From THE DEPARTMENT FOR CLINICAL SCIENCE INTERVENTION AND TECHNOLOGY, DIVISION OF ANAESTHESIOLOGY AND INTENSIVE CARE
Karolinska Institutet, Stockholm, Sweden

GLUTAMINE TO ICU PATIENTS

Agneta Berg

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Klassisk är en bok som folk berömmer men ingen läser.
MARK TWAIN

ABSTRACT

Intravenous glutamine supplementation to intensive care patients using a glutamine containing dipeptide is now widely accepted in clinical practice. There are evidences that glutamine supplementation of ICU patients in need of parenteral nutrition improves mortality and morbidity. The beneficial effects may be extended also to ICU patients on combined enteral and parenteral nutrition and perhaps also to ICU patients on enteral nutrition only. The effect of intravenous and/or enteral glutamine supplementation to these patient groups is presently not conclusive.

To facilitate clinical use of glutamine supplementation a number of safety aspects are considered in this thesis work. The possibility to administer the concentrated glutamine containing dipeptide solution in peripheral vessels, and the metabolic tolerance of the dipeptide formulation in ICU patients. Metabolic tolerance was assessed as the absence of accumulation of the dipeptide, the absence of urinary losses of the dipeptide, and the clearance of dipeptide and constituent amino acids over time. Furthermore, the possible losses of the dipeptide and the constituent amino acids during continuous renal replacement therapies in ICU patients with kidney failure have not been clarified. The extent of glutamine losses into the ultrafiltrate and the possibility that exogenous supplemented glutamine will be lost into the ultrafiltrate to a high degree have been addressed. Finally the concern that exogenous provided glutamine may be converted into glutamate in the brain of head trauma patients has been a concern. Head trauma patients some times suffer from multiple organ failure and hence maybe treated with exogenous glutamine. The suggestion of a connection between elevated intracerebral glutamate levels and an unfavourable outcome in the head trauma patients has been suggested. Therefore the possible connection between exogenous glutamine supplementations and the level of free glutamate interstitially in the brain and the balance of free glutamate and glutamine across the brain was investigated.

The results show that a glutamine containing dipeptide in a concentrated solution may be administered in the peripheral vein without any signs of inflammatory reaction. In addition, the glutamine containing dipeptide is metabolically well tolerated in ICU patients. The losses of exogenously provided glutamine into the ultrafiltrate during continuous renal replacement therapy are not different from a situation where no exogenous supply is given. The increased loss into the ultrafiltrate is on the contrary an argument to increase the exogenous supplementation of glutamine. Finally, there was no connection between glutamine supplementation and the level of free glutamate interstitially in the brain and the balance of glutamate across the brain in head trauma patients.
In addition, posthoc analyses were performed demonstrating that the endogenous rate of appearance of glutamine (estimate of glutamine production) in ICU patients is of the same magnitude as in healthy individuals. And it is suggested that this endogenous production is not inhibited by exogenous glutamine supplementation.

The results presented in this thesis work provide evidence that studies to elucidate outcome advantages for ICU patients in relation to intravenous glutamine supplementation are safe and can be encouraged. Furthermore new insights in glutamine production and handling of exogenous glutamine supplementation were gained.

LIST OF PUBLICATIONS

I. **The local vascular tolerance to an intravenous infusion of concentrated glutamine solution in ICU patients.** Berg A, Forsberg E, Wernerman. Clinical Nutrition, (21) 135-139, 2002.

II. **Elimination kinetics of L-alanyl-L-glutamine in ICU patients.** Berg A, Rooyackers O, Norberg Å, Wernerman J. Amino Acids. (29) 221-228, 2005.

III. **Glutamine kinetics during intravenous glutamine supplementation in ICU patients on continuous renal replacement therapy.** Berg A, Norberg Å, Martling CR, Gamrin L, Rooyackers O, Wernerman J. Intensive Care Med. (33) 660-666, 2007.

IV. **Intravenous glutamine supplementation to head trauma patients leaves cerebral glutamate concentration unaffected.** Berg A, Bellander BM, Wanecek M, Gamrin L, Elving Å, Rooyackers O, Ungerstedt U, Wernerman J. Intensive Care Med. (32) 1741-1746, 2006.

V. **The pattern of amino acids exchanges across the brain is unaffected by intravenous glutamine supplementation in head trauma patients**. Berg A, Bellander BM, Wanecek M, Norberg Å, Ungerstedt U, Rooyackers O, Wernerman J. Submitted to Clinical Nutrition 2007.

CONTENTS

LIST OF ABBREVIATIONS

AA	Amino acids
APACHE II	Acute physiology, age, chronic health evaluation
ATP	Adenosine triphosphate
AUC	Area under the curve
av-diff	Arterio venous difference
b.w.	body weight
C	Concentration
CL	Clearance
CRRT	Continue renal replacement therapy
CVVH	Continuous veno-venous haemofiltration
CVVHDF	Continuous veno-venous haemodiafiltration
DNA	Deoxy ribonucleic acid
EN	Enteral nutrition
GCS	Glasgow coma scale
Gln	Glutamine
Glu	Glutamate
GOS	Glasgow outcome scale
HPLC	High performance liquid chromatography
ICU	Intensive Care Unit
NCA	Non compartmental analysis
NICU	Neurosurgery Intensive Care Unit
NIH	National Institute of Health
PN	Parenteral nutrition
R_a	Rate of appearance
RNA	Ribonucleic acid
SD	Standard deviation
SOFA	Sepsis-related Organ Failure Assessment
TPN	Total parenteral nutrition
V_d	Volume of distribution

1 INTRODUCTION

Glutamine is a non-essential amino acid with a special role in metabolism and nutrition. At the time when development commercial products for intravenous nutrition started, glutamine represented technical problems. It has a limited solubility and stability in aqueous solution. Therefore glutamine was not included in commercially available amino acid solutions for intravenous use. The thought was that endogenous production of glutamine would be sufficient for the patient. Later on evidence accumulated that this may not be true. On the contrary, several signs of glutamine depletion are present in patients with stressed metabolism (Roth et al. 1982, Vinnars et al. 1975). In particular for intensive care unit patients the depletion of glutamine is reported to be a predictor of a poor outcome (Oudemans-van Straaten et al. 2001). Furthermore, supplementation of intravenous nutrition with glutamine improves outcome in terms of mortality and morbidity in ICU patients (Goeters et al. 2002, Griffiths et al. 1997, Novak et al. 2002). Today the technical difficulties to include glutamine in parenteral nutrition have been solved by the introduction of dipeptides. Glutamine containing dipeptides are readily soluble in aqueous solution and stable. They are immediately hydrolysed after intravenous administration and the glutamine part of the dipeptide becomes available to the patients (Albers et al. 1989, 1988). When glutamine containing intravenous nutrition came into clinical use, several questions were raised concerning the handling of glutamine supplemented nutrition in ICU patients. Among these questions were the vascular and metabolic tolerance of the dipeptide infusions, the handling of glutamine during renal replacement therapy in ICU patients and also the safety of glutamine administration in head trauma patients. These are the questions addressed in the present thesis work.

1.1 PHYSIOLOGICAL BACKGROUND

1.1.1 Glutamine metabolism in health

Glutamine is a non-essential amino acid with a molecular weight of 146 dalton (Fig. 1). It is build up by a carbon skeleton, alfa-ketoglutarate, and two amino groups. Alfa-ketoglutarate is a constituent of the Citric Acid Cycle, and therefore a substance directly involved in aerobic energy production in the mitochondria. Alfa-ketoglutarate, glutamate and glutamine are closely related by the carbon skeleton they have in common (Fig. 2). It is by the action of transaminases and glutamine synthase as well as

glutaminase that these three substances are synthesised from each other by the addition or removal of amino groups. Amino groups appear on their own as ammonia, which is preferably not transported outside the cells in the general circulation, because of the potential harmful effects in the central nervous system. In the splanchnic area on the other hand, free ammonia can be captured in the liver working as a filter of the portal blood-stream, and be metabolised to urea and excreted via the urine. Skeletal muscle is the tissue that produces the major part of the glutamine synthesised in the body. Smaller amounts of glutamine are also synthesised in other tissues. Estimations of the endogenous synthesis rate of glutamine are in the magnitude of 60–80 g/24 h in healthy adult human subjects (Hankard et al. 1995, Van Acker et al. 1998).

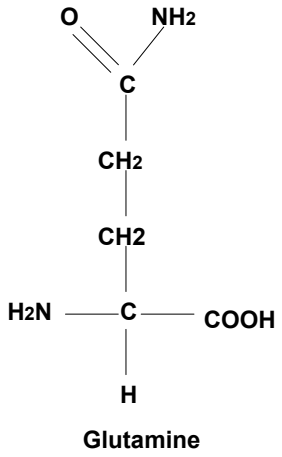

Figure1. Structural formula of glutamine. Glutamine has a carbon skeleton with 5 carbons and one amino group and one amide group.

Glutamine has a number of key functions in metabolism (Table 1). It is a constituent of proteins, where it together with other amino acids forms the brick stones building up proteins (Vinnars 1990, Newsholme et al. 1997, Wernerman et al. 1999). Glutamine is also a precursor for the synthesis of nucleotides, which build up the RNA and DNA involved in protein synthesis and cell division. It is also a precursor for alfa-ketoglutarate and energy production in mitochondria-containing cells. A 4th major function for glutamine is to be an inter-organ transporter of amino groups or ammonia.

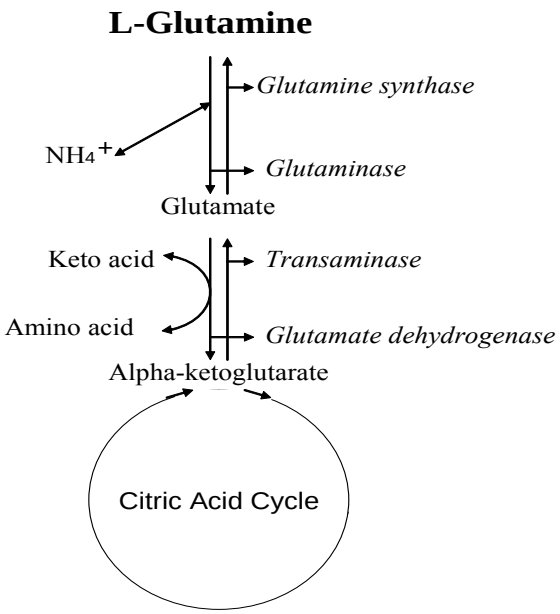

Figure 2. The pathway of glutamine metabolism in the cells. The relation between glutamine, glutamate and alpha-ketoglutarate with the enzymes involved indicated.

In addition there are numbers of others functions associated with glutamine, but these are the major functions.

