Glutamine Appearance Rate in Plasma Is Not Increased after Gastrointestinal Surgery in Humans

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ABSTRACT The metabolic response to surgical stress is characterized by muscle protein breakdown and mobilization of amino acids and has been postulated to furnish glutamine and other amino acids to the immune system, gut and liver. The present study was undertaken to investigate whether the whole body appearance rate (Ra) of glutamine in plasma is increased after major elective surgery. Fourteen patients (8 males, 6 females) were measured prior to laparotomy and on the second postoperative day. Patients received a primed continuous 6-h infusion of L-[5,15-N]glutamine and L-[1,13C]leucine, and arterial blood samples and muscle biopsies were taken for concentration and enrichment measurements. As expected, the metabolic response to surgery was characterized by a rise in whole body protein breakdown (n = 14, P < 0.001) and a decreased concentration of glutamine in plasma (n = 14, P < 0.001) and muscle (n = 8, P < 0.01). However, these catabolic changes were not reflected by an increase in the plasma Ra of glutamine: 246 ± 8 μmol·kg⁻¹·h⁻¹ before surgery vs. 241 ± 10 μmol·kg⁻¹·h⁻¹ on the second postoperative day. We conclude that the whole body Ra of glutamine in plasma is not increased 2 d after elective gastrointestinal surgery. Further studies are warranted to establish whether the lack of an increase in plasma glutamine Ra provides a rationale for glutamine supplementation.

KEY WORDS: glutamine metabolism, trauma, humans, stable isotopes, protein degradation

The metabolic response to surgical trauma is characterized by increased peripheral protein breakdown. Amino acids are mobilized and taken up by visceral organs for gluconeogenesis and synthesis of acute phase proteins (Clowes et al. 1983, Deutz et al. 1992, Douglas and Shaw 1989, Wannemacher 1977). It has been postulated that these processes serve the purpose of supplying fuel for energy generation and building blocks for the synthesis of specific proteins involved in the immunological response and in the restoration of damaged tissue.

Glutamine has a pivotal role as major gluconeogenic precursor and vehicle for interorgan carbon and nitrogen transport (Nur- 
nization method, using a primed continuous infusion of stable isotopes, to assess whole body protein turnover in order to relate changes in glutamine Ra to surgery-induced alterations in whole body protein breakdown.

PATIENTS AND METHODS

Patients. Fourteen patients (aged 58–78 y, 8 men and 6 women) admitted to the hospital for elective abdominal surgery participated in the study. Underlying diseases were colorectal cancer (n = 6), gastric cancer (n = 1), pancreatic cancer (n = 1), villous adenoma (n = 2), diverticular disease (n = 3) and Crohn’s disease (n = 1). No clinical signs of inflammatory activity were present. Body weight averaged 73 ± 4 kg and was stable in all subjects, except in three patients who had suffered a recent weight loss of ~10% over the last 6 mo. Body mass index was 25.9 ± 1.1 kg/m². Written informed
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consent was obtained from all patients, and the protocol was approved by the Medical Ethics Committee of the University Hospital Maastricht.

Study design. The measurements were performed 1 or 2 d before surgery and on the second postoperative day. Preoperatively, the patients were fasted overnight and remained fasted until the study was completed. In the period between surgery and the postoperative measurement, fluid balance was maintained with normal saline and glucose 50 g/L (2–3 L/d, 850–1250 kJ per 24 h) according to standard protocol. Patients had a zero nitrogen intake during this period.

The patients were operated in epidural analgesia with local anesthetics (bupivacaine and sufentanil) in addition to general anesthesia. The epidural catheter was left in place during 1–4 d after surgery. In 9 patients the epidural infusion of local anesthetics and opiates was continued during the postoperative measurements. In the other subjects the measurements were carried out after the epidural block had been withdrawn.

