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Glutamine kinetics during intravenous glutamine supplementation in ICU patients on continuous renal replacement therapy

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Abstract Objective: To investigate glutamine kinetics during continuous renal replacement therapy (CRRT) in multiple organ failure (MOF) patients with and without exogenous intravenous glutamine supplementation. **Design and patients:** In a pragmatic clinical study 12 patients without urine production receiving CRRT were prospectively randomized in a cross-over design to receive glutamine intravenously for 20 h before placebo or placebo before glutamine on two consecutive days. Alanyl-glutamine or placebo (saline) was infused. **Measurements:** Plasma glutamine concentration was measured in artery, femoral vein, and filtration fluid. Blood flow across the leg was measured and the efflux of glutamine calculated. The rate of appearance of glutamine was calculated from the plasma decay curve of glutamine concentration on the day of treatment.

Results: Glutamine supplementation increased plasma concentrations from 570 ± 252 to $831 \pm 367 \mu\text{mol l}^{-1}$. Glutamine losses into the filtration fluids were similar during treatment and control days: 25 ± 13 vs. $24 \pm 11 \text{ mmol } 24 \text{ h}^{-1}$, corresponding to 3.6 ± 1.9 and $3.5 \pm 1.6 \text{ g } 24 \text{ h}^{-1}$, respectively. Net glutamine balance across the leg was also similar on treatment and control days: 150 ± 138 and $188 \pm 205 \text{ nmol min}^{-1} 100 \text{ ml}^{-1}$, respectively. The rate of appearance of glutamine was $54 \pm 17 \text{ g } 24 \text{ h}^{-1}$. **Conclusion:** The loss of glutamine into the ultrafiltrate during CRRT in MOF patients suggests a greater need for exogenous glutamine than in patients without renal failure. The leg efflux and the filtration losses of glutamine were not affected in response to intravenous glutamine supplementation.

Introduction

A loss of glutamine into the ultrafiltrate/dialysate can be anticipated during continuous renal replacement therapy (CRRT) in ICU patients, but how much exogenously supplemented glutamine is lost in the ultrafiltrate during CRRT is not known. ICU patients have a shortage of glutamine, which for the individual patient is related to outcome [1]. Furthermore, intravenous glutamine supplementation as a part of nutrition is beneficial to ICU patients in terms of decreased mortality, morbidity, and length of stay [2, 3, 4]. During intermittent hemodialysis glutamine losses via the dialysate are $2.7 \pm 0.6 \text{ mmol/h}$,

corresponding to $0.4 \pm 0.1 \text{ g/h}$ [5, 6]. For ICU patients with multiple organ failure, including acute renal failure, CRRT is an alternative to intermittent hemodialysis. CRRT carries the risk of high loss of glutamine into the ultrafiltrate/dialysate, particularly when exogenous intravenous glutamine supplementation is given.

The primary aim of this pilot study was to quantify the loss of amino acids, particularly glutamine, during CRRT treatment in critically ill patients. A pragmatic protocol was used that left the choice of CRRT treatment modality to the attending intensivist. The protocol included a cross-over design with and without intravenous glutamine supplementation over two consecutive days of

CRRT treatment. As secondary parameters, amino acid exchange across the leg was determined, and the decay curve after cessation of the glutamine infusion was used to calculate the rate of appearance for endogenous glutamine.

Preliminary results of this study were presented at the 26th ESPEN Congress, in September 2004 in Lisbon, Portugal.

Patients and methods

We studied 12 patients on ventilator treatment in a general ICU with multiple organ failure including acute renal failure requiring CRRT. Patient characteristics are presented in Table 1. The nature and purpose of the study and the risk involved were explained to the subjects or their next of kin orally and in writing before obtaining informed consent. The study protocol was approved by the Ethics Committee of Karolinska Institutet at Karolinska University Hospital, Stockholm, Sweden. The patients' illness severity was assessed on admission by the Acute Physiology and Chronic Health Evaluation (APACHE) II and daily by the Sepsis-Related Organ Failure Assessment (SOFA) scoring system [7].

