Hormonal Control of Renal and Systemic Glutamine Metabolism\textsuperscript{1,2}

John E. Gerich,\textsuperscript{3} Christian Meyer and Michael W. Stumvoll\*  
Department of Medicine, University of Rochester, Rochester, NY 14642 and \textsuperscript{*}Department of Medicine, University of Tubingen, 72076 Tubingen, Germany

\textbf{ABSTRACT}  
Glutamine is the most abundant free amino acid in the human body. Recent studies indicate that it may be an important vehicle for interorgan nitrogen and carbon transport. However, relatively little is known about hormonal factors regulating its metabolism in humans. We review here our recent work on the effects of insulin, glucagon and epinephrine on plasma glutamine kinetics and its conversion to glucose by liver and kidney. J. Nutr. 130: 995S–1001S, 2000.

\textbf{KEY WORDS:} \textbullet{} glutamine \textbullet{} gluconeogenesis \textbullet{} kidney \textbullet{} liver \textbullet{} glucose metabolism

Glutamine is the most abundant free amino acid in the human body (Smith and Panico 1985). It not only participates as an important intermediate in numerous metabolic pathways, but also acts as a regulator of certain key physiologic processes (e.g., glycogen synthesis, gluconeogenesis and lipolysis) (Stumvoll et al. 1999). Preeminent among these functions is its role as an interorgan carrier of nitrogen and carbon (Nurjhan et al. 1995, Welbourne 1987).  

Until quite recently, studies in humans have used \textsuperscript{15}N-labeled glutamine to access its fluxes; consequently, virtually nothing was known about the fate of its carbon. During the past few years, we have used a combination of isotopic (\textsuperscript{14}C-labeled glutamine) and net-balance techniques to assess various aspects of glutamine carbon metabolism in humans (Nurjhan et al. 1995, Perriello et al. 1995 and 1997, Stumvoll et al. 1996). These studies indicate the following: 1) in postabsorptive, normal volunteers, plasma glutamine turnover (rates of appearance and disappearance) averages 5–6 \textmu{}mol/(kg min), slightly greater than that calculated using a \textsuperscript{15}N-labeled glutamine tracer (Kreider et al. 1997); and 2) 40–60% of plasma glutamine disappearance is due to oxidation (Nurjhan et al. 1995, Perriello et al. 1997, Stumvoll et al. 1996), 10–20% to gluconeogenesis (Nurjhan et al. 1995, Perriello et al. 1997, Stumvoll et al. 1996) and most of the remainder (~15%) to protein synthesis (Perriello et al. 1997) and incorporation into other macromolecules. Skeletal muscle (~50%) (Nurjhan et al. 1995, Stumvoll et al. 1996), kidney (~15%) (Stumvoll et al. 1998b) and liver (5–10%) (Stumvoll et al. 1998b) account for nearly 75% of glutamine uptake from plasma. The bulk of glutamine entering plasma (~70%) is due to de novo synthesis (Nurjhan et al. 1995, Stumvoll et al. 1996), some of which is the consequence of the conversion of glucose to glutamine (Perriello et al. 1995). Skeletal muscle is the predominant source of plasma glutamine, accounting for 65–75% of its rate of appearance in plasma (Nurjhan et al. 1995, Stumvoll et al. 1996).

In this paper, we review our recent work (Meyer et al. 1998, Stumvoll et al. 1998a and 1998b) concerning the effects of insulin, epinephrine and glucagon on systemic glutamine kinetics, the incorporation of glutamine into glucose by liver and kidney and the metabolism of glutamine by the kidney.

\textbf{SUBJECTS AND METHODS}

\textbf{Subjects and study design.} The data presented here were published previously (Meyer et al. 1998, Stumvoll et al. 1998a and 1998b); the reader is referred to these papers for a detailed description of the study designs and analytical procedures. In brief, all subjects were normal volunteers of both genders who had fasted overnight. At ~0730 h, a renal vein catheter was inserted under fluoroscopy; shortly thereafter, a primed infusion of \textsuperscript{p}-aminohippuric acid was started for calculation of renal blood flow. After the collection of at least three baseline blood samples at 30-min intervals for relevant substrate and hormone concentrations and for \textsuperscript{[3H]}glucose, \textsuperscript{[14C]}glucose and \textsuperscript{[14C]}glutamine specific activities from an arterialized dorsal hand vein and the renal vein, subjects were depending on the protocol, infused with insulin [0.6 mU/(kg min) for 4 h] along with sufficient glucose to maintain euglycemia using the glucose clamp technique (Meyer et al. 1996), or glucagon [5 ng (kg min) for 3 h] (Stumvoll et al. 1998a), or epinephrine [270 pmol/(kg min) for 3 h] (Stumvoll et al. 1998b).  