In the morning of each study, patients received a catheter in an antecubital vein for isotope infusion and another in the radial artery for blood sampling. The arterial catheter was kept patent by a slow saline infusion. Glucose infusion was stopped during the experiments. At 0830 h a primed constant intravenous infusion of L-[1-15N]glutamine (0.68 μmol · kg⁻¹ · h⁻¹; prime 0.68 μmol/kg) and L-[1-13C]leucine (7.63 μmol · kg⁻¹ · h⁻¹; prime 7.63 μmol/kg) was given for 6 h. The tracers were purchased from Cambridge Isotope Laboratories (Woburn, MA). Blood samples were drawn in chilled-on-ice heparinized tubes before the start of the tracer infusion for measurement of baseline enrichment and at 2, 3, 4, 5 and 6 h after onset of the infusion. Plasma was obtained by centrifugation of whole blood at 2,200 × g at 4°C for 5 min. For the determination of plasma glutamine concentration, plasma was deproteinized with sulfosalicylic acid (van Eijk et al. 1994), mixed with a vortex mixer, frozen in liquid nitrogen and stored at −80°C. For tracer enrichment measurements, plasma was frozen and stored at −80°C until analysis. In 8 patients, percutaneous muscle biopsies were taken from the anterior tibial muscle once or twice during each tracer infusion to measure glutamine enrichment and concentration in the intracellular free glutamine pool in muscle. Biopsies were taken using the conchothome technique (Dietrichson et al. 1987). Blood, visible fat and connective tissue were quickly removed from the specimen, and the tissue was immediately frozen in liquid nitrogen and subsequently stored at −80°C for later analysis. To limit the number of biopsies in each patient, baseline enrichment in muscle was assumed to be equal to the enrichment in plasma. Support for this assumption comes from a previous study in a similar group of preoperative patients. By extrapolating back to time zero the glutamine enrichment data in successive measurements performed on the second postoperative day, Values of intracellular enrichment were calculated in a similar manner.

Calculations. The Ra of glutamine into plasma (Ra,Gln,in, in μmol · kg⁻¹ · h⁻¹) was calculated as:

\[ R_{a,Gln} = \frac{\text{tracer flux}}{2} = \frac{[\text{E}_{Gln},p]}{t} \]

where \( [\text{E}_{Gln},p] \) is the tracer enrichment rate in μmol · kg⁻¹ · h⁻¹, \( E_{Gln} \) is the glutamine enrichment in the tracer infused, expressed in mole percentage excess (MPE), and \( E_{Gln} \) is the mean plasma glutamine enrichment between 2 and 6 h of tracer infusion. As demonstrated before, the rate of glutamine appearance obtained in this way overestimates the true appearance rate of glutamine in plasma by at least 20% because of slow equilibration of the glutamine tracer with the large muscle glutamine pool (van Acker et al. 1998). Proteolysis was measured using the whole body Ra, of leucine (Ra,leu, in μmol · kg⁻¹ · h⁻¹). Ra,leu was calculated using plasma KIC enrichments (Horbert et al. 1989):

\[ R_{a,leu} = \frac{[\text{E}_{leu},p]}{t} \]

where \( [\text{E}_{leu},p] \) is the leucine enrichment rate in μmol · kg⁻¹ · h⁻¹, \( E_{leu} \) is the leucine enrichment (MPE) in the tracer infused and \( E_{leu,KIC} \) is the average plasma KIC enrichment from 2 to 6 h of tracer infusion. In the absence of exogenous amino acids, the calculated Ra by definition equals the endogenous Ra.

Glutamine arising from protein degradation (PD,Gln) was calculated as:

\[ PD_{Gln} = R_{a,leu} - R_{a,Gln}, \]

assuming the plasma concentration [Gln]p to be equal to the concentration in the interstitial fluid. Regarding the preoperative measurements, the extracellular (H2Oe) and intracellular (H 2Oi) water contents (mL/kg muscle) were assumed to amount to 13 and 87% of the total water content of muscle tissue (Bergström et al. 1974). Concerning the increased extracellular and decreased intracellular water content observed after surgical trauma (Vinnars et al. 1975) values of 18% (H2Oe) and 82% (H 2Oi) were used for the measurements performed on the second postoperative day. Values of intracellular enrichment were calculated in a similar manner.

