UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA

FINAL DEGREE PROJECT

Clinical evidence of parenteral glutamine supplementation in critical illness

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Main Field:
CLINICAL PHARMACY AND PHARMACOTHERAPY
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Abstract

Glutamine is a non-essential amino acid mostly synthetized and released to the blood stream by the skeletal muscle and the lungs. The enterocytes and the immune system cells are great glutamine consumers and glutamine is crucial for their correct function. Due to metabolic changes occurred during critical illness, the plasma level of glutamine becomes low and skeletal muscle becomes depleted. Treatment with glutamine tries to normalize plasma levels and improve the immune cell response. Glutamine's chemical properties are unfavorable for their storage and so new ways of glutamine delivery are enveloped. The most current used alternative source are glutamine dipeptides. Administration of glutamine can be supplying the artificial nutrition support or being administrated independently; by parenteral or by enteral route. Traditional trials of glutamine supplementation demonstrate favorable results in patients with critical illness. However, newer trials which included more study population, among which the REDOX trial, where not able to confirm these results. A trend towards favorable outcomes were found in surgical and oncological patients with intravenous glutamine supplementation on physiological doses without renal and/or liver dysfunction. In contrast, there was also found high glutamine baseline levels in a subgroup of critically ill patients. With all the available clinical research information, it is still not clear if glutamine should be included in the routine clinical use.

Resumen

La glutamina es un aminoácido no esencial sintetizado y liberado a la circulación sanguínea mayoritariamente por la musculatura esquelética y los pulmones. Los enterocitos y las células del sistema inmunitario son grandes metabolizadores de la glutamina y ésta es crucial para su funcionamiento óptimo. Debido a cambios metabólicos sufridos en el paciente crítico, los niveles sanguíneos de glutamina disminuyen significativamente y se observa una depleción de glutamina en la musculatura esquelética. El tratamiento con glutamina tiene como objetivo normalizar los niveles plasmáticos y mejorar la respuesta del sistema inmunitario. Las propiedades químicas de la glutamina son desfavorables para su almacenaje y por eso se han desarrollado nuevas estructuras químicas de glutamina para ser administradas. La fuente alternativa más utilizada actualmente son los dipéptidos de glutamina. La administración de glutamina se puede realizar suplementando a la nutrición artificial o administrándose de forma independiente; por vía parenteral o bien por vía enteral. Los estudios tradicionales sobre la suplementación con glutamina demuestran resultados favorables en el paciente crítico. En cambio, estudios posteriores que incluyen un mayor número de pacientes, entre los cuales destaca el estudio REDOX, no han sido capaces de confirmar dichos resultados. Los estudios demuestran una tendencia favorable en pacientes quirúrgicos y oncológicos con suplementación de glutamina por vía parenteral en dosis fisiológicas que no presentaban insuficiencia renal y/o hepática. En cambio, también se han detectado en un subgrupo de pacientes niveles elevados de glutamina. Aun así, con toda la información clínica disponible actualmente, sigue siendo poco concluyente la inclusión de la glutamina en la práctica clínica habitual.

Integrating educational fields

Clinical pharmacy is one of the pharmacy specialties where pharmacists take care of the correct and safe utilization of drugs and other medical substances through pharmaceutical care and intervention; inform and advise patients about their treatment, support physicians and other healthcare professionals by informing about the rational use of medication, conduct observational studies and clinical trials of therapeutic effects and educate civilization to promote health and prevent disease. These are just some of the clinical pharmacist responsibilities.

Hospital pharmacy, a branch of clinical pharmacy, has further specific functions in the hospital ambit taking charge of hospital treated diseases, drugs for hospital use or hospital diagnostic, toxicology and pharmacokinetics and elaboration of parenteral and enteral nutrition. Prescribing and preparing parenteral nutrition individualized for every patient, including glutamine supplementation, is part of their daily practices. Therefore, the main education field of this final degree project is clinical pharmacy and pharmacotherapy.

In order to understand the implications of glutamine supplementation in the critically ill patients, physiology and physiopathology is the second education field integrated in this literature review. Glutamine physiological functions, the patients' metabolic adaptation response to critical illness and glutamine requirements and utilization during a stressful situation are some of the studied and mentioned contents of this field.

Finally, pharmaceutical technology is integrated as part of the clinical hospital pharmacy activities in elaboration of glutamine solutions and glutamine-supplemented parenteral nutrition. This part has some particular interest as glutamine has chemical properties which make the routine clinical use difficult and could have an impact on the treatment effects.

Introduction

In the last years, the importance of nutrition support has been raised. The prevalence of hospital malnutrition was a common finding and appears to be an independent predictor of outcomes and associated with a higher rate of morbidity and mortality. Nutrient deficiency is one of the most common causes of immunodeficiency as nutrition is a critical determinant of the immune system response. Therefore, physicians start to consider adequate nutrition support crucial for recovery[1].

Critically ill patients are mostly not able to eat (enough) by themselves, so artificial nutrition support, defined as the administration of nutrient solutions by the enteral or intravenous (parenteral) routes, is required to supply all or the remainder part of the patients' needs. Enteral nutrition support is preferred. Parenteral nutrition is only indicated when enteral route is contraindicated (e.g. in case of gastric complications) or fails to cover the patients' requirements[1].

Until now, controversy persists in defining adequate nutrition support. Guidelines between hospital settings vary in timing, caloric quantity, protein needs, monetarization, use of adjuvant therapies such as probiotics or immunonutrition, support during specific conditions, etc. Many clinical trials are performed in order to clarify findings and to develop recommendations for adequate nutrition support practice, including for particular nutrients, such as glutamine.

Glutamine is the most abundant free amino acid found in the human body pool and blood stream. In a healthy man, the plasma values range between 450 and 650 μmol/L although intracellular levels are 25-30 times greater. Glutamine is considered a non-essential amino acid; it is not a required dietary nutrient for humans as it can be synthesized endogenously[2].

The physiological pathway and metabolism of glutamine have been studied during years and glutamine has showed to be an important substrate for different tissues, especially for the gut and the immune system[3,4]. When the immune system is activated (e.g. during illness) different human defense systems increase their glutamine consumption and overcome the endogenous synthesis capacity, causing glutamine muscle depletion and low plasma levels. Glutamine becomes essential and therefore it's considered a conditionally essential amino acid[2].

In order to cover the metabolic deficiency and to let critically ill patients benefit from different glutamine functions, randomized clinical trials have been performed with glutamine supplementation. Most of the earlier small trials using parenteral glutamine supplementation could prove the glutamine efficacy by enhancement of clinical outcomes in critically ill patients.

Over time, the field of nutrition support progressed from simply solving the problem of providing enough micronutrients to meet metabolic demands to the current search for individual nutrient regimes to optimize immune function and cell recovery. Those nutrients which stimulate the cellular defense system and reduce infection or other complications in pharmacologic doses, like glutamine, are called “pharmaconutrients” or “immunonutrients”[5].

