Renal Substrate Metabolism and Gluconeogenesis During Hypoglycemia in Humans

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To examine the potential contribution of precursor substrates to renal gluconeogenesis during hypoglycemia, 14 healthy subjects had arterialized hand vein and renal vein (under fluoroscopy) catheterized after an overnight fast. Net renal balance of lactate, glycerol, alanine, and glutamine was determined simultaneously with systemic and renal glucose kinetics using arteriovenous concentration differences and 6-[$^2H_2$]glucose tracer dilution. Renal plasma flow was measured by para-aminohippurate clearance and was converted to blood flow using the mathematical value (1-hematocrit). Arterial and renal vein samples were obtained in the postabsorptive state and during a 180-min hyperinsulinemic period during either euglycemia or hypoglycemia. Insulin increased from 49 ± 14 to 130 ± 25 pmol/l (hypoglycemia) and to 102 ± 10 pmol/l (euglycemia). Arterial blood glucose decreased from 4.5 ± 0.2 to 3.0 ± 0.1 mmol/l during hypoglycemia but did not change during euglycemia (4.3 ± 0.2 mmol/l). After 150 min, endogenous glucose production reached a plateau value that was higher during hypoglycemia (10.3 ± 0.6 μmol · kg^{-1} · min^{-1}) than during euglycemia (5.73 ± 0.6 μmol · kg^{-1} · min^{-1}, $P < 0.001$). Hypoglycemia was associated with a rise in renal glucose production (RGP) from 3.0 ± 0.7 to 5.4 ± 0.6 μmol · kg^{-} · min^{-1} ($P < 0.05$), although glucose utilization remained the same (2.0 ± 0.8 vs. 2.1 ± 0.6 μmol · kg^{-1} · min^{-1}). As a result, net renal glucose output increased from 1.0 ± 0.3 to 3.3 ± 0.40 μmol · kg^{-1} · min^{-1}. Elevations in net renal uptake of lactate (2.4 ± 0.5 to 3.5 ± 0.7 vs. 2.8 ± 0.4 μmol · kg^{-1} · min^{-1}), glycerol (0.6 ± 0.3 to 1.3 ± 0.5 vs. 0.4 ± 0.2 μmol · kg^{-1} · min^{-1}), and glutamine (0.7 ± 0.2 to 1.1 ± 0.3 vs. 0.1 ± 0.3 μmol · kg^{-1} · min^{-1}) during hypoglycemia versus euglycemia ($P < 0.05$) could account for nearly 60% of all glucose carbons released in the renal vein during hypoglycemia. Our data indicate that extraction of circulating gluconeogenic precursors by the kidney is enhanced and responsible for a substantial fraction of the compensatory rise in RGP during sustained hypoglycemia. Increased renal gluconeogenesis from circulating substrates represents an additional physiological mechanism by which the decrease in blood glucose concentration is attenuated in humans. *Diabetes* 49:1186–1193, 2000

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DW10, 10% dextrose in water; EGP, endogenous glucose production; FE_g, fractional extraction of glucose; INF, para-aminohippurate infusion rate; PAH, plasma para-aminohippurate concentration; PE, plasma glucose enrichment; RBF, renal blood flow; RGP, renal glucose production; RGU, renal glucose utilization; RPF, renal plasma flow.

During hypoglycemia, various counterregulatory hormones are released that blunt insulin action by simultaneously limiting peripheral glucose utilization and increasing glucose production (1). Earlier studies have demonstrated that hepatic glycogenolysis is responsible for the initial increase in glucose production, whereas gluconeogenesis is the primary process by which glucose production is sustained when hypoglycemia is prolonged (2–4). Recent findings (5,6) have indicated that the kidney, in addition to the liver, makes a significant contribution to glucose counterregulation. This is not surprising because glucose production in the kidney (analogous to that in the liver) is suppressed by insulin (7) and stimulated by catecholamines (8). Although substantial in vitro evidence exists that cells of the proximal convoluted tubule are able to efficiently convert 3-carbon precursors and glutamine to glucose (9,10), the in vivo data are less conclusive.

The ability of the mammalian kidney to form glucose from noncarbohydrate precursors was first described in humans by Aber et al. (11). The metabolic pathways and enzymatic steps of renal gluconeogenesis reflect largely those found in the liver of the same species; however, several differences between the liver and the kidney regarding substrate specificity are apparent. Although lactate and glycerol are common substrates for both organs (7,12–15), alanine conversion to glucose takes place almost exclusively in the liver, whereas glutamine conversion to glucose occurs predominantly in the kidney (16,17). The potential contribution of these precursors to renal gluconeogenesis during prolonged hypoglycemia has not yet been determined in humans. The present study represents an extension of previously published data (5) and was therefore undertaken to examine the role of lactate, glycerol, alanine, and glutamine uptake by the kidney in gluconeogenesis during insulin-induced hypoglycemia in humans using arteriovenous balance combined with a 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. Subjects were randomized to hypoglycemic-hyperinsulinemic ($n = 6$) or euglycemic-hyperinsulinemic ($n = 8$) clamp studies and were matched for age (33 ± 2 vs. 32 ± 4 years, respectively), sex (5 male and 3 female vs. 3 male and 3 female, respectively), body weight (73 ± 4 vs. 67 ± 4 kg, respectively), and BMI (23.8 ± 0.7 vs. 22.8 ± 1.3 kg/m^2, respectively). All individuals studied had normal fasting glucose levels, blood chemistry, and urine analysis and had no personal or family history of diabetes, hypertension, or renal disease. For 3 days before the study, all subjects had been on a weight-maintaining diet containing at least 200 g carbohydrate and had abstained from alcohol.

