Plasma glutamine levels in critically ill intensive care patients

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Mini-dissertation submitted in partial fulfilment of the requirements for the degree *Magister Scientiae* in Dietetics at the Potchefstroom Campus of the North-West University

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May 2015

ABSTRACT

Background

Nutritional treatment in the intensive care unit (ICU) has evolved from meeting nutritional requirements to manipulating patient outcome. Pharmaconutrition, referring to nutrients that are applied for their pharmacological properties, forms part of the standard nutritional care plan. The most abundant amino acid in the body, glutamine, is also the most-researched pharmaconutrient. It is an independent predictor of mortality in ICU patients, at both deficient and very high levels. Glutamine supplementation is recommended in the ICU setting for its proven outcome benefits. However, recent data showed that glutamine supplementation increases mortality risk in certain patient groups. Moreover, it suggested that not all ICU patients are glutamine deficient. Therefore, the main aim of this study was to investigate the plasma glutamine levels of adult ICU patients, on admission to the ICU. In addition, to elucidate the profile of ICU patients that can be expected to present with a glutamine deficiency or excess, with regards to gender, diagnosis and inflammatory markers.

Methods

In this observational, cross-sectional study, 60 mixed ICU adult patients admitted to two hospitals in the North West province were included in the study group. Blood sampling was conducted within 24 hours following ICU admission, to determine plasma glutamine, interleukin (IL)-6 and C-reactive protein (CRP) levels. Plasma glutamine levels were compared with those of a control group of healthy individuals, matched by age, race, and gender. Gender-related differences in plasma glutamine levels were investigated, as well as differences between patients with various medical conditions. The relationship between plasma glutamine levels and IL-6 or CRP was examined. Additionally, a CRP concentration cut-off point at which glutamine becomes deficient was determined by means of a receiver operating characteristic (ROC) curve.

Results and discussion

Intensive care unit patients had significantly lower plasma glutamine levels than healthy individuals on day one of ICU admission ($p < 0.0001$). However, only 38.3% ($n = 23$) had deficient plasma glutamine levels (< 420 µmol/L), while 6.7% ($n = 4$) presented with supra-normal levels (> 930 µmol/L). No significant difference could be detected between the plasma glutamine levels of male and female ICU patients ($p = 0.116$). Likewise, levels between diagnosis categories were also not significantly different ($p = 0.325$). There was a significant inverse association between plasma glutamine levels and CRP concentrations ($r = -0.44$,

$p < 0.05$), and a trend towards an inverse association with IL-6 ($r = - 0.23$, $p = 0.08$). A CRP cut-off value of 95.5 mg/L was determined, above which plasma glutamine values became deficient; however, more research is needed to confirm this result.

Conclusion and recommendations

This research therefore showed that ICU patients, when compared with healthy individuals, had lower plasma glutamine levels on day one of admission to the ICU. However, not all were glutamine deficient, as the majority had normal and some presented with supra-normal plasma glutamine levels. An individualised approach should therefore be followed in identifying candidates for glutamine supplementation. The patients' condition alone may not be sufficient to predict glutamine status, but an association between plasma glutamine levels and CRP was firmly established, as well as a cut- off CRP-value above which glutamine can be expected to become deficient, which could be of use in this regard.

KEYWORDS

Glutamine, intensive care unit, C-reactive protein, interleukin-6, gender

OPSOMMING

Agtergrond

Voedingsbehandeling in die intensiewesorgeenheid (ISE) het ontwikkel vanaf die bereiking van ‘n pasiënt se voedingsbehoeftes, tot die manipulering van pasiëntuitkomste deur middel van nutriënte. Farmakovoeding verwys na nutriënte wat aangewend word op grond van hulle farmakologiese eienskappe en word tans toegedien as deel van ‘n standaard voedingsorgplan. Glutamien is die mees algemene aminosuur in die liggaam en is daarby ook die mees nagevorsde farmakonutriënt. In beide hoë en lae vlakke is daar bewys dat glutamien mortaliteitsrisiko kan voorspel. Glutamienaanvulling word aanbeveel in die ISE as gevolg van die reeds bewese voordele wat daaruit verkry kan word. Glutamienaanvulling is egter onlangs bewys om mortaliteit te verhoog in sekere pasiëntgroepe. Daar word ook postuleer dat nie alle intensiewesorgpasiënte verlaagde vlakke het nie. Die hoofdoel van die studie was daarom om plasma glutamienvlakke in volwasse intensiewesorgpasiënte met toelating te ondersoek. Verder was daar ook gepoog om die profiel van intensiewesorgpasiënte, wat moontlik kan presenteer met lae of hoë plasma glutamienvlakke te bepaal, in terme van geslag, diagnose en inflammatoriese merkers.

Metode

In hierdie waarnemings-, dwarsdeursnitstudie is 60 volwasse, gemengde, intensiewesorgpasiënte ingesluit vanaf twee hospitale in die Noordwes Provinsie. Bloedmonsters is geneem binne 24uur vanaf opname en die plasma glutamien-, interleukin-6 (IL-6)- en C-reaktiewe proteïenvlakke (CRP) is bepaal. Plasma glutamienvlakke van die pasiënte is met vlakke van ‘n gesonde kontrolegroep, van gelykwaardige ouderdom, ras en geslag vergelyk. Geslagsverwante glutamienvlakverskille, asook verskille ten opsigte van mediese diagnose was ondersoek. Verder is die korrelasie tussen plasma glutamienvlakke en IL-6 asook CRP bepaal. ‘n CRP afsnypunt, waarbo glutamienvlakke onder die normale grens daal, is ook bepaal.

Resultate en bespreking

Intensiewesorgpasiënte het statisties betekenisvolle laer glutamienvlakke, as die gesonde kontrolegroep, op dag een van opname gehad ($p < 0.0001$). Ten spyte hiervan het slegs 38.3% ($n = 23$) van die pasiënte baie lae (< 420 µmol/L) en 6.7% ($n = 4$) baie hoë (> 930 µmol/L) plasma glutamienvlakke gehad. Geen verskil kon in die plasma glutamienvlakke van manlike en vroulike pasiënte, gevind word nie ($p = 0.116$). Dit was ook die geval met die plasma glutamienvlakke tussen verskillende mediese diagnosis ($p = 0.325$). Daar was ‘n statisties betekenisvolle, omgekeerde verwantskap tussen plasma glutamien- en CRP-vlakkke ($r = -0.44$,

$p < 0.05$), asook ‘n neiging tot ‘n omgekeerde verwantskap met IL-6 vlakke ($r = - 0.23$, $p = 0.08$). ‘n CRP-vlak afsnypunt van 95.5 mg/L is bepaal, waar plasma glutamienvlakke onder normale vlakke gedaal het, maar verdere navorsing word benodig om die bevinding te bevestig.

Gevolgtrekking en aanbevelings

Die navorsing het getoon dat pasiënte verlaagde glutamienvlakke, in vergelyking met ‘n gesonde kontrolegroep, alreeds op dag een van opname in die ISE gehad het. Al die pasiënte het egter nie ‘n tekort gehad nie, aangesien die meerderheid normale vlakke en sommige baie hoë plasma glutamienvlakke getoon het. Daarom moet ‘n geïndividualiseerde benadering gevolg word in die identifisering van pasiënte wat aanvulling benodig. Die pasiënt se mediese toetstand kan nie alleenlik gebruik word om glutamienstatus te voorspel nie, maar ‘n omgekeerde verwantskap tussen CRP en glutamien was bepaal, asook ‘n CRP-vlak waarbo ‘n glutamientekort verwag kan word en dit kan moontlik bruikbaar wees in die opsig.

SLEUTELWOORDE

Glutamien, intensiewesorgeenheid, C-reaktiewe proteïen, interleukin-6, geslag

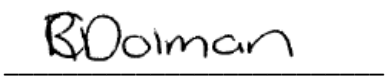
PREFACE

This mini-dissertation will be presented in article format. Arista Nienaber the *Magister Scientiae* (MSc) student, wrote the article: “Plasma glutamine levels in adult ICU patients: a cross-sectional study” in accordance with the authors instructions of the journal *Critical Care* to which the article (Chapter 3) will be submitted.

The co-authors of this article (Chapter 3) Dr R.C. Dolman, Dr A.E. van Graan and Prof R. Blaauw provided permission that the article may be submitted for examination purposes. The article is still to be submitted to the journal; therefore, no permission was obtained from the editor of the journal.

The following signatures and statement confirm the co-authors’ role as mentioned in the article (Chapter 3) and their permission to include the article “Plasma glutamine levels in adult ICU patients: a cross-sectional study”, in this mini-dissertation for examination purposes in partial fulfilment of the requirements for the degree *Magister Scientiae in Dietetics*.

“I declare that I have approved the above-mentioned article, and that my role in the study, as indicated in the article, is representative of my contribution. I hereby give my consent that the article may be published as part of the *Magister Scientiae in Dietetics* mini-dissertation of Mrs A. Nienaber.”



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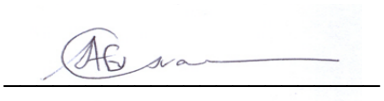
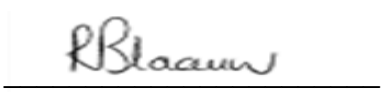


Dr A.E. van Graan



Prof. R. Blaauw

ACKNOWLEDGEMENTS

First and most importantly I would like to thank God for the talents and opportunities that He has bestowed upon me. His grace and protection has let every part of this study fall into place. There is no way I would have been able to complete even a small part of this on my own.

With regards to this project and completion of the mini-dissertation there are certain individuals that have played an irreplaceable role. I would like to thank:

- My study leader, Dr R.C. Dolman. Thank you for all the support and providing me with the tools and motivation to plan and execute the project. Additionally for all the help to successfully complete this mini-dissertation. I have learned so much from you and you are an inspiration to me.