Nevertheless, when this new concept was putted in practice by a scientific strong multi-center randomized clinical trial, known as the Reducing Death due to Oxidative Stress (REDOX) trial[6], the results were not as expected. This trial fails in finding any benefit from glutamine supplementation and could even associate an increased 28-day mortality to the intervention group. This study raised new questions around the actual evidence of glutamine support in critical illness.

Objectives

The aim and principal objective of this literature review was to discuss the current clinical evidence related to parenteral glutamine supplementation in the intensive care unit (ICU) since the results of the REDOX trial have opened a new discussion around this subject.

Secondary study objectives were:

- To learn about the metabolic pathways and physiological functions of glutamine in a healthy human body.
- To figure out the potential beneficial implications of glutamine in critically ill patients.
- To understand the process of glutamine supplementation in the routine clinical practice, from preparation to administration, and the different associated problems.
- To have knowledge of the current clinical guidelines recommendations of glutamine supplementation in the critically ill patients.
- To analyze the existing scientific evidence of glutamine supplementation during the years and interpret their results according to different identified variables.
- To interpret the results of the REDOX trial and to know why these differ from the earlier realized studies.

Material and methods

This original literature review about intravenous glutamine supplementation in the ICU was performed between February and May 2017. PubMed, a free research website connected to the Medical Literature Analysis and Retrieval System online (MEDLINE) database, which contains the most relevant publications in the setting of life science and biomedics, was used to explore and acquire scientific articles of interest. The bibliographic review was completed in English. Only free and facilitated by the *Centre de Recursos per a l'Aprenentatge i la Investigació* (CRAI) articles could be consulted and included. The REDOX trial, which promotes to produce the actual review, was provided by my mentor of this final degree project.

First, an exhaustive bibliographic search of background information about glutamine and critical illness was carried out to understand and interpret the results of the later included clinical trials. Some of the main keywords utilized in this research were "glutamine" in combination with "physiology", "functions", "critical illness", "metabolism", "preparation" and "kinetics". From these first found articles, new sources of interest were provided from its bibliography.

Second, the most relevant current guidelines of glutamine supplementation were extracted from the official websites of the American Society for Parenteral and Enteral Nutrition (ASPEN), The European Society for Clinical Nutrition and Metabolism (ESPEN) and the Critical Care Nutrition at the Clinical Evaluation Research Unit (CERU).

Third, clinical trials and meta-analysis were obtained using the search terms "glutamine" in combination of "parenteral", "supplementation", "administration", "nutrition", "support", "intravenous", "critically ill patients", "critical illness" and "ICU", applying the search filters "clinical trial" and "meta-analysis". More clinical trials were extracted from these consulted meta-analysis. Clinical trials and meta-analysis were included if they focused mostly on parenteral glutamine support and on patients of the intensive care unit. These studies were classified in if they were performed before the REDOX trial (pre-REDOX) or after the REDOX trial (post-REDOX).

Finally, commentaries on the REDOX trial and other recent literature reviews of glutamine supplementation in critically ill patients were explored and interpreted as inspiration for my own point of view.

Results and discussion

1 Glutamine

1. 1 Endogen synthesis

In physiological conditions, dietary glutamine coming from different protein sources only represents 12% of the whole-body glutamine appearance so glutamine availability depends more on the endogen synthesis[7].

The glutamine synthetase is responsible for the endogenous formation of glutamine by catalyzing the condensing reaction of glutamate and ammonia (NH_4^+) in presence of adenosine triphosphate (ATP). This cytosolic enzyme is mostly found in the skeletal muscle and lungs[2].

$$Glutamate + ATP + NH_4^+ \rightarrow Glutamine + ADP + Pi$$

On the other hand, glutamine is hydrolyzed by the mitochondrial glutaminase obtaining glutamate and ammonia. Rapidly dividing cells, like enterocytes and some immune system cells, present a high activity of this enzyme and are consequently great glutamine consumers[2].

$$Glutamine + H_2O \rightarrow Glutamate + NH_4^+$$

The metabolism of glutamine depends on the presence and activity of both enzymes. Both are dispersed differently along the human body and so glutamine production and consumption differs between tissues. In physiological conditions, there is a balance between both reactions and the homeostasis of glutamine is maintained. However, some psychopathological conditions can lead to a shortage of glutamine with consequently harmful effects on high-consume tissues[2,7].

1.2 Metabolic pathways

Glutamine acts as nitrogen and carbon skeletal source in order to maintain the cell function. Glutamine and glutamate are important substrates in the intermediary metabolism of the cells and are precursors of many compounds (Figure 1)[3,7].

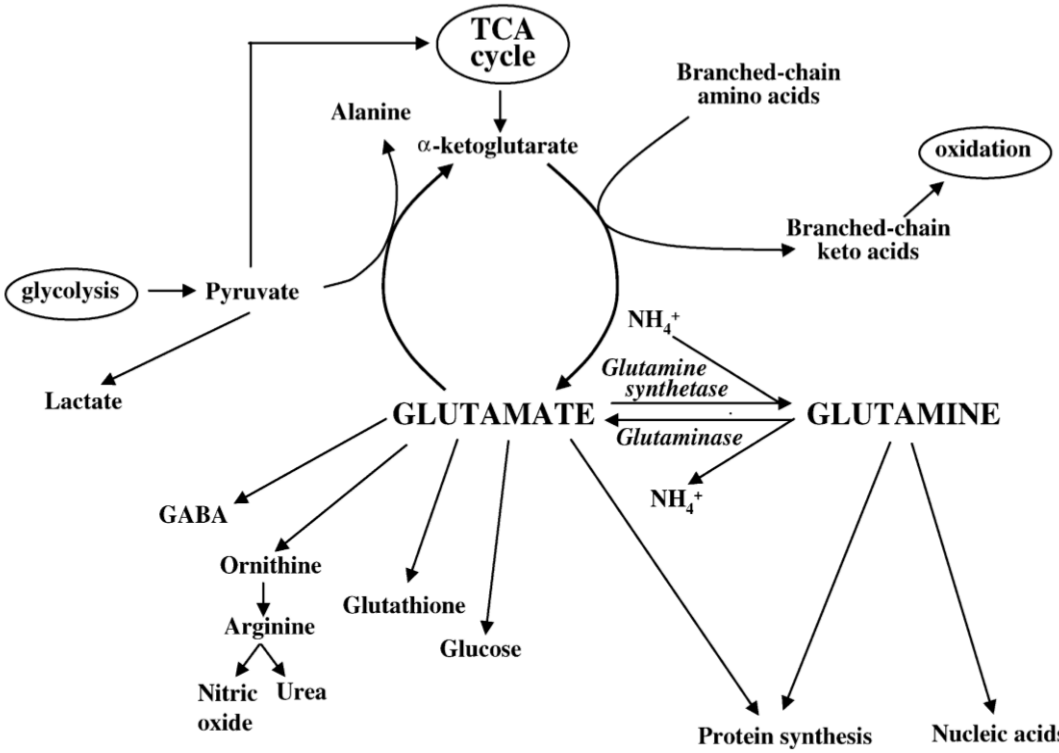

Figure 1. Overview of glutamine and glutamate metabolism. Glutamate is produced from glutamine through glutaminase activity. Glutamate can be converted into glutamine through glutamine synthesase activity. TCA: tricarboxylic acid. GABA: gamma-aminobutyric acid. [Adapted Biolo et al.[7]].

