Insulin regulation of renal glucose metabolism in humans

EUGENIO CERSOSIMO, PETER GARLICK, AND JOHN FERRETTI

Departments of Medicine, Surgery and Radiology, State University of New York at Stony Brook, Stony Brook, New York 11794

Cersosimo, Eugenio, Peter Garlick, and John Ferretti. Insulin regulation of renal glucose metabolism in humans. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E78–E84, 1999.—Eighteen healthy subjects had arterialized hand and renal veins catheterized after an overnight fast. Systemic and renal glucose and glycerol kinetics were measured with [6,6-2H2]glucose and [2-13C]glycerol before and after 180-min peripheral infusions of insulin at 0.125 (LO) or 0.25 (HI) mU·kg⁻¹·min⁻¹ with variable [6,6-2H2]dextrose or saline (control). Renal plasma flow was determined by plasma p-aminohippurate clearance. Arterial insulin increased from 37 ± 8 to 53 ± 5 (LO) and to 102 ± 10 pm (HI; P < 0.01) but not in control (35 ± 6 pm). Arterial glucose did not change and averaged 5.2 ± 0.1 (control), 4.7 ± 0.2 (LO), and 5.1 ± 0.2 (HI) µmol/ml; renal vein glucose decreased from 4.8 ± 0.2 to 4.5 ± 0.2 µmol/ml (LO) and from 5.3 ± 0.2 to 4.9 ± 0.1 µmol/ml (HI) with insulin but not saline infusion (5.3 ± 0.1 µmol/ml).

Endogenous glucose production decreased from 9.9 ± 0.7 to 6.9 ± 0.5 (LO) and to 5.7 ± 0.5 (HI) µmol·kg⁻¹·min⁻¹; renal glucose production decreased from 2.5 ± 0.6 to 1.5 ± 0.5 (LO) and to 1.2 ± 0.6 (HI) µmol·kg⁻¹·min⁻¹, whereas renal glucose utilization increased from 1.5 ± 0.6 to 2.6 ± 0.7 (LO) and to 2.9 ± 0.7 (HI) µmol·kg⁻¹·min⁻¹ after insulin infusion (all P < 0.05 vs. baseline). Neither endogenous glucose production (10.0 ± 0.4), renal gluconeogenesis (1.1 ± 0.4), nor renal glucose utilization (0.8 ± 0.4) changed in the control group. During insulin infusion, systemic gluconeogenesis from glycerol decreased from 0.67 ± 0.05 to 0.18 ± 0.02 (LO) and from 0.60 ± 0.04 to 0.20 ± 0.02 (HI) µmol·kg⁻¹·min⁻¹ (P < 0.01), and renal gluconeogenesis from glycerol decreased from 0.10 ± 0.02 to 0.02 ± 0.02 (LO) and from 0.15 ± 0.03 to 0.09 ± 0.03 (HI) µmol·kg⁻¹·min⁻¹ (P < 0.05). In contrast, during saline infusion, systemic (0.66 ± 0.03 vs. 0.82 ± 0.05 µmol·kg⁻¹·min⁻¹) and renal gluconeogenesis from glycerol (0.11 ± 0.02 vs. 0.41 ± 0.04 µmol·kg⁻¹·min⁻¹) increased (P < 0.05 vs. baseline). We conclude that glucose production and utilization by the kidney are important insulin-responsive components of glucose metabolism in humans.

