

# Renal Glucose Production During Insulin-Induced Hypoglycemia in Humans

Eugenio Cersosimo, Peter Garlick, and John Ferretti

We investigated the effects of hypoglycemia on renal glucose production (RGP) and renal glucose uptake (RGU) using arteriovenous balance combined with tracer technique in humans. Our 14 healthy subjects had arterialized hand veins (artery) and renal veins (under fluoroscopy) catheterized after an overnight fast. Systemic and renal glucose kinetics were measured with infusion of [ $6\text{-}^2\text{H}_2$ ]glucose, and renal plasma flow was measured by para-aminohippurate clearance. After a 150-min equilibration period, artery and renal vein samples were obtained between -30 and 0 min, and subjects received a 180-min peripheral insulin infusion ( $0.250 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) with a variable infusion of [ $6\text{-}^2\text{H}_2$ ]dextrose adjusted to maintain plasma glucose at either  $\sim 60 \text{ mg/dl}$  (hypoglycemic clamp) or  $\sim 90 \text{ mg/dl}$  (euglycemic clamp). Blood samples were obtained between 150 and 180 min during the study period. Insulin increased from  $49 \pm 14$  to  $130 \pm 25$  (hypoglycemia) and to  $102 \pm 10$  (euglycemia) pmol/l. Glucose decreased from  $5.32 \pm 0.11$  to  $3.58 \pm 0.07 \mu\text{mol/ml}$  during hypoglycemia, but it did not change during euglycemia ( $5.20 \pm 0.19$  vs.  $5.05 \pm 0.15 \mu\text{mol/ml}$ ). Endogenous glucose production decreased ( $9.30 \pm 0.70$  vs.  $5.65 \pm 0.50$ ) during euglycemia but not during hypoglycemia ( $9.80 \pm 0.50$  vs.  $10.25 \pm 0.60 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). During hypoglycemia, net renal glucose output increased from  $0.54 \pm 0.30$  to  $2.31 \pm 0.40$ , RGP increased from  $1.88 \pm 0.70$  to  $3.65 \pm 0.50$  ( $P < 0.05$ ), and RGU did not change ( $1.34 \pm 0.50$  vs.  $1.34 \pm 0.60 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). During euglycemia, renal glucose balance switched from a net output of  $0.72 \pm 0.20$  to a net uptake of  $1.70 \pm 0.92$ , RGP decreased from  $2.31 \pm 0.50$  to  $1.20 \pm 0.58$ , and RGU increased from  $1.59 \pm 0.50$  to  $2.90 \pm 0.70 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P < 0.05$ ). During hypoglycemia, arterial glucagon increased from  $105 \pm 6$  to  $129 \pm 8$ , epinephrine increased from  $116 \pm 28$  to  $331 \pm 33$ , norepinephrine increased from  $171 \pm 9$  to  $272 \pm 9$  (all  $P < 0.05$ ), and renal vein norepinephrine increased from  $236 \pm 13$  to  $426 \pm 50$  ( $P < 0.001$ ). These data indicate that, in addition to counterregulatory hormones, activation of the autonomic nervous system during hypoglycemia stimulates glucose production by the kidney, which may represent

an important additional component of the body's defense against hypoglycemia in humans. *Diabetes* 48:261-266, 1999

The role of the kidney in glucose metabolism is not well understood. Substantial in vitro evidence indicates that the kidney is capable of both glucose production and utilization (1-5). Krebs and colleagues (1,2) have demonstrated that cells of the proximal convoluted tubule are able to efficiently convert three-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, glycogen storage, and glucose oxidation (3-5). The in vivo data, however, are less conclusive. Most review articles and textbooks emphasize the dominant role of the liver in regulating glucose appearance (6-8) and indicate that the net contribution of the kidney to glucose production is not significant, except in prolonged fasting (9) and perhaps uncontrolled diabetes (10). We have recently demonstrated that postabsorptive tracer-determined renal glucose production (RGP) accounts for  $\sim 30\%$  of systemic glucose appearance in dogs, although net renal glucose balance was neutral (11). Subsequent studies confirmed these findings in humans by demonstrating that despite negligible net contribution, the kidney is responsible for  $\sim 25\%$  of glucose production in the postabsorptive state (12). Of additional interest, glucose production by the kidneys, analogous to the liver, appears to be suppressed by insulin (11) and stimulated by catecholamines (12). The observations that hypoglycemic episodes in hospitalized patients occur in nondiabetic individuals with renal insufficiency (13) and that hypoglycemia is relatively common among uremic patients subject to protein-calorie deprivation (14) underscore the potential importance of the kidney in glucose homeostasis. Although human studies are not available, the kidney was recently shown to make a substantial net contribution to glucose production in hypoglycemic dogs (15). These findings have raised the possibility that RGP and utilization may play an important role in glucose counterregulation. The present studies were, therefore, undertaken to evaluate the contribution of the kidney to systemic glucose production and utilization rates in humans during insulin-induced hypoglycemia using arteriovenous balance combined with tracer technique.