Glutamine metabolism occurs in all tissues and cells although some metabolic pathways preferentially occur in some specific organs. Glutamine use for gluconeogenesis occurs typically in kidneys and liver whereas gamma-aminobutyric acid (GABA) synthesis is more observed in neurons.

Furthermore, glutamine is used as energy source by the rapidly dividing cells, mostly of the immune system, and enterocytes.

1.3 Physiological functions

As the implied enzymes in the metabolism of glutamine and the related metabolic pathways vary hardly between tissues, glutamine has not the same importance to maintain their correct cell function. Some tissues consume large quantities and glutamine is an essential metabolic precursor. These tissues are the most affected when shortage of glutamine occurs.

Lungs and skeletal muscle are the most important storages of glutamine of the human body. Both release big quantities of glutamine to plasma and are an important source of glutamine in physiological, postabsorptive and hypercatabolic states, characterized in metabolic stress states. This is possible due to the high glutamine synthesis activity present in both organs.

Glutamine represents more than 50 per cent of the intracellular free amino acid pool of the skeletal muscle and is a normal constituent of many muscle proteins. Physiological intracellular glutamine levels are significantly higher than extracellular levels. Glutamine is obtained from *in novo* synthesis and, in less proportion, by proteolysis. A large amount of glutamine is released to plasma and a small part is used by the own muscle tissue, mostly for protein synthesis[7].

In the liver, bidirectional flux of glutamine exists as both synthesis and hydrolysis occurs. The net flux depends on the body needs and state at each moment, as glutamine metabolism adapts itself and regulates glutamine homeostasis. The perivenous scavenger cells of the liver are responsible for the synthesis of glutamine, cleaning up the bloodstream from ammonia. In the periportal cells, glutamine is broken up and used especially for gluconeogenesis and urea synthesis[3].

On the other hand, gut and immune system are the biggest consumers of glutamine, making glutamine an especially important factor for the correct cell function. Gut obtains glutamine mostly from the lumen in postpandrial state and from the bloodstream in postabsorptive state and is required in order to conserve the integrity, function and metabolism of the gut, used as major fuel by the enterocytes[2,3]. As a result, glutamine helps reducing the bacterial translocation and supports the immunological function of the intestine tract, being beneficial to reduce infection complications in critically ill patients[8].

Lymphocytes and macrophages are great glutamine-spending immune cells during their proliferation processes, which occurs when the immune system is activated due to a need of protecting against disease and foreign bodies. In fact, proliferating lymphocytes have a 10-fold greater glutamine utilization compared with resting cells[9]. Glutamine provides the immune system metabolic intermediates for synthesis of purines and pyrimidines and is used as energy source[4].

Nevertheless, glutamine has other essential functions related to cell survival during stress. Glutamine stimulates the expression of heat shock proteins (HSP), which are a group of proteins which enhance cellular survival in stressful situations[10]. Glutamine and glutamate are also precursors for glutathione synthesis which play a role in the antioxidant defenses of the cells[11].

Kidneys are small consumers of glutamine, where it's used mostly for renal gluconeogenesis and ammoniagenesis. New synthetized ammonia is excreted and linked to increased acid excretion, helping to sustain the acid-base balance. In acidosis conditions, glutamine renal absorption becomes higher satisfying the higher need of ammonia excretion[3].

Generally, liver and kidneys are the principal organs implied in glutamine metabolism. Therefore, when liver and renal functions are impaired, the level of glutamine and ammonia in plasma increases and becomes harmful[4].

2 Critical illness

2.1 Metabolic changes

Critical illness is paired with an adaptive stress response. This typical and complicate response implies changes in neuroendocrine pathways, including an activation of the sympathetic nervous system and the hypothalamus-pituitary axis which results in increased circulating levels of stress hormones, such as corticoids and catecholamines. Besides, inflammation mediators and cytokines are produced in high quantities. All these chemical changes affect the metabolic state of the patient, leading to a generalized catabolic state with resistance to anabolic signals (such as insulin)[12].

Clinically, those patients with stress induced metabolism have variations in their energy expenditure, use different sources of energy substrates, suffer from stress hyperglycemia (due to excessive gluconeogenesis and glycogenesis and insulin resistance), experience alterations in lactate metabolism and have an increased rate of lipolysis and proteolysis (therefore lean body mass is loosed). The nitrogen balance of these patients is negative, which reflect the higher protein breakdown than synthesis[12].

2.2 Glutamine's role

Glutamine plays a vital role in critically ill patients by modulating the inflammatory response, inducing glutathione synthesis and promoting HSP expression. The demands of glutamine are therefore increased and deficiency is especially harmful as rapid cell proliferation is required[11].

Skeletal muscle and lungs are responsible to maintain the glutamine plasma levels by releasing higher levels of glutamine to the extracellular medium in intention to subserve the demands of glutamine. The associated metabolic changes to the adaptive stress response affect consequently the cellular glutamine metabolism. The increased levels of stress hormones, primarily glucocorticoids, can up-regulate the glutamine synthetase expression and increase glutamine release to plasma[7,11].

However, the endogenous glutamine production becomes insufficient and skeletal muscle spends glutamine from the intracellular pool and by breaking down own muscle proteins, promoted by the overall catabolic state. The human body reservoir of glutamine becomes depleted as the increased requirements overcome the muscle and lung releasing and glutamine becomes essential[2,7]. In consequent, blood glutamine levels fall[13].

Low glutamine plasma and tissue levels impair the cellular stress response and altered immune function. Glutamine deficiency has been associated to the severity of illness and hospital mortality as independent variables[13,14]. Critically ill patients may benefit from glutamine supplementation in order to normalize glutamine levels and promote several metabolic cell functions (Figure 2)[15]. Nevertheless, critical illness is not necessarily associated with a low plasma glutamine[13].

