Regulation of Splanchnic and Renal Substrate Supply by Insulin in Humans

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To determine the effects of peripheral insulin infusion on total, hepatic, and renal glucose production and on the percent contribution to glucose production of gluconeogenesis versus glycogenolysis, 10 healthy subjects had arterialized hand and hepatic vein catheterization after an overnight fast and the results were compared with data from 12 age- and weight-matched subjects with renal vein catheterization during a 180-minute infusion of either insulin (0.25 mU/kg · min) with dextrose, or saline. Endogenous, hepatic, and renal glucose production was measured with [6,6-$^2H_2$]glucose, regional lactate, alanine, and glycerol balance by arteriovenous difference; hepatic blood flow by indocyanine green clearance; and renal blood flow by *p*-aminohippurate clearance, before and every 30 minutes during each infusion period. Insulin increased from about 42 to 98 pmol/L and blood glucose remained constant in all studies (3.8 ± 0.2 *v* 4.4 ± 0.1 μmol/ml, hepatic *v* renal vein). In response to insulin infusion, endogenous, hepatic, and renal glucose production decreased immediately (30 minutes) and reached a lower plateau value (10.8 ± 0.8 *v* 6.4 ± 0.7, 10.4 ± 1.1 *v* 7.8 ± 1.0, and 2.8 ± 0.6 *v* 1.5 ± 0.6 μmol/kg · min, respectively) between 120 and 180 minutes (all $P < .05$). Net renal uptake of lactate (2.4 ± 0.4 *v* 0.9 ± 0.6) decreased earlier (30 minutes) and returned to baseline between 120 and 180 minutes (2.4 ± 0.5 μmol/kg · min), whereas net splanchnic uptake of lactate (5.7 ± 0.7 *v* 0.7 ± 0.6) and alanine (1.8 ± 0.1 *v* 1.0 ± 0.5 μmol/kg · min) decreased later (120 to 180 minutes). Net renal (0.3 ± 0.1 *v* 0.1 ± 0.1) and splanchnic (0.7 ± 0.3 *v* 0.4 ± 0.2 μmol/kg · min) glycerol uptake decreased 90 to 180 minutes after insulin and increased ($P < .05$) with saline infusion (0.4 ± 0.1 *v* 0.6 ± 0.3 and 1.0 ± 0.5 *v* 1.8 ± 0.4 μmol/kg · min, respectively). These data indicate that the rapid suppression of endogenous glucose production by insulin reflects primarily a decrease in hepatic glucose release, most likely due to inhibition of net glycogenolysis, combined with suppression of renal gluconeogenesis. Inhibition of hepatic gluconeogenesis presumably occurs later during hyperinsulinemia. We conclude that peripheral insulin, in addition to its inhibition of glycogen degradation, regulates endogenous glucose production, in part, by modifying the splanchnic and renal substrate supply.

GLUCONEOGENESIS plays a central role in the maintenance of glucose homeostasis in humans and contributes to approximately 50% of glucose production in the postabsorptive state and nearly all glucose production during prolonged fasting.[1,2] Based on prior experiments in humans[3,4] and animals[5] that have demonstrated a close correlation between the rate of glucose production and substrate flux,[3] it is believed that control of the gluconeogenic precursor supply represents an important mechanism in the regulation of glucose production. Although there is ample evidence indicating that insulin can immediately reduce glucose release by the liver,[6-9] more recent studies have shown that glucose production is regulated by both direct hepatic and extrahepatic effects of insulin.[10] Actually, it has been suggested that peripheral insulin dominates the suppression of glucose production and that this effect is mediated by plasma free fatty acids.[11,12] These observations support the notion that peripheral insulin regulates glucose production by modifying the availability of energy and substrates for gluconeogenesis. Most studies[4,6-9] evaluating the relationship between substrate supply and glucose production have focused on hepatic glucose production and ignore the potential contribution of the kidney to systemic gluconeogenesis.

It has long been recognized that in addition to the liver, the kidney is the only other organ capable of releasing glucose into the circulation.[3,13] Renal glucose production derives primarily from the conversion of precursors such as lactate, glycerol, and glutamine to glucose in the proximal tubules.[13] The physiological reserve of the kidney with regard to glucose production is well documented[3] and is not in question. More recent observations in humans in some studies,[14,15] but not all,[16] indicating that renal glucose production may be responsible for 5% to 25% of postabsorptive glucose production and that glucose production and utilization by the kidney are sensitive to hormone action[15,17] have reawakened our interest in renal carbohydrate metabolism. Furthermore, these findings raise the possibility that changes in substrate availability affect both hepatic and renal gluconeogenesis. The current studies were therefore undertaken to investigate the extent to which insulin suppression of endogenous glucose production reflects a modification in the substrate supply to the liver and kidney in normal subjects using arteriovenous balance measurements combined with an isotope dilution technique during euglycemic-hyperinsulinemic conditions.

SUBJECTS AND METHODS

Subjects

Informed written consent was obtained from 22 healthy subjects after the protocol was approved by our local Institutional Review Board. All subjects had normal fasting glucose and no personal or family history of diabetes, hypertension, or renal disease (Table 1). For 3 days before the study, all were on a weight-maintenance diet containing at least 200 g carbohydrate and abstained from alcohol.

Protocol

Subjects were admitted to the University Hospital General Clinical Research Center at SUNY, Stony Brook, after an overnight fast between 6 and 7 on the morning of the experiments. An antecubital vein was cannulated and a primed-continuous infusion of [6,6-$^2H_2$]glucose (1.8

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Submitted July 19, 1999; accepted October 20, 1999.

Supported in part by grants from the American Diabetes Association and National Institutes of Health (DK49861) and General Clinical Research Center NIH Grant No. M01-RR10710.