## RESEARCH DESIGN AND METHODS

**Subjects.** Informed written consent was obtained from 14 healthy volunteers after the protocol had been approved by our local institutional review board. All subjects (Table 1) had normal fasting glucose, blood chemistry, and urine analysis and had no personal or family history of diabetes, hypertension, or renal dis-

From the Departments of Medicine (E.C.), Surgery (P.G.), and Radiology (J.F.), State University of New York at Stony Brook, Stony Brook, New York.

Address correspondence and reprint requests to Eugenio Cersosimo, MD, PhD, Department of Medicine, Division of Diabetes and Endocrinology, Health Science Center T15-060, SUNY at Stony Brook, Stony Brook, NY 11794-8154. E-mail: ecersosi@mail.som.sunysb.edu.

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CV, coefficient of variation; EGP, endogenous glucose production;  $FE_g$ , fractional extraction of glucose; HGP, hepatic glucose production; PAH, para-aminohippurate;  $R_a$ , rate of appearance; RGO, renal glucose output; RGP, renal glucose production; RGU, renal glucose uptake; RPF, renal plasma flow.

ease. For 3 days before the study, all had abstained from alcohol and had been on a weight-maintaining diet containing at least 200 g of carbohydrate.

**Protocol.** Subjects were admitted to the SUNY University Hospital General Clinical Research Center at Stony Brook after an overnight fast between 6:00 and 7:00 A.M. the morning of the experiments. An antecubital vein was cannulated, and a primed-continuous infusion of  $[6\text{-}^2\text{H}_2]\text{glucose}$  (20–24  $\mu\text{mol}/\text{kg}$ ,  $0.20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; Cambridge Isotope Laboratories, Andover, MA) and a continuous infusion of para-aminohippurate (PAH) (12 mg/min; Merck, West Point, PA) were started. Subsequently, a dorsal hand vein was retrogradely cannulated and kept in a thermoregulated Plexiglas box at  $65^\circ\text{C}$  for arterialized venous blood sampling (16). During the 150-min equilibration period, subjects had left ( $n = 12$ ) 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 ( $4.0 \text{ U}/\text{min}$ ) to maintain patency.

During the baseline period (–30 to 0 min), three consecutive blood samples were simultaneously collected from the dorsal hand vein and the renal vein at 15-min intervals for the determination of PAH, insulin, glucagon, catecholamines, plasma glucose concentration, and percent enrichment. At 0 min, upon completion of baseline collections, subjects were randomized to receive a 180-min continuous peripheral infusion of insulin at the rate of  $0.250 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  with a concomitant variable infusion of  $[6\text{-}^2\text{H}_2]\text{dextrose}$  (2% APE DW10) to achieve and maintain the plasma glucose concentration at either  $-5.0 \mu\text{mol}/\text{ml}$  (euglycemic clamp,  $n = 8$ ) or  $-3.3 \mu\text{mol}/\text{ml}$  (hypoglycemic clamp,  $n = 6$ ) and to keep glucose enrichment constant (17). Insulin infusion rate was selected to produce physiological hyperinsulinemia with mild-to-moderate hypoglycemia so as to mimic the evolution of clinical hypoglycemia (18). Blood samples were again collected from the dorsal hand vein and the renal vein at 15-min intervals from 150 to 180 min.