\textbf{Calculations.} Renal plasma flow (RPF) was determined by the \textsuperscript{p}-aminohippuric acid clearance technique (Brun 1951) and renal blood

\textsuperscript{1}Presented at the International Symposium on Glutamate, October 12–14, 1998 at the Clinical Center for Rare Diseases Aldo e Cele Dacco, Mario Negri Institute for Pharmacological Research, Bergamo, Italy. The symposium was sponsored jointly by the Baylor College of Medicine, the Center for Nutrition at the University of Pittsburgh School of Medicine, and Silvio Garattini, the Mario Negri Institute for Pharmacological Research.  

\textsuperscript{2}Supported in part by National Institutes of Health/DRR/GCRC grants SM01-RR0044 and NIDDK-20411.  

\textsuperscript{3}To whom correspondence should be addressed.

\textsuperscript{4}Abbreviations used: FFA, free fatty acids; FX, fractional extraction; HGR,
flow (RBF) was calculated as RPF/(1 - hematocrit). Fractional extraction (FX) of glucose across the kidney was calculated as ([6-3H] glucose specific activity \( SA_{\text{renal vein}} \) \times \( [6-3H] \) glucose\( SA_{\text{renal vein}} \) \times \( [6-3H] \) glucose\( SA_{\text{renal vein}} \)). Renal glucose uptake (RGU) was calculated as RBF \times \( [6-3H] \) glucose\( SA_{\text{renal vein}} \) \times FX, and renal glucose net balance (NB) as RBF \times \( [6-3H] \) glucose\( SA_{\text{renal vein}} \) - NB. Analogous equations were used for glutamine, except that renal plasma flow was used.

Systemic appearance (Ra) and removal (Rd) of glucose from the circulation was determined with steady-state equations under basal conditions and subsequently during infusion of epinephrine with non-steady–state equations. Hepatic glucose release (HGR) was calculated as the difference between the overall plasma appearance of glucose and renal glucose release. Systemic glutamine rates of appearance and disappearance were calculated using steady-state equations under basal conditions and a modification of DeBodo’s equation (DeBodo et al. 1963) during the non-steady–state using a pool fraction of 0.75 and a volume of distribution of 430 mL/kg body weight (Kreider et al. 1997).

The proportion of systemic glucose appearance in the steady state due to whole-body glutamine gluconeogenesis was calculated as \((1^{14}C\)glucose\( SA_{\text{art}} \)/\((1^{14}C\)glutamine\( SA_{\text{art}} \)) \times 100/1.2 using the standard precursor/product calculation (Kreisberg et al. 1970). The division by 1.2 corrects for differences in carbons (i.e., glutamine has 5, glucose has 6 carbons). Total glutamine gluconeogenesis was calculated as the proportion of glucose Ra due to glutamine multiplied by glucose Ra. During the non-steady state, whole-body glucose gluconeogenesis was calculated using the equation of Chiasson et al. (1977). These calculations assume that all glutamine carbons are incorporated into glucose, that there is no fixation of \(^{14}\)CO\(_2\) derived from oxidation of glutamine and that there is no dilution of \(^{14}\)C derived from glutamine by unlabeled glucose in the Krebs cycle. The first and last assumptions are not valid and will result in underestimates that could be considerable (~40%) (Consoli et al. 1987, Katz 1985, Krebs et al. 1966). On the other hand, Hankard et al. (1997) showed recently that only 4% of \(^{14}\)C glutamine incorporation into plasma glucose involves fixation of \(^{14}\)CO\(_2\) derived from glutamate.