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0026-0495/00/4905-0007$10.00/0

Table 1. Characteristics (mean ± SEM) of 22 Subjects Studied in the Postabsorptive State Following Peripheral Infusion of Either Saline (control) or Insulin 0.250 mU/kg · min

Characteristic	Saline		Insulin	
	HV Cath (n = 4)	RV Cath (n = 4)	HV Cath (n = 6)	RV Cath (n = 8)
Age, yr (range)	32 ± 4 (23-46)	30 ± 5 (21-44)	34 ± 4 (22-45)	33 ± 3 (27-42)
Gender (male/female)	2/2	2/2	3/3	4/4
Weight (kg)	72 ± 5	71 ± 6	74 ± 6	81 ± 4
BMI (kg/m^2)	22 ± 2	23 ± 1	24 ± 1	26 ± 2

Abbreviations: HV cath, hepatic vein catheterization; RV cath, renal vein catheterization; M, male; F, female; BMI, body mass index.

mmol, 18.0 μmol/min; Cambridge Isotope Laboratories, Andover, MA) and either indocyanine green (0.24 mg/min; Becton-Dickinson, Cockeysville, MD) or *p*-aminohippurate (12.0 mg/min; Merck, West Point, PA) was started. The tracer infusion rate was chosen to produce steady-state arterial, hepatic, and renal venous plasma glucose enrichment to permit detection of a difference as low as 4% across the kidney.[18] Subsequently, a dorsal hand vein was cannulated retrogradely and kept in a thermoregulated Plexiglas (Rohm and Haas, Philadelphia, PA) box at 65°C for sampling arterialized venous blood.[19] During the 150 minute equilibration period, the subjects had the left renal vein (n = 12) or right hepatic vein (n = 10) 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 (−30 to 0 minutes), 3 consecutive blood samples were collected simultaneously from the dorsal hand vein and either the renal or hepatic vein at 15-minute intervals for determination of plasma *p*-aminohippurate, indocyanine green, and insulin, blood lactate, alanine, glycerol, and glucose, and plasma [6,6-$^2H_2$]glucose enrichment. At 0 minutes, upon completion of the baseline collections, subjects were randomized to receive a 180-minute continuous peripheral infusion of either insulin 0.25 mU/kg · min with a concomitant variable infusion of [6,6-$^2H_2$]dextrose mixed in a solution containing 10 g/100 mL dextrose (DW10) (2% atom percent excess) to maintain the plasma glucose concentration and enrichment constant, or saline. The insulin infusion rate was selected to produce elevations in plasma insulin to reduce "hepatic glucose production" by less than 50%, based on a previous report.[20] Normal saline (0.9% NaCl) was infused at a rate of 50 mL/h throughout the entire 180-minute experimental period in 8 individuals, 4 with hepatic vein catheterization and 4 with renal vein catheterization, representing the control group. Blood samples were collected from the dorsal hand, renal, or hepatic vein at 30-minute intervals from 0 to 180 minutes. The total radioactive exposure of the subjects during the catheterization procedure did not exceed 200 mrad.

Analytical Techniques

Plasma glucose was measured at the bedside with the Beckman II Glucose Analyzer (Fullerton, CA). Indocyanine green[21] and *p*-aminohippurate[22] were determined by a colorimetric method and insulin by radioimmunoassay.[23] Blood concentrations of glucose, lactate, alanine, and glycerol were determined in samples deproteinized with 6% (wt/vol) perchloric acid (1 mL blood and 1 mL perchloric acid) by enzymatic assays.[24] Plasma enrichment 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 evaporated to dryness. Derivatization was performed with butane boronic acid in pyridine and acetic anhydride.[25] The glucose derivative was quantified by selective ion monitoring at *m/z* 297, *m/z* 299, and *m/z* 303 for natural [$^{12}C_1$], [$^2H_2$], and [U-^{13}C]glucose, respectively. One set of standards were measured containing known amounts of [$^2H_2$]glucose. Isotopic enrichment was calculated by multiple linear regression.[26,27] For selected samples, a set of standards containing 0 to 10 mmol glucose and 5 mmol [U-^{13}C]glucose internal standard were used to calculate plasma glucose.

Calculations

Hepatic plasma flow was calculated by indocyanine green clearance using the equation,

$$HPF = INF/([ICG]_{(a)} - [ICG]_{(hv)}), \quad (1)$$

where HPF is hepatic plasma flow in milliliters per minute, INF is the indocyanine green infusion rate in milligrams per minute, and [ICG] is the plasma indocyanine green concentration in milligrams per milliliter in the hepatic vein (hv) and artery (a). Renal plasma flow was calculated by *p*-aminohippurate clearance using the analogous equation,

$$RPF = INF/([PAH]_{(a)} - [PAH]_{(rv)}), \quad (2)$$

where RPF is renal plasma flow in milliliters per minute, INF is the *p*-aminohippurate infusion rate in milligrams per minute, and [PAH] is the plasma *p*-aminohippurate concentration in milligrams per minute. Hepatic and renal plasma flow were converted to hepatic (HBF) and renal (RBF) blood flow by the (1 hematocrit) factor. The whole-body glucose rate of appearance (Ra) was calculated using the steady-state formula,

$$Ra = INF/[^2H_2]PE_{(a)}, \quad (3)$$

where INF is the rate of [6,6-$^2H_2$]glucose infusion in micromoles per kilogram per minute and [$^2H_2$]PE$_{(a)}$ is the percentage of arterial plasma glucose enriched with [$^2H_2$]glucose. During the experimental period (0 to 180 minutes), INF represents the time-varying rate of infusion of [6,6-$^2H_2$]dextrose in micromoles per kilogram per minute at each time point, according to the "hot-GINF" method.[20,30] Underestimation of the unlabeled glucose Ra in the systemic circulation related to deficiencies in the monocompartmental equations was minimized by maintenance of near-isotopic steady state during the entire experiment. The endogenous glucose production rate was calculated by subtracting the infusion rate of exogenous dextrose, including [6,6-$^2H_2$]glucose tracer, from the Ra in Eq 3. Net splanchnic glucose, lactate, alanine, and glycerol balance were calculated by the product of the arterial-hepatic venous blood concentration difference for each metabolite and hepatic blood flow. Similarly, net renal glucose, lactate, alanine, and glycerol balance were calculated by the product of the arterial-renal venous blood concentration difference for each metabolite and renal blood flow. Splanchnic fractional extraction of glucose (Spl-FE$_g$) was calculated using the formula,

