Effects of Glucagon on Renal and Hepatic Glutamine Gluconeogenesis in Normal Postabsorptive Humans

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Glutamine is an important gluconeogenic amino acid in postabsorptive humans. To assess the effect of glucagon on renal and hepatic glutamine gluconeogenesis, we infused six normal healthy postabsorptive subjects with glucagon at a rate chosen to produce circulating glucagon concentrations found during hypoglycemia and, using a combination of isotopic and net balance techniques, determined the systemic, renal, and hepatic glucose release and renal and hepatic production of glucose from glutamine. Infusion of glucagon increased systemic and hepatic glucose release (both $P < .02$), but had no effect on renal glucose release ($P = .26$). Systemic and hepatic glutamine gluconeogenesis increased from 0.45 ± 0.3 and 0.11 ± 0.02 μmol · kg^{-1} · min^{-1}, respectively, to 0.61 ± 0.04 ($P = .002$) and 0.31 ± 0.03 μmol · kg^{-1} · min^{-1} ($P = .001$), respectively, whereas renal glutamine gluconeogenesis was unchanged (from 0.33 ± 0.03 to 0.30 ± 0.04 μmol · kg^{-1} · min^{-1}, $P = .20$). The hepatic contribution to systemic glutamine gluconeogenesis increased from 25.2% ± 6.2% to 51.6% ± 5.5% ($P = .002$), while that of the kidney decreased from 74.8% ± 6.2% to 48.4% ± 5.5% ($P = .003$). Glucagon had no effect on the renal net balance, fractional extraction, or uptake and release of either glucose or glutamine. We thus conclude that glucagon stimulates glutamine gluconeogenesis in normal postabsorptive humans, predominantly due to an increase in hepatic glutamine conversion to glucose. Thus, under certain conditions such as counterregulation of hypoglycemia, the liver may be an important site of glutamine gluconeogenesis.

GLUTAMINE is the most abundant free amino acid in human plasma and tissues.[1,2] It subserves many functions,[3] prominent among which are its roles as a transporter of carbon and nitrogen between tissues[4,5] and as both a substrate and regulator of gluconeogenesis.[6] Glutamine plasma turnover,[5,7] its conversion to glucose,[5,8] and its net addition of carbon to the glucose pool[9] generally exceed that of alanine, which has been considered the most important gluconeogenic amino acid.[10] The recent report by Hankard et al,[8] demonstrating that glutamine itself could account for nearly 30% of all gluconeogenesis in overnight-fasted normal subjects, suggests that glutamine may rank second only to lactate as a gluconeogenic precursor in humans.

Most studies of glutamine conversion to glucose have focused on the liver[11-13] because it was thought that this organ was the exclusive site for gluconeogenesis in overnight-fasted humans. However, it is now apparent that the human kidney is also an important gluconeogenic organ, even in the postabsorptive state.[14,15] Moreover, recent studies indicate that the kidney uses glutamine preferentially as a gluconeogenic precursor.[16]

There is considerable evidence that both renal and hepatic gluconeogenesis are under hormonal control.[17-19] Studies in dogs[20] and humans,[14,15,21,22] for example, indicate that renal and hepatic gluconeogenesis are suppressed by insulin and stimulated by epinephrine. Glucagon, considered the major gluconeogenic hormone in humans,[23] has been shown to stimulate hepatic glucose production from a variety of amino acids.[24] However, to our knowledge, its specific effect on glutamine conversion to glucose by either the liver or the kidney has not been investigated. Therefore, in the present studies we used a combination of isotopic and net balance techniques to assess the effect of glucagon on renal and hepatic glutamine gluconeogenesis in postabsorptive subjects.