**Analytical techniques.** Plasma glucose was measured at the bedside with the Beckman Glucose Analyzer II (Fullerton, CA), and PAH concentration was determined by a colorimetric method (19). Plasma insulin (20) and glucagon (21) were determined by radioimmunoassays, and catecholamines were determined by a radioenzymatic method (22). Plasma concentration and enrichment of  $[^2\text{H}_2]\text{glucose}$  were measured by gas chromatography/mass spectrometry. In brief,  $150 \mu\text{l}$  of plasma was added to  $150 \mu\text{l}$  of glucose internal standard solution ( $5 \text{ mmol}/\text{l}$   $[\text{U}\text{-}^{13}\text{C}]\text{glucose}$ ). Samples were deproteinized with acetonitrile and evaporated to dryness. Derivatization was carried out with butane boronic acid in pyridine and acetic anhydride (23,24). The glucose derivative was quantified by selective ion monitoring at masses  $m/z$  297, 298, 299, and 303 for natural  $[\text{U}\text{-}^{13}\text{C}]\text{glucose}$ ,  $[^2\text{H}_2]\text{glucose}$ , and  $[\text{U}\text{-}^{13}\text{C}]\text{glucose}$ , respectively. Two sets of standards were measured containing known amounts of  $[^2\text{H}_2]\text{glucose}$  and  $[\text{U}\text{-}^{13}\text{C}]\text{glucose}$ . Isotope enrichments were calculated by multiple linear regression (25). A set of standards containing 0–10 mmol of glucose and 5 mmol of  $[\text{U}\text{-}^{13}\text{C}]\text{glucose}$  internal standard were used to calculate plasma concentration of glucose.

**Calculations.** Renal plasma flow was calculated by PAH clearance using the following:

$$\text{RPF} = \text{INF}/([\text{PAH}]_{(a)} - [\text{PAH}]_{(rv)}) \quad (1)$$

where RPF is renal plasma flow in milliliters per minute, INF is the PAH infusion rate in milligrams per minute, PAH concentration is measured in milligrams per milliliter, a is artery, and rv is renal vein. Whole-body glucose rate of appearance ( $R_a$ ) was calculated using the steady-state formula:

$$R_a = \text{INF}/[^2\text{H}_2]\text{PE}_{(a)} \quad (2)$$

where INF is the rate of  $[6\text{-}^2\text{H}_2]\text{glucose}$  infusion in  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and  $[^2\text{H}_2]\text{PE}_{(a)}$  is the percentage of the arterial plasma glucose enriched with  $[^2\text{H}_2]\text{glucose}$ . During the experimental period (150–180 min), INF represents the time-varying rate of infusion of  $[6\text{-}^2\text{H}_2]\text{dextrose}$  in  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at each time point, according to the “Hot-GINF” method (26). Underestimation of the  $R_a$  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  $R_a$  in Eq. 2. Renal fractional extraction of glucose ( $\text{FE}_g$ ) was calculated using the following:

$$\text{FE}_g = ([\text{Glu}]_a \times \text{PE}_a - [\text{Glu}]_{rv} \times \text{PE}_{rv})/([\text{Glu}]_a \times \text{PE}_a) \quad (3)$$

where Glu is the plasma glucose concentration and PE refers to the  $[^2\text{H}_2]\text{glucose}$  plasma enrichment. Renal glucose uptake (RGU) was calculated using the following:

$$\text{RGU} = \text{FE}_g \times [\text{Glu}]_a \times \text{RPF} \quad (4)$$

Because glycosuria was not present, RGU was assumed to be equal to glucose utilization. RGP was calculated using the following:

$$\text{RGP} = \text{RGU} + ([\text{Glu}]_{rv} - [\text{Glu}]_a) \times \text{RPF} \quad (5)$$

Because glucose is extracted into whole blood and there is rapid equilibration between red cell and plasma glucose concentrations, Eqs. 4 and 5 will underestimate RGP and RGU.

**Statistics.** All values obtained during the baseline and during the experimental period were averaged, and mean  $\pm$  SE is expressed for each period. Data obtained at baseline in each group were compared with those from the experimental period using a paired Student's  $t$  test; data between groups after insulin infusion were compared using a nonpaired Student's  $t$  test. All  $P$  values  $< 0.05$  were considered statistically significant (27).