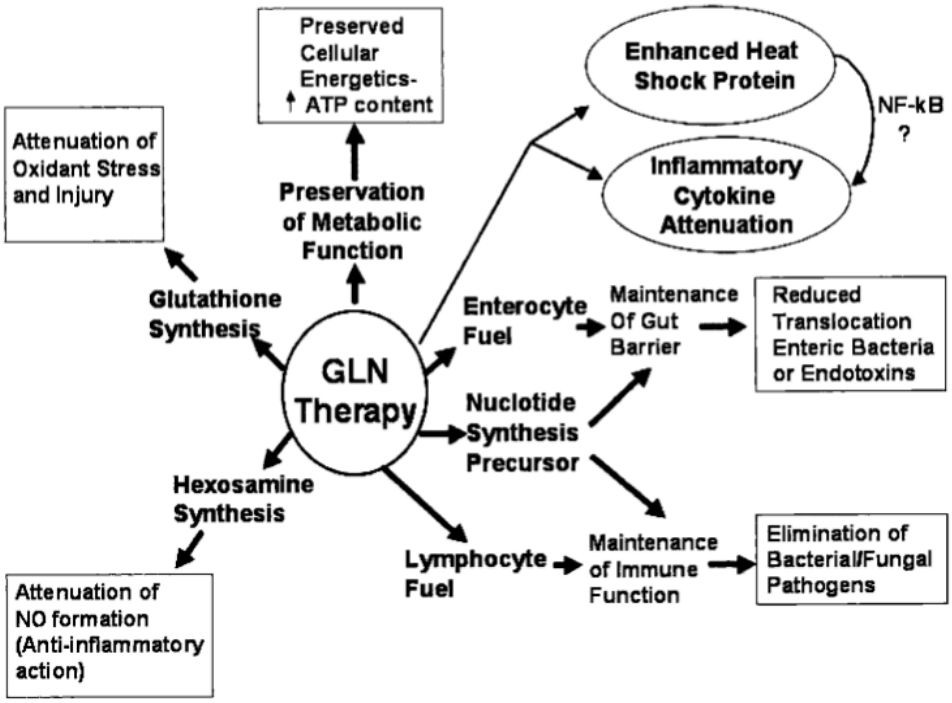

Figure 2. Potential mechanisms of GLN's beneficial effects in critically ill patients. NO: nitric oxid. NF-kB: nuclear transcription factor. ATP: adenosine triphosphate. [Adapted from Wischmeyer et al.[15]].

Doses of glutamine administration above the patient requirements or glutamine supplementation to those patients suffering from liver or renal failure can result in strongly elevated amino acids and ammonia levels and consequently have toxic effects[4].

3 Glutamine preparation

Glutamine preparation for intravenous administration has several complications due to its unfavorable chemical properties. The solubility of free glutamine is approximately 35 g/L and considerably increases the volume of fluid to infuse in order to achieve a beneficial dose and avoid precipitation. At the same time, free glutamine is decomposed to potentially toxic pyroglutamic acid and ammonia during storage in aqueous solutions (Figure 3). Even short periods of time are enough to accumulate toxic compounds and produce damage. Increased temperatures, as applied during heat sterilization, fasten the breakdown process[16].

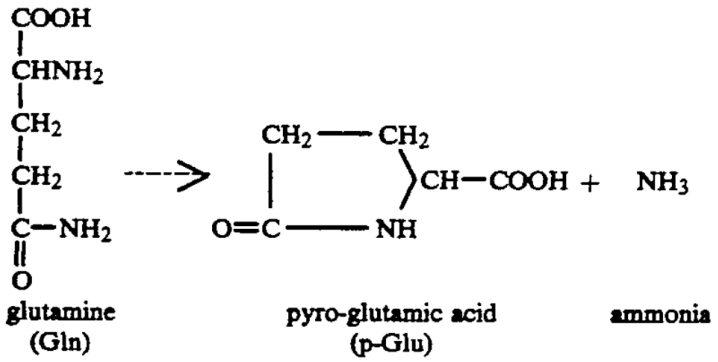

Figure 3. Schematic illustration of glutamine degradation to pyroglutamic acid and ammonia.

In order to preserve stability, glutamine is provided as white and crystalline powder or as frozen solution. Dry powder of l-glutamine is used to form a glutamine solution, which must be freshly prepared under strict septic conditions before administration due to its poor stability. Preparation instructions of l-glutamine solution from powder could be the following:

1. Determine the amount of glutamine and water for injection needed, taking into account the solubility of glutamine (the concentration of glutamine should not exceed 2.5%), the amount of glutamine required to produce the desired effect and the volume of solution able to be administrated to the patient.

2. User water on room temperature and do not heat to stimulate solubility, as this speed up the degradation of glutamine.

3. Add water to a clean and dry mixing vessel and add the powder slowly to the water and mix. Ensure that there are no visible particles in the solution.

4. Sterilize by filtration through a sterile 0.22 μm filter into a sterile collection vessel.

Glutamine solution can be administrated independently through an intravenous infusion line or added to the parenteral nutrition bag as the standard amino acids formulas and “all-in-one” parenteral nutrition bags do not contain free glutamine.

Sometimes, stock solutions are made. In this case the glutamine solution should be frozen in small aliquots into sterile containers. When needed, these small aliquots can be thawed in the refrigerator before use.

However, this process is time-consuming and adding glutamine to a parenteral nutrition bag increases the risk of contamination and errors in formulation. These limitations in its clinical use initiated research for new ways of glutamine delivery[17]:

- Dipeptides are the most frequently used alternative source of glutamine and are stable and soluble in aqueous solutions. The synthetic glutamine-containing

dipeptides, alanyl-glutamine or glycyl-glutamine, are hydrolyzed rapidly after intravenous administration, being an adequate source of free glutamine. Those dipeptides are stable during prolonged storage periods in aqueous solutions. Some well-balanced amino acid solutions contain alanyl-glutamine or glycyl-glutamine and can be added easily to any parenteral solutions or to the all-in-one multichamber bag[17].

- Ornithine alfa-ketoglutarate is a salt formed of two molecules of ornithine and one molecule of alfa-ketoglutarate. Ornithine alfa-ketoglutarate has showed to improve the protein status in patients with protein depletion by restoring glutamine pools, as both molecules (ornithine and alfa-ketoglutarate) increased glutamate levels and so can be considered as glutamine precursors. However, their stability and solubility are still poor[18].

- Branched-chain amino acids are essential amino acids that serve as essential substrates and regulators in the synthesis of body proteins and represent the major nitrogen source for glutamine and alanine synthesis in muscle. During critical illness, the endogenous use of branched-chain amino acids for glutamine synthesis is increased[19].

- N-acetyl-glutamine: are high soluble and stable, taken up rapidly and hydrolyzed by acylases after their parenteral administration. However, they are poorly used due to restricted acylase capacities.

- Glutamate is maybe the most direct related substance with glutamine and stable in water solutions. Nonetheless, glutamate is not used as it hardly passes through cell membranes and high doses can cause neurotoxic side effects.

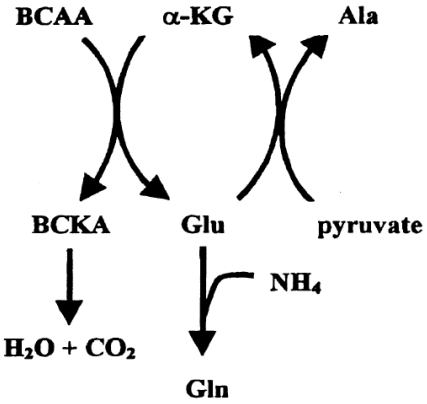

Figure 4. Relation between glutamine production, branched-chain amino acids, alfa-ketoglutarate and glutamate. BCAA: branched-chain amino acids. BCKA: branched-chain keto acid. α-KG: alfa ketoglutarate. Glu: glutamate. Gln: glutamine. Ala: alanine. [Adapted from Holecek et al.[19]].