Table 1. Glutamine functions

- Precursor for DNA/RNA
- Constituent for proteins
- Energy substrate for immunocompetent cells and enterocytes
- Substrate for gluconeogenesis
- Precursor for glu in the brain, glu is an important excitatory neurotransmitter in the brain.
- A pathway for glu transport out of the brain
- Via glu a precursor for glutathione which is an antioxidant
- A substrate for renal ammoniagenesis and acid-base regulation.

In plasma glutamine concentration is 0.5–0.8mmol/L. This is 20–40 % of the total amino acid content in plasma, which is of the magnitude 2.0–2.5mmol/L. Also in the intracellular compartment of many tissues, glutamine is the most abundant free amino acid constituent. In particular in skeletal muscle, where the intracellular concentration is around 20mmol/L (Bergstrom et al. 1974, Vinnars et al. 1975). Consequently there is a gradient from intra- to extra- cellular space in muscle of approximately 30:1. In other

tissues, like intestinal mucosa and the liver, glutamine concentration is not as high as in muscle (Ahlman et al. 1993, Barle et al. 1996). These tissues are mainly consumers of glutamine, while skeletal muscle is the main producer. The glutamine content as a constituent in various proteins is usually between 5 and 10 % of the total amino acid content. This is less than for leucine, which is the most abundant amino acid in most proteins, approximately 10–12 %. Proteins particularly rich in glutamine are for example gluten, which has a glutamine content of as much as 20–30 %.

The contribution from glutamine, glutamate and alfa-ketoglutarate in energy production is particularly important in cells that can export free ammonia. The theoretical maximum energy exchange from glutamine is of the same magnitude as the energy exchange from of glucose. This makes glutamine a valuable energy source in certain situations. For example most cultured cells in the laboratory use glutamine as energy substrate, and it is well known that it is difficult to grow cells in culture medium that is not well enriched in glutamine. The rational is the combined role of glutamine in energy production and in nucleotide synthesis.

Being a precursor for nucleotide synthesis makes glutamine availability a key factor in cell growth. Outside experimental systems, in the human body, glutamine availability is crucial for intestinal mucosal cells and also for immune-competent cells (Newsholme et al. 1987). These are cells, which in real life sometimes need to multiply rapidly with short notice. In that situation it is necessary to increase nucleotide synthesis many-fold over a short period of time. Such an alteration in metabolic demand is problematic to handle for the cell. A common solution to this problem is to use a metabolic pathway with a high flux, from which a smaller fraction can be diverted with short notice, a so-called futile cycle (Newsholme et al. 1997). When glutamine is used as an energy source, a high flow rate is at hand, furthermore a small fraction of that flux will be sufficient to dramatically increase the nucleotide synthesis rate. Therefore many cells use glutamine as an energy substrate. It has been demonstrated that stressed cells have a particular preference for glutamine (Ardawi et al. 1990).

The role of glutamine for interorgan transport is also well established. There is a constant export of glutamine from skeletal muscle, and this export is taken up in the organs within the splanchnic area. In the basal state all free amino acids, except glutamate, are exported from skeletal muscle, but in the fed state this is reversed into an

uptake of all amino acids into skeletal muscle except for glutamine, which is constantly exported out of muscle (Fig. 3). In this way glutamine is the major inter-organ carrier of amino groups. The rational is to keep the plasma concentration of ammonia low in the systemic circulation. Low ammonia is necessary because ammonia is toxic to neurons in the central nervous system. In the portal circulation this is of less importance because the liver acts as a filter, which captures the free ammonia present in portal blood. Glutamate exported from the liver and taken up by skeletal muscle is a major source for the de novo synthesis of glutamine in skeletal muscle (Wernerman et al. 1985). By the action of glutamine synthase an amino group is added to glutamate to produce glutamine. In this manner there is a glutamine-glutamate cycle between muscle and the splanchnic area in human subjects.

In addition the carbon-skeleton of glutamine can serve as interorgan transport of energy from the muscle to other tissues.

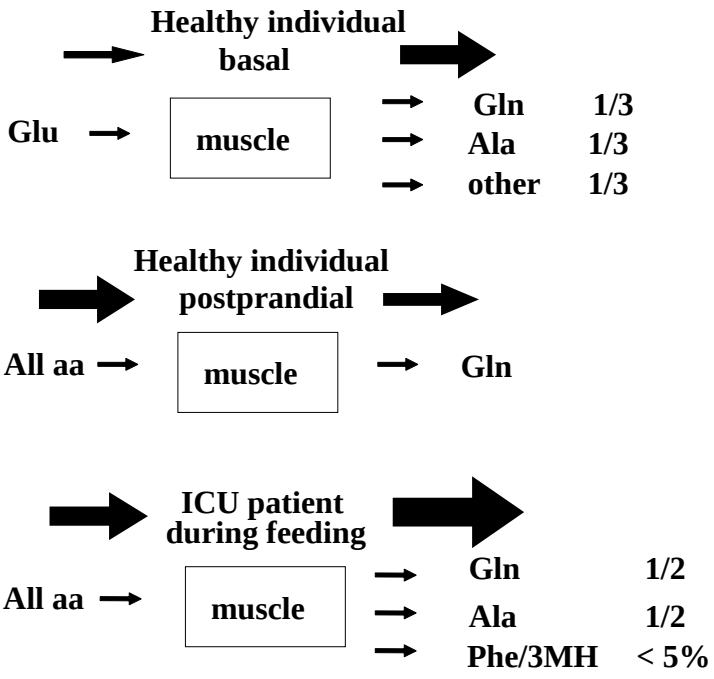

Figure 3. Muscle amino acid fluxes in health and disease. The relative quantities of amino acid, influx and efflux occurs muscle tissue are illustrated by the bold arrows. The small arrows indicate the most important constituents in the different fluxes.

1.1.2 Glutamine metabolism during metabolic stress

During metabolic stress there is an increased efflux of glutamine from skeletal muscle (Fig. 3), and there is an intracellular depletion of glutamine in muscle. The increased

efflux from muscle is present also after trauma without infection, but it is more pronounced in septic states (Clowes et al. 1980, Vesali et al. 2002). The intracellular free glutamine depletion in muscle occurs after trauma and elective surgery, but is further accentuated in sepsis and in multiple organ failure (Gamrin et al. 1996, Roth et al. 1982, Tjäder et al. 2004b). Following elective surgery the glutamine depletion in muscle is proportional to the size of the trauma (Hammarqvist et al. 1989, Stehle et al. 1989, Vinnars et al. 1976). Hence after a bigger trauma a more pronounced depletion is seen and the lowest point in glutamine concentration comes later in time. Restitution back to normal occurs during 2-3 weeks (Petersson et al. 1990). In ICU patients the profound depletion, often down to 20 % of the normal level, usually is at hand already on the first day of stay in the ICU (Gamrin et al. 1996, Roth et al. 1982). The natural time-course of this depletion is not well characterised, but the depletion remains unaltered during the ICU stay. It is demonstrated in healthy volunteers subjected to an endotoxin challenge, that glutamine levels in plasma and muscle start to decline within a few hours (Vesali et al. 2005). It has been suggested that the magnitude of the depletion in muscle free glutamine may be predictive of outcome (Roth et al. 1985), however this has so far not been confirmed.

There are several reports of plasma free glutamine concentration following surgical trauma. In medium size surgery of non-malnutriated subjects, plasma free glutamine stays within the normal range postoperatively (Hammarqvist et al. 1990, Vinnars et al. 1975). When malnutriated subjects are studied, for example in cancer surgery, there are low plasma glutamine levels also preoperatively. Postoperatively following major surgical procedures there are several reports of decreases in plasma free glutamine (Morlion et al. 1998, Mertes et al. 2000). In ICU patients, plasma free glutamine is constantly low, and the low plasma glutamine is a predictor of mortality (Oudemans-van Straaten et al. 2001). The efflux of glutamine from skeletal muscle is slightly increased in the basal state following trauma. It is more increased in the fed state. It is therefore important to differentiate between studies of patients in the basal unfed state and in the fed state postoperatively. In ICU patients there is basically no information available about the basal state, but in the fed state the glutamine efflux from skeletal muscle is elevated, although not dramatically (Vesali et al. 2002). Measurements of glutamine production measured as the rate of appearance(R_a) of glutamine show an unaltered de novo production of glutamine in the posttraumatic state of metabolically

healthy subjects undergoing elective surgery (van Acker et al. 2000b, Van Acker et al. 1998).

Glutamine kinetics in other tissues during metabolic stress is less well charecterized (Biolo et al. 2005, Hulsewe et al. 2003). The concentration of free glutamine in intestinal mucosa is not very different in ICU patients as compared to healthy subjects (Ahlman et al. 1993, Ahlman et al. 1995). A condition which is markedly different is acute liver failure. Subjects with an acute failing liver have extremely high plasma concentrations of glutamine (Clemmesen et al. 2000). In contrast this is not seen in patients with a chronic liver failure, even if an acute exacerbation is present. The significance of this descriptive finding is presently not clear. Probably, liver failure is accompanied by a low urea production and consequently the high glutamine levels may be a reflection of an increased glutamine synthesis in order to capture ammonia. This explanation is however purely hypothetical.

1.2 GLUTAMINE SUPPLEMENTATION TO PATIENTS

Conventional amino acid solutions for intravenous use do not contain any glutamine at all, while conventional enteral feeding contains glutamine as a part of the ordinary protein sources used in those formulations, out of which 5-10 % of the amino acid content is glutamine. Commercial formulas supplemented with extra glutamine have so far not become available in Sweden. In healthy subjects undergoing a short time fasting, there is a decrease in muscle free glutamine concentration (Hammarqvist et al. 2005). This is immediately normalised when subjects are re-fed. Following elective surgery this is not the case. Instead a 40-50 % decrease in muscle free glutamine is seen (Hammarqvist et al. 1989, Petersson et al. 1994). However when intravenous feeding is supplemented with glutamine, 20 g/24 h, starting immediately after the surgical procedure, of the decrease is attenuated; (Stehle et al. 1989, Neri et al. 2001). In fact this is a prevention of the decrease, when a similar supplementation is given after muscle free glutamine has already decreased, no immediate restitution can be seen (Januszkiewicz et al. 1996). Furthermore, if the intravenous supplementation is discontinued after a few days, a drop in muscle free glutamine of the same magnitude as it should have been without glutamine supplementation occurs (Petersson et al. 1994). In intensive care patients, several unsuccessfully attempts have been made to attenuate the decrease in muscle free glutamine, (Tjäder et al. 2004b, Gamrin et al. 1996). For plasma free glutamine on the other hand, a normalisation is always possible,

also in ICU patients (Tjäder et al. 2001). On the other hand provision of exogenous glutamine by the intravenous route does not decrease the endogenous glutamine production as reflected by the glutamine rate of appearance, either in healthy volunteers or in trauma patients (van Acker et al. 2000a, Boza et al. 2001)

The clinical effects of glutamine supplementation to ICU patients have been summarised in a meta-analysis (Heyland et al. 2003, Novak et al. 2002). It shows that intravenous glutamine supplementation gives advantages in terms of mortality as well as morbidity (Goeters et al. 2002, Oudemans-van Straaten et al. 2001). For intensive care patients receiving glutamine supplementation by the enteral route the results are less conclusive (Hall et al. 2003, Garrel et al. 2003, Novak et al. 2002, Jones et al. 1999). For mortality as end-point, the individual studies of enteral glutamine supplementation are to small in size to be conclusive and no singular study shows a mortality advantage within the ICU The two studies studying intravenous glutamine supplementation that have a 6-months follow up show a reduction in mortality at that time point (Griffiths et al. 1997, Goeters et al. 2002). The other studies involving intravenous glutamine supplementation may show a non-significant tendency towards a decreased mortality in the ICU (Powell-Tuck et al. 1999, Wischmeyer et al. 2001, Déchelotte et al. 2006). The patients recruited to these studies are multiple organ failure patients with long ICU stay. This is a patient group that carries a very high mortality during ICU stay as well as during the post ICU period. In additions all studies also show beneficial effects in terms of infectious morbidity.