$$SpI\text{-}FE_g = ([GIc]_a \times PE_a - [GIc]_{hv} \times PE_{hv})/([GIc]_a \times PE_a), \quad (4)$$

where $[Glc]_{hv}$ is the blood glucose concentration in the hepatic vein and PE_{hv} refers to $[^2H_2]$glucose plasma enrichment in the hepatic vein. Splanchnic glucose uptake (SGU) was calculated using the formula,

$$SGU = Spl\text{-}FE_g \times [Glc]_a \times HBF, \quad (5)$$

and hepatic glucose production (HGP) was calculated using the formula,

$$HGP = SGU + \{([Glc]_{hv} - [Glc]_a) \times HBF\}. \quad (6)$$

Renal fractional extraction of glucose ($Ren\text{-}FE_g$), renal glucose uptake (RGU), and renal glucose production (RGP) were calculated using the analogous formulae,

$$Ren\text{-}FE_g = ([Glc]_a \times PE_a - [Glc]_{rv} \times PE_{rv})/([Glc]_a \times PE_a), \quad (7)$$

$$RGU = Ren\text{-}FE_g \times [Glc]_a \times RBF, \quad (8)$$

$$\text{and } RGP = RGU + \{([Glc]_{rv} - [Glc]_a \times RBF\}, \quad (9)$$

where $[Glc]_{rv}$ is the blood glucose concentration in the renal vein and PE_{rv} refers to $[^2H_2]$glucose plasma enrichment in the renal vein. Since glycosuria was not present, renal glucose utilization was assumed to be equal to glucose uptake. Data for systemic and renal glucose tracer kinetics obtained in the renal vein catheterization group are discussed in detail elsewhere[17]; however, the data are presented here to enable direct comparison to results obtained in the hepatic vein catheterization studies.

Statistics

All values for plasma glucose enrichment and blood glucose and substrate concentrations in each study during the baseline and experimental periods were used in the calculations and are expressed as the mean ± SEM. The coefficient of variation for the blood glucose measurement was between 3% and 6%; for plasma glucose enrichment, about 3%; and for hepatic/renal blood flow, between 6% and 8%. As a consequence, the calculated coefficient of variation for hepatic and renal glucose production was 8.85%. All values obtained for splanchnic and renal glucose fractional extraction, positive and negative, were used in the calculations. Mean data at baseline in each group were compared with those from the last 30 minutes of the study period using a paired *t* test. Changes in each group over time were assessed using 1-way ANOVA, and mean differences between the baseline and experimental periods between groups were compared with repeated-measures 2-way ANOVA.[28] A P value less than .05 was considered statistically significant.

RESULTS

The arterial plasma insulin concentration increased from 46 ± 8 to a mean of 94 ± 7 pmol/L ($P < .01$) in the hepatic vein catheterization group and from 37 ± 8 to a mean of 102 ± 10 pmol/L ($P < .01$) in the renal vein catheterization group following insulin infusion at a rate of 0.250 mU/kg · min. Arterial insulin did not change in either the hepatic vein catheterization (32 ± 5 *v* 29 ± 6 pmol/L) or renal vein catheterization (30 ± 7 *v* 35 ± 8 pmol/L) saline control groups. Mean hepatic blood flow was 20.0 ± 0.7 mL/kg · min at baseline and remained constant during either the insulin (19.6 ± 1.5 mL/kg · min) or saline (19.1 ± 1.1 mL/kg · min) infusion. Similarly, mean renal blood flow was 14.4 ± 0.9 mL/kg · min at baseline and did not change significantly during the study period (16.1 ± 1.5 and 13.0 ± 1.0 mL/kg · min in the insulin and saline infusion groups, respectively). The blood glucose concentration and plasma $[^2H_2]$glucose enrichment were maintained nearly constant during the entire 180-minute experimental period in both hepatic and renal vein catheterization studies.

Endogenous glucose production decreased by about 65% and hepatic glucose production by about 75% after 180 minutes of insulin infusion, whereas neither changed significantly with saline infusion. The mean exogenous glucose infusion rate during the euglycemic-hyperinsulinemic clamp was 5.6 ± 0.8 μmol/kg · min. Endogenous and hepatic glucose production rates during insulin infusion are shown in Table 2. Splanchnic glucose utilization increased from 1.37 ± 0.40 at baseline to 2.38 ± 0.50 μmol/kg · min ($P < .05$) after 180 minutes of insulin infusion, but it did not change with saline infusion (1.23 ± 0.50 *v* 0.94 ± 0.40 μmol/kg · min). The arterial blood lactate concentration did not change significantly following 180 minutes of either insulin (970 ± 109 *v* 1,021 ± 60 μmol/L) or saline (932 ± 56 *v* 1,065 ± 129 μmol/L) infusion. Nevertheless, the splanchnic fractional extraction of lactate decreased from a mean of 0.33 ± 0.04 at baseline to 0.04 ± 0.02 ($P < .05$) and net splanchnic uptake of lactate decreased by about 90% during the last 30 minutes of the insulin infusion period, whereas neither net splanchnic fractional extraction (0.32 ± 0.06 *v* 0.33 ± 0.10) nor splanchnic uptake of lactate changed with saline infusion. Similarly, although arterial blood alanine did not change significantly following either insulin (280 ± 25 *v* 252 ± 18 μmol/L) or saline (315 ± 20 *v* 282 ± 18 μmol/L) infusion, net splanchnic fractional extraction of alanine decreased from a mean at baseline of 0.35 ± 0.06 to 0.19 ± 0.10 ($P < .05$) and net splanchnic uptake of alanine decreased by about 45% during the last 30 minutes of the insulin infusion period. Neither net splanchnic fractional extraction (0.40 ± 0.10 *v* 0.37 ± 0.17) nor splanchnic uptake of alanine changed with saline infusion. Arterial blood glycerol decreased from a mean of 71 ± 8 at baseline to 39 ± 5 μmol/L ($P < .01$) and splanchnic uptake of glycerol decreased by about 40%, although net splanchnic fractional extraction of glycerol did not change (0.54 ± 0.25 *v* 0.49 ± 0.20), during the last 30 minutes of the insulin infusion period. In contrast, arterial blood glycerol increased from 95 ± 22 to 167 ± 32 μmol/L ($P < .01$) and splanchnic glycerol uptake increased by about 60%, although net splanchnic fractional extraction of glycerol did not change (0.58 ± 0.30 *v* 0.56 ± 0.33), after 180 minutes of saline infusion (Table 2).