4 Glutamine administration

Glutamine can be administrated by both enteral and intravenous route, provided alone or integrated in the artificial nutrition solution. Intolerance to enteral feeding is a common finding in critically ill patients and the glutamine absorption is low due to the strong first pass metabolism in the intestine; consequently, enteral glutamine supplementation is not so beneficial because most of the administrated glutamine doesn't reach the systemic circulation. In fact, enteral glutamine delivery preferentially increases glutamine disposal in the enterocytes and intestine immune cells[8].

When given intravenously, plasma glutamine concentration increases by 70% by intravenous infusion of exogenous glutamine dipeptide[20]. In spite the fact that glutamine is able to normalize plasma glutamine concentration, glutamine supplementation does not attenuate depletion of muscle free glutamine neither increases the intracellular storage[20,21].

5 Guidelines and recommendations

The European Society for Clinical Nutrition and Metabolism (ESPEN) is a scientific organization dedicated to all relevant issues in the field of clinical nutrition and metabolism and promotes the clinical research of parenteral and enteral nutrition support. The ESPEN publishes guidelines and position papers about recommendations for clinical nutrition practices at a regular basis.

The ESPEN guideline on parenteral nutrition in the intensive care published in 2009 recommended adding 0.2-0.4 g/kg/day of intravenous glutamine (0.3-0.6 g/kg/day of alanyl-glutamine dipeptide) when parenteral nutrition is indicated, which is given a grade A of evidence. When glutamine cannot be incorporated within the parenteral nutrition itself, it can be administered through an intravenous line[22].

The American Society for Parenteral and Enteral Nutrition (ASPEN) published a position paper of parenteral nutrition glutamine supplementation in 2011, giving strong evidence that parenteral nutrition glutamine supplementation has beneficial effects in certain patient population, including the critically ill patients, and has not shown to be harmful although it should be used in caution in patients with hepatic and renal failure[23].

However, past 2016, the ASPEN published new guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient where they recommended with moderate evidence based on the newer reported results of randomized trials that parenteral glutamine supplementation should not be used routinely in the critical care setting[24].

The Canadian Clinical Practice Guidelines (CCPG) from 2003 of nutrition support in mechanically ventilated critically ill adult patients recommended parenteral

supplementation with glutamine but not intravenous glutamine supplementation in critically ill patients receiving enteral nutrition due to insufficient data[25]. The updated guideline of 2015, however, changes their setting to: “when parenteral nutrition is prescribed to critically ill patients, we recommend parenteral supplementation with glutamine NOT be used”.

Evidence-based guidelines are systematically developed statements based on published clinical trials and meta-analyses. The older ESPEN and CCPG recommend glutamine supplementation, however the recent ASPEN and CCPG guidelines do not support this point of view. This transition in the recommendations of glutamine supplementation in the guidelines suspects that new findings do not support the traditional evidence.

6 Clinical evidence of parenteral glutamine supplementation

Glutamine supplementation has been evaluated in more than 20 years in many clinical trials and meta-analysis to determine if there is a positive impact on clinical outcomes. As low plasma glutamine levels were found in critical illness and were associated with poor outcomes and glutamine may improves the immune system function, it seems to be logical that critically ill patients should obtain some benefit on exogenous replacement.

Many of the early studies demonstrated significant improvement on outcomes, including lower mortality rates, reduction in infection rates and less hospital length of stay when total parenteral nutrition was supplied with glutamine. However, more recently studies failed to demonstrate such benefit, including the RDOX trial of 2013, currently the greatest study in evaluating glutamine supplementation efficacy[6].

6.1 Pre-REDOX studies

Small clinical trials were performed since early 90s in order to discover possible benefits of glutamine supplementation with parenteral nutrition. These studies, due to their similar study protocol, can be considered as “traditional glutamine trials”.

For example, in 2001, Wischemeyer et al.[26] conducted a randomized trial of glutamine supplementation in twenty-six burn patients and concluded a significant fewer incidence of bacteremia in the glutamine supplemented group. In addition, decreased inflammation and a trend towards lower mortality were found.

In 2006 Dechelotte et al.[27] demonstrated a reduction of infectious complications and a better metabolic tolerance in glutamine supplemented total parenteral nutrition in one-hundred fourteen ICU patients in 16 different hospitals in France.

Other similar studies realized during this period found different beneficial results including reduction of hospital mortality[28] and six-month mortality[29], serum heat shock protein increasing and significant decreased ICU length of stay[30].

Larger clinical trials were performed in order to consolidate the results that were found until then, as most realized studies were too small to detect significant differences in mortality or infectious complications and could mostly just concluded a trend towards better outcomes. However, against their study hypothesis, these newer and more scientific strength studies did not confirm previous conclusions.

The Scottish Intensive care Glutamine or seleNium Evaluative Trial (SIGNET)[31] and the Scandinavian glutamine trial[32], two large multi-center well-conduced trials, published in 2011, found a lack of benefit in glutamine supplementation with parenteral nutrition. Where the Scandinavian study observed a reduced ICU mortality, although not in the 6-month mortality and sequential organ failure assessment (SOFA), the SIGNET trial showed neither statistical significance on the ICU and 6-month term mortality nor new infection rates between the supplemented group and the control group. However, the SIGNET study used low doses of glutamine (mean of 20.2 g/day).

These new findings paid attention to some clinical researchers and were consequently examined in different meta-analysis to determine a more precise estimation of the effects of glutamine treatment on patients' outcomes.

Bollhalder et al.[33] included forty randomized clinical trials (RCT) that were published in the period between 1990 and 2012. The results concluded a significant reduction of infectious complications, a shortening in hospital LOS but no significant reduction of the short-term and 6-month mortality. Although, when they considered only doses above 0.2 g/kg/day with a duration of more than nine days of glutamine administration, a significant reduction of short term mortality, infections and hospital length of stay were found, concluding that glutamine dose and duration of administration influences the clinical outcomes of the patients. However, the exclusion of the SIGNET trial in the analysis improves all outcomes.

The meta-analysis of Wischemeyer et al.[34] was conducted after the launch of the REDOX trial in order to contrast new findings with prior results by focusing only on the previous published articles. Twenty-six traditional glutamine trials were finally included. The main finding was a significant reduction in hospital mortality. Secondary outcomes showed significant reductions of hospital length of stay and trends towards reduction in infectious complications and ICU length of stay with a more significant signal in higher quality studies than lower quality trials.

6.2 The REDOX trial

A totally different and new regimen of glutamine supplementation was tested in this, until now, largest multicenter randomized clinical trial in evaluating the efficacy of glutamine[6]. Glutamine was considered a pharmaconutrient and administrated separately from the standard nutrition support, which could be enteral or parenteral.

The field of pharmaconutrition can be understood as using nutrients in a pharmacological way and not simply for restoring nutrient deficiency.

In total, 1223 critically ill patients around the world which present multiorgan failure and received mechanical ventilation were included in this study. Glutamine, given as dipeptide alanyl-glutamine in pharmacological doses in combination with intravenous and enteral supplements, was provided together with antioxidants and administration was started within 24 hours after admission to the ICU.