## RESULTS

RPF was  $10.8 \pm 1.5$  and  $10.7 \pm 1.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at baseline and remained constant during the study periods. It was  $11.3 \pm 1.7$  and  $11.0 \pm 1.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P = 0.80$ ), respectively, during the euglycemic and hypoglycemic clamps. Arterial plasma insulin concentration increased from an average of  $49 \pm 14$  to  $102 \pm 10$  and to  $130 \pm 25 \text{ pmol}/\text{l}$  ( $P < 0.001$ ) after insulin infusion at the rate of  $0.250 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, during the euglycemic and hypoglycemic clamps. Table 2 summarizes plasma glucose concentrations (coefficient of variation [CV] = 2.9%), plasma  $[^2\text{H}_2]\text{glucose}$  enrichments (CV = 2.8%), and renal  $\text{FE}_g$  in eight individuals in the postabsorptive state and during the final 30 min of the euglycemic-hyperinsulinemic clamp period. Mean arterial plasma glucose concentration was  $5.20 \pm 0.19 \mu\text{mol}/\text{ml}$  in the postabsorptive period and did not change during the study period ( $5.05 \pm 0.15 \mu\text{mol}/\text{ml}$ ,  $P = 0.55$ ). Mean renal vein glucose concentration was  $5.27 \pm 0.23$  and  $4.90 \pm 0.12 \mu\text{mol}/\text{ml}$  ( $P = 0.12$ ), respectively, during the postabsorptive and study periods. Mean renal  $\text{FE}_g$  doubled from  $2.83 \pm 0.54$  to  $5.08 \pm 1.08\%$  ( $P = 0.027$ ) during the euglycemic clamp. As a result, postabsorptive renal glucose balance switched from a net output of  $0.72 \pm 0.20$  to a net uptake of  $1.70 \pm 0.92 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $0.0016$ ), RGP decreased from  $2.31 \pm 0.50$  to  $1.20 \pm 0.58 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P = 0.037$ ), and RGU increased from  $1.59 \pm 0.50$  to  $2.90 \pm 0.70 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P = 0.028$ ). Table 3 summarizes plasma glucose concentrations (CV = 2.3%), plasma  $[^2\text{H}_2]\text{glucose}$  enrichments (CV = 3.7%), and renal  $\text{FE}_g$  in six individuals in the postabsorptive state and during the final 30

TABLE 1

Characteristics of 14 subjects studied in the postabsorptive state and during either euglycemic- or hypoglycemic-hyperinsulinemic clamp with peripheral insulin infusion at the rate of  $0.250 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$

	Euglycemia	Hypoglycemia
<i>n</i>	8	6
Age (years)	$33.0 \pm 1.9$ (23–42)	$32.2 \pm 4.2$ (20–47)
Sex (M/F)	5/3	3/3
Weight (kg)	$73 \pm 4$	$67 \pm 4$
BMI ( $\text{kg}/\text{m}^2$ )	$23.8 \pm 0.7$	$22.8 \pm 1.3$
Ethnicity		
Caucasian	4	4
African-American	2	1
Hispanic	2	1

Data are means  $\pm$  SE (range) or *n*.

TABLE 2

Plasma glucose concentrations and plasma [ $^2\text{H}_2$ ]glucose enrichments in arterialized hand vein and in renal vein, and renal  $\text{FE}_g$  in normal healthy subjects in the postabsorptive state and the last 30 min of a 180-min euglycemic-hyperinsulinemic clamp

Subjects	Postabsorptive state			Euglycemic clamp		
	Artery	Renal vein	$\text{FE}_g$	Artery	Renal vein	$\text{FE}_g$
1						
Glu	5.11 ± 0.03	5.07 ± 0.07	2.10	4.26 ± 0.07	4.35 ± 0.08	1.81
PE	2.28 ± 0.01	2.25 ± 0.05		2.08 ± 0.12	2.00 ± 0.01	
2						
Glu	5.48 ± 0.07	6.02 ± 0.05	-0.28*	5.11 ± 0.03	5.33 ± 0.07	2.97
PE	2.41 ± 0.01	2.20 ± 0.14		2.15 ± 0.03	2.00 ± 0.02	
3						
Glu	5.41 ± 0.05	5.59 ± 0.01	0.93	5.14 ± 0.01	4.94 ± 0.01	1.88
PE	2.43 ± 0.01	2.33 ± 0.01		1.91 ± 0.02	1.95 ± 0.01	
4						
Glu	4.87 ± 0.04	4.85 ± 0.06	4.55	4.75 ± 0.03	4.65 ± 0.01	3.36
PE	2.65 ± 0.01	2.54 ± 0.02		2.34 ± 0.01	2.31 ± 0.02	
5						
Glu	6.02 ± 0.03	6.00 ± 0.01	2.33	5.59 ± 0.06	5.35 ± 0.02	5.54
PE	2.00 ± 0.02	1.96 ± 0.01		2.30 ± 0.01	2.27 ± 0.01	
6						
Glu	5.57 ± 0.05	5.76 ± 0.02	0.90	4.78 ± 0.01	4.65 ± 0.02	6.70
PE	2.16 ± 0.01	2.07 ± 0.02		2.20 ± 0.01	2.11 ± 0.02	
7						
Glu	4.44 ± 0.04	4.33 ± 0.03	5.73	5.44 ± 0.05	5.00 ± 0.02	8.77
PE	2.40 ± 0.02	2.32 ± 0.01		2.68 ± 0.01	2.66 ± 0.01	
8						
Glu	4.67 ± 0.02	4.56 ± 0.02	6.11	5.33 ± 0.05	4.89 ± 0.01	9.57
PE	2.60 ± 0.01	2.50 ± 0.02		2.80 ± 0.01	2.76 ± 0.02	
Mean Glu	5.20 ± 0.19	5.27 ± 0.23	2.83 ± 0.54	5.05 ± 0.15	4.90 ± 0.12	5.08 ± 1.08
± SE PE	2.37 ± 0.08	2.27 ± 0.07		2.31 ± 0.11	2.26 ± 0.11	