Studies of ICU patients given glutamine supplementation by the enteral route, commonly involve patients with a lower rate of mortality (Hall et al. 2003, Peng et al. 2005). The majority of these studies show a beneficial effect upon infectious morbidity, while others do not confirm that result. Some of the studies included a high fraction of trauma patients (Schulman et al. 2005). In that particular patient group the results are non-conclusive. So far there are no studies reporting negative effects attributable to exogenous glutamine supplementation given to ICU patients.

For patients treated in a neuro intensive care there are two studies in the literature involving glutamine supplementation. In these studies glutamine was given via the enteral route (Falcao de Arruda et al. 2004, Schulman et al. 2005). One of these studies shows a shorter length of stay related to glutamine supplementation. Another specialised form of intensive care is patients with burns. These patients are routinely fed by the enteral route (Garrel et al. 2003, Juang et al. 2007), nevertheless one study

gives glutamine supplementation intravenously (Wischmeyer et al. 2001)., while two other studies give it enterally.
All studies show a decrease in infectious morbidity or length of stay. In one study there was an effect upon mortality, but the exceptionally high mortality in the control group of that particular study and the small size of the study, makes that result difficult to interpret.

For postoperative patients, a more favourable nitrogen balance and a prevention of muscle glutamine depletion can be achieved by intravenous glutamine supplementation (Hammarqvist et al. 1989, Hammarqvist et al. 1991, Petersson et al. 1990, Stehle et al. 1989). Due to the low rate of mortality and morbidity following elective surgery, no singular studies include sufficient number of patients to evaluate possible effects upon these parameters. As a surrogate parameter length of hospital stay has been used. A number of studies addressing this point have been published, which have been summarised in a meta-analysis (Heyland et al. 2003). Overall these studies show that patients requiring parenteral nutrition following elective surgery have a shorter period of hospital stay if the parenteral nutrition is supplemented with glutamine (Jiang et al. 1999, Novak et al. 2002).

In haematological patients there are several studies reporting beneficial effects attributable to glutamine supplementation (Schloerb et al. 1999, Ziegler et al. 1992). This patient group often requires parenteral nutrition as chemotherapy treatment frequently results in an impairment of the gastrointestinal tract. A lower rate of infectious morbidity as well as a lower incidence of fungal colonisation has been reported when extra glutamine is given (Anderson et al. 1998a). The same is also reported in oncological patients (Cerchietti et al. 2006). In premature children supplementation with glutamine is reported to result in a better tolerance to enteral feeding, and in a lower rate of morbidity (Neu et al. 1997). In two large NIH supported multi-centre studies in North America, these results were not possible to confirm (Poindexter et al. 2004, Vaughn et al. 2003). The level of glutamine depletion in the patients in these studies was, however, not documented.

From the role of glutamine in physiology, it can be anticipated that glutamine may have a beneficial effects upon cells that are rapidly turning over. In man, and in particular in diseased states, this is true for immune-competent cells as well as the gastrointestinal

mucosa. This has been reported in numerous animal experiments, but there are also good evidences from human studies. Following administration of intravenous glutamine supplementation the absorption of carbohydrates from the gastrointestinal tract is improved in patients with a compromised gut (Tremel et al. 1994, van der Hulst et al. 1996). Also the histology of the gastrointestinal tract is improved (van der Hulst et al. 1996). The effect upon immune-competent cells could be the background to the beneficial effect on infectious morbidity reported in a number of patient groups such as intensive care patients (Goeters et al. 2002, Houdijk et al. 1998, Jones et al. 1999), haematological patients (Schloerb et al. 1993, Ziegler et al. 1992), postoperative patients (Jiang et al. 1999, Mertes et al. 2000), oncological patients (Anderson et al. 1998b, Cerchietti et al. 2006), premature children (Dallas et al. 1998, Neu et al. 1997) and gastrointestinal patients (van der Hulst et al. 1996).

2 SPECIFIC AIMS

It can be summarised that there is good evidence for glutamine supplementation of intravenous nutrition in the ICU (Goeters et al. 2002, Griffiths et al. 1997, Heyland et al. 2003). However, there are a number of questions unresolved in this context. Many patients are given a combination of enteral and parenteral nutrition, and a central venous line is not always at hand. Could a glutamine containing dipeptide solution of high osmolality also be given in a peripheral vein?

Glutamine containing dipeptides are readily hydrolysed and utilised when given to healthy volunteers. However, the knowledge if this also happens in multiple organ failure in the ICU patients is limited. Therefore it is necessary to document that a glutamine containing dipeptide is not accumulating and that the plasma concentration of glutamine is not cumulating to very high levels. A specific question following that is whether or not exogenous glutamine supplementation has an inhibitory effect upon the endogenous glutamine production?

ICU patients with multiple organ failure are frequently subjected to continuous renal replacement therapy (CRRT). It is known that small molecules like amino acids are filtered out and/or dialysed. However, it is not known to what extent exogenously provided glutamine is lost into the ultrafiltrate in this situation. Therefore the indication and the dosage of glutamine supplementation to ICU patients requiring continuous renal replacement therapy must be elucidated.

In neuro intensive care and in particular in patients with head trauma there are concerns over the reports of a high free glutamate level interstitially in the brain. There are reports that a high interstitial glutamate concentration is associated with an unfavourable outcome. The close biochemical relationship between glutamine and glutamate has raised a question if exogenous glutamine supplementation resulting in an elevated plasma concentration of glutamine would also result in an elevated concentration of glutamate interstitially in the brain? This question has to be sorted out before prospective studies may be performed in neuro ICU patients to answer the question if exogenous glutamine supplementations may improve outcome also in this subgroup of ICU patients.

The **specific aims** of this project may be formulated as follows.

1. Can a glutamine containing dipeptide be safely administered also in peripheral veins?
2. Can glutamine be administered safely in ICU patients from a metabolic point of view? In particular will there be an accumulation of the glutamine containing dipeptide or of the constituent amino acids?
3. What are the losses of glutamine during continuous renal replacement therapy with or without exogenous glutamine supplementation?
4. Will exogenously supplemented glutamine increase the interstitial glutamate concentration in the brain or interfere with the glutamine balance across the brain in head trauma patients?
5. How is exogenously supplemented glutamine metabolically handled in head trauma patients?

3 MATERIAL & METHODS

3.1 PATIENTS

The five studies of the thesis are comprised of 4 totally separate groups of ICU patients (Table 2). As defined by the inclusion and exclusion criteria these four groups are subgroups out of the total population of ICU patients. In studies I and II two separate groups of patients were studied. The inclusion criteria selected patients with only very moderate dysfunction of liver and kidney. Some of the patients from this subgroup were actually postoperative patients, although undergoing major surgery. The patients included in study III were multiple organ failure patients, and in particular all of them were on mechanical ventilation and continuous renal replacement therapy (CRRT). In studies IV and V exactly the same group of patients was studied and these patients were selected from patients with head trauma. Some of these patients, but not all, were multiple trauma patients. All of them had a cerebral dysfunction (GCS < 8), and all of them were on mechanical ventilation, but none had primary pulmonary failure. In the individual articles the patients are characterized in details. When the endogenous and exogenous glutamine utilisation and kinetics are discussed the key question is of course if these groups of patients may represent ICU patients in general, and what findings may be confined to subgroups of patients only.

Table 2. Patients in the studies

Study	n	Age	Gender m/f	Kg bw	ICU day Study start	APACHE-II, At admission	SOFA score	GCS
I	20	57±17	15/5	76±14	4 (1-14)	23±7		
II	20	58±11	15/5	87±18	10 (1-47)	23±7		
III	12	53±16	10/2	77±13	7 (2-20)		9±2	
IV-V	15	46±18	11/4	73±10	1 (1-6)			4 (3-8)

Mean ± SD, Medium (range)

All studies were approved by the Ethics Committee of Karolinska Institutet and the studies were performed in agreement with the Helsinki declaration. In all studies there were patients who were screened but not included into the study protocol due to absence of informed consent. All patients studied per protocol were also included in the results. In a few cases, clearly defined in each article, results were not included due to

failure of analysis, and otherwise discussed in terms of adherence to the inclusion and exclusion criteria. No intention to treat analysis was performed, related to those physiological parameters rather than outcome parameters were studied. In summary the patients were selected within prospectively defined inclusion and exclusion criteria, but beyond that no bias in selection of patients or selection of the results shown are present.

3.2 STUDY DESIGNS

Study I and II involved an intravenous infusion of a concentrated dipeptide solution during 4 hours. The total dose of dipeptide during these 4 hours was the same as the total dose infused during 20 hours in studies III-V. The latter infusion rate is more in accord with the clinical practice in the ICU. The shorter infusion period was chosen to study the vascular and pharmacological tolerance in a worst-case scenario. In studies I and II the infusion was followed by sampling of arterial blood for plasma dipeptide and amino acid analyses and check up of the infused vessel for vascular tolerance at regular time interval (Fig. 4-5). In studies III-V a different study setup was used (Fig. 6). Two consecutive days were studied. One day was treatment with dipeptide, one day was control (no dipeptide). The patients were randomly assigned to have treatment before control or control before treatment. There was always a 24 h wash-out period before the study in terms of intravenous glutamine-containing nutrition. Within the study protocol the wash-out period was 4 h. The results from study II gave reasonable evidence that this limited wash-out period is sufficient for the purposes of the studies. The 20 h infusion period was chosen to mimic conditions of clinical practice. At the end of the infusion period kinetic measurements were performed in terms of AV-concentration differences across the leg, from which uptake and release calculations were done using blood flow assessments of the leg. Immediately after the stop of the infusion a decay curve of the concentration in arterial plasma was produced for further kinetic calculations. The same procedure was used in study II following the 4 h dipeptide infusion. Another reason for choosing the 20 h infusion period was to obtain a clear steady state in terms of dipeptide and glutamine concentrations in plasma. In study III the loss of glutamine in the ultrafiltrate during CRRT was studied. Studies IV and V included the same head trauma patients and the intracranial and whole body handling of the extra given glutamine in were studied in studies IV and V.

