8. Imbalance of arginine and asymmetric dimethylarginine is associated with markers of circulatory failure, organ failure and mortality in shock patients

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British Journal of Nutrition. 2012 May;107(10):1458-65

Abstract

In shock, organ perfusion is of vital importance because organ oxygenation is at risk. Nitric oxide (NO), the main endothelial-derived vasodilator, is crucial for organ perfusion and coronary patency. The availability of NO might depend on the balance between substrate (arginine) and inhibitor (asymmetric dimethylarginine, ADMA) of NO synthase. Therefore, we investigated the relation of arginine, ADMA and their ratio with circulatory markers, disease severity, organ failure, and mortality in shock patients. In forty-four patients with shock (cardiogenic n17, septic n 27), we prospectively measured plasma arginine and ADMA at intensive care unit admission, Acute Physiology and Chronic Health Evaluation (APACHE) II-(predicted mortality) and Sequential Organ Failure Assessment (SOFA) score, and circulatory markers to investigate their relationship. Arginine concentration was decreased (34.6 (SD 17.9) µmol/l) while ADMA concentration was within the normal range (0.46 (SD 0.18) µmol/l), resulting in a decrease in the arginine:ADMA ratio. The ratio correlated with several circulatory markers (cardiac index, disseminated intravascular coagulation, bicarbonate, lactate and pH), APACHE II and SOFA score, creatine kinase and glucose. The arginine:ADMA ratio showed an association (OR 0.976, 95% CI 0.963, 0.997, P=0.025) and a diagnostic accuracy (area under the curve 0.721, 95% CI 0.560, 0.882, P=0.016) for hospital mortality, whereas the arginine or ADMA concentration alone or APACHE II-predicted mortality failed to do so. In conclusion, in shock patients, the imbalance of arginine and ADMA is related to circulatory failure, organ failure and disease severity, and predicts mortality. We propose a pathophysiological mechanism in shock: the imbalance of arginine and ADMA contributes to endothelial and cardiac dysfunction resulting in poor organ perfusion and organ failure, thereby increasing the risk of death.

Introduction

Arginine, the sole nitric oxide (NO) precursor and a semi-essential amino acid, is thought to become essential in shock patients. Septic [1] and cardiogenic shock [2] are characterized by low arginine and excessive NO levels. Several studies have tried to boost arginine levels in critically ill patients by supplementing this amino acid alone or in combination with other immunomodulating substances. Results of the studies are controversial. While some have shown beneficial or no effects [2-4] others have in fact suggested that arginine might increase the risk of mortality [5, 6].

A possible negative effect of arginine might have been mediated by arginine-induced increased NO production by inducible NO synthase (NOS), which in turn could have led to detrimental systemic vasodilation [5] or to increased formation of peroxynitrite due to concomitant oxyradical production inducing cellular damage [6]. On the other hand, arginine may have positive effects by enhancing NO-mediated microvascular vasodilatation facilitated by endothelial NOS, which is crucial for organ perfusion and coronary patency. Probably NO availability needs to be perfectly balanced [7].

Quantitatively, arginine consumption by NOS is partly determined by the availability of NOS inhibitors, such as asymmetric dimethylarginine (ADMA) that facilitates vasoconstriction and deteriorates endothelial and cardiac function [8]. In critically ill patients, highly elevated levels of ADMA were observed, and high ADMA predicted mortality and correlated with organ severity [9].

The above findings suggest that NO availability might depend on the balance of a NOS substrate (arginine) and an inhibitor (ADMA). Indeed, in a recent study by our group, low arginine and high ADMA levels reduced cardiac output in rats [10].

Based on previous studies, we hypothesize that an imbalance of arginine and ADMA contributes to poor organ perfusion in patients with shock. Therefore, we investigated the relationship between arginine, ADMA and their ratio at admission to the intensive care unit (ICU) and circulatory markers, disease severity, organ failure and mortality in critically ill shock patients.

Materials and Methods

Patients

The present prospective cohort study was conducted in a twenty-bed closed format general ICU of a teaching hospital in the city of Amsterdam, The Netherlands. The study includedadult patients with persistent septic or cardiogenic shock within 24 h after ICU admission, requiring mechanical ventilation. Other inclusion criteria were predicted ICU treatment and intention to treat for at least 5 d. Exclusion criteria were active massive bleeding, pregnancy, HIV with less than fifty CD4 cells, hematologic malignancy, metastatic malignancy, Child C liver cirrhosis, hepatic coma and therapeutic hypothermia after cardiac arrest.

For inclusion into the study, shock was defined as persistent hypotension despite adequate fluid resuscitation and the need of dopamine at a dose of more than 6 mg/kg per min and anydose of additional noradrenaline in the presence of perfusion abnormalities, manifest by oliguria, reduced peripheral perfusion and organ dysfunction. Hypotension was defined asa systolic pressure <90 mmHg; oliguria as a urinary output <20 ml/min despite fluid infusion; reduced peripheral perfusion as ΔT (difference between the core and peripheral temperature), <4°C, skin colour not pink, poor capillary refill; and organ dysfunction was defined according to the Sequential Organ Failure Assessment (SOFA) score [11]. Septic shock was defined as the form of acute circulatory shock occurring secondary to severe infection [12]. Cardiogenic shock is the form of circulatory shock occurring secondary to heart failure as evidenced by low cardiac output or ejection fraction accompanying cardiac disease (supported by, for example, echocardiography).

To optimize the circulation, we used fluids in a mixture of crystalloids and colloids (Gelofusinew; B. Braun, Melsungen, Germany) and dopamine as a first-line inotropic and vasopressor agent. Noradrenaline was added if higher doses of dopamine were needed and/or if the patient developed tachycardia. Enoximone was added when the cardiac index remained <2.5 l/kg per h at a standard dose of 8 mg/h. Nitroglycerin was used in patients with cardiogenic shock, cardiac ischemia and/or persistent poor peripheral perfusion [13]. Fluids were infused in amounts considered to be necessary to restore circulating volume, and to optimize cardiac output and peripheral circulation. Endpoints of the circulation treatment were DT, skin colour, capillary refill, blood pressure (target >90 mmHg), central venous pressure, cardiac index (target >2.5 l/kg per min), pulse pressure variation (target <10% if appropriate) and mixed venous oxygen saturation (target >70mmHg if feasible).

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the medical ethics committee of the Onze Lieve Vrouwe Gasthuis. Written informed consent was obtained from all patients or their legal representative. Patients were followed until they died (non-survivors) or were discharged from the hospital (survivors).

Physiological and laboratory parameters

Blood samples were taken after the inclusion criteria were met in the first 24 h of ICU admission and were immediately placed on ice and centrifuged. Plasma was pipetted and immediately put in liquid N2 and stored at -80°C before analysis. The concentrations of arginine and ADMA were measured by HPLC on a monolithic column as described previously [14, 15]. Intra- and inter-assay CV for both arginine and ADMA were <2 and <3%, respectively. In addition, the arginine:ADMA ratio was calculated. Normal values for arginine and ADMA concentrations were 80 (SD 20) [16] and 0.497 (SD 0.063) mmol/l [15], respectively.

Laboratory parameters indicating hepatic and renal function (bilirubin, alanine aminotransferase, creatinine (clearance) and urea), muscle degradation (creatine kinase) and acid–base physiology (bicarbonate, pH and lactate) were analysed by standard laboratory methods. Diagnosis of disseminated intravascular coagulation was done by calculation of the global coagulation test score [17].
At ICU admission, cardiac output was assessed invasively with the Swan Ganz catheter based on the thermodilution method, ornon-invasively with cardiac output (NICO; Novametrix Medical Systems, Inc., Wallingford, CT, USA) that continuously measures pulmonary perfusion based on the Fick principle.

NICO was preferred, especially i006E patients with sepsis, since it offers the benefit of continuous monitoring and avoids the risks of invasive monitoring. Cardiac index was calculated by dividing the cardiac output by the body surface area. Furthermore, severity of illness was scored using the Acute Physiology and Chronic Health Evaluation (APACHE) II system over the first 24 h of ICU admission [18], and APACHE II-predicted mortality was used as a reference for the predictive values of arginine, ADMA and their ratio for mortality [19]. The SOFA score as defined by the Dutch National Intensive Care Evaluation (www.stichting-nice.nl) was measured daily [11].

Statistical analysis

Data are expressed as means and standard deviations in the case of normally distributed data and as medians and interquartile ranges when data are not normally distributed. Normality was tested by the Shapiro–Wilk normality test. Likewise, we used Pearson's correlation and Spearman's rank correlation coefficient to determine whether clinical and biochemical variables were significantly related to arginine, ADMA and their ratio. Predictors of mortality were studied by calculation of the OR in a logistic regression model with one variable (arginine, ADMA, arginine:ADMA ratio or APACHE II-predicted mortality). Our sample size did not permit a multiple regression analysis. Receiver-operating characteristic curves were estimated using the non-parametric method to

further evaluate the association of arginine, ADMA, the arginine:ADMA ratio and APACHE II-predicted mortality with hospital mortality. The area under the curve (AUC) was calculated to determine the accuracy of arginine, ADMA, the arginine:ADMA ratio and APACHE II-predicted mortality as predictors of hospital mortality. The further the curve lies above the reference line and the higher the AUC, the more accurate is the test. Coordinates of the curve were examined across the full range of potential arginine: ADMA cut-off values in an attempt to select an optimal arginine:ADMAcut-off value that properly balanced the needs of sensitivity and specificity. Furthermore, positive and negative predictive cut-off values of arginine:ADMA were calculated. The χ^2 test was used to analyse the difference in mortality between patients with an arginine:ADMA ratio below the cut-off value and those with an arginine:ADMA ratio above the cut-off value. A P value of <0.05 (two-tailed) was considered to be statistically significant. Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Patients

Patient characteristics and biochemical values are presented in Table 1. Of the forty-four shock patients, twenty-seven had septic and seventeen had cardiogenic shock. At admission, mean APACHE II-score was 26.2 (SD 7.4) and SOFA-score was 10.0 (SD 3.0). Mean levels of arginine and ADMA were 34.6 (SD 17.9) µmol/l and 0.46 (SD 0.18) µmol/l respectively, and mean arginine:ADMA was 83.2 (SD 42.5). The levels of arginine and ADMA, the arginine:ADMA ratio, APACHE II- and SOFA-score and cardiac index did not differ significantly between septic and cardiogenic shock patients (arginine: 32.2 (SD15.9) and 38.4 (SD 20.7), P=0.269; ADMA: 0.43 (SD 0.16) and 0.50 (SD 0.20), P=0.185; arginine/ADMA: 79.9 (SD 33.7) and 88.4 (SD 54.4), P=0.571; APACHE II-score: 26.5 (SD 7.7) and 25.6 (SD 7.), P=0.691; SOFA-score: 9.9 (SD 3.2) and 10.3 (SD 1.7), P=0.887; cardiac index: 2.5 (SD 1.1) and 2.0 (SD 1.0), P=0.150).

Arginine, asymmetric dimethyl arginine and their ratio in shock patients

Arginine correlated negatively with ICU length of stay, APACHE II predicted mortality, SOFA-score, and urea. ADMA (tended to) correlate(d) positively with alanine aminotransferase, urea, and glucose, and negatively with pH. The arginine:ADMA ratio correlated with markers of circulation (Figure 1: cardiac index (Figure 1A), bicarbonate (Figure 1B), lactate score (Figure 1C), and pH (Figure 1D)), and negatively with APACHE II and SOFA-score (Table 2). The arginine:ADMA ratio also correlated positively with creatine kinase and glucose (Table 2).

Predictors of hospital mortality

In a logistic regression model, the arginine:ADMA ratio predicted hospital mortality (OR: 0.98, 95% confidence interval (CI): 0.963, 0.997, P = 0.025), while arginine, ADMA and APACHE II

Data are expressed as Pearson's correlation or as [a]: Spearman rank correlation coefficient. predicted mortality were not significantly related to hospital mortality (OR: 0.976, 95% CI:0.940, 1.013, P = 0.205; OR: 40.9, 95% CI:0.867, 1930, P = 0.059; OR: 3.47, 95% CI: 0.267, 45.2, P = 0.342, respectively), although the relation with ADMA tended to significance (Table 3). The receiver operating characteristics curves in Figure 2 reveals that the arginine:ADMA ratio (AUC: 0.721, 95% CI: 0.560, 0.882, P = 0.016, Figure 2C) provides better diagnostic accuracy to predict mortality

compared to arginine (AUC: 0.621, 95% CI: 0.443, 0.798, P = 0.188) (Figure 2A), ADMA (AUC: 0.706, 95% CI: 0.533, 0.880, P = 0.024) (Figure 2B), and APACHE II predicted mortality (AUC: 0.583, 95% CI: 0.401, 0.765, P = 0.367) (Figure 2D). The optimal arginine:ADMA cut-off value was 93.4 (sensitivity: 0.875, specificity: 0.571) with a positive predictive of 0.W54 and a negative predictive value of 0.89 (Table 4). Hospital mortality was significantly higher in patients with an arginine:ADMA ratio below the cut-off value compared to patients above the cut-off value (14:24 vs. 2:18, χ^2 P =0.004).

Table 1 - Patient characteristics and biochemical values.

ICU, intensive care unit; IQR interquartile ranges; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ALAT, alanine aminotransferase; DIC, disseminated intravascular coagulation; ADMA, asymmetric dimethylarginine.

