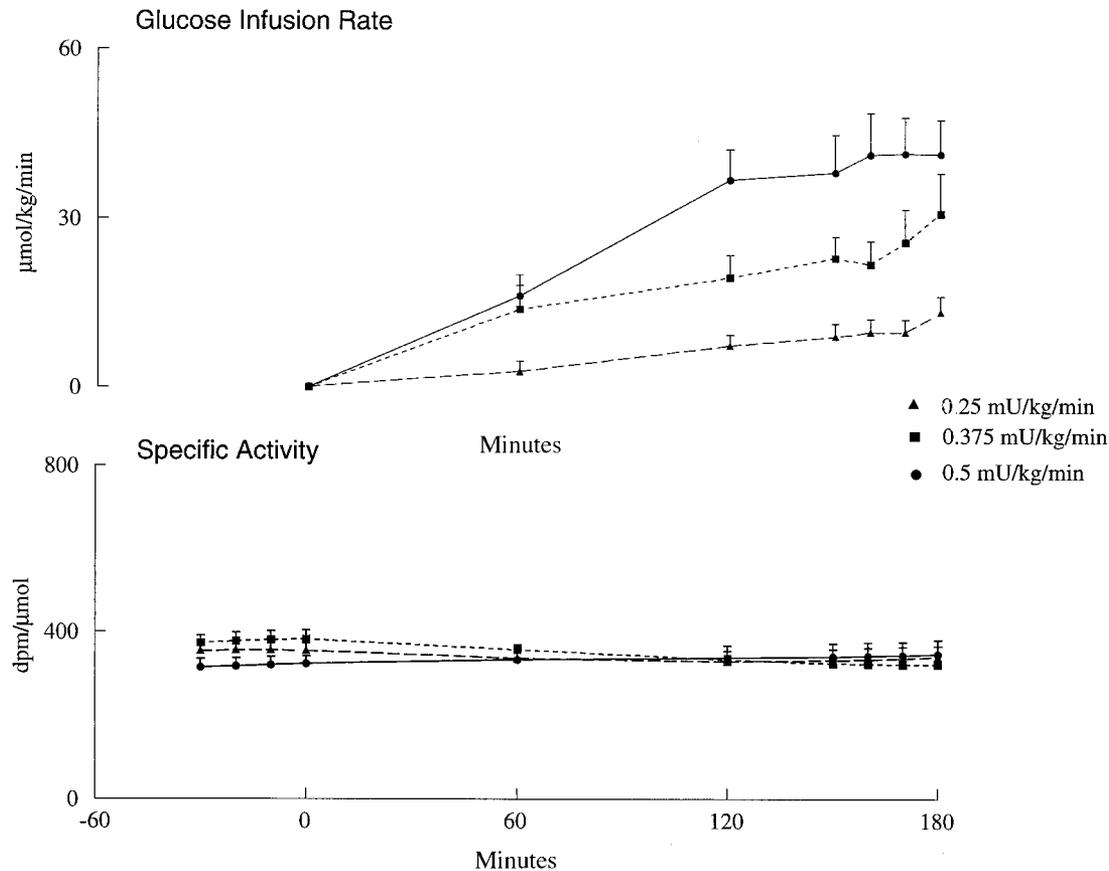
FIG. 2. The glucose infusion rates required to maintain euglycemia (*upper panel*) and the plasma [3-³H]glucose specific activity (*lower panel*) observed when insulin was infused at rates of 0.25 or 0.375 or 0.5 mU · kg^{-1} · min^{-1}.

min^{-1} insulin infusions to 13.1 ± 1.4 vs. 11.7 ± 1.0 vs. 6.4 ± 2.15 μmol · kg^{-1} · min^{-1}, respectively (Fig. 3, *upper panel*). The decrease in EGP was entirely attributable to progressive suppression ($P < 0.05$) of the contribution of glycogenolysis to EGP (4.9 ± 1.9 vs. 3.4 ± 1.2 vs. -2.1 ± 1.3 μmol · kg^{-1} · min^{-1}). On the other hand, whereas the contribution of gluconeogenesis to EGP was lower ($P < 0.002$) during the 0.25 mU · kg^{-1} · min^{-1} insulin infusion than during the basal state, rates of gluconeogenesis did not differ during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions (8.2 ± 1.0 vs. 8.3 ± 1.1 vs. 8.5 ± 1.3 μmol · kg^{-1} · min^{-1}).