Protocol. Subjects were admitted to the University Hospital General Clinical Research Center at the State University of New York 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-[$^2H_2$]glucose (20–24 μmol/kg, 0.20 μmol · kg^{-1} · min^{-1}; Cambridge Isotopes, Andover, MA) and a continuous infusion of para-aminohippurate (12 mg/min; Merck, West Point, PA) were started. Subsequently, a dorsal hand vein was cannulated retrogradely and was kept in a thermoregulated Plexiglas box at 65°C for arterialized venous blood sampling (5). During the 150-min equilibration period, subjects had the 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. Catheters were advanced as far into the renal vein as possible to minimize blood sampling from left adrenal and gonadal veins. The catheter was then continuously infused with a heparinized saline solution (4.0 U/min) to maintain patency. During the baseline period (–30 to 0 min), 3 consecutive blood samples were collected simultaneously from the dorsal hand vein and the renal vein at 15-min intervals for the determination of para-aminohippurate, insulin, glucagon, catecholamines, plasma glucose concentration, and percentage of enrichment. 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 0.250 mU · kg^{-1} · min^{-1} with a concomitant variable infusion of 6-[$^2H_2$]dextrose (2% atoms percent excess 10% dextrose in water [DW10]) to achieve and maintain the plasma glucose concentration at either ~5.0 μmol/ml (euglycemic clamp) or ~3.3 μmol/ml (hypoglycemic clamp) and to keep glucose enrichment constant (18). The insulin infusion rate was selected to produce physiological hyperinsulinemia with mild to moderate hypoglycemia to mimic the evolution of clinical hypoglycemia (2). Blood samples were again collected from the dorsal hand vein and the renal vein at 15-min intervals between 150 and 180 min.

Analytical techniques. Plasma glucose was measured at the bedside with the Beckman II glucose analyzer (Fullerton, CA), and plasma para-aminohippurate concentration (PAH) 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 glucose enrichment (PE) of [$^2H_2$]glucose was measured by gas chromatography/mass spectrometry. In brief, 150 μl plasma was added to 150 μl glucose internal standard solution (5 mmol/l [U-^{13}C]glucose). Samples were deproteinized with acetonitrile and were 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 of mass of isotopomer/total mass of glucose (m/z) 297, m/z 298, m/z 299, and m/z 303 for natural [$^{13}C_1$]glucose, [$^2H_2$]glucose, and [U-^{13}C]glucose, respectively. Two sets of standards were measured containing known amounts of [$^2H_2$]glucose and [$^{13}C_1$]glucose. Isotope enrichments were calculated by multiple linear regression (25). Blood concentrations of glucose, lactate, alanine, glycerol, and glutamine were determined in triplicate samples treated with 6% perchloric acid (1:1 vol/vol) by an enzymatic assay adapted for the Technicon (Tarrytown, NY) autoanalyzer (26). The coefficient of variation for single determinations was 2.1% for blood glucose, 2.7% for lactate, 2.6% for alanine, and 3% for glutamine.

Calculations. Renal plasma flow (RPF) was calculated by para-aminohippurate clearance using the following (Eq. 1):

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

where RPF is in milliliters per minute, INF is the para-aminohippurate infusion rate in milligrams per minute, PAH is in milligrams per milliliters, a is artery, and rv is renal vein. Plasma was converted to renal blood flow (RBF) by dividing the RPF (Eq. 1) by the factor (1-hematocrit). Whole-body glucose rate of appearance (R_a) was calculated using the following steady-state formula:

$$R_a = INF/[^2H_2]PE_{(a)} \quad (2)$$

where INF is the rate of 6-[$^2H_2$]glucose infusion in micromoles · kilogram^{-1} · minute^{-1} and [$^2H_2$]PE$_{(a)}$ is the percentage of the arterial plasma glucose enriched with [$^2H_2$]glucose. During the experimental period (150–180 min), INF represents the time-varying rate of infusion of 6-[$^2H_2$]dextrose in micromoles · killigram^{-1} · minute^{-1} at each time point according to the Hot-GINF method (simultaneous infusion of deuterated glucose and DW10) (28). Underestimation of the rate of appearance of unlabeled glucose (R_a) in the systemic circulation related to deficiencies in the monocompartmental equations was minimized by maintenance of an 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 Equation 2. Renal fractional extraction of glucose (FE_g) was calculated using the following:

$$FE_g = ([Glu]_a \times PE_a - [Glu]_{rv} \times PE_{rv})/([Glu]_a \times PE_a) \quad (3)$$

where [Glu] is the blood glucose concentration, and PE is the [$^2H_2$]glucose PE. Renal glucose utilization (RGU) was calculated using the following:

$$RGU = FE_g \times [Glu]_a \times RBF \quad (4)$$

Net renal substrate balance was calculated according to the following:

$$\text{Net balance} = ([SUB]_a - [SUB]_{rv}) \times RBF \quad (5)$$

where SUB represents blood concentrations of substrates (i.e., glucose, lactate, glycerol, alanine, or glutamine). Positive values indicate net renal uptake, and negative values indicate net renal output. Net renal fractional extraction of substrates was obtained by dividing the arterial substrate concentration by the arterial–renal vein concentration difference. Renal glucose production (RGP) was calculated using the following:

$$RGP = RGU - \text{net [Glu] balance} \quad (6)$$

where net [Glu] balance is either net renal glucose uptake or output.

Statistics. All values obtained at baseline and during the experimental period were averaged, and the data are means ± SE for each period. Data obtained at baseline in each group were compared with those in the experimental period with a paired t test. Differences between baseline and study periods in each group were compared using a nonpaired t test. $P < 0.05$ was considered statistically significant (28).

RESULTS

RBF was 18.6 ± 2.6 and 17.8 ± 2.0 ml · kg^{-1} · min^{-1} at baseline and remained constant during the euglycemic and hypoglycemic clamp studies (18.8 ± 2.8 and 18.3 ± 2.6 ml · kg^{-1} · min^{-1}, respectively, P = 0.80). Arterial plasma insulin concentration increased from an average of 49 ± 14 to 102 ± 10 pmol/l and to 130 ± 25 pmol/l ($P < 0.001$) after insulin infusion at the rate of 0.250 mU · kg^{-1} · min^{-1}, respectively, during the euglycemic and hypoglycemic clamp studies. During hypoglycemia, the arterial plasma glucagon concentration increased from 105 ± 6 to 129 ± 8 ng/l ($P < 0.05$), epinephrine increased 3-fold from 116 ± 28 to 331 ± 33 ng/l ($P < 0.001$), and norepinephrine increased from 171 ± 9 to 272 ± 9 ng/l ($P < 0.01$). The arterial plasma glucagon concentration decreased from 95 ± 3 ng/l in the postabsorptive period to 82 ± 3 ng/l ($P < 0.05$) during the final 30 min of the euglycemic clamp study. Neither arterial plasma epinephrine (74 ± 8 vs. 84 ± 16 ng/l) nor norepinephrine (165 ± 20 vs. 152 ± 22 ng/l) changed significantly during the euglycemic clamp study.