	Mean/median/n(SD/IQR/%)
Demographics	
Gender: female/male n (%)	21/23 (47.7/52.3)
Age (years)	65.7 (13.8)
Height (cm)	172.5 (8.9)
Weight (kg)	75.3 (17.4)
Clinical assessment	
ICU admission type: medical/surgical (%)	30/14 (68.2/31.8)
ICU stay (days) Median (IQR)	5.9 (3.7-9·3)
ICU mortality n (%)	8 (18.2)
Hospital mortality n (%)	16 (36.4)
APACHE II-score	26.2 (7.4)
APACHE II predicted mortality	0.56 (0.25)
SOFA-score	10.0 (3.0)
Cardiac index (L/min/m2)	2.3 (1.1)
Shock: septic/cardiogenic n (%)	27/17 (61·4/38·6)
Site of infection	
Lung n (%)	13 (29.5)
Abdomen n (%)	8 (18.2)
Urogenital n (%)	1 (2.3)
Other n (%)	5 (11.4)
None (cardiogenic shock) n (%)	17 (38.6)
Dose of vasoactive agents at day 1	
Dopamine (µg/kg/min)	8.1 (2.8)
Noradrenalin (µg/kg/min)	0.04 (0.04)
Nitroglycerin (µg/kg/min)	0.39 (0.18)
Laboratory measurements	
Bilirubine (µmol/L)	14.8 (11.3)
ALAT (U/L) Median (IQR)	29.0 (15-67)
Creatinine (µmol/L) Median (IQR)	124.5 (87.3-167.8)
Creatinine clearance Median (IQR)	52.3 (32.2-67.2)
Urea (mmol/L) Median (IQR)	9.4 (7.1-17.4)
Creatine Kinase (U/L) Median (IQR)	99.5 (60.3-458.5)
DIC	2.8 (1.2)
Bicarbonate (mmol/L)	18.7 (5.9)
Lactate (mmol/L) Median (IQR)	3.6 (1.9-6.4)
pH	7.3 (0.13)
Glucose (mmol/L) Median (IQR)	9.5 (5.0)
Arg (µmol/l)	34.6 (17.9)
ADMA (µmol/l)	0.46 (0.18)
Arg:ADMA	83.2 (42.5)

Table 2. – Correlations of arginine, ADMA and arginine / ADMA ratio and clinical and biochemical variables

	Arginine		ADMA		Arginine / ADMA	
	r	P	r	P	r	P
ICU stay[a]	-0.412	0.027	-0.040	0.794	-0.070	0.652
APACHE II-score	-0.198	0.302	0.086	0.581	-0.314	0.038
APACHE II-predicted mortality	-0.386	0.010	-0.062	0.688	-0.256	0.093
SOFA-score	-0.391	0.009	0.160	0.301	-0.362	0.016
Cardiac index	0.176	0.320	-0.279	0.110	0.364	0.034
Bilirubine	-0.004	0.978	0.261	0.087	-0.154	0.317
ALAT[a]	-0.126	0.516	0.303	0.045	-0.790	0.612
Creatinine[a]	-0.011	0.944	-0.027	0.863	-0.021	0.891
Creatinine clearance[a]	0.044	0.775	0.005	0.974	0.123	0.427
Urea[a]	-0.436	0.018	0.285	0.061	-0.095	0.540
Creatine kinase[a]	-0.319	0.092	-0.048	0.755	0.371	0.013
Bicarbonate	0.156	0.420	-0.176	0.253	0.552	<0.001
pH	0.125	0.518	-0.380	0.011	0.498	0.001
Lactate[a]	0.070	0.717	0.267	0.084	-0.467	0.002
DIC	-0.097	0.625	0.245	0.118	-0.409	0.007
Glucose	-0.220	0.151	0.395	0.008	0.337	0.025

ADMA, asymmetric dimethylarginine; ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ALAT, alanine aminotransferase; DIC, disseminated intravascular coagulation.

Table 3 – Risk factors for hospital mortality

	OR	95% CI	P
Arg	0.976	0.940, 1.013	0.121
ADMA	40.9	0.867, 1930	0.135
Arg / ADMA	0.980	0.963, 0.997	0.539
APACHE II predicted mortality	3.47	0.267, 45.2	0.026

ADMA, asymmetric dimethylarginine; APACHE, acute physiology and chronic health evaluation.

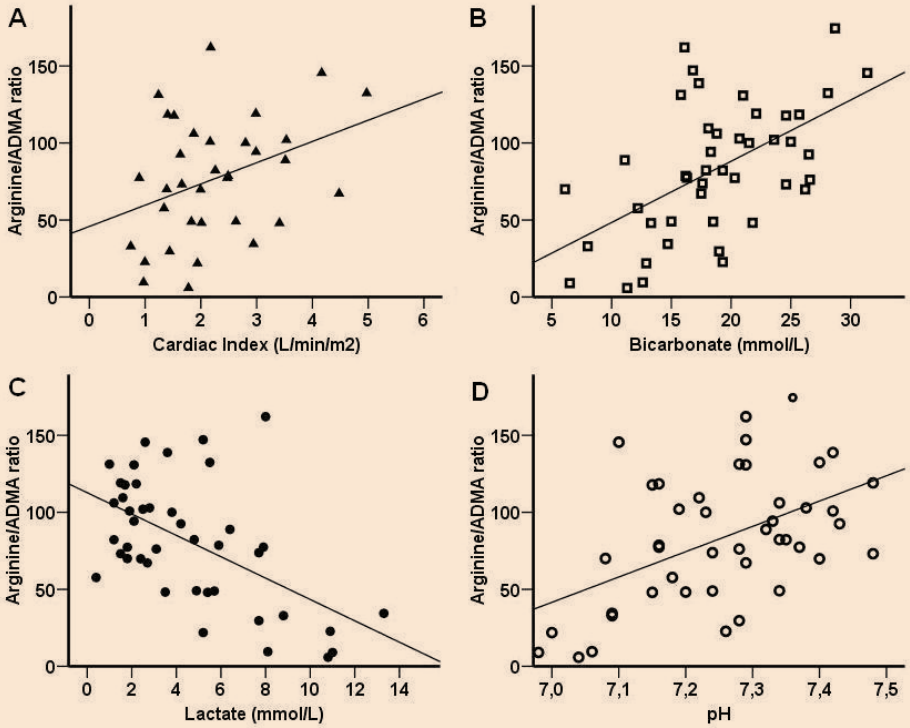
Table 4 – Predictive values of the arginine / ADMA ratio for hospital mortality

	Arginine / ADMA ratio		
	< 93.4	≥ 93.4	P*
Survivors	12	16	0.004
Non-survivors	14	2	
Positive predictive value			
Mean	0.54		
95% CI	0.36, 0.71		
Negative predictive value			
Mean		0.89	
95% CI		0.67, 0.97	

* χ^2 test

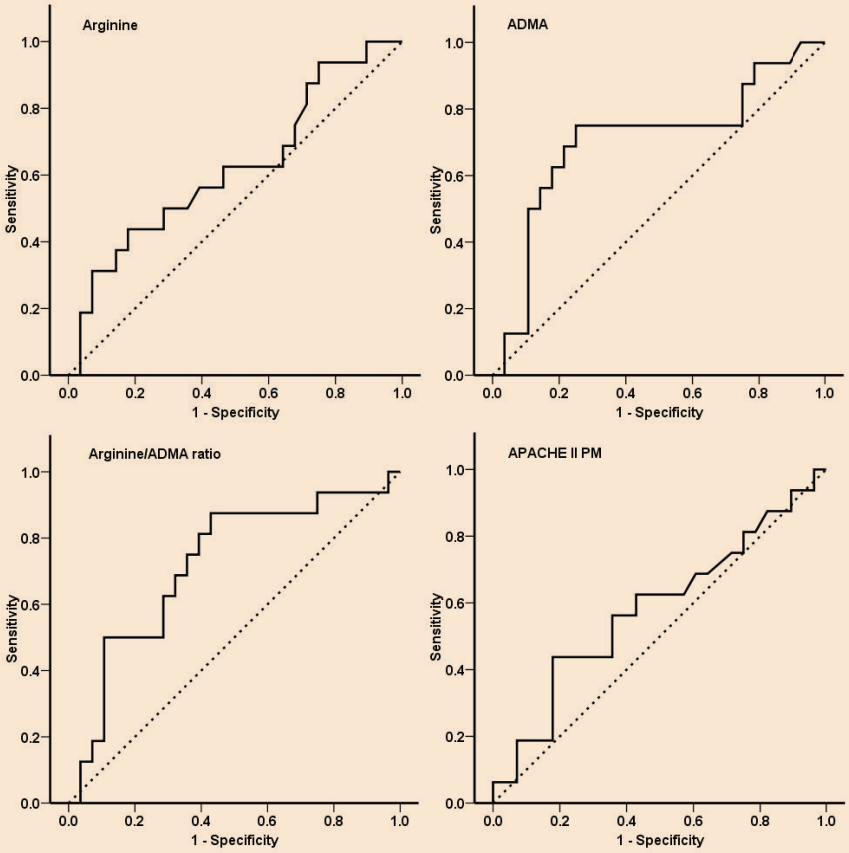
ADMA, asymmetric dimethylarginine; APACHE, acute physiology and chronic health evaluation.

Figure 1



Arg:ADMA ratio correlates with the markers of circulation. Cardiac index:r2 0.132; bicarbonate r2 0.305; lactate: r2 0.218; pH: r2 0.248

Figure 2



Receiver-operating characteristic curves of (a) arginine, (b) ADMA, (c) arg:ADMA and (d) APACHE II predicted mortality.ADMA – asymmetric dimethylarginine; APACHE II PM – acute physiology and chronic health evaluation II predicted mortality; ROC – receiver operating characteristic.

Discussion

The present observational cohort study in critically ill patients with shock shows that an imbalance of arginine and ADMA at ICU admission is associated with severity of disease and organ failure, and predicts mortality. The imbalance of arginine and ADMA in this study is mainly the result of decreased arginine levels as ADMA levels were within the normal range. Furtermore, the arginine:ADMA ratio was related to all markers of circulation while arginine and ADMA separately were not. In addition, the ratio showed a stronger association and better diagnostic accuracy for hospital mortality in the present study, whereas the arginine or ADMA concentration alone failed to do so. It therefore seems that the balance of arginine and ADMA might be important for proper micro and macrocirculation and not their individual concentrations. When arginine is depleted, a normal ADMA concentration may be relatively high. In the present shock patients, this imbalance between the NO substrate (arginine) and the inhibitor (ADMA) may result in poor organ perfusion

Since poor organ perfusion is the main cause of organ failure, and both arginine and ADMA influence endothelial and cardiac function [8], the present observations support the role of an imbalance between the NO substrate and the inhibitor in the pathophysiological mechanism of circulatory failure, organ failure and mortality in patients with shock. We propose a mechanism in which a combination of reduced flow to the organs and diminished microcirculation within these organs as a result of an imbalance between arginine and ADMA will end up in organ failure and an increased risk of death in shock patients. The associations with mortality and disease severity in septic patients with low arginine:ADMA ratios, as reported in the literature [20, 21] and the associations found in the present study, support this mechanism. The magnitude of the OR of 0.98 can be illustrated by an example: for a patient with an arginine:ADMA ratio of 80, the odds of dying are 1.50 (1/(0.9820)) times higher than for a patient with an arginine:ADMA ratio of 100.
The present results are in accordance with an experimental study done by our group in which a low arginine:ADMA ratio reduced cardiac output and diminished flow in the microcirculation of major organs in animals [10]. In addition, exogenous arginine increased coronary blood flow and restored perfusion in the ischaemic areas in the endotoxin-treated heart [22], and increased cardiac output in sepsis [23, 24]. On the other hand, safety of arginine administration remains controversial because two studies have shown increased mortality rates with supplementation of this amino acid in sepsis [25, 26]. However, both studies have used immunonutrition with a low-arginine formula, which makes it difficult to attribute this effect to a single nutrient. Furthermore, the strength of the design of one of these studies [26] can be doubted as different routes of feeding were used and the statistical significance was quite thin since one more deceased subject would have made the difference non-significant.
The influence of ADMA has been supported by studies in which administration of NOS inhibitors reduced cardiac output in healthy volunteers [27] and in septic shock patients [28], and reduced coronary flow [22] and induced local ischaemia [22] in endotoxaemic rats.

Furthermore, a study with cardiogenic shock patients has suggested that ADMA can regulate flow to the lung as the NOS inhibitor was associated with pulmonary capillary wedge pressure and with both systolic and diastolic pulmonary artery pressures [29]. Nevertheless, NOS inhibitors have been proposed as a treatment for the overproduction of NO in sepsis [28]. The hypothesis underlying the use of NOS inhibitors is that the increased production of NO by inducible NO synthase in sepsis contributes to hypotension and multipleorgan dysfunction [30]. However, results of these experiments are conflicting [23, 28, 31]. Moreover, a non-selective NOS inhibitor increased mortality rate of septic shock patients, which was associated with decreased cardiac output and heart failure [31]. Overcorrection of vascular tone by the non-selective NOS inhibitor might have hampered organ perfusion, resulting in myocardial and vascular dysfunction.
The observed low arginine:ADMA ratio in patients with high severity of disease and organ failure, and low cardiac index may be the result of decreased production or increased degradation of arginine and/or increased production or decreased clearance of ADMA, or both of these. In our patients, ADMA levels (0.44 (SD 0.15)mmol/l) at ICU admission were not elevated compared with normal values (0.5mmol/l) [15], while arginine levels were decreased (32.2 (SD 16.6) compared with 80mmol/l in healthy subjects [16]). Similar results were found in a study by Mittermayer et al. [32] in healthy males after the injection of Escherichia coli endotoxin: arginine concentration decreased while ADMA levels were not affected, resulting in a decline in the arginine:ADMA ratio. These observations suggest that the effect of the arginine: ADMA ratio on clinical outcome can mainly be due to low arginine levels.
Arginine depletion in shock is probably the result of diminished production of arginine [33] or its precursor citrulline [1].

The limitations of the present study need to be addressed. Unfortunately, we could not measure NO or its oxidation products nitrite and nitrate. NO is highly reactive and has a shorthalf-life (<0.1 s in the human circulation) [34]. Furthermore, plasma levels of NO oxidation products are influenced by several factors such as endogenous NO synthesis, dietary intakeand excretion, and clinical and therapeutic interventions. Therefore, the production of NO, nitrite and nitrate may not be reliably assessed in shock patients. Second, cardiac output was not uniformly measured, because we used both the pulmonary artery catheter and NICO for cardiac output measurement. This choice is based on the absence of clinical evidence that the use of a pulmonary artery catheter reduces morbidity or mortality and that a continuous measurement, as provided by NICO, improves optimisation of the circulation at the bedside and reduces the risk of

infection in septic patients [35]. However, cardiac output is only one of the markers of circulation. We also found a significant relationship with other markers of circulation such as disseminated intravascular coagulation score, bicarbonate, lactate and metabolic acidosis. Finally, because of the small sample size and the observational design of the present study, it is not possible to draw conclusions about the cause-effect relationship of the present results.