Statistics. Data are given as means ± SEM, unless stated otherwise. Comparisons between pre- and postoperative values were made using the Wilcoxon matched pairs signed rank sum test. The Mann-Whitney U test was used for comparing data from different patient groups (i.e., patients with and without cancer, patients with and without epidural anesthesia). Regarding the time course of glutamine and KIC enrichment in plasma, a repeated measures ANOVA was performed to detect effects of time and surgical treatment. A P-value of < 0.05 was considered significant.

RESULTS

Plasma [15 N]glutamine and [1-13C]KIC enrichments before and after surgery are shown in Figure 1. A plateau in enrichment was achieved for KIC but not for glutamine. Plasma glutamine enrichment increased slowly but significantly with tracer infusion time (P < 0.05), indicating that isotopic steady state had not been reached. Similar values were obtained for pre- and postoperative glutamine enrichment in plasma. On the other hand, plasma KIC enrichment decreased after surgery (P < 0.001), reflecting increased dilution of the leucine
tracing by endogenous amino acids. When calculating whole body protein breakdown rates, individual patients showed higher values on the second postoperative day than during the preoperative study. On average, leucine $R_a$ increased by 26 ± 5%, from 91 ± 3 μmol·kg⁻¹·h⁻¹ before operation to 115 ± 6 μmol·kg⁻¹·h⁻¹ 2 d after surgery (Fig. 2, P < 0.001). Despite the rise in whole body protein breakdown, no increase was observed in the $R_a$ of glutamine in plasma (Fig. 3). Whole body glutamine $R_a$ in plasma averaged 246 ± 8 and 241 ± 10 μmol·kg⁻¹·h⁻¹ during the pre- and postoperative measurement, respectively, with some patients showing no change, some showing an increase and some a decrease in glutamine $R_a$. The calculated amount of glutamine $R_a$ arising from proteolysis increased in each patient, and averaged 45 ± 1 μmol·kg⁻¹·h⁻¹ before and 57 ± 3 μmol·kg⁻¹·h⁻¹ on d 2 after surgery ($P < 0.001$).

The rates of protein breakdown and glutamine $R_a$ were not different between patients with cancer and those without, either before or after the surgical trauma (Table 1). Also, similar values were obtained in patients with recent weight loss and patients with a stable weight. The presence of epidural anesthesia did not affect the postoperative changes in leucine and glutamine $R_a$; leucine $R_a$ increased by 30 ± 6% and 20 ± 6% in the patients with and without epidural anesthesia, respectively, whereas glutamine $R_a$ did not change in either group (−4 ± 6% and −1 ± 3%).

After surgery, the concentration of glutamine in plasma decreased in every patient. On the second postoperative day, the average decrease in plasma glutamine concentration was 31 ± 3%, from 625 ± 22 μmol/L to 431 ± 17 μmol/L (Fig. 4, $P < 0.001$). Hematocrit decreased by 12 ± 2%, from 0.40 ± 0.01 to 0.35 ± 0.01 ($P < 0.001$), indicating that part of the reduction in plasma glutamine may be attributed to postsurgical hemodilution. A decrease also occurred in the concentration of intramuscular free glutamine: from 10.82 ± 0.75 to 7.69 ± 0.59 mmol/L intracellular water, a decline of 27 ± 6% (Fig. 5, $P < 0.01$). No change was observed in the total water content in muscle tissue: 759 ± 19 mL/kg muscle before surgery vs. 756 ± 16 mL/kg muscle 2 d after surgery.