Renal gluconeogenesis from glutamine was calculated as RBF \times \( (1^{14}C\)glucose\( SA_{\text{renal vein}} \)/\( [14C]\)glutamine\( SA_{\text{renal vein}} \)) \times (1 - FX) \times (\( [14C]\)glucose\( SA_{\text{renal vein}} \)/\( [14C]\)glutamine\( SA_{\text{renal vein}} \))) (BenGalim et al. 1980). Hepatic gluconeogenesis was calculated as the difference between systemic and renal gluconeogenesis for glutamine.

**Statistical analysis.** Data are expressed as means ± SEM. Unless stated otherwise, paired two-tailed Student’s t tests were used to compare data obtained before and after hormone infusions using the mean of baseline determinations and generally the mean of determinations during the last hour of the hormone infusions.

**RESULTS**

**Effects of insulin.** During infusion of insulin, plasma insulin concentrations averaged 219 ± 13 pmol/L (P < 0.001 vs. basal 36 ± 4 pmol/L) and plasma glucose concentrations were maintained at 4.97 ± 0.03 mmol/L. Systemic glucose release decreased ∼50% (P < 0.001) (Fig. 1). Although the absolute decrement in HGR was greater than that for RGR, in terms of the percentage of suppression, HGR decreased to a lesser extent than did RGR (47.3 ± 6.0% vs. 60.7 ± 4.3%, P = 0.027).

During insulin infusion, arterial glutamine concentration (Fig. 2) decreased (P = 0.001) because systemic glutamine disposal increased to a greater extent than systemic glucose release. Systemic glutamine clearance increased ∼25% from 10.0 ± 0.8 to 12.8 ± 0.6 mL/(kg · min) (P < 0.001). Insulin had no effect on renal glutamine net balance, fractional extraction, uptake and release of glutamine (Table 1).

Before infusion of insulin, renal and hepatic glucose gluconeogenesis accounted for 60.6 ± 2.8 and 39.4 ± 2.8%, respectively, of systemic glucose gluconeogenesis (Fig. 3). Insulin decreased systemic glucose gluconeogenesis ∼50%. As was the case for glucose release, insulin suppressed renal glutamine gluconeogenesis to a greater extent than hepatic glucose gluconeogenesis (71.6 ± 5.2 vs. 24.7 ± 4.9%, P < 0.001).

**Effects of epinephrine infusion.** During infusion of epinephrine, arterial epinephrine concentrations averaged 3550 ± 387 pmol/L; arterial glucose and insulin increased significantly (P < 0.001), whereas arterial glucagon remained unchanged (Fig. 4). Systemic glucose release, RGR and HGR all increased (Fig. 5). Arterial glutamine (Fig. 6) decreased slightly (P < 0.01) because glutamine removal from plasma increased to a greater extent than did its release into plasma.

During infusion of epinephrine, renal glucose gluconeogenesis (Fig. 7) increased nearly twofold (P < 0.001) and accounted for 90.3 ± 4.0% of systemic glucose gluconeogenesis and 67.3 ± 7.9% of renal glucose uptake during this interval. The contribution of renal glucose gluconeogenesis to overall renal glucose release remained unchanged (12.7 ± 1.7%, P = 0.13), suggesting that gluconeogenesis from other precursors was increased. Hepatic glucose gluconeogenesis decreased 50% during infusion of epinephrine (P = 0.01) and accounted for <10% of overall glutamine glu-
coneogenesis and <1% of overall hepatic glucose release during this period.

Renal glutamine fractional extraction increased; 60% (P < 0.001) and glutamine uptake increased nearly 80% (P < 0.005), whereas renal glutamine release increased about twofold (P < 0.001) (Table 1).

**Effects of glucagon.** Infusion of glucagon increased plasma glucagon to ~270 ng/L (Fig. 8). Plasma glucose increased transiently to a peak at 60 min, and then decreased to ~4.8 mmol/L at the end of the glucagon infusion (P = 0.16). Plasma insulin also increased transiently.

Systemic glucose release (Fig. 9) increased transiently to a peak at 30 min and subsequently decreased to values not different from baseline (P = 0.48). HGR increased to a peak at 30 min and subsequently decreased to rates that were not different from baseline. RGR did not change during infusion of glucagon.