In conclusion, the main finding of the present observational study is that an imbalance of arginine and ADMA at ICU admission is associated with circulatory failure, organ failure and mortality in patients with septic or cardiogenic shock. In the present study, the imbalance was caused by arginine depletion, while ADMA concentrations were normal. However, the ratio was related to the markers of circulation and outcome, while arginine and ADMA levels alone were not. These results support the present hypothesis that an imbalance of arginine and ADMA, being the substrate and the inhibitor of NO synthase, respectively, might contribute toendothelial and cardiac dysfunction with subsequent poor organ perfusion and organ failure, thereby increasing the risk of mortality. Future studies should focus on the role of arginine supplementation or ADMA removal in a specific subgroup of early shock with persisting circulatory failure.

Acknowledgments:

M.V. was supported by the Egbers Foundation. M.V. and M.A.R.V. contributed equally to this work and share first authorship. They both substantially performed the statistical analysis and drafted the manuscript. H.M.O.S. conceived of the design and execution of the study, contributed to the interpretation of the data and the writing of the manuscript, and is responsible for all parts of the research. T.T. carried out the samples analyses. P.J.K. helped performing and interpreting the statistical analysis. M.C.R., A.P.J.H., W.W., B.A.J.M.M. and P.A.M.L. critically analyzed and interpreted the data and helped writing the manuscript. All authors read and approved the final manuscript. The authors have no conflicts of interest to declare.

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9. Taurine concentrations decrease in critically ill patients with shock given enteral nutrition

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Journal of Parenteral and Enteral Nutrition.2016 Feb; 40(2): 264-72

Abstract

Background Nutritional studies in the intensive care unit (ICU) have shown that adequate enteral nutritional support has clinical benefits. However, the course of amino acid concentrations in plasma was never investigated in patients admitted with shock receiving enteral nutrition. We hypothesized that plasma concentrations when deficit, increase during enteral nutrition and that persistent deficiency is associated with poor outcome.

Methods In 33 septic or cardiogenic shock patients receiving enteral nutrition, plasma amino acid concentrations were measured during 5 days. Changes in amino acid concentrations, correlations with clinical outcome variables and regression analyses were studied.

Results On ICU admission, several plasma concentrations were deficient. Plasma concentrations of almost all amino acids increased. In contrast, taurine decreased by >50%, from 47.6 on admission to 20.0 μmol/L at day 1, and remained low at day 5. Taurine (admission) correlated with time on mechanical ventilation (R= -0.42 p= 0.015). Taurine decrease within 24 hours correlated with APACHE II predicted mortality (0.43; p= 0.017) and sequential organ failure score (R 0.36 p= 0.05). Regression analyses confirmed correlations.

Conclusions Several amino acids were deficient in plasma on ICU admission, but increased during enteral nutrition. Taurine concentrations declined and were associated with longer periods of mechanical ventilation and ICU support. Fast taurine decline correlated with severity of organ failure. These findings support the role of taurine during ischemia, reperfusion and inflammation. Taurine may be an essential candidate to enrich nutritional support for critically ill patients, although more research is required.

Introduction

The intensive care population is known for its high prevalence of disease-related malnutrition, associated with prolonged hospital stay, increased infectious morbidity and mortality [1]. Inflammation further increases the metabolic demand due to protein catabolism. Evidence has shown that nutritional support can improve wound healing, decrease the catabolic response to injury, enhance immune system function, improve gastrointestinal structure and function, and improve clinical outcome [2].

Amino acid metabolism in critically ill patients has been topic of extensive research over the past decades [3-5]. However, little is known about plasma concentrations in critically ill patients on enteral nutrition. Nowadays, enteral nutrition is the preferred way of feeding especially to maintain intestinal barrier function in the critically ill patient. It is recommended for Intensive Care Unit (ICU) patients who are not expected to take full oral diet within three days [6].

The primary aim of this study was to evaluate the course of plasma amino-acids concentrations, whether there are persistent plasma depletions and as a secondary aim, to study the relation between persistent low plasma amino acid concentration and clinical outcome in critically ill patients given enteral nutrition. On account of the results, we have specifically focussed on taurine. Since enteral nutrition is given continuously and fasting should be avoided in ICU patients, we studied plasma amino acids during continuous feeding, which seems the most representive situation.

Subjects and methods

Patients and setting

This prospective cohort pilot study was conducted in a 20-bed closed format general ICU of a Teaching Hospital in the city of Amsterdam, The Netherlands. The study was designed as a pilot for possible future amino acid intervention studies, aiming to include 30 consecutive adult patients, conform earlier studies [5, 7], with septic or cardiogenic shock within 24 hours after ICU admission, requiring mechanical ventilation. Primary endpoints were plasma amino-acids concentrations on admission and their course during enteral nutrition. Secondary endpoints were the relation between persistent low plasma amino acid concentration and clinical outcome (severity of organ failure and intensity and duration of intensive care support).

Inclusion criteria were: predicted ICU treatment and intention to treat for at least five days. Exclusion criteria were: active massive bleeding, pregnancy, HIV with less than 50 CD4 cells, hematologic malignancy, metastatic malignancy, Child C liver cirrhosis, hepatic coma, and therapeutic hypothermia after cardiac arrest. Subjects were recruited between August 2004 and June 2006.

For inclusion of the study, shock was defined as persistent hypotension despite adequate fluid resuscitation and the need of dopamine in a dose of more than 6 µg/kg/min and any dose of additional noradrenalin in the presence of perfusion abnormalities, manifest by oliguria, reduced peripheral perfusion and organ dysfunction. Hypotension was defined as a systolic pressure <90 mmHg; oliguria as a urinary output <20 ml/hour despite fluid infusion; reduced peripheral perfusion as delta T (difference between core and peripheral temperature) <4∞C, skin color not pink, poor capillary refill; and organ dysfunction was defined according to the Sequential Organ Failure Assessment (SOFA) score [8]. Septic shock (SS) was defined as acute circulatory shock occurring secondary to severe infection [9], cardiogenic shock (CS) as circulatory shock as occurring secondary to heart failure as evidenced by low cardiac output or ejection fraction accompanying cardiac disease (supported by e.g. ECG, echocardiography). To optimize circulation we used fluids in a mixture of crystalloids and colloids (Gelofusine®, B Braun), and dopamine as a first line inotropic and vasopressor agent. Noradrenalin was added if higher doses of dopamine were needed and/or if the patient developed tachycardia. Enoximone was added when cardiac index remained <2.5 L/kg/h in a standard dose of 8 mg/h. Nitroglycerin was used in patients with CS, cardiac ischemia and persistent poor peripheral perfusion [10]. Fluids were infused in amounts considered to be necessary to restore circulating volume, and to optimize cardiac output and peripheral circulation. Endpoints of circulation treatment skin color and temperature, capillary refill, blood pressure (target >90mmHg), central venous pressure, cardiac index (target >2.5 L/kg/min, pulse pressure variation (target < 10%) if appropriate and mixed venous oxygen saturation (target > 70 % if available).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the medical ethical committee of the Onze Lieve Vrouwe Gasthuis. Written informed consent was obtained from all patients or his legal representative. Patients were followed until they deceased or were discharged from the hospital.

Physiological and laboratory parameters

At ICU admission, blood samples were taken after patients were stabilized and inclusion criteria were met (T0), after 24 hours (T1) and after 5 days (T2) if and when still in the ICU. Samples were immediately placed on ice and centrifuged at 3000 r.p.m. for 10 min at 4°C. Plasma was pipetted and immediately put in liquid nitrogen and stored at -80°C before analysis. The concentrations of glutamine, citrulline, taurine, arginine, threonine, glutamate, asparagine, serine, glycine, valine, cysteine, methionine, isoleucine, leucine, proline, lysine, tyrosine, alanine, asparagine acid, phenylalanine and histidine were measured by high-performance liquid chromatography. Concentrations were compared to normal ranges [11].

Severity of illness was scored using the Acute Physiology and Chronic Health Evaluation (APACHE) II system over the first 24-hours of ICU admission [12], and APACHE II predicted mortality (APIIPM) was used as reference for predictive value for mortality [13]. The SOFA score as defined by the Dutch National Intensive Care Evaluation (www.stichting-nice.nl) was measured daily[14]. Length of mechanical ventilation (LOMV) was monitored in hours and length of ICU stay at the ICU (LOS-ICU) was monitored in days. These standard clinical data were routinely stored in the patient data management system (IMDsoft®, Tel Aviv, Israel).

Nutrition

All patients received enteral nutrition, which was initiated in the first hours of ICU admission at 20 ml/h and dose was increased by 20 ml if gastric retention remained < 150 ml/6h. If gastric retention was >150 ml/6h twice, intravenous erythromycin was started at a dose of 200 mg twice daily. If gastric retention remained after the start of erythromycin, a nasoduodenal tube was inserted. The protein target was 1.2 g protein/kg actual body weight and energy amounts were adjusted daily to the calculated energy expenditure (EE), using the mean of the continuously measured VCO_2/24h. EE (kcal/24 hours) was calculated with the Weir equation (EE= $3.941*VO_2+1.106*VCO_2$). VO_2 was extrapolated from mean VCO_2, supposing a respiratory quotient (RQ) of 0.85 ($VO_2 = (1/RQ)* VCO_2$). Accordingly, EE (kcal/ 24 hours) approximates $8*VCO_2$ (ml/min) [15]. Patients additionally received ascorbic acid (1g) and vitamin B complex (containing thiamine 50 mg, nicotinamide 100 mg, riboflavin 10 mg, pyridoxine 10 mg

and dexpanthenol 5 mg) intravenously once daily. Additionally, patients received a mixture of the following trace elements daily: chromium (0,2 µmol), copper (20 µmol), manganese (5 µmol), zinc (100 µmol), fluor (50 µmol), iodine (1 µmol), molybdate (0.2 µmol), seleniumoxide (0,4 µmol). All septic patients additionally received selenium 0,5 mg/day for five days.

According to protein and energy targets, patients received either Isosource Protein® or Novasource GI Control® (both Nestle Nutrition HealthCare, Oosterhout, the Netherlands), or Nutrison Concentrated® (Danone/Numico Zoetermeer, the Netherlands) (if fluid intake had to be restricted). According to the local guidelines at the time of the study, Impact® 1.0 (Nestle Nutrition HealthCare, Oosterhout, the Netherlands) was used if sepsis was suspected (Table 1). All enteral formula contained whole protein in the form of caseinate; Nutrison Concentrated also contained whey protein. Impact® 1.0 contained extra arginine. None of the formulas contained taurine.

Statistics

Data are expressed as mean ± SD 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 changes in plasma amino acid concentrations. Likewise, we used the Pearson and the Spearman rank correlation coefficient to determine whether clinical and biochemical variables were significantly related. In addition, significant correlations were tested in a simple regression model.

Chi square test was performed to test determinants for independence. Missing data were handled pairwise.

A p-value of <0.05 (2-tailed) was considered as statistical significant. Statistical analysis was performed with SPSS 15.0 for Windows® (SPSS Inc., Chicago, IL, USA).

Table 1 - Enteral Nutrition

	Energy	Protein(g/L)	Protein per kcal %	Freqency
Isosource Protein®	1220	54	18%	7 (21.2)
Novasource GI Control®	1060	41	15%	
Nutrison Concentrated®	2000	75	15%	
Impact®	1000	55	22%	12 (36.4)
Isosource Protein® & Novasource GI Control®				2 (6.1)
Isoscource Protein® & Nutrison Concentrated®				2 (6.1)
Isosource Protein®, Impact®				4 (12.1)
Isosource®, Impact®, Novasource GI Control®				2 (6.1)
Impact® & Novasource GI Control®				3 (9.1)
Impact® & Nutrison Concentrated®				1 (3.0)

Results

Patients

Patient characteristics are presented in Table 2. Of the 33 shock patients, 19 had SS and 14 had CS. Baseline characteristics were similar for SS and CS patients, apart from ALT which was significantly higher in CS (68 U/L, IQR: 29;239) than in SS (26 U/L, IQR: 14;56); p=0.046. All patients received enteral nutrition (Table 1). Target amounts of enteral nutrition were reached at a mean of 49 (SD 21.5) hours after ICU admission.

Length of mechanical ventilation (LOMV) and ICU stay were longer in the SS than in the CS patients, LOMV: SS 141.8 (IQR 68.3;189.7) vs. CS: 64.1 (IQR: 31.4;97.3). p=0.009; ICU stay: SS 6.5 (IQR 4.2;9.4) vs. CS: 3.7 (IQR 2.5;6.5), p=0.017. Hospital mortality was 4/15 (26.6%) in the SS group and 1/13 (7.7% in the CS patients, p=0.271 (Pearson χ^2). 13 Patients had an ICU stay of less than 5 days and data of day 1 (2 patients) and day 5 (13 patients) were therefore not available. Reasons were ICU death (5 patients) or discharge (8 patients)

Amino acids

Of all amino acids, concentrations of asparagine, citrulline, ornithine, serine and threonine were deficient on ICU admission (below normal range). Almost all plasma amino acid increased after ICU admission, none increased to supranormal concentrations (Figure 1).

In contrast, median taurine concentration decreased (Figure 2) by more than 50%, from 49.0 µmol/L (day 0) to 21.4 µmol/L (day 5) (p<0,000), with a significant decrease already in the first 24 hours (27.5 µmol/L at day 1) (p=0,001). Within 24 hours patients were taurine deficient. The decrease in taurine concentration was not related to fluid balance and none of the other amino acids decreased significantly. Histidine concentrations were below normal level at day 5, however the decline was not significant.