SUBJECTS AND METHODS

Subjects

Informed written consent was obtained from six normal subjects (four men and two women) after the protocol was approved by the local Institutional Review Board. The subjects were aged 24 ± 1 (mean ± SEM) years, weighed 75 ± 4 kg (body mass index, 23.9 ± 0.9 kg/m^2), and had normal glucose tolerance tests according to World Health Organization criteria[25] and no family history of diabetes. For 3 days before the study, all subjects were on a weight-maintaining diet containing at least 200 g carbohydrate and abstained from alcohol.

Protocol

Subjects were admitted to the University of Rochester General Clinical Research Center between 6:00 and 7:00 PM on the evening before the experiments. They consumed a standard meal (10 kcal/kg, 50% carbohydrate, 35% fat, and 15% protein) between 6:00 and 8:00 PM and fasted overnight until the experiments were completed.

At approximately 5:30 AM, an antecubital vein was cannulated and primed-continuous infusions of [6-^{3}H]glucose (30 μCi, 0.3 μCi/min), [U-^{14}C]glutamine (25 μCi, 0.25 μCi/min; both Amersham International, Amersham, UK) were started. Between 8:00 and 9:00 AM, a renal vein was catheterized through the femoral vein under fluoroscopy, and the position of the catheter tip was verified by injecting a small amount of iodinated contrast material. The catheter was then continuously infused with a saline solution to maintain patency.

At 9:00 AM, a dorsal hand vein was cannulated and kept in a thermoregulated Plexiglas box at 65°C for sampling of arterialized venous blood.[26] An antecubital venous infusion of *p*-aminohippuric acid (12 mg/min) was then started for determination of renal blood flow. After allowing approximately 4 hours to achieve isotopic steady state, three blood samples were collected simultaneously from the dorsal hand vein and the renal vein at 30-minute intervals (−60, −30, and 0 minutes). At 0 minutes, a continuous infusion of glucagon (5 ng · kg^{-1} · min^{-1}) was started via the antecubital infusion line, and

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blood was collected as already described at 30-minute intervals for 3 hours.

Analytical Procedures

Blood samples for substrate concentrations and specific activities were collected in oxalate-fluoride tubes; samples for insulin and glucagon were collected in EDTA tubes containing a protease inhibitor. Whole blood glucose was immediately determined in triplicate with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). For other determinations, samples were placed immediately in a 4°C ice bath and the plasma was separated within 30 minutes by centrifugation at 4°C. An internal standard (25 nmol *p*-fluoro-phenylalanine) was added to 4 mL plasma, the pH was adjusted to 4.8 to 5.0, and samples were frozen for later analysis of the glutamine concentration and [^{14}C]glutamine specific activity by high-performance liquid chromatography (HPLC) as described previously,[27] with an intraassay coefficient of variation of approximately 5%. Plasma [^{3}H] and [^{14}C]glucose specific activities were determined in duplicate by HPLC[28] with an intraassay coefficient of variation of approximately 2%. Plasma insulin and glucagon concentrations were determined by standard radioimmunoassays as previously described.[29] The plasma *p*-aminohippuric acid concentration was determined by a colorimetric method.[30]

Calculations

After 4 hours of isotope infusion (−60 minutes), isotopic steady state was achieved for [^{3}H]glucose and [^{14}C]glutamine, as evidenced by the fact that values at −60 minutes were not significantly different from those at 0 minutes (paired Student's *t* test). However, isotopic steady state was not closely approximated for [^{14}C]glucose until −30 minutes, as evidenced by the fact that the specific activity at −60 minutes but not at −30 minutes was significantly different versus 0 minutes (paired Student's *t* test). Consequently, for measurements involving [^{14}C]glucose in the basal state, only values at −30 and 0 minutes were used.