The contribution of gluconeogenesis to EGP during the insulin infusions was also calculated using the plasma C5:C2 glucose ratio. Data from the two subjects in whom the plasma C2 glucose enrichment was undetectable (observed during the 0.5 mU · kg^{-1} · min^{-1} insulin infusion) were excluded from analysis since the resultant ratio was infinite. Although the absolute gluconeogenic rates were somewhat lower, the conclusions were the same. The contribution of gluconeogenesis to EGP did not differ during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions (7.2 ± 1.0 vs. 7.0 ± 1.0 vs. 7.9 ± 2.2 μmol · kg^{-1} · min^{-1}) with the decrease in EGP being accounted for by a decrease ($P < 0.05$) in glycogenolysis. Of note, when C2 was detectable, gluconeogenic rates calculated with the plasma C5:^{2}H$_2$O ratio were highly correlated with those calculated with the plasma C5:C2 ratio (Fig. 4). The correlation between the two methods was significant when all insulin infusion rates were considered as a single group

($r = 0.88$; $P < 0.001$) or when calculated separately for the 0.25 mU · kg^{-1} · min^{-1} ($r = 0.94$; $P < 0.01$), 0.375 mU · kg^{-1} · min^{-1} ($r = 0.86$; $P < 0.01$), or 0.5 mU · kg^{-1} · min^{-1} ($r = 0.95$; $P < 0.01$) insulin infusions.

UDP-flux and flux via the direct and indirect pathways. Total UDP-glucose flux (5.0 ± 0.4 vs. 5.0 ± 0.3 vs. 4.0 ± 0.5 μmol · kg^{-1} · min^{-1}) and UDP-flux via the indirect (intracellular) pathway (4.4 ± 0.3 vs. 4.1 ± 0.4 vs. 3.8 ± 0.5 μmol · kg^{-1} · min^{-1}) did not differ during the 0.25, 0.375, and 0.5 mU · kg^{-1} · min^{-1} insulin infusions (Fig. 3, *bottom panel*). UDP-flux via the direct (extracellular) pathway was minimal during all three insulin infusions (0.6 ± 0.2 vs. 0.9 ± 0.4 vs. 0.1 ± 0.1 μmol · kg^{-1} · min^{-1}, respectively).