When comparing taurine concentrations with other amino acids measured simultaneously, concentrations of taurine correlated with glutamate (T0: R 0.44; p=0.010 / T1 R 0,60; p= 0.000), aspartate (T0: R 0.44; p=0,019 / T1: R 0.42; p= 0,019), cysteine (T0: R 0.45 p=0.008 / T1: R 0.32 p= 0.079 (trend)) and citrulline (T0: R 0.35 p=0.045 / T1: R 0.55 p= 0.001). The correlations of all amino acids with taurine are presented in a supplementary table.

None of the deficient amino acid concentrations were related to clinical variables. Furthermore, none of the other (not deficient) amino acid concentrations at admission correlated positively to length of stay, length of ventilation or to organ severity scores. Instead, some were significantly inversely related, as we expected (serine, glycine, alanine, ornithine, histidine, proline, glutamate). Of these amino acids, glutamate was one of the amino acids that significantly increased during ICU stay.

Taurine plasma concentration and clinical variables

Taurine plasma concentration declined significantly in both types of shock. In SS patients, taurine declined from 49.0 µmol/L (IQR14.4;59.3) on admission to 15.1 µmol/L (IQR 9.3;31.8) at T1 and 19.4 µmol/L (IQR: 13.3;28.5) at T5; (p=0.019). In CS patients, taurine decline from 49.0 µmol/L (IQR 31.1-91.5) on admission to 33.1 µmol/L (IQR 22.0-60.0) at T1 and 21.4 µmol/L (IQR: 19.4-37.4) at D5, (p = 0.028). Taurine decline from T0 until T5 was not different between SS and CS patients (SS: 16.4 µmol/L (IQR 1.6;30.4) vs. CS: 13.4 µmol/L (IQR 8.6;43.5), p= 0.78).

Taurine plasma concentrations on admission correlated inversely to LOMV (R= -0.42 p= 0.015) and LOS-ICU (R -0.33 p= 0.064). Even so, at T1 and T5 an inverse correlation was observed between the concentration of taurine and LOMV (T1: R -0.43 p=0.017 / T5: R -55 p=0.011) and LOS-ICU (T1 R -0.32 p=0.082 / T5: R -0.66 p 0.002). Regression analysis was not significant.

Plasma taurine concentrations at admission also correlated with glucose concentration (R=0.46, p=0.007), and correlated inversely with lactate (R=-0.41, p=0.02). The decline in plasma taurine (%) concentrations within 24 hours after admission correlated with APACHEII score (R 0.35; p= 0.053: trend), APIIPM (0.43; p= 0.017) and SOFA score on ICU admission (R 0.36 p= 0.05) . Regression analysis evidenced these correlations (Table 3). Taurine concentrations on admission were not significantly different between survivors and non-survivors (57.5 µmol/L±8.0 vs. 46.5 µmol/L).

Table 2 - Baseline characteristics

	n (%)	Mean / median (SD / IQR)
Demographics		
Gender: male/female (%)	16/17 (48.5/51.5)	
Age (y)		64 (12.7)
Height (cm)		171 (9.3)
Weight (kg)		72 (62;85)
BMI (kg/m2)		24.5 (22.2;28.2)
Clinical Assessment		
ICU admission		
type: medical/surgical (%)	20/13 (61/39)	
Shock: septic/ cardiogenic (%)	19/14 (58/42)	
APACHE II-score		27.2 (7.8)
APACHE II predicted mortality		0.58 (0.26)
SOFA-score admission		10 (8;12)
SOFA day 1		8 (7;11)
SOFA day 5		7 (4.5;11)
Site of infection (%)		
Lung	7 (21)	
Abdomen	7 (21)	
Urine	1 (3)	
Other	4 (12)	
Origin of cardiogenic shock		
After cardiac surgery	7 (21)	
Medical	7 (21)	
(presented at ER/ CCU/ward)		
Laboratory measurements		
Creatine Kinase (U/L)		240 (104;1058)
ALT (U/L)		38 (20;91)
Bicarbonate (mmol/L)		18.3 (6.1)
Bilirubin (µmol/L)		10 (8;17)
CRP (mg/L)		138 (54;258)
Glucose (mmol/L)		7.7 (6.0;11.6)
Creatinine (µmol/L)		129 (95;184)
Lactate (mmol/L)		3.55 (2.1;7.4)
pH		7.25 (0.13)
Urea (mmol/L)		9.5 (7.3;17.0)
Cardiac Output (L/ min)		3.7(2.6;5.4)
ICU Mortality (%)	5 (15)	
-Septic shock patients	4 (21)	
-Cardiac shock patients	1 (7)	
Hospital mortality (%)	11 (33)	
-Septic shock patients	6 (31)	
-Cardiac shock patients	5 (35)	
Length of Stay / Mechanical ventilation		
Length of stay ICU (days)		4.8 (3.2;9.0)
Length of stay Hospital (days)		24 (11;35)
Length of mechanical ventilation (hours)		82 (51;165)

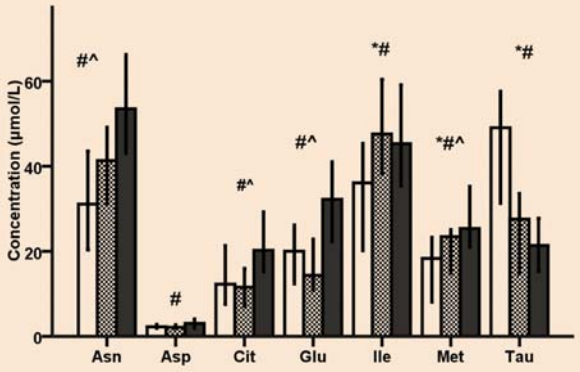
Baseline characteristics. ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ALT, alanine aminotransferase

Tabel 3 - Taurine decrease and organ severity score

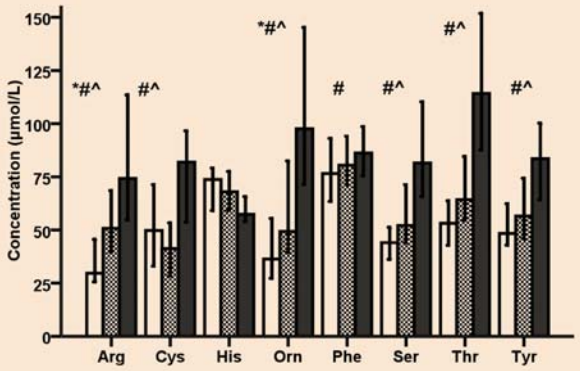
	Day 0-1				Day 0-5			
	b	95% CI	R^2	P	b	95% CI	R^2	P
Apache II	1.24	0.08-2.40	0,14	0.036	2.6	-0.11-5,33	0,18	0,059
Apache II PM	39.3	4.5-74.0	0.16	0.028	68.0	-25.3-161.3	0.12	0.143
SOFA admission	3.5	0.9-6.1	0.20	0.011	5.3	-1.4-12.0	0.12	0.112

Regression analysis indicates significant relations with early taurine decline (<24h: Day 0-1) and organ severity scores. Regression analysis did not reach significance for overall decline in taurine (admission to day 5: Day 0-5). APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment. Apache II PM : APACHE II Predicted Mortality. CI: Confidence Interval.

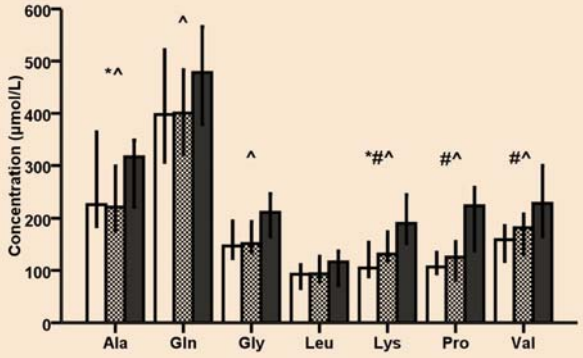
Figure 1



1A



1B

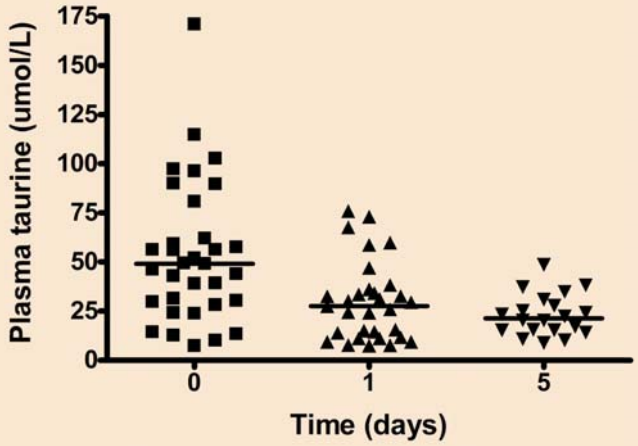


1C

Median plasma amino acids concentrations (µmol/l) on admision (day 0) and days after admission. * p≤0.05 D0 vs. D1; # p≤0.05 D0 vs. D5; ^ p≤0.05 D1 vs. D5.

Normal ranges (µmol/l) are: Alanine (177;583), Arginine (15;128), Asparagine (35;74), Aspartic acid (1;25), Citrulline (15;55), Cysteine (5;82), Glutamine (205;756), Glutamate (10;131), Glycine (151;490), Histidine, (72;124), Isoleucine (30;108), Leucine (72;201), Lysine (82;236), Methionine (10;42), Ornithine, (48;195), Phenylalanine, (35;85), Proline, (97;329), Serine, (58;181), Taurine (27;210), Threonine (60;225), Tyrosine (34;112), Valine (119;336)

Figure 2 - Taurine concentrations.



Median plasma concentrations decreased significantly (p=0.000) by more than 56.3% with a significant (p=0.001) decrease in the first 24 hours.

Discussion

The present observational cohort study in critically ill patients with shock, shows that several amino acids were deficient in plasma on admission. However almost all plasma amino acid concentrations increased from admission to day 5 while targeted enteral nutrition was provided. In contrast, taurine behaved differently: plasma taurine concentrations declined by more than 50% at day 1 and this decline persisted at day 5.
Low plasma taurine on admission correlated with high lactate, and a longer duration of mechanical ventilation and ICU stay. In addition, the decline in taurine concentration within the first 24 hours was more severe in patients with a higher severity of disease, higher degree of organ failure and higher predicted mortality on admission, and in those needing a longer duration of mechanical ventilation and ICU support. Furthermore, taurine concentrations were related to glucose concentrations. The decline in taurine concentrations was not related to fluid balance. Since ischemia, reperfusion and inflammation are the underlying pathophysiological mechanisms of organ failure in cardiogenic shock, these findings support a role of taurine in ischemia, reperfusion and inflammation, whether causative, consequence or both.

Low plasma taurine concentrations have been reported in critically ill patients with sepsis [4] and after trauma [16]. However, the present study is the first to present a series of plasma amino acid concentrations in critically ill shock patients receiving enteral nutrition and showing that plasma taurine did not behave differently in septic or cardiogenic shock.

Role of taurine in critical illness

Taurine is a non-essential sulfonic amino acid that is not incorporated into protein. It can be synthesized from methionine and cysteine in the liver [17]. Taurine is derived from dietary intake, de novo synthesis, and kidney reabsorption [18]. Intracellular taurine concentrations are maintained 50- to 100-fold higher than extracellular concentration[19]. However the relation between intra- and extracellular taurine concentrations is complex [20]. Taurine seems to play a role in a variety of processes which are crucial during critical illness.
First, taurine is used by the cells for cell-volume regulation to adapt to an osmotic imbalance. Taurine uptake into the cell is stimulated by hypertonicity and vice versa. Cellular taurine uptake is a saturable and active process, whereas its release is a volume-sensitive leak [19]. A drop in plasma taurine concentration as seen in our patients could have been caused by cellular uptake and subsequent extracellar deficiency might imbalance osmoregulation and cellular hydration state. This may especially be harmful during shock states with volume imbalance. Second, taurine has anti-oxidant and anti-inflammatory effects and may therefore mitigate sepsis and ischemia/reperfusion induced membrane damage [21, 22]. Among other mechanisms, taurine neutralizes hypochlorous acid, which is generated by activated neutrophils for microbial killing, but overwhelming production causes cellular damage in systemic inflammation. Taurine binding generates the biologically active but less toxic taurine-chloramine and thereby limits an overwhelming inflammatory response [22] or even protects the cell form self-destruction by inducing the expression of cytoprotective anti-oxidant enzymes [23].
Third, taurine is crucial for mitochondrial function, since taurine provides substrate for one of the posttranslational modifications of transfer-RNA, encoding for the synthesis of complex 1 of the respiratory chain. Taurine deficiency leads to impaired respiratory chain function, resulting in reduced ATP generation, enhanced oxidative stress, mitochondrial permeability transition and apoptosis [24].
The association low taurine with high lactate may reflect the mitochondrial dysfunction due to taurine deficiency as explained above [24].

We also found that hyperglycaemia at admission was associated with higher taurine concentrations. This may be due to osmoregulation. An other explanation could be that hyperglycemia decreases intracellular taurine content, taurine transport activity, and taurine transporter mRNA, possibly through protein kinase C-mediated transcriptional and posttranslational pathways [25].

The low plasma taurine concentration as seen in the present population with shock could be an intracellular shift and or a plasma depletion due to rapid consumption, and the subsequent depletion may reduce cytoprotection against inflammation and oxidative stress and contribute to cellular injury.

Metabolic relations

Taurine can be synthesized by the methionine cysteine pathway in the liver [26], in the presence of vitamin B6. Involved enzymes cysteine sulphinic acid decarboxylase and cysteine dioxygenase are also found extrahepatically: in the kidney, astrocytes and testes [17, 27, 28]. Although vitamin concentrations were not measured in our patients, all patients routinely received additional intravenous vitamins (see methods section). We found that concentrations of taurine correlated with cysteine, which can be well explained by above mentioned pathway. In agreement with Chiarla et al., we observed associations with glutamate and aspartate [4]. Glutamate and aspartate are metabolically related with cysteine sulfinic acid, an intermediate for taurine synthesis from cysteine, explaining these correlations. Taurine, cysteine and glutamate are all used for glutathione synthesis, a crucial antioxidant during critical illness.