Systemic and renal glucose kinetics in the postabsorptive state and after insulin infusion with either euglycemia or hypoglycemia were analyzed using blood glucose concentrations and RBF (vs. plasma glucose and plasma flow) (5) and are summarized in Tables 1–3 and Figs. 1 and 2. Individual data on blood glucose concentration, PE of glucose, and renal FE_g are shown in Tables 1 (euglycemic clamp, n = 8) and 2 (hypoglycemic clamp, n = 6). RGP increased from an average 3.0 ± 0.7 μmol · kg^{-1} · min^{-1} in the postabsorptive state to 5.4 ± 0.5 μmol · kg^{-1} · min^{-1} during hypoglycemia but decreased to 1.7 ± 0.6 μmol · kg^{-1} · min^{-1} during euglycemia (Table 3). RGU in the postabsorptive state (2.0 ± 0.6 μmol · kg^{-1} · min^{-1}) did not change significantly during hypoglycemia (2.1 ± 0.7 μmol · kg^{-1} · min^{-1}) but increased to 4.1 ± 0.8 μmol · kg^{-1} · min^{-1} during euglycemia (Table 3). As a consequence, net renal glucose output increased from 1.0 ± 0.3 to 3.3 ± 0.4 μmol · kg^{-1} · min^{-1} during hypoglycemia, whereas it reversed to a net uptake of 2.4 ± 0.7 μmol · kg^{-1} · min^{-1} during euglycemia ($P < 0.01$ hypoglycemia vs. euglycemia). Figures 1 and 2 depict changes in mean arterial and renal vein blood glucose concentrations and PE during the entire 180-min period of insulin infusion with either euglycemia (Fig. 1) or hypogly-

TABLE 1
Blood glucose concentrations and plasma [$^2H_2$]glucose enrichments in the arterialized hand vein and in the renal vein in healthy subjects in the postabsorptive state and during the last 30 min of a 180-min euglycemic-hyperinsulinemic clamp

	Postabsorptive state		Euglycemic clamp	
Subject	Artery	Renal vein	Artery	Renal vein
1				
Glc	4.30 ± 0.10	4.30 ± 0.10	3.60 ± 0.10	3.70 ± 0.10
PE	2.28 ± 0.01	2.20 ± 0.14	2.08 ± 0.12	2.00 ± 0.01
2				
Glc	4.70 ± 0.10	5.10 ± 0.10	4.30 ± 0.10	4.50 ± 0.10
PE	2.41 ± 0.01	2.20 ± 0.14	2.15 ± 0.03	2.00 ± 0.02
3				
Glc	4.60 ± 0.10	4.80 ± 0.10	4.40 ± 0.10	4.20 ± 0.10
PE	2.43 ± 0.01	2.33 ± 0.01	1.91 ± 0.02	1.95 ± 0.01
4				
Glc	4.10 ± 0.10	4.10 ± 0.10	4.10 ± 0.10	4.00 ± 0.10
PE	2.65 ± 0.01	2.54 ± 0.02	2.34 ± 0.01	2.31 ± 0.02
5				
Glc	5.10 ± 0.10	5.10 ± 0.10	4.80 ± 0.10	4.60 ± 0.10
PE	2.00 ± 0.02	1.96 ± 0.01	2.30 ± 0.01	2.27 ± 0.01
6				
Glc	4.70 ± 0.10	4.90 ± 0.10	4.10 ± 0.10	4.00 ± 0.10
PE	2.16 ± 0.01	2.07 ± 0.02	2.20 ± 0.01	2.11 ± 0.02
7				
Glc	3.80 ± 0.10	3.70 ± 0.10	4.60 ± 0.10	4.30 ± 0.10
PE	2.40 ± 0.02	2.32 ± 0.01	2.68 ± 0.01	2.66 ± 0.01
8				
Glc	4.00 ± 0.10	3.90 ± 0.10	4.50 ± 0.10	4.20 ± 0.10
PE	2.60 ± 0.01	2.50 ± 0.01	2.80 ± 0.01	2.76 ± 0.02
Glc	4.40 ± 0.20	4.50 ± 0.20	4.30 ± 0.10	4.17 ± 0.10
PE	2.37 ± 0.08	2.27 ± 0.07	2.31 ± 0.11	2.26 ± 0.11

Data are means ± SE of 3 values performed in triplicate. Blood glucose concentrations (Glc) are expressed in millimoles per liter. PEs are expressed as molar percent excess [tracer/(tracer + tracee)].

TABLE 2
Blood glucose concentrations and plasma [$^2H_2$]glucose enrichments in arterialized hand vein and in renal vein in healthy subjects in the postabsorptive state and during the last 30 min of a 180-min hypoglycemic-hyperinsulinemic clamp

	Postabsorptive state		Hypoglycemic clamp	
Subject	Artery	Renal vein	Artery	Renal vein
1				
Glc	4.60 ± 0.10	4.50 ± 0.10	3.20 ± 0.10	3.40 ± 0.10
PE	1.80 ± 0.02	1.78 ± 0.03	1.65 ± 0.02	1.50 ± 0.03
2				
Glc	4.80 ± 0.10	4.90 ± 0.10	3.10 ± 0.10	3.20 ± 0.10
PE	1.58 ± 0.02	1.52 ± 0.04	1.40 ± 0.04	1.28 ± 0.04
3				
Glc	4.20 ± 0.10	4.30 ± 0.10	2.80 ± 0.10	3.00 ± 0.10
PE	1.71 ± 0.02	1.64 ± 0.03	1.58 ± 0.02	1.42 ± 0.04
4				
Glc	4.60 ± 0.10	4.50 ± 0.10	3.10 ± 0.10	3.30 ± 0.10
PE	1.78 ± 0.03	1.75 ± 0.05	1.63 ± 0.06	1.48 ± 0.03
5				
Glc	4.70 ± 0.10	4.70 ± 0.10	3.20 ± 0.10	3.30 ± 0.10
PE	1.60 ± 0.03	1.54 ± 0.06	1.48 ± 0.04	1.34 ± 0.03
6				
Glc	4.30 ± 0.10	4.40 ± 0.10	2.90 ± 0.10	3.10 ± 0.10
PE	1.72 ± 0.04	1.64 ± 0.06	1.50 ± 0.04	1.40 ± 0.06
Glc	4.50 ± 0.10	4.50 ± 0.10	3.04 ± 0.10	3.22 ± 0.10
PE	1.70 ± 0.04	1.65 ± 0.04	1.54 ± 0.04	1.40 ± 0.04