Data are means ± SE of three values performed in triplicate. Plasma glucose concentrations (Glu) are expressed in micromoles per milliliter. Plasma [ $^2\text{H}_2$ ]glucose enrichments (PE) are expressed as molar percent excess (tracer/[tracer + tracee]). Renal  $\text{FE}_g$  is expressed in percent. \*Assumed to be equal to zero.

min of the hypoglycemic-hyperinsulinemic clamp period. Mean arterial plasma glucose concentration was  $5.32 \pm 0.11 \mu\text{mol/ml}$  in the postabsorptive period and decreased to  $3.58 \pm 0.07 \mu\text{mol/ml}$  ( $P < 0.001$ ) during the study period. Mean renal vein glucose concentration decreased from  $5.37 \pm 0.11$  to  $3.79 \pm 0.08 \mu\text{mol/ml}$  ( $P < 0.001$ ) and was consistently higher than arterial glucose ( $P < 0.001$ ) during hypoglycemia. Mean renal  $\text{FE}_g$  did not change during the hypoglycemic clamp ( $2.36 \pm 0.33$  vs.  $3.41 \pm 0.64\%$ ,  $P = 0.084$ ). Postabsorptive net renal glucose output (RGO) increased fivefold from  $0.54 \pm 0.30$  to  $2.31 \pm 0.50$  ( $P < 0.001$ )  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . RGP doubled from  $1.88 \pm 0.70$  to  $3.65 \pm 0.50 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P = 0.012$ ), and RGU did not change ( $1.34 \pm 0.50$  vs.  $1.34 \pm 0.60 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P = 0.978$ ).

Mean EGP was  $9.51 \pm 0.66 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the postabsorptive period and decreased to  $5.65 \pm 0.50$  ( $P < 0.01$ ) during the final 30 min of the euglycemic-hyperinsulinemic clamp; it averaged  $10.25 \pm 0.60 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  during the last 30 min of the hypoglycemic clamp. Whole-body glucose disposal rates were  $11.12 \pm 0.80$  and  $12.70 \pm 0.80 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, during the euglycemic and hypoglycemic clamps. Assuming the liver is the only other source of endogenous glucose, we have estimated (Fig. 1) that hepatic glucose production (HGP) ( $\sim 7.40 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) is responsible for  $\sim 78\%$  and RGP ( $\sim 2.10 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) accounts for  $\sim 22\%$  of tracer-determined  $R_a$  in the postab-

sorptive state. During the final 30-min period of the euglycemic-hyperinsulinemic clamp, this ratio is maintained, because HGP is responsible for  $\sim 4.45 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (78%) and RGP accounts for  $\sim 1.20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $\sim 22\%$ ) of glucose appearance. During the final 30-min period of the hypoglycemic clamp, however, the contribution of the kidney increases significantly, and RGP ( $\sim 3.65 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) accounts for  $\sim 36\%$ , whereas HGP ( $\sim 6.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) is responsible for  $\sim 64\%$  of tracer-determined  $R_a$ .

During hypoglycemia, arterial plasma glucagon concentration increased from  $105 \pm 6$  to  $129 \pm 8$  ( $P < 0.05$ ), epinephrine increased threefold from  $116 \pm 28$  to  $331 \pm 33 \text{ pg/ml}$  ( $P < 0.001$ ), and norepinephrine increased from  $171 \pm 9$  to  $272 \pm 9 \text{ pg/ml}$  ( $P < 0.01$ ). Renal vein plasma epinephrine and norepinephrine concentrations increased from  $84 \pm 4$  to  $213 \pm 10$  and from  $236 \pm 13$  to  $426 \pm 50$  (all  $P < 0.001$ ), respectively. As a consequence, the arteriovenous concentration difference of norepinephrine ( $65 \pm 11$  vs.  $154 \pm 30 \text{ pg/ml}$ ) increased significantly, whereas that of epinephrine decreased from  $-32 \pm 16$  to  $-118 \pm 22 \text{ pg/ml}$  during the final 30-min period of the hypoglycemic clamp ( $P < 0.01$ ). Arterial plasma glucagon concentration decreased from  $95 \pm 3$  in the postabsorptive period to  $82 \pm 3 \text{ pg/ml}$  ( $P < 0.05$ ) during the final 30-min period of the euglycemic clamp. Plasma catecholamines were available in four subjects, and neither arterial plasma epinephrine ( $74 \pm 8$  vs.  $84 \pm 16 \text{ pg/ml}$ ) and norepinephrine ( $165$