Arterial glutamine decreased progressively from 0.58 ± 0.03 to 0.51 ± 0.04 mmol/L (P < 0.02) at the end of the glucagon infusion (Fig. 10) despite no detectable change in plasma glutamine rates of appearance and disappearance.

Before infusion of glucagon, systemic glutamine gluconeogenesis (Fig. 11) accounted for 3.7 ± 0.3% of systemic glucose release. Renal glutamine gluconeogenesis accounted for 74.8 ± 6.2% of systemic glutamine gluconeogenesis and 12.3 ± 1.4% of RGR. Hepatic glutamine gluconeogenesis accounted for 25.2 ± 6.2% of systemic glutamine gluconeogenesis and 1.2 ± 0.3% of HGR.

During infusion of glucagon, systemic glutamine gluconeogenesis increased progressively and accounted for an increased proportion of systemic glucose release (5.2 ± 0.3%, P < 0.002). Renal glutamine gluconeogenesis remained unchanged, whereas hepatic glutamine gluconeogenesis increased nearly threefold and accounted for increased proportions of systemic glutamine gluconeogenesis (51.6 ± 5.5%, P = 0.002) and HGR (3.8 ± 0.5%, P = 0.002). Infusion of glucagon had no effect on renal glutamine net balance, fractional extraction, uptake and release (Table 1).

**TABLE 1**

<table>
<thead>
<tr>
<th>Effect of insulin, epinephrine and glucagon on renal glutamine metabolism</th>
<th>Net balance</th>
<th>Fractional extraction</th>
<th>Uptake</th>
<th>Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/min</td>
<td>%</td>
<td>µmol/min</td>
<td>µmol/min</td>
<td></td>
</tr>
<tr>
<td>Baseline²</td>
<td>20 ± 4</td>
<td>8.0 ± 1.9</td>
<td>38 ± 7</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Insulin³</td>
<td>15 ± 4</td>
<td>5.2 ± 1.1</td>
<td>29 ± 8</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>P =</td>
<td>0.38</td>
<td>0.25</td>
<td>0.45</td>
<td>0.54</td>
</tr>
<tr>
<td>Baseline²</td>
<td>36 ± 8</td>
<td>9.2 ± 1.2</td>
<td>54 ± 10</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Epinephrine³</td>
<td>59 ± 12</td>
<td>15.1 ± 1.4</td>
<td>96 ± 13</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>P =</td>
<td>0.01</td>
<td>0.001</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>Baseline²</td>
<td>31 ± 9</td>
<td>8.2 ± 1.7</td>
<td>40 ± 8</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Glucagon³</td>
<td>39 ± 4</td>
<td>10.1 ± 1.1</td>
<td>48 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>P =</td>
<td>0.46</td>
<td>0.32</td>
<td>0.48</td>
<td>0.44</td>
</tr>
</tbody>
</table>

¹ The data are from Kreider et al. (1997), Miles et al. (1984) and Perriello et al. (1995).
² Mean of 3 baseline values.
³ Mean of values during last hour of infusions.
DISCUSSION

These studies provide some insight into the hormonal regulation of systemic and renal glutamine metabolism. Insulin decreased arterial glutamine while increasing both its release into and removal from plasma and suppressing its conversion to glucose by liver and kidney. The increase in release of glutamine into plasma was unexpected because insulin suppression of proteolysis should have reduced the release of glutamine into the circulation. However, the increased glucose disposal with subsequent conversion of glucose to glutamine (Perriello et al. 1995) could readily account for this. The increase in systemic glutamine disposal could reflect stimulation of protein synthesis or the substitution of glutamine for free fatty acids as an oxidative fuel in certain tissues.

Although hepatic glutamine uptake was not measured, renal glutamine uptake was not affected by insulin. This indicates that the increased systemic glutamine disposal was due to extrarenal tissues and, furthermore, that the decrease in renal glutamine gluconeogenesis was not due to decreased availability of glutamine. Regarding the latter, inhibition of gluconeogenic enzymes by insulin and suppression of plasma free fatty acids (FFA) (known stimulators of renal gluconeogenesis) (Krebs et al. 1965) leading to diversion of glutamine carbon into the oxidative pathway could be possible explanations. Clearly it would have been of interest to measure glutamine oxidation in those experiments.