To our knowledge, the strong relation with citrulline is unknown. An explanation could be that glutamate can be synthesized from glutamine by glutaminedeaminase in the intestines. A small part of glutamate is subsequently converted to citrulline [29].
An alternative explanation could be the role of citrulline as a marker for intestinal malaborption [30]. Since bile malabsorption could also lead to diminished taurine levels,

theoretically this oculd be the link between the two amino acids (bearing in mind that citrulline is used as a malabsorption marker after glutamine is given, not on its own).

Although we did not observe a relation between glutamine and taurine, Boelens et al. observed a relation between glutamine and taurine in glutamine supplied trauma patients and rats [16, 31]. However, they did not observe similar effects in their control fed group.

Comparison of taurine concentrations in the literature

Our results are in line with previous studies. Chiarla et al. found low taurine plasma concentrations in septic patients [4].
We showed that also critically ill cardiogenic shock patients develop a comparable drop in plasma taurine concentrations. It was already known that taurine plasma concentrations are lower in trauma patients compared to healthy controls [32]. The relation between plasma taurine depletion, severity of disease, high lactate and length of mechanical ventilation corresponds to previous observations in which low taurine in sepsis correlated with pulmonary artery pressure, pulmonary vascular resistance, worsening of pulmonary dysfunction and high lactate [33].

Our results show that, despite optimal enteral nutrition, taurine concentrations still drop from sub-normal at admission (50 µmol/L) to depletion on day 2 and 5 (27 and 21 µmol/L). Interestingly, Paauw et al. indicated normal concentrations at 126 µmol/L and trauma concentrations at 50 µmol/L, concentrations that are almost twice compared to the concentration in our shock patients at day 2 and 5 [32] , and to those in the trauma population of Boelens et al. [16] and a mixed critically ill population by Ahlman [34]. Thus, plasma taurine concentrations seem to be specifically low in shock.

Plasma taurine depletion was also found in neonates and adults receiving home parenteral nutrition (36-38).

Taurine and nutritional formulas

Taurine is naturally abundant in fish, poultry and meat. Since 1980 taurine is added to most neonate TPN formulas. In neonates the taurine biosynthetic pathway is immature, leading to deficiency if taurine is not provided [32]. In adult enteral formulas, taurine is not routinely added, although sometimes in specific kidney formulas and formulas that contain eicosapentaenoic acid and gamma-linolenic acid. About a quarter of our patients received such a formula (Impact®), apparently not enough for restoration of taurine plasma concentration. Parenteral nutrition is sometimes taurine enriched. Taurine added to total parenteral nutrition (TPN) increased its serum concentrations, although concentrations remained lower compared to healthy controls [35]. In neonates and in adults receiving home TPN, intravenous supplementation of taurine increeased plasma concentration [36 37]. We could consider adding taurine to hospital enteral nutrition as well [38], especially since taurine is non-toxic even in high pharmacological doses[38].
Critically ill patient might benefit most.

The study has several limitations. First, the sample size of this pilot study was relatively small. Second, we did not include all consecutive cardiogenic and septic shock patients during the study period, but unfortunately did not document reasons for non-participation. Main reasons were work overload and failure to get informed consent within 24-h of ICU admission. We do not know whether these shortcoming have caused an inclusion bias. Third, we used different nutritional formulas to attain our nutritional goals. This is however a practical clinical strategy to attain energy and protein targets in individual patients [39]. Unfortunately we did not have exact data on day to day amino acid intake. Fourth, we do not know whether low plasma taurine concentrations reflect underlying nutritional state, reduced de novo synthesis, an intracellular shift and or increased needs as osmoregulator or antioxidant. Dilution as a result of fluid loading is likely not the explanation, because the decline in taurine was not related to fluid balance and the other amino acids increased during the days.
Altered metabolic pathways, such as impaired or inadequate taurine synthesis by the methionine-cysteine pathway, could play a role. The continued fall in taurine could indicate rapid consumption and or an increased transport into the cell. Finally, the extracellular concentrations measured do not necessarily reflect intracellular deficits. Measuring gradients and intracellular concentrations would be interesting but requires a different scientific setup, which is not feasible for a large sample size.

Conclusions

This hypothesis generating pilot study in critically ill patients with shock receiving targeted enteral nutrition shows that plasma all amino acid concentrations increased during the first 5 days. However, plasma taurine concentrations declined. Low taurine on admission was associated with high lactate and a longer duration of mechanical ventilation and ICU support. A fast decline of taurine correlated with severity of organ failure.

Acknowledgements and contributions:

M.A.R.V. and M.F.M.S. contributed equally to this work and share first authorship. They both substantially performed the statistical analysis and drafted the manuscript. H.M.O.S. conceived of the design and execution of the study, contributed to the interpretation of the data and the writing of the manuscript, and is responsible for all parts of the research and therefore the guarantor of the study. M.V. and S.M.P.L. helped performing the statistical analysis and drafted the manuscript, A.P.J.H. and P.A.M.L. helped interpreting the data and writing the manuscript. All authors read and approved the final manuscript. This project received a financial grant for the amino acid determinations from Fresenius Medical Nederland BV. All authors approved the final version of the article, including the authorship list

Statement of interest:

P.A.M.v.L.WW has served as a speaker, a consultant and an advisory board member for Fresenius Kabi.

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10. Published letters

10.1 Glutamine: precursor or nitrogen donor for citrulline synthesis?

Introduction

The relation between glutamine and arginine is known since the publication of Windmueller and Spaeth in which they found evidence that the small intestine is the major source of circulating citrulline for endogenous synthesis of arginine in the rat [1]. This relation is not only of importance for better metabolic understanding, but just as well for nutritional interventions.

How exactly does glutamine exert its beneficial effects? Hypotheses are described extensively in the general introduction of this thesis and among these one theory is that glutamine potentiates its substrates citrulline and arginine. Bearing in mind the amino acid deficiencies in the ICU and the association between these deficiencies and mortality [2, 3] it seemed plausible to supply both. However, before debate on glutamine started, debate on arginine arose. Bertolini et al. stopped their trial with arginine (and omega-3fatty acids, vitamin E, beta carotene, zinc and selenium) in sepsis after interim analysis showed higher mortality rates in the intervention group [4].

Since it was believed that there were no downsides to glutamine supplementation, indirect arginine supplementation through glutamine and citrulline seemed the right way of doing.

However, scientifically editing nutritional formulas requires quantitative evidence based medicine.

Pitfalls as such are interspecies differences, the route of administration, duration of therapy and clinical aspects of the patient population of choice.

In the mean time, methodological aspects of quantitative amino acid research are ever evolving. While AV flux measurements are easier to perform, stable isotope research precisely differentiates exogenous from endogenous metabolism. LCMS (Liquid Chromatography Mass Spectrometry) is a technique that combines liquid chromatography with mass spectrometry. Currently, LCMS/MS (tandem MS) enables sequencing individual peptides. Tissue investigation using Nuclear Magnetic Resonance (NMR) technique facilitates identification of molecular structures.

But even while using similar tracer determination techniques, results are determined by the choice of study design. For example, enteral tracers undergo first pass effect; enteral nutrition enlarges tracee pool. Parenteral tracer administration to study enteral effects only addresses apical effects. A double tracer pooling study design allows measuring both the enteral and parenteral administrative effects but then different tracers are needed potentiating interference of metabolic products and rechanneling of labeled molecules.

Furthermore, the current concerns about tracer methodology involve a discussion about the definition of a precursor. De facto every molecule contributing to the formation of a new molecule is a precursor. Nevertheless the subtlety is to what extent the contribution of the origin metabolite adds to the new formation. Multiple stable isotope studies (with both C and N tracers) showed that the glutamine molecule is used for arginine synthesis through citrulline, pinpointing its precursor role.

In the comment published in American Journal of Physiology, we discuss the translation from mice to men [5].

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10.2 Glutamine: precursor or nitrogen donor for citrulline synthesis?

Gerdien C. Ligthart-Melis, Mechteld A. R. Vermeulen,
Paul A. M. van Leeuwen, Nicolaas E. P. Deutz

American Journal of Physiology. Endocrinology and Metabolism. 2010;299:E683.

To the editor

We would like to comment on the recent publication by Marini et al. [1]. The authors present an impressive, meticulous analysis of the metabolic pathways by which glutamine contributes to the synthesis of citrulline. Their study in mice shows that glutamine predominantly donates nitrogen and only a limited amount of carbon to the synthesis of citrulline. Therefore, the authors conclude that glutamine is not a true precursor of citrulline and that L-[2-15N]glutamine is an invalid tracer to study this precursor relationship.

Marini et al. [1] do not discuss the mechanism by which glutamine supplementation is able to enhance plasma levels of citrulline in humans [2, 7–10, 12] if not by providing (a substantial part of) its carbon skeleton. We think that a possible explanation for their contradictive findings could be the difference between species. To our knowledge, only two stable isotope studies on the metabolic relationship between glutamine and citrulline have been performed in mice [1, 2]. The L-[2-15N] glutamine and L-[13C-ureido-5,5,2H2] citrulline tracers used in mice were also used by us to quantify the turnover of glutamine into citrulline in humans [2-5]. Noteworthy is that the mice studies were performed using large tracer (mass) dosages, whereas we used very small tracer dosages in the human studies. The results of these studies were strikingly different. The studies in mice showed that glutamine contributed only between 15 and 36% to the turnover of citrulline[2], whereas glutamine contributed ~83% to the turnover of citrulline in humans [3]. Furthermore, in mice, despite the large amount of administered glutamine, the rate of appearance of citrulline was not enhanced when compared with a control group not receiving glutamine [6]. This is in contrast with the response in humans[7-12]. Together these observations provide an argument against the legitimacy of direct translation of results in mice to humans.

With respect to the second conclusion, the authors clearly showed that L-[2-15N] glutamine may be a less valid tracer to establish the contribution of the carbon skeleton of glutamine to the synthesis of citrulline when positional information on the label is not provided. Our studies were performed with liquid chromatography-mass spectrometry equipment and analysis techniques that could not differentiate between the possible positions of the isotope in citrulline. Marini et al. [6] did an excellent job by providing this information in their study in mice, although human metabolism may treat the L-[2-15N]glutamine differently.
We agree that for future in vivo stable isotope studies it may be necessary to provide positional information on the isotopes present in the infused labeled molecule and its products.

In summary, we would like to state that caution is warranted when results in mice are translated to humans and that knowledge of the position of the label in the conversion products of a stable isotope tracer will be pivotal for future in vivo stable isotope studies.

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10.3 Glutamine and antioxidants in critically ill patients

&

Consequences of REDOX and METAPLUS trials: The end of an era of glutamine and antioxidant supplementation for critically ill patients?

Introduction

Gutamine supply to intensive care patients has been topic of investigations since the early nineties. Different patient populations have been investigated and numerous end point have been taken into account. Glutamine, whether in mono-or dipeptide form has been administrated in several dosages, by different routes of administration and often in combination with other nutritional additives. Heyland et al. performed a multicenter clinical trial in which critically ill patients received either placebo (A), high dose glutamine enriched parenteral and enteral nutrition (B), antioxidants (C) and glutamine and antioxidant enriched nutrition (D). The primary endpoint was mortality.

An intervention dose of 30g enteral glutamine was combined with a parenteral dose of 0.35g/kg glutamine. In advance a safety study was performed[1] in which increasing dosages of glutamine were administered. In the highest dose glutamine groups (aimed at 0.35 g/kg/day iv and 30g enterally, with a total actually received daily glutamine of 41.1 ± 15.2 g) a urea increase post treatment was observed. In 3 (out of 7) patients receiving the highest dose, study nutrients had to be interrupted due to safety concerns about a high urea level. Yet the authors concluded that despite these concerns in patients that only received approximately 40 g of glutamine, glutamine supplementation of 0.35 g/kg plus 30 g via the combined route was safe in critically ill patients.

The results of their multicenter trial are published in New England Journal of Medicine[2]. We addressed the change of end points[3] and invalid grouping in the same journal (see beneath).

Importantly, the authors changed the primary endpoints in the clinical trial database. The fact that patients were quantitatively and qualitatively malnourished was addressed by Bistrian et al[4].

Another glutamine trial by Van Zanten et al.[5] evaluates whether high protein enteral nutrition enriched with immune modulating nutrients compared with an isocaloric enteral feed reduced infectious morbidity in mechanically ventilated ICU patients and affected long-term morbidity and mortality. We commented on this trial in JAMA in which we discuss statistical power, the higher adjusted predicted mortality in the intervention group and the imbalance in protein, fat and carbohydrate content amongst the used formulas[6]. The authors comment on the findings of the mentioned studies in JPEN and discuss a possible "End of an era of glutamine and antioxidant supplementation for critically ill patients" [7]. We argue their statements in this journal [8], as displayed in this chapter.

Nevertheless with the inclusion of Heylands results (not included in infectious complications analysis), a Cochrane meta-analysis states that [there is] "moderate evidence that glutamine supplementation could reduce

[ed.] days on mechanical ventilation, and low quality evidence that glutamine supplementation reduced length of hospital stay in critically ill or surgical participants. However, the differences in risk of mortality, length of ICU stay and quality of life were not significant between the intervention and control groups" [9]. There was moderate evidence that glutamine supplementation could reduce the infection rate, but Heylands results were not included in this analysis due to their grouped data presentation. These two trials could have made statement or maybe changed the current opinion on glutamine supplementation in the ICU patient. However due to statistical flaws and the use of invalidly high dosages, the split opinion is still present for glutamine believers and non-believers.