In the renal vein catheterization group, baseline endogenous glucose production decreased by about 60% and renal glucose production by about 50% in the last 30 minutes of the insulin infusion period, whereas neither changed significantly during saline infusion (Table 3). The mean exogenous glucose infusion rate during the euglycemic-hyperinsulinemic clamp was 5.4 ± 0.6 μmol/kg · min. Renal glucose utilization increased from a mean of 1.97 ± 0.50 to 3.38 ± 0.70 μmol/kg · min ($P < .05$) in the last 30 minutes of the insulin infusion period, but it did not change (1.25 ± 0.40 *v* 1.10 ± 0.30 μmol/kg · min) during saline infusion. Arterial blood lactate did not change during 180 minutes of either insulin (814 ± 55 *v* 875 ± 50 μmol/L) or

Table 2. Mean Blood Glucose Concentration (μmol/mL), Plasma Glucose Enrichment (%), Endogenous and Hepatic Glucose Production (μmol/kg · min), and Splanchnic Balance of Lactate, Alanine, and Glycerol (μmol/kg · min) in the Postabsorptive State (baseline) and During 180 Minutes of Peripheral Infusion of Either Insulin (n = 6) or Saline (n = 4) in Healthy Subjects

		Infusion Period					
Parameter	Baseline	30 min	60 min	90 min	120 min	150 min	180 min
Insulin							
Glc	3.78 ± 0.21	3.34 ± 0.18*	3.73 ± 0.20	3.93 ± 0.15	3.60 ± 0.18	3.70 ± 0.20	3.64 ± 0.25
PE	2.08 ± 0.06	2.15 ± 0.10	2.11 ± 0.12	1.99 ± 0.10	2.07 ± 0.05	2.18 ± 0.06	2.24 ± 0.06
Saline							
Glc	3.77 ± 0.15	3.62 ± 0.12	3.60 ± 0.10	3.64 ± 0.10	3.70 ± 0.15	3.63 ± 0.12	3.69 ± 0.10
PE	2.22 ± 0.08	2.30 ± 0.04	2.33 ± 0.05	2.41 ± 0.08	2.45 ± 0.08	2.50 ± 0.08*	2.45 ± 0.10
Insulin							
EGP	10.8 ± 0.8	6.1 ± 1.0*	6.6 ± 1.1*	6.9 ± 0.8*	6.5 ± 0.7*	6.7 ± 1.0*	7.5 ± 0.8*
HGP	10.4 ± 1.1	6.6 ± 1.2*	4.9 ± 1.4*	4.5 ± 1.0*	5.6 ± 1.0*	7.2 ± 0.8*	8.3 ± 1.2*
Saline							
EGP	10.8 ± 0.6	10.6 ± 0.8	10.4 ± 1.0	9.6 ± 1.2	9.2 ± 1.0	9.0 ± 0.8	9.5 ± 1.0
HGP	8.8 ± 0.8	9.2 ± 0.6	8.2 ± 1.4	10.2 ± 1.2	8.9 ± 1.2	7.9 ± 1.1	8.1 ± 0.8
Insulin							
LAC	5.7 ± 0.7	6.1 ± 1.0	5.4 ± 1.2	3.7 ± 1.6*	1.8 ± 0.8*	0.7 ± 0.6*	0.7 ± 0.5*
ALA	1.8 ± 0.1	2.2 ± 0.3	2.5 ± 0.2	2.0 ± 0.2	1.0 ± 0.5*	0.9 ± 0.6*	1.0 ± 0.4*
GLY	0.7 ± 0.3	0.9 ± 0.3	0.6 ± 0.3	0.4 ± 0.2*	0.3 ± 0.2*	0.3 ± 0.2*	0.4 ± 0.2*
Saline							
LAC	6.7 ± 0.9	5.8 ± 1.0	4.3 ± 0.8*	4.5 ± 1.8	5.6 ± 1.2	6.5 ± 1.1	6.9 ± 1.2
ALA	2.8 ± 0.3	2.8 ± 0.6	1.0 ± 0.2*	1.9 ± 1.2	1.5 ± 1.0	1.9 ± 1.0	2.1 ± 0.8
GLY	1.0 ± 0.5	0.7 ± 0.4	0.8 ± 0.2	1.2 ± 0.4	1.4 ± 0.8	1.6 ± 0.3*	2.0 ± 0.6*

Abbreviations: Glc, glucose; PE, plasma enrichment.
*$P < .05$ by repeated-measures ANOVA *v* baseline.

saline (780 ± 60 *v* 890 ± 110 μmol/L) infusion. However, the net renal uptake of lactate decreased initially (30 to 90 minutes), but it was comparable to the baseline and the saline control in the last 30 minutes of the insulin infusion period. Similarly, net renal fractional extraction of lactate at baseline (0.16 ± 0.05) decreased initially to a mean of 0.05 ± 0.05 (30 to 90 minutes), but it was comparable to baseline (0.14 ± 0.08) and to the saline control (0.16 ± 0.10) in the last 30 minutes of insulin

