7. Enteral glutamine supplementation in optimally fed critically ill patients does not affect protein metabolism

Mechteld A.R. Vermeulen, Albertus Beishuizen,
Peter J. Weijs, Pierre M. Bet, Henk Schierbeek,
Helena M. Oudemans van Straaten,
Johannes B. van Goudoever and Paul A.M.van Leeuwen

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Abstract

Background During critical illness muscle and protein wasting occurs, but the effect of optimal enteral nutrition in critically ill patients on protein synthesis remains unclear. Critically ill patients have significantly decreased muscle glutamine levels sometimes accompanied by reduced plasma glutamine concentrations. Attempting to counteract catabolism glutamine has been supplied in the past decades. The objective was to investigate the effect of targeted enteral nutrition with or without enteral glutamine, on protein synthesis.

Materials and Method This was an open label randomized clinical trial including 2 groups of 10 critically ill patients, assigned to receive full enteral nutrition (CON), or isocaloric isonitrogenous enteral nutrition including 0.5 g/kg L-alanyl-L-glutamine per day (ALA-GLN). Tracer methodology determined rate of appearance and turnover of phenylalanine and tyrosine. Groups received intravenous and enteral tracers in a cross-over design on separate days to calculate splanchnic extraction.

Results Most intravenously measured experiments were significantly different from the enteral experiments. Protein breakdown was not different for both groups. Phenylalanine hydroxylation was significantly higher in the ALA-GLN group (CON 0.75 μmol/kg/h; ALA-GLN 1.72 μmol/kg/h, p=0.028). Protein synthesis was not different between groups. Protein balances were nearly zero and were more negative in the ALA-GLN group (CON -0.75 (SEM±0.36) μmol/kg/h; ALA-GLN -1.72 (SEM±0.17) μmol/kg/h, p=0.028). Splanchnic extraction of tyrosine and phenylalanine was not different between groups.

Conclusion This study proves that in critically ill stable non-septic patients with optimal nutrition and a target protein intake of 1.2-1.7 g/kg/day, protein balances approach zero. Enteral glutamine supplementation has no positive effect on protein synthesis in this population.

Introduction

Nutritional status regarding energy and protein intake influences outcome of ICU patients [1-5]. However, up to now randomized studies on optimal protein intake, the main driver of anabolism, are not available yet [1-5]. While muscle and protein wasting may occur, the effect of optimal enteral nutrition in critically ill patients on protein synthesis remains unclear. In order to counteract catabolism, glutamine has been supplied in the past decades. Evidently, compared to healthy volunteers, critically ill patients have significantly decreased muscle glutamine levels accompanied by reduced plasma glutamine concentrations[6]. Furthermore, glutamine deficiency correlates with hospital mortality [7, 8]. Intravenous more than enteral supplementation improved plasma glutamine concentrations. However, iv supplementation might affect intestinal availability, while the gut is a major immunological organ and has a high metabolic turnover. Many clinical studies have demonstrated that supplementation with glutamine as free molecule or dipeptide resulted in a favourable clinical outcome as reflected by a reduction in infectious morbidity [9], mortality and a reduction in length of hospital stay [10-18] in severely ill patients [19-21], although others found no effect [22].

The potential mechanism on clinical outcome is unclear. Repeated muscle biopsies proved that glutamine enriched parenteral nutrition could not alter muscle glutamine content in severely ill patients [6, 23] . However ever since, we learnt that when on adequate glutamine supply, glutamine metabolism (into arginine) takes approximately 3 days and reduction of skeletal muscle catabolism by glutamine could take the same amount of time [9, 24]. Therefore, we aimed to quantify amino acid kinetics in critically ill patients following a three day supplementation of a therapeutically relevant dose of L-glutamine, provided as L-alanyl-L-glutamine along with adequate enteral nutritional supply. We hypothesize that exogenous, enterally provided L-glutamine improves substantially to whole body protein turnover.

Patients and methods

Patients and methods

To investigate this hypothesis, we performed an open labelled randomized trial in which twenty critically ill patients were randomly assigned to one of the two groups: ten patients (ALA-GLN) received an enteral dose of 0.5 g/kg L-alanyl-L-glutamine (=0.325 g/kg glutamine/day) simultaneously with enteral nutrition, through a nasogastric or duodenal tube; ten control patients received isonitrogenous enteral nutrition without the additional glutamine. To achieve a similar nitrogen intake, nutritional nitrogen was compensated by using different enteral nutritional formulas containing different amounts of protein. Both groups received tracers both intravenously and enterally, in a cross-over randomized order on separate days, enabling splanchnic extraction calculations.