As a result of the reduced free glutamine pool in muscle, on the second postoperative day higher levels of intramuscular glutamine enrichment were observed toward the end of each tracer infusion: 0.055 ± 0.006 vs. 0.038 ± 0.005 MPE preoperatively (Fig. 6, $P < 0.05$). When the postoperative reduction in the size of the intramuscular free glutamine pool was taken into account, similar amounts of labeled glutamine were found in muscle after 6 h of tracer infusion: 4.0 ± 0.6 and 4.1 ± 0.5 μmol/L intracellular water during pre- and postoperative measurements, respectively. As shown in Figure 6, in four postoperative patients an additional biopsy was taken 3 h after start of tracer infusion. The rate of rise of labeled glutamine in muscle calculated from the consecutive biopsies was 0.56 ± 0.05 μmol·L⁻¹ intracellular water⁻¹·h⁻¹ between 3 and 6 h of tracer infusion. When, in these patients, the rate of rise of labeled glutamine in muscle was calculated on the basis of a single biopsy at 6 h assuming equal enrichment in plasma and muscle at baseline, a similar value was obtained (0.58 ± 0.09 μmol·L⁻¹ intracellular water⁻¹·h⁻¹), suggesting that the assumption was valid.
DISCUSSION

The changes in protein breakdown and in plasma and intramuscular glutamine concentration observed in this study are consistent with the literature concerning the metabolic effects of elective abdominal surgery (Carli et al. 1990, Vinnars et al. 1975). The catabolic stress response was not arrested by continuous epidural infusion of local anesthetics, presumably as a result of the 60–70% lower dose of bupivacaine used in the present study as compared to previous investigations on the effect of epidural anesthesia (Carli et al. 1996). Proteolysis of skeletal muscle is a major contributor to the increase in whole body protein breakdown observed 2 d after surgery (Clowes et al. 1983). The decrease in the concentration of glutamine in skeletal muscle is a highly consistent finding after trauma (Essén et al. 1992, Vinnars et al. 1975). Quantitatively, this is a considerable reduction, because muscle glutamine represents more than half of the total free amino acid pool in the human body. Essén et al. observed a 21% decline in the concentration of glutamine in muscle during the first 24 h after surgery (Essén et al. 1992). This would indicate that the losses of glutamine from the intracellular free glutamine pool occur mainly during the first postoperative day, and to a smaller extent during the following days.

The rise in protein breakdown and the shrinking plasma and intracellular glutamine pools 48 h after laparotomy is consistent with the concept of an increased metabolic demand of glutamine in the postoperative period. Nevertheless, this remains conjectural because it is not fully known how intracellular glutamine levels are maintained and how this process is regulated. Other possibilities may include an impairment of

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| **Whole body glutamine appearance rate and whole body protein breakdown before and 2 d after surgery in patients with and without gastrointestinal cancer**

<table>
<thead>
<tr>
<th></th>
<th>Preoperative</th>
<th>Postoperative</th>
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<tr>
<td></td>
<td>Cancer</td>
<td>Noncancer</td>
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<tr>
<td>Glutamine $R_a$</td>
<td>248 ± 12</td>
<td>244 ± 14</td>
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<tr>
<td>Leucine $R_a$</td>
<td>88 ± 3</td>
<td>96 ± 4</td>
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1 Means ± SEM, $n = 8$ (cancer) or 6 (noncancer). $R_a =$ appearance rate. Statistical analysis by Mann-Whitney $U$ test and Wilcoxon signed ranks test. Preoperative cancer vs. noncancer: Glutamine $R_a$, ns; Leucine $R_a$, ns; Postoperative vs. preoperative, cancer: Glutamine $R_a$, ns; Leucine $R_a$, $P < 0.05$. Postoperative vs. preoperative, noncancer: Glutamine $R_a$, ns; Leucine $R_a$, $P < 0.05$, ns, $P \geq 0.05$.
membrane integrity, a loss of the Na\(^+\) electrochemical gradient and/or changes in the activity of the glutamine transporter by trauma (Hundal et al. 1987, Rennie et al. 1986). Evidence exists that tissues involved in the immune system, such as liver and spleen, take up increased amounts of glutamine in response to trauma (Deutz et al. 1992). Previous studies in humans have shown an increased net release of glutamine from muscle during periods of elevated metabolic stress, such as during sepsis (Clowes et al. 1980), 3 to 5 d after major trauma (Clowes et al. 1980), immediately after elective cholecystectomy (Stjernström et al. 1986), 4 d after hysterectomy (Carli et al. 1990) and on the d 2 after elective gastrointestinal surgery in a group of patients similar as the present study group (Mjaaland et al. 1993). On the other hand, utilizing tracer kinetic techniques, we did not observe a rise in the \( R_a \) of glutamine in plasma 2 d after surgery. Our findings are in agreement with a recent study in critically ill patients, showing an unaltered plasma glutamine \( R_a \) when compared to matched healthy controls, despite a major increase in proteolysis and decrease in plasma glutamine concentration (Jackson et al. 1999). A discrepancy between the \( R_a \) of glutamine in plasma and the net release of glutamine from muscle has previously been observed in our laboratory; in rats, the surgery-induced rise in the net release of muscle glutamine was not matched by an increased plasma \( R_a \) (de Blaauw et al. 1998).