TABLE 3

Plasma glucose concentrations and plasma [ $^2\text{H}_2$ ]glucose enrichments in arterialized hand vein and in renal vein, and renal  $\text{FE}_g$  in normal healthy subjects in the postabsorptive state and the last 30 min of a 180-min hypoglycemic-hyperinsulinemic clamp

Subjects	Postabsorptive state			Hypoglycemic clamp		
	Artery	Renal vein	$\text{FE}_g$	Artery	Renal vein	$\text{FE}_g$
1						
Glu	5.35 ± 0.02	5.28 ± 0.03	2.41	3.78 ± 0.06	4.04 ± 0.10	2.84
PE	1.80 ± 0.02	1.78 ± 0.03		1.65 ± 0.02	1.50 ± 0.03	
2						
Glu	5.65 ± 0.05	5.78 ± 0.08	1.58	3.60 ± 0.05	3.80 ± 0.07	3.49
PE	1.58 ± 0.02	1.52 ± 0.04		1.40 ± 0.04	1.28 ± 0.04	
3						
Glu	4.96 ± 0.10	5.06 ± 0.01	2.16	3.33 ± 0.03	3.54 ± 0.02	4.46
PE	1.71 ± 0.02	1.64 ± 0.03		1.58 ± 0.02	1.42 ± 0.04	
4						
Glu	5.35 ± 0.02	5.30 ± 0.03	2.60	3.66 ± 0.05	3.87 ± 0.06	3.99
PE	1.78 ± 0.02	1.75 ± 0.05		1.63 ± 0.06	1.48 ± 0.03	
5						
Glu	5.56 ± 0.05	5.56 ± 0.01	3.75	3.71 ± 0.06	3.89 ± 0.01	5.07
PE	1.60 ± 0.03	1.54 ± 0.04		1.48 ± 0.04	1.34 ± 0.03	
6						
Glu	5.06 ± 0.08	5.22 ± 0.05	1.64	3.39 ± 0.03	3.61 ± 0.04	0.61
PE	1.72 ± 0.04	1.64 ± 0.06		1.50 ± 0.04	1.40 ± 0.06	
Mean Glu	5.32 ± 0.11	5.37 ± 0.11	2.36 ± 0.33	3.58 ± 0.07	3.79 ± 0.08	3.41 ± 0.64
± SE PE	1.70 ± 0.04	1.65 ± 0.04		1.54 ± 0.04	1.40 ± 0.04	

Data are means ± SE of three values performed in triplicate. Plasma glucose concentrations (Glu) are expressed in micromoles per milliliter. Plasma [ $^2\text{H}_2$ ]glucose enrichments (PE) are expressed as molar percent excess (tracer/[tracer + tracee]). Renal  $\text{FE}_g$  is expressed in percent.

± 20 vs. 152 ± 22 pg/ml) nor renal vein epinephrine (82 ± 10 vs. 76 ± 8 pg/ml) and norepinephrine (144 ± 14 vs. 128 ± 12 pg/ml) changed significantly during the euglycemic clamp.

## DISCUSSION

The present studies confirm previous findings in dogs (11,15) and indicate that tracer-determined RGP, which accounts for ~20% of systemic glucose production in postabsorptive humans, is suppressed by physiological hyperinsulinemia and increases significantly during insulin-induced hypoglycemia. Our results are in agreement with recently published data in healthy subjects (12), although Ekberg et al. (28) were unable to detect the kidney's contribution to glucose production in the postabsorptive state. The reason(s) for these controversial findings is not yet entirely clear, but limitations in the current techniques used to address these questions are likely to play a role. Differences in both glucose concentrations and plasma enrichments (or specific activity) across the kidney are small and may lead to error. In our series, for example, renal  $\text{FE}_g$  measured with [ $^2\text{H}_2$ ]glucose shows a considerable interindividual variation (range from -0.28 to 6.11 in postabsorptive, 1.81 to 9.57 in euglycemia, and 0.61 to 5.07 in hypoglycemia). The potential for error is further amplified when arteriovenous differences are multiplied by renal blood flow, which represents ~20% of cardiac output (~16 ml · kg<sup>-1</sup> · min<sup>-1</sup> in our series). In addition, by assuming that negative renal glucose fractional extraction equals zero in one subject (Table 2), we have introduced a bias and overestimated mean fractional extraction and renal glucose utilization by ~2% (renal glucose production was underestimated by ~1%) in the postabsorptive state in the euglycemic group. Thus, our estimated rates of RGP and RGU should not be interpreted in absolute terms, but as a near-quantitative