Renal plasma flow (RPF) was determined by the *p*-aminohippuric acid clearance technique,[31] and renal blood flow (RBF) was calculated as RPF/(1 − hematocrit). Fractional extraction (FX) of glucose across the kidney was calculated as ([6-^{3}H]glucose $SA_{art} \cdot [glucose]_{art}$ − [6-^{3}H]glucose $SA_{renal\ vein} \cdot [glucose]_{renal\ vein}$)/([6-^{3}H]glucose $SA_{art} \cdot [glucose]_{art}$),[32] where SA is specific activity. Renal glucose uptake (RGU) was calculated as $RBF_{art} \cdot [glucose]_{art} \cdot FX$, and renal glucose net balance (NB) as $RBF \cdot ([glucose]_{art} - [glucose]_{renal\ vein})$.[32] Renal glucose release (RGR) was calculated as RGU − NB.[32] Analogous equations were applied for glutamine, except that renal plasma flow was used because glutamine is predominantly transported by plasma.[33,34]

The systemic appearance and removal of glucose from the circulation was determined with steady-state equations before glucagon infusion[35] and subsequently with the non–steady-state equations of DeBodo et al[36] as described previously[14] using a pool fraction of 0.65 and a volume of distribution of 200 mL · kg^{-1}. Since the liver and kidney are the only organs capable of releasing glucose into the circulation, hepatic glucose release (HGR) was calculated as the difference between the overall appearance of glucose and renal glucose release.

The glutamine rate of appearance was calculated with steady-state equations before glucagon infusion[5] and subsequently with DeBodo's equation.[36] A pool fraction of 0.65 and a volume of distribution of 400 mL/kg body weight was used.[37,38]

The percent of the systemic glucose rate of appearance (Ra) due to glutamine gluconeogenesis at steady state was calculated as ([^{14}C]glucose SA_{art}/[^{14}C]glutamine SA_{art}) · 100/1.2.[39] The division by 1.2 corrects for differences in carbon (ie, glutamine has five and glucose has six carbons). Total glutamine gluconeogenesis was calculated as the percent of glucose Ra from glutamine multiplied by glucose Ra. During the non–steady state, whole-body glutamine gluconeogenesis was calculated using a modification of the equation of Chiasson et al[40] as ($Rd_{gluc} \cdot$ [^{14}C]glucose $SA_{art} + p \cdot V \cdot$ ([^{14}C]glucose $SA_{art}(t_2)$ − [^{14}C]glucose $SA_{art}(t_1)$)/30 min)/(1.2 · [^{14}C]glutamine SA_{art}), where t_1 and t_2 refer to the beginning and end of every 30-minute interval and p and V refer to the pool fraction and volume of distribution for glucose.

Renal gluconeogenesis from glutamine was calculated as $RBF \cdot ([^{14}C]glucose\ SA_{renal\ vein} \cdot [glucose]_{vein} - (1 - FX) \cdot ([^{14}C]glucose\ SA_{art} \cdot [glucose]_{art})/(1.2 \cdot ([^{14}C]glutamine\ SA_{art}))$,[20] where FX is the renal fractional extraction of glucose obtained from [^{3} H]glucose data as already described. Hepatic glutamine gluconeogenesis was calculated as the difference between total glutamine gluconeogenesis and renal glutamine gluconeogenesis. Krebs cycle carbon exchange was assumed to be equal in the liver and kidney.[41]

Statistical Analysis

Data are expressed as the mean ± SEM. Unless stated otherwise, comparisons were made between the averaged baseline time points and the average of the six time points during the glucagon infusion using paired two-tailed Student's *t* tests. A *P* value less than .05 was considered statistically significant.

RESULTS

Arterial Concentrations of Glucagon, Insulin, Glucose, and Glutamine

Infusion of glucagon increased plasma glucagon from a basal level of 135 ± 25 ng/L to approximately 270 ng/L, concentrations observed in humans during insulin-induced hypoglycemia.[42] Plasma glucose increased from 4.48 ± 0.13 mmol/L to approximately 5.5 mmol/L ($P < .001$) at 60 minutes, and then decreased to approximately 4.8 mmol/L at the end of glucagon infusion ($P = .16$). Plasma insulin increased from 43 ± 3 pmol/L to a peak at 60 minutes of 83 ± 12 pmol/L ($P = .01$) and then decreased to 45 ± 3 pmol/L ($P = .29$) at the end of glucagon infusion. Arterial glutamine decreased progressively from 0.58 ± 0.03 to 0.51 ± 0.04 mmol/L ($P = .02$) at the end of glucagon infusion (Figs 1 and 2).

