Absence of glutamine isotopic steady state: implications for the assessment of whole-body glutamine production rate


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ABSTRACT

1. During infusion of [5-15N]glutamine in patients with gastrointestinal cancer we unexpectedly observed a gradual decrease in time of the appearance rate ($R_a$) of glutamine in plasma. Here we investigate whether the failure to achieve a plateau isotopic enrichment in plasma is, among other factors, due to incomplete equilibration of the glutamine tracer with the large intramuscular free glutamine pool.

2. Plasma and intramuscular glutamine enrichment were measured during 6–11 h infusions of L-[5-15N]glutamine and L-[1-13C]glutamine in post-absorptive patients admitted to hospital for elective abdominal surgery. L-[1-13C]Leucine and L-[ring-2H5]phenylalanine were infused to measure the proportion of glutamine appearing in plasma directly due to its release from protein.

3. The glutamine tracer entered muscle, but the rise in intramuscular glutamine enrichment was small, presumably as a result of the enormous size of the intramuscular glutamine pool and the limited speed of entry of glutamine into muscle. In each patient the intramuscular glutamine enrichment was lower than that in plasma ($P < 0.001$), and both increased with tracer infusion time ($P < 0.001$), indicating incomplete equilibration of the glutamine tracer.

4. A comparison of the results obtained by the two glutamine tracers indicated that recycling of the nitrogen label contributed to about 15% of the decrease in $R_a$.

5. There was a gradual reduction in the glutamine release from proteolysis, which contributed to 16–21% of the decline in $R_a$.

6. We conclude that slow equilibration of the glutamine tracer with the large muscle glutamine pool significantly contributes to the absence of isotopic steady state. Consequently, the appearance rate of glutamine in plasma measured during short tracer infusion periods (hours) considerably overestimates the whole-body glutamine flux.

Key words: glutamine kinetics, muscle, stable isotopes.

Abbreviations: APE, atom percentage excess; KIC, α-ketoisocaproate; MPE, mole percentage excess; TBDMS, tert-butyldimethylsilyl.

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INTRODUCTION

Glutamine is the most abundant free amino acid in the human body and can be synthesized from glutamate and ammonia by the enzyme glutamine synthetase in various tissues. Muscle tissue is a major site for glutamine synthesis in the human body [1–3] and contains over 90% of the large whole-body glutamine pool [4,5]. Glutamine has several pivotal biochemical properties which suggest it plays an important role in health and disease. Among these are the transport of nitrogen from the periphery to visceral organs, the maintenance of acid/base balance, the provision of fuel for rapidly dividing cells and of nitrogen for de novo synthesis of nucleotides [6].

The rationale for clinical dietary regimens to include glutamine stems from the hypothesis that severely or chronically ill patients, who often have a reduced muscle mass, produce insufficient amounts of glutamine to meet their increased needs [7]. However, actual data on glutamine production and utilization in vivo in humans are scarce and are limited primarily to studies in healthy individuals. Most of the current knowledge of glutamine metabolism has been gained from invasive studies using determination of arteriovenous concentration differences across different organ beds and from whole-body flux measurements with stable isotope tracers. To judge whether endogenous glutamine production was depressed in patients with gastrointestinal malignancies, we measured whole-body glutamine fluxes by the traditional approach. In contrast to previous studies in healthy subjects we did not observe a steady state in plasma glutamine enrichment between 2 and 6 h of infusion with L-[1-15N]glutamine. Instead, glutamine enrichment in plasma gradually increased for the duration of the study.

Therefore, the aim of the present study was to investigate the mechanism behind this ongoing rise in plasma enrichment. We specifically investigated the potential role and relative contribution of (1) incomplete equilibration of the glutamine tracer with the large muscle compartment, (2) recycling of the labelled amide nitrogen, and (3) gradual changes in the contribution of protein breakdown to glutamine production. Patients were infused with L-[5-15N]glutamine for periods of up to 11 h. Arterial blood samples and percutaneous muscle biopsies were taken and analysed for glutamine enrichment to determine to what extent and at what rate the intravenously administered glutamine tracer mixes with the large glutamine pool in skeletal muscle. To investigate recycling of the amide-15N of glutamine a simultaneous infusion of L-[1-13C]glutamine was given to a subgroup of patients. Two essential amino acid tracers, L-[1-13C]leucine and L-[ring-2H6]phenylalanine, were used to determine the contribution of protein degradation to glutamine production throughout the study.