In the absence of exogenous glutamine, the sources that contribute to the \( R_a \) of glutamine are proteolysis, de novo synthesis and glutamine losses from the free intracellular pool. Because glutamine derived from proteolysis was increased after surgery, a decline in the rate of glutamine de novo synthesis and/or a reduction in the loss from the free intracellular pool are factors that might explain the absence of any change in the whole body glutamine \( R_a \). Hankard et al. recently observed an \( \sim 11\% \) decline in whole-body glutamine \( R_a \) between 18 and 42 h of fasting, entirely accounted for by a drop in the estimated rates of glutamine de novo synthesis (Hankard et al. 1997). In the present study, the preoperative measurements were conducted after an overnight fast, whereas during the postsurgical measurements patients were in a near-fasted state for about 80 h. Only a limited amount of glucose was provided in the immediate postoperative days, which is the postoperative clinical routine in patients undergoing gastrointestinal surgery. In theory, therefore, this period of fasting may have played a role in our observation that the plasma \( R_a \) of glutamine was not increased on the second postoperative day.

Although skeletal muscle is the main glutamine-producing tissue [in humans \( >60\% \) of endogenous glutamine \( R_a \) is released by muscle (Nurjhan et al. 1995, Stumvoll et al. 1996)], other sources such as liver, brain, lung and adipose tissue also contribute to the \( R_a \) of glutamine in plasma measured by tracer-dilution techniques. An alternative explanation for the unchanged whole body glutamine \( R_a \) after laparotomy, therefore, is that any surgery-induced rise in muscle glutamine release might have been offset by a decreased release from sources other than muscle, such as liver. Increased consumption of glutamine produced within the tissue itself, before exchange has occurred with the systemic circulation, may contribute to the diminished release. In a previous study in pigs, we showed that the liver switches from net production of glutamine in the preoperative setting to net consumption of glutamine after surgery (Deutz et al. 1992). Part of the glutamine consumed by the liver may be used for proliferation of Kupffer cells and synthesis of acute phase proteins, another part for gluconeogenesis.

With respect to the muscle data, we assumed similar glutamine baseline enrichment values in muscle and plasma. Support for this assumption comes from the consecutive muscle biopsies taken here and in a previous study (van Acker et al. 1998). By extrapolating the successive glutamine enrichment data back to time zero, it was shown that the baseline enrichment in muscle approximated that in plasma, both in pre- and postoperative patients. As a result of the slow equilibration of the glutamine tracer with the large muscle glutamine pool, the \( R_a \) of glutamine in plasma overestimates the whole-body glutamine flux (van Acker et al. 1998). In this study, too, isotopic steady-state conditions were not achieved during the 6-h infusion of glutamine tracers, neither before nor after surgery. However, similar amounts of glutamine tracer were retained in the Intramuscular free glutamine pool during the two measurements, i.e., before and 2 d after surgery. This indicates that the degree in which the true glutamine flux is estimated rates of glutamine de novo synthesis in vivo.
undergoing gastrointestinal surgery will benefit from glutamine derived amino acids). In that case it is likely that patients undergoing gastrointestinal surgery will benefit from glutamine supplementation in the first few days after surgery.

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**LITERATURE CITED**