Table 3. Mean Blood Glucose Concentration (μmol/mL), Plasma Glucose Enrichment (%), Endogenous and Renal Glucose Production (μmol/kg · min), and Renal Balance of Lactate, Alanine, and Glycerol (μmol/kg · min) in the Postabsorptive State (baseline) and During 180 Minutes of Peripheral Infusion of Either Insulin (n = 8) or Saline (n = 4) in Healthy Subjects

		Infusion Period					
Parameter	Baseline	30 min	60 min	90 min	120 min	150 min	180 min
Insulin							
Glc	4.42 ± 0.10	4.34 ± 0.08	4.25 ± 0.08	4.20 ± 0.08	4.30 ± 0.10	4.24 ± 0.08	4.34 ± 0.12
PE	2.37 ± 0.03	2.10 ± 0.06*	2.22 ± 0.05*	2.25 ± 0.06	2.28 ± 0.03	2.30 ± 0.06	2.32 ± 0.06
Saline							
Glc	4.44 ± 0.10	4.42 ± 0.08	4.00 ± 0.10*	4.16 ± 0.10	4.25 ± 0.08	4.41 ± 0.08	4.45 ± 0.10
PE	2.72 ± 0.03	2.82 ± 0.06	2.78 ± 0.06	2.81 ± 0.10	2.91 ± 0.06*	2.80 ± 0.05	2.86 ± 0.07
Insulin							
EGP	9.5 ± 0.7	6.0 ± 0.9*	5.7 ± 1.0*	5.1 ± 1.0*	5.9 ± 0.8*	5.6 ± 0.7*	5.8 ± 0.5*
RGP	2.8 ± 0.6	0.5 ± 0.6*	2.0 ± 0.7	0.9 ± 0.7*	3.3 ± 0.7	1.8 ± 0.6	1.2 ± 0.5*
Saline							
EGP	9.9 ± 0.5	10.5 ± 0.6	9.9 ± 0.8	10.0 ± 0.7	8.7 ± 1.5*	9.9 ± 0.8	9.5 ± 1.0
RGP	1.6 ± 0.4	1.5 ± 0.5	0.9 ± 0.8	0.7 ± 0.7*	1.3 ± 0.5	1.6 ± 0.8	1.9 ± 0.5
Insulin							
LAC	2.4 ± 0.4	0.9 ± 0.6*	1.2 ± 0.3*	0.3 ± 0.1*	1.8 ± 1.0	2.3 ± 0.4	2.4 ± 0.5
ALA	0.2 ± 0.1	0.4 ± 0.4	0.1 ± 0.2	0.2 ± 0.4	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.1
GLY	0.3 ± 0.1	0.2 ± 0.3	0.4 ± 0.3	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1*	0.1 ± 0.1*
Saline							
LAC	1.6 ± 0.3	2.6 ± 0.8	1.2 ± 0.3	0.8 ± 0.8	2.4 ± 1.0	2.1 ± 0.8	2.5 ± 0.4
ALA	0.4 ± 0.2	0.1 ± 0.3	0.9 ± 0.5*	0.4 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.9 ± 0.4*
GLY	0.4 ± 0.1	0.1 ± 0.2	0.3 ± 0.3	0.5 ± 0.2	0.3 ± 0.2	0.5 ± 0.2	0.7 ± 0.3*

NOTE. See Table 2 for abbreviations.
*$P < .05$ by repeated-measures ANOVA *v* baseline.

infusion period. Arterial blood alanine did not change during 180 minutes of either insulin (267 ± 13 *v* 248 ± 12 μmol/L) or saline (279 ± 25 *v* 217 ± 28 μmol/L) infusion. Neither the net renal fractional extraction (0.05 ± 0.05 *v* 0.02 ± 0.04) nor net renal uptake of alanine changed significantly during insulin infusion, although net renal fractional extraction of alanine increased from 0.11 ± 0.04 to 0.23 ± 0.13 ($P < .05$) and net renal alanine uptake doubled in the last 30 minutes of the saline infusion period (Table 3). Arterial blood glycerol decreased from a mean of 60 ± 8 μmol/L at baseline to 32 ± 5 μmol/L ($P < .01$) and renal uptake of glycerol decreased by about 70% in the last 30 minutes of the insulin infusion period, although net renal fractional extraction of glycerol did not change (0.27 ± 0.12 *v* 0.23 ± 0.15). In contrast, arterial blood glycerol increased from a mean of 89 ± 6 μmol/L at baseline to 114 ± 13 μmol/L ($P < .05$) and renal glycerol uptake increased by about 50% in the last 30 minutes of the saline infusion period, although renal fractional extraction of glycerol did not change (0.28 ± 0.20 *v* 0.28 ± 0.12).

Assuming that the sum of net lactate, alanine, and glycerol uptake by the splanchnic tissues represents the maximum gluconeogenic capacity of the liver, and considering that 2 mol of each of these 3-carbon precursors is necessary to form 1 mol glucose, we have estimated the rate of net hepatic glycogen degradation by subtracting maximum gluconeogenesis from hepatic glucose production (Fig 1). Following insulin infusion, the contribution of net glycogenolysis to postabsorptive hepatic glucose production decreased immediately ($P < .05$ by ANOVA) from 6.28 ± 0.51 μmol/kg · min to 2.04 ± 0.43 (30 minutes), 0.65 ± 0.52 (60 minutes), and 1.44 ± 0.51 (90 minutes), and returned to near-baseline values of 4.07 ± 0.63 (120 minutes), 6.24 ± 0.17 (150 minutes), and 7.23 ± 0.62 (180 minutes) μmol/kg · min. In contrast, the contribution of gluconeogenesis to postabsorptive hepatic glucose production (4.12 ± 0.59 μmol/kg · min) initially remained constant at 4.56 ± 0.77 (30 minutes), 4.25 ± 0.88 (60 minutes), and 3.06 ± 0.49 (90 minutes) μmol/kg · min, but it decreased to 1.53 ± 0.37 (120 minutes), 0.96 ± 0.63 (150 minutes), and 1.07 ± 0.55 (180 minutes) μmol/kg · min. In the saline infusion control studies, hepatic gluconeogenesis did not change (5.20 ± 0.83 *v* 5.25 ± 0.72 μmol/kg · min) and the estimated contribution of net glycogenolysis to postabsorptive hepatic glucose production decreased from 3.60 ± 0.80 to 3.0 ± 0.60 μmol/kg · min, although these did not reach statistical significance. Analogous to the liver, assuming that the net uptake of lactate, alanine, and glycerol by the kidney is entirely diverted into glucose formation, and considering that 2 mol of each of these 3-carbon precursors is necessary to form 1 mol glucose, we have estimated the rate of renal gluconeogenesis and (Fig 2). The contribution of renal gluconeogenesis to postabsorptive endogenous glucose production decreased initially from 1.45 ± 0.30 to 0.75 ± 0.65 (30 minutes), 0.85 ± 0.40 (60 minutes), and 0.35 ± 0.40 (90 minutes) μmol/kg · min, but returned to baseline values of 1.15 ± 0.70 (120 minutes), 1.25 ± 0.25 (150