METHODS

Patients

Twenty patients (12 males and 8 females, aged 62 ± 13 years) admitted to the hospital for elective abdominal surgery participated in the study. The patients had a normal body mass index (23.4 ± 0.7 kg/m²) and most had a stable body weight. Underlying diseases were colorectal cancer (n = 8), gastric cancer (n = 4), intra-abdominal carcinoid (n = 1), villous adenoma (n = 2), gastric volvulus (n = 1), diverticular disease (n = 1), ulcerative colitis (n = 1) and Crohn’s disease (n = 2). No clinical signs of inflammatory activity were present. Written informed consent was obtained from all patients under the protocol as approved by the Medical Ethics Committee of the University Hospital Maastricht.

Materials

L-[5-15N]glutamine (99% 15N in the amide nitrogen), L-[1-13C]glutamine (99% 13C), L-[1-13C]leucine (99% 13C) and L-[ring-2H6]phenylalanine (99% 2H) were purchased from Cambridge Isotope Laboratories (Woburn, MA, U.S.A.).

Study design

The measurements were performed 1 or 2 days before surgery. Patients were fasted overnight and remained fasted until the study was complete. On the morning of the study two catheters were inserted: one into an antecubital vein for isotope infusion and the other into the radial artery for blood sampling. The arterial catheter was kept patent by a slow 0.9% NaCl infusion. At 08:30 h, a primed constant intravenous infusion of L-[5-15N]glutamine (priming dose 0.68 µmol/kg; infusion rate 0.68 µmol h⁻¹ kg⁻¹), L-[1-13C]leucine (priming dose 7.63 µmol/kg; infusion rate 7.63 µmol h⁻¹ kg⁻¹) and L-[ring-2H6]phenylalanine (priming dose 3.03 µmol/kg; infusion rate 3.03 µmol h⁻¹ kg⁻¹) was given to 15 patients for 6 h. The other five patients received an 11-h infusion including 13C-labelled glutamine: L-[5-15N]glutamine (priming dose 0.82 µmol/kg; infusion rate 0.82 µmol h⁻¹ kg⁻¹), L-[1-13C]glutamine (priming dose 0.82 µmol/kg; infusion rate 0.82 µmol h⁻¹ kg⁻¹), L-[1-13C]leucine (priming dose 9.20 µmol/kg; infusion rate 9.20 µmol h⁻¹ kg⁻¹) and L-[ring-2H6]phenylalanine (priming dose 3.64 µmol/kg; infusion rate 3.64 µmol h⁻¹ kg⁻¹). Blood samples were drawn in chilled-on-ice heparinized tubes before the start of the tracer infusion for measurement of baseline enrichment and then at 0.5–2-h intervals throughout the study. Plasma was obtained by centrifugation of whole blood at 3500 r.p.m. (2200 g) at 4 °C for 5 min. For the determination of glutamine concentration plasma was deproteinized with sulphosalicylic acid [8], vortexed, frozen in liquid nitrogen and stored at −80 °C. For tracer enrich-
ment measurements plasma was frozen and stored at −80 °C until analysis. Percutaneous muscle biopsies were taken from the anterior tibial muscle at one to three time points during the tracer infusion to measure glutamine enrichment and concentration in the intracellular free glutamine pool in muscle. Biopsies were taken using the conchotome technique as described previously [9]. 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 equal that in plasma.

**Analytical procedures**
The biopsies were freeze-dried and further freed from adherent blood and connective tissues. The water content of the biopsies was calculated from the weight difference before and after freeze-drying and used for conversion from wet to dry weight. The muscle specimens were powdered and deproteinized using a Mini-Beadbeater (Biospeck Products, Bartlesville, U.S.A.). Approximately 30 mg of the pulverized tissue was added to 350 µl of 5% sulphosalicylic acid and 0.1-g glass beads (diameter 1 mm, Biospeck Products) and beaten for 30 s. The supernatant was frozen in liquid nitrogen and stored at −80 °C until later analysis. The concentration of glutamine in supernatant, plasma and tracer infusate was determined by fully automated HPLC [10].