Patients

Twenty critically ill patients considered stable with an expected ICU or medium care stay of a minimum of 5 days were studied: All subjects met with the following inclusion criteria: Age: 18 years, BMI [3] 18,5 and £ 35, ability to tolerate enteral nutrition, provided by nasogastric or duodenal tube, meeting full protein/energy requirements based on indirect calorimetric measurements (energy) and a protein goal of 1.2-1.7 g/kg/day.

Exclusion criteria were as previously described [25]: septic shock [26], high dose vasoactive medication; admission after elective surgery; pregnancy or lactation, liver failure; kidney failure; urea cycle defects; chronic corticosteroids; gastrointestinal malabsorption; parenteral nutrition; use of medium chain triglycerides or glutamine/citrulline supplements.

Informed consent was obtained from all included patients or their legal representative. The Medical Ethical Committee of the VU University Medical Hospital approved the study protocol (MEtC VUmc 2009.083). The study complied with the Declaration of Helsinki.The study was registered at the Nederlands Trial Register (Dutch trial register), trial number NTR2285.

Methods

All ICU patients received enteral nutrition via nasogastric or postpyloric tube. Ten critically ill patients received an additional enteral dose of 0.5 g/kg/day L-alanyl-L-glutamine (=0.325 g/kg glutamine/day) (Fresenius Kabi. Nederland B.V. Den Bosch, the Netherlands) simultaneously with enteral nutrition. The critically ill control group received isonitrogenous enteral nutrition without additional glutamine. Total nitrogen was adjusted by using different enteral nutritional formulas containing different amounts of protein, to obtain equal levels. Patients were investigated while being fed continuously. Resting energy expenditure (REE) was measured during one hour with the Deltatrac I Metabolic Monitor (Datex-Engstrom Division, Helsinki, Finland), daily calibrated. Energy requirement was studied within 24h hours before study or control feeding was started. Nutritional intake from continuous feeding was not interrupted for the purpose of this measure. Body weight and height were (self) reported at admission. Total Energy Expenditure (TEE) was calculated by adding 10 % (activity factor) above REE [3]. Nutrition was based on TEE. Total protein was aimed for at 1.2-1.7 g/kg/day [3, 27] but at least not under 1.2 g-kg-day.
To achieve these nutritional goals we used enteral feeding containing different amounts of protein: Nutrison Protein Plus® Nutrison Standaard®, (both Nutricia, Zoetermeer, the Netherlands), Promote® (Abbott, Columbus, Ohio, US). Data on nutrition and nutritional requirements are listed in Table 1. Baseline characteristics were measured. Acute Physiology and Chronic Health Evaluation (APACHE) II scores were calculated as measures of severity of disease in ICU patients [28]. A blood sample was taken for baseline amino acid concentration.

All patients received both enteral and parenteral tracers at day three or four in random order. After 3 and 4 days of glutamine enriched or control feeding, but before the start of the tracer infusion, at 10.00am an arterial baseline sample was collected to measure natural enrichment, followed by a primed continuous intravenous tracer infusion (dosages in table 2). Blood samples were collected at 30 minute intervals for the subsequent 2.5 hours, sample handling is previously described [25] .

Stable isotopes

Stable isotopic tracers of L-[ring-$^2H_4$]-tyrosine (TYR M+4)), L-[ring-$^2H_2$]-tyrosine (TYR M+2) and L-[ring-$^2H_5$]-phenylalanine (PHE M+5) were used to investigate the effect of the enteral supplementation of ALA-GLN on total protein turnover. Tracers were purchased from Cambridge Stable Isotope Laboratory (Woburn, MA, USA). The Department of Clinical Pharmacy at the Erasmus Medical Center in Rotterdam, the Netherlands prepared the sterile and pyrogen-free stock solutions of the tracers. The tracers were administered as a primed continuous infusion except for L-[ring-$^2H_4$]-tyrosine, which was only administered as a primer. Tracers and amounts are listed in Table 2.