assessment of true rates. Furthermore, it should be recognized that, although the observation that the kidney contributes to glucose production in postabsorptive humans contrasts with the prevailing notion that EGP is equal to HGP, our findings are entirely supported by the available data (29–36). Simultaneous measurements of glucose production combining tracers and hepatic vein catheterization performed in 37 postabsorptive healthy individuals (32) demonstrate an unequivocal difference of ~1 μmol · kg<sup>-1</sup> · min<sup>-1</sup> between

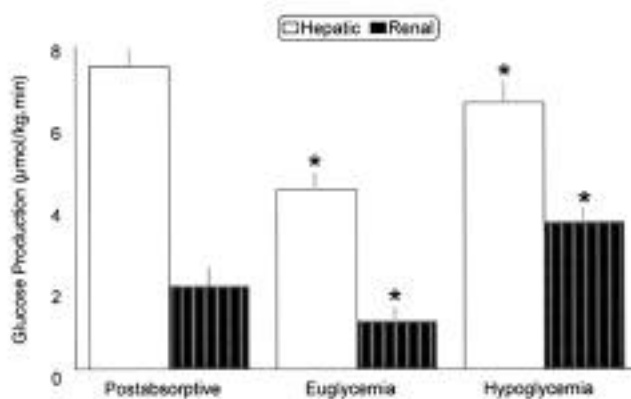


FIG. 1. HGP and RGP in the postabsorptive state and during the final 30 min of a 3-h hyperinsulinemic period with either euglycemia or hypoglycemia in healthy subjects. HGP is estimated as the difference between calculated EGP (Eq. 2 in text) and RGP (Eq. 5 in text). Postabsorptive values represent an average of all 14 subjects. \* $P < 0.05$  vs. postabsorptive.

tracer-determined glucose appearance ( $\sim 12.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and HGP ( $\sim 11.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), i.e., after splanchnic glucose uptake is factored out. The possibility that the kidney contributes to postabsorptive gluconeogenesis is further suggested by the fact that using hepatic venous catheterization to measure the uptake of gluconeogenic amino acids, pyruvate, and lactate across the splanchnic bed, Wahren et al. (34) estimated that the contribution of gluconeogenesis to total glucose production was only  $\sim 20\%$  during the first 12–14 h of fasting. Even though this method underestimates the contribution of gluconeogenesis to whole-body glucose production to the extent that it does not take into consideration gluconeogenic precursors released from the gut and intrahepatic pools of gluconeogenic substrates, data from dogs (37) and humans (38), in which portal vein blood was obtained, suggest that there is relatively little gluconeogenic substrate release from the gut. More recently developed methods to estimate gluconeogenesis, either as the difference between tracer-determined whole-body glucose production and net hepatic glycogenolysis with  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy (39) or using deuterated  $\text{H}_2\text{O}$  (40), minimize the error associated with intraportal and intrahepatic gluconeogenic precursor availability but cannot partition hepatic and renal gluconeogenesis. Thus, using combined arterial-renal vein difference and tracer technique to assess renal gluconeogenesis, our studies provide evidence that the kidney makes a small, albeit significant, contribution to postabsorptive systemic glucose turnover in humans, and that insulin suppresses RGP and stimulates glucose utilization under euglycemic conditions. Reversal of renal glucose balance to net uptake during the euglycemic clamp and the substantial increase in net RGO during hypoglycemia are readily apparent from the arteriovenous concentration difference data alone; although, the use of tracer provides additional information on individual rates of glucose production and utilization by the kidney. That is, insulin suppresses tracer-determined RGP and simultaneously stimulates glucose uptake, and the kidney, which accounts for  $\sim 15\%$  at baseline, becomes responsible for  $\sim 25\%$  of systemic glucose disposal during euglycemia. In contrast, since there is no change in RGU, the percent contribution of the kidney to systemic glucose disposal in hypoglycemia decreases from  $\sim 15$  to  $\sim 10\%$ ; doubling of RGP results in a fivefold increase in net RGO.