The $^{15}$N and $^{13}$C enrichment of plasma and muscle glutamine was determined using GC combustion isotope ratio MS (Finnigan MAT 252, Bremen, Germany). In brief, deproteinized plasma and muscle extracts were passed over an ion-exchange column and the eluted amino acids were freeze-dried and then derivatized with N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) (Pierce Europe, The Netherlands) into the tert-butyldimethylsilyl (TBDMs) derivative as described previously [11]. The resulting molecule (C$_{60}$H$_{111}$N$_5$O$_2$Si$_2$) contains three TBDMS groups attached to the carboxy, the 2-amino and the 5-amide group of glutamine respectively. After injection and baseline separation in the gas chromatograph the amino acid derivative is combusted in the capillary-sized combustion interface and the pulses of N$_2$ and CO$_2$ generated are introduced on-line into the IRMS in a stream of helium. The enrichment of glutamine standards was linear from natural abundance up to +1.0 APE (atom percentage excess) both for $^{13}$C and $^{15}$N. The reproducibility of seven repeated measurements was ±0.0002 APE or better for carbon and ±0.001 APE or better for nitrogen over the specified enrichment range both for standards and biological samples. The precision of the measurements was checked with standards by comparing enrichments obtained after straight elemental analyser combustion (Carlo Erba-MAT 252 combination, Bremen, Germany) and measurement of the same standards as TBDSMS derivatives and found to be of the same magnitude as the reproducibility over the entire abundance range. The measured APE was converted to MPE (mole percentage excess) by multiplying the $^{15}$N enrichment for the $^{1-}$[5-$^{15}$N]glutamine tracer by 2 (two N-atoms per molecule of derivatized glutamine), and by multiplying the $^{13}$C enrichment for the $^{1-}$[1-$^{13}$C]glutamine tracer by 23 (23 C-atoms per molecule of derivatized glutamine).

Plasma [1-$^{13}$C]α-ketoisocaprate (KIC) enrichment was measured using a quinoxalinotrimethylsilyl derivative and a GC–MS system (Finnigan Incos XL, San Jose, CA, U.S.A.) as previously described [12]. Plasma phenylalanine was derivatized to its TBDMS derivative and its deuterium enrichment was determined by GC–MS (Finnigan Incos XL), in a similar manner to that described previously [13]. Final values for phenylalanine and KIC determinations were corrected using calibration curves. The within-assay coefficient of variation for enrichment determinations was less than 3% for both [3H]phenylalanine and [13C]KIC.

**Calculations**

The appearance rate of glutamine, phenylalanine and leucine into plasma was calculated by the standard isotope expression for dilution of the infused tracer amino acid in plasma: $R_{a} = i(E_{p}/E_{i} − 1)$, where $R_{a}$ is the rate of amino acid appearance (µmol h$^{-1}$·kg$^{-1}$), $E_{i}$ and $E_{p}$ are the amino acid tracer enrichments (in MPE) in the infusate and in plasma respectively, and $i$ is the tracer infusion rate (µmol h$^{-1}$·kg$^{-1}$). This expression is based on a single pool model and describes the appearance rate of the amino acid into the sampled compartment. In a single pool model it is assumed that the $R_{a}$ of tracer and the infusion of tracer is into, and sampling is from, a single, homogenous, instantly mixing pool.

We have assumed that amino acids are released from proteolysis in proportion to their relative content in muscle protein. Rates of protein catabolism were calculated by assuming the phenylalanine and leucine content in protein are 4.3 g and 8.0 g/100 g protein respectively, based on averages of amino acid composition of mammalian tissues as previously described [14]. The $R_{a}$ of phenylalanine was calculated from arterial [3H]phenylalanine enrichments, which were corrected to mixed venous values as described before [15]. The glutamine appearance from protein breakdown was calculated by using the average of the appearance rates of leucine and phenylalanine. It was assumed that glutamine residues contributed to half of the total glutamine and glutamate content of 13.9 g/100 g protein, i.e. 6.95 g/100 g protein [16].