DISCUSSION

In the presence of euglycemia, an increase in insulin concentration from ~75 to ~150 pmol/l for 3 h resulted in progressively greater suppression of EGP. The contribution of glycogenolysis to EGP concomitantly decreased, whereas the contribution of gluconeogenesis to EGP remained essentially unchanged, as did both hepatic UDP-glucose flux and flux via the indirect (intracellular) pathway. Flux through the direct (extracellular) pathway was minimal and remained unchanged during all of the insulin infusions. These data indicate that in the presence of euglycemia, a small increase in insulin suppresses EGP by inhibiting glycogenolysis without altering flux into glycogen. They also indicate that suppression of glucone-
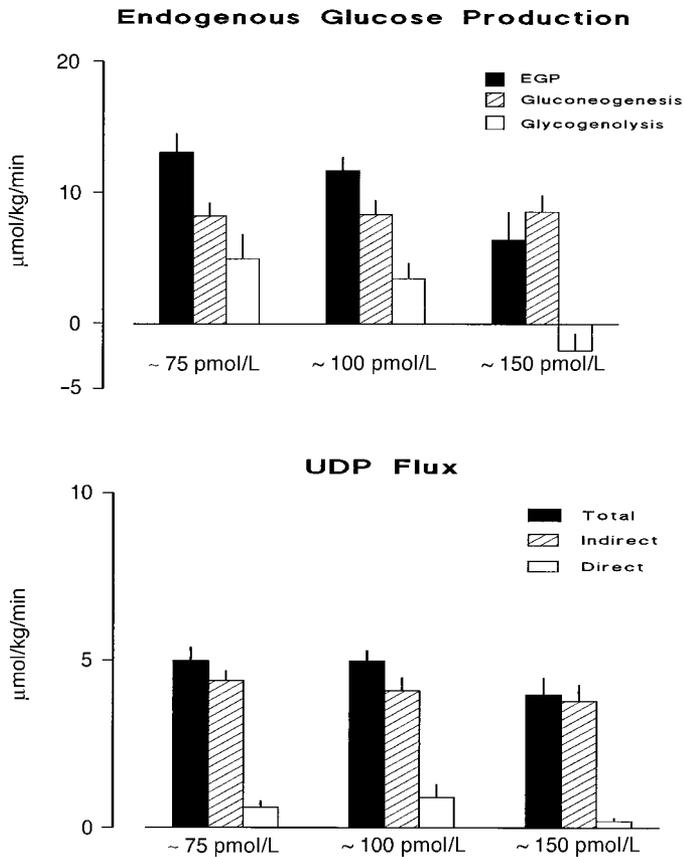
Endogenous Glucose Production

UDP Flux

FIG. 3. Rates of EGP, gluconeogenesis, and glycogenolysis observed during 0.25, 0.375, or 0.5 mU · kg^{-1} · min^{-1} insulin infusions are shown in the upper panel. Total hepatic UDP-glucose flux and the contributions of the direct and indirect pathways to UDP-glucose flux observed during 0.25, 0.375, or 0.5 mU · kg^{-1} · min^{-1} insulin infusions are shown in the lower panel.

ogenesis in humans requires higher insulin concentrations than suppression of glycogenolysis. The net effect is that in healthy nondiabetic humans, an increase in insulin concentrations within the physiologic range suppresses glycogenolysis and directs glucose-6-phosphate into hepatic glycogen.

The results of the present experiments in humans are remarkably similar to those previously reported by Chiasson et al. (13) in dogs. Insulin concentrations of ~600 pmol/l resulted in essentially complete suppression of EGP but had a minimal effect on the rate of incorporation of [^{14}C]alanine into hepatic glycogen. Although the intrahepatic [^{14}C]gluconeogenic precursor enrichment was not measured, those data strongly suggested that insulin suppressed EGP but not flux through the gluconeogenic pathway. Similar results were subsequently observed in three healthy men using a similar experimental design (14). More recently, Edgerton et al. demonstrated that an increase in hepatic sinusoidal insulin concentrations to ~20 µU/ml decreased EGP but did not alter gluconeogenesis simultaneously measured using the arterial venous difference, phosphoenolpyruvate, and deuterated water methods (12). The absence of a decrease in the contribution of gluconeogenesis to EGP or UDP-glucose flux observed in the present studies during the insulin infusions despite progressive suppression of EGP indicates that higher insulin concentrations are also required in humans to suppress gluconeogenesis than are required to suppress glycogenolysis.

Several processes in addition to gluconeogenesis contribute to UDP-glucose flux, including uptake of extracellular glucose and cycling of glucose-1-phosphate derived from glycogenolysis back into glycogen (19,20,28,29). UDP-glucose flux could have remained unchanged if at each insulin concentration, an increase in either one or both of these processes was sufficient to offset any decrease in rate of formation of glucose-6-phosphate via gluconeogenesis. Uptake of extracellular glucose, measured as the contribution of the direct pathway to UDP-glucose flux, was minimal and constant at all insulin concentrations, consistent with prior data indicating that hyperglycemia rather than hyperinsulinemia is the primary determinant of hepatic glucose uptake (9,30). Hepatic