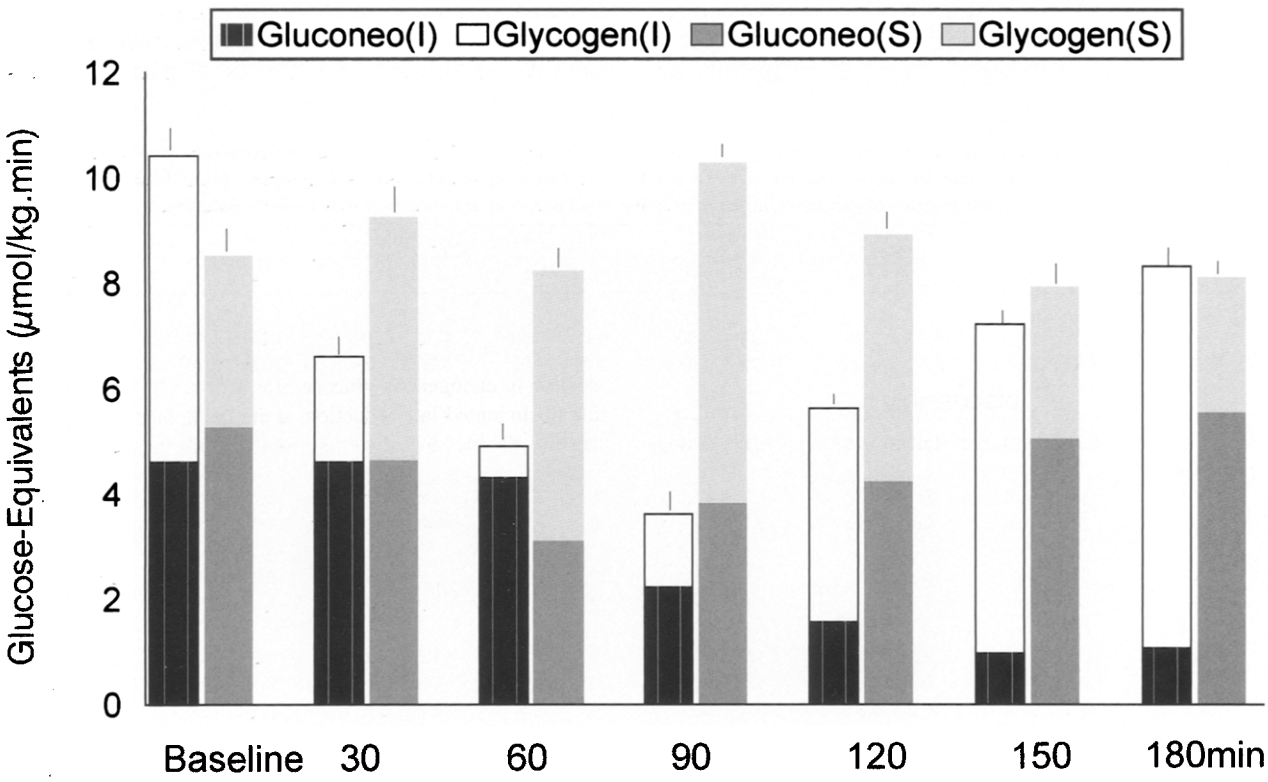

Fig 1. Estimated contribution of splanchnic uptake of lactate, alanine, and glycerol to hepatic glucose production (gluconeo) in μmol/kg · min during baseline (−30 to 0 minutes) and every 30 minutes during a 180-minute peripheral infusion of either saline (S;n = 4) or insulin (I;n = 6) at 0.25 mU/kg · min in postabsorptive healthy subjects. The amount of glucose production unaccounted for is presumed to be derived from net hepatic glycogen breakdown (glycogen).