- My co-supervisors, Prof R. Blaauw and Dr A.E. van Graan, for your significant contribution and help in the planning of the project, as well as the completion of my mini-dissertation. Both of your expertise have helped me so much and have left a lasting impression on me.

- The Nutricia Research Foundation and the National Research Foundation, for providing me with scholarships to be able to study full-time and fund the research project.

- My most important supporter, my husband Conrad. Thank you for letting me study full-time and for your support, encouragement, prayers and all the little things you did. I will never be able to say thank you enough.

- All my friends and family for their support and encouragement. A special thanks to a remarkable women, my mother, who has taught me what it means to work hard and supported me immensely throughout this project. Thank you for all the opportunities that you have given me throughout the years, that has shaped my future and for encouraging me to always do my best.

- The dietitians of Tshepong and Potchefstroom hospital for your assistance in the completion of this project. Specifically to Miss D. Hartmann, Miss M. de Koker and Miss M. Slabbert for the time and effort they put into the data collection at Tshepong hospital.

- The staff of the intensive care units of Tshepong and Potchefstroom hospital. Gideon Witbooi that assisted with the blood sampling and Prof Variava that assisted with the planning and implementation of the project.

- I would further like to acknowledge the staff of the Wits Perinatal HIV Research Unit for their help with blood preparation and storage at Tshepong hospital.
- Sr Chrissie Lessing, for the blood sampling of the healthy control group participants, as well as the provision of all the necessary items for blood collection in the ICU's and contact with the nursing agency.
- Mrs E. Rossouw and Prof M. Pieters for their help with all the laboratory analysis aspects of this study, as well as the analysis of interleukin-6 concentrations.
- The National Health Laboratory of Tshepong hospital and the Potchefstroom Laboratory for inborn Errors of Human metabolism for the analysis of other markers.
- Dr Suria Ellis and Marike Cockeran for assistance in reviewing my statistics and providing suggestions.
- Mary Hoffman for the language editing.

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LIST OF ABBREVIATIONS

AIDS	acquired immune deficiency syndrome
ALA-GLN	alanyl-glutamine
ANCOVA	analysis of covariance
ANOVA	analysis of variance
A.S.P.E.N.	American Society for Parenteral and Enteral Nutrition
ATB°	amino acid transporter system B
ATP	adenosine triphosphate
AUC	area under curve
BCAA	branched-chain amino acid
CCPG	Canadian Critical Care Clinical Practice Guidelines
CEN	Centre of Excellence for Nutrition
CI	confidence interval
CRP	C-reactive protein
EN	enteral/ enteral nutrition
ESPEN	European Society for Clinical Nutrition and Metabolism
GABA	*gamma*-aminobutyric acid
GALT	gut-associated lymphoid tissue
GC-MS	gas chromatography-mass spectrometry
GIT	gastrointestinal tract
GLY-GLN	glycyl-glutamine
GS	glutamine synthetase

LIST OF ABBREVIATIONS (Continued)

HIV	human immunodeficiency virus
HLA-DR	human leukocyte antigen-DR
HPLC	high-performance liquid chromatography
HSP	heat-shock protein
ICU	intensive care unit
IgA	immunoglobulin A
IL	interleukin
iNOS	inducible nitric oxide synthase
IV	intravenous
LBM	lean body mass
LOHS	length of hospital stay
MOF	multiple organ failure
mRNA	messenger ribonucleic acid
PN	parenteral/ parenteral nutrition
n	number of/ sample size
Na^+/K^+- ATPase	sodium/ potassium adenosine triphosphatase
NH_3	ammonia
NH_4^+	ammonium
NIDDM	noninsulin-dependent diabetes mellitus
NO	nitric oxide
NWU	North-West University

LIST OF ABBREVIATIONS (Continued)

OR	odds ratio
REDOXS	Reducing Deaths due to Oxidative Stress
SIGNET	Scottish Intensive care Glutamine or SeleNium Evaluative Trial
SIRS	systemic inflammatory response syndrome
Sn	sensitivity
Sp	specificity
TNF- α	tumour necrosis factor alpha
TPN	total parenteral nutrition

LIST OF SYMBOLS AND UNITS

r	correlation
°C	degrees Celsius
g	gram
g/d	gram per day
g/kg	gram per kilogram body weight
g/kg/d	gram per kilogram body weight per day
>	greater than/ above
↑	increased
kg	kilogram
<	less/ lower than
µmol/L	micromoles per litre
mg/L	milligram per litre
mmol/L	millimoles per litre
_	negative
%	percentage
pg/mL	picograms per millilitre
↓	reduced

CHAPTER 1 – INTRODUCTION

1 CHAPTER 1- INTRODUCTION

1.1 General introduction

Intensive care unit (ICU) patients require a specific, individualised, multidisciplinary treatment approach, one important aspect of which includes nutritional care. Nutritional treatment in this setting has evolved, as the emphasis has shifted from meeting nutritional requirements by the provision of sufficient nutrients to applying nutritional care in order to manipulate patient outcome (Prins & Visser, 2012). In this regard, immunonutrition has become a well-established practice in the clinical setting.

Immunonutrition is the term used for the provision of nutrients above what would be found in a normal diet, thereby modulating a patient's immune function and inflammatory processes (Grimble, 2001). It should be kept in mind that the patient's nutritional requirements must still be met and that these individual nutrients or nutrient combinations are additives to a nutritionally complete dietary regime (Wernerman, 2003). Immunonutrition regimes are currently implemented in clinical environments both nationally and internationally for their potential contribution to improved patient outcomes and have therefore been extensively researched in order to justify their benefit. However, owing to contradictory data reported and heterogeneous groups included in the different trials, there is still great uncertainty in this field, which needs to be further explored in order to provide more concrete recommendations (Dupertuis *et al*., 2009).

In the last few years there has been a paradigm shift from the concept of immunonutrition to pharmaconutrition, the latter referring to nutrients that are applied in the clinical setting for their pharmacological properties (Dupertuis *et al*., 2009). The reasoning behind this shift was to rule out the uncertainty that has been created by the immunonutrition concept. Pharmaconutrition refers to the provision of nutrients as pharmacological agents by applying the correct administration schedule, with the correct combination of nutrients and administering it to the correct patients, based on sound scientific trials (Dupertuis *et al*., 2009). Glutamine is one such pharmaconutrient that has been subject to a significant amount of scrutiny in recent years.

Glutamine, the most researched pharmaconutrient, is the most abundant non-essential amino acid in the body, contributing more than 50% of the free amino acid pool (Askanazi *et al*., 1980; Bergström *et al*., 1974; Oudemans-van Straaten *et al*., 2001; Roth, 2008). The rationale behind the interest in glutamine as a pharmaconutrient is based firstly on the supposed benefits that it can provide to the human body, especially in stressed states. Glutamine's important functions include the following: it serves as a metabolic substrate for enterocytes and immune cells; it is important in anabolic activities; it replenishes the citric cycle; it is involved in nucleic acid synthesis; it functions as the rate-limiting precursor of glutathione; it plays an important role in

acid-base homeostasis of the kidney; it attenuates hyper-inflammation; and acts as a signalling molecule by inducing heat-shock protein (HSP) expression, thus providing cellular protection against stress and injury (Amores-Sánchez & Medina, 1999; Boza *et al*., 2000; Le Bacquer *et al*., 2001; Wischmeyer, 2003; Wischmeyer *et al*., 2001).

The second reason why glutamine is described as an important nutrient to be provided in the clinical setting, is that it is believed to become deficient under circumstances of critical illness. In critically ill ICU patients, the protein content of muscle can decrease by up to 10% within five days of illness, with continued protein degradation for at least two weeks (Gamrin *et al*., 1997; Vesali *et al.,* 2002). Decreased glutamine levels in tissue and plasma have previously been reported in these patients as well as in post-operative patients (Déchelotte *et al*., 2006; Gamrin *et al*., 1997; Gottschalk *et al*., 2013; Oudemans-van Straaten *et al*., 2001; Parry-Billings *et al*., 1992; Pérez-Bárcena *et al*., 2014; Rodas *et al*., 2012; Van Acker *et al*., 2000; Vesali *et al*., 2002; Viggiano *et al*., 2012). This is thought to be due to an increased glutamine demand, together with a reduced production, insufficient to meet these demands. Glutamine is therefore termed a "conditionally essential" amino acid under circumstances of critical illness, where its deficiency can lead to an impaired immune function and an inappropriate response to stress and injury (Soeters & Grecu, 2012; Vesali *et al*., 2002). Moreover, deficient levels have been associated with an increased mortality risk and poor outcomes (Oudemans-van Straaten *et al*., 2001; Rodas *et al*., 2012). This, therefore, indicates the importance of a patient's glutamine status in critical illness.

It is thought that glutamine supplementation will refill the deficient pool, exert its beneficial functions and thereby improve patient outcomes. Glutamine supplementation is well researched and a search of the available literature delivers a large body of evidence suggesting its benefit in patients in a variety of clinical settings. Meta-analyses on glutamine supplementation, reported outcome benefits in severely ill, ICU, burns, pancreatitis and surgical patients (Asrani *et al*., 2013; Bollhalder *et al*., 2013; Lin *et al*., 2013; Wang *et al*., 2010; Wischmeyer *et al*., 2014; Yue *et al*., 2013). In these patients, outcome benefits such as reduced length of hospital stay (LOHS) and ICU stay, as well as reductions in mortality risk and infectious complications and an improvement in nitrogen balance, have been reported, depending on the patient diagnosis (Asrani *et al.,* 2013; Bollhalder *et al*., 2013; Lin *et al*., 2013; Wang *et al*., 2010; Wischmeyer *et al*., 2014; Yue *et al*., 2013). The beneficial effects of glutamine supplementation are further thought to be largely dependent on the dose and route of administration, more successful results being reported when it is administered parenterally (Bolhalder *et al*., 2013; Novak *et al*., 2002; Wischmeyer, 2003). When considering the evidence, it seems that glutamine administration in the clinical setting should be established practice; and therefore the question arises as to why its use would need any further investigation

1.2 Rationale for the study

Until very recently, glutamine supplementation was thought to be an efficient and safe practice to be implemented as part of patient care. However, in 2013, the Reducing Deaths due to Oxidative Stress (REDOXS) study was published with alarming results (Heyland *et al*., 2013). In this study glutamine supplementation was shown to increase mortality, bearing in mind that the study included patients with multiple organ failure (MOF), which is generally a contra-indication for glutamine supplementation. In addition, high dosages of both enteral and intravenous (IV) glutamine were administered from day one of admission to the ICU (Heyland *et al*., 2013). Nevertheless, this study questioned the safety of glutamine supplementation in all patient groups. Moreover, when analysing the plasma glutamine levels of a sub-study of 66 patients, it was found that only 31% had deficient baseline glutamine levels (less than 420 µmol/L), while 15% had supra-normal levels (greater than 930 µmol/L) (Heyland *et al*., 2013).