Relationship Between Gluconeogenesis determined with C2 Glucose and ^{2}H$_2$O
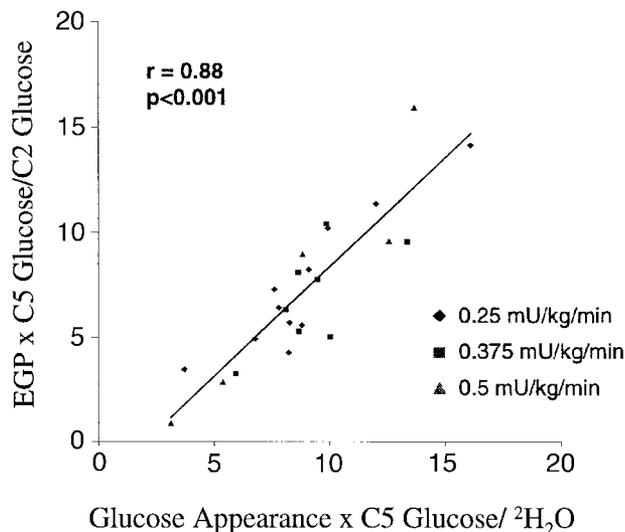

FIG. 4. Correlation between gluconeogenesis calculated by multiplying the plasma C5 glucose–to–C2 glucose ratio times EGP and gluconeogenesis calculated by multiplying the plasma C5 glucose–to–^{2}H$_2$O ratio times total glucose appearance.

glycogen cycling could not be measured in the present experiments. Petersen et al. (9), using ^{13}C nuclear magnetic resonance spectroscopy, have recently reported that hyperinsulinemia in the presence of euglycemia increases hepatic glycogen cycling. It is therefore possible that glycogen cycling progressively increased as insulin concentrations increased, directing glucose-1-phosphate derived from glycogen back into glycogen, thereby maintaining UDP-glucose flux constant. This would require that glycogenolysis progressively increases as insulin concentrations increase. Although limited, available data indicate that insulin inhibits rather than stimulates glycogenolysis, making this scenario unlikely (1,7,8,31).

The relative contributions of gluconeogenesis and glycogenolysis to EGP were measured using the deuterated water method (18). Gluconeogenesis labels the fifth carbon of glucose with deuterium. On the other hand, glycogenolysis, gluconeogenesis, and phosphorylation of extracellular glucose all label the second carbon of glucose with deuterium. Therefore, the plasma C5:C2 ratio provides an index of the percent of plasma glucose derived from gluconeogenesis. Previous studies have shown that plasma ^2H_2O and C2 glucose enrichment are equal after an overnight fast, indicating equilibration of plasma ^2H_2O with the intra-hepatic glucose-6-phosphate pool (23). This enables the more easily measured plasma ^2H_2O to be used in place of the more difficult to measure plasma C2 glucose enrichment. The present data indicate that a similar equilibration appears to occur during infusion of small amounts of exogenous glucose used in the current study. The contribution of gluconeogenesis to EGP calculated using the C5:^2H_2O ratio was highly correlated with that calculated using the plasma C5:C2 ratio measured in the same individuals. Perhaps more importantly, both methods indicated that low doses of insulin suppressed EGP without altering the contribution of gluconeogenesis to EGP.

Infusion of glucose dilutes both the plasma C5 and C2 glucose enrichment. In the present experiments, the plasma C5 and C2 glucose enrichment fell below the limit of detection in some individuals, particularly at the highest insulin infusion rate (i.e., 0.5 mU · kg^{-1} · min^{-1}). While it is theoretically possible that plasma C5 enrichment can decrease to zero due to complete inhibition of gluconeogenesis, plasma should continue to be enriched in C2 glucose, albeit at low levels, as long as EGP persists. However, the limited sensitivity of existing methods makes accurate measurement of low levels of enrichment of C2 glucose difficult, thereby introducing additional variability into the calculation of gluconeogenesis. In contrast, the plasma ^2H_2O enrichment remains essentially constant during exogenous glucose infusion and can be readily measured. Under these circumstances, accurate measurement of C5 glucose enrichment becomes the limiting factor. Taken together, the present data indicate that the plasma C5–to–C2 glucose ratio and the plasma C5 glucose–to–^2H_2O ratio provide an equivalent assessment of gluconeogenesis during low glucose infusion rates when steady state is approached and when plasma C5 glucose, C2 glucose, and plasma ^2H_2O can all be accurately measured. Whether this also occurs during higher glucose infusion rates is currently unknown.