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10.4 Glutamine and antioxidants in critically ill patients

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New England Journal of Medicine. 2013;369:484

To the editor

Heyland et al. [1] report a trend toward higher 28-day mortality in critically ill patients who received glutamine. However, we have major concerns about many aspects of the study, including the inadequate nutritional prescription and the statistical adjustment in which the glutamine groups are combined, showing an imbalance in baseline variables. The number of patients with more than two failing organs at baseline was much higher in the two groups receiving glutamine than in the two groups not receiving glutamine (187 vs. 148), which obviously resulted in higher mortality. Neither 6-month mortality nor in-hospital mortality was predefined as a primary or secondary end point. The reported 6-month mortality is trivial and not properly documented, since many patients were lost to follow-up. In addition, no 6-month mortality significance is reported for the original groups. Sepsis-related Organ Failure Assessment (SOFA) scores were not provided for separate organs (including the liver), which would have allowed for comparisons. In all, we suggest that more severely ill patients were allocated to the glutamine groups as a result of randomization error and believe that patients were not adequately fed, which may explain the observed higher mortality in the groups receiving glutamine. Complementary data are needed to support the scientific value of this study.

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10.5 Consequences of REDOX and METAPLUS trials: The end of an era of glutamine and antioxidant supplementation for critically ill patients?

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Journal of Parenteral and Enteral Nutrition. 2016;40:12

Dear Sir,

We read with interest the statements of Arthur van Zanten et al. [1]. We cannot agree on their analysis for the following reasons. In the manuscript of van Zanten et al. [2] it is clearly stated at page 518 that there were no statistically significant differences between the IHMP and HP groups in any clinical outcome parameter. It can also be seen in Table 4 page 520 that 6 month survival had a p-value of 0.21. Only within a secondary analysis in the medical subgroup there was an increase in mortality. Secondary analysis has no statistical power and therefore is of no value but only hypothesis-generating. Moreover, the study was underpowered [3] to show a difference in mortality. Van Zanten et al. compare their 6 month mortality graph with the graph of Heyland et al [4]. It is clearly written in the protocol in 2006[5] that 6 month mortality was neither a primary nor secondary endpoint. However, Heyland et al. changed the endpoints during the course of the study [6]. Thus, the 6 month mortality as published by Heyland is a secondary analysis and also has no statistical power. Moreover a serious number of patients are still lost for follow up in the REDOX study. The difference in mortality, as seen in Figure 11 is solely explained by adding two groups who have a randomization error within the glutamine groups [7]. More sick patients were present in the glutamine group. This was also substantiated by the fact that there were no differences in the original four study groups [8]. Physiologically, glutamine dipeptide (alanyl- or glycyl-glutamine) given intravenously or enterally are very rapidly (halflife <3 min) hydrolyzed releasing the constituent amino acids; dipeptides, thus, cannot exhibit "own" harmful effects [9].

In conclusions, the statements done by the authors are not true. They are not scientifically proven and can be very misleading to the readers.

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11. Summary of the main results and conclusions

Introduction

This thesis approaches glutamine and protein metabolism in critically ill patients from bench to bedside. Attempting to better understand the function and fate of glutamine in this group of patients started with studying glutamine on intestinal function in vivo, followed by quantitative alanyl-glutamine studies in non critically ill (surgical) patients introduced by a methodological study. Consecutively, glutamine and protein metabolism in stable intensive care patients was studied in a double blind manner. After that, the relation between amino acids and clinical parameters in enterally fed shock patients was investigated. While working on these trials and this thesis, related newly published studies were addressed and commented in the final chapter. Chapter 1 is an introduction on glutamine scoping on metabolism and clinical properties of glutamine, citrulline and arginine, followed by the rational for this thesis.

Summary of the main results

To underpin the potential beneficial effects of glutamine, first we had to gain insight in the effect of glutamine on intestinal cell function. Glutamine has been ascribed several properties that are supportive of intestinal cell function and that are relevant to cell survival [1] . On the other hand sepsis, bacteraemia and multiple organ failure could be related to intestinal hyperpermeability [2, 3], In vivo experiments, however, had not yet provided definitive evidence to support the claim that glutamine supplementation has a beneficial effect on gut permeability, in contrast to in vitro experiments [4, 5].
In Chapter 2[6] we found that both glutamine and glutamate reduced an induced form of hyperpermeability in human colon derived cell lines. Glutamine and glutamate seemed to reduce this hyperpermeability by acting on the tight junction (paracellular permeability) as opposed to endocytosis (transcellular permeability). The effect of glutamine could be nullified by blocking the extracellular converting enzyme, γ-GT, whereas the effect of glutamate was nullified by blocking the glutamate transporters EAAT1-5.
These results suggest that the transamination of glutamine to glutamate is essential for its beneficial effect on permeability. Additionally, transport of glutamate into the cell is essential for the beneficial effect of glutamate on permeability. These insights were already proven for the proximal tubulelike LLC-PK_1-F^+ cells[7], but not for intestinal cells. This provides evidence that the protective effect of glutamine on gut mucosa does not only result from cell proliferation and attenuation of apoptosis, as was often advocated [8].
To further explore these mechanisms, we suggest focussing on multiple concentrations of apically applied glutamine and glutamate in different cell lines including tubulelike cells, paralleling research on intracellular conversion.

In Chapter 3 [6] an introduction is made into stable isotope methodology by questioning organ flow measurements, required for flux calculations (net flux = ([Vein] – [Artery] * plasma flow). These flux calculations were used in chapter 4 [9] and 5 [10]. This chapter describes problems that arise while performing flux studies. On its own, measuring (tracer) concentrations in organ vessels can be challenging in all respects. Next to this, the validity and reliability of the calculated flux depends on the flow measured.
This is technically possible with DDUS (Duplex Doppler Ultrasound), yet difficulties are involved: the perioperative approach of flow measurement can prolong the duration of surgery and measurements naturally have to be performed within a sterile setting. Theoretically, MRI is superior to DDUS because of more pronounced accuracy and less interobserver variability [12].

MRI, unfortunately, is currently not possible during surgery. We compared setting of measurement (DDUS perioperatively with preoperatively) and technique (DDUS vs. MRI). We observed equality of mean for all measurements, however with widespread margins restricting individual comparison. Therefore using mean values of MRI or DDUS in either setting could be used for experimental setups.

Chapter 4 [9] describes the fate of glutamine that is embedded in alanyl-glutamine, the glutamine dipeptide that is stable in aqueous solutions. We studied postabsorptive surgical patients receiving isotopically labeled dipeptide alanyl-glutamine [9]. Similar to the glutamine tracer studies, glutamine enrichment was significantly lower when the alanyl-glutamine tracer was administered enterally, compared to intravenous administration. Also in line with previous results, enrichments of citrulline [M+1] and arginine [M+1] were significantly higher in the enteral group. Whole body rate of appearance of glutamine was lower in the IV group compared to the enteral group (201 vs. 281 µmol/kg/h) reflecting 29% splanchnic extraction. Whole body rates of appearance of citrulline and arginine were comparable for both administered routes. De novo synthesis of arginine was 85% in both groups (fraction of citrulline used for arginine synthesis). However in enterally administered groups twice as much glutamine was used for the synthesis of citrulline. Hence, double of the glutamine amounts were invested in the arginine synthesis.

On organ level, the intestines were observed to take up significant amounts of glutamine with subsequent significant amounts of citrulline release. A small non-significant release of arginine was observed. Renal or hepatic release of citrulline [M+1] or arginine was not significantly different in both groups, failing to explain the higher arterial enrichment of citrulline [M+1] and arginine [M+1].

In Chapter 5 [10] a similar approach was attempted to quantify arginine production from glutamine while an intervention dose of intravenous alanyl-glutamine was given to postabsorptive abdominal surgery patients. Significant increases of concentrations of glutamine, citrulline and arginine were observed. By using isotopically labeled glutamine, a calculated 91% of total citrulline turnover was derived from glutamine, while 49% of whole body citrulline turnover was used for de novo synthesis of arginine. Furthermore, the kidneys were again identified as the main production site for endogenous arginine with 75% of the whole body arginine production originating from citrulline. We noticed an excessive whole body arginine [M+1] production derived from glutamine [M+1] (>100% of the citrulline to arginine conversion rate), matching the theory of overestimation caused by nitrogen channeling or recycling. The results of this study suggest that compared with other studies, intravenous glutamine supplementation accounts for doubled renal arginine production from citrulline, also reflected in arginine concentrations. Unfortunately, due to study design (aorta surgery patients) glutamine metabolism of the portally drained viscera could not be quantified.

Chapter 6 [11] studied the effect of an enteral addition of 0.5 g/kg alanyl-glutamine to adequately fed stable intensive care patients. In isocaloric patients, there was no extra citrulline or arginine synthesis. The splanchnic uptake of glutamine and arginine was not increased. This suggests that for arginine synthesis enhancement there is no need for an additional dose of glutamine when this population is adequately fed. Contrarily, in control patients, more citrulline was derived from glutamine (47.8% versus 24.8% in the alanyl-glutamine group). We noticed lower citrulline to arginine turnover and glutamine to arginine turnover compared to our previous experiments, with a conversion rate of 0-6.5% and 0.7-1.3% respectively, differing with a factor of 5-10% compared to earlier experiments[9]. In the control group, the TTR% of the metabolic product of citrulline [M+5] metabolism – arginine [M+5] - was below detection level in most of the control patients. Therefore median conversion of citrulline to arginine was calculated zero while showing higher glutamine to arginine conversion rates. It is plausible that the arginine synthesis probably finds is origin in the gut by the enzymes argininosuccinate synthase and –lyase. Circumstantial induction seems evident since this has been subject of discussion earlier [9, 11].

In Chapter 7 in the same population protein synthesis was studied. In both critically ill stable non-septic patients with optimal nutrition and a target protein intake of 1.2-1.7 g/kg/day, protein breakdown and protein synthesis was not different. Protein balances were nearly zero and were more negative in the alanyl-glutamine group. Splanchnic extraction of tyrosine and phenylalanine was not different between groups. Thus enteral glutamine supplementation has no positive effect on protein synthesis in this population.

Chapter 8 [12] and 9 [13] investigated enterally fed critically ill shock patients. Patients received no extra glutamine. We observed several deficient plasma amino acids on ICU admission, but almost all increased during enteral nutrition. In contrast, taurine decreased by >50% during ICU stay. This was associated with longer periods of mechanical ventilation and ICU support. Fast taurine decline correlated with severity of organ failure. These findings support the role of taurine during ischemia, reperfusion and inflammation. We also observed that in shock patients, imbalance of arginine and ADMA is related to poor circulatory markers, disease severity, organ failure, and that it is predictive for hospital mortality. The imbalance of arginine and ADMA was mainly the result of decreased arginine levels as ADMA levels were within the normal range, possibly due to disequilibrium of NO substrate and inhibitor. We suggest that an imbalance of arginine and ADMA hampers endothelial and cardiac dysfunction with subsequent poor organ perfusion and organ failure thereby increasing the risk of mortality.

Chapter 10 [14-16] discusses recent debate on glutamine (tracer) metabolism and glutamine supply to critically ill patients. Marini et al [17]. concluded that glutamine was not a true precursor of citrulline and that L-[2-15N] glutamine was an invalid tracer to study this precursor relationship, we argued this addressing earlier studies that showed quantitative interspecies differences in glutamine to citrulline metabolism (mice vs. humans).

Heyland et al. [19] concluded that there was a trend for higher mortality for glutamine supplied patients in a large multicenter trial

in critically ill patients. This was stated after secondary analysis in the medical subgroup and has therefore no statistical power.
In the Heyland trial glutamine groups showed significantly more than two failing organs at baseline than the two groups not receiving glutamine (187 vs. 148), obviously resulting in higher mortality [19]. Furthermore, patients were not adequately fed, which may explain the observed higher mortality in the groups receiving glutamine. Thirdly, the fact that they studied shock patients (also with liver failure) prelimits the potential beneficial effect of nutritional intervention. We commented on this trial in the New England Journal of Medicine[15].

Van Zanten et al. [20] state in their trial comparing high protein enteral nutrition (enriched with immune modulating nutrients) with an isocaloric enteral feed that the enriched nutrition did not improve infectious complications or clinical end points and that the enriched formula could be harmful because of increased adjusted mortality at 6 months.

We commented on the Van Zanten trial in JAMA in which we discussed statistical power, the nutritional content of the formulas and the higher adjusted predicted mortality in the intervention group [21] . Furthermore, we published a comment on a joint article in JPEN [17, 22] in which we addressed the statistical underpower for mortality and we discussed the value of secondary analysis in the published results.

Future Perspectives

Glutamine metabolism in health and disease had now been quantified in specific subgroups. It was already mentioned that approximately 50% of glutamine is extracted and metabolized by the splanchnic organs after enteral administration of glutamine in humans [23]. We discovered in a non critically ill population a 100% extraction of enterally administered alanyl-glutamine by the intestines and liver [24] with concomitant higher plasma glutamine concentrations after parenteral administration. This finding illustrates the first pass splanchnic extraction of enterally provided glutamine. Splanchnic metabolism of enterally provided glutamine was further supported by the finding that the plasma concentration of glutamate was only enhanced with enteral administration of the dipeptide.

The fact that the glutamine to glutamate conversion is essential for reversing intestinal hyperpermeability (this thesis) illustrates the potential benefit of enteral glutamine rather than parenteral administration in specifically the critically ill population, since they often suffer from intestinal dysfunction. However when we consider enteral alanyl-glutamine administration in critically ill non septic patients (this thesis), we did not observe higher glutamine plasma concentrations in the dipeptide group. Even so, we did not observe higher turnover into citrulline and arginine in this group. Furthermore, in the alanyl-glutamine group protein synthesis was not promoted. All this does not imply that extra enteral glutamine in this group is useless. It could be that most of it is used for internal use of the gut, including enterocytes and gut associated lymphoid tissue. Should alanyl-glutamine be advised to critically ill patients? This thesis shows that in critically ill patients that neither have multiple organ failure nor sepsis, adequate enteral feeding including a small dose of glutamine (currently included in most enteral formulas) is probably optimal nutrition, considering metabolic parameters. It would be of interest to further zoom into parameters of gut function in ICU patients with or without enteral glutamine. For sure, this study does not tell whether mortality, infectious complications or hospital or ICU stay are affected by enteral glutamine administration. Neither did we investigate whether severely ill or septic patients metabolize enteral alanyl-glutamine differently. Since a lot of the positive outcome studies on glutamine were investigated on intravenously administered patients, it would be interesting to see whether the parenteral route of administration would stimulate citrulline or arginine synthesis, or would promote protein synthesis. In that case we would suggest starting with a low dose of glutamine (discussed below).