Plasma glutamine levels are commonly used as a marker of glutamine status. Tjäder *et al*. (2004) suggested that muscle free glutamine status may not be the crucial factor for immediate survival, but that the availability of glutamine for other cells (i.e. plasma glutamine levels) is more important to ICU patients. Additionally, Rodas *et al.* (2012) reported an increased mortality risk with low as well as very high (> 930 µmol/L) plasma glutamine levels. The authors of a recent review article concluded that there is still a lack of evidence for claims that glutamine becomes deficient in certain disease states (Soeters & Grecu, 2012). Subsequent to this, Heyland & Dhaliwal (2013) published a commentary on the REDOXS study results and recommended that future research be aimed at the determination of baseline plasma glutamine levels, which should then guide glutamine supplementation studies. This highlights the important need to determine first whether all ICU patients are glutamine-deficient, in order to justify the necessity of supplementation as routine practice in ICUs. Although there is a substantial amount of literature available on the metabolism and supplementation of glutamine, a gap exists between claims of glutamine deficiency in certain disease states and the evidence confirming a supplementary benefit.

However glutamine levels are not routinely measured in the hospital setting and therefore other markers can be of aid in the determination of glutamine status. A possible link between glutamine and inflammation has previously been established (Andreasen *et al*., 2009; Parry-Billings *et al.*, 1992; Suliman *et al*., 2005). In this regard, two well-known inflammatory markers namely interleukin-6 (IL-6) and C-reactive protein (CRP) are early indicators of inflammation and predictors of the severity of the injury as well as complications (Mihara *et al.*, 2012). Establishing a relationship between glutamine and these biomarkers will therefore be of use in the in the critical care setting, possibly serving as proxy indicators for glutamine status.

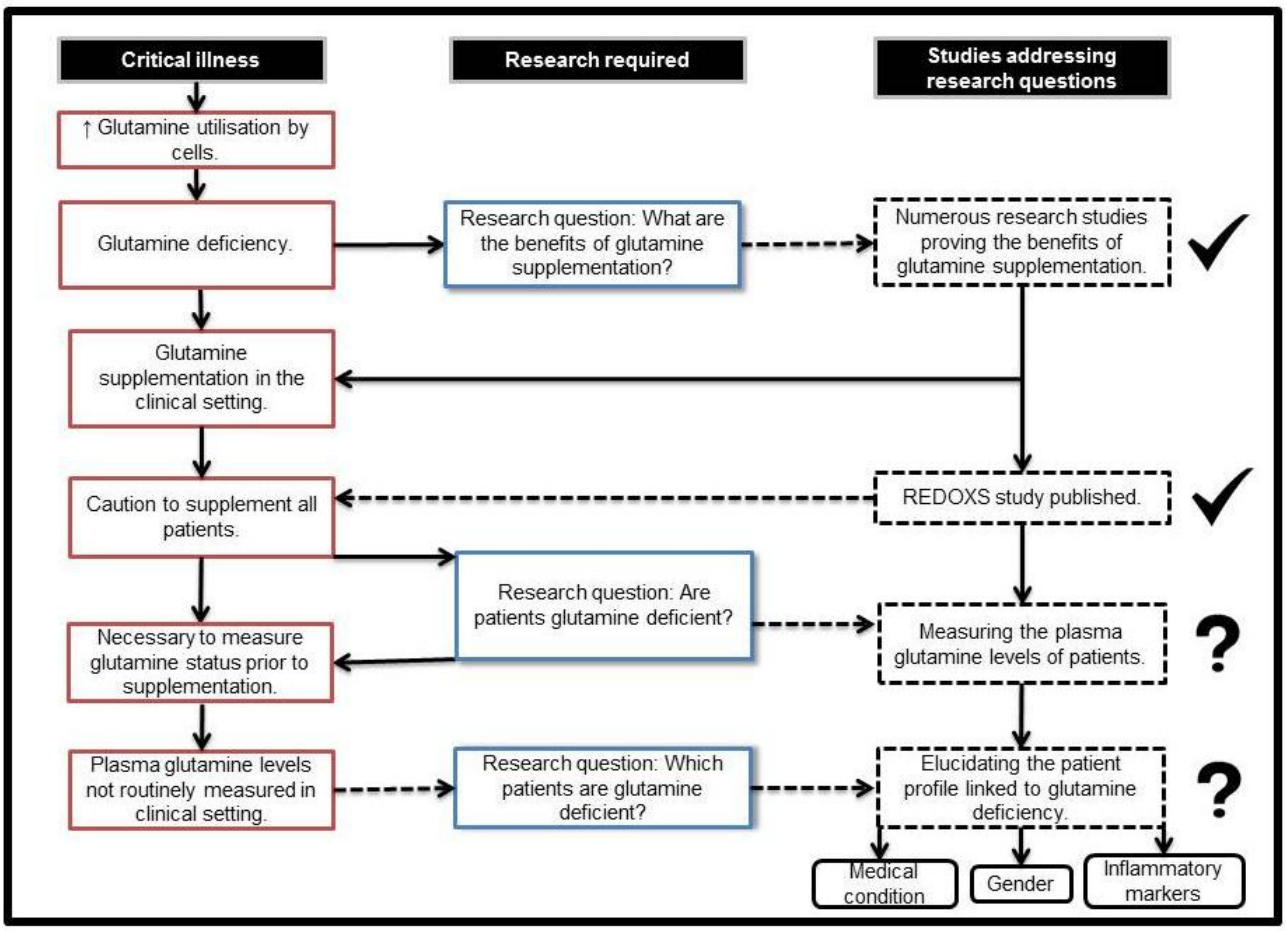

Figure 1.1 A conceptual framework linking the rationale for the research with the outcomes to be measured

1.3 Research aim

The aim of this cross-sectional, observational study was to examine plasma glutamine levels of adult ICU patients in order to establish whether a deficiency exists (plasma levels less than 420 µmol/L), as well as to investigate possible associations between selected inflammatory markers (CRP and IL-6) and low plasma glutamine levels. In addition, the influence of gender and different diagnoses on plasma glutamine levels was examined.

1.4 Research objectives

The objectives of this research were to:

1.4.1 measure the plasma glutamine levels of adult ICU patients on admission to the ICU to determine whether they were deficient (< 420 µmol/L) and to compare these levels with those of a healthy control group;

1.4.2 determine whether there was an association between glutamine and selected inflammatory markers, namely CRP and IL-6;

1.4.3 establish whether there was a difference between the plasma glutamine levels of medical, trauma and surgical ICU patients; and

1.4.4 determine whether gender influenced plasma glutamine levels of ICU patients.

1.5 Structure of this mini-dissertation

This mini-dissertation will be presented in article format according to the postgraduate guidelines of the North-West University (NWU). The structure of this mini-dissertation takes the form of four chapters. Decimal numbers are used to ensure that the headings follow a logical sequence. The directives of the NWU were strictly followed for the language format and referencing in this mini-dissertation. Relevant references will be provided at the end of each chapter. The references used in the unpublished chapters one, two and four are presented as stipulated by the mandatory referencing style of NWU.

Chapter one provides a brief introduction to the research, states its aim and objectives, and describes the research outputs that will emanate from this research. It also gives details of the contributions of the different research team members.

Chapter two consists of a review of the available literature on glutamine in the critical care setting. This is intended to ensure a sufficient understanding of the background of the topic and to help in the interpretation of the data presented in the article in Chapter three. The literature review focuses on the physiology of glutamine, glutamine status in critically ill patients and the scientific evidence regarding the benefits of glutamine supplementation.

Chapter three includes the article containing the data output of this research project. This article, titled “Plasma glutamine levels in adult ICU patients: a cross-sectional study”, will be submitted for publication to the journal *Critical Care*. In Chapter three the headings are not numbered, and the tables and figures are numbered according to the guidelines of the journal *Critical Care.* The paragraphs are however justified and line spacing of one-and-a-half used, contradicting guidelines of this journal, to ensure uniformity with other chapters. The references of the article in Chapter three will be provided at the end of the chapter according to the instructions provided to authors by the specific journal to which the article will be submitted for publication.

Chapter four completes this mini-dissertation, providing a summary of the work and a conclusion, as well as recommendations for further research. This chapter is based on the key objectives that have been identified.

1.6 Research outputs emanating from this study

An article will be submitted for approval to the journal *Critical Care*. Feedback will be provided on the study results to both hospitals where the study was based, as well as to the North West Province Department of Health. Results of this study will also be presented at a national or international congress.

1.7 Contributions of members of the research team

The contributions of the researchers listed as authors in the article and that were part of this research project are presented in Table 1.1.