The patients received CRRT via venovenous central dialysis catheter using PRISMA equipment (Gambro, Sweden). The mode of CRRT used was left to the discretion of the attending intensivists. Continuous venovenous hemofiltration (CVVH) and continuous venovenous hemodiafiltration (CVVHDF) were used. In both cases replacement fluid was given as predilution. All subjects except two were given heparin as anticoagulant; subject no. 11 was given no anticoagulant and subject no. 12 was given citrate. All details of the CRRT protocols were recorded, and the dialysis doses were calculated. Blood flow rates (Qb) varied between 100–150 ml/min. The

given dialysis dose was calculated both as estimated urea clearance and Kt/V, which is the clearance related to treatment time and distribution volume for urea. Estimated urea clearance was calculated differently depending on the mode in use. The equations used for estimating clearance (K) and dialysis dose (Kt/V) are presented below, where Quf = ultrafiltration rate, Qdo = dialysate outflow rate, Qr = predilution fluid replacement rate, Qb = blood flow rate, and V = body weight × 0.6:

$$K_{CVVH} = Q_{uf}/[1 + (Q_r/Q_b)] \quad (1)$$

$$K_{CVVHDF} = Q_{do}/[1 + (Q_r/Q_b)] \quad (2)$$

$$Kt/V = K \times \text{treatment time}/V. \quad (3)$$

All patients were fed enterally in accord with the routines of the unit, occasionally in combination with parenteral nutrition to reach the nutritional target defined for each individual patient, usually 20–25 kcal/kg per 24 h. The parenteral nutrition used did not contain glutamine on the day before the study or during the 2 days of the study. The enteral nutrition was standard formulas containing approx. 0.3 g/100 ml glutamine but without any additional supplementation. The patients were randomized in a cross-over design to receive treatment before placebo or placebo before treatment. Glutamine was given as a 20 h intravenous infusion of 20% l-alanyl-l-glutamine, 0.5 g/kg bodyweight (Dipeptiven, Fresenius-Kabi) corresponding to 0.32 g/kg bodyweight glutamine (2.2 mmol/kg bodyweight) or an identical volume of placebo (saline). The components of the nutrition (enteral as well as parenteral) were given continuously over 24 h.

Blood samples were taken from an arterial line three times before the beginning of the infusion to obtain a baseline concentration of glutamine in plasma. Thirty minutes before cessation of the infusion the arteriovenous

Table 1 Patient characteristics (ARDS acute respiratory distress syndrome)

Subject no.	Diagnosis	Sex	Age (years)	Body-weight (kg)	Total Gln (g)	CRRT indication	SOFA day 1	SOFA day 2
1	Sepsis, kidney transplantation	M	38	92	31	Uremia	6 ^a	5
2	Sepsis, Mb Hodgkin, bone marrow transplantation	M	41	80	27	Organ failure	15	15 ^a
3	Peritonitis, sepsis	F	54	61	22	Anuria	10 ^a	10
4	Sepsis	M	60	70	24	Anuria	10	12 ^a
5	Trauma, sepsis	M	58	100	34	Organ failure	8 ^a	8
6	Pneumonia	M	52	85	28	Anuria	10	9 ^a
7	Sepsis	F	72	76	26	Anuria	5 ^a	5
8	Pneumonia, ARDS	M	20	58	20	Sepsis	7	10 ^a
9	Pneumonia	M	75	65	22	Anuria	9 ^a	10
10	Peritonitis, sepsis	M	78	78	26	Sepsis	9	5 ^a
11	Pancreatitis	M	36	85	28	Anuria	12 ^a	10
12	Gastroenteritis	M	61	69	23	Anuria	7	5 ^a

^aThe day randomized to glutamine infusion

concentration difference across the leg was measured by samples taken from the femoral vein and the radial artery. Samples were taken on three occasions 5 min apart within 10 min. Immediately following the blood sampling the blood flow over the leg was measured by venous occlusion plethysmography [8]. An occluding cuff (venous occlusion) was placed around the thigh about 15 cm above the knee, and the pressure used was 40 mmHg. Mercury strain gauge plethysmography equipment was used (Electro Medicine, Sweden). This mercury strain gauge was wrapped around the calf at the level of maximum circumference. The blood flow was measured ten times in 10 min and the mean value of these readings was used. When the infusion was stopped after 20 h, a decay curve of plasma glutamine concentrations was established by sampling six times during 15 min from the arterial line and thereafter another six times up to 4 h after end of the infusion. In parallel the bags with filtration fluid were collected during the two 24-h study periods and analyzed for amino acid concentration.