The role of the kidney in glucose metabolism is thought to be minor under most circumstances. Cahill and coworkers (11, 12) demonstrated that net renal glucose output was negligible in the postabsorptive state, but it increased substantially with prolonged fasting, contributing nearly one-half of daily systemic glucose appearance after 7–10 wk of fasting in humans. More recently, however, although studies in postabsorptive dogs (13) and in humans (39) have confirmed that net glucose output is minimal, partition of glucose production and utilization across the kidney indicates that renal glucose production equals glucose utilization and accounts for ~15–25% of endogenous glucose production. These recent in vivo experiments are in agreement with the original observations under fasting conditions (22, 32) and further suggest that glucose released by the kidney in the postabsorptive state appears to reflect renal gluconeogenesis, primarily from lactate (16), glycerol (13), and circulating amino acids (38). They are also consistent with the abundant in vitro evidence indicating that the kidney is capable of simultaneous glucose production and utilization. Several years ago, Krebs et al. (28) demonstrated that the biochemical machinery is in place in cells of the proximal convoluted tubule to efficiently convert 3-carbon precursors to glucose. At the same time, cells of the distal nephron and those in the interstitial medulla are very active in glucose uptake and oxidation (43, 44). The mechanisms that regulate glucose production and utilization by the kidney, however, are presently unknown.

Most in vitro experiments have led us to believe that renal glucose production is hormone insensitive and regulated primarily by substrate availability (44). In contrast, recent studies in dogs demonstrate that physiological hyperinsulinemia can simultaneously suppress glucose production and stimulate glucose utilization by the kidney (13). Insulin-induced hypoglycemia is associated with a twofold increase in renal glucose production that cannot be abolished by normalization of renal plasma glucose (15). These observations strongly suggest that glucose production by the kidney, analogous to the liver, is inhibited by insulin and stimulated by counterregulatory hormones, particularly catecholamines. In support, epinephrine infusion in postabsorptive humans has recently been shown to enhance renal glucose production (39). The regulatory role of insulin on renal glucose metabolism in humans, however, has not yet been investigated. If, similar to our observations in dogs (13), renal glucose production and utilization in humans are divergently regulated, these processes would be of potential significance. The present studies were therefore undertaken to determine whether renal glucose production and utilization are responsive to physiological hyperinsulinemia in postabsorptive humans, using arteriovenous balance measurements combined with a tracer technique.

MATERIALS AND METHODS

Subjects. Informed written consent was obtained from 18 healthy volunteers after the protocol had been approved by our local institutional review board. All subjects (Table 1) had normal fasting glucose and no personal or family history of diabetes, hypertension, or renal disease. For 3 days before the study, all had been on a weight-maintaining diet containing ≥200 g of carbohydrate and had abstained from alcohol.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. Characteristics of eighteen subjects studied in postabsorptive state and after peripheral insulin infusion of either saline (control group) or insulin.

<table>
<thead>
<tr>
<th></th>
<th>Saline (n = 4)</th>
<th>Low Insulin (n = 6)</th>
<th>High Insulin (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr (range)</td>
<td>30 ± 5 (21–44)</td>
<td>36 ± 4 (23–46)</td>
<td>33 ± 3 (27–42)</td>
</tr>
<tr>
<td>Gender</td>
<td>2M/2F</td>
<td>4M/2F</td>
<td>4M/4F</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71 ± 6</td>
<td>78 ± 7</td>
<td>81 ± 4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23 ± 1</td>
<td>24 ± 1</td>
<td>26 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Insulin was infused at rate of 0.125 (low insulin) or 0.250 (high insulin) mU·kg⁻¹·min⁻¹. M, male; F, female; BMI, body mass index.

Protocol. Subjects were admitted to the University Hospital General Clinical Research Center at State University of New York at Stony Brook after an overnight fast between 6:00 PM and 7:00 AM in the morning of the experiments. An antecubital vein was cannulated, and a primed continuous infusion of [6,6-²H₆]glucose (24 µmol/kg, 0.24 µmol·kg⁻¹·min⁻¹; Cambridge Isotope Laboratories, Andover, MA) and [2-¹³C]glycerol (45 µmol/kg, 0.45 µmol·kg⁻¹·min⁻¹; Cambridge Isotope Laboratories) and a continuous infusion of p-aminohippurate (12 mg/min; Merck, West Point, PA) were started. Tracer infusion rates were chosen and changed accordingly during each experiment to produce steady-state arterial and renal vein plasma glucose and glyceral enrichment levels to permit detection of a difference as low as 5% across the kidney (13, 15). Subsequently, a dorsal hand vein was cannulated retrogradely and kept in a thermoregulated Plexiglas box at 65°C for sampling arterialized venous blood (14). During the 150-min equilibration period, subjects had left (n = 16) or right (n = 2) renal vein catheterized through the right femoral vein under fluoroscopy, and the position of the catheter tip was ascertained by injecting a small amount of iodinated contrast material. The catheter was then continuously infused with a heparinized saline solution (4.0 U/min) to maintain patency.