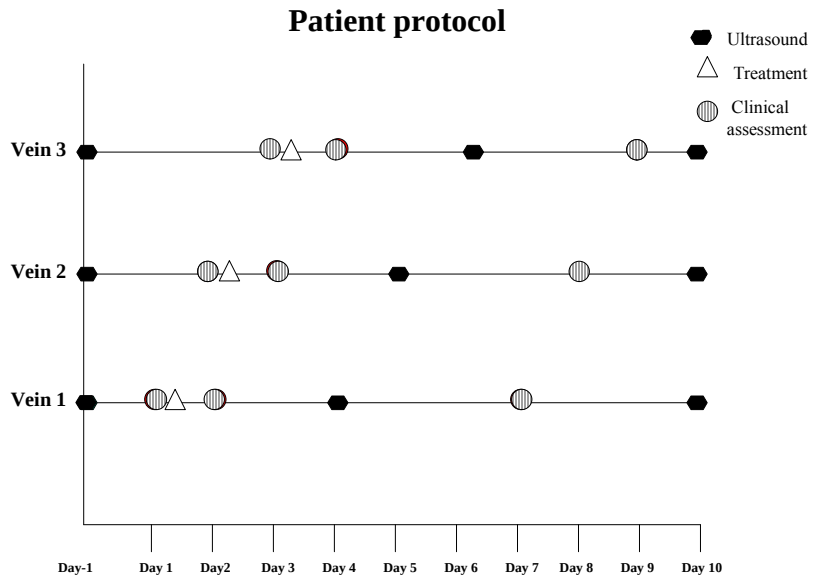

Figure 4. Protocol for study I. Filled symbols represent when the ultrasounds was performed, open symbols represent when the infusion of dipeptide or placebo was given and the striped symbols represent when the clinical assessment was done of the three difference veins.

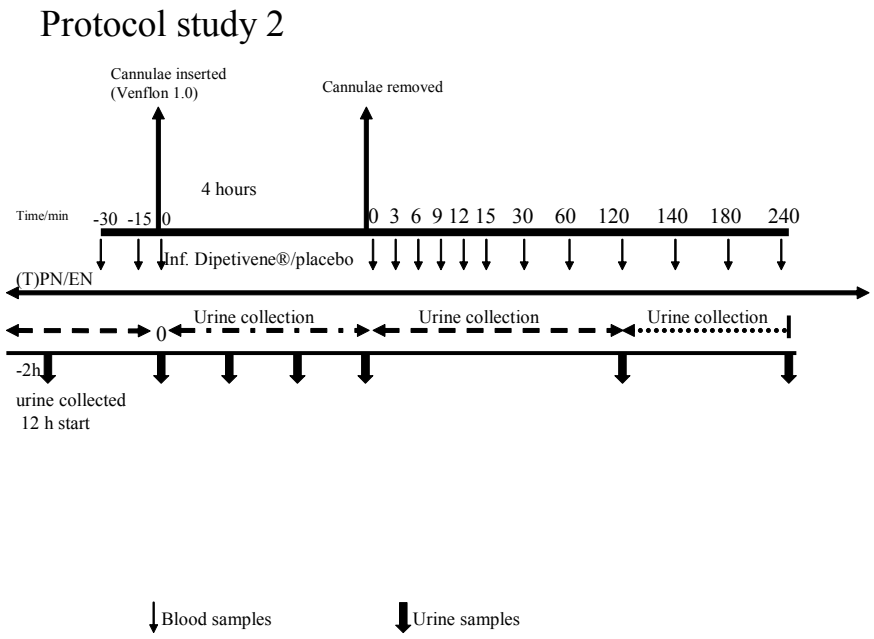

Figure 5. Protocol for study II. The protocol was identical in the two groups. The figure shows the time schedule of the study, and when the cannuale was inserted and removed. Blood samples (indicated by arrows) were taken 30 minutes before to 240 minutes after the infusion. Dipeptiven® or saline was infused for 4 hours. Nutrition (either parenteral, enteral or combined) was given throughout the day. Urine was collected with times intervals indicated at the bottom of the figure.

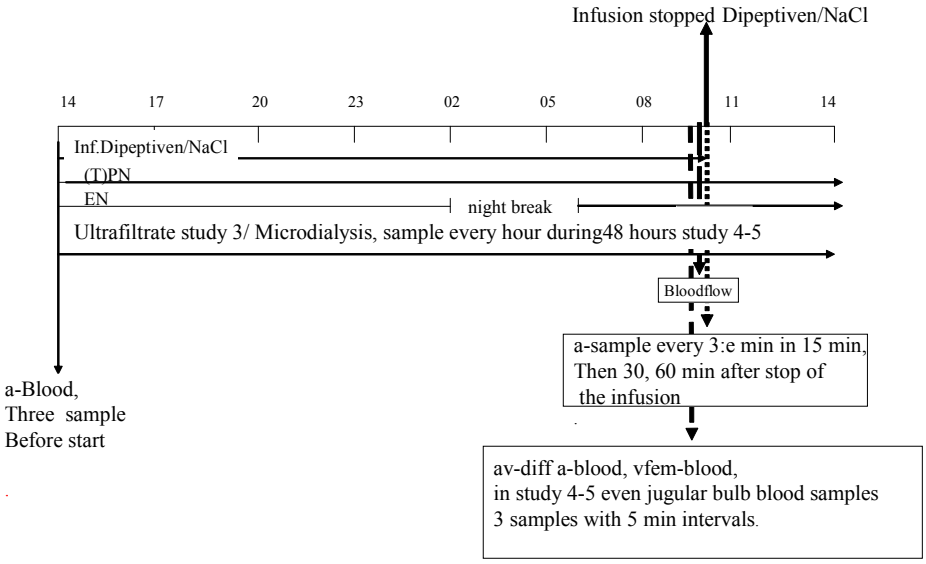

Figure 6. Protocol for studies III and IV-V. The protocol was identical on the two days. Before start three blood samples were taken for the baseline of glutamine. Dipeptiven® or saline (2.5 ml/kg which corresponds to 0.34g glutamine/ kg) was infused during 20 hours (from 14.00 till 10.00 next day). Parenteral and enteral nutrition was given according to the routines of the unit during 24 hours. From 09.30 till 10.00, blood flow of the leg was measured together with collection of 3 consecutive sampling from the artery (a-sample), femoral vein (v-sample) and jugular bulb (study IV and V) to allow measurement of glutamine fluxes. In study III, ultrafiltrate was collected continuously during the 2 study days. In study IV and V microdialysis sample from the brain were collected hourly during the 2 study days.

3.3 VASCULAR TOLERANCE

The vascular tolerance was evaluated using the Maddox score described in detail in article I. The arguments for using the modified Maddox score are also given in article I. The protocol chosen for the peripheral venous lines used in studies I and II, which is in accord within instruction for Swedish Health Care, no problems of vascular tolerance were revealed. It is obvious that these instructions are not adhered to in such detail in everyday clinical practice. Also the design of studies I and II, with a comparatively short infusion time, is a deviation from clinical practise. So the tolerance test was more

focused upon the acidity and the hyperosmolality of the dipeptide solution than persistence of an intravenous access over time.

3.4 LEG BLOOD FLOW MEASUREMENTS

Leg blood flow was measured using venous occlusion pletysmography. The plasma flow used in the calculations was obtained by multiplying the blood flow with the heamatocrit level. The argument for using occlusion pletysmography instead of other techniques of leg blood flow measurements are given in article III. Most importantly occlusion pletysmography is a non-invasive method with a reasonable accuracy and reproducibility. Sometimes the use of indiocyanine green infusion into the femoral artery using the Fick principle is advocated to be the "golden standard". After some consideration it was decided not to use that technique because of the invasiveness and also because that the physiological variation in leg blood flow may introduce more uncertainty than can be gained by using a more exact method. This physiological variation may be present in a short term as well as a longer term time perspective. The main purpose of the leg blood flow measurements was to give evidence that the two consecutive days studied were as comparable as possible. It could be discussed whether or not an agreement in the leg blood flow between the two days should be strict prerequisite for final inclusion of the subject into the study. It is quite obvious that if the two days differ considerably in terms of the leg blood flow a quite unpredictable error will be introduced. The reproducibility between singular readings using the occlusion plethysmography technique is not impressive. However, these readings are usually normally distributed and the mean value of 10 readings becomes quite reproducible (Fig. 7). Therefore we used this precaution of repetitive readings and calculation of the mean value. Furthermore it was always the same individual doing the readings on the two consecutive days. To further improve the reproducibility of the readings placement of the cuff and the measurement wire were meticulously indicated on the skin of the patient and placement of the leg involved on pillows and supporters was done exactly the same way on the two days.

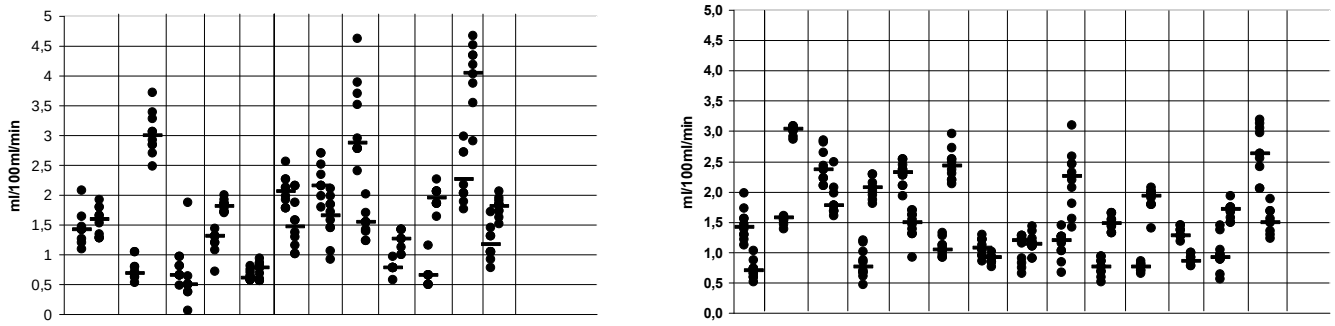

Figure 7. Plasma flow measurements. The left-hand figure shows leg plasma flow in study III and the right-hand figure shows leg plasma flow in study IV-V. Each point represents a single measurement and the line represents the medium value. Values from the two consecutive days from each patient are shown in each box.