The deuterated water method measures the glucose C5:^2H_2O ratio in plasma. Therefore, it only assesses the percent of plasma glucose derived from gluconeogenesis rather than the actual rate of glucose-6-phosphate formation from gluconeogenesis (18). The fact that the contribution of glycogenolysis to EGP decreased in parallel with EGP itself during the insulin infusions, whereas the contribution of gluconeogenesis to EGP and UDP-glucose flux remained unchanged, indicates that glycogenolysis is more sensitive to insulin than gluconeogenesis. This conclusion is consistent with the recent report by Gastaldelli et al. (15) that the contribution of gluconeogenesis to EGP, measured after 2.5 h of insulin concentrations of ~700 pmol/l in obese nondiabetic volunteers, averaged 4.5 ± 1 μmol · kg^{-1} · min^{-1}, whereas EGP averaged 3.6 ± 0.5 μmol · kg^{-1} · min^{-1}, indicating complete suppression of glycogenolysis despite ongoing gluconeogenesis. Similarly, Boden et al. (16) reported that contribution of gluconeogenesis to EGP and EGP after 4 h of insulin concentrations of ~450 pmol/l averaged 3.7 ± 1.4 and 2.0 ± 2.5 μmol · kg^{-1} · min^{-1}, respectively. Since the present study as well as these previous studies measured the enrichment of deuterium in the fifth carbon of plasma glucose, none can exclude the possibility that a portion of the C5-enriched glucose first passed through glycogen. If so, C5-enriched glucose released from glycogenolysis would be considered gluconeogenesis (12,15,18). However, if this process were making a substantial contribution to EGP, progressive suppression of glycogenolysis presumably would have resulted in progressively less release of C5 glucose from glycogen, leading to an apparent decrease in the contribution of gluconeogenesis to EGP. This did not occur. On the other hand, since the C5 enrichment of glycogen is not known, estimates of the contribution of glycogenolysis and gluconeogenesis to EGP should be considered qualitative rather than quantitative. Nevertheless, taken together with the unchanged rates of UDP-glucose flux, the present data provide strong evidence that, consistent with previous reports in animals (12,13), lower insulin concentrations are required to suppress glycogenolysis in humans than are required to suppress gluconeogenesis.

Suppression of EGP in the absence of a decrease in either UDP-glucose flux or the contribution of gluconeogenesis to EGP could have been due to several factors. It is likely that insulin, by stimulating glycogen synthetase (i.e., a "pull" mechanism), decreased intra-hepatic glucose-6-phosphate concentration (2,32,33). This decrease, alone or in combination with a decrease in glucose-6-phosphatase activity (33), could have caused a decrease in EGP. Gluconeogenesis continued unchanged with the same amounts being partitioned between glycogen and EGP. Therefore, UDP-flux and the contribution of gluconeogenesis to EGP also remained unchanged. Of note, glucagon concentrations were clamped at constant levels in all experiments in order to assess the effect of insulin in the absence of a concurrent change in glucagon. Since glucagon can increase gluconeogenesis and glucose-6-phosphatase activity (34,35), it is likely that an even greater effect of insulin on EGP may have been observed if glucagon concentrations had been permitted to fall as insulin concentrations increased.