Chapter 10 discusses the outcome of performed ICU glutamine trials. Besides the

points of discussion outlined in the published letters, concerns have raised about glutamine administration in septic or multiple organ failure patients. Nevertheless and despite all pitfalls, a glutamine supplementation of 0.35 g/kg plus 30 g via the combined (enteral and parenteral) route is a very enthusiastic and never before investigated dose of glutamine to a severely ill group of patients. We opt for a careful approach since we observed a doubled arginine production by the kidneys while receiving "only" 0.5 g/kg alanyl-glutamine intravenously per day (corresponding with 0.35 g/kg/day glutamine) in surgical patients (this thesis). Our surgical patients results imply that glutamine could certainly serve for (citrulline and) arginine promotion which could be beneficial for a whole range of surgical patients [24-27] It would also be valuable to study the effect of an enteral dose of glutamine in surgical patients, as this would translate these results more easily towards the intensive care population.

The dispute is now whether arginine promotion is wished for in critically ill patients. On one had we know that during sepsis and trauma arginine concentrations are decreased [28]. On the other hand, observed adverse events of arginine administration might be due to increased nitric oxide production and subsequent hemodynamic instability [29]. Until now this remains uncertain. Since performed studies with just intravenous glutamine 0.35 g/kg/day have never shown adverse events we advocate performing an ICU clinical trial as performed in chapter 6, but then with intravenous alanyl-glutamine.

The understanding of the importance of clinical nutrition in the ICU is ever increasing. While back in the (not so very) old days patients were not nourished, lately nourished, or malnourished (in quantity or quality), the perspective nowadays is different. Still, targets are not reached, but at least they are approaching nutritional goals. This evolvement should influence the way we interpret older studies. While in those days studies focused on single nutrients (such as glutamine and arginine), the context of embedded optimal nutrition is different (although the definition of optimal is also evolving). The effect of nutrition on glutamine metabolism is described in this thesis. Different groups of interest could be identified for a similar approach of metabolic investigation.

Critically ill patients that are in shock show multiple amino acid deficiencies. They have a low arginine/ADMA ratio and perform worse when they are also persistently taurine deficient (this thesis). While receiving an optimal nutritional approach, amino acid deficiencies mostly disappear. These results are interesting but also start the discussion about 1) whether amino acid deficiencies are cause or effect of the clinical situation and outcome and 2) whether these deficiencies should be all be countered (individually). Septic patients probably behave differently and should be investigated carefully with respect to nutritional interventions. Nevertheless, we advocate performing stable isotope studies in this exact group, concerning general amino acid and protein metabolism, as well as glutamine and arginine metabolism. Paradigm shifts in intensive care medicine are as old as the profession. In the future, the use of nutrition, enriched nutrition and specific nutrients will probably be more nuanced and more tailored than we were used to in the pro-glutamine era.

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12. Nederlandse samenvatting

Samenvatting

In dit proefschrift wordt het metabolisme van glutamine, citrulline en arginine beschreven tegen de achtergrond van ernstige ziekte, waarbij enterale voeding en suppletie een centrale rol spelen. De relatie tussen glutamine, citrulline en arginine is reeds decennia bekend: Glutamine wordt in de darm omgezet in citrulline, waarna citrulline in de nieren wordt omgezet in arginine[1]. Dit is de zogenaamde intestinale-renale as. De omzetting van glutamine naar citrulline volgt via glutamaat als tussenstap. Deze relatie is belangrijk om te begrijpen wanneer het gaat om het toedienen van voeding bij klinische patiënten (Hoofdstuk 1). Het is de vraag op welke manier de darm belangrijk is bij deze omzetting. Bij ernstig zieke patiënten geniet enterale voeding de voorkeur. Echter, het is in deze groep patiënten niet bekend hoe functioneel de darm is. Het is belangrijk om te weten of de omzetting van glutamine naar citrulline (en arginine) anders is ten opzichte van patiënten die niet ernstig ziek zijn en of optimaal enteraal voeden ook zorgt voor optimale aminozuurspiegels en navenante kliniek.

Het effect van glutamine vs. glutamaat op darmpermeabiliteit

Allereerst hebben we in celonderzoek aangetoond dat zowel glutamine als glutamaat een toegenomen permeabiliteit van de darm kunnen reduceren. Dit wordt waarschijnlijk via tight junctions bewerkstelligd (paracellulaire permeabiliteit). Omdat γ-GT toevoeging buiten de cel (hetgeen voor transaminering van glutamine naar glutamaat zorgt) het effect van glutamine reduceerde, hebben we aangetoond dat de omzetting van glutamine naar glutamaat buiten de cel bepalend is voor het effect op de darmpermeabiliteit (Hoofdstuk 2).

Methodologische benadering van flowmetingen ten behoeve van metabole studies

Om klinisch flux metingen over de organen te kunnen verrichten was het nodig de beperkingen te onderkennen van de instrumenten die orgaanflow kunnen meten om op die manier de flux te kunnen berekenen. De gangbare methode Duplex Doppler echografie kent als nadeel een grote inter- en intra-observer variabililiteit. MRI zou een concurrerende methode kunnen zijn, echter hiermee zouden tijdens de operatie geen metingen verricht kunnen worden.

In Hoofdstuk 3 wordt aangetoond dat zowel de chirurgische ingreep als de methode (echo vs. MRI) geen invloed heeft op groepsresultaten van flowmetingen.

Stabiele isotopenonderzoek in chirurgische patiënten

Aangezien glutamine als therapeutische toediening in monomoleculaire vorm niet goed mogelijk is gezien de slechte oplosbaarheid wordt in de praktijk een dipeptide gebruikt: alanyl-glutamine. Het was echter lange tijd onduidelijk of de dipeptide zich metabool gezien met de monomoleculaire vorm liet vergelijken. In Hoofdstuk 4 tonen we aan dat dit voor een deel zo is: Enteraal aangeboden alanyl-glutamine geeft –net als gluatmine- een lagere plasma glutamine verrijking in het bloed ten opzichte van toediening via de bloedbaan. Daarnaast laten enteraal glutamine en alanyl-glutamine beide een hogere omzetting naar citrulline liet zien. Er was echter meer arginine-omzetting in de enterale dipeptide groep, anders dan in de eerdere studie.

Door deze resultaten kon de vertaalslag naar een interventiedosis gemaakt worden (Hoofdstuk 5). Door minimaal 15 uur preoperatief te starten met intraveneus alanyl-glutamine 0.5 g/kg/dag kon als het ware in steady state toediening perioperatief gemeten worden wat de rol van de nieren was in de omzetting van citrulline (en glutamine) naar arginine. Het bleek dat 91% van de citrulline afkomstig was uit glutamine en 49% van de citrulline werd gebruikt voor de arginine synthese. De nieren namen 75% van de synthese van arginine voor rekening. In vergelijking met Hoofdstuk 4 blijkt dat een interventiedosis de glutamineflux verdubbelt en dat hierdoor ook de absolute citrulline spiegels en de de novo argininesynthese verhoogd werden, hoewel in percentages conform eerdere studies. Deze studies tonen aan dat er inderdaad een relatie is tussen glutamine, citrulline en arginine, en dat het toedienen van een therapeutische dosering zich hetzelfde gedraagt als een tracerdosering en dus kan leiden tot citrulline en argininеproduktie.

Stabiele isotopenonderzoek in stabiele intensive care-patiënten

De voorgaande studies waren uitgevoerd in niet zieke electieve chirurgische patiënten. De volgende stap was om te kijken naar de situatie in ernstig zieke patiënten. Bij deze groep onderzochten we in Hoofdstuk 6 wat er met het glutaminemetabolisme gebeurt bij optimaal enteraal gevoede patiënten. Aan de helft van deze groep werd isocalorisch en isonitrogeen een enterale dosis van 0.5 g/kg gegeven. Ten opzichte van de controlegroep vond er in de alanyl-glutaminegroep geen extra omzetting naar citrulline en arginine plaats. In tegendeel: er werd in de controlegroep meer citrulline uit glutamine gesynthetiseerd. Ook was er geen verhoogde opname van glutamine en arginine door o.a. de darm in de alanyl-glutamine gesuppleerde groep. Er werd in beide groepen minder arginine uit citrulline gevormd ten opzichte van eerdere studies in niet ernstig zieke populaties.

In Hoofdstuk 7 wordt het effect van enteraal alanyl-glutamine in therapeutische dosering beschreven op eiwitmetabolisme. Patiënten hadden een eiwitinname van 1.2-1.7 g/kg/dag. We zagen dat eiwitafbraak en synthese in beide groepen hetzelfde waren. Eiwitbalans was nagenoeg nul, maar significant meer negatief in alanyl-glutamine gesuppleerde patiënten. Hieruit kan geconcludeerd worden dat enterale alanyl-glutaminesuppletie in optimaal gevoede niet-septische ernstig

zieke stabiele patiënten de eiwitsynthese niet verder stimuleert.

Observationele studies in optimaal gevoede IC-patiënten

Hoewel in het verleden veel studies verricht zijn met als doel aminozuren als reflectie van ziekte en voedingstoestand bij ernstige ziekte te begrijpen, werden deze studies niet verricht in gevoede toestand. Daarnaast is er het laatste decennium een inhaalslag gemaakt wanneer het gaat om kwantitatieve en kwalitatieve enterale voeding in deze groep patiënten. Bij ernstig zieke patiënten was het niet duidelijk in hoeverre plasma aminozuren veranderen tijdens de optimaal gevoede opname. Wij zagen in Hoofdstuk 9 dat er bij aanvang van de opname veel aminozuurdeficiënties zichtbaar waren in het plasma. Bijna alle aminozuurconcentraties tijdens 3-5 dagen voeden. Echter taurine daalde. Deze daling ging gepaard met een langere beademingsduur en IC-ligduur. Wanneer er meer orgaanfalen was correleerde dit met een snelle daling van taurinespiegels gedurende de eerste dagen.

Gezien de goede potentie van glutaminetoediening bij ernstig zieke patiënten en de hypothese dat dit voor een deel op arginine-omzetting zou kunnen berusten was het noodzakelijk arginine verder te bekijken bij ernstige ziekte. Immers: arginine is een stikstof oxide-donor (NO) en NO speelt een grote rol in orgaanoxygenatie bij de ernstig zieke patiënt. In Hoofdstuk 8 werden arginine en de competitieve NO-inhibitor asymmetrisch dimethylarginine (ADMA) gecorreleerd aan klinische paramaters in septische en cardiogene shock patiënten. Wanneer de balans tussen arginine en ADMA meer ten faveure van ADMA ligt, is dit geassocieerd met ernst van de ziekte en slechte circulatoire markers.

Een kritische noot ten aanzien van klinische glutaminestudies en glutamine traceronderzoek

Stabiele isotopenstudies (tracers) worden beschouwd als gouden standaard binnen metabool onderzoek. Hierbij is echter niet gezegd dat deze methode een snelle vertaalslag van dier naar mens verantwoord. Ook is het belangrijk goed na te blijven denken of metabole berekeningen een uitkomst zijn van een op voorhand verwachte omzetting of van een aannemelijke vertroebeling van de verzwaarde atomen (Hoofdstuk 10).

Terwijl glutamine lange tijd een onschendbare reputatie leek te hebben zijn er sinds 2013 ook negatieve studies gepubliceerd. Belangrijk is te beseffen in welke groepen het geven van bepaalde voedingssupplementen zinnig is en in welke groepen potentieel discutabel. Daarnaast dienen een goede powerberekening en een heldere presentatie van resultaten klinische verstaanbaarheid ten goede (Hoofdstuk 10).

Dankwoord

Dankwoord

Geen promotie zonder dankwoord.
En ook in mijn geval, geen promotie zonder patiënten. Bij de chirurgische studies ging dit om patiënten die met een ernstige buik- of vaataandoening een grote ingreep zouden ondergaan, niet altijd vanuit de meest optimistische setting. Dank dat er ruimte was voor het openstaan voor deelname aan wetenschappelijk onderzoek. Bij de IC patiënten is de toestemming verleend door familieleden. Ook voor hen geldt dat dit een situatie is waarin veel speelt en ik kan me voorstellen dat het beslissen voor een ander op welk gebied dan ook ontzettend moeilijk is. Die dapperheid heeft mij een schat aan data opgeleverd.

Mijn promotor, beste Paul. Ik wist na mijn wetenschappelijke stage bij de interne dat ik ooit wilde promoveren. Dat jij onderzoek deed naar voeding was voor mij een reden om eens binnen te wandelen. Dat ik uiteindelijk internist zou gaan worden wisten wij toen beiden nog niet. Ik loof je creativiteit, ambitie en doorzettingsvermogen. Ik ben de laatste die wat over koppigheid mag zeggen. Vaak had je toch gelijk.

Mijn copromotor Hans. Het was verfrissend om iemand tegen te komen die in mogelijkheden denkt en handelt. De kamikaze die het traceronderzoek dreigde te ondergaan door het labyrint aan regelgeving werd gecoupeerd na onze ontmoeting in Rotterdam. Dank voor je steun.

Beste hooggeleerde heren en vrouw van de leescommissie. Beste Kees, de uitjes naar Maastricht waren altijd leerzaam en zonder meer gezellig. De misère van het opstarten heeft verdere samenwerking de das omgedaan, maar dat lag niet aan intenties. Beste Heleen, je kennis van zaken en kritische noot hebben het onderzoek telkens op scherp gezet. Misschien had er zelfs nog meer ingezeten. Beste Bert, je hebt het onderzoek op de IC ontzettend gefaciliteerd, je relaxte houding heeft de inclusie op de IC veel snelheid gegeven. Beste Yvo, opleider, specialist en onderzoeker. Op een uitgebluste dag is het goed mezelf te reanimeren door je pleidooi voor zolderkamernieuwsgierigheid weer eens kracht bij te zetten. Ik wil allround blijven groeien en hoop nog veel van je te blijven leren. Beste Henk, ik leerde jou en je kennis pas kennen toen je al weer bijna weg was. Fijn dat je alsnog betrokken bent.