Data are means ± SE of 3 values performed in triplicate. Blood glucose concentrations (Glc) are expressed in millimoles per liter. PEs are expressed as molar percent excess [tracer/(tracer + tracee)].

TABLE 3
Systemic and renal glucose kinetics in the postabsorptive period and during the final 30 min of a 180-min hyperinsulinemia with either hypoglycemia or euglycemia in 14 healthy subjects

	Postabsorptive	Hypoglycemia	Euglycemia
n	14	6	8
EGP	9.5 ± 0.7	10.3 ± 0.6*	5.7 ± 0.5
GDR	9.5 ± 0.7	12.7 ± 0.8	11.1 ± 0.8
RGU	2.0 ± 0.6	2.1 ± 0.7*	4.1 ± 0.8
RGP	3.0 ± 0.7	5.4 ± 0.5*	1.7 ± 0.6

Data are means ± SE in millimoles per kilogram^{-1} per minute^{-1}. EGP and rates of glucose disposal (GDR) are calculated according to Equation 2, and RGU and RGP are calculated according to Equations 4 and 6. *P < 0.05 hypoglycemia vs. euglycemia.

cemia (Fig. 2). Blood glucose concentration is consistently higher and PE is consistently lower in the renal vein than in the artery because blood glucose concentrations fell from an average postabsorptive value of 4.5 ± 0.2 mmol/l to a plateau value of 3.0 ± 0.1 mmol/l during the hypoglycemic period. In contrast, during the steady-state euglycemic period (150–180 min), blood glucose concentration in the artery (4.30 ± 0.10 mmol/l) was slightly higher (P < 0.05) than in the renal vein (4.17 ± 0.10 mmol/l), whereas plasma glucose enrichment in the artery (2.31 ± 0.11%) and in the renal vein (2.26 ± 0.11%) were comparable (NS).

Table 4 summarizes arterial and renal venous blood concentrations of lactate, alanine, glycerol, and glutamine. An ~20% increase was evident in arterial lactate, and an ~20% decrease was evident in arterial glycerol concentration during euglycemia, both of which were blunted by hypoglycemia; arterial alanine and glutamine concentrations did not change significantly during either the euglycemic or hypoglycemic clamps. Net renal fractional extraction of lactate did not change during euglycemia (0.13 ± 0.03 vs. 0.13 ± 0.05%, NS) but increased to 0.19 ± 0.5% (P < 0.05) during hypoglycemia. Similarly, net renal lactate uptake did not change during euglycemia (2.4 ± 0.5 vs. 2.8 ± 0.6 μmol · kg^{-1} · min^{-1}, NS), but increased to 3.5 ± 0.6 μmol · kg^{-1} · min^{-1} (P < 0.05) during hypoglycemia. Net renal fractional extraction of glycerol did not change during euglycemia (0.33 ± 0.08 vs. 0.30 ± 0.07%, NS) but increased to 0.49 ± 0.05% (P < 0.05) during hypoglycemia. Net renal glycerol uptake decreased from 0.6 ± 0.1 to 0.4 ± 0.1 μmol · kg^{-1} · min^{-1} (P < 0.05) during euglycemia but increased to 1.3 ± 0.2 μmol · kg^{-1} · min^{-1} (P < 0.05) during hypoglycemia. Both net renal fractional extraction of alanine (~0.03 ± 0.01%) and net renal alanine balance (range –0.07 to 0.31 μmol · kg^{-1} · min^{-1}) were negligible in the postabsorptive state and did not change significantly during either the euglycemic or hypoglycemic clamp periods. Net renal fractional extraction of glutamine decreased from 0.07 ± 0.02 to 0.01 ± 0.02% (P < 0.05) during euglycemia but increased to 0.14 ± 0.05% (P < 0.05) during hypoglycemia. Net renal glutamine uptake decreased from 0.7 ± 0.2 to 0.1 ± 0.1 μmol · kg^{-1} · min^{-1} (P < 0.05) during eugly-