**Statistics**

Data are given as means ± S.E.M. Comparisons between different time points were made using the Wilcoxon
matched pairs signed rank sum test. For comparison of \(^{15}\text{N}\) and \(^{13}\text{C}\) enrichment data linear regression analysis was applied to quantify the slope and intercept of the enrichment curves. A \(P\) value of \(< 0.05\) was considered statistically significant.

**RESULTS**

The arterial \(^{15}\text{N}\) enrichment of glutamine increased for the duration of the study (Figure 1): from 0.245 ± 0.015 (\(t = 2\) h) to 0.275 ± 0.016 MPE (\(t = 6\) h) in the 6-h studies (\(n = 15, P < 0.001\)), and from 0.338 ± 0.024 (\(t = 2\) h) to 0.429 ± 0.032 MPE (\(t = 11\) h) in the 11-h experiments in which a higher rate of tracer infusion was used (\(n = 5, P < 0.05\)). The intramuscular glutamine \(^{15}\text{N}\) enrichment also increased with tracer infusion time (Figure 2, \(P < 0.001\)), indicating that mixing occurred of the glutamine tracer with the intracellular free glutamine pool in skeletal muscle. In each patient the intramuscular enrichment was lower than the arterial enrichment, but relative rises in glutamine enrichment were consistently larger for muscle than for plasma. Consequently, the ratio between intramuscular and arterial enrichment significantly increased during tracer infusion: average values were 0.076 ± 0.006 at 2 h (\(n = 10\)), 0.106 ± 0.016 at 4 h (\(n = 4\)), 0.157 ± 0.011 at 6 h (\(n = 20\)), and 0.263 ± 0.022 at 11 h (\(n = 5\)).

Inspection of the rise in enrichment with time of both plasma and intramuscular free glutamine indicated that isotopic steady state was not achieved after 11 h infusion in any patient. The whole-body pool in which the glutamine tracer equilibrated was not a single, instantly mixing compartment, and therefore conditions for application of the \(R_n = (E_i/E_0 − 1)\) equation were not fulfilled. However, if, for the sake of comparison with previous data in the literature, we used the isotopic data to calculate the appearance rate of glutamine into plasma, the values obtained would have been lower by the end of the tracer infusion than shortly after the onset of infusion (Table 1).

When \([1-^{13}\text{C}]\)glutamine was simultaneously infused with \([5-^{15}\text{N}]\)glutamine the rate of rise in enrichment observed for \([1-^{13}\text{C}]\)glutamine in both plasma and muscle was lower than that observed for the \(^{15}\text{N}\)-labelled amino acid (Figure 3). Linear regression revealed that the \(^{15}\text{N}\) enrichment of glutamine in plasma increased by 0.012 ± 0.002 MPE/h, whereas the \(^{13}\text{C}\) enrichment rose by 0.008 ± 0.002 MPE/h (\(n = 5, P < 0.05\)). Increments similar to those in plasma were observed for the enrichment of glutamine in muscle: 0.011 ± 0.002 and 0.008 ± 0.001 MPE/h for \(^{15}\text{N}\) and \(^{13}\text{C}\) respectively (\(P < 0.05\)). When the rate of rise in each patient was divided by the tracer enrichment, values were obtained of 0.040 ± 0.007 per hour for plasma glutamine \(^{15}\text{N}\) and 0.028 ± 0.006 per hour (\(P < 0.05\)) for glutamine \(^{13}\text{C}\). Extrapolating the enrichment data in plasma to time 0 yielded glutamine \(R_n\) values that were not different for the \(^{13}\text{C}\) and \(^{15}\text{N}\) labels: 261 ± 16 and 249 ± 17 \(\mu\)mol·h\(^{-1}\)·kg\(^{-1}\) respectively. By the end of the 11-h tracer infusion period, glutamine \(R_n\) averaged 205 ± 18 \(\mu\)mol·h\(^{-1}\)·kg\(^{-1}\) for the \(^{13}\text{C}\) tracer compared with 185 ± 14 \(\mu\)mol·h\(^{-1}\)·kg\(^{-1}\) for the \(^{15}\text{N}\) tracer (Table 1, \(P = 0.08\)).