Table 1.1 List of members and their contribution to this research project

Name and signature	Affiliation	Role in the study
Mrs A. Nienaber (MSc student)	CEN within the School for Physiology, Nutrition and Consumer Science of the NWU	Responsible for the planning, execution and management of this project. Compiled the literature review, conducted the statistical analysis, interpretation of data and writing up of this mini-dissertation.
Dr. R.C. Dolman (Supervisor)	CEN within the School for Physiology, Nutrition and Consumer Science of the NWU	Supervisor of Mrs A. Nienaber in the completion of this mini-dissertation. Played a supervisory role in the planning and execution of the research project as well as the statistical analysis and interpretation of data.
Prof. R. Blaauw (Co-supervisor)	University of Stellenbosch	Co-supervisor of Mrs A. Nienaber in the completion of this mini-dissertation. Played a supervisory role in the planning of the research project and interpretation of data.
Dr. A.E. van Graan (Co-supervisor)	CEN within the School for Physiology, Nutrition and Consumer Science of the NWU	Co-supervisor of Mrs A. Nienaber in the completion of this mini-dissertation. Played a supervisory role in the planning of this research project and interpretation of data.

CEN: Centre of Excellence for Nutrition; NWU: North-West University

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CHAPTER TWO – LITERATURE REVIEW

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2.1 Introduction

Nutritional care forms an important part of the multidisciplinary treatment of intensive care unit (ICU) patients. However, these patients are a heterogeneous group and therefore one nutritional regime will not fit all. Over the last two decades, the aim of clinical nutrition has shifted from merely meeting nutritional requirements to treating individual conditions therapeutically (Prins & Visser, 2012). Currently, immunonutrition forms an important part of the nutritional treatment plan and is a fast-growing field, both nationally and internationally, aimed at promoting better outcomes in a variety of patients (Dupertuis *et al.*, 2009; Prins & Visser, 2012). Immunonutrition is a collective term that describes the provision of significant amounts of individual or a combination of nutrients in order to modulate a patient's immune and inflammatory status (Grimble, 2001). These nutrients, therefore, may rather be described as pharmacological agents or pharmaconutrients, targeting mainly the immune system, muscles, and intestines (Dupertuis *et al.*, 2009). In South Africa, several constraining factors in the use of pharmaconutrients, such as limited finances, may affect the successful implementation of specialised nutrition regimes. However, through improved patient outcomes, hospital costs may be reduced and therefore balance the financial implications of such treatment. Consequently, pharmaconutrition is currently implemented in South Africa in the clinical setting (Prins & Visser, 2012). Glutamine is the most studied pharmaconutrient to date.

Glutamine is the most abundant non-essential amino acid in the blood and the free intracellular amino acid pool (Askanazi, Carpentier, *et al*., 1980; Essen *et al*., 1992; Oudemans-van Straaten *et al*., 2001; Roth, 2008; Van Acker *et al*., 2000). It constitutes more than half (more or less 61% in healthy men) of the total free amino acids, as well as 5–6% of bound amino acids (Askanazi, Carpentier, *et al*., 1980; Bergström *et al*., 1974; Essen *et al*., 1992; Roth, 2008; Van Acker *et al*., 2000). Glutamine supplementation has gained significant interest for its application in athletes and critically ill patients.

Twenty years ago, researchers started investigating glutamine's use in critical illness and today it is applied in many clinical settings, either added to frequently used parenteral and enteral nutrition formulations or supplemented via the intravenous (IV) or oral route. A large amount of literature exists on this nutrient, elucidating the science of glutamine's metabolism, as well as the benefits of supplementation in certain disease states (Cynober & De Bandt, 2014; Gottschalk *et al*., 2013). Published South African-based studies are, however, still limited or unavailable, so that evidence in this population group is lacking. This literature review will focus

on the available literature, providing an overview of the metabolism and functions of glutamine and summarising the evidence on glutamine kinetics and supplementation in critical illness.

2.2 The physiology of glutamine

In order to better understand the relationship between endogenous glutamine production, plasma glutamine levels, glutamine transport between tissues and its utilisation, it is important that glutamine kinetics in the human body first be explained. The two enzymes that play a significant role in glutamine metabolism, namely glutaminase and glutamine synthetase (GS), are found predominantly in the liver and skeletal muscles (Labow *et al*., 2001; Roth, 2008). Glutamine is degraded by glutaminase, while GS is responsible for its *de novo* synthesis and they are regulated by short- and long-term factors (Newsholme & Carrié, 1994; Watford *et al*., 2002). Two long-term factors that cause decreased glutaminase and GS activity include a low-protein diet and insulin secretion, while diabetes and glucocorticoids will up-regulate both these enzyme activities. Furthermore, a long-term high-protein diet, starvation and acidosis all cause increased glutaminase activity, in other words, enhanced glutamine degradation, while long-term starvation will have a down- regulating effect on GS (Watford *et al*., 2002).

Endogenous glutamine is derived mainly from muscle proteolysis and synthesis via GS. In healthy subjects, endogenous glutamine production has been reported to fall between 50g and 80g per day, contributing significantly to the maintenance of glutamine homeostasis (Darmaun *et al*., 1994; Kuhn *et al.,* 1999). It is produced in the cell cytoplasm, predominantly from branched-chain amino acids (BCAA) and glutamate provided by proteolysis and uptake in the skeletal muscles (Häussinger *et al*., 1985; Labow *et al*., 2001; Roth, 2008; Vesali *et al*., 2002). Glutamine synthesis is dependent on the availability of precursors, but mostly on the activity of GS as the rate-limiting factor (Häussinger *et al*., 1985; Labow *et al*., 2001; Vesali *et al*., 2002). It has been reported that skeletal muscle produces more than 60% of synthesised glutamine, owing to its large available free amino acid pool and high GS activity. The skeletal muscles also contain 90% of stored glutamine (Bergström *et al*., 1974; Darmaun *et al*., 1994; Ytrebø *et al*., 2006). Furthermore, the muscles contribute to detoxification by taking up ammonia (NH_3) and converting it to glutamine (Cahil *et al*., 1972; Ytrebø *et al*., 2006). To a lesser extent, most other tissues such as the liver, brain, and adipose tissues, but especially the kidneys and pulmonary tree, are also able to synthesise glutamine (Hulsewé *et al*., 2003; Iqbal & Ottaway, 1970; Nurjhan *et al*., 1995; Van Acker *et al*., 2000; Vesali *et al*., 2002). Glutamine can therefore be described as a non-essential amino acid.

The synthesised glutamine can then be exported from cells (Newsholme *et al*., 2003; Vesali *et al*., 2002). Following glutamine's release from the periphery, it is taken up mainly by the splanchnic bed (i.e. liver and gastrointestinal tract (GIT)) (Felig *et al*., 1973; Marliss *et al*., 1971;

Nurjhan *et al*., 1995). As reviewed by Soeters & Grecu (2012), active transport, predominantly mediated by the sodium/ potassium adenosine triphosphatase (Na^+/K^+- ATPase)-driven ion pump, is responsible for the movement of the glutamine between tissues and plasma. Transport systems are then available for the uptake of glutamine into the mitochondria of cells (Häussinger *et al*., 1985; Molina *et al*., 1995). Felig *et al.* (1973) reported that the GIT, rather than the liver, absorbs most of the glutamine from the circulation. However, a more recent study demonstrated that the liver consumes almost half of the exported glutamine (Watford *et al.*, 2002). Nevertheless, the liver can export or remove glutamine as regulated by GS and glutaminase activity and is therefore thought to play a significant role in the conservation of glutamine homeostasis in the body (Watford *et al*., 2002). Additionally, it has been found that glutamine is also removed from the circulation by the kidney, which indicates that the kidneys also play a role in the maintenance of blood glutamine levels (Marliss *et al*., 1971). The removal of glutamine from the circulation is therefore largely dependent on the functioning of different organs, especially the GIT, liver and kidneys. The dysfunction of these organs can then lead to the accumulation of glutamine in the blood.

Following glutamine uptake, the pathway of its oxidation is termed glutaminolysis (Figure 2.1) (Curi *et al*., 1999). Glutamine metabolism begins with its deamination, producing glutamate and ammonium (NH_4^+) (Figure 2.1) (Newsholme & Carrié, 1994; Quesada *et al*., 1988; Soeters & Grecu, 2012). The immediate product of glutamine metabolism is, consequently, glutamate, which is considered the most abundant intracellular amino acid (Newsholme *et al*., 2003). Glutamate can then be transported back to the cell cytosol for the production of glutathione (Figure 2.1) (Newsholme & Carrié, 1994; Quesada *et al*., 1988; Soeters & Grecu, 2012). Adequate amounts of glutamine will therefore maintain the intracellular glutamate pool and thus avoid glutathione depletion (Amores-Sánchez & Medina, 1999). Glutamate can also yield α-ketoglutarate, which serves as an intermediate, replenishing the Krebs cycle (Figure 2.1) (Soeters & Grecu, 2012). Alanine and carbon dioxide are the final end products of glutamine metabolism (Newsholme & Carrié, 1994).

Glutamine can also be consumed exogenously either via food intake or in the form of supplementary enteral, parenteral or oral glutamine. Dietary intake of glutamine typically varies between four and 8g per day, which is significantly less than that which is produced endogenously (Palmer *et al*., 1996). Exogenous glutamine consumption from dietary sources is absorbed predominantly in the small intestine by the epithelial sodium-dependent neutral amino acid transporter system B (ATB°), but also via the sodium-independent neutral amino acid transport system L (Choudry *et al*., 2006; Minami *et al*., 1992). A large proportion of the glutamine that is provided from protein digestion in the GIT is absorbed and utilised by the cells of the intestines and is found only in small amounts in the blood. Therefore other rapidly

proliferating cells are mostly dependent on muscle amino acid metabolism for glutamine provision (Newsholme, 2001). An increase in glutamine luminal uptake in the small intestine has been observed with increased enteral or oral glutamine consumption, the driving force for transport being a proton gradient with no saturation point (Choudry *et al*., 2006; Déchelotte *et al*., 1991; Minami *et al*., 1992). Consequently, supplemented glutamine will be continuously absorbed even if high concentrations of glutamine are already present in intestinal cells.