3.5 MICRODIALYSIS

In study IV results of microdialysis in brain tissue were used. Microdialysis is a technique that is, in general, used to assess changes of glucose, lactate, pyruvate and glycerol in the interstitial space of brain tissue. The clinical routine for head trauma patients at the Neurosurgical ICU at Karolinska University Hospital Solna, involves the use of intracerebral microdialysis monitoring, whenever a surgical procedure is performed. The surgical procedure could be craniotomy or placement of intracerebral pressure sensors or intraventricular drainages. For clinical reasons the catheters are placed in the penumbral zone", representing tissue that may die or may survive during the course of neurointensive care. The position of the tip of the microdialysis catheter was documented by CT scan. There was also placement of a microdialysis catheter on the other, non-injured side. The microdialysis technique is described in detail elsewhere (Ungerstedt et al. 1987), but in summary standard pumps (CMA 106, CMA Microdialysis, Stocholm, Sweden) were used, a standard perfusion solution (Perfusion fluid T1, CMA Microdialysis, Stockholm, Sweden) was used as well and a standard pumping velocity (0.3 μL/min). Microdialysis fluid was collected and sampled every hour, but for the amino acid analysis involved in study IV only every fourth hour samples were analysed.

3.6 CONTINUOUS RENAL REPLACEMENT THERAPY (CRRT)

The patients treated with CRRT were given a veno-venous central dialysis catheter and PRISMA equipment (Gambro, Sweden) was used. The mode of CRRT in the induvidual patient was left to the discretion of the attending intensivists. Continuous veno-venous haemofiltration (CVVH) and continuous veno-venous hemodiafiltration

(CVVHDF) were used. The given dialysis dose was calculated both as estimated urea clearance and Kt/V, which is the clearance related to treatment time and distribution volume for urea. Estimated urea clearance was calculated differently depending on the mode in use.

3.7 AMINO ACID ANALYSIS

In studies II-V amino acid concentration analyses were done. The analyses were performed in three different laboratories employing very similar although not identical techniques. Plasma analyses for study II were done at the University of Bonn (Prof Peter Stehle, Graser et al. 1985).The microdialysis samples in study IV were analysed at the Institution of Pharmacology, Karolinska Institutet (Prof Urban Ungerstedt) (Lindroth et al. 1979). All other plasma and kidney dialysis samples in studies III, IV and IV were analysed at KFC, Karolinska University Hospital Huddinge (Doc Olav Rooyackers) (Vesali et al. 2002). All laboratories used modern HPLC equipment, with the same pre-column derivatisation with *o*-Phthaldialdehyde and fluorenscence detection (excitation at 330 nm and emission at 450 nm). The only difference is that the method to analyse the microdialysis samples (study IV) was adapted for small volumes and low concentrations (Dabrosin et al. 1997). This latter method was different in that only external standards were used and that the gradients for the HPLC analyses consisted of an acetate buffer and methanol instead of a phosphate buffer and a mixture of methanol and acetonitrile. Coefficients of variation for all the methods and all amino acids below 5%.

3.8 PHARMACOKINETIC CALCULATIONS

In study II clearance was calculated using Non-compartmental analysis (NCA) by dividing the dose with the area under the concentration-time curve (AUC). This area is estimated by the trapezoidal rule. In the case of endogenous compounds, the baseline can be subtracted. Therefore, poor precision of the baseline estimation is an important source of error. Another error arises from the part of the area that comes after the last measurement point, i.e. it is important to continue to measure concentrations until the difference from baseline is negligible. Fluctuations of baseline or measurement errors can easily confound the late phase of body equilibration and cause underestimation of AUC. Non-compartment analyses is particularly useful when clearance mainly relies upon area eliminates in the estimated.

Endogenous compounds can also be studied by turnover models that look at mass flow which often also the possibility to estimate glutamine Ra. Hence in studies III and V the baseline was used to calculate not only volume of distribution (V_d) and clearance (CL) but also rate of appearance (R_a) that contains valuable information for glutamine kinetics in ICU patients. Unfortunately the possible error introduced by the uncertainly in the baseline estimate is the risk when using turnover modelling. Future studies will be needed to evaluate if the assumptions mode in our calculation, such as stable baseline concentration and an decay unchanged endogenous glutamine Ra, can be justified**.**

4 RESULTS

4.1 VASCULAR TOLERANCE

There are two commercially available preparations containing glutamine. In both cases the stability and solubility of glutamine is guaranteed by a dipeptide formulation. The amino acid solution containing glutamine is Glavamin® (Fresenius Kabi), containing glycine glutamine. In addition there is a more concentrated dipeptide solution Dipeptiven® (Fresenius Kabi) containing 20 g alanyl glutamine (containing 13g glutamine)/ 100 ml. The comparatively low concentration of glutamine in relation to the other amino acids and the nitrogen content in Glavamin, makes Dipeptiven the primary source of glutamine by the intravenous route in most clinical situations. The concentrated formulation is pH neutral, but is hyperosmolaric, 920 milliosmole. The initial question in this research program was whether or not it was possible to give an intravenous infusion of a concentrated dipeptide in a peripheral vein. Given directly in a central vein, or supplemented into an amino acid or a glucose solution has earlier been demonstrated to be safe way. A difficulty when looking upon vasculature tolerance in peripheral veins is that clinical practice and the guidelines for health care (Metodboken, Stockholms läns landsting 1994) differ considerably. In the guidelines it is recommended that intravenous lines are exchanged every 24 h in order to preserve peripheral veins for iterated use (Lundgren et al. 1993). In practice, peripheral intravenous lines are often kept up to one week resulting in thrombophlebitis and occlusion, more or less destroying the vessel. When the study was designed to test vasculature tolerance, it was not reasonable to deviate from the guidelines. Consequently a protocol was designed where three different vessels in the same arm were used on three consecutive days. The possible inflammatory insult to the vessels was evaluated clinically by a slightly modified Maddox score on three occasions during one week following the infusion. In addition possible subclinical sings of inflammation was looked for by the use of ultrasound. The ultrasound also made it possible to evaluate the size of the veins in the non-occluded state.

The result was that no veins showed any signs of inflammation according to the Maddox score in the treatment group or control group. Also the ultrasound evaluation relieved no sign of inflammation. The diameter of the vessels in non-occluded state was

2.2±0.8mm in the treatment group and 2.2±0.5mm in the placebo group, demonstrating that the size of the vessels used is within the range used in clinical practice.

The absence of any sign of thrombophlebitis or perivascular inflammation in the both treatment and control groups can of course not totally exclude the possibility of thrombophletic events. However, it demonstrates that such an event will occur at a very low rate if the rules of the guidelines for intravenous lines are adhered to. This is slightly different from the literature of intravenous lines, but these rarely adhere to the guidelines for Swedish healthcare. The obvious conclusion is that the ph neutrality although combined with a high osmolality is a minor problem in peripheral veins.

4.2 METABOLIC TOLERANCE

The metabolic tolerance was studied in a group of ICU patients with only marginal organ failure in kidney and liver. Approximately 50% of the ICU patients in study III were postoperative patients following major surgery. The protocol was aiming at a short infusion period, the rational for that was to disclose any tendency for accumulation of dipeptide or constituents of the dipeptide. Urinary sampling was also performed to reveal any losses of dipeptide and/or the constituents of amino acids. The treatment group was compared to a control group given saline as placebo. The infusion rate was 0.625ml/kg/h (corresponding to 0.125g dipeptide/kg/h or 0.085g glutamine/kg/h) during 4h. This infusion rate gives the daily allowance during only four hours. All subjects reached a steady state level in dipeptide concentration during the 4h and a rapid elimination phase during the first 2h after the infusion was stopped (Fig. 8). In addition, there were no losses of dipeptide in the urine. The elimination constants for the dipeptide, were slightly longer than what has been reported in healthy subjects (Albers et al. 1988), but the values seen are still indicating complete removal of the dipeptide within two hour following the end of infusion.

In addition to the dipeptide, the constituent amino acids glutamine and alanine were analyzed together with glutamate. For these amino acids, plasma concentration reaches a steady state in some subjects but not in others. The elimination kinetics as reflected by half-life clearance demonstrates that basal plasma concentration was re-established in all subjects within eight hours after termination of the infusion. The study included one extreme outlayer, discussed in detail in the paper II. The statistics of the original publication, performed by a professional statistician did not contain calculations of the

endogenous rate of appearance, possible to deduct from the obtained results. However such calculations were made and are presented later in this summary under the appropriate heading.

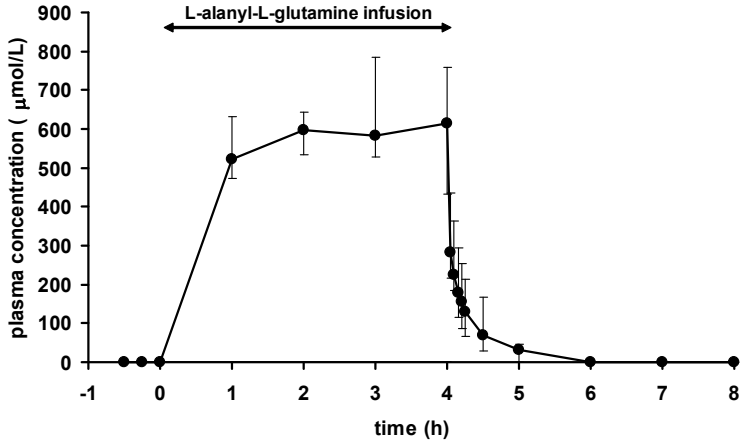

Figure 8. Plasma concentration of alanyl-glutamine. The plasma concentration of L-alanyl-L-glutamine before, during and after 4 hours infusion of L-alanyl-L-glutamine 0.125g ·kg $^{-1}$ h $^{-1}$ in ICU patients (n=10). None alanyl-glutamine was detected in the patients receiving placebo. Values are given as medians and (25, 75) percentiles.

4.3 INTRAVENOUS GLUTAMINE SUPPLEMENTATION DURING CRRT

A question often discussed in the context of ICU nutrition is how to handle patients on continuous renal replacement therapy (CRRT). It is well known that any dialysis treatment aiming at the removal of waste products such as urea and creatinine also will eliminate free amino acids. In intermittent hemodialysis the losses are in the magnitude of 10g amino acids during a 4h session (Lofberg et al. 2000). In the intensive care setting using continuous hemodialysis often combining with hemofiltration and hemodiafiltration, the flow through the dialysis filter is lower per time unit, but the elimination continues around the clock.