During the baseline period (0 to 30 min), three consecutive blood samples were collected simultaneously from the dorsal hand vein and the renal vein at 15-min intervals for the determination of p-aminohippurate, insulin, free fatty acid, glucose, and glycerol concentrations and plasma glucose and glycerol enrichments. At 0 min, on completion of baseline collections, subjects were randomized to receive a 180-min continuous peripheral infusion of insulin at the rate of either 0.125 (n = 6) or 0.250 (n = 8) mU·kg⁻¹·min⁻¹ with a concomitant variable infusion of [6,6-²H₆]dextrose (2% excess, dextrose in water, 10% solution) to maintain plasma glucose concentration and enrichment constant, assuming a reduction in endogenous glucose production between 25 and 50% (10, 23). These insulin infusion rates were selected to produce elevations in plasma insulin concentration comparable to low and high postprandial hyperinsulinemia in humans and to reduce presumed hepatic glucose production by ~50% on the basis of previous publications by Katz et al. (27). Four additional individuals were infused with normal saline (0.9% NaCl) at the rate of 50 ml/h throughout the entire 180-min experimental period, and these individuals represent the control group. Blood samples were collected from the dorsal hand and renal veins at 30-min intervals from 0 to 150 min and at 15-min intervals from 150 to 180 min.

Analytical techniques. Plasma glucose at the bedside was measured with the Beckman II glucose analyzer (Fullerton, CA); p-aminohippurate concentration was determined by a calorimetric method (9), and insulin was determined by radioimmunoassay (24). Plasma free fatty acid concentration was determined by the colorimetric method described by Bergman et al. (6) with a commercially available kit (Wako, Osaka, Japan). Plasma concentration and enrichment of [²H₅]glucose, [¹³C₁]glycerol, and [¹³C₁]glycerol were measured by gas chromatography-mass spectrometry. In brief, 150 µl of plasma were added to 150 µl of glucose internal standard solution (5 mmol/l [U-¹³C]glucose). Samples were deproteinized with acetonitrile and evaporated to dryness. Derivatization was carried out with butane boronic acid in pyridine and acetic anhydride (40). The glucose derivative was quantified by selective ion monitoring at mass-to-charge ratios (m/z) 297, 298, 299, and 303 for natural [¹²C₁]-, [¹³C₁]-, [²H₅]-, and [U-¹³C]glucose, respectively. Two sets of standards were measured containing known amounts of [²H₅]- and [¹³C₁]-glucose. Isotope enrichments were calculated by multiple linear regression (41). A set of standards containing 0 to 10 mmol of glucose and 5 mmol of [U-¹³C]glucose internal standard was used to calculate plasma concentration of glucose. To determine plasma glycerol enrichment and concentration, 100 µl of plasma were added to 100 µl of glycerol internal standard solution (60 µmol [²H₅]glycerol). After samples were deproteinized and dried, derivatization was carried out with acetic anhydride (7), and the glycerol derivative was quantified in the positive ion chemical ionization mode with methane as the reagent gas. Separation was achieved with selective ion monitoring at m/z 159, 160, and 164 for natural [¹²C₁]-, [¹³C₁]-, and [²H₅]-glycerol, respectively. Isotope enrichments were calculated by multiple linear regression (41). A set of standards containing 0 to 350 µmol/l of glycerol and 60 µmol/l of [²H₅]-glycerol internal standard was used to calculate plasma concentration of glycerol.