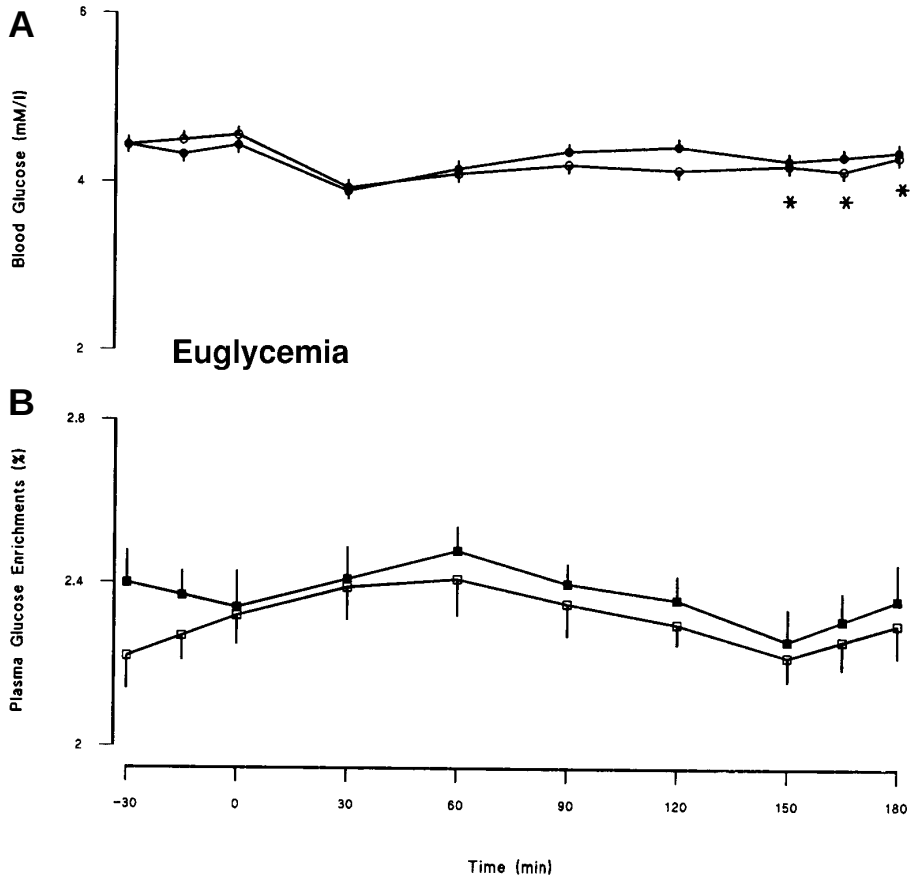

FIG. 1. Blood glucose concentration (*A*) and plasma glucose enrichment (*B*) in the artery (● and ■) and renal vein (○ and □) in the postabsorptive period (–30 to 0 min) and during a 180-min euglycemic-hyperinsulinemic clamp period in 8 healthy subjects. Data are means ± SE and are derived from all measurements performed in triplicate (concentrations) and duplicate (enrichments) at each time point. *P < 0.05 vs. baseline.

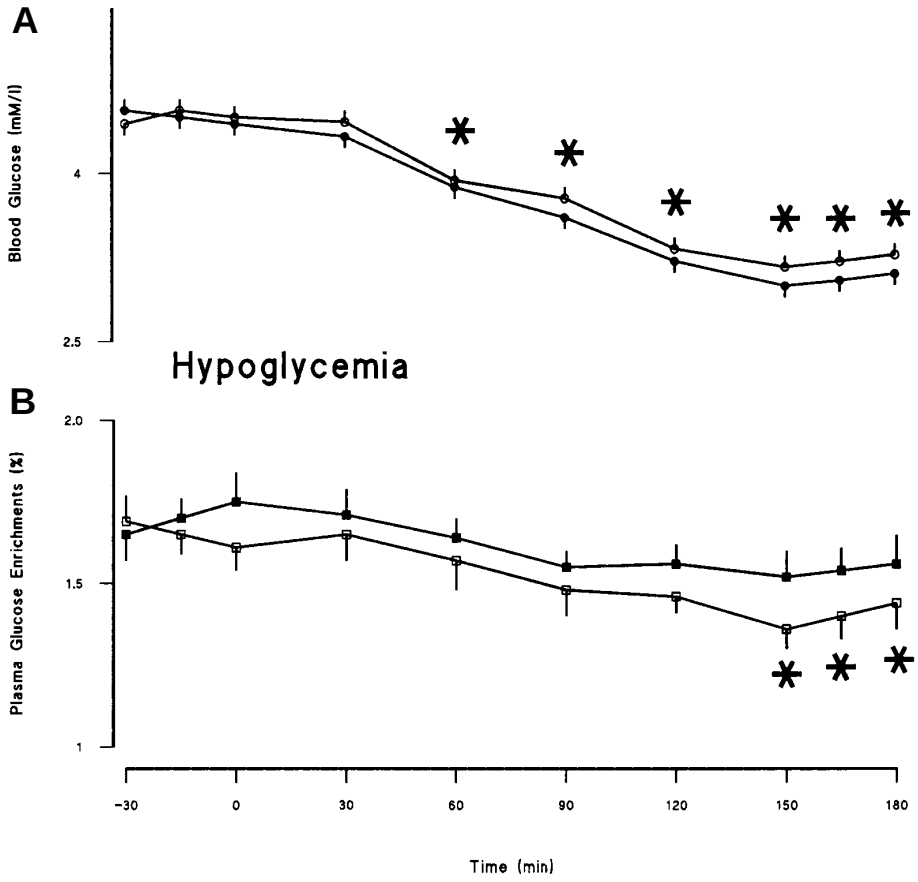

FIG. 2. Blood glucose concentration (*A*) and plasma glucose enrichment (*B*) in the artery (● and ■) and renal vein (○ and □) in the postabsorptive period (–30 to 0 min) and during a 180-min hypoglycemic-hyperinsulinemic clamp period in 6 healthy subjects. Data are means ± SE and are derived from all measurements performed in triplicate (concentrations) and duplicate (enrichments) at each time point. *$P < 0.05$ vs. baseline.

cemia but increased to 1.1 ± 0.2 μmol · kg^{-1} · min^{-1} ($P < 0.05$) during hypoglycemia.

Assuming that renal extraction of these gluconeogenic precursors is entirely diverted toward gluconeogenesis in the kidney, and considering that 2 mol each of lactate, glycerol, and alanine and 1.2 mol glutamine (9) are required for every mole of glucose synthesized de novo, we have estimated the potential contribution of these circulating substrates to RGP (Fig. 3) under these experimental conditions. In the postabsorptive state, circulating lactate could account for 1.2 μmol · kg^{-1} · min^{-1}, glycerol for 0.3 μmol · kg^{-1} · min^{-1}, alanine for 0 μmol · kg^{-1} · min^{-1}, and glutamine for 0.6 μmol · kg^{-1} · min^{-1} of renal glucose equivalents. Although the contributions of lactate (1.4 μmol · kg^{-1} · min^{-1}) and alanine (0.02 μmol · kg^{-1} · min^{-1}) did not change significantly during euglycemia, those of glycerol (0.2 μmol · kg^{-1} · min^{-1}) and glutamine

TABLE 4
Blood concentrations in arterialized hand vein and arterial-renal vein ([A-RV]) concentration differences in lactate, alanine, glycerol, and glutamine in the postabsorptive state and during the final 30 min of a 180-min hyperinsulinemic period with either hypoglycemia or euglycemia in 14 healthy subjects

	Postabsorptive	Hypoglycemia	Euglycemia
n	14	6	8
Arterial lactate	968 ± 227	974 ± 28	1,123 ± 50
[A-RV]	127 ± 24	190 ± 46*	151 ± 21
Arterial alanine	450 ± 20	529 ± 34	592 ± 18
[A-RV]	–4 ± 19	17 ± 42	2 ± 24
Arterial glycerol	100 ± 18	144 ± 17†	78 ± 6
[A-RV]	33 ± 18	70 ± 17†	27 ± 6
Arterial glutamine	506 ± 23	397 ± 11	423 ± 15
[A-RV]	35 ± 18	55 ± 27*	3 ± 26

Data are *n* or means ± SE in micromoles per liter. *$P < 0.05$; †$P < 0.01$, hypoglycemia vs. euglycemia.