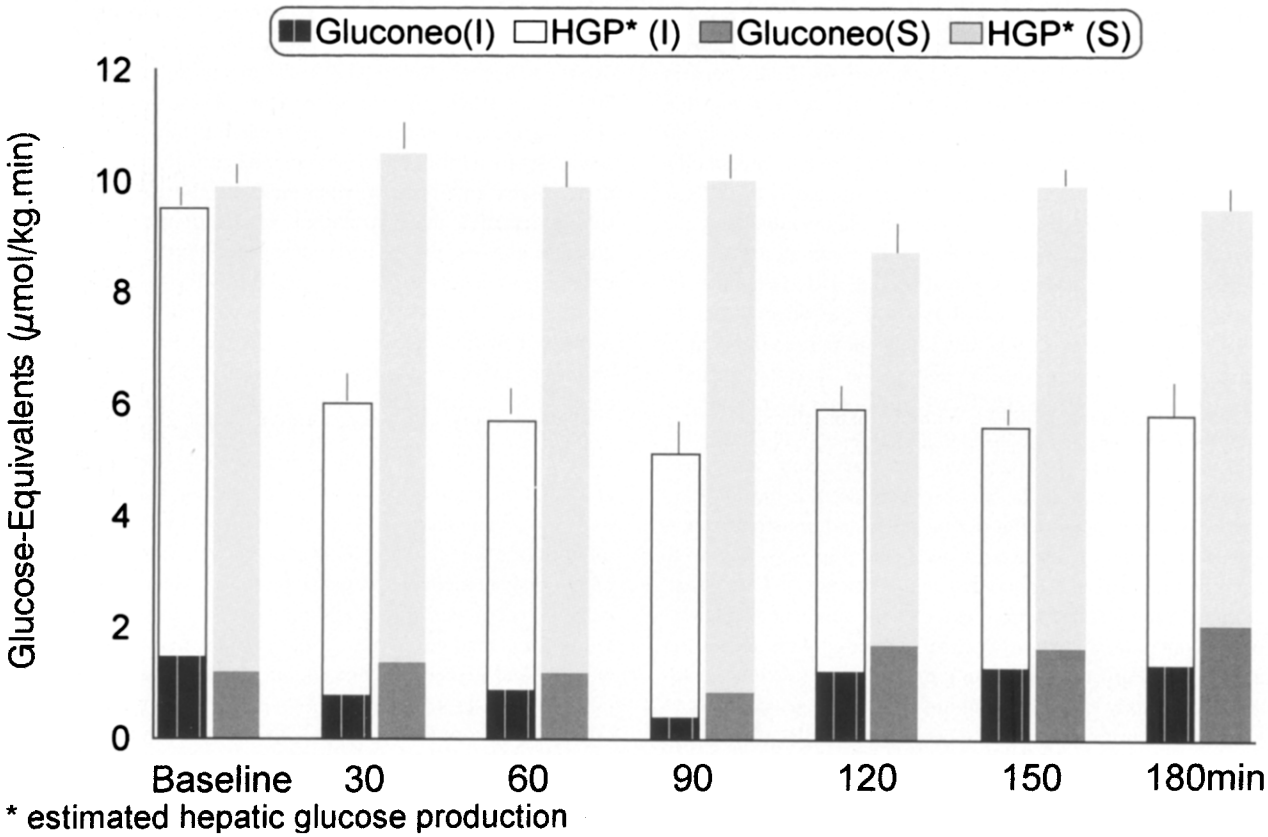

Fig 2. Estimated contribution of renal uptake of lactate, alanine, and glycerol to endogenous glucose production (gluconeo) in μmol/kg · min during baseline (−30 to 0 minutes) and every 30 minutes during a 180-minute peripheral infusion of either saline (S;n = 4) or insulin (I;n = 8) at 0.25 mU/kg · min in postabsorptive healthy subjects. Net uptake of glutamine, glutamate, and other TCA cycle intermediates by the kidney and their potential contribution to renal gluconeogenesis are not included. Hepatic glucose production (HGP*) is estimated as the difference between renal gluconeogenesis and tracer-determined endogenous glucose production.

minutes), and 1.30 ± 0.35 (180 minutes) μmol/kg · min following insulin infusion ($P < .05$ by ANOVA). The estimated contribution of renal gluconeogenesis to postabsorptive endogenous glucose production increased from 1.20 ± 0.30 to 1.85 ± 0.42 μmol/kg · min ($P < .05$) during the last 30 minutes of the saline infusion period.

DISCUSSION

The present studies confirm that insulin suppresses endogenous glucose production primarily by reducing hepatic glucose production,[29] and further demonstrate that although the inhibition of endogenous and hepatic glucose production occurs in parallel, simultaneous adjustments in renal glucose production play a role in determining the characteristic response of systemic glucose appearance to a physiologic insulin dose. Based on the arteriovenous balance of gluconeogenic precursors, our results indicate that the kidney, which may be responsible for about 13% (1.30 of 9.80 μmol/kg · min) of endogenous glucose production in the postabsorptive state, accounts for about 22% (1.23 of 5.70 μmol/kg · min) of endogenous glucose production after 180 minutes of continuous peripheral insulin infusion. According to the data in Tables 2 and 3, immediate reductions in hepatic glucose release and net gluconeogenic precursor uptake by the kidney together may account for the initial (30 to 90 minutes) 40% decrease in endogenous glucose production. The fact that net splanchnic gluconeogenic precursor uptake does not change significantly during the initial 90 minutes following insulin infusion strongly suggests that simultaneous inhibition of net hepatic glycogenolysis and renal gluconeogenesis is responsible for the early rapid decline in endogenous glucose production. On the other hand, the documented late reduction in the net splanchnic uptake of lactate, alanine, and glycerol could account for the decrease in endogenous glucose production that occurs between 90 and 180 minutes following insulin infusion. At this time, either the intraportal or intrahepatocyte precursor contribution to gluconeogenesis increases or the net hepatic glycogen degradation is partially restored, both of which may be responsible for up to 75% of glucose release by the liver. In addition, the reduction in the net uptake of glycerol by the kidney could account for a fraction of the 30% decrease in renal glucose production measured by tracer dilution, and may have contributed to sustain the low rate of endogenous glucose production during the last 90 minutes of the hyperinsulinemic period. These data suggest that insulin exerts a sudden inhibitory effect on net hepatic glycogenolysis and renal gluconeogenesis, whereas suppression of hepatic gluconeogenesis is a later event.