Our studies indicate that glucose production by the kidney is responsible for  $\sim 36\%$  of tracer-determined glucose appearance during sustained mild-to-moderate hypoglycemia in humans. The contributions of glycogenolysis and gluconeogenesis to glucose production during similar hypoglycemic conditions have been previously reported (18,29), but the individual contribution of the liver and the kidney has not yet been investigated. In animals, a discrepancy of  $\sim 1.1 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $\sim 6.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) between systemic glucose appearance measured by the tracer dilution technique ( $\sim 6.1 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and net hepatic glucose balance measured by arteriovenous difference ( $\sim 5.0 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), and the fact that maximum estimated percent contribution of three-carbon precursor uptake and conversion to glucose by the liver could account for only  $\sim 60\%$  of tracer-determined glucose produced (12% of net hepatic output and 22% of tracer-determined glucose production were unaccounted for) after 3 h of hypoglycemia, raised the possibility of an extrahepatic source of glucose (41). Moreover, a difference of  $\sim 1.2 \text{mg} \cdot \text{kg}^{-1} \cdot$

$\text{min}^{-1}$  ( $\sim 6.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) between tracer-determined glucose production and net hepatic glucose output during high-dose ( $8 \text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) insulin-induced hypoglycemia in dogs, and the observation that hepatic precursor uptake and conversion to glucose could account for only 3.8 of  $5.2 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of tracer-determined glucose production (42), are consistent with our findings and lend further support to the theory that the kidney plays a major role in glucose counterregulation. The biochemical capacity and physiological reserve of the kidney to produce glucose is not in question (1–3,9,43). Several recent publications have expanded on the original work reported by Cahill (9), emphasizing the role of the kidney in glucose metabolism during hyperinsulinemia (11), hypoglycemia (15), and hyperglycemia (44), and in conditions of high levels of circulating catecholamines (12). In view of the fact that renal insufficiency is associated with frequent hypoglycemic episodes (13,14), it is not surprising that the kidney is responsible for a considerable fraction of endogenously produced glucose during hypoglycemia. The net contribution of the kidney to glucose production ( $\sim 2.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) results from a combined absolute increase in tracer-determined RGP ( $\sim 3.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and unchanged RGU rates ( $\sim 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Considering that delivery of precursor substrates to the liver may be rate-limiting for gluconeogenesis, it is conceivable that hypoglycemia in uremic individuals could result from an inadequate compensatory increase in HGP with a progressive loss of RGP. Nonetheless, these hypotheses must be verified by additional studies, since our findings do not elucidate the pathogenesis of hypoglycemic episodes in uremia.

Similar to previous observations, our studies demonstrate that plasma glucagon increases (18,29) and circulating levels of catecholamines are substantially elevated (45) during prolonged mild-to-moderate hypoglycemia. Additionally, arteriovenous norepinephrine concentration differences across the kidney increased threefold. Renal venous overflow of norepinephrine indicates that the autonomic sympathetic nervous system is activated in the kidney (46,47) and, analogous to the pancreas (48) and to skeletal muscle and adipose tissue (49), suggests that early activation of the sympathetic nervous system in response to hypoglycemia (50) may be responsible for stimulating renal gluconeogenesis. The observation that elevated plasma catecholamines are associated with increased RGP during hypoglycemia agrees with an earlier demonstration that glucose production by the kidney is responsive to epinephrine (12) and is consistent with the well-documented hierarchy among redundant glucose counterregulatory factors (50).

In summary, we have demonstrated that the kidney is responsible for  $\sim 20\%$  of endogenous glucose turnover in postabsorptive healthy subjects. Physiological hyperinsulinemia suppresses RGP and stimulates glucose uptake. Mild sustained hypoglycemia is associated with a twofold increase in RGP and a fivefold increase in net RGO, as the kidney becomes responsible for  $\sim 36\%$  of tracer-determined systemic glucose appearance. Elevations in renal vein plasma norepinephrine far above arterial concentrations during hypoglycemia document catecholamine spillover and suggest that, in addition to the potential effects of circulating counterregulatory hormones, activation of the autonomic nervous system may directly stimulate RGP. We conclude that glucose production by the kidney represents an important component of the body's defense against hypoglycemia in humans.

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Author Queries (please see Q in margin and underlined text)

Q1: In footnote, are the initials after departments placed correctly?

Q2: The abbreviation RGU was used for renal glucose uptake in text and renal glucose utilization in the abstract. OK to define it as uptake and not utilization?

Q3: Sentence OK as edited?