Epinephrine had effects similar to those of insulin on arterial glutamine and its plasma kinetics but increased glutamine conversion to glucose selectively by the kidney. The increase in release of glutamine into plasma, which was also found by Matthew et al. (1990) using N\(^{15}\)-labeled glutamine, could reflect increased proteolysis as well as increased conversion of glucose to glutamine. However, Miles et al. (1984) reported that proteolysis, as reflects by leucine fluxes, decreases during infusion of epinephrine and that the increase in plasma alanine appearance during infusion of epinephrine is due to de novo synthesis. The increase in systemic glutamine disposal was due to some extent to an increase in renal glutamine uptake, but this could explain only a small proportion leaving plasma. The other tissue sites remain to be determined.

The stimulation of glutamine gluconeogenesis was wholly accounted for by the increase in renal gluconeogenesis, which in turn was almost nearly equal to the increase in renal glutamine uptake. The latter was explained largely by an increase in renal glutamine fractional extraction. Whether this completely explains the stimulatory effects of epinephrine remains to be determined because other mechanisms are possible (e.g., increased FFA or activation of gluconeogenic enzymes).

The lack of effects of epinephrine on hepatic glutamine gluconeogenesis was unexpected because epinephrine stimulates hepatic alanine gluconeogenesis (Stumvoll et al. 1998b). These different responses of liver and kidney could involve differences in hepatic and renal amino acid transport systems (Shotwell et al. 1983) and glutaminase activities (Joseph and McGivan, 1978) as well as the relative sensitivity of liver and kidney to epinephrine (e.g., receptor density).
FIGURE 6  Effects of epinephrine on arterial glutamine concentration and rates of plasma glutamine appearance (Ra) and disappearance (Rd). Data from Stumvoll et al. (1998b).

FIGURE 7  Effects of epinephrine on systemic, renal and hepatic glutamine gluconeogenesis. Data from Stumvoll et al. (1998b).

FIGURE 8  Effects of glucagon on plasma glucose and insulin concentrations. Data from Stumvoll et al. (1998a).

FIGURE 9  Effects of glucagon on systemic, renal and hepatic glucose release. Data from Stumvoll et al. (1998a).
Glucagon infusion decreased arterial glutamine concentration without a detectable change in either the rate of plasma glutamine appearance or disappearance, and increased hepatic, but not renal glutamine conversion to glucose. We believe that lack of statistical power probably explains the failure to detect changes in systemic glutamine kinetics. Presumably, glutamine removal from plasma during the glucagon infusion must have exceeded its release into plasma. Simulation of glutamine conversion to glucose in liver could reflect the effects of glucagon on hepatic amino acid transport and/or its stimulation of hepatic glutaminase (Ochwadt et al. 1965).

In summary, previous studies have shown that cortisol affects glutamine metabolism in humans (Darmaun et al. 1988); the present studies demonstrate that insulin, glucagon and epinephrine also affect glutamine metabolism in humans. Furthermore, glucagon and epinephrine show selectivity in their stimulation of glutamine conversion to glucose by liver and kidney. The mechanism for these differences and their physiologic significance remain to be determined. The studies reported here provide evidence that the human kidney makes more than a trivial contribution to overall gluconeogenesis under postabsorptive conditions, e.g., 40–50%, assuming that gluconeogenesis represents ~50% of overall glucose production (Landau et al. 1995). A similar conclusion can be drawn from the recent work by Cersosimo et al. (1999a, 1999b and 1998). Nevertheless, Ekberg et al. (1999) recently reported a contribution of only ~10%. The latter findings should be interpreted with caution, however, because negative values for renal glucose uptake and renal glucose fractional extraction were found using [6H]glucose, but not using [U-13C]glucose. Indeed, their 95% confidence limits would be consistent with a renal contribution to overall gluconeogenesis as great as 20–30%.

ACKNOWLEDGMENTS

We thank the staff of the General Clinical Research Center for their excellent technical help and Mary Little for her superb editorial support.

LITERATURE CITED

production in the postabsorptive state and after 60 h of fasting. Diabetes 48: 292–298.