Blood samples were immediately put on ice and centrifuged within 30 min (4 °C, 2,800 g for 10 min). The protein-free supernatant was transferred to an aliquot kept at -80 °C until analysis. Concentrations of free amino acids in plasma and in filtration fluid were determined by HPLC analysis with fluorescence detection after postcolumn derivatization, as described elsewhere [8]. Concentration-time data of the decay curve were analyzed by using WinNonlin Professional 4.0.1 (Pharsight, Cary, N.C., USA). A one-compartment turnover model comprising three model parameters was fitted to all experimental concentration-time data for each subject. V_d is the volume of distribution, CL is clearance, and R_a is the turnover rate, that is, the rate of appearance of endogenous glutamine in the observed compartment [9, 10]. All parameters were presumed to be constant over the study time. C is the plasma glutamine concentration representing the single compartment. The turnover of glutamine in the single compartment is then:

$$V_d \times (dC/dt) = (D_{iv}/T_{inf}) + R_a - CL \times C, \quad (4)$$

where D_{iv} is total intravenous glutamine dose and T_{inf} is infusion time. The term D_{iv}/T_{inf} is zero before and after the infusion. C_0 is the concentration at steady state calculated as R_a/CL and the half-life of the exogenous glutamine was calculated from $\ln(2) \times V_d/CL$. The clearance by continuous renal replacement treatment (CL_{CRRT}) is a fraction of CL calculated from the excretion rate of glutamine in dialysate divided by the mean plasma concentration. The area under the curve of the model predicted time-concentration plot divided by observation time was taken as the mean plasma concentration.

Values are given as mean \pm SD unless otherwise indicated. Control and treatment periods were compared using Student's t -test for paired samples. Correlations were tested using Spearman's test.

Results

The two study days in the cross-over protocol were compared by SOFA scores, leg blood-flow, and dialysis dose. None of these differed significantly between the 2 days. SOFA scores were 8.8 ± 3.2 and 8.8 ± 2.7 ($p=0.99$) on treatment and control days, respectively. The plasma flow through the leg was 1.51 ± 0.78 and 1.71 ± 1.02 ml min⁻¹ 100 ml⁻¹ leg tissue ($p=0.48$) on treatment and control days, respectively. The volume of the ultrafiltrate/dialysate was 40 ± 18 and 46 ± 12 L/24 h ($p=0.21$) on treatment and control days, respectively. Median effective CRRT time was 20 h (range 7–24) on treatment and 20 h (range 16–24) on control days ($p=0.29$). Two of the patients were treated with the CVVH mode and the other ten with CVVHDF. The patients were studied on median day 3 (range 1–13) of CRRT treatment. Dialysis dose calculations showed that on the day of glutamine infusion the estimated urea clearance and Kt/V were 30.1 ± 6.4 and 0.85 ± 0.25 ml/min, respectively. During the placebo infusion the corresponding values were 31.7 ± 7.4 ($p=0.15$) and 0.90 ± 0.24 ml/min ($p=0.27$). Thus the dialysis dose did not differ between the glutamine and placebo sessions.

Glutamine concentration in plasma was higher following 20 h of glutamine infusion than the control period, 831 ± 367 vs. 570 ± 252 μ mol/l, respectively