Laboratory analyses

Amino acid concentrations in deproteinized samples and infusates were measured using high-performance liquid chromatography [29]. The phenylalanine and tyrosine enrichments were determined by liquid chromatography-mass spectrometry [30].

Isotopic enrichment was expressed as tracer-to-tracee (labeled vs. unlabeled substrate) ratio (TTR, %), which was corrected for background TTR determined in the baseline sample and for contribution for lower masses.

Calculations

Isotopic enrichment was corrected for natural enrichment and for contribution of

overlapping isotopomer distributions of the tracee and tracers with lower masses to the measured TTR. [31]. Conversions and metabolic fluxes were calculated by using established formulas [32] .

For the estimation of individual steady state values, arterial enrichment curves were fitted for each patient with the use of PRISM software (version 4.03; GraphPad Software Inc, San Diego, CA). Steady state calculations are described previously [25].

Whole body plasma rate of appearance of phenylalanine and protein synthesis

Because phenylalanine cannot be synthesized de novo, the whole body rate of appearance (WBRA: μmol*kg^{-1}*h^{-1}) of phenylalanine can be used as an indication of whole body protein breakdown. WBRA of tyrosine and phenylalanine, and the known infusion rate of these tracers are based on the following equation:

1. WBRA = I(tracer) / TTR

Where I(tracer) is the known infusion rate of the tracers and TTR is the tracer/tracee ratio. Because enteral feeding affects the RA, the WBRA includes the exogenous infusion rate of tracee:

2. WBRA = RA(endogenous) + I(tracee)

In which I(tracee) reflects the exogenous amino acid (AA) supply after splanchnic extraction:

3. I(tracee) = I(AA) * TTR(EN)/TTR(IV)

With TTR(EN) being the TTR with enterally administered tracers, and TTR(IV) the TTR with intravenously administered tracers, hereby correcting for the splanchnic extraction of amino acid, reflected by splanchnic tracer extraction.

True RA [RA(endogenous)] is therefore calculated as follows:

4. RA(endogenous)= [I(tracer) / TTR(IV)] – [I(AA) * TTR(EN)/TTR(IV)]

In which RA(endogenous) of phenylalanine reflects whole body protein breakdown.

Calculation of the rate of hydroxylation (turnover Q: μmol*kg^{-1}*h^{-1}) of phenylalanine into tyrosine was performed by using the following equation in which the conversion of PHE M+5 into TYR M+4 is calculated [33] :

5. Q(phenylalanine→tyrosine) = RA (endogenous)TYRM+2 * TTR(TYR M+4) / TTR(PHE M+5)

Protein synthesis (PS) can be calculated by subtracting phenylalanine hydrolysis from the protein breakdown [34].

6. PS=RA(endogenous)PHE – Q(PHE→TYR)

Hence, protein balance can be calculated as follows:

Protein balance = Protein synthesis – Protein breakdown

First pass effect (%) of tyrosine and phenylalanine was calculated as follows:

7. [1-(TTR(EN)/TTR(IV))] * 100

Statistical analyses

From previous surgical experiments we know that a number of 6-8 patients is sufficient to study our goals [35], but due to suspected heterogeneity and dropout we aimed at a larger population

Data are expressed as mean ± standard deviation (SEM) in case of normally distributed data, and as median ± interquartile range (IQR) when data were not normally distributed (tested by Shapiro-Wilk normality test). Paired T-Tests or Wilcoxon signed rank tests were used to compare intravenously with enterally obtained data. Likewise, independent sample T-Test or Mann Whitney Test was used to compare control group with ALA-GLN group.

One sample T-Test was used to test whether steady state of metabolic products differed from zero. Plasma values over time were compared using ANOVA and Bonferroni to determine specific time differences.
A p-value of <0.05 (2-tailed) was considered as statistical significant. Statistical analysis was performed with SPSS 17.0 for Windows® (SPSS Inc., Chicago, IL, USA).

Results

Twenty patients were included, ten received ALA-GLN isocalorically, isonitrogenously, compared to 10 control patients. One patient had his tracer administration accidently interrupted, therefore results could not be obtained. Patient characteristics are summarized in Table 3. Baseline characteristics were not significantly different when comparing the two groups.