Of interest, while the contribution of gluconeogenesis to

EGP did not decrease as insulin was increased from ~75 to 150 pmol/l, rates were lower than those present in the basal state when insulin concentrations averaged ~25 pmol/l. While it is possible that a small increase in insulin concentration (i.e., from 25 to 75 pmol/l) maximally suppressed gluconeogenesis, this is unlikely since both Gastaldelli et al. (15) and Boden et al. (16) have shown that gluconeogenesis is suppressed by higher doses of insulin. It is more likely that the decrease in gluconeogenesis that was evident at the lowest insulin infusion was due to a fall in portal glucagon concentrations (34). Portal venous glucagon concentrations are generally ~40% higher than peripheral concentrations (36,37). Infusion of somatostatin abolishes the normal portal venous-peripheral gradient by inhibiting endogenous secretion. Therefore comparable plasma glucagon concentrations before and during the somatostatin and "replacement" glucagon infusion indicate a fall in portal venous glucagon concentrations. It is interesting to speculate that in the presence of constant basal insulin concentrations, small changes in portal glucagon concentrations have a greater effect on the contribution of gluconeogenesis to EGP than small changes in insulin in the presence of constant basal glucagon concentrations. Since insulin normally suppresses glucagon secretion (38), a proportionately larger increase in the portal insulin-to-glucagon ratio may enhance the ability of insulin to modulate gluconeogenesis under the conditions of daily living. Further study of the effects of changes in glucagon in the presence and absence of concomitant changes in insulin will be of interest in this regard.

The present studies suffer from certain limitations. Use of the deuterated water method to measure the contribution of gluconeogenesis to EGP requires the presence of steady state (12,15,18). Each dose of insulin was infused for 3 h in an effort to approximate steady state. However, it is possible that gluconeogenesis was inhibited, but the plasma C5 glucose enrichment was still falling at the time of measurement. If so, this presumably would have accelerated the apparent suppression of the contribution of gluconeogenesis to EGP as insulin concentrations increased due to more rapid turnover of the C5-enriched glucose in the glucose pool. This was not the case since the calculated contribution of gluconeogenesis to EGP did not differ during the 0.25, 0.375 and 0.5 mU · kg^{-1} · min^{-1} insulin infusions.

Urine for measurement of UDP-glucose flux was collected from 0 to 180 min. Therefore, the calculated UDP-glucose flux rates represent an average of that entire period. Since the time for the hepatic [^{14}C]glucuronide pool to reach equilibrium is finite but unknown, it is possible that glucuronide enrichment during the initial portion of an insulin infusion was lower than that during the latter portion of the infusion. This would artificially decrease urinary glucuronide specific activity and thereby increase apparent UDP-glucose flux. Therefore, the UDP-glucose flux rates should be considered relative rather than absolute. Finally, we have not established the full insulin dose response curve for glycogenolysis, gluconeogenesis, or UDP-glucose flux since insulin concentrations <75 pmol/l and >150 pmol/l were not examined. It is likely that, in the absence of hyperglycemia, very high insulin concentrations by suppressing gluconeogenesis, will eventually reduce hepatic glycogen synthesis (13). However, the applicability of such extreme elevation of insulin to the conditions of daily living remains uncertain.

In summary, the present studies show that in the presence of euglycemia and constant glucagon concentrations, increasing plasma insulin from ~75 to ~150 pmol/l (i.e., from ~12.5 to 25 μU/ml) resulted in progressive suppression of both EGP and the contribution of glycogenolysis to EGP. On the other hand, these concentrations of insulin did not decrease either UDP-glucose flux or the contribution of gluconeogenesis to EGP. These data indicate that in healthy nondiabetic humans, higher concentrations of insulin are required to suppress gluconeogenesis than are required to suppress glycogenolysis. They also strongly suggest that in the presence of euglycemia, physiologic insulin concentrations decrease divert glucose-6-phosphate derived from gluconeogenesis into glycogen, thereby maintaining near constant rates of hepatic glycogen synthesis. The extent to which alterations in these actions of insulin contribute to the pathogenesis of disease states such as type 2 diabetes remains to be determined.

ACKNOWLEDGMENTS

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