Renal Blood Flow, Net Balance, Fractional Extraction, and Uptake and Release of Glucose and Glutamine

Renal blood flow was 1,489 ± 122 mL · min^{-1} in the baseline period and remained unaltered during glucagon infusion (1,463 ± 143 mL · min^{-1}, $P = .29$). Similarly, infusion of glucagon had no effect on the renal net balance, fractional extraction, or uptake and release of either glucose or glutamine (Table 1).

Systemic, Renal, and Hepatic Glucose Release and Systemic Glutamine Turnover

During infusion of glucagon, systemic glucose release increased from a basal rate of 12.5 ± 0.7 μmol · kg^{-1} · min^{-1} to a peak at 30 minutes (17.5 ± 1.1 μmol · kg^{-1} · min^{-1}, $P = .002$), and subsequently decreased to values not different from baseline ($P = .48$). Rates of systemic glucose disposal followed a similar pattern (data not shown), increasing to approximately 14.5 μmol · kg^{-1} · min^{-1} between 30 and 90 minutes ($P = .02$) and then decreasing to 12.5 ± 0.7 μmol · kg^{-1} · min^{-1} at the end of glucagon infusion ($P = .76$). Hepatic glucose release increased from a baseline rate of 9.7 ± 0.6 μmol · kg^{-1} · min^{-1} to a peak at 30 minutes of 14.9 ± 0.2 μmol · kg^{-1} · min^{-1} ($P = .005$), and subsequently decreased to 8.7 ± 0.8 μmol ·

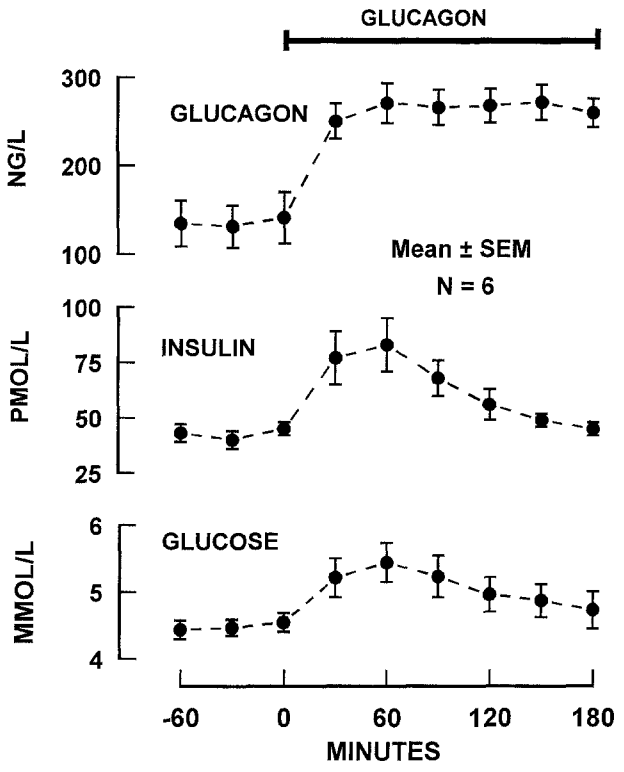

Fig 1. Plasma concentrations of glucagon, insulin, and glucose.