More patients in the ALA-GLN group received Nutrison Standard® (lowest in protein enteral formula we used), due to correcting the administered amount of ALA-GLN with the total amount of protein within the enteral formula (Table 4).

Patients received the same amount of calories and energy expenditure was similar in both groups. Nitrogen intake corrected for body weight was not significantly different. Glutamine administration was higher in the ALA-GLN group. Administered tyrosine and phenylalanine amounts were not significantly different (Table 4).

Arterial plasma concentrations of glutamine, tyrosine and phenylalanine were not different at D0 for both groups. Glutamine supplementation did not result in higher plasma glutamine concentrations and glutamine plasma levels did not differ between groups. Arterial phenylalanine levels dropped significantly after 3-4 days in the ALA-GLN group (D3: 71 µmol/ml ±7.1, D4: 69 µmol/ml ±10.0 p=0.029), specifically reaching statistical significance between baseline and D3 (p=0.022), without differences between groups. Tyrosine plasma levels did not differ over time or between groups.

Arterial plasma enrichment (TTR) was in steady state for PHE M+5 and TYR M+2 tracers and for the conversion substrate TYR M+4. All tracer enrichments differed from zero (p ≤0.001).

The TTR% of the metabolic products of L-[ring-$^2H_5$]-phenylalanine metabolism—L-[ring-$^2H_4$]-tyrosine was significantly different from zero in both groups with both ways of administration (Table 5).

TTR% of infused PHE5 and TYR2 tracers and TTR% of the metabolic product TYR4 were not different between control and ALA-GLN group (table 5).

RA and TTR% for tyrosine and phenylalanine in intravenously measured experiments were mostly significantly different from the enteral experiments (Table 5). RA corrected for splanchnic extraction and exogenous amino acids (endogenous RA) of TYR M+2 and PHE M+5 (equaling protein breakdown) were not different between groups, although large SEMs and IQRs may cover higher TTRs in the ALA-GLN group (Table 5). Phenylalanine hydroxylation (whole body plasma turnover PHE→TYR) was significantly higher in the ALA-GLN group versus the control group (CON 0.75 µmol/kg/h; ALA-GLN 1.72 µmol/kg/h, p=0.028; Figure 1). Protein synthesis was not different between groups (CON 14.0 (IQR12.4-23.6) µmol/kg/h; ALA-GLN 19.5 (SEM±2.7) µmol/kg/h, p=0.354, Figure 1).

Protein balance was significantly more negative in the ALA-GLN group (CON -0.75 (SEM±0.36) µmol/kg/h; ALA-GLN -1.72 (SEM±0.17) µmol/kg/h, p=0.028; Figure 1). Protein balance in the ALA-GLN group was significantly negative (p= 0.000; compared to zero) but not in the control group. Splanchnic extraction of tyrosine and phenylalanine was not different between groups (Table 6).

Table 1. Enteral nutrition

	Energy (kcal/l)	Protein (g/l)	Glutamine (g/l)
Nutrison Standard®	1000	40g/l	4.6
Nutrison Protein Plus	1250	63g/l	7.19
Promote®	1000	63g/l	2.3

Table 2. Tracer dosages

	Prime (mg)		Infusate (µmol/kg/h)	
	CON	ALA-GLN	CON	ALA-GLN
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
TYR M+4	3.6 (0.6)	4.0 (0.4)		
PHE M+5	21.3 (3.6)	23.4 (2.3)	2.0 (0.8)	1.8 (0.8)
TYR M+2	10.1 (1.7)	11.1 (1.1)	0.6 (0.2)	0.6 (0.2)