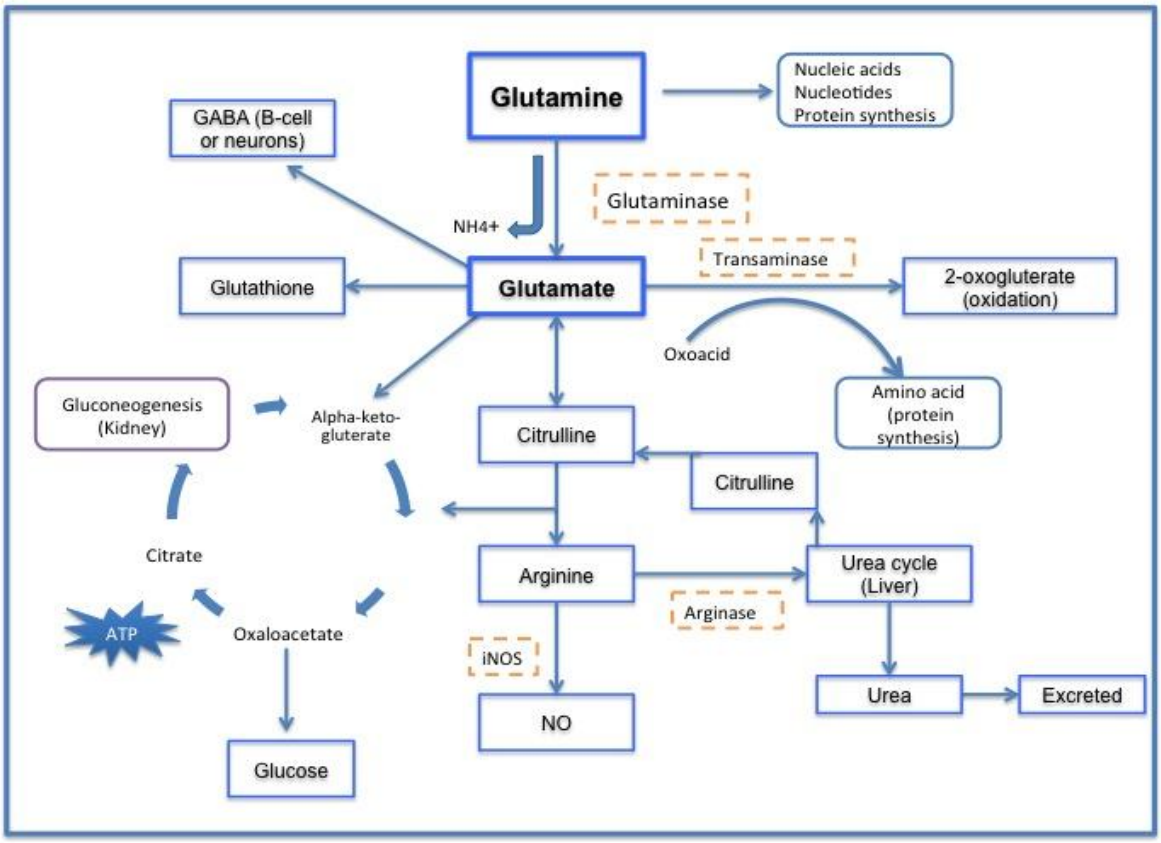

Figure 2.1 Glutamine metabolism in cells (adapted from Dupertuis *et al.* (2009), Newsholme *et al.* (2003) and Stumvoll *et al.* (1999))

GABA: *gamma*-aminobutyric acid; iNOS: inducible nitric oxide synthase; NH_4^+: Ammonium; NO: nitric oxide

Intravenously supplemented glutamine has been shown to increase plasma glutamine levels correspondingly (Mori *et al*., 2014). In healthy individuals, alanyl-glutamine (ALA-GLN) dipeptide has a half-life of five minutes, which may result in a steady state in plasma glutamine levels within an hour after supplementation (Berg *et al*., 2005). In critically ill patients, however, ALA-GLN was shown to have a median half-life of 16 minutes. Nevertheless, the time in which a steady state in plasma levels is reached after supplementation varies among critically ill patients and is influenced by several factors (Berg *et al*., 2005).

When the excretion rate of exogenous supplemented glutamine was measured, it was found that only 0.2% of ingested glutamine was excreted and that increased plasma glutamine levels did not result in higher excretion rates (Berg *et al*., 2005). Additionally, tracer bolus methods investigating ALA-GLN infusion demonstrated that glutamine production is not controlled by circulating glutamine levels (Mori *et al*., 2014). Increased exogenous glutamine intakes, therefore, will neither reduce endogenous glutamine production and release, nor lead to a higher excretion rate, and, in turn, can lead to a significant increase in circulating glutamine levels. However, an increased parenteral intake of other precursor amino acids will increase the glutamine synthesis rate proportionally (Mori *et al*., 2014). The physiology behind glutamine has been provided as background, its resulting functions will now be briefly discussed in order to demonstrate its significant role in the human body.

2.3 Functions of glutamine, with emphasis on its role in critically ill patients

Glutamine serves important functions in the human body and is involved in multiple biochemical processes. It is thought to play a significant role in the functioning of different tissues and cells, including the kidneys, lungs, central nervous system, heart, hepatocytes, enterocytes, immune cells, white adipocytes and the pancreas (β-cells) (Curi *et al.*, 2005; Newsholme *et al*., 2003; Wischmeyer, 2003). Figure 2.2 demonstrate glutamine's potential functions in critically ill patients.

2.3.1 Glutamine's role in anabolic activities and nitrogen metabolism

One of the major concerns in critically ill patients is malnutrition, which is seen as a contributor to mortality and morbidity in this patient group (Giner *et al*., 1996; Singh *et al*., 2006). Here glutamine may play a significant role by improving nutritional status, as was evident when investigating the effect of parenteral glutamine supplementation on the pre-albumin and transferrin concentrations of burns patients (Wischmeyer, Lynch, *et al*., 2001). Furthermore, Le Bacquer *et al.* (2001) found the availability of glutamine to be an important contributing factor to the rate of protein synthesis. Glutamine assists in the non-toxic transfer of nitrogen from peripheral tissues, where it is synthesised, to the splanchnic bed and other organs, including the kidneys, neurons, and immune cells (Amores-Sánchez & Medina, 1999; Avenell, 2006; Darmaun *et al*., 1994; Newsholme *et al*., 2003; Nurjhan *et al*., 1995). Here it donates nitrogen for many anabolic activities, including the production of non-essential amino acids, peptides, proteins, purines, pyrimidines, and, therefore, the synthesis of nucleic acids (Figure 2.1 and 2.2) (Avenell, 2006; Boza *et al*., 2000; Oliveira *et al.*, 2010; Wischmeyer, 2003). Parenteral glutamine supplementation may improve glutamine concentrations in plasma and skeletal muscles, thereby promoting protein synthesis and leading to a better whole-body nitrogen balance (Andreasen *et al*., 2009; Berg *et al*., 2005; Fuentes-Orozco *et al*., 2008; Hammarqvist

et al., 1989; Ockenga *et al*., 2002). In contrast, Tjäder *et al.* (2004) reported that supplemental parenteral glutamine has no effect on muscle glutamine levels in ICU patients, even when plasma glutamine levels were restored or supra-normal. The authors concluded that supplemental parenteral glutamine does not increase muscle protein synthesis (Tjäder *et al*., 2004). More research is therefore still needed to ascertain the role of glutamine supplementation in the improvement of protein synthesis and nutritional status in ICU patients.

Another important function of glutamine supplementation in this regard is that it may enhance hepatic energy levels by increasing adenosine triphosphate (ATP) (Figure 2.1 and 2.2) (Dhar *et al*., 2003). Here it provides nitrogen to refill the intermediates of the Krebs cycle, as mentioned in Section 2.2 (Yuneva *et al*., 2007)**.** High rates of glutaminase activity have further been found in adipose tissue, comparable with its activity in other tissues such as lymphocytes. Therefore glutamine is also used in these cells, but its exact role still needs to be confirmed (Kowalchuk *et al*., 1988). In addition, glutamine has an anti-lipolytic effect, thereby preserving fat stores (Déchelotte e*t al*., 1991).

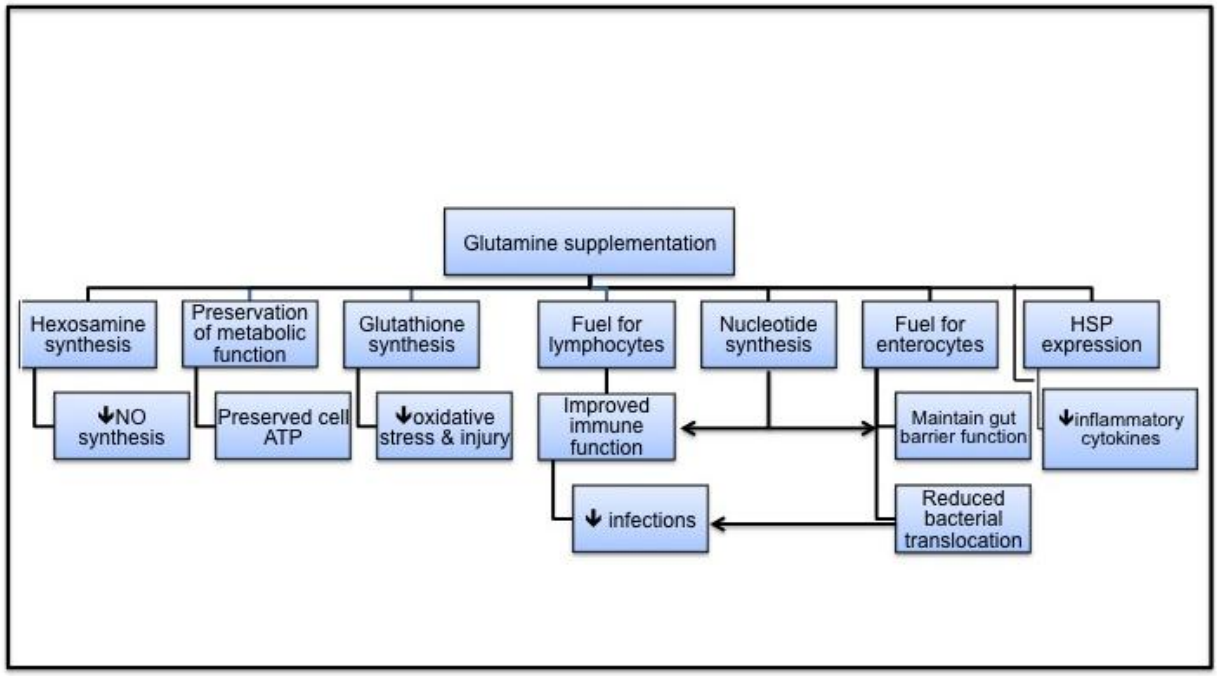

Figure 2.2 Potential functions of glutamine in critically ill patients (adapted from Wischmeyer (2003))