Mijn paranimfen, Marlieke en Lidwien. Lieve Marlieke, het was een feest dat je mijn collega werd. We hebben echt waanzinnig leuke congressen gedaan en je bent een ongelooflijke pro. Fijn om binnenkort weer eens af te spreken. Lieve Lidwien, 2 artsen, 2 loopbanen. Wij zouden de laatste zijn die zouden verwachten eenzelfde carrière te hebben. Hoewel jij de jongere van ons bent wist jij toch eerder welke kant je op wilde (en welke niet). Vergeet niet je eigen ambitie te zien!

Ik wil de afdelingen Heelkunde (Vumc en MCA), diëtetiek, interne geneeskunde, intensive care, apotheek, anesthesie, orthopedie en (endo- en klinisch chemisch) lab bedanken. De dames van het planbureau, Ingrid en Erna van het research centrum. Rob en Sigrid, jullie hebben me echt ondersteund en gesteund op het lab. Het feit dat mijn hormonale research tak het heeft afgelegd tegen de aminozurentak was alleen een gedwongen poging tot prioriteren. Gelukkig ga ik de schade inhalen in mijn aandachtsgebied. Dank Annemieke, Annelies, Rachid, Marelise en wijlen Michaela voor jullie betrokkenheid bij dit ongewone project. Pierre, bedankt voor je inzet, het was zeker niet altijd gemakkelijk!

Professeur Cynober and Jean Pascal de Bandt, thank you for collaborating, Your in-depth knowledge and commitment to the subject is extraordinary. Shame I did not make it to the last couple of ASPEN and ESPEN meetings. We will meet again, for sure!

Ik bedank de onderzoeksgroep: Gerdien, Milan, Berbel, Barbara, Lenny, Mireille, Joanna, Nikki en Saskia voor de gezelligheid, praktische hulp, wijze lessen en af en toe een schouder. Er waren onvergetelijke congressen, bizarre uitjes en fijne borrels. Mireille, als tandem begonnen en toch ook onze eigen projecten gedaan. We hebben veel onzekerheden gehad in het begin en ik prijs je loyaliteit aan de studietelefoon waardoor je toch iedere keer bij nacht en ontij je Ford Ka van stal haalde. Lieve Nikki en Saskia, veel onderzoekstijd hebben jullie ingezet naast je studie. Na ieder tentamen, in de vakanties en gewoon na college waren jullie er. Het heeft jullie geen windeieren gelegd en er volgen spoedig hele mooie boekjes. Nikki, ons uitje naar de VS heeft ons op waanzinnige plekken gebracht. Ik heb wel laatst wat onduidelijke contacten van Facebook verwijderd.. Saskia, ik vind het onwijs knap hoe je stralend je doelen plaatst, in het vizier houdt, en met niets minder dan een 180 binnenhaalt. Berbel en Milan dank voor de fijne start, jullie hebben mooie boekjes om op terug te kijken! Lieve Bar, heel veel succes in Australië, je open blik naar je carrière en je leven is een voorbeeld. Lieve Joanna, you did it baby! Work hard play hard, wat een mooi boekje! Leuk dat we weer in hetzelfde ziekenhuis werken. Lieve Lenny, na jouw promotie heb ik je in Suriname pas echt beter leren kennen, het was echt een fantastische tijd, die nog warmer werd door jullie.

De onderzoekskamer met Jorgos, Kakkhee, Paul, Pieter-Jan, Dieuwertje, Marjolein: dank voor de gezelligheid (die die kamer van nature niet kende).
Mijn collega´s, toen nog studenten René, Mathijs, Fransje, Stéphanie en Thomas: het was een leerproces voor ons allen, dank voor jullie inzet, die zeker niet in 3 maanden te proppen was. En het studentenbedankuitje was echt een succes!

Vakgroep interne geneeskunde, dank voor jullie opleiding, I love my job! Mark, Yvo, Abel, dank voor jullie inzet zo'n heterogene grote groep te sturen en mij toch het gevoel te geven dat er aandacht is voor het persoonlijke. Lieve AIOS-collega´s: dank jullie voor de fijne tijd op de VU. Het is een periode van continu scoren en shinen naast 30-ersdilemma's. Ik ben inmiddels het halve ziekenhuis door en vele deurcodes verder, en zonder wat relativering en vooral gezelligheid was die appel misschien af en toe toch wat zuur geweest. Never let go! Linde, Carianne, Astrid en Marleen: de Benjamin van ons groepje gaat snel weer een datum regelen.

Ik dank mijn lieve vrienden en vriendinnen, ik voel me gelukkig dat ik jullie ken en kijk uit naar de komende tijd.
Mijn jaarclub met in het bijzonder Sis, Coroon en Eef: wat was het een feest in 19XX! Dank voor de fijne vriendschap en jullie steun die nu al de helft van ons leven beslaat. Mijn studievriendinnen, Mariette, Danielle, Carolijn, Ví: onze drukke levens geven ons te weinig tijd om reflexie en soms een beetje intervisie te combineren met het aangename des levens.
Gaat veranderen! Lieve Anke en Floor, vanaf de amazonenstraat hebben we eerste baan + frustraties, liefdes + perikelen en zwangerschappen + baby's gedeeld.
Heel bijzonder en waardevol hoe onze vriendschap zich heeft ontwikkeld.
Ik haat jullie natuurlijk wel om jullie nieuwe postcodes.

Multitude, jullie hebben met een esthetisch oog naar wetenschapscommunicatie gekeken. Wat fantastisch dat het zo mooi is geworden!

Familie, de vanzelfsprekendheid die de warmte van jullie is, is de grootste rijkdom.

Mijn ouders, jullie hebben ons altijd alles gegund en ervoor gezorgd dat onze blikken breed werden. Lieve mama, door je carrière heb je mij ongemerkt ook een natuurlijke dosis emancipatie meegegeven. Onze levens veranderen, zijn rijker en complexer geworden en het is fijn om te zien en voelen dat je er nog steeds altijd voor me bent. Lieve mama en Bram, ik hou van jullie en ik heb zin om jullie deur ook weer eens wat vaker plat te lopen! Lieve papa, internist en epidemioloog van de dieren, de stap naar geneeskunde was in theorie eerder gezet dan in de praktijk. En nu in de praktijk, denk ik vaak aan jouw praktijk. Papa en Maris, ik heb jullie lief en ik geniet ervan als jullie er zijn!

Wim en Trix, ik heb altijd het gevoel gehad in mijn handen te mogen knijpen met jullie als schoonouders. Ik knap helemaal op als jullie in de buurt zijn, of ik in de buurt van kuuroord Kotten/Winterswijk. Dank voor jullie steun, interesse en liefde. Dank lieve Floor en Anna, Lucas en Todd: de koude kant heeft een hele aangename gevoelstemperatuur.

Lieve Alard, grote broer, ik zal nooit je advies vergeten net voor ik begon met dit project. Terwijl wij in de intercity of boemel naar de Achterhoek zaten coupeerde jij mijn laatste twijfels door te stellen dat ook ik altijd uit een trein kon stappen. Een gebaand pad wordt pas interessant als er vanaf geweken kan worden. Love you bro! Elsbeth, lief zusje. Hoe het leven loopt is niet te voorspellen.

You win some, you lose some, door dik en dun. Je bent er altijd bij geweest, van meegebrachte broodjes in de hortus tot en met de sample delivery in Parijs, van de Loveboat tot en met bankhangen van zuid naar centrum naar west. Forevever my sweet sister! Lidwien, babysister, de zonneschijn, het komt toch wel goed. Je hebt het dik voor elkaar in 010, maar het is vreselijk je hier te missen. Je tijd voor mij heb ik niet altijd goed terug kunnen geven.
Het wordt tijd binnenkort weer eens onze Vermeulen-kroegmores onder de loep te nemen..! Lieve Oscar en Stijn, jullie zijn niet meer weg te denken, pas goed op die twee mirakels!

Lieve Jorre, het is heel fijn om samen te zijn met iemand die stevig in zijn schoenen staat en de wereld en mij aankijkt.
De keren dat je gezegd hebt: "Laten we het gewoon doen" hebben ook mijn vizier verder geopend. Met twee drukke carrières en samen een gezin, is het gunnen en geven van vrijheid aan de ander niet altijd gemakkelijk. Dat jij dat kan geeft mij mijn vleugels. De tropenjaren hebben we misschien eens wat letterlijk genomen, maar als ondernemer begrijp je dat er nog een keer eens wat moet volgen!
Je verrast me iedere dag en ik blijf van je leren. Ik hou van je.

Lieve Krijn en Toon, jullie zijn de allerleukste mannen. Krijn, vanaf je geboorte blijk je gezegend met een ongekende dosis sociale vaardigheden. Het is fantastisch om te zien hoe jij leren met lachen combineert. Toon, je beschouwende blik is even mysterieus en daarna ga je recht op je doel af. Je hebt je eerste stapjes op Surinaamse bodem gezet en ik ben benieuwd naar al je volgende stappen. Jullie zijn een gouden duo, ik hou van jullie en kan niet wachten op alles dat komen gaat.

Curriculum Vitae

Curriculum Vitae

Mechteld Vermeulen was born on the 10th of April 1980 in Winterswijk, the Netherlands. She completed high school successfully at "Scholengemeenschap De Driemark" in Winterswijk after which she followed a coarse in English Language and Literature at the University of Exeter (UK) and studied Pharmacy in Utrecht. In 2000 she started Medicine at the Vrije Universiteit Amsterdam (VU) which was completed in 2007. During those years she worked as a research assistant at the departments of vascular medicine of the Academic Medical Center and the internal medicine department of the Medical Center Sloter-vaart and she participated in medical student education at her home university. In 2007 she started her PhD project concentrating on amino acid metabolism in critically ill patients at the department of surgery of the VU University Medical Center (professor dr. P.A.M. Van Leeuwen, co-supervised by professor dr. J.B. van Goudoever).

Next to this she studied carbohydrate metabolism in surgical patients. She presented parts of her work at various national and international conferences such as the European Society of Parenteral and Enteral Nutrition (ESPEN), Symposium Experimental Research in Surgery (SEOHS) and NWO Conference on Nutrition. She won the prestigious Norman Yoshimura award by the American Society of Enteral and Parenteral Nutrition (ASPEN) twice and won the best abstract award by the Netherlands Society for Enteral and Parenteral Nutrition (NESPEN) in 2009.

She also worked as a travel medicine consultant and volunteered as buddy. In 2011 she started as a resident in internal medicine at the VUmc (professor dr. Y.M. Smulders, dr. A. Thijs and professor dr. M.H.H. Kramer). In 2016 she worked as an infectious diseases resident at the Academic Medical Center Paramaribo in Suriname for four months (AZP; dr. S. Vreden). In 2016 she started a fellowship in endocrinology (VUmc, professor dr. M.L. Drent).

Mechteld lives with her partner in Amsterdam, they have two sons.

Curriculum Vitae, NL

Mechteld Vermeulen (1980), behaalde haar VWO diploma in 1998 aan Scholengemeenschap De Driemark, te Winterswijk. In Exeter (VK) haalde zij haar propedeuse Engels en daarna studeerde zij farmacie in Utrecht. Uiteindelijk werd zij na 2 jaar ingeloot en begon zij de studie geneeskunde aan de Vrije Universiteit in Amsterdam alwaar ze haar artsendiploma in 2007 haalde. Tijdens haar studie deed zij wetenschappelijk onderzoek bij de afdeling vasculaire geneeskunde in het Academisch Medisch Centrum en de afdeling interne geneeskunde van het Medisch Centrum Slotervaart. Daarnaast gaf zij onderwijs aan medisch studenten.

Zij startte daarna haar promotieonderzoek naar klinische voeding bij de vakgroep heelkunde van het VU medisch centrum onder leiding van professor dr. P.A.M. Van Leeuwen en onder begeleiding van professor dr. J.B. Van Goudoever, waarbij zij naast aminozuurmetabolisme bij zieke patiënten door middel van stabiele isotopen ook koolhydraatmetabolisme onderzocht bij de afdeling diëtetiek.
Zij presenteerde haar werk op nationale en internationale congressen zoals de European Society of Parenteral and Enteral Nutrition (ESPEN), Symposium Experimenteel Onderzoek Heelkundige Specialismen (SEOHS) en het NWO Voedingscongres. Voor haar werk won zij twee maal de prestigieuze Norman Yoshimura grant bij de American Society of Enteraland Parenteral Nutrition (ASPEN) en de prijs voor beste abstract bij de Netherlands Society for Enteral and Parenteral Nutrition (NESPEN) in 2009.

Zij werkte tevens als reizigersgeneeskundige en was werkzaam als buddy. In 2011 startte zij de opleiding tot internist in het VUmc (opleiders professor dr. Y.M. Smulders, dr. A. Thijs en professor dr. M.H.H. Kramer). In 2016 werkte zij enkele maanden in Suriname teneinde ervaring op te doen op het gebied van infectieziekten (Academisch Ziekenhuis Paramaribo; dr. S. Vreden). Gezien haar interesse in voeding en metabolisme koos zij het deelspecialisme endocrinologie, waarmee zij in 2016 startte (opleider professor M.L. Drent).

Mechteld woont samen in Amsterdam, zij heeft twee zoons.

Publications and Awards

List of publications

(1) S.J.H. Brinkmann / N. Buijs, M.A.R. Vermeulen, E. Oosterink, H. Schierbeek, A. Beishuizen, J. de Vries, W. Wisselink, P.A.M. van Leeuwen - Perioperative glutamine supplementation restores the disturbed renal arginine synthesis after open aortic surgery, a randomized controlled clinical trial –Am J Physiol – Renal Physiol. 2016; Sep; 1:311(3):F567-75

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Awards

Norman Yoshimura Award 2010

Beste Research Aanvraag American Society for Parenteral and Enteral Nutrition: 'The Contribution of L-glutamine to L-citrulline and L-arginine synthesis when alanyl-glutamine is supplied in an enteral dose of 0.5 g/kg, in critically ill patients'

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