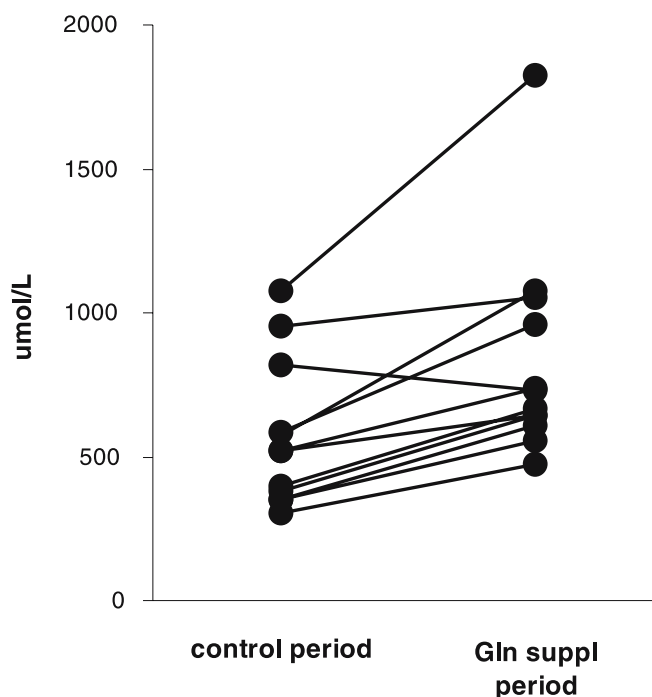


Fig. 1 Arterial concentration of glutamine at the end of the 20-h infusion period of alanyl-glutamine or placebo in ICU patients on CRRT ($n=12$), randomized to receive glutamine before placebo or placebo before glutamine on two consecutive days

Fig. 2 The correlation between the glutamine concentrations ($\mu\text{mol/l}$) in dialysate fluid and in venous plasma at the end of the 20-h infusion period of alanyl-glutamine or placebo and the in ICU patients on CRRT ($n = 12$), randomized to receive glutamine before placebo or placebo before glutamine on two consecutive days

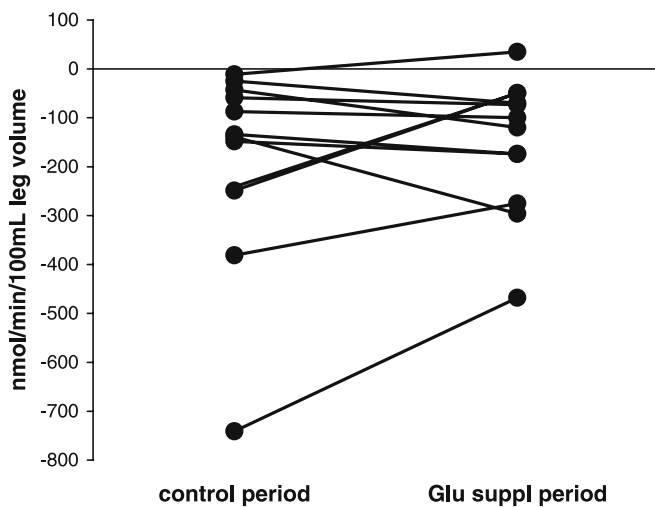
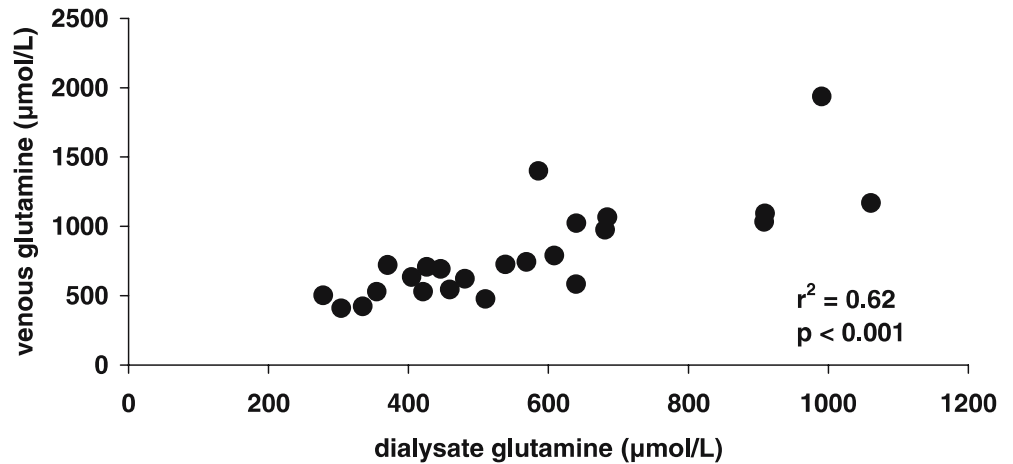


Fig. 3 The flux of glutamine across the leg at the end of the 20-h infusion period of alanyl-glutamine or placebo in ICU patients on CRRT ($n = 12$), randomized to receive glutamine before placebo or placebo before glutamine during 20 h on two consecutive days