Table 3. Baseline characteristics

	CON	ALA-GLN
	N (%) / mean (SEM) / median (IQR)	N (%) / mean (SEM) / median (IQR)
Demographics		
Sex: male/female (%)	6/4 (60/40)	6/4 (60/40)
Age (y)	65 (6.4)	57 (5.4)
Length (cm)	172.6 (3.7)	176.8 (3.0)
Weight (kg)	73.2 (5.8)	77.7 (4.1)
BMI (kg/m2)	24.2 (1.0)	24.5 (3.3)
Clinical Assessment		
Type of ICU admission		
Respiratory insufficiency	6(60)	3(30)
Cardiogenic shock	1(10)	4(40)
Neurotrauma	1(10)	0
Multitrauma	1(10)	2(20)
Other	1(10)	1(10)
Apache II-score	27.0 (2.2)	25.3 (3.0)
Laboratory measurements at inclusion		
pH	7.42 (0.048)	7.44 (0.027)
pCO2	41.4 (10.3)	43.9 (9.7)
Bicarbonate (mmol/L)	32.9 (22.0-33.0)	30.5 (5.3)
Glucose (mmol/L)	6.6 (0.83)	7.5 (1.4)
Leukocytes (10*9 µmol/l)	11.0 (3.1)	11.8 (4.4)
Bilirubin (µmol/L)	8.5 (5.0-16.8)	9 (4.0-11.3)
Creatinine (µmol/L)	78 (9.7)	81.8 (13.7)
Urine production (ml/24h)	2301 (361)	2333 (295)

ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation

Table 4. Nutrition characteristics

	CON Mean (SEM)	ALA-GLN Mean (SEM)	Sig. between groups (P)
Carlorimetry			
REE (kcal/24h)	1847 (165)	1998 (85)	0.429
TEE (kcal/24h)	1998 (165)	2176 (80)	0.350
VCO_2 (ml/min)	223 (17.0)	234 (10.7)	0.587
VO_2 (ml/min)	269 (24.8)	292 (12.4)	0.577
RQ	0.84 (0.03)	0.81 (0.016)	0.237
Nutrition			
Nutrison Protein Plus®/ Nutrison	3/5/2	0/1/9	
Standard®/ Promote®	(30/50/20)	(0/10/90)	
Energy (kcal/d)	1832 (230)	2181 (110)	0.33
Nitrogen (g/d)	94.5 (10.8)	123.2 (7.2)	0.034
(g/kg/d)	1.33 (0.05)	1.58 (0.05)	0.066
Glutamine (g/d)	10.4 (1.4)	36.5 (2.3)	<0.000
(mmol/kg/d)	1.06 (0.08)	3.19 (0.22)	(<0.000)
Tyrosine (g/d)	5.5 (0.7)	5.1 (0.3)	0.145
(mmol/kg/d)	0.45 (0.03)	0.36 (0.01)	(0.014)
Phenylalanine (g/d)	5.1 (0.7)	4.7 (0.3)	0.128
(mmol/kg/d)	0.46(0.03)	0.37(0.01)	(0.011)
Baseline plasma			
glutamine (µmol/ml)	536 (77)	497 (37)	0.743
Baseline plasma			
tyrosine (µmol/ml)	62 (8)	78 (8)	0.110
Baseline plasma			
phenylalanine (µmol/ml)	84 (12)	86 (5)	0.868

Table 5. Tracer dynamics

Tracer dynamics and calculations		CON Mean (SEM)/ Median (IQR)	ALA-GLN Mean (SEM)/ Median (IQR)	Difference CON vs. ALA-GLN (p)
Tyrosine				
RA TYR M+2	IV	47.5 (6.0)	39.1 (28.7-63.9)	0.529
	EN	74.7 (6.7)	54.7 (5.9)	0.039
IV vs. EN	(p)	0.032	0.037	
Endogenous RA TYR M+2		7.36 (4.17)	18.39 (11.26-19.97)	0.102
TTR% TYR M+2	IV	1.33 (0.51)	1.55 (0.12)	0.275
	EN	0.92 (0.12)	0.91 (0.09)	0.974
IV vs. EN	(p)	0.078	<0.000	
TTR% TYR M+4	IV	0.28 (0.05)	0.36 (0.04)	0.263
	EN	0.23 (0.05)	0.24 (0.05)	0.880
IV vs. EN	(p)	0.377	0.066	
Splanchnic extraction TYR		31.3 (13.3)	36.0 (-3.8;39.8)	0.514
Phenylalanine				
RA PHE M+5	IV	64.0 (8.0)	67.3 (6.4)	0.284
	EN	108.8 (19.3)	76.6 (8.4)	0.112
IV vs. EN	(p)	0.021	0.413	
Endogenous RA PHE M+5		14.26 (13.18-20.66)	21.21 (2.72)	0.223
TTR% PHE M+5	IV	2.91 (0.27)	3.12 (0.31)	0.630
	EN	2.04 (0.26)	2.04 (0.23)	0.995
IV vs. EN	(p)	<0.000	<0.000	
Splanchnic extraction PHE		41.8 (12.9)	27.9 (-16.6-32.2)	0.258

RA in µmol/kg/h, endogenous RA in µmol/kg/h, Splanchnic extraction in %, TTR in %, Q in µmol/kg/h.