Infusion of \(^{3}\text{H}_2\)phenylalanine and \([1-^{13}\text{C}]\)leucine permitted the calculation of whole-body proteolysis. Any observed reduction in the rate of protein breakdown (and thus a reduction in glutamine release from the
Glutamine kinetics and isotopic steady state

Table 1 Glutamine metabolism

<table>
<thead>
<tr>
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<th>6-h study (n = 15)</th>
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<td></td>
<td>t = 2</td>
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<td>5-¹⁵N label</td>
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<td>86 ± 5</td>
<td>80 ± 4</td>
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<td>P value †</td>
<td>–</td>
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|                |                   |              |                   |              |
| Percentage of overall Ra |                   |              |                   |              |
| 5-¹⁵N label    | 30 ± 1            | 31 ± 1       | < 0.05            | 34 ± 2       | 39 ± 3        | < 0.05         |
| ¹³C label      | –                 | –            | –                 | 32 ± 3       | 35 ± 3        | 0.08           |
| P value †      | –                 | –            | –                 | 0.35         | 0.08          | –              |

![Figure 3](image_url) Composite time course of plasma and intramuscular glutamine ¹⁵N and ¹³C enrichments

Enrichments were averaged at each time point for the five subjects studied during 11 h. ▲, plasma glutamine ¹⁵N enrichments; △, plasma glutamine ¹³C enrichments; ■, intramuscular glutamine ¹⁵N enrichments; ○, intramuscular glutamine ¹³C enrichments. The values of muscle glutamine enrichment were not corrected for the water distribution between the intracellular and extracellular spaces.

![Figure 4](image_url) Composite time course of plasma enrichments of [¹³C]KIC and [²H₅]phenylalanine ([D₅]PHE) during a primed continuous infusion of l-[¹–¹³C]leucine and l-[ring-²H₅]-phenylalanine

Enrichments were averaged at each time point for the five subjects studied during 11 h (upper line) and for the 15 subjects studied during 6 h (lower line)

Figure 3 and Figure 4 show the composite time courses of plasma and intramuscular glutamine ¹⁵N and ¹³C enrichments. The data are presented for the 6- and 11-h studies as depicted in Figure 4. In the 6-h experiments protein breakdown was calculated with the phenylalanine tracer averaged 179 ± 16 and 163 ± 13 mg protein·h⁻¹·kg⁻¹ at t = 2 and t = 6 h respectively (P < 0.05). When the leucine tracer was used, protein breakdown amounted to 184 ± 8 mg protein·kg⁻¹·h⁻¹ at t = 2 h and 174 ± 6 mg protein·kg⁻¹·h⁻¹ at t = 6 h (P < 0.05). In the 11-h studies, proteolysis was 161 ± 11 (t = 2 h) and 153 ± 5 (t = 11 h) mg protein·h⁻¹·kg⁻¹ for the phenylalanine tracer, and 169 ± 14 (t = 2 h) and 142 ± 6 (t = 11 h) mg protein·h⁻¹·kg⁻¹ for the leucine tracer, (P < 0.05). The corresponding estimates of glutamine release from proteolysis are shown in Table 1. Calculation revealed that the reduction in glutamine...
release directly from protein breakdown accounted for 16±6% (11-h study) and 21±4% (6-h study) of the decline observed in overall glutamine $R_a$.

Plasma glutamine concentration averaged 606±19 $\mu$mol/l. The concentration of glutamine in the intracellular free glutamine pool in muscle was 12.8±0.6 mmol/l intracellular water (assuming that 15% of the total tissue water is extracellular water [4]). In four patients, arterial glutamine concentrations were measured throughout the course of their 6-h study. No significant change in glutamine concentration was observed. Average values were 587±64 $\mu$mol/l before onset of the tracer infusion and 615±55 $\mu$mol/l at 6 h. Muscle free glutamine concentration did not change either during the course of the study: 12.1±0.8 ($t = 2$ h) versus 11.2±0.6 ($t = 6$ h) mmol/l intracellular water in the 6-h study, and 14.4±1.6 ($t = 4$ h), 12.7±0.9 ($t = 6$ h) and 12.9±1.3 ($t = 11$ h) mmol/l intracellular water in the 11-h study.