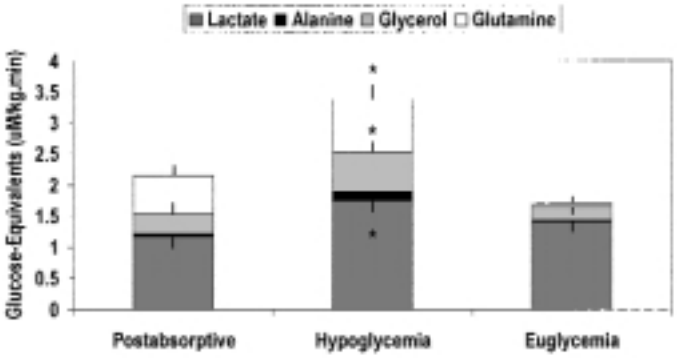

FIG. 3. Net contribution of lactate, alanine, glycerol, and glutamine to tracer-determined RGP in micromoles · kilogram^{-1} · minute^{-1} in the postabsorptive state and during the final 30 min of a 180-min hyperinsulinemic period with either hypoglycemia or euglycemia in healthy subjects. Net renal substrate balance was calculated using Equation 5, and RGP was calculated using Equation 6. Precursor contribution to glucose production assumes that 2 mol each of lactate, glycerol, and alanine, and 1.2 mol glutamine are required for each mole of glucose and that substrates extracted from the circulation are entirely diverted toward gluconeogenesis in the kidney. Postabsorptive values represent an average of all 14 subjects. *P < 0.05 hypoglycemia vs. euglycemia.

(0.1 µmol · kg^{-1} · min^{-1}) decreased (P < 0.05). During hypoglycemia, however, the contributions of lactate (1.8 µmol · kg^{-1} · min^{-1}), glycerol (0.7 µmol · kg^{-1} · min^{-1}), and glutamine (0.9 µmol · kg^{-1} · min^{-1}) increased significantly (all P < 0.05) and together may account for ~60% of renal gluconeogenesis. The contribution of alanine to RGP remained negligible (0.1 µmol · kg^{-1} · min^{-1}) during hypoglycemia.

DISCUSSION

The present studies confirm previous findings in dogs (14,15) that indicated that mild to moderate hypoglycemia caused by sustained insulin infusion in humans is accompanied by enhanced renal gluconeogenesis and further demonstrated that utilization of gluconeogenic precursors by the kidney is increased during the process of glucose counterregulation. Renal conversion of circulating lactate, glycerol, and glutamine could account for nearly 60% of all glucose carbons released in the renal vein after 3 h of insulin infusion with sustained blood glucose concentrations of ~3.0 mmol/l. Moreover, our data indicate that alanine conversion to glucose by the kidney (in contrast with that in the liver) is negligible, whereas renal glutamine gluconeogenesis appears to be enhanced in these hypoglycemic conditions. These observations are consistent with the documented limited biochemical capacity of the kidney to produce glucose from alanine (29) and further support the notion of organ substrate selectivity (i.e., that alanine is converted to glucose exclusively by the liver and that glutamine is utilized preferentially by the kidney) (16). Preferential utilization of alanine by the liver and of glutamine by the kidney may reflect differences in amino acid uptake and transport in each organ. Glutamine transport across tubular cell membranes is hormone sensitive (A system), whereas the N system in hepatocytes is not (30). Thus, renal (but not hepatic) glutamine transport may be responsive to counterregulatory hormones. Furthermore, in contrast with alanine, glutamine supply to the liver is substantially decreased, considering that portal blood flow accounts for ~70% of total hepatic blood flow (31) and that rates of glutamine utilization by portal-draining viscera (particularly the intestines) are very high (32). The extent to which this reduced load of glutamine (and the correspondingly increased load of alanine) to the liver contributes to organ selectivity is not known. Even though the mechanisms for organ substrate selectivity are not entirely clear, our findings support the notion that systemic tracer measurements of alanine conversion to glucose reflect primarily hepatic gluconeogenesis and that similar measurements of glutamine provide estimates of renal gluconeogenesis.

By measuring substrate concentration in blood sampled directly from the renal vein simultaneously with the tracer infusion technique, our data complement previous findings (2) and provide strong evidence that, in humans (analogous to dogs), renal utilization of circulating substrates becomes an additional source of glucose during sustained hypoglycemia. According to our data, circulating lactate represents a major contributor to RGP both in the postabsorptive state and during hypoglycemia. Despite the fact that arterial blood lactate concentrations remained unaltered during hypoglycemia, renal fractional extraction of lactate increased by 50%, which suggests that the increase in net lactate uptake by the kidney results from changes in transport across plasma membranes most likely mediated by counterregulatory hormones. Additionally, an increase in the overall gluconeogenic efficiency in the kidney (15) may have sustained augmented rates of RGP. The contribution to systemic gluconeogenesis of lactate conversion to glucose by the human kidney suggested in these experimental conditions is of particular importance because increased lactate recycling in diabetes is presumed to occur exclusively in the liver (33), and lactate conversion to glucose in the liver has been shown to be inhibited by antihyperglycemic agents used in the treatment of diabetes (34). Whether renal gluconeogenesis contributes to lactate recycling in diabetes and whether the process in the kidney (analogous to that in the liver) is responsive to these drugs are currently unknown.