The results found were in contradiction with the initial propounded hypothesis. The group receiving glutamine supplementation shows a trend towards an increased 28-day mortality and significant higher hospital mortality and 6-month mortality. On side, glutamine had no beneficial effect on rates of organ failure or infectious complications.

The REDOX trial was followed by a post-hoc analysis published by the same authors[35]. The aim of this study was to evaluate the effect of glutamine supplementation in different subgroups of baseline characteristics.

The 28-day mortality, the primary outcome of the study, was only significantly increased in patients who presented renal failure at baseline. Glutamine intervention was not associated with harm or benefit in patients without baseline renal dysfunction. The harm caused of glutamine supplementation in patients with renal impairment was attenuated in the presence of dialysis, with could help in preventing the accumulation of glutamine or other harmful metabolites derivate from glutamine.

In conclusion, the authors of the REDOX trial recommended to be cautious when providing high-dose glutamine supplementation to critically ill patients with multiorgan failure, particularly to patients with renal failure but their results should not be generalized to the rest of ICU patients who can have some benefit in lower doses.

6.3 Understanding the REDOX trial

The findings of the REDOX trial were not as expected and differed from previous findings. However, these new results cannot be ignored due to its scientific strength and high-quality design, being prospective enveloped, randomized, double blind controlled and carefully performed and executed among a large number of patients from different major centers in North America and Europe.

The REDOX trial is based on a completely new way of glutamine utilization as some points of their study protocol differed most of the previous studies which could have promoted the discrepancies in results found between them:

- A combination of glutamine supplementation by enteral and intravenous pathway was used and glutamine was administrated separately from the artificial nutrition support. As it was suggested that enteral supplementation has

beneficial effects on splanchnic tissues and intravenous supplementation on the overall tissues, it seems rational that co-administration would be more beneficial on the patient's health. However, this regimen was never used before and could lead to an excessive glutamine delivery.

- Glutamine was administrated in high doses, arriving up to 0.6-0.8 g/kg/day. This supraphysiologic dose, understood as delivering glutamine in amounts that exceed the naturally glutamine production of the body, was justified with the underlying idea of glutamine being a pharmaconutrient or immunonutrient rather than just a part of the nutrition support. Nonetheless, this dosage can lead to unknown adverse effects and have a negative impact on the patient's health.

- Glutamine treatment started within 24 hours after the ICU admission corresponding to the acute phase of critical illness. The intention was preventing the development of glutamine deficiency. In contrast, in the traditional trials, glutamine was administrated when parenteral nutrition was required, mostly in later phases of ICU stay. Nutrition, stress and metabolic states of the patients vary during the different phases of the illness process and could have influenced the response to glutamine.

- Glutamine was mixed with other antioxidants as both seem to potentiate the defense system and benefit the critically ill patients. The combination of both treatments, however, confuse as findings can be caused by glutamine, antioxidants or by the interaction of supplying both together (e.g. glutamine's positive effects could have been blocked or countered by antioxidants).

- Only patients with at least two organ failures were included in their study without exclusion of renal and liver failure. Heyland et al. suggested that patients with organ dysfunction would most likely have low plasma glutamine levels and therefore would benefit the most from supplementation. However, kidneys and liver are central organs in glutamine metabolism and consequently impairment of one or both organs could increase the glutamine and ammonia blood levels above the physiological upper limit and being toxic.

- On average, the amount of energy and proteins administrated to the patients was less than 50% of patients' needs and so quite insufficient. Patients may not benefit from glutamine supplementation when they are underfed because inadequate clinical nutrition significantly affects the outcomes in case of ICU patients.

- Most of the included patients below to medical ICU wards though most trials have demonstrated that surgical and oncological ICU patients may benefit most from glutamine supplementation.

Considering these points, the differences between traditional glutamine trials and the REDOX trial can be resumed as shown in Table 1[6,34]:

Table 1. Principal differences between traditional glutamine supplementation trials and the REDOX trial.

	Traditional glutamine trials	The REDOX trial
Study design	Small trials	Large multicenter trial
Artificial nutrition administration	Patients mostly requiring parenteral nutrition	Patients with enteral or parenteral nutrition regimens
Glutamines' role	Supplement of the artificial nutrition support	Pharmaconutrient
Glutamine administration	Mixed together with artificial nutrition support (mostly parenteral)	Separate from the nutrition support through both enteral and parenteral route
Start of glutamine administration	Later in the course of ICU admission	Within 24 hours of ICU admission
Glutamine dosage	Physiological doses	Supraphysiological doses
Patient type	Mostly surgical	Mostly medical
Patients with renal or liver failure	Excluded	Included

6.4 Post-REDOX studies

After publishing the results of the REDOX trial, new meta-analyses were performed in a need to update the evidence of glutamine supplementation in critically ill patients. These new meta-analyses included and analyzed together traditional glutamine trials and the REDOX trial.

Chen et al.[36] included eighteen randomized clinical trials. The primary outcome, hospital mortality, was not significantly different between the glutamine intervention and the control group. In contrast, the incidence of nosocomial infections in the glutamine group was significantly lower than in the control group. No shortening was observed in the hospital length of stay in the glutamine supplemented group.

Sub-group meta-analyses were performed simultaneously to estimate the effects of glutamine in specific patient populations, the effects related to a specific dosage and the effects related to the nutritional supplementation method. Glutamine effects differed by ICU setting, where patients from surgical ICU wards most benefit from glutamine supplementation by an observed reduction of nosocomial infection rate and a tendency towards decreased mortality, in contrast to patients from medical or mixed ICU wards. Benefits of glutamine were found in patients to whom parenteral nutrition was supplemented with glutamine, but not were glutamine was administrated enterally. The high dose subgroup (above the 0.5 g/kg/day), as used in the REDOX trial, were associated with an increased mortality risk in ICU patients.

The Cochrane database review of Tao et al.[37] found moderate evidence that glutamine supplementation reduces the infection rate and days of mechanical ventilation and low evidence that glutamine supplementation reduces the length of hospital stay. Besides, glutamine showed no or very little effect on mortality and ICU length of stay.

The meta-analysis of Oldani et al.[38] of thirty randomized clinical trials suggested that glutamine supplementation given to critically ill patients does not significantly reduce hospital and ICU mortality. Furthermore, they could not demonstrate a protective effect of glutamine on infection rates. In their subgroup analysis, they could observe a protective roll of glutamine administrated intravenously for more than five days in patients with a low Acute Physiology and Chronic Health disease Classification System II (APACHE) score below 15.

The differences in findings between the pre-REDOX and post-REDOX realized meta-analyses were mostly due to the inclusion of results of the REDOX trial. All three meta-analyses suggest a need for new large clinical trials to confirm or deny the potential protective or harmful effects of glutamine in more specific subgroups of patients.

Only few new clinical trials have been published since the REDOX trial in a try to answer the new questions raised and to fill the gap of the lack of good clinical evidence in the use of glutamine supplementation in critically ill patients.

Perez-Barcena et al.[39] used an intravenous glutamine supplementation (0.35 g/kg/day) to trauma patients with enteral or parenteral nutrition support. No evidence of glutamine efficacy could be found in any measured outcome (new infections, length of stay and mortality).