Calculations. Renal plasma flow was calculated by p-aminohippurate clearance with the equation

\[ RPF = \frac{INF \times [PAH]_a - [PAH]_v}{INF} \]  

where RPF is renal plasma flow in milliliters per minute, INF is p-aminohippurate infusion rate in milligrams per minute, [PAH] is plasma p-aminohippurate concentrations in milligrams per minute, subscript v is renal vein, and subscript a is artery. Whole body glucose rate of appearance (Ra) was calculated with the steady-state formula

\[ R_a = \frac{INF \times [H_2] \times PE_a}{[H_2] \times PE} \]  

where INF is the rate of [6,6-²H₆]glucose infusion in micromoles per kilogram per minute, and [²H₅]PE is the percentage of the arterial plasma glucose enriched with [²H₅]glucose. Before the experimental period (0–180 min), INF represents the time-averaging rate of infusion of [6,6-²H₆]dextrose in micromoles per kilogram per minute at each time point, according to the "Hot-GINF" method (23). Underestimation of Rₐ of unlabeled glucose in the systemic circulation related to deficiencies in the monocompartmental equations was minimized by maintenance of isotopic steady state during the entire experiment (see RESULTS). Endogenous glucose production (EGP) rate was calculated by subtracting the rate of exogenous dextrose infusion from Rₐ in Eq. 2. Systemic glycerol Rₐ was calculated by an equation similar to Eq. 2, except that the infusion rate of [²H₅]glycerol was divided by arterial plasma enrichment of [¹³C₁]glycerol. Net renal glucose balance was calculated by the product of the arteriovenous glucose concentration difference and renal plasma flow. Renal fractional extraction of glucose (FEg) was calculated with the following formula

\[ FE_g = \frac{(Glc_a \times PE_a - Glc_v \times PE_v) / (Glc_a \times PE_a)}{1 - (Glc_v \times PE_v) / (Glc_a \times PE_a)} \]
where \([\text{Glc}]\) is plasma glucose concentration, and \(\text{PE}\) refers to the \([\text{H}_2]\)glucose plasma enrichment. Renal glucose uptake (RGU) was calculated with the following formula

\[
\text{RGU} = \text{FE}_g \times [\text{Glc}]_a \times \text{RPF}
\]  

(Because glycosuria was not present, renal glucose utilization was assumed to be equal to glucose uptake. Renal glucose production (RGP) was calculated with the following formula)

\[
\text{RGP} = \text{RGU} + ( [\text{Glc}]_v - [\text{Glc}]_a ) \times \text{RPF}
\]  

Because glucose is extracted into whole blood and there is rapid equilibration between red cell and plasma glucose concentration, Eqs. 4 and 5 will underestimate renal glucose production and utilization. The percentage of systemic glucose production derived from glycerol was calculated by the formula

\[
\%\text{Glc} \text{Ra from glycerol} = \frac{( [\text{Glc}]_a - [\text{Glc}]_v )}{[\text{Glc}]_a} \times 100
\]

where \([\text{Glc}]_v\) and \([\text{Glc}]_a\) represent arterial plasma enrichment of \([\text{13C}]\)glucose and \([\text{13C}]\)glycerol, respectively. This is a standard product-precursor calculation that takes into account the fact that 2 moles of glycerol are required to produce 1 mole of glucose. The rate of systemic glyceral-derived gluconeogenesis (GNGk) was obtained from the following formula

\[
\text{GNGk} = \%\text{Glc} \text{Ra from glycerol} \times \text{Glc Ra} \times \text{RPF}
\]

The numerator in the formula represents the renal vein plasma \([\text{13C}]\)glucose enrichment in excess of that anticipated from the known arterial plasma \([\text{13C}]\)glucose enrichment and the fractional extraction of \([\text{H}_2]\)glucose, i.e., that \([\text{13C}]\)glucose that has been newly generated in the kidney. The denominator in the formula is the precursor pool (glycerol) percent enrichment, again taking into account the fact that 2 moles of glycerol are required to generate 1 mole of glucose. This formula will underestimate actual renal gluconeogenesis from glycerol to the extent that the kidney metabolizes newly synthesized \([\text{13C}]\)glucose. The use of arteriovenous balance measurements and tracer techniques in the above equations to assess renal glucose and glycerol kinetics has been previously validated in animals (13, 15).