Figure 1A

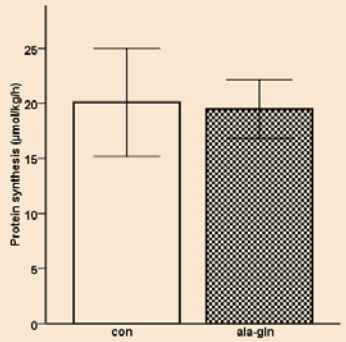


Figure 1B

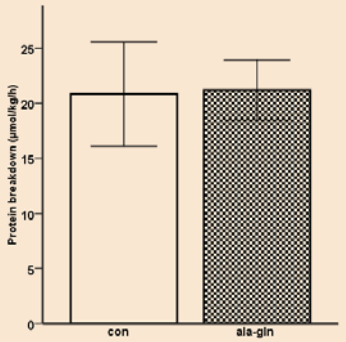


Figure 1C

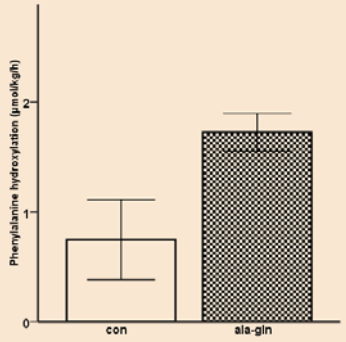
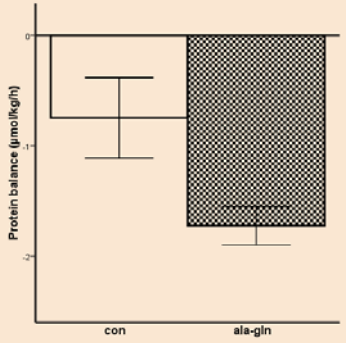


Figure 1D



Protein synthesis (A), protein breakdown (B), phenylalanine hydroxylation (C) and protein balance (D) (µmol/kg/h). Phenylalanine hydroxylation was significantly higher in the alanyl-glutamine group versus the control group (p=0.028). Protein balance was significantly more negative in the alanyl-glutamine group (p=0.028).

Discussion

This study clearly shows that critically ill patients, despite receiving full caloric enteral nutrition based on energy expenditure, did not reach an anabolic status.
This is in contrast to other age groups, such as premature infants and children at neonatal and paediatric intensive cares. Supplementation with enteral ALA-GLN did not improve protein balance; it even increased phenylalanine hydroxylation rates. This is in contrast with early assumptions that exogenous glutamine would protect against muscle wasting, but in line with other experiments (short term or intravenous administration) [24, 36, 37].

Glutamine and protein kinetics

The concentrations of plasma glutamine in the patients we studied were not as low as seen in earlier publications, despite severity of disease [9].Supplementation did not result in higher plasma levels. As protein synthesis did not increase, it is not clear where glutamine was used for. A number of explanations for both observations may be postulated: primarily, the strict enteral feeding regime providing optimal nutrition based on REE may have prevented glutamine depletion from the beginning. Secondly, the gut preferentially takes up enterally provided glutamine, therefore arterial concentrations may have been attenuated [38, 39]. And thirdly, following scientific results, modern enteral formulas contain a fair amount of glutamine compared to previous formulas: in our control patients, exogenous glutamine administration was 10.4 g/d (see also Table 1 and 4).
Finally, increased glutamine consumption caused by a selection of rapidly dividing cells such as macrophages could also have attributed to these glutamine levels [40].