Although the mechanisms for this biphasic response are not

entirely clear, it is conceivable that these reflect direct and indirect effects of insulin[10,12] on net hepatic glycogen degradation and on renal and hepatic gluconeogenesis. Reductions in the renal (early) and splanchnic (late) net uptake of lactate and alanine are due to either a decrease in tissue utilization or an increase in tissue production of these gluconeogenic precursors. The observation that changes in net lactate uptake by the kidney precede similar changes in net lactate and alanine uptake by the splanchnic tissue raises the possibility that although glycogen content in the liver and its degradation rate may determine the timing[5] and the relative contribution of gluconeogenesis to hepatic glucose release,[31] this may not be the case in the kidney. Throughout the entire insulin and saline infusion periods, alterations in tracer-determined renal glucose production tend to parallel changes in renal gluconeogenic precursor uptake by the kidney. Moreover, the fact that the renal and splanchnic fractional extraction of lactate decrease despite the constant arterial lactate concentration during insulin administration suggests that these are hormonally mediated and may be due to decreased intracellular gluconeogenic efficiency.[4,5] In contrast, although fractional extraction remained the same, both splanchnic and renal net uptake of glycerol decreased with decreasing arterial blood glycerol, indicating that reduced substrate availability secondary to insulin inhibition of adipose tissue lipolysis was partially responsible for the decline in net glycerol uptake, which may account for the reduced rate of conversion of glycerol to glucose in the liver and kidney. It should be pointed out that the infusion of equal amounts of heparin (4.0 U/min) in the renal and hepatic vein catheters during the study period may have been partly responsible for the increases in arterial glycerol, and perhaps even organ glycerol extraction, in the saline control subjects, which may have blunted the effect of insulin on the blood glycerol concentration and organ balance. Of additional interest, these observations are entirely consistent with previous findings indicating that although systemic glucose production rates do not change significantly, the predominant availability of one substrate determines the precursor utilization for gluconeogenesis,[1] and lend further support to the recently proposed role for peripheral insulin in the regulation of glucose production.[10,12] Theoretically, a decrease in peripheral glucose oxidation with a reduced availability of pyruvate for lactate and alanine formation could have limited the substrate supply to the liver. However, the extent to which the initial reduction in hepatic glucose production and in renal gluconeogenesis can be attributed to insulin-induced suppression of glucagon secretion[20] and inhibition of adipose tissue lipolysis[10,11] and whether additional peripheral factors mediate the response of endogenous glucose production to insulin administration were not evaluated. Furthermore, the extent to which right hepatic vein samples reflect glucose metabolism in the liver as a whole is unknown.

In these studies, we have estimated that net hepatic glycogenolysis is responsible for about 48% and gluconeogenesis (hepatic and renal combined) for about 52% of tracer-determined systemic glucose appearance in postabsorptive humans, and that after peripheral insulin infusion, net hepatic glycogenolysis makes a larger contribution (up to 75%) to hepatic glucose production and systemic glucose appearance. Following 180 minutes of saline infusion, net hepatic glycogenolysis can account for up to 30% and gluconeogenesis for about 70% of systemic glucose appearance. These findings are in close agreement with data reported for the use of NMR spectroscopy to directly measure net hepatic glycogen degradation in humans.[2] Unlike previous experiments,[4] nonetheless, our studies partition the contribution of the liver and kidney to gluconeogenesis and provide strong additional evidence that both in the postabsorptive state and following insulin infusion, endogenous glucose production results from the interaction between hepatic glycogen degradation and renal and hepatic gluconeogenesis. Using the arteriovenous concentration difference, these experiments demonstrate that the kidney may account for about 25% (1.3 of 6.0 μmol/kg · min) of whole-body gluconeogenesis in the postabsorptive state, about 50% (1.23 of 2.40 μmol/kg · min) following peripheral insulin administration, and about 26% (1.85 of 7.10 μmol/kg · min) after 180 minutes of saline infusion. However, considering that measurements of gluconeogenesis using the net balance of gluconeogenic precursors have several limitations, our data should not be viewed in absolute terms. First, the estimation of lactate, alanine, and glycerol incorporation into glucose by arteriovenous concentration differences represents a maximum possible contribution from the circulation and assumes that all circulating gluconeogenic precursors extracted by the splanchnic tissues and kidney are diverted into gluconeogenesis. Second, although the amount of intracellularly derived lactate, alanine, and glycerol available for gluconeogenesis is likely negligible in the kidney,[13] the potential contribution of the portal system and of intrahepatocyte precursors to hepatic gluconeogenesis is not considered. Finally, the fact that circulating pyruvate[32] and other potential gluconeogenic precursors, such as glutamine, glutamate, and TCA cycle intermediates, are not included in the calculation could have caused further underestimation of hepatic and renal gluconeogenesis.[13,33] These methodological limitations may be responsible for some discrepancies noted between tracer-determined glucose kinetics and arteriovenous-difference measurements. In addition, although unlikely, the fact that net renal glycogenolysis was assumed to be negligible could help to explain some minor differences between tracer-determined glucose production and the estimated rate of gluconeogenesis in the kidney.

In summary, we have demonstrated that insulin suppresses endogenous glucose production primarily by reducing hepatic glucose production, and although the inhibition of endogenous and hepatic glucose production occurs in parallel, changes in renal glucose production play a role in determining the characteristic response of systemic glucose appearance to insulin. Our observations using either hepatic or renal arteriovenous-concentration differences combined with tracer dilution to determine glucose production by the liver and kidney and to partition hepatic and renal gluconeogenesis in comparable groups of healthy subjects indicate that net hepatic glycogenolysis and renal gluconeogenesis are reduced immediately and could account for the initial rapid decline in endogenous glucose production in response to insulin administration. Inhibi-

tion of the hepatic gluconeogenesis dependent on precursors in the systemic circulation occurs later when intraportal and intrahepatocyte precursor availability, net glycogenolysis, and renal gluconeogenesis could be responsible for most of the residual glucose produced endogenously. These findings provide additional evidence that peripheral insulin suppresses endogenous glucose production, in part, by modifying the gluconeogenic precursor supply to the liver and kidney, and support the hypothesis that hepatic glycogenolysis and renal and hepatic gluconeogenesis are under common regulation.

ACKNOWLEDGMENT

We thank E. Hayes, I. Zaitseva, M. Ajmal, S. Pacheco, and J. Vasek for excellent technical assistance and Dr Alan Cherrington for the substrate assay and helpful discussion of the arteriovenous concentration-difference data.

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