Statistics. All values obtained in each study in the baseline and study periods were used in the calculations and are expressed as means ± SE. Mean data at baseline in each study group were compared by one-way ANOVA. Results obtained during the experimental periods were compared among groups with repeated-measures two-way ANOVA. All P values <0.05 were considered statistically significant (36).

Results

Arterial plasma insulin concentration increased from \(37 ± 8\) to an average of \(53 ± 5\) and \(102 ± 10\) pmol/l (\(P < 0.01\)) after insulin infusion at the rates of \(0.125\) and \(0.250\) mU·kg\(^{-1}\)·min\(^{-1}\), respectively, but it did not change in the saline control group (\(30 ± 7\) vs. \(35 ± 8\) pmol/l). Renal plasma flow was \(10.0 ± 1.6\) and \(10.8 ± 1.5\) ml·kg\(^{-1}\)·min\(^{-1}\) at baseline and remained constant during the study period, \(10.6 ± 1.7\) and \(11.3 ± 1.7\) ml·kg\(^{-1}\)·min\(^{-1}\) (\(P =\) nonsignificant), in the low and high insulin infusion groups, respectively; renal plasma flow did not change during saline infusion (\(9.40 ± 1.2\) vs. \(9.10 ± 1.4\) ml·kg\(^{-1}\)·min\(^{-1}\)). Figure 1 shows plasma glucose concentration during baseline and 180-min experimental periods. Arterial plasma glucose concentration averaged \(5.23 ± 0.10\) and \(5.22 ± 0.05\) µmol/ml in the saline control group, \(4.70 ± 0.10\) and \(4.50 ± 0.20\) µmol/ml in the low insulin infusion group, and \(5.20 ± 0.20\) and \(5.05 ± 0.15\) µmol/ml in the high insulin infusion group, respectively, during baseline and in the final 30 min of the infusion period. Renal vein glucose concentration did not change during saline infusion (\(5.25 ± 0.10\) vs. \(5.27 ± 0.10\) µmol/ml) but decreased from \(4.80 ± 0.20\) at baseline to \(4.52 ± 0.20\) µmol/ml (\(P < 0.05\) vs. baseline) in the last 30 min of the study period in the low insulin group and from \(5.27 ± 0.23\) to \(4.90 ± 0.12\) µmol/ml (\(P < 0.05\) vs. baseline) in the high insulin group. As a consequence, although net glucose balance did not change significantly during saline infusion (\(-0.19 ± 0.10\) vs. \(-0.46 ± 0.30\) µmol·kg\(^{-1}\)·min\(^{-1}\)), it changed from a net output of \(-1.00 ± 0.40\) to net uptake of \(1.06 ± 0.50\) µmol·kg\(^{-1}\)·min\(^{-1}\) (\(P < 0.05\) vs. baseline) after low insulin infusion and from a net output of \(-0.74 ± 0.15\) to net uptake of \(1.70 ± 0.20\) µmol·kg\(^{-1}\)·min\(^{-1}\) (\(P < 0.05\) vs. baseline) after high insulin infusion.