Some interesting point of this study was the monetarization of glutamine blood levels. 60% presented low baseline glutamine which significant increase in the glutamine supplementation group compared to the non-glutamine supplementation group after six days of treatment. Nevertheless, glutamine supplementation within the recommended guidelines dose was insufficient to normalize the levels of plasma glutamine in many patients which could explain the lack of effect in glutamine administration. Maintained low glutamine levels on day six was associated with an increased number of infected patients and a longer hospital length of stay.

In the randomized clinical trial of Grintescu and al.[40], glutamine efficacy was measured by the incidence of hyperglycemic episodes and insulin consumption as poor glycemic control and insulin resistance have been paired with complications and mortality. They report beneficial effects on parenteral nutrition supplemented with glutamine in trauma patients.

The most important trial published since the REDOX trial, the Glutamine Dipeptid (GLND) trial of Ziegler et al.[41], demonstrated that glutamine supplemented parenteral

nutrition is safe on 0.35 g/kg/day (0.5 g/kg/day of alanyl-glutamine dipeptide) but they could not demonstrate any improvement in clinical outcomes in surgical ICU patients. At time, low glutamine plasma levels were not correlated with hospital mortality.

6.5 The ESPEN meta-analysis

Last February 2017, the ESPEN study group published the currently most recent meta-analysis of glutamine supplementation. In this new study, they included 15 randomized clinical trials which all strictly delivered glutamine supplementation according to the current clinical ESPEN guidelines: critically ill patients without hepatic and/or renal failure, hemodynamically and metabolically stablished were administrated glutamine dipeptides via parenteral route at 0.3-0.5 g/kg/day in combination with adequate nutrition for at least 3 days.

In contrast to other meta-analysis published after the REDOX trial, this particular study using precise and specific inclusions criteria and excluding the REDOX trial, proved that glutamine administration according to the 2009 ESPEN guidelines[22] reduces infection complications, the ICU and hospital length of stay, the number of days of mechanical ventilation and hospital mortality rate.

7 The role of glutamine in the ICU

Positive evidence in support of intravenous glutamine supplementation comes from small single-center trials which are individually inconclusive. These traditional glutamine trials include small sample sizes of population and are so exposed to the small study bias. A reduced study population can make findings insensitive to possible harmful effects that can be identified in larger trials. Consequently, these small trials combined in meta-analysis are not able to detect those negative effects.

On side, most of this meta-analysis included a broad range of different studies leading to design weakness and many potential confounder factors as different patient population, different administration route and glutamine form, etc. The ESPEN meta-analysis, although using more accurate inclusion criteria and therefore less exposed to a bias due to differences in the way of glutamine supplementation, is still based on small studies so the individual statistical power can be questioned.

Newer and larger multi-center trials with more scientific power couldn’t support the same beneficial findings, starting by the SIGNET and Scandinavian trials. The REDOX trial was the first in suggesting harmful effects to glutamine supplementation, however this trial has to be interpreted with caution due to its alternative study design.

Several causes are suggested as related to the distinctive results reported in the performed trials, based on characteristics of the different patients included and to the regimen of glutamine administration used. The most relevant items are shown in Table

2. Nevertheless, more aspects can be implicated and must be further investigated to extract final conclusions of glutamine supplementation.

Table 2. Patient characteristics and regimen of glutamine utilization related to different treatments.

	Probably potentiate beneficial treatment effects	Probably contribute to insignificant or prejudicial treatment effects
Baseline glutamine level	Low	High
Patient type	Surgical and oncological	Medical
Organ dysfunctions	No renal and/or liver impairment	Renal and/or liver impairment
Administration route	Intravenous	Enteral
Dose	Physiological	Supraphysiological

7.1 Patient characteristic

Not all critically ill patients are at the same degree of illness. The baseline condition, adaptive response and stress levels influence the final response to glutamine. Not all patients may experience the same advantage of exogenous glutamine administration. Glutamine supplementation could not be sufficient to improve health and support the recovery in those most severe ill as its protective role may is not significant to attenuate damage caused by the underlying disease. Meanwhile, sicker patients could be earlier suffering from the toxicity effects of glutamine.

Besides, positive effects of glutamine are found in trials where patients with renal or liver impairment are excluded. Patients with baseline renal impairment were significantly associated with an increased risk for mortality in the REDOX trial which was not observed in the normal renal function subgroup. However, not all types of organ failure would have the same effect on glutamine supplementation as kidney and liver are those implied in its metabolism.

Surgical and medical patients have also shown to react on a distinct way to glutamine. Surgical ICU patients, including burns injury and trauma patients, are more likely to benefit from glutamine. It has been suggested that surgical patients may be more susceptible to glutamine depletion due to higher stress levels, mechanical obstruction, cachexia or blood loss or limitation of food intake and absorption as their intestine track may be impaired. Oncology patients are also hypothesized to have a greater chance of benefit from glutamine treatment as tumors are great consumers of glutamine.

Maybe the most impacting finding is that critical illness is not necessarily associated with low baseline glutamine levels. Several newer studies started to record glutamine baseline levels but could only confirm glutamine deficiency in part of the study population. Low baseline glutamine levels have been associated as an independent

predictive risk factor for mortality and mobility but this fact is not so consistent, as it could not be concluded in all trials. Curiously, some critically ill patients present remarkably elevated baseline levels, above the physiological upper limit maybe due to the increased muscle release or decreased liver uptake and metabolism. High baseline glutamine has also been found to be a risk factor for mortality. Glutamine supplementation in patients with high baseline glutamine levels would probably potentiate toxicity and lead to worse outcomes. Figure 5 present a simulation of additional prediction of mortality when glutamine plasma concentration at admittance to ICU is outside the physiological range.

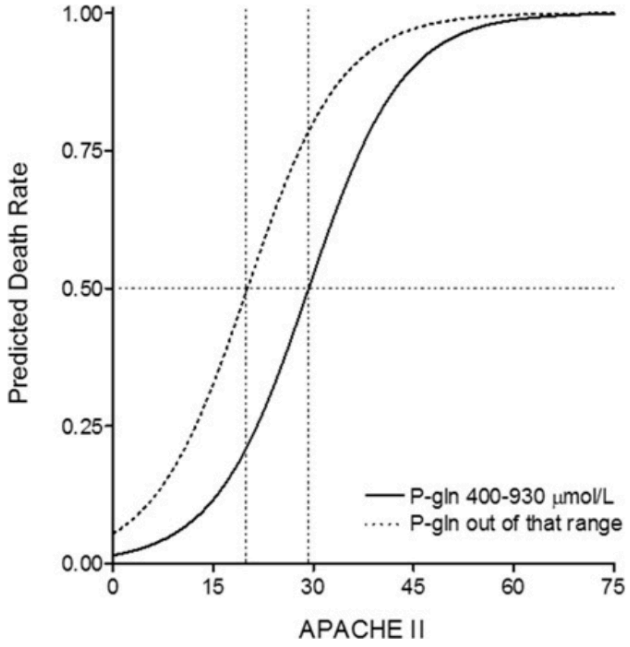

Figure 5. Simulation of the additional prediction of mortality rate from an out of range glutamine concentration at admittance. The black line is the APACHE-predicted rate if the plasma glutamine concentration at admittance is within the range 400-930 μmol/l. With an admittance concentration outside of that range, the dotted curve represents the predicted mortality rate, suggesting a mortality rate of 50% at APACHE 20 in contrast with APACHE 29.5 if the glutamine concentration is not considered. [Adapted from Rodas et al.[14]].