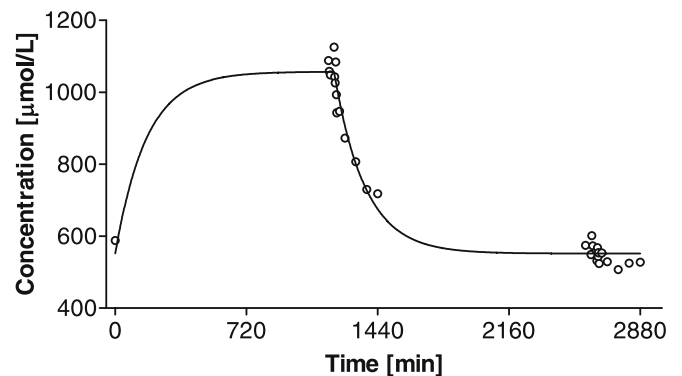


Fig. 4 Concentration-time data of subject no. 9 after an intravenous infusion of 2.2 mmol glutamine/kg body weight during the initial 1200 min

($p < 0.001$; Fig. 1). The concentration of glutamine in ultrafiltrate/dialysate was 609 ± 220 and $525 \pm 217 \mu\text{mol/l}$ ($p < 0.05$) on treatment and control days, resulting in a total daily glutamine loss of 25 ± 13 vs. 24 ± 11 mmol, corresponding to 3.6 ± 1.9 and 3.5 ± 1.6 g ($p = 0.75$) on

Table 2 Individual estimates for the three model parameters (R_a rate of appearance, V_d volume of distribution, CL clearance), the calculated clearance by continuous renal replacement therapy (CL_{CRRT}), and two secondary parameters (C_0 concentration at base-

line $t_{1/2}$ half-life of exogenous glutamine) in a one-compartment turnover model when glutamine was given as an intravenous infusion over 20 h (parentheses coefficient of variation, calculated as $100 \times \text{SD}/\text{estimate}$, of the regression analysis)

Subject no.	R_a ($\mu\text{mol}/\text{min}$)	V_d (l)	CL (l/min)	CL_{CRRT} (l/min)	C_0 ($\mu\text{mol}/\text{l}$)	$t_{1/2}$ (min)
1	331 (4)	26 (14)	0.90 (3)	0.027	370	20
2	253 (8)	112 (26)	0.80 (6)	0.022	316	96
3	399 (10)	22 (39)	0.78 (9)	0.016	512	20
5	300 (7)	82 (23)	0.51 (5)	0.039	589	111
6	229 (9)	79 (30)	0.63 (7)	0.024	363	87
7	329 (9)	10 (31)	0.64 (8)	0.019	514	11
9	129 (4)	40 (11)	0.23 (3)	0.031	551	118
10	220 (7)	13 (23)	0.56 (6)	0.023	393	16
11	222 (5)	47 (16)	0.21 (4)	0.017	1081	158
12	163 (6)	7 (19)	0.44 (5)	0.024	371	11
Mean \pm SD	258 ± 83	44 ± 36	0.57 ± 0.23	0.024 ± 0.007	506 ± 223	60 ± 54

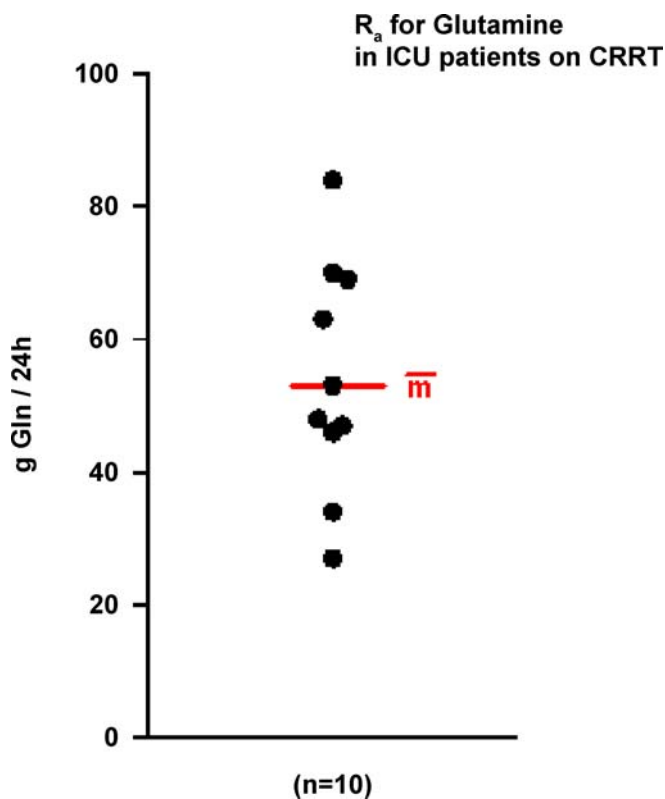


Fig. 5 The endogenous rate of appearance (R_a) in ICU patients on CRRT ($n=10$) as calculated from the decay curve of plasma glutamine concentration