Fig. 1. Plasma glucose concentration in µmol/ml in artery (solid symbols) and in renal vein (open symbols) during baseline (30 to 0 min) and study (0–180 min) periods, after either saline (upper) or insulin infusion at the rates of 0.125 mU·kg\(^{-1}\)·min\(^{-1}\) (middle) and 0.250 mU·kg\(^{-1}\)·min\(^{-1}\) (lower). Arterial plasma glucose was maintained constant in all experiments, whereas renal vein glucose concentration decreased in the last 30 min of study period (*\(P < 0.05\) vs. baseline) in both insulin, but not saline, infusion groups.
insulin infusion. As shown in Fig. 2, arterial plasma enrichment of \([^3H_2]\)glucose was stable at 2.83 ± 0.03% in the saline group, at 2.32 ± 0.03% in the low insulin group, and at 2.28 ± 0.03% in the high insulin group during the entire experimental period and was consistently higher than in the renal vein (2.75 ± 0.03% in the saline group, 2.20 ± 0.02% in the low insulin group, and 2.25 ± 0.02% in the high insulin group). Endogenous glucose production (Fig. 3) remained constant at 10.0 ± 0.4 µmol·kg\(^{-1}\)·min\(^{-1}\) during the entire saline infusion period. After insulin infusion, however, it decreased from 9.9 ± 0.7 at baseline to 6.9 ± 0.5 µmol·kg\(^{-1}\)·min\(^{-1}\) during the last 30 min (P < 0.01) of the low dose and from 9.5 ± 0.7 at baseline to 5.7 ± 0.5 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.01) during the last 30 min of the high dose. Whole body glucose disappearance rates were 10.8 ± 0.60 and 11.12 ± 0.80 µmol·kg\(^{-1}\)·min\(^{-1}\) in the last 30 min of the low and high insulin doses, respectively. Renal glucose production (Fig. 3) remained constant at 1.1 ± 0.4 µmol·kg\(^{-1}\)·min\(^{-1}\) during the entire saline infusion period, but when insulin was infused at the rate of 0.125 mU·kg\(^{-1}\)·min\(^{-1}\), renal glucose production decreased from 2.5 ± 0.6 to an average of 1.5 ± 0.5 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.05) in the last 30 min of the experimental period. Similarly, when insulin was infused at the rate of 0.250 mU·kg\(^{-1}\)·min\(^{-1}\), renal glucose production decreased from 2.6 ± 0.6 to an average of 1.2 ± 0.6 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.05) in the last 30 min of the experimental period. Mean renal glucose utilization did not change during saline infusion (0.91 ± 0.40 vs. 0.65 ± 0.30 µmol·kg\(^{-1}\)·min\(^{-1}\)), but it increased from 1.50 ± 0.60 to 2.60 ± 0.70 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.05) in the low insulin group and from 1.60 ± 0.50 to 2.90 ± 0.70 µmol·kg\(^{-1}\)·min\(^{-1}\) (P < 0.05) in the high insulin group in the last 30 min of the experimental period.

Table 2 summarizes data on systemic and renal glycerol appearance and on gluconeogenesis from glycerol. Plasma \([^{13}C]\)glucose and \([^{13}C]\)glycerol enrichments were stable during the baseline and in the last 30 min of study periods in all experiments, indicating steady-state conditions had been achieved. In the saline control study, plasma enrichment of \([^{13}C]\)glucose increased from 1.20 ± 0.03 to 1.45 ± 0.08% (P < 0.05) in the artery and from 1.21 ± 0.02 to 1.52 ± 0.02% in the renal vein (P < 0.05), whereas after insulin infusion at the rate of 0.125 mU·kg\(^{-1}\)·min\(^{-1}\), it decreased from 1.50 ± 0.02 to 0.93 ± 0.02% in the artery (P < 0.01) and from 1.45 ± 0.01 to 0.89 ± 0.02% in the renal vein. Similarly, after insulin infusion at the rate of 0.250 mU·kg\(^{-1}\)·min\(^{-1}\), plasma enrichment of \([^{13}C]\)glycerol decreased from 1.23 ± 0.03 to 0.92 ± 0.02% in the artery (P < 0.01) and from 1.26 ± 0.02 to 0.94 ± 0.02% (P < 0.01) in the renal vein. Arterial plasma enrichment of \([^{13}C]\)glycerol did not change significantly during saline infusion (9.5 ± 0.4 vs. 8.9 ± 0.2%, P = 0.10), but it increased from an average of 12.3 ± 0.2 to 16.3 ± 0.3% (P < 0.01) and from 8.8 ± 0.4 to 15.1 ± 0.2% (P < 0.01), respectively, in the low and high insulin groups. Arterial glycerol concentration increased from 105 ± 11 in the baseline to 134 ± 3 µmol·kg\(^{-1}\)·min\(^{-1}\) after 180
min of saline infusion ($P < 0.05$), whereas it decreased from 60 ± 5 to 34 ± 10 µmol/l and from 71 ± 6 to 38 ± 5 µmol/l ($P < 0.01$) after insulin infusion at low and high doses, respectively. Similarly, arterial plasma free fatty acid concentration increased from 771 ± 40 to 917 ± 34 µmol/l ($P < 0.05$) after 180 min of saline infusion, but it decreased from 710 ± 85 to 248 ± 22 µmol/l and from 692 ± 71 to 282 ± 37 µmol/l (all $P < 0.01$) after insulin infusion, respectively, at low and high doses.