**DISCUSSION**

In this study we unequivocally showed that isotopic steady-state conditions were not achieved in patients with gastrointestinal disease during an 11-h primed constant intravenous infusion of glutamine tracers. Therefore, the relative contribution of some processes that might have contributed to the ongoing rise in plasma glutamine $^{15}N$ enrichment was investigated.

To establish whether recycling of labelled nitrogen contributed to the rise in plasma glutamine $^{15}N$ enrichment, a comparison was made with the enrichment curve of simultaneously infused [1-$^{13}$C]glutamine. Recycling of the labelled C1-carbon between the glutamate and glutamine pool is probably negligible because oxidation is the predominant fate of an infused [1-$^{13}$C]glutamate tracer [17]. Recycling via the Krebs cycle does not occur because the labelled C1-carbon is released as $^{13}$CO$_2$ in the conversion of α-ketoglutarate to succinate. Insignificant differences, in line with minimal C1-carbon cycling, were also observed by other researchers between plasma glutamine $R_a$ measured using either a [1-$^{13}$C]- and [3,4-$^2$H]glutamate tracer [16], or a [U-$^{14}$C]- and [3,4-$^2$H]glutamine tracer [18]. Recycling of the amide nitrogen label used in the present study could have occurred through reincorporation of $^{15}$N ammonia released by glutaminase action back into glutamine via glutamine synthetase. Recycling of the nitrogen label into the amino nitrogen of glutamine is probably minimal, since insignificant enrichment in either plasma glutamine or glutamate amino nitrogen has been observed by us (results not shown) and by others [5] during infusion of [5-$^{14}$N]glutamine. Comparison of the increase in plasma glutamine $^{15}N$ and $^{13}C$ enrichment observed in the period from 2 to 11 h of tracer infusion revealed that recycling of the nitrogen label could explain only a minor part (15±5%) of the observed rise in plasma glutamine $^{15}N$ enrichment (Table 1).

A small increase occurred in the plasma enrichment of leucine and phenylalanine during the course of the study. As a result the rate of whole-body protein breakdown calculated from these enrichment data slightly decreased throughout the study, as did the release of glutamine from proteolysis (Table 1). By comparing the reduction in glutamine from proteolysis with the observed decrease in whole-body glutamine $R_a$, we estimated that 16–21% of the decline in overall $R_a$ could be attributed to a reduction in the release of glutamine from proteolysis. The decline in the contribution of protein breakdown to glutamine production and recycling of the $^{15}$N label together contributed approximately 30–35% to the decrease in glutamine $R_a$.