treatment and control days. The range of glutamine loss was 0.5–6.8 g per 24 h. The concentration of glutamine in the dialysate was significantly related to the concentration in plasma ($R^2=0.62$, $p < 0.001$; Fig. 2). In addition, the glutamine loss was significantly correlated to the flow rate of the CRRT. A high filtration volume increased the losses of glutamine independently of exogenous glutamine supplementation ($R^2 = 0.46$, $p < 0.001$).

Arteriovenous concentration differences across the leg were -104 ± 74 and $-113 \pm 130 \mu\text{mol/l}$ ($p = 0.74$) on treatment and control days, respectively. The calculated efflux of glutamine from the leg was -150 ± 132 and $-188 \pm 196 \text{ nmol min}^{-1} 100 \text{ ml}^{-1}$ ($p = 0.34$) on treatment and control days (Fig. 3).

Model fit was good in most cases (Fig. 4). Two subjects (nos. 4 and 8) were excluded from the pharmacokinetic analysis because of poor precision in the parameter estimates and very skewed distribution of residuals in the model fit, suggesting a significant alteration in baseline plasma glutamine concentration over the study time. The mean value of R_a was $258 \pm 82 \mu\text{mol/min}$, corresponding to $189 \pm 83 \mu\text{mol/kg}$ per hour, or $54 \pm 17 \text{ g/24}$ (Fig. 5), and V_d was $44 \pm 36 \text{ l}$, corresponding to 0.48 l/kg bodyweight (Table 2). The correlation was high in the regression analysis between the parameters CL and R_a

($R^2 = 0.94$, $p < 0.01$). The parameter estimates of CL and R_a were also correlated ($R^2 = 0.51$, $p < 0.05$). Bodyweight was significantly correlated to V_d ($r^2 = 0.49$, $p < 0.05$). Mean CL_{CRRT} was $0.024 \pm 0.007 \text{ l/min}$, or $5.3 \pm 3.5\%$ of the total clearance from the single model compartment.

Discussion

The amino acid loss during CRRT in ICU patients with multiple organ failure must not be overlooked. This study focused upon glutamine, and the loss into the ultrafiltrate/dialysate ranged from 0.5 to 6.8 g over a period of 24 h. The loss was related both to plasma concentration and to the filtrated volume, the latter being by far the most important. Although the plasma concentration of glutamine increased during intravenous glutamine supplementation, this did not prove a major determinant of loss. Therefore glutamine depletion in patients on CRRT not supplemented with glutamine is accelerated by the loss in the ultrafiltrate/dialysate, while intravenous supplementation was demonstrated to be retained within the patient to meet the glutamine need in patients on CRRT during supplementation.

The design of this pilot study was pragmatic and left both general clinical and CRRT management to the attending intensivist. Consequently the loss of glutamine in the combined ultrafiltrate and dialysis fluid measured may not be representative for all CRRT patients. Such factors as higher blood flow rate, longer CRRT time, and postdilution may increase glutamine loss further. The cross-over design of the study aimed to use the patients as their own controls. The two consecutive days studied were comparable in terms of mean values for SOFA score, leg blood flow, filtration volume, and CRRT time, which was a prerequisite to obtain comparable data. Nevertheless, 2 of the 12 patients lacked a stable baseline of plasma glutamine concentrations, disabling the kinetic model calculations performed. All patients received nutrition to their caloric target, and the difference between glutamine treatment day and control day was solely the intravenous glutamine supplementation. The pragmatic approach resulted in that the time of CRRT actually delivered and the mode of CRRT were not identical in all the patients. It has previously been shown that, although urea clearance is similar, clearance of amino acids is greater on predilution CVVH than on CVVHD [11], indicating that convection is related to greater glutamine loss than diffusion. In both cases $21 \text{ h}^{-1} 1.73 \text{ m}^{-2}$ replacement fluid or dialysate was used. The sieving coefficient for glutamine was 1.53 ± 0.29 in that study. The authors speculated that the high sieving coefficient was explained by the positively charged amino acid being attracted by the negatively charged polysulfone membrane in use. In our study an AN69 filter was used. The AN69 membrane is even more negatively charged than polysulfone, which is in keeping

with the greater glutamine loss in this study than in the study by Maxvold et al. [11]. In our study CVVHDF was the most common modality, and only two patients received pure CVVH. In both modalities a convective flow is present.