**DISCUSSION**

The present studies confirm previous findings in dogs (13) and humans (39) indicating that renal glucose production approximates glucose utilization and accounts for ~10–25% of endogenous glucose production in postabsorptive healthy subjects, and they further document a reversal in renal glucose balance to net uptake during physiological hyperinsulinemia. Net uptake of glucose by the kidney is due to simultaneous suppression of renal glucose production and stimulation of glucose utilization by insulin. Low and high physiological insulin infusions reduce endogenous glucose production by ~30 and ~40% and suppress renal glucose production by ~40 and 50%, respectively, whereas renal glucose utilization nearly doubles after either insulin dose. Whether insulin suppresses renal glucose production in a dose-dependent fashion, however, analogous to its effect on hepatic (endogenous) glucose production (10), cannot be determined in these studies. The fact that neither endogenous or renal glucose production nor renal glucose utilization changes significantly during saline infusion confirms that these are not merely time-dependent effects but secondary to insulin infusion. Plasma free fatty acid, glycerol concentration, and glycerol turnover decrease by ~50%, and systemic and renal glycerol conversion to glucose are substantially reduced with either insulin dose. On the basis of these findings with a combination of arteriovenous balance and tracer dilution across the kidney, it appears that a more complicated model of glucose regulation, which takes into account possible different effects on the kidney and liver of the large number of hormones (17, 35, 37) and substrates (2, 18, 33) known to affect systemic glucose metabolism, is required.

The combination of arteriovenous balance and tracer dilution with stable isotopes can effectively partition uptake and release in a tissue bed and has previously been applied to the study of glucose (5, 15, 25), lipids (26), and amino acid metabolism (4). It should be recognized, however, that differences in both glucose concentrations and plasma enrichments across the kidney are small and may lead to error. In these experiments, for example, rates of renal fractional extraction of glucose measured with $[2H_2]$glucose show considerable interindividual variation, ranging from 0 to 6.11% in the postabsorptive period and during saline infusion and from 1.81 to 9.57% during the euglycemic-hyperinsulinemic clamp period (individual data not presented). As a consequence, renal glucose production, i.e., tracer dilution across the kidney, is detected in some, but not all, individuals studied. The potential for error is further amplified when arteriovenous differences are multiplied by renal blood (plasma) flow, which represents ~20% of cardiac output (~16 ml·kg$^{-1}$·min$^{-1}$, in our series). In addition, by assuming that negative fractional extraction rates are equal to zero in 4 of 18 postabsorptive subjects, we have introduced a bias and overestimated mean fractional extraction and renal glucose utilization rates in these studies by ~4% (mean renal glucose production is not significantly affected). Thus our estimated rates of renal glucose production and utilization should not be interpreted in absolute terms but as a near-quantitative assessment of true rates. Nonetheless, even though the observations that the kidney contributes to tracer-determined glucose production and is insulin sensitive contrast with the prevailing notion that the liver is the only significant source of glucose production under insulin regulation in postabsorptive humans (10, 29), our findings are entirely supported by the available data (3, 21, 42). The possibility that the kidney makes a contribution to gluconeogenesis in the postabsorptive state is further supported by the fact that nearly 20% of systemic glycerol conversion to glucose (see Table 2) can be detected by measuring $[13C]$glucose percent enrichment in the renal vein in postabsorptive individuals.