7.2 Regimen of glutamine utilization

High doses of any substance can lead to toxic effects and in contrast low doses can be insignificant to produce positive effects on the patient's health. The trick is finding the adequate dose to improve the patient's outcomes without being harmful. Nearly all positive glutamine studies used physiological doses of glutamine within the different guidelines recommendations and have not been paired to any harmful effect. However, high glutamine doses regarding to the immunonutrition concept result in poor clinical outcomes. The glutamine toxicity mechanism is still unknown but too high glutamine doses may result in metabolic imbalances.

Administrated glutamine doses must be able to normalize plasma glutamine as this is the suggested mechanism whereby glutamine can support the immune system and normalize cell function. Only few studies have determined glutamine levels at baseline and after some days of glutamine treatment. These could find significant differences between the intervention and control group in restoring plasma glutamine levels with physiological dose. But it's not clear if this is enough to normalize glutamine related physiological functions and to improve the immune system. Besides, depletion of intracellular muscle glutamine is not attenuated by an exogenous glutamine infusion.

The used route of glutamine administration affects the subsequent plasma concentration and consequently the response to supplementation. In this bibliographic review, intravenous glutamine administration was analyzed as this is the most evidence-based route. Positive effects on enteral glutamine administration have never been as consistent as parenteral administration and do not increase that much the plasma levels. The recent published MetaPlus trial[43] didn't help to firm benefits of enteral administration as the intervention group, fed with enriched enteral nutrition with immune-modulating nutrients (including glutamine, antioxidants and fish oils) was associated with a higher six-month mortality without any improvement in other studied endpoints (such as infection complications).

Glutamine supplementation with parenteral nutrition is mostly started after some days of ICU admission, as parenteral nutrition is not the first chosen option to cover nutritional requirements in the critically ill patients. Pharmacological glutamine administration is designed to be started at admission to the ICU, without taking account if the patient receives enteral or parenteral nutrition. Patient's state and degree of illness differ in early and later phases of ICU stay and so the glutamine efficacy and toxicity can vary between "early" and "late" start of supplementation. Onside, by choosing only patients needing parenteral nutrition support, a subgroup of critically ill patients could be selected to whom glutamine is especially beneficial (e.g. their gastrointestinal tract is more impaired leading to less proper defense support which is improved by exogenous glutamine delivery).

Early studies have used l-glutamine and later studies glutamine dipeptide compositions due to their better stability. It's not clear if and how this could influence the results of the glutamine treatment, however differences between these different sources in their kinetics and final release of free glutamine in plasma are observed.

8 An alternative point of view

Trials are performed on the hypothesis that apparent glutamine deficiency compromises an adequate immune system function and cell survival and therefore glutamine supplementation should improve the outcome in severely ill patients. Nevertheless, this suggesting could be erroneous and low glutamine levels during acute

critical illness could reflect just an adaptive and beneficial stress response rather than a conditional deficiency. Administration of exogenous glutamine could interfere in this favorable response and so worsen outcomes.

Maybe glutamine plays a role in several prior mentioned physiological functions and its depletion is a marker of disease severity but exogenous supplementation and enhancement of plasma levels is not determinant to improve outcomes. Maybe decreased glutamine levels just reflect the typical global protein depletion without having any essential function for critically ill patients.

In these cases, the significant differences between the control and the intervention groups that were found in some trials could be caused by an unknown or undetermined study bias.

9 Further research

With all the available information, it's still uncertain if glutamine should be included in the routine clinical use. New trials have only lead to more confusion and current clinical evidence interpretation vary widely between different authors. The REDOX trial has mostly contributed to question the scientific recommendations published until 2013. Currently, glutamine's use according to the ESPEN guidelines seems to be the most effective and safe way of glutamine delivery.

In this same line, optimal nutrition support for critically ill patients remains controversial. It's difficult to predict energy expenditure and protein needs in those patients due to the complex and variable metabolic alterations suffered. Artificial nutrition support and intravenous glutamine supplementation are still unphysiological ways of feeding and not without complications. In overall, the optimal amount, route and timing of artificial nutritional support and glutamine supplementation are uncertain.

New research should explain if glutamine supplementation is harmful, a complete waste and insignificant or only relevant for some subgroups of patients. And so, it's important to define safe and effective regimens of glutamine supplementation and the right specific subgroups of patients who potentially may benefit from this treatment. The next questions should be further investigated in order to propose new valid recommendations:

- Which patients present low baseline glutamine levels, taking into account the underlying disease, illness severity and/or nutritional baseline status?
- How should glutamine supplementation be used in routine clinical practice (dosage, days of treatment, route of administration, given with or separately of

nutrition support…) in order to restore glutamine plasma levels within the healthy physiological range?

- Can glutamine supplementation really restore low glutamine plasma levels?
- Can restoration of the plasma glutamine level enhance the clinical situation of the patient and so improve the prognostic and the clinical outcomes?
- Can high doses of glutamine be toxic? Which underlying mechanism is implied and how should it be avoid or treated?
- Which subgroup of critically ill patients are at risk to show high glutamine baseline levels and which underlying mechanism is implied?

Conclusions

- Glutamine is an important amino acid for the human body in health and disease, supporting mostly enterocytes and immune system cells.
- Glutamine can be synthetized endogenously, but during illness, when plasma levels can become low and muscle depletion is observed, it needs probably to be supplied exogenously. And so, gutamine is considered a conditionally essential amino acid.
- Glutamine has difficult chemical characteristics, such as low aqueous solubility and stability. New delivery sources, like glutamine containing dipeptides included in commercial amino acid solutions, are currently more popular and are easy ways to include glutamine supplementation to the regular use.
- Traditional studies associate glutamine supplementation with beneficial treatment effects, however newer and more scientific robust trials, as the REDOX trial, found contradictory results, questioning glutamine supplementation efficacy. Consequently, some big and important guidelines have changed their recommendations.
- Some patients' characteristics and glutamine regimen aspects promote beneficial treatment effects of glutamine while other aspects show more prejudicial effects. New trials should further investigate how these different points influence the glutamine efficacy.
- Not all critically ill patients have low baseline glutamine and some have conversely an increased plasma level, being both an independent predictor of mortality. Correcting the deficient state seems to be logically a better target than over supplementing non-deficient patients. Therefore, determining glutamine baseline levels and identifying patients with glutamine deficiency should be indispensable before any glutamine supplementation.
- Besides, it is still unclear if the depletion in glutamine levels contributes to death or is a simple marker of disease severity and if glutamine contributes to enhance clinical outcomes.

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