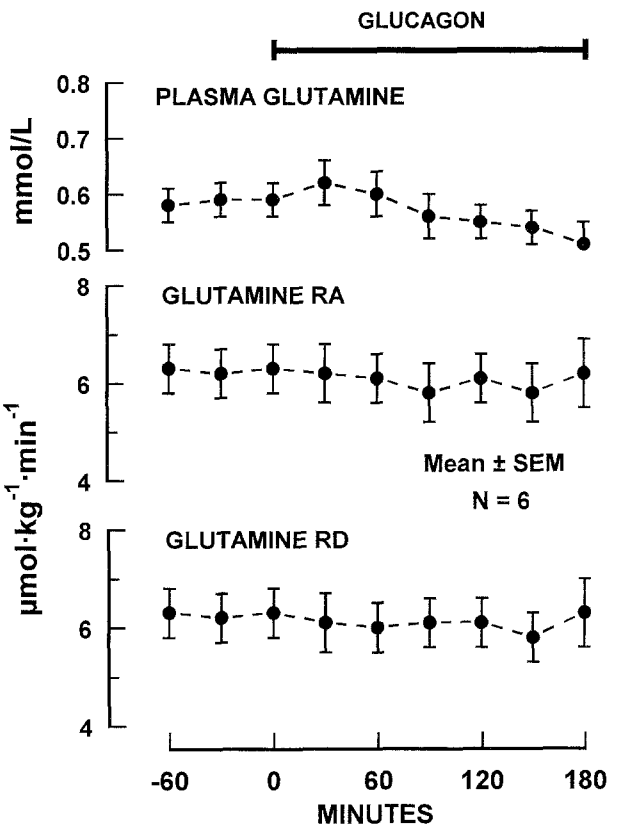

Fig 2. Concentration and rates of appearance (RA) and disappearance (RD) of plasma glutamine.

$kg^{-1} \cdot min^{-1}$, which was not different from baseline ($P = .36$). Renal glucose release was 2.8 ± 0.1 μmol · kg^{-1} · min^{-1} and did not change during infusion of glucagon (3.1 ± 0.2 μmol · kg^{-1} · min^{-1}, $P = .26$). Basal plasma glutamine rates of appearance and disappearance were 6.3 ± 0.5 μmol · kg^{-1} · min^{-1} and did not change significantly during glucagon infusion (Figs 2 and 3).

Systemic, Renal, and Hepatic Glutamine Gluconeogenesis

Before infusion of glucagon, systemic glutamine gluconeogenesis was 0.45 ± 0.03 μmol · kg^{-1} · min^{-1} and accounted for 3.7% ± 0.3% of systemic glucose release. Renal glutamine gluconeogenesis was 0.33 ± 0.03 μmol · kg^{-1} · min^{-1} and accounted for 74.8% ± 6.2% of systemic glutamine gluconeogenesis and 12.3% ± 1.4% of renal glucose release. Hepatic glutamine gluconeogenesis, calculated as the difference between systemic and renal gluconeogenesis, was 0.11 ± 0.02 μmol · kg^{-1} · min^{-1} and accounted for 25.2% ± 6.2% of systemic glutamine gluconeogenesis and 1.2% ± 0.3% of hepatic glucose release (Fig 4).

During infusion of glucagon, systemic glutamine gluconeogenesis increased progressively to 0.61 ± 0.04 μmol · kg^{-1} · min^{-1} ($P = .002$) and accounted for an increased proportion of systemic glucose release (5.2% ± 0.3%, $P = .002$). Renal glutamine gluconeogenesis remained unchanged during infusion of glucagon (0.30 ± 0.04 μmol · kg^{-1} · min^{-1}, $P = .20$), and consequently, its contribution to systemic glutamine gluconeogenesis decreased to 48.4% ± 5.5% ($P = .003$). Its contribution to renal glucose release remained unchanged (9.8% ± 1.5%, $P = .13$). Hepatic glutamine gluconeogenesis increased nearly threefold to 0.31 ± 0.03 μmol · kg^{-1} · min^{-1} ($P = .001$) during infusion of glucagon, and accounted for the increased proportions of systemic glutamine gluconeogenesis (51.6% ± 5.5%, $P = .002$) and hepatic glucose release (3.8% ± 0.5%, $P = .002$) (Fig 4).