Our studies provide strong evidence that the kidney is responsible for a small and variable, albeit significant, fraction of systemic glucose turnover in postabsorptive humans and that insulin suppresses renal glucose production and stimulates glucose utilization under euglycemic conditions. Maintenance of arterial plasma glucose and enrichment constant during prolonged insulin infusion periods with the Hot-GINF method (23) enables evaluation of the rate of onset as well as the eventual maximal response to a given insulin

**Table 2. Whole body glycerol $R_a$ and systemic and renal GNG in baseline and last 30 min of experimental period during either saline or insulin infusion**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Study</th>
<th>Baseline</th>
<th>Study</th>
<th>Baseline</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body $R_a$</td>
<td>4.7 ± 0.4</td>
<td>5.1 ± 0.2</td>
<td>3.7 ± 0.3</td>
<td>1.8 ± 0.2†</td>
<td>5.1 ± 0.3</td>
<td>1.4 ± 0.2†</td>
</tr>
<tr>
<td>Whole body GNG</td>
<td>0.66 ± 0.03</td>
<td>0.82 ± 0.05†</td>
<td>0.67 ± 0.05</td>
<td>0.18 ± 0.02†</td>
<td>0.60 ± 0.04</td>
<td>0.20 ± 0.02†</td>
</tr>
<tr>
<td>Renal GNG</td>
<td>0.11 ± 0.02</td>
<td>0.41 ± 0.04‡</td>
<td>0.10 ± 0.02</td>
<td>0.02 ± 0.02*</td>
<td>0.15 ± 0.03</td>
<td>0.09 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE. $R_a$, rates of appearance; GNG, gluconeogenesis from glycerol; baseline, 0–30 min. Insulin was infused at rates of 0.125 (low) or 0.250 (high) mU·kg$^{-1}$·min$^{-1}$. Whole body $R_a$ and systemic and renal GNG are measured in µmol·kg$^{-1}$·min$^{-1}$ during the saline control experimental period, systemic and renal GNG increased, whereas these decreased after insulin infusion at either dose ($P < 0.05$ vs. same group baseline and control; †$P < 0.05$ vs. same group baseline and control).
concentration of glucose production and utilization rates. Comparable to recent studies in healthy subjects (10), it is apparent from Fig. 3 that the rate of fall of endogenous glucose production at both insulin doses is greater during the 1st h than in the subsequent 2 h. Considering that the two insulin infusion doses were used in small samples of different subjects and that there is variability in insulin action even in lean normal individuals, however, one should use caution in comparing the effects of the two insulin doses in these experimental conditions. Although changes in renal glucose production show considerable variation during the initial 120 min of insulin infusion, steady values lower than baseline in the final 30 min of the hyperinsulinemic period were achieved when each insulin dose has had its full effect in suppressing endogenous glucose production. It must be emphasized that insulin action on renal glucose metabolism is readily apparent from the arteriovenous concentration difference data alone, although the use of tracers provides additional information on individual rates of glucose production and utilization. The observations that glucose balance switches to net uptake and that renal glucose utilization after either insulin dose may have been secondary to a reduction in uptake of circulating free fatty acid and in systemic and renal gluconeogenesis from glycerol suggest that insulin exerts its peripheral effects on systemic glucose production and utilization, in part, by reducing renal glucose production and enhancing renal glucose utilization via inhibition of adipose tissue lipolysis. We conclude that glucose production and utilization by the kidney are important insulin-responsive components of glucose metabolism in humans.

We thank E. Hayes, I. Zaitseva, S. Pacheco, and J. Vasek for excellent technical assistance and J. Boshard for editorial help. This work was supported in part by grants from the American Diabetes Association and National Institute of Diabetes and Digestive and Kidney Diseases (DK 40656).

Address for reprint requests: E. Cersosimo, Dept. of Medicine, Division of Endocrinology, Health Science Center T15–060, SUNY at Stony Brook, Stony Brook, NY 11794–8154.

Received 6 July 1998; accepted in final form 14 September 1998.

REFERENCES