An important reason for the observed lack of isotopic steady state appeared to be incomplete equilibration of the glutamine tracer with a large second pool. Skeletal muscle is a major contributor to this second pool, but other tissues and cells may also be part of it. For instance, equilibration of glutamine between erythrocytes and plasma was found to be far from complete after 4 h of glutamine tracer administration [5]. Direct measurement of the glutamine enrichment in muscle clearly demonstrated that glutamine tracers do enter the large intracellular free glutamine pool in muscle, but the increase in enrichment is slow. The linearity observed in the rise in intramuscular enrichment indicated that near-isotopic steady state was not even approached by 11 h of glutamine tracer infusion. The slow equilibration with the muscle pool is probably due to the enormous size of the intramuscular free glutamine pool and the speed of entry of glutamine into muscle. From the expected size of the muscle free glutamine pool and the reported kinetics of glutamine transport in human skeletal muscle [19] it can be deduced that the process of isotope equilibration with the muscle pool will take many hours. Compartmentalization of glutamine within cells, as suggested by the limited erythrocyte glutamine exchange [5] and the slow mixing of plasma and intramuscular glutamine demonstrated here, may further slow down the process of equilibration. It is impossible to quantify exactly the contribution of incomplete equilibration to the decrease in glutamine $R_a$ as other unquantified factors (such as changes in glutamine synthesis rate) may also be important. However, our finding that the muscle glutamine pool equilibrates so slowly interferes with the interpretation of glutamine kinetics measured by the tracer dilution method, i.e. the measured $R_a$ of glutamine into plasma does not reflect the whole-body flux but rather reflects transport rates through plasma [5]. Similar considerations may apply to the flux measurements of other amino acids with large muscle pools such as alanine and glutamate.
A relevant question is why the gradual increase in plasma glutamine enrichment that we see in this patient population may have remained unnotice by previous researchers. First, steady-state conditions for the tracer infused have usually been determined on the basis of a quick succession of plasma enrichment measurements within a sampling period of 2 h or less [14,16,18,20–29]. This period may have been too short to assess the fractional change in glutamine enrichment shown here over a period of 6–11 h: 0.028 and 0.040 per hour for the \(^{13}\)C and \(^{15}\)N tracer respectively. Second, the traditional GC–MS methods which have been used previously for measuring plasma and muscle glutamine enrichments [5,14,16,18,20–27] may not have had the required precision to accurately measure low enrichments such as in muscle or to detect small systematic increases in plasma enrichment. The combination of very precise GC combustion isotope ratio MS measurement with prolonged tracer infusion periods has enabled us to conclude that steady-state conditions for the glutamine tracer were not achieved.

Although the subjects included in this study had gastrointestinal disease and in general were older than those in other studies, the plasma glutamine \(R_s\) measured after 2 h of \([^{15}\text{N}]\)glutamine infusion was in the range previously observed in young healthy volunteers: mean \(R_s\) of 276 compared with 270–305 \(\mu\)mol h\(^{-1}\) kg\(^{-1}\) [5,24,30]. Darmaun et al. [26] previously showed a 20% reduction in the glutamine \(R_s\) in lean but weight-stable patients with a short bowel, whereas an increased glutamine \(R_s\) has been observed in weight-losing patients with malnutrition due to non-neoplastic gastrointestinal disease, including short bowel patients [27]. The majority of our patients were well-nourished and had a stable body weight. Their intramuscular glutamine concentration was slightly below normal (12.8 versus 14–20 mmol/l intracellular water) [4,30,31], which could be due to either different muscle types (tibialis anterior versus quadriceps femoris) or disease. It is widely recognized that a decline of intramuscular glutamine occurs in a wide variety of conditions of disease and injury [32]. Although we do not know how widely applicable the present finding of incomplete equilibration of the glutamine tracer is, it is not likely to be specific for the patient population involved, because little mixing of plasma and intramuscular glutamine has also been suggested in healthy subjects [5]. Therefore, care should be taken in the interpretation of plasma glutamine \(R_s\) as it may not reflect the whole-body glutamine flux. In conclusion, continued net uptake of the glutamine tracer in skeletal muscle contributes to the absence of isotopic steady state in patients with gastrointestinal disease. As a consequence, calculation of the plasma glutamine \(R_s\) during a short tracer infusion, when intracellular muscle has little enrichment, overestimates the whole-body glutamine flux. We continue our attempts to estimate the whole-body production rate of glutamine with a tracer dilution method, as this is the physiological variable desired to judge whether patients with a variety of diseases are in need of glutamine supplementation.

**ACKNOWLEDGMENTS**

This study was supported by the Dutch Cancer Foundation (93-521). We acknowledge the technical assistance of Mrs M. Meers, Mrs A. P. Gijsen, Mr F. van de Vegt, Mr H. M. van Eijk and Mr D. R. Rooyackers.

**REFERENCES**

glutamine metabolism to exogenous glutamine in humans. Am. J. Physiol. 269, E663–E670


32 Rennie, M. J., Hundal, H. S., Babij, P. et al. (1986) Characteristics of a glutamine carrier in skeletal muscle have important consequences for nitrogen loss in injury, infection, and chronic disease. Lancet 2, 1008–1012

Received 28 January 1998; 9 April 1998; accepted 28 April 1998