In the study protocol the kinetics of endogenous and exogenously supplied glutamine was also investigated. ICU patients are known to have a constant and high efflux of glutamine from muscle tissue [8]. Here we had the possibility to compare the efflux during the last hour of the 20 h of treatment to that of the control situation. As the glutamine balance across the leg did not change, it can be concluded that the patient retained a major portion of the exogenously supplied glutamine. Of 20 g approx. 15 g was retained, and it was not disposed in muscle. The retained glutamine went to other organs, and it can be hypothesized that it ended up in the splanchnic region. Also the kinetic calculations demonstrated CL_{CRRT} (glutamine clearance via the dialysate) to be small compared with CL (total glutamine clearance). The design of the study did not offer the possibility to determine whether it was immediately oxidized. Another important point is that the present results reflect only the initial 20 h of intravenous glutamine supplementation. It may very well be that continuous supplementation for a longer period of time would result in a different use pattern. However, in a longitudinal study of amino acid exchange across the leg a low level of glutamine supplementation (< 10 g/24h) also did not alter glutamine exchange across the leg [8].

The plasma concentration of glutamine was very variable but was low in the majority of patients. This is in accord with earlier reports [1, 12, 13]. Here an increase in plasma glutamine concentration during the intravenous infusion was seen in all patients but one. This patient showed an increase in SOFA score between the 2 days, of which the initial day was control and the second day was treatment. Plasma glutamine concentration decreased on both days, which may have been due to the deterioration in the patient's general condition. The supplementation may have attenuated the decrease, but it did not prevent it. Still this is merely a case report and no conclusions are possible from this singular patient.

As expected, the distribution volume obtained from the glutamine kinetic calculations was extremely variable between patients, which is in accord with earlier reports [13, 14]. From the decay curve it was possible to calculate the

turnover rate, which equals the rate of appearance. This proved to be 27–84 g/24 h, which is in line with reports in the literature using an isotope dilution technique [15]. The rather high scatter was not related to bodyweight or dose of glutamine. This type of kinetics calculations needs to be validated and compared to other techniques employing stable isotopes. There was an increase in plasma glutamine concentration during the infusion time, but clearance was sufficient to return the plasma concentration to the initial concentration within 4 h after cessation. This demonstrated that the clearance of exogenous glutamine is rapid also in ICU patients on CRRT. In particular no accumulative effect over time following intravenous glutamine supplementation was seen. On the other hand, the high clearance rate further supports the suggested high demand of glutamine in ICU patients. In this study a high clearance rate was shown in multiple organ failure patients with acute renal failure, in accord with what has been demonstrated before for ICU patients with only marginal renal insufficiency [14].

Low plasma glutamine is associated with severity of disease and poor outcome in the ICU [1]. Baseline plasma glutamine decreased over time in two subjects, suggesting that they failed to meet one of the prerequisites of the turnover model, namely that model parameters remain constant over the study time. In subject no. 8 this change was concomitant with an increased SOFA score from 5 to 10. Longitudinal measurements in ICU patients are necessary to elucidate variations in baseline plasma glutamine. If great variations over time prove to be a recurrent finding, this suggests that shorter study protocols are preferable in the ICU setting.

In summary, exogenous glutamine supplementation to ICU patients on CRRT was not wasted into the filtrate/dialysate to any greater extent than in a situation without exogenous supplementation. On the contrary, patients on CRRT not given exogenous glutamine are likely to be at a higher risk of developing severe glutamine depletion. The retained glutamine was not distributed to muscle tissue, and it was hypothesized to be distributed towards the splanchnic area. Calculations of glutamine kinetics estimated the endogenous rate of appearance of glutamine at 27–84 g per 24 h, demonstrating that the 20 g exogenous supply of glutamine corresponds to 25–75% of the totally available glutamine. Therefore this addition may be crucial.

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