Although hormonal changes in these hypoglycemic conditions are rather mild, increases in net renal fractional extraction of glycerol (50%) and glutamine (100%) (despite minimal changes in blood levels) suggest that enhanced renal gluconeogenesis from circulating substrates during hypoglycemia in humans may be hormonally mediated and not secondary to changes in substrate supply (10). Our data are consistent with earlier in vivo human studies that indicated that net renal glucose output is increased during fasting (35) and in acidosis (36) despite reduced substrate availability, and our data lend further support to the hypothesis that hepatic and renal gluconeogenesis are regulated by common neurohormonal regulation. Of additional interest, however, is the fact that, because renal glutamine gluconeogenesis is invariably accompanied by ammonia formation (which facilitates acid excretion and restores bicarbonate) (37), the possibility that glutamine gluconeogenesis is driven by mechanisms involved in pH regulation during hypoglycemia cannot be ruled out.

Our calculated rates of renal gluconeogenesis from lactate, glycerol, and glutamine should not be viewed in absolute terms, for various reasons. First, estimation of substrate incorporation into glucose by arteriovenous concentration differences represents a maximum possible contribution and assumes that all circulating precursors extracted by the kidney are diverted into glucose production de novo. Second,

although the amount of intracellularly derived substrates available for gluconeogenesis is likely to be negligible, glycerol and glutamine are not formed to a significant extent, and lactate is generated in the distal nephron whereas gluconeogenesis takes place in proximal tubules (10), these techniques do not account for any intracellular sources. Finally, by limiting our analyses to circulating lactate, glycerol, alanine, and glutamine, we were unable to identify the source of the 30–40% of glucose carbons released in the renal vein both in the postabsorptive period and during hypoglycemia. Because the distal nephron lacks glucose-6-phosphatase activity (10), the contribution of glycogen degradation to glucose production by the kidney is presumably insignificant. On the other hand, tracer-determined RGP rates may be systematically overestimated by our methods, which is suggested by discrepancies between our findings and those of other investigators (38).

Considering that RGP rates are higher in our studies than in those reported by Ekberg et al. (38) in the postabsorptive state, we may have conceivably overestimated RGP rates during hypoglycemia. The fact, however, that increases in RGP detected by the tracer dilution technique closely agree with data obtained by measurements of gluconeogenic precursor extraction during hypoglycemia supports our hypothesis that glucose production by the kidney is enhanced in response to a decrease in blood glucose. According to our data, the kidney may contribute between 30% (gluconeogenic precursor extraction) and 50% (glucose tracer data) of EGP during counterregulation in these mild to moderate hypoglycemic conditions. Similar observations by an independent group of investigators (39) further support our original hypothesis. Our measurements based on whole blood rather than plasma analyses yielded greater rates of glucose production and utilization by the kidney in the same group of individuals. When considering that glucose is exchanged across organs in whole blood, the current data are more likely to reflect true rates of glucose production and utilization than those published earlier (5). Although the latter represents a significant improvement in our method, a discrepancy between our findings and those of others (38) still remains.

The reasons for discrepant findings in postabsorptive human subjects are not yet entirely clear but can be partially explained by differences in isotopes used (given that the small arterial–renal venous differences in disintegrations per minute counts and in glucose enrichment infusion of higher doses of isotopes with maintenance of higher plasma glucose specific activity and/or enrichments are more likely to detect label dilution than conventional doses), catheterization, and blood sampling techniques (e.g., contamination of the left renal vein by blood draining from the adrenal and gonadal veins could have led to overestimation of left and birenal glucose utilization and production rates, whereas contamination by backflow from the inferior vena cava could have the opposite effect). Moreover, in contrast with a population of male Caucasian subjects studied by Ekberg et al. (38), in our series, women represented 50% of the study population, and African-Americans and Hispanics together accounted for 40% of all subjects studied. Our subjects are an average of 5 years younger than those reported by Ekberg et al. (38). The extent to which differences in techniques, age, sex, and ethnic background among subjects in these studies are responsible for some of these discrepant findings cannot be determined at this point. Nonetheless, although the exact contribution of the human kidney to glucose production and utilization in the postabsorptive state remains controversial, the fact that the kidney makes a contribution to postabsorptive EGP has been recently confirmed with the use of highly sensitive isotopes ([U-^{13}C]glucose) by 2 independent groups of investigators (38,40).

In summary, we have demonstrated that extraction of circulating lactate, glycerol, and glutamine (but not alanine) by the human kidney is enhanced and may account for ~60% of the compensatory rise in RGP, which is equivalent to ~20% of systemic glucose production during sustained mild to moderate hypoglycemia. These findings strongly suggest that increased utilization of circulating substrates directed toward gluconeogenesis by the kidney is an additional mechanism by which the decrease in blood glucose concentration can be attenuated. Our observations further support the notion of organ selectivity with preferential utilization of alanine by the liver and of glutamine by the kidney. We conclude that utilization of circulating gluconeogenic precursors by the kidney is stimulated during counterregulation and represents an additional physiological mechanism in defending against hypoglycemia in humans.