In the study protocol the choice was to use a pragmatic design. Hence the prescription of renal replacement therapy was entirely left to the attending intensivist. This means that the mode of CRRT as well as the dialysis dose, were not uniform among the patients studied. The pragmatic design also means that the time of dialysis or hemofiltration treatment during each 24h period was not standardized. To compensate for this a design was used where patients were studied on two consecutive days randomized to intravenous glutamine supplementation or placebo. The randomization was performed by lottery. In this type of protocol means, that each patient will serve as their own control. The necessary prerequisite to attain interpretable results is that the two days were similar. Similar in terms of legcirculation, in terms of dialysis dose, and in terms of as many other clinical parameters as possible Sepsis-Related Organ Failure Assessment (SOFA score) (Vincent et al. 1996) . If the two days come too far apart in terms of various clinical background data, the studied manipulation, the infusion of glutamine containing dipeptide or placebo will not be detectible.

The protocol includes a 20h infusion of alanyl-glutamine 0.5mg/kg/h. At the end of this infusion the exchange of glutamine across the leg was studied by use of the arteriovenous concentration difference combined with a measurement of the leg blood flow. Following the termination of the infusion, the decay curve over the following hours was studied in order to enable calculation of clearance parameters and the endogenous rate of glutamine appearance. The diafiltrate from the CRRT was sampled, and the glutamine content analyzed.

Plasma concentration of glutamine showed a 30% increase during the infusion. The losses into the ultrafiltrate correspond to 0.6-6.8g/24h. The level of glutamine loss in the ultrafiltrate was related to both the blood flows through the dialysis machine and the plasma concentration (Fig. 9, 10). The blood flow through the dialysis machine and filter was the stronger determinant of the two. The glutamine exchange across the leg, representing a constant export of glutamine was not different between treatment day and control day. Data from the decay curve including calculation of the endogenous rate of appearance will be commented on under a separate heading below.

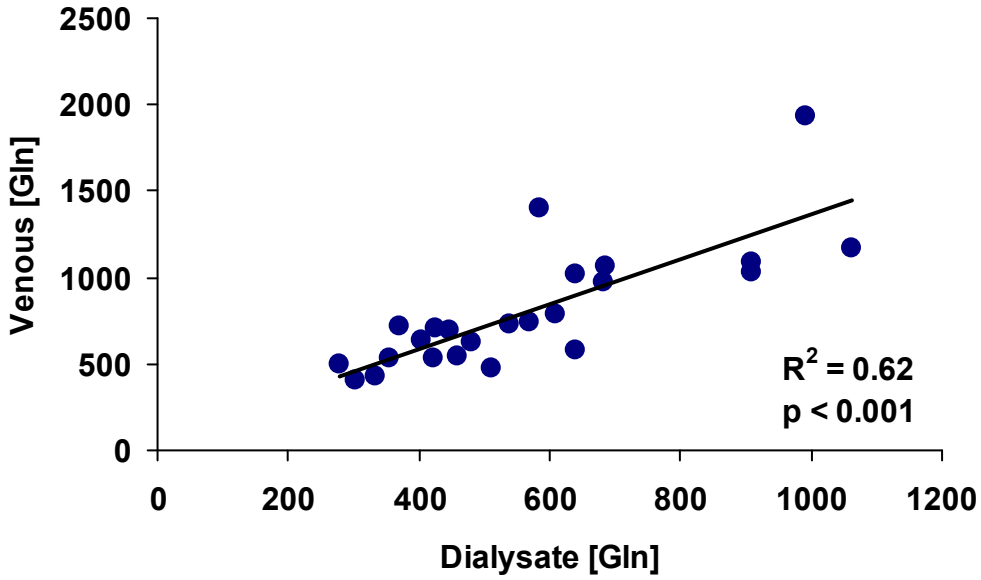

Figure 9. Correlation between the glutamine plasma concentration and glutamine losses in the ultrafiltrate. Mean concentration of venous glutamine of both treatment (alanyl-glutamine) and control day were correlated with the mean concentration of glutamine in the ultrafiltrate in 12 ICU patients treated with CRRT.

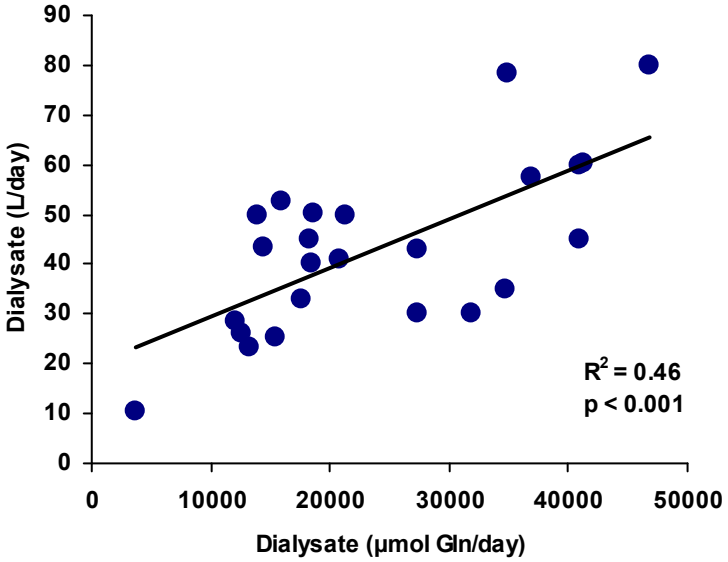

Figure 10. Correlation between the CRRT flow and the glutamine losses in the ultrafiltrate. Total loss of glutamine in the ultrafiltrate per day for both the treatment (alanyl-glutamine) and control day was correlated with the total CRRT volume per day in 12 ICU patient treated with CRRT.

4.4 GLUTAMATE CONCENTRATION AND EXCHANGE ACROSS THE BRAIN IN HEAD TRAUMA PATIENTS

The role of glutamate as a neurotransmitter is well characterized. There are a number of reports suggesting that severe brain damage, in particular with an unfavorable outcome, are associated with high free glutamate concentration in cerebral spinal fluid and in the interstitial fluid of the brain. The purpose was therefore to study patients following head trauma with GCS<8, who routinely are given a microdialysis catheter in penumbra zone of the injured brain. The purpose of the study was to provide exogenous intravenous glutamine supplementation and to monitor interstitial brain concentration of glutamine and glutamate together with AV-difference of glutamine and glutamate across the brain and the leg. In addition the endogenous glutamine rate of appearance way calculated. The protocol used was identical to that used in the CRRT patients above, with the same emphasis upon the need to study two comparable days for the evaluation.

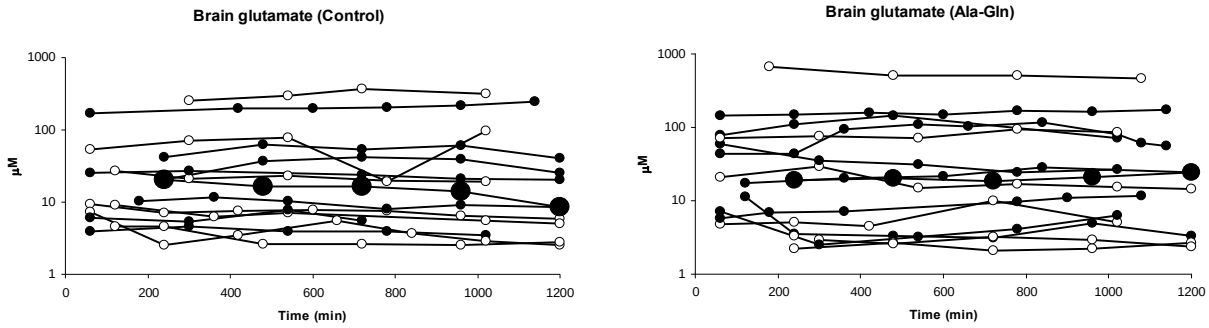

Figure 11. Interstitial cerebral glutamate concentrations in head trauma patents, during treatment and placebo day. Interstitial cerebral glutamate concentrations obtained via a microdialysis catheter placed adjacent to the injured area in head trauma patients given L-alanyl-L-glutamine (right-hand) or placebo (left-hand) during 20 h. Filled symbols represents patients randomized to be given glutamine before placebo, while open symbols represents patients given placebo before glutamine. The bold line represents median values.

The results show interstitial glutamate and glutamine concentrations in the brain which are unaffected during the treatment day as well as during the control day. In particular for glutamate there is a large interindividual variation, irrespective whether exogenous

glutamine was provided or not (Fig. 11). The subjects studied were too few to make any statement on possible relation between outcome and the level of glutamate in the microdialysate. The overall outcome of the patients included into the study was favorable with 14/15 patients with a Glasgow outcome score of 3 or better at hospital discharge, and only one death (Kaye et al. 2000). However, the whole discussion of the level of glutamate and favorable or unfavorable outcome is beyond the scope of this particular study.

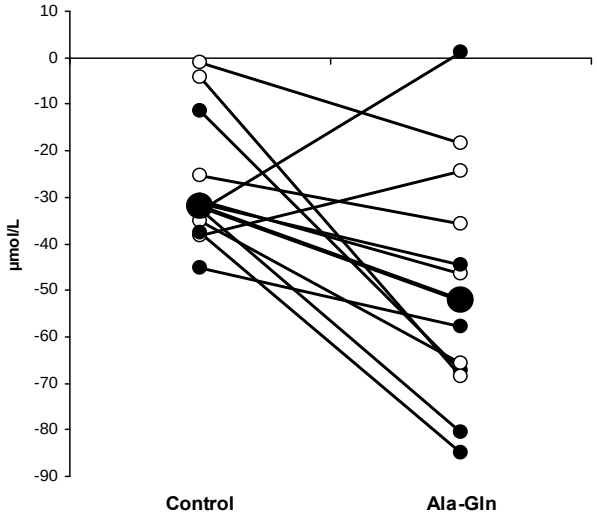

Figure 12. A-v difference between arterial and jugular vein of glutamine over the brain. Glutamine a-v difference across the brain in head trauma patient (n=15) studied on 2 consecutive days with or without exogenous gluatmine supplementation in a cross-over design. The filled symbols represent patients randomized to treatment before placebo, open symbols represents placebo before treatment. The bold line shows the median value. There is a negative a-v balance for glutamine ($p<0.0001$) during control as well as treatment periods.