Different effects of intravenous glutamine supplementation on muscle glutamine content and muscle protein synthesis have been reported. In elective abdominal surgery patients receiving glutamine-enriched parenteral nutrition, plasma glutamine concentration decreased, while muscle glutamine content increased and the fal in muscle protein synthesis as seen in the control group receiving conventional parenteral nutrition was prevented in the glutamine group [24]. In ICU patients, the intravenous glutamine (0.28, 0.57 and 0.86 mmol/kg) supply for five days normalized plasma glutamine concentrations [36]. Despite restoration to normal levels, muscle protein content [36] nor muscle protein synthesis rates were altered [37], although patients in the latter study were only given a three hour enteral infusion of glutamine as well as a short term identical nutrition. In the present study we show that also on the long run (three days or more enteral supplemental) glutamine supplementation to critically ill patients in addition to enteral nutrition according to the guidelines had no effect on protein synthesis rates as measured by phenylalanine kinetics.
The amount of hydroxylation of phenylalanine (the conversion of phenylalanine into tyrosine) was more pronounced in the ALA-GLN group. This response was not described earlier. An explanatory argument could be that the lower phenylalanine and tyrosine supply did not meet the demands. Our glutamine supplied group received significantly less phenylalanine and tyrosine in their diet. Lower tyrosine could have triggered higher phenylalanine hydroxylation rates.

The amount of protein delivered was set between 1.2 to 1.7 g/kg/g, approaching the criteria of the European Society of Enteral and Parenteral Nutrition and the European Society of Intensive Care Medicine. Evidently, it is unknown which amounts of protein are optimal for critically ill patients [1, 2, 41], but there appears to exist a correlation between the amount of amino acids administered and protein balance[42]. Our study shows that for this population, whether glutamine added or not, a protein delivery of 1.2 up until 1.7 g/kg/d ensures protein synthesis.

Strengths and weaknesses of this study

We aimed for an approach in which patients would be in metabolic steady state as ideally as possible by supplying continuous enteral nutrition (based on REE and protein delivery according to recommendations) and glutamine for a minimum of 3 days.
However all results of metabolic studies using stable isotope techniques, enteral feeding or enteral administration of nutrients and/or tracers, should be treated with caution. The cross over study design is an approach to enable correcting for splanchnic extraction and enteral feeding. Nonetheless even this method is not rock solid:
the administration on two different days is fed by the assumption that clinical conditions are stable. Within the ICU population, one single day can matter: one patient was suddenly discharged from ICU, one was detubated. Furthermore, we know that critically ill patients run different metabolic phases with different energy needs [41]. Therefore adaptation in energy demands based on frequent REE might have been preferred, or may have gained insight in the specific metabolic phases of the patients included. By randomizing the administration order we tried to minimize prominent differences, however an alternative, or maybe a better design would have been a two way administration at the same time. However that approach would imply using different tracers, often yielding different metabolic results.

Interpretation of splanchnic extraction

Enteral administration is sometimes regarded as a black box: Nutrients entering the gut are taken up in different amounts, in a range from 0-100%. The uptake determines the faith of the nutrients: some are metabolized into other products, some are delivered to the blood in unaltered condition. Others may be used as fuel for the enterocyte, for gut associated lymphoid tissue, or other metabolic processes and may therefore be hard to detect systemically. Importantly, after systemic delivery basolateral uptake by the

gut can take place, however not necessarily in similar proportions compared to apical uptake. This means that even though a thorough calculation was made to determine the exact amount of phenylalanine and tyrosine after splanchnic extraction, the exact behavior of those amino acids is hard to determine unless splanchnic sampling is allowed. This is not feasible in critically ill patients.

Within an ICU population experiencing catabolism because of critical illness accompanied by additive muscle wasting due to immobility [43], anabolic conversion has long been aimed for. On the other hand, protein breakdown can be considered an adaptive mechanism. Although severe sarcopenia should obviously be avoided, a positive protein balance will probably remain utopia. This study shows that with targeted energy nutrition and an actual protein intake of 1.2-1.7 g/kg/day, protein balance approaches zero. Enteral glutamine supplementation has no positive effect on protein synthesis in this population.

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Contributions of the authors:

M.A.R.V. designed and performed the study, performed calculations and statistical analyses and wrote the manuscript. A.B. helped designing the study and performed the study, P.M.B. was responsible for ad hoc tracer preparation and pharmaceutical handling of the tracer solutions. P.J.W. en H.M.O.v.S. critically reviewed the manuscript, J.B.v.G. helped designing the study, performed calculations and critically reviewed the manuscript. P.A.M.v.L. was responsible for all parts of the study. All authors read and approved the final manuscript.

Conflicts of interest:

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