ATP: adenosine triphosphate; HSP: heat-shock protein; NO: nitric oxide

The nitrogen obtained from glutamine is disposed of via ammoniagenesis in the kidney and ureagenesis in the liver (Nurjhan *et al*., 1995). Therefore glutamine plays a significant role in ammonia metabolism and is the most important nitrogen donor and precursor of renal NH_3 formation and detoxification (Newsholme *et al*., 2003; Oliveira *et al*., 2010; Roth, 2008). Consequently, it is also important in maintaining the acid-base homeostasis in the kidney

(Oliveira *et al*., 2010). Hence glutamine can be described as an anti-catabolic amino acid and plays a significant role in nitrogen metabolism (Santora & Kozar, 2010).

2.3.2 Glutamine's role in the immune system and inflammatory processes

Macrophages, lymphocytes and neutrophils all have important functions in immunity and inflammatory processes (Melis *et al*., 2004; Newsholme, 2001). Glutamine's protective effect on the immune system is seen as one of its main purposes (Amores-Sánchez & Medina, 1999). This is related to the fact that glutamine, as is now known, acts as the primary fuel for rapidly proliferating cells such as the enterocytes and immune cells, although it was initially thought that glucose provided the main energy source for these cells (Curi *et al*., 1999; De-Souza & Greene, 2005; Wilmore, 2001; Wischmeyer *et al.*, 2003). Moreover, the rate of glutamine utilisation seems to be the same as, or even higher than, that of glucose in these cells, providing an important source of energy in the form of ATP (Figures 2.1 and 2.2). This is especially true in macrophages, where the activity of glutaminase in an immune challenge is fourfold higher than in lymphocytes (Ardawi & Newsholme, 1983; Curi *et al*., 1999; Newsholme *et al*., 1985; Newsholme *et al*., 1986; Newsholme, 2001; Zellner *et al*., 2003).

In lymphocytes glutamine is important for cell proliferation, energy production and as a precursor for the synthesis of macromolecules, while in macrophages it plays a role in messenger ribonucleic acid (mRNA) production, phagocytosis and arginine synthesis (Newsholme, 2001; Newsholme *et al*., 1985; Newsholme *et al*., 1986; Roth, 2008; Spittler *et al*., 1995). Additionally, glutamine controls the functioning and expression of cell surface molecules in macrophages (Roth, 2008; Spittler *et al*., 1995). It is also a recognised precursor of purine and pyrimidine synthesis, which is required when lymphocytes and macrophages are activated (Roth, 2008). Neutrophils function as the first line of defence in infection, and glutamine is required for their phagocytic activity and superoxide production (Newsholme, 2001). Hence many immune cells use glutamine at high rates and their activity has also been linked to glutamine availability (Newsholme, 2001).

Yeh *et al.* (2008) described how peri-operative glutamine supplementation contributes to a less pronounced decrease in lymphocyte count in gastrointestinal surgery patients. This then contributes to a significantly lower depression of the cellular immunity in these patients (Yeh *et al*., 2008). In support of this, an increase in lymphocyte counts as well as CD4, CD8, and immunoglobulin A (IgA) concentrations was observed in another study supplementing parenteral glutamine to acute pancreatitis patients (Fuentes-Orozco *et al.*, 2008).

Human leukocyte antigen-DR (HLA-DR) receptor expression may be one of the explanations for the beneficial immune effects of supplemental glutamine. The expression of HLA-DR has been found to be lower in trauma patients than in healthy controls, which may impair their cellular

immune function and cause increased susceptibility to infections (Boelens *et al*., 2002). Boelens *et al.* (2002) demonstrated that enteral glutamine supplementation induced a higher expression of HLA-DR in trauma patients, thereby improving immune functioning.

With regard to glutamine's role in inflammatory processes, the beneficial effects of glutamine supplementation have also been attributed to the possible reduction of the acute inflammatory response by the modulation of pro-inflammatory markers (Figure 2.2) (Wischmeyer, Kahana, *et al*., 2001). Glutamine availability is a determining factor in the rate of interleukin (IL)-2 production by T-lymphocytes, as well as superoxide, IL-1 and IL-6 production by macrophages (Newsholme, 2001; Yassad *et al*., 1997). Furthermore, it has been proven that glutamine supplementation reduces the release of pro-inflammatory cytokines. Fuentes-Orozco *et al.* (2008) found that parenteral glutamine administration decreased pro-inflammatory IL-6 levels, while increasing IL-10 levels as an anti-inflammatory cytokine (Wischmeyer *et al*., 2003). The findings of another study are consistent with this, reporting that administered parenteral nutrition containing glutamine recovered IL-6 and IL-10 levels in rodent sepsis (O'Leary *et al*., 2007). Its effect on tumour necrosis factor alpha (TNF-α), however, is still uncertain as Andreasen *et al.* (2009), could not detect any changes in TNF-α in glutamine-supplemented groups.

Another well-known inflammatory marker to consider is C-reactive protein (CRP). Glutamine administered parenterally has been found to significantly decrease CRP concentrations in various patient groups (Fuentes-Orozco *et al*., 2008; Ockenga *et al*., 2002; Sahin *et al*., 2007; Wischmeyer, Lynch, *et al*., 2001; Yeh *et al*., 2008). Yeh *et al.* (2008) supplemented glutamine pre- and post-operatively, and found significantly lower CRP concentrations in those receiving glutamine. Therefore a possible inverse relationship between glutamine stores and CRP levels can be predicted.

From the above literature it is clear that this amino acid plays a significant role in both immune balance and anti-inflammatory processes, thereby contributing to better patient outcomes. The GIT is not only important for the digestion and absorption of food, but is also considered an immunological organ and therefore glutamine's effect in intestinal cells should also be clarified (Melis *et al*., 2004).

2.3.3 Glutamine's role in the gastrointestinal tract

Increased intestinal permeability is proposed as a contributor to systemic infectious complications in critically ill patients, which is associated with a higher frequency of systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF), but this is yet to be confirmed in humans (De-Souza & Greene, 2005; Doig *et al*., 1998). In addition, changes in GIT immunity, in other words, the gut-associated lymphoid tissue (GALT), have been reported to lead to an increased risk of infections in patients (Kudsk *et al*., 2000). The protection of the GIT

may, therefore, improve the outcome of critically ill patients by providing a barrier to prevent the entry of bacteria or toxins (Amores-Sánchez & Medina, 1999; Jones *et al*., 1999).

As previously mentioned, glutamine is the predominant fuel for enterocytes and colonocytes, promoting ATP synthesis (De-Souza & Greene, 2005; Newsholme *et al.,* 2003). For this reason, glutamine supplementation could contribute to decreased gut atrophy, resulting in improved intestinal absorption, together with a lower risk diarrhoea and malabsorption, as well as reduced intestinal permeability in critical illness (Leite *et al*., 2013; Oliveira *et al*., 2010; Wilmore, 2001; Wischmeyer, Lynch, *et al*., 2001). The findings of previous research are consistent with this, showing that parenteral glutamine supplementation resulted in significantly reduced intestinal permeability of patients undergoing major abdominal surgery and those with chronic intestinal inflammatory disease or intestinal neoplasia (Jiang *et al*., 1999; Van der Hulst *et al*., 1993). Likewise, enteral glutamine supplementation was also found to reduce and normalise intestinal permeability in burns patients (Zhou *et al*., 2003). However, Conejero *et al.* (2002) could not demonstrate that enteral glutamine supplementation reduced intestinal permeability in critically ill patients with SIRS. Therefore more research is required in this regard.

Wischmeyer, Lynch, *et al.* (2001) hypothesised that glutamine enhances gut barrier function and thereby prevent bacterial translocation, but could not positively confirm this. Glutamine supplementation in the critically ill has been shown to maintain the intestinal barrier function and the GALT (De-Souza & Greene, 2005). Lai *et al.* (2004) reported an increase and maintenance in the total Peyer's patch lymphocyte yield in GALT when supplementing septic rats with glutamine enterally and parenterally. This study also found higher plasma and gut IgA levels, as well as lower secretions of IL-10 by lymphocytes in glutamine-supplemented groups. In support of this, Coëffier *et al.* (2003) and Kudsk *et al.* (2000) reported that parenteral glutamine supplementation improved mucosal immunity, as well as intestinal IgA, IL-4, and IL-10 levels, while attenuating pro-inflammatory IL-6 and IL-8 in the mucosa. Whether these effects translate into reduced bacterial translocation is still to be confirmed. On the other hand, Clark *et al.* (2003) established that glutamine depletion in inflammation led to the unavailability of fuel substrate for the gut barrier, which can lead to bacterial translocation. Lastly, glutamine preserves glutathione levels in the GIT, which further helps to protect against gastric damage (Amores-Sánchez & Medina, 1999).