In theory, exogenous glutamine supplementation may hinder the efflux of glutamine from the brain. The background to the importance of glutamine efflux from the brain is that possible the elimination pathways of intracerebral free glutamate are 1) re-uptake in the neuron, 2) degradation, 3) uptake into astrocytes and conversion into glutamine, which is then exported out of the brain. Quantitatively the third alternative is by far the most important. Hence there is a constant export of glutamine from the brain (Fig. 12). Theoretically an elevation of plasma glutamine concentration may compromise this

export. The results from the study indicate that the arterial venous concentration difference was not decreasing during glutamine treatment. As there was no estimate of cerebral blood flow, the assumption is that the cerebral blood flow was unaltered and therefore the export of glutamine was unaffected. In comparison, the exchange of glutamine across the leg was also included, and no difference between the treatment and the control days were seen, similar to that for the CRRT patients.

4.5 ENDOGENOUS GLUTAMINE RATE OF APPEARANCE

The advantage of NCA is that it is rather assumption free compared with compartmental models. However, it is assumed that systemic elimination takes place from the measured central compartment and that the model parameters are constant over time. In study II drug safety was the main issue and therefore focus was set on elimination. However, in studies III and V R_a also caught our interest and we therefore turned to turnover models.

All compartmental models are over simplifications, but could still be useful. The simplest model that describes data well should be chosen. This means that the one-compartment model was the model of choice even if it is known that amino acids are neither produced into nor eliminated directly from the central compartment.

A too short study time can give falsely high values of R_a and CL in both NCA and turnover models and also in isotope tracer studies (Van Acker et al. 1998).

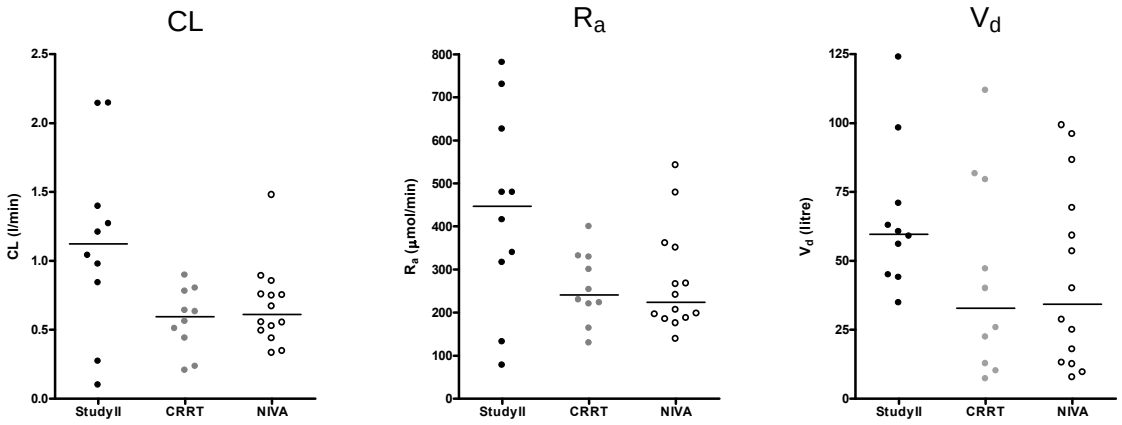

Figure 13. Clearance, rate of appearance and distribution volume in studies II, III and V. Individual estimates of pharmacokinetic model parameters from study II (filled symbols), study III (grey symbols), and study V (open symbols). All data were analyzed by a one-compartment turnover model. Median value is marked.

In several studies of this thesis the endogenous rate of appearance for glutamine was calculated. The hypothesis that exogenous glutamine supplementation may be beneficial for intensive care patients rests upon the assumption that the endogenous production is insufficient. So far the endogenous glutamine production, usually measured as endogenous glutamine rate of appearance has not been quantified. There is literature in healthy individuals or in individuals undergoing elective surgery (van Acker et al. 2000a, Jackson et al. 2000), but measurements among intensive care unit patients, in particular with multiple organ failure, have so far not been available. The values presented in the studies over metabolic tolerance, CRRT treatment and in head trauma patients show a uniform pattern (Fig. 13). The mean and median values are well in accord with the estimates from healthy individuals mean values 283±31, (Darmaun et al. 1986) 280±23 (Kreider et al. 1997).The healthy individuals in the literature are not comparable to ICU patients in terms of age or underlying health status. Nevertheless the endogenous production of glutamine seems to be on the same level in ICU patients, mean value.271±120 in head injury patients and 258±83 in CRRT patients. It can also be seen that the scatter in between individuals is large, but it is not possible to conclude whether the scatter is larger than expected or if it is a reflection of physiology. Again the references to healthy individuals and to elective surgery patients are not sufficiently matched to allow any conclusions regarding the interindividual scatter.

5 GENERAL DISCUSSION

The metabolic tolerance to an exogenous supply of glutamine containing dipeptides was addressed in this project. In studies III–V the daily dose was administered during 20 h resulting in a mean increase in plasma concentration of approximately 50% (Fig. 14). In all subjects it was within the physiological range during the supplementation. In study II a similar dose was infused during 4 h, resulting in supraphysiological levels in individual subjects. All these levels were cleared back to baseline within 8 h, but the upper limit to clear glutamine in some individuals. As pointed out in the discussion in the article (study II) approximately 50% of the subjects included in that study achieved a steady state of plasma glutamine, usually within the physiological range or at a slightly elevated level. Some subjects, however, did not attain a steady state in plasma glutamine level during the 4 h. This indicates that an infusion rate at this level cannot be recommended beyond that time period. In clinical practice in the ICU a continuous infusion of glutamine containing dipeptide for 24h has several advantages. All patients, so far studied, attain a steady state within the physiological range, and the plasma concentration will be kept within the physiological range, witch may have implications for glutamine availability. The mechanisms for glutamine availability to explain the beneficial effects of glutamine supplementations are, however, beyond the scope of this research program.

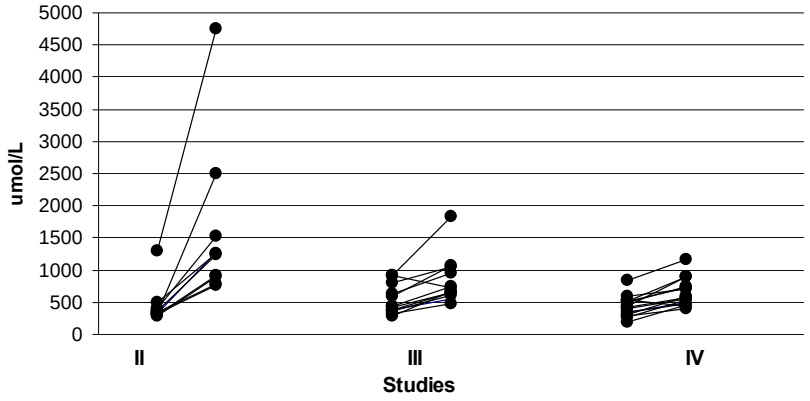

Figure 14. The increase of glutamine in ICU patents. The figure demonstrates the glutamine concentration in plasma before and after glutamine supplementation in studies II, III and IV.

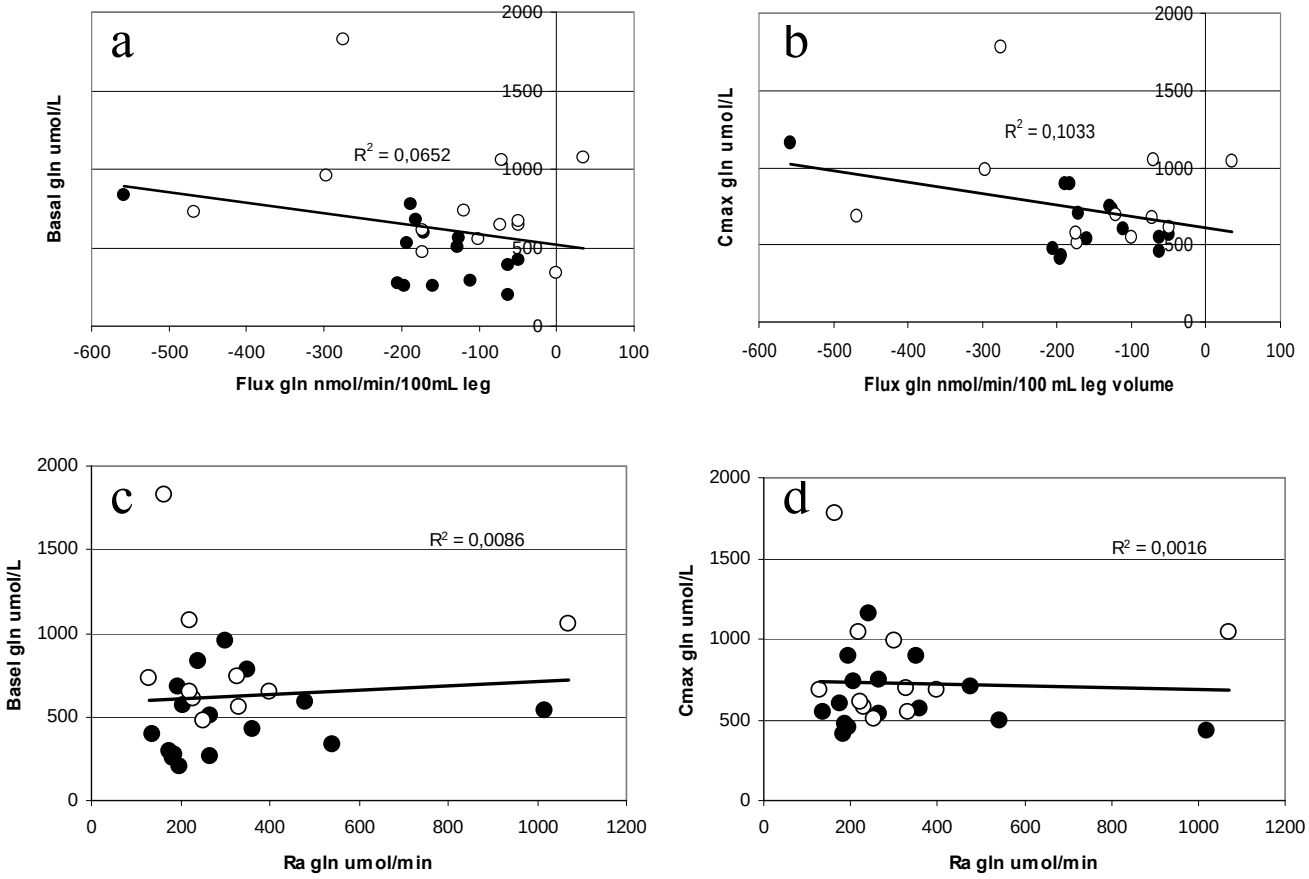

Figure 15. <u>Correlation glutamine concentration and glutamine kinetics.</u> a) The glutamine concentration in plasma before alanyl-glutamine (C_0) and b) max concentration of glutamine at the end of the 20h infusion period off alanyl-glutamine (C_{max}) correlated with the flux of glutamine across the leg in study III and IV. c) The glutamine concentration in plasma before alanyl-glutamine (C_0) and d)max concentration of glutamine at the end of the 20h infusion period of alanyl-glutamine (C_{max}) correlated with the rate of appearance (R_a) in study III and V. The open symbols represent patients in study III and the filled symbols represent study V.