Table 1. Renal Net Balance, Fractional Extraction, and Uptake and Release of Glucose and Glutamine at Baseline and During Glucagon Infusion (mean ± SEM)

Parameter	Glucose	Glutamine
Net balance (μmol · min^{-1})		
Baseline	−29 ± 12	31 ± 9
Glucagon*	−35 ± 7	39 ± 4
P v baseline	.97	.46
Fractional extraction (%)		
Baseline	2.8 ± 0.2	8.2 ± 1.7
Glucagon*	2.7 ± 0.3	10.1 ± 1.1
P v baseline	.90	.32
Uptake (μmol · min^{-1})		
Baseline	179 ± 7	40 ± 8
Glucagon*	192 ± 12	48 ± 3
P v baseline	.59	.48
Release (μmol · min^{-1})		
Baseline	208 ± 9	10 ± 2
Glucagon*	227 ± 8	7 ± 2
P v baseline	.13	.44

*Mean of 6 time points during glucagon infusion.

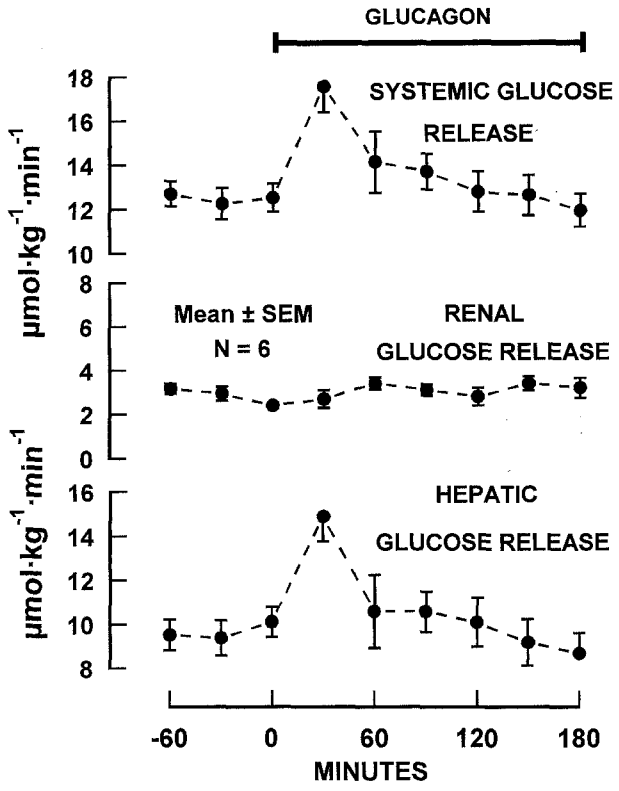

Fig 3. Systemic, renal, and hepatic glucose release.

DISCUSSION

The present studies demonstrate that an infusion of glucagon resulting in physiological concentrations found during counterregulation of hypoglycemia[42] produces a sustained increase in glutamine gluconeogenesis in normal postabsorptive humans while causing only a transient increase in systemic glucose release. The latter has been previously observed and attributed to an evanescent effect on hepatic glycogenolysis.[43,44] The increases in glutamine gluconeogenesis and systemic glucose release were wholly attributable to the liver, since neither renal glutamine gluconeogenesis nor renal glucose release were altered during the glucagon infusion.

This lack of effect of glucagon on the kidney contrasts with a sustained stimulation of renal glucose release observed during infusion of epinephrine in normal postabsorptive humans.[14] However, the present findings are consistent with observations that the suppression of glucagon secretion does not affect the increased renal glucose release of 60-hour-fasted humans or alter the augmentation of renal glucose release during infusion of glycerol in these subjects.[45]

Although the kidney possesses glucagon receptors, these appear to be limited to cells in the distal nephron.[46] Virtually all renal glucose release is attributable to gluconeogenesis, and this occurs almost exclusively in the proximal tubules where gluconeogenic enzymes and those affecting glutamine metabolism are located.[47,48] The lack of glucagon receptors in proximal tubules may thus explain the failure of glucagon to affect renal glucose release and renal glutamine metabolism. In a previous study, 70% to 90% of systemic glutamine gluconeogenesis was attributable to the kidney postabsorptively and during infusion of epinephrine. The present results thus indicate that under certain circumstances, the liver may be an important site of glutamine gluconeogenesis.