Glutamine therefore has a significant role in the GIT. The exact mechanism behind the manner in which glutamine preserves the gut is still unknown, but is probably attributable to multiple factors, including that it serves as a substrate for enterocytes and maintains gut immunity, while reducing oxidative stress and inflammation in the GIT.

2.3.4 Glutamine and the regulation of cells

Glutamine's non-nutritive effects have been well established and one of these includes its osmo-regulatory effect, which is not only confined to glutamine-consuming cells (Roth, 2008). Furthermore, it is known as a signalling molecule for the activation of genes and signalling pathways. In this regard it has a cyto-protective effect via the expression of heat-shock proteins (HSPs). Heat-shock proteins provide protection for cells and tissues against injury, allowing for the correct refolding of denatured proteins, and are essential to ensure the survival of cells under conditions of injury and stress (Oliveira *et al*., 2010; Santora & Kozar, 2010; Wischmeyer, Kahana, *et al*., 2001; Ziegler *et al*., 2005). Major HSPs in cellular protection include HSP70 and HSP25 (Santora & Kozar, 2010; Wischmeyer, Kahana, *et al*., 2001; Ziegler *et al.,* 2005). Heat-shock protein 70 is important specifically for the refolding of proteins, while other HSPs have other important functions in cells (Lightfoot *et al*., 2009). Increased HSP expression has also been shown to inhibit nuclear factor kappa activation and decrease pro-inflammatory cytokine levels in plasma, thereby exerting an anti-inflammatory effect (Yoo *et al*., 2000). Decreased expression of HSPs was previously reported in critically ill, stressed patients, possibly attributed to deficient glutamine levels (Lightfoot *et al*., 2009; Wischmeyer, 2005; Wischmeyer, 2006).

Glutamine supplementation has been proved to increase HSP25, HSP27, HSP32, HSP70, and HSP72 expression in stressed and unstressed animals, cell cultures and human organs, specifically in the heart, kidney, lungs and colon (Figure 2.2) (Santora & Kozar*,* 2010; Wischemeyer, 2006; Wischmeyer, Kahana, *et al*., 2001; Wischmeyer *et al*., 2003; Ziegler *et al*., 2005). This amplified expression has been found at dosages of glutamine thought to be harmless for human consumption and that correlate with improved clinical outcomes (Wischmeyer, Kahana, *et al*., 2001; Ziegler *et al*., 2005). However, a study conducted by Andreasen *et al.* (2009) on isolated blood mononuclear cells could not demonstrate any effect of glutamine supplementation on HSP70 or cytokine concentrations. These results may, however, have been due to insufficient dose and duration of administration used in this particular study. The beneficial effects attributed to glutamine supplementation can thus possibly be ascribed to its direct anti-inflammatory effects on cytokine production or indirect anti-inflammatory, and cellular protective effects, by inducing increased HSP expression.

2.3.5 Glutamine and glutathione production

Glutathione is a major intracellular anti-oxidant providing protection against reactive oxygen species and oxidative injury in cells (Denno *et al*., 1996; Hong *et al*., 1992; Oliveira *et al*., 2010). It plays a significant role in ensuring normal cell functioning and replication, and protects against glucose-deprivation-activated protein (Denno *et al*., 1996; Lee, Galoforo, *et al*., 1998). Glutamine is one of the precursors of glutathione via glutamate synthesis, where a strong

relationship between glutathione levels and glutamine supply has been established (Figures 2.1 and 2.2) (Hong *et al*., 1992; Roth, 2008).

As the simple administration of glutamate or glutathione in humans is not feasible, glutamine can be supplemented as precursor to exert glutathione's beneficial anti-oxidant effects (Amores-Sánchez & Medina, 1999; Belmonte *et al*., 2007; Fläring *et al*., 2003). Parenteral glutamine supplementation has been found to enhance glutathione production in animal studies by increasing its translocation from the liver in support of plasma concentrations (Denno *et al*., 1996). Furthermore, human studies have demonstrated that post-operative parenteral glutamine supplementation attenuated the diminished glutathione levels found after surgery (Fläring *et al*., 2003). Intravenous glutamine administered to enterally fed, critically ill trauma patients has also been shown to increase plasma glutathione levels when administered at dosages of 0.5 g/kg per day (Eroglu, 2009). Enteral glutamine can also reduce intestinal oxidative stress by increasing mucosal glutathione levels, as mentioned earlier (Hong *et al*., 1992; Joon Suh *et al*., 2003).

Similarly, taurine may become a conditionally essential amino acid in critical illness (Chiarla *et al.,* 2000). Glutamine supplementation leads to the increased availability of taurine to organs, which, in addition to glutathione, serves as an endogenous anti-oxidant in stressed states (Boelens *et al*., 2003; Melis *et al*., 2005). However, further investigation is required to confirm this. It can be concluded that glutamine has an anti-oxidant capacity by means of glutathione and possibly taurine production, thereby protecting tissues against oxidative stress in critical illness (Amores-Sánchez & Medina, 1999; Belmonte *et al*., 2007; Santora & Kozar, 2010).

2.3.6 Glutamine's effect on glucose metabolism

Glutamine further plays a role in glucose production and metabolism (Felig & Wahren, 1971; Newsholme & Carrié, 1994). Besides plasma glucose, there are four sources of carbon involved in maintaining the glucose pool. These include hepatic glycogen, triglycerides, lactate, and amino acids (Stumvoll *et al*., 1996). In this regard, glutamine is an important carrier of carbon in plasma and therefore expands the glucose pool (Nurjhan *et al*., 1995). Furthermore, Nurjhan *et al.* (1995) reported that glutamine was an important producer of glucose, comparable to alanine, the other major gluconeogenic amino acid.

Glutamine is also thought to improve the insulin sensitivity of adipose tissue, decreasing lipolysis and improving glucose homeostasis. Hence glutamine may be useful as an anti-obesity and anti-diabetic agent, especially in pre-diabetic patients (Roth, 2008). However, the enteral administration of glutamine has been found to have no effect on either plasma glucose levels, the glucose appearance rate or plasma insulin levels in a study conducted by Déchelotte *et al.*

(1991). Further investigation is therefore required to ascertain the exact role of exogenously administered glutamine in glucose metabolism.

2.3.7 The relationship between glutamine and arginine

The seventh mechanism by which glutamine supplementation exerts its beneficial effects is by increasing arginine synthesis through the intestinal-kidney pathway (Figure 2.1). In the intestine glutamine is converted to citrulline and then further used in the kidney for arginine synthesis (Coëffier & Déchelotte, 2010). Glutamine can therefore provide a protective effect in critical illness by means of arginine production (Houdijk *et al*., 1998; Murphy & Newsholme, 1998). This was found to be true particularly for supplementation via the enteral route, which has been reported to produce the highest levels of arginine (Houdijk *et al*., 1998). In contrast, Melis *et al.* (2005) found increased plasma arginine levels only with parenteral glutamine supplementation, but not with enteral glutamine. Thus more research is required to explore the effect of parenteral versus enteral glutamine supplementation on arginine concentrations.

In addition, arginine degradation can lead to the formation of various products, including nitric oxide (NO), ornithine, urea, polyamines, proline, glutamate, polyamines, and creatine, which exert important functions in the body (Coëffier & Déchelotte, 2010). It would then be assumed that glutamine acts as a precursor to NO and increases its production by means of arginine synthesis (Murphy & Newsholme, 1998). However, the opposite is true, as glutamine dose-dependently inhibits NO's release in endothelial cells and macrophages, via a glucosamine-dependent mechanism causing decreased inducible NO synthase (iNOS) (Figure 2.2). Therefore it plays a governing role in the biosynthesis of NO and can aid in the prevention of ischemia or reperfusion injury (Arnal *et al*., 1995; Coëffier & Déchelotte, 2010; Dupertuis *et al*., 2009; Joon Suh *et al*., 2003; Roth, 1998).

In summary, glutamine supplementation in the critically ill patient is important for HSP expression, better intestinal integrity, anti-inflammatory processes, maintenance of ATP levels, up-regulation of insulin sensitivity and enhanced glutathione levels and may also play a role in the maintenance of nutritional status, thereby improving patient outcomes. Considering all the functions that glutamine can fulfil in the human body, it is now important to discuss the way in which the glutamine status of patients can be determined.

2.4 Plasma glutamine levels as marker of glutamine status

Plasma glutamine levels are frequently used as a measure to quantify glutamine availability in the body. Normal plasma glutamine levels in healthy individuals have been reported to fall between 400 µmol/L and 700 µmol/L (Vinnars *et al*., 1975; Wischmeyer, Lynch, *et al*., 2001). Other articles reported the reference ranges for plasma glutamine levels as between 482 µmol/L

and 938µmol/L, or an average of 570 µmol/L (Bergström *et al*., 1974; Boelens *et al*., 2002; Houdjik *et al*., 1998). Nevertheless, plasma levels lower than 420 µmol/L can be labelled as deficient, while those above 930 µmol/L can be considered supra-normal (Oudemans-van Straaten *et al*., 2001; Rodas *et al*., 2012). Muscle and liver concentrations of glutamine are much higher and fall between 5 mmol/L and 20 mmol/L or, more specifically, 19.45 mmol/L, in healthy adults (Bergström *et al*., 1974; Vinnars *et al*., 1975). The question then arises as to whether plasma glutamine is reflective of a patient's glutamine status and whether it can be used to determine the need for supplementation.

2.4.1 Muscle versus plasma glutamine levels

The biggest part of the total free amino acid pool in the human body is found within the cells of the muscles and not in the extracellular space, as plasma amino acids account only for as much as 10% of the free amino acid pool (Askanazi, Carpentier, *et al*., 1980; Bergström *et al*., 1974; Vente *et al*., 1989). Glutamine is freely held in the skeletal muscle in concentrations of 32:1 above plasma glutamine levels, mediated by active transport (Lightfoot *et al*., 2009). Intracellular glutamine concentrations are influenced by various factors, including glutamine production via endogenous synthesis, the uptake of glutamine from amino acid breakdown and protein synthesis, as well as glutamine transport (Soeters & Grecu, 2012). On the other hand, plasma levels will be influenced by glutamine export from the cells to the extracellular space, the free amino acid pool and exogenous glutamine supply (Soeters & Grecu, 2012).