The characteristic decrease in glutamine plasma concentration and also the decrease in muscle glutamine concentration are well described (Biolo et al. 2005, Gamrin et al. 1996, Roth et al. 1982**,** Tjäder et al. 2004a). Nevertheless, the mechanisms behind these changes in concentrations have not been explained satisfactorily. Despite the low tissue concentration of glutamine in skeletal muscle, the export of glutamine from leg muscle as well as the rate of appearance as calculated within the framework of this research program, are normal or perhaps even slightly elevated (Darmaun et al. 1986, Kerin et

al. 1991). Also the low plasma concentration does not seem to hinder the export of glutamine from muscle, to the splanchnic area to be kept on the same level as when normal plasma glutamine concentration is at hand (Boza et al. 2001). Using the data from the studies in this research program shows no correlation between plasma concentrations of glutamine in the basal state, or during exogenous supplementation and the efflux of glutamine from the leg or the calculated rate of appearance were seen (Fig. 15). The independence of glutamine efflux from the leg in relation to plasma concentration has been demonstrated before (Vesali et al. 2002). This is in contrast to the concentration dependent uptake of glutamate in skeletal muscle and cardiac muscle (Vesali et al. 2002**,** Svedjeholm et al. 1990). Also the results from this research program demonstrate such a plasma concentration dependent glutamate uptake (Fig. 16). There is data from animal experiments indicative of a concentration dependent uptake of glutamine within splanchnic area (Bruins et al. 2003). This is probably not the case in intensive care patients, as the export from the leg is independent of plasma concentration, and the reciprocal uptake in splanchnic area will be of the same magnitude as the export from the leg (Boza et al. 2000). Nevertheless, the increase in plasma concentration seen after exogenous supplementation must result in a higher uptake, most likely in the splanchnic area, but without any negative feedback on the export from muscle.

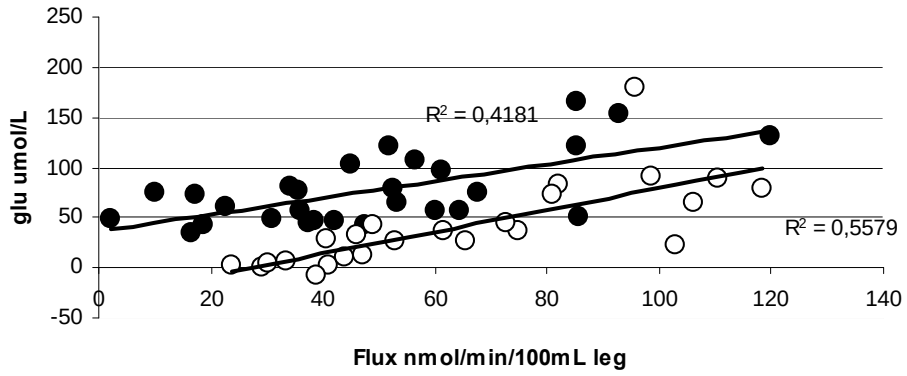

Figure 16. <u>Correlation between glutamate concentration in plasma and glutamate flux from muscle tissue.</u> Glutamate concentration in plasma during both the placebo and the treatment day correlated and glutamate flux across the leg in ICU patients in study III and V. The open symbols represent study III and the filled represent study V.

The experimental designs of the studies in this research program do not permit any evidence over how plasma concentration of glutamine may be regulated. Still, as pointed out before, the decrease in concentration in plasma as well as in tissue is established quite early in the cause of disease (Tjäder et al. 2004b). The majority of the patients studied within this research program demonstrate plasma concentrations of glutamine below the normal range (Fig. 17). This low level is stabile over time and is established quite early in the cause of disease. In studies where the initiating event can be controlled, such as the temporal pattern following elective surgical trauma, or the temporal pattern following an endotoxin challenge in healthy volunteers, a decrease in plasma glutamine concentration starting up at 4–6 hours and to be well established within 12 h is seen (Vesali et al. 2005, Hammarqvist et al. 1990). The decrease in muscle tissue concentration comes in parallel. The most likely explanation in that the transmembrane transport of glutamine, that maintains the concentration gradients across cell membranes, is somehow set at another level. As a result dramatic changes in concentrations can be seen, but not in the interorgan exchange of glutamine. This is an argument against that the concentration in itself may be a regulatory mechanism.

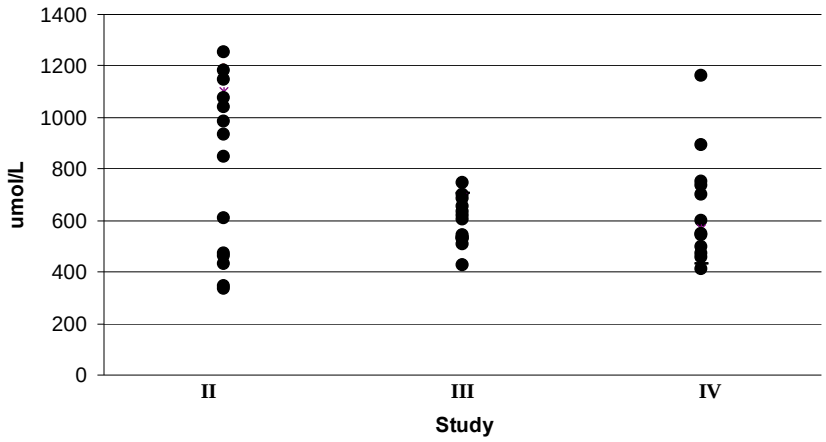

Figure 17. Plasma glutamine at baseline. The figure shows the baseline concentration of glutamine value for study II, III and IV. Each point represents one patient.

In studies III and V the export of glutamine from muscle tissue is measured quantitatively. The no change in muscle export has been indirectly demonstrated before (Jackson et al. 1999, van Acker et al. 2000b), but this is the first time such measurements were performed prospectively using the patients as their own controls. As the study was confined to only one day of glutamine supplementation, it cannot

totally be ruled out that the picture may change after several consecutive days of glutamine supplementation. That remains to be studied. However in earlier studies providing an posthoc analysis of patients given exogenous glutamine supplementation over a longer time period, no such difference was seen (Vesali et al. 2002).

The design of study II was partly to achieve a plasma glutamine concentration well above the basal level to allow for a well defined decay curve to calculate clearance. The results in studies III and V show that also the decay curve from an increase within physiological range give the same opportunity for these calculations in addition the rate of appearance may be calculated. The accuracy of these calculations are more related to the stability of the basal concentration level, the number of concentration determinations in the decay curve and to whether or not a steady state has been achieved in plasma concentration during the period of glutamine supplementation (Cmax). Under the assumption that rate of appearance of glutamine did not change in relation to glutamine plasma concentration, rate of appearance was calculated (Fig. 13). The rational for these assumptions was of course the no change in glutamine export from muscle. In spite of this, the underlying assumption needs to be validated in future studies. The requisite for a steady state in plasma glutamine concentration during the exogenous infusion was well illustrated by the results from study II, which for the subjects that did not attain a plasma glutamine steady state gave erroneously high estimates of their glutamine rate of appearance. The results from studies III and V demonstrate a glutamine rate of appearance on the level of what is seen in the literature (Van Acker et al. 1998). The relation between glutamine rate of appearance and the factors that may influence the rate of appearance is another fascinating research area that needs to be addressed in future studies.

As stated above, the aim of this research program was mainly to document safety of glutamine administration in ICU patients. Beneficial effects have been demonstrated from intravenous glutamine supplementation by others (Griffiths et al. 1997, Goeters et al. 2002). The patient groups where the positive effect upon mortality has been demonstrated are ICU patients with need of total parenteral nutrition. The indicating for glutamine supplementation by the enteral or parenteral route to ICU patients on enteral nutrition is less well substantiated. It has been suggested to use plasma glutamine concentration to settle the indication for exogenous glutamine supplementation (Wernerman 2004). This suggestion is hypothetical and needs to be verified by

prospective studies. The evidence demonstrate that intravenous glutamine is beneficial, but so far it has not been demonstrated that normalisation of plasma glutamine level as a result of the intravenous supplementation is connected to beneficial effects. The reasons for the less convincing results in enterally fed ICU patients may be related to several issues. Enteral supplementation with glutamine is not as effective as parenteral to increase plasma glutamine level (Fish et al. 1997). This is related to a relatively high first pass extraction through the splanchnic area and liver when glutamine is provided by the enteral route (Haisch et al. 2000). The number of studies giving parenteral glutamine to enterally fed patients are so far few, of small size, and therefore not conclusive (Wischmeyer et al. 2001). The studies where enterally fed ICU patients are given enteral supplementation with glutamine contain very different groups of ICU patients. The largest study, comprising more patients than all other studies of enteral supplementation to ICU patients put together, has a very low mortality rate and a very short mean ICU stay time (Hall et al. 2003). The majority of patients included in that particular study are therefore different from the patients given intravenous glutamine supplementation where a beneficial effect upon outcome has been demonstrated.

6 CONCLUSIONS

In conclusion, in this thesis work intravenous glutamine supplementation by the use of a glutamine containing dipeptide has been studied in 3 different groups of ICU patients. Results of the studies can be summarised in the following conclusions.

1. A glutamine containing dipeptide can be safely administered in a peripheral vein as a concentrated solution with a high osmolality. No signs of trombophelebitis or any other damage to the veins could be detected.
2. A glutamine containing dipeptide can be safely administrated intravenously also at a high infusion rate. In particular no tendency of dipeptide accumulation in plasma could be detected, and the losses in the urine were below the detection level. Both for the dipeptide and for the constituent amino acids plasma concentration were back to baseline within 8 hours after termination of the infusion..
3. Intravenous supplementation with a glutamine containing dipeptide during continuous renal replacement therapy resulted in an increase in plasma glutamine concentration, but the losses in to the ultrafiltrate were not different during the treatment day as compared to the control day. Nevertheless the losses of glutamine into the ultrafiltrate which occur regardless of intravenous glutamine supplementation are not neglectable.
4. Exogenously supplemented glutamine does not interfere with interstitial glutamate concentration in the brain following head trauma. Furthermore, the balances of glutamine and glutamate across the brain are also left unaltered during glutamine supplementation in these patients.

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