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REFERENCES

1. Cryer P: Glucose counterregulation in man. *Diabetes* 30:261–264, 1981
2. Lecavalier L, Bolli G, Cryer PE, Gerich J: Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. *Am J Physiol* 256:E844–E851, 1989
3. Frizzell RT, Hendrick GH, Biggers DW, Lacy DB, Donohue DP, Green DR, Carr RK, Williams PE, Stevenson RS, Cherrington AD: Role of gluconeogenesis in sustaining glucose production during hypoglycemia caused by continuous insulin infusion in conscious dogs. *Diabetes* 37:749–759, 1988
4. Davis SN, Dobbins R, Tarumi C, Neal JD, Cherrington AD: Paradoxical insulin-induced increase in gluconeogenesis in response to prolonged hypoglycemia in conscious dogs. *Am J Physiol* 268:E521–E530, 1995
5. Cersosimo E, Garlick P, Ferretti J: Renal glucose production during insulin-induced hypoglycemia in humans. *Diabetes* 48:261–266, 1999
6. Meyer C, Dostou JM, Gerich JE: Role of the human kidney in glucose counterregulation. *Diabetes* 48:943–948, 1999
7. Cersosimo E, Garlick P, Ferretti J: Insulin regulation of renal glucose metabolism in humans. *Am J Physiol* 276:E78–E84, 1999
8. Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierrez O, Gerich J: Uptake and release of glucose by the human kidney: postabsorptive rates and responses to epinephrine. *J Clin Invest* 96:2528–2533, 1995
9. Krebs HA, Hems R, Gascoyne T: Renal gluconeogenesis IV: gluconeogenesis from substrate combinations. *Acta Biol Med Ger* 11:607– 615, 1963
10. Wirthensohn G, Guder W: Renal substrate metabolism. *Physiol Rev* 66:469–497, 1986
11. Aber GM, Morris LO, Housley E: Gluconeogenesis by the human kidney. *Nature (Lond)* 212:1589–1590, 1966
12. Ahlborg G, Felig P: Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *J Clin Invest* 69:45–54, 1982
13. Tayek JA, Katz J: Glucose production, recycling and gluconeogenesis in normals and diabetics: a mass isotopomer [U-^{13}C]glucose study. *Am J Physiol* 270: E709–E717, 1996
14. Cersosimo E, Judd RL, Miles JM: Insulin regulation of renal glucose metabo-

lism in conscious dogs. *J Clin Invest* 93:2584–2589, 1994
15. Cersosimo E, Molina PE, Abumrad NN: Renal lactate metabolism and gluconeogenesis during insulin-induced hypoglycemia. *Diabetes* 47:1101–1106, 1998
16. Stumvoll M, Meyer C, Perriello G, Kreider M, Welle S, Gerich J: Human kidney and liver gluconeogenesis: evidence for organ substrate selectivity. *Am J Physiol* 274:E817–E826, 1998
17. Cersosimo E, Zaitseva IN, Ajmal M: β-Adrenergic mechanisms and substrate availability to splanchnic tissues and kidney in hypoglycemia (Abstract). *Diabetes* 48 (Suppl. 1):A121, 1999
18. Finegood D, Bergman R, Vranic M: Modeling error and apparent discrimination confound estimation of endogenous glucose production during euglycemic glucose clamps. *Diabetes* 37:1025–1034, 1988
19. Brun C: A rapid method for the determination of para-aminohippurate acid in kidney function tests. *J Lab Clin Med* 37:955–958, 1951
20. Herbert V, Lau K, Gotlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
21. Aguillar-Parada E, Eisentraut AM, Unger UH: Pancreatic glucagon secretion in normal and diabetic subjects. *Am J Med Sci* 257:415–419, 1969
22. Cryer PE, Santiago J, Shah D: Measurement of norepinephrine and epinephrine in small volumes of human plasma by a single isotope derivative method: response to the upright posture. *J Clin Endocrinol Metab* 39:1025–1029, 1974
23. Van Goudoever JB, Sulkers E, Chapman TE, Carnielli V, Efstatopoulos T, Degenhart HJ, Sauer PJ: Glucose kinetics and glucoregulatory hormone levels in ventilated preterm infants on the first day of life. *Pediatr Res* 33:583–589, 1993
24. Vogt JA, Chapman TE, Wagner DA, Young VR, Burke JF: Determination of the isotope enrichment of one or a mixture of two stable labelled tracers of the same compound using the complete isotopomer distribution of an ion fragment: theory and application to in vivo human tracer studies. *Biol Mass Spectrom* 22:600–612, 1993
25. Beylot MC, Martin C, Beaufrere B, Riou JP, Morneaux R: Determination of steady-state and nonsteady-state glycerol kinetics in humans using deuterium-labeled tracer. *J Lipid Res* 28:414–422, 1987
26. Lloyd B, Burrin P, Smythe P, Alberti KGGM: Enzymatic fluorometric continuous flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. *Clin Chem* 24:1724–1729, 1978
27. Butler PC, Cuomo A, Zerman A, O'Brien PC, Cobelli C, Rizza RA: Methods of assessment of the rate of onset and offset of insulin action during nonsteady state in humans. *Am J Physiol* 27:E548–E560, 1993
28. Rosner B: *Fundamentals of Biostatistics*. Boston, MA, PWS-Kent, 1990, p. 245–265
29. DeRosa G, Swick R: Metabolic implications of the distribution of the alanine aminotransferase isoenzymes. *J Biol Chem* 2501:7961–7967, 1975
30. Christensen H: Role of amino acid transport and counter-transport in nutrition and metabolism. *Physiol Rev* 70:43–77, 1990
31. Leevy CM: Estimation of hepatic blood flow with indocyanine green. *J Clin Invest* 41:1169–1179, 1962
32. Felig P, Wahren J, Karl I, Cerasi E, Luft R, Kipnis D: Glutamine and glutamate metabolism in normal and diabetic subjects. *Diabetes* 22:573–576, 1973
33. Reichard GA Jr, Moury NF, Hochella NJ, Patterson AL, Weinhouse S: Quantitative estimation of the Cori cycle in the human. *J Biol Chem* 238:E463–E470, 1985
34. Radziuk J, Zhang Z, Wiensperger N, Pye S: Effects of metformin on lactate uptake and gluconeogenesis in the perfused rat liver. *Diabetes* 46:1406–1413, 1997
35. Cahill GF Jr: Starvation in man. *N Engl J Med* 282:668–675, 1970
36. Goodman AD, Fuisz RE, Cahill GF Jr: Renal gluconeogenesis in acidosis, alkalosis and potassium deficiency: its possible role in regulation of renal ammonia production. *J Clin Invest* 45:612–619, 1966
37. Halperin ML, Jungas RL: Metabolic production and renal disposal of hydrogen ions. *Kidney Int* 24:709–713, 1983
38. Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J: Contributions by the kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes* 48:292–298, 1999
39. Meyer C, Dostou JM, Gerich JE: Role of the human kidney in glucose counterregulation. *Diabetes* 48:943–948, 1999
40. Moller N, Andrews J, Bigelow M, Rizza R, Nair KS: Assessment of postabsorptive renal glucose metabolism in humans with multiple glucose tracers (Abstract). *Diabetes* 48 (Suppl. 1):A291, 1999