Accompanying the glucagon-induced increase in hepatic glutamine gluconeogenesis was a decrease in arterial glutamine concentrations, but no change was observed in either the plasma glutamine appearance or disappearance. The most likely cause for this discrepancy is insufficient analytical sensitivity and/or statistical power. Glucagon can stimulate hepatic amino acid uptake[49,50] and activate hepatic glutaminase[51] and other gluconeogenic enzymes,[18,52] all of which could be involved in the increase in hepatic glutamine gluconeogenesis. An increase in hepatic glutamine uptake would be expected to be accompanied by an increase in glutamine disappearance from the plasma. Our methods simply may have been insufficiently sensitive to detect subtle changes in glutamine turnover. It is also possible that glucagon stimulation of glutamine gluconeogenesis may have involved merely an alteration in the intrahepatic fate of glutamine rather than an augmentation of its uptake by the liver, although the latter cannot be entirely excluded.

Glucagon and catecholamines are considered the most important counterregulatory hormones.[53] During counterregulation of insulin-induced hypoglycemia, there is a substantial increase in renal glucose release.[54-57] This response has been shown to be hormonally mediated, since it occurs even when the kidney is sheltered from hypoglycemia by infusion of glucose in the renal artery.[54] Our results suggest this increase in renal glucose release cannot be attributed to glucagon. Although growth hormone and glucocorticoids have been shown to increase renal glucose release in vitro,[17,58] a prime candidate would be epinephrine, since it has been shown in humans to increase

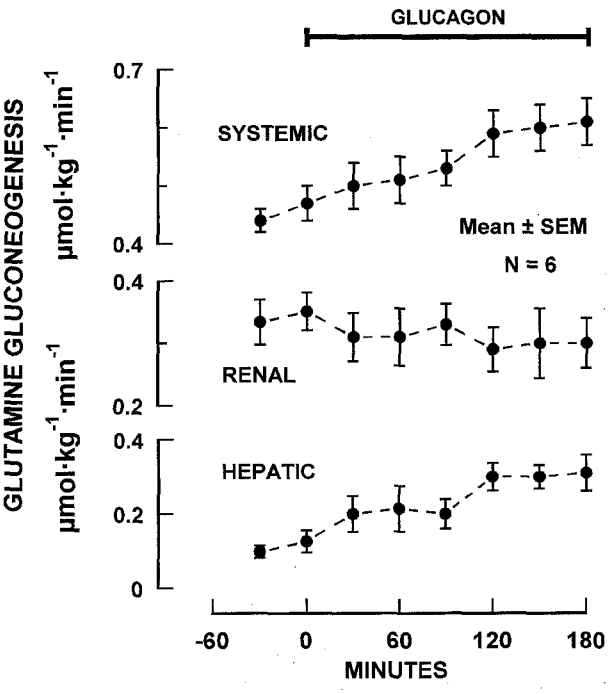

Fig 4. Systemic, renal, and hepatic glutamine gluconeogenesis.

renal glucose release at concentrations observed during hypoglycemia.[14]

In conclusion, the present studies demonstrate that physiologic concentrations of glucagon stimulate glutamine gluconeogenesis in normal postabsorptive human subjects, and that this is solely attributable to an increase in hepatic glutamine conversion to glucose since neither renal glucose nor renal glutamine gluconeogenesis were altered. Thus, under certain conditions, the liver may be an important site of glutamine gluconeogenesis.

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