In earlier studies it was suggested that one should not draw any conclusions about the amino acid pool by measuring plasma amino acid levels. Plasma levels were described as a poor reflection of amino acid status, to be interpreted with caution and that deficient muscle glutamine levels would not be evident in measured plasma concentrations (Essen *et al*., 1992; Vente *et al*., 1989; Vinnars *et al*., 1975). Vente *et al.* (1989) further mentioned that plasma amino acid levels are poor indicators of disease severity. Contradicting this later, Vesali *et al.* (2002) found that muscle amino acid balances and fluctuations were virtually comparable with those of whole blood and that whole-blood and plasma glutamine levels, in turn, were practically identical (Vesali *et al*., 2002). Moreover, in a study conducted by Tjäder *et al.* (2004), it was suggested that the muscle free glutamine status might not be a crucial factor for immediate survival in ICU patients, but that the availability of glutamine for other cells (i.e. plasma glutamine levels) was more important. Oudemans-van Straaten *et al.* (2001) agree, describing plasma glutamine levels as an important limiting factor in critical illness, as other cells depend on it for their glutamine supply. Plasma glutamine levels have further been reported to predict outcome in ICU patients (Oudemans-van Straaten *et al*., 2001; Rodas *et al*., 2012). Therefore it currently seems to be the best marker of glutamine status, especially in critical illness (Oudemans-van Straaten *et al*., 2001; Rodas *et al.,* 2012). This highlights the fact that plasma

glutamine levels can be used to determine adequate glutamine supply in the body. The available methods of measuring plasma glutamine levels will now be briefly mentioned.

2.4.2 Measurement of plasma glutamine levels

Obtaining a blood sample is the first step in the determination of plasma glutamine levels. Following sampling, blood glutamine levels will remain stable in samples for one hour at room temperature, after which it will increase by 10% per hour. However, in plasma it will remain stable for at least a month when stored at -70°C (Oudemans-van Straaten *et al*., 2001). Earlier Grossie *et al.* (1999) found plasma glutamine levels to be stable for over one year when kept at -70°C pending analysis. The following step is then to conduct the plasma glutamine analysis.

The measurement of plasma glutamine levels is not routinely available in the ICU setting (Oudemans-van Straaten *et al*., 2001). Plasma glutamine levels can be determined by different methods. Initial methods used for the analysis of amino acids, such as ion-exchange chromatography in combination with post-column ninhydrin detection, were expensive, labour intensive and inefficient (Terrlink *et al*., 1994; Tjäder *et al*., 2004). Alternative methods have since been developed and are being utilised in more recent trials. High-performance liquid chromatography (HPLC) is the preferred method of analysis used in several studies (Berg *et al*., 2005; Berg *et al*., 2006; Fläring *et al*., 2003; Goeters *et al*., 2002; Houdjik *et al*., 1998; Hulsewé *et al*., 2004; Melis *et al*., 2005; Nurjhan *et al*., 1995; Rodas *et al*., 2012; Schroeder *et al*., 2005; Stumvoll *et al*., 1996; Vesali *et al*., 2002). This method is accurate in the determination of amino acids and characterised by its speed of analysis, as well as its low manual sample handling (Terrlink *et al*., 1994). Another laboratory method that has been used in previous trials is an automated amino acid analyser (Askanazi, Carpentier, *et al*., 1980; Askanazi, Furst *et al*., 1980; Blomqvist *et al*., 1995; Hammarqvist *et al*., 2001). Furthermore, micro-fluorometric enzymatic assays can also be applied in the determination of plasma amino acid concentrations (Choudry *et al.*, 2006; Oudemans-van Straaten *et al*., 2001). Likewise, some trials have used gas chromatography-mass spectrometry (GC-MS) for the determination of plasma glutamine levels (Biolo *et al*., 2000; Darmaun *et al*., 1994; Matthews & Campbell, 1992). This method was also applied in the current research project for laboratory analysis (Chapter three). When now interpreting plasma glutamine levels, there are certain factors that should be taken into account that may influence results. The following section will aim to elucidate these factors.

2.4.3 Factors influencing plasma glutamine levels

There are several factors, which can influence plasma amino acid levels (Askanazi, Furst *et al*., 1980; Darmaun *et al*., 1994). The effect of selected factors on plasma glutamine levels will now be discussed in further detail.

2.4.3.1 Nutrition and insulin secretion

Nutrition leads to the availability or absence of substrate, which can then alter glutamine synthesis and release, in turn affecting plasma glutamine levels. Fasting has been reported to alter plasma glutamine levels, as glutamine and arginine are the main amino acids released from muscle into the circulation during brief starvation (Cahill *et al*., 1972). The differing plasma levels found in the fasted and nourished state can be partly ascribed to the effects of insulin, as its presence is known to decrease endogenous proteolysis (Engelen *et al*., 2000). Increased circulating glucose and amino acid concentrations lead to an increased release of insulin, thereby decreasing the rate of muscle proteolysis, with reduced plasma glutamine levels and increased muscle synthesis (Cahill *et al*., 1972). The opposite is thus also true for starvation, where insulin levels will drop, causing proteolysis, together with a release of amino acids from tissue into the circulation (Aoki *et al*., 1972). Amino acid levels have also been reported to normalise instantly during refeeding following a period of fasting (Hammarqvist *et al*., 2001).

In contrast, Darmaun *et al.* (1994) studied the changes in glutamine metabolism when administering enteral and parenteral nutrition versus the fasted state and found no effect on plasma glutamine levels in healthy men. Castel and Newsholme (1997) also reported no change in plasma glutamine levels from normal with a 21-hour fast. The glutamine appearance was reported to increase, however, in the nourished state compared with the fasted state, especially with enteral nutrition administration (Darmaun *et al.*, 1994). This was supported by the findings of Hankard *et al.* (1997) and Marliss *et al.* (1971), who reported that plasma glutamine decreased only slightly during a fasting period, but that an 11% decline in the glutamine appearance rate was observed between 18 and 42 hours of fasting.

It can therefore be concluded that there will be a decreased release of glutamine with prolonged fasting, but that this will not affect plasma glutamine levels. The decreased glutamine appearance rate found as result of extended fasting periods has been attributed to the eventual reduced *de novo* glutamine synthesis (Hankard *et al*., 1997). Glutamine can also be converted to glucose after 18 and 42 hours of fasting, with the contribution of glutamine to glucose production increasing proportionately with the duration of the fast. Therefore glutamine can be seen as a precursor of glucose in the fasted state (Hankard *et al*., 1997). However, the reason for the increased glutamine appearance rate after feeding is still unknown, but it could be due to enhanced endogenous glutamine synthesis, release from protein breakdown, the conversion of enteral glutamate to glutamine or the absorption of free exogenous glutamine from enteral feeds (Darmaun *et al*., 1994). Nevertheless, this information proves that fasting versus nourishment will not have a pronounced effect on plasma glutamine levels, but the contradictory findings indicate that this still requires further investigation.

As glutamine is an amino acid, it can be anticipated that glutamine concentrations will be influenced by dietary protein intake. Interestingly, an inverse association has been proved between plasma glutamine levels and protein intake in the post-absorptive state (Matthews & Campbell, 1992; Swendseid *et al*., 1966). A study investigating the effect of dietary protein intake on plasma glutamine levels found that reduced protein consumption did not markedly influence glutamine levels where an adequate nitrogen balance was maintained. However, a large reduction in protein intake, as low as 3.5 g of nitrogen, caused a negative nitrogen balance, with decreased essential amino acid levels and increased plasma glutamine levels (Swendseid *et al*., 1966). This study concluded that pronounced changes in dietary protein intake influences glutamine status. Matthews and Campbell (1992) agree, reporting that plasma glutamine was increased by 25% with decreased protein consumption in healthy adult men. In this study the glutamine flux also decreased when going from deficient to adequate protein intakes (Matthews & Campbell, 1992). Focusing specifically on glutamine administration, it was found that after administering ALA-GLN parenterally, deficient plasma glutamine levels returned to normal within eight hours after the start of infusion (Berg *et al*., 2005). The specific effects of glutamine supplementation will be further discussed in Section 2.6, but it is clear from the results mentioned above that the protein content of the diet could have an effect on plasma glutamine levels. Whether this is also true in critical illness requires further research.

In the available literature on the effect of carbohydrate intake on plasma glutamine levels, it has been reported that small amounts administered to fasting men decrease proteolysis and plasma amino acid concentrations (Cahill *et al*., 1972). Similarly, Felig and Wahren (1971) found a decreased release of amino acids after a high-dose glucose infusion, associated with hyper-insulinemia, thus reducing plasma glutamine levels. In conclusion, it seems that neither fasting nor the provision of nutrition influences plasma glutamine levels as such, but that the nutrient content and distribution provided could have an effect, more specifically with reference to protein and carbohydrate content. Whether this can directly be applied to the critically ill population group still needs to be determined.

2.4.3.2 Body composition

Lower plasma amino acid levels have been found in malnourished patients, especially those who have some form of inflammation (Suliman *et al*., 2005). Decreased muscle mass, as a result of wasting, can contribute to glutamine deficiency by altering muscle glutamine flux, as the skeletal muscles are the main producers of glutamine (Hulsewé *et al*., 2004; Suliman *et al*., 2005). However, in surgical patients, no association could be found between plasma glutamine levels and the patients' percentage ideal body weight, fat free mass or percentage weight loss (Hulsewé *et al*., 2004). The latter study, however, was not conducted in critically ill patients, and therefore the relevance to this population is unknown and the results cannot be directly

extrapolated. Further evidence is required regarding the influence of body composition on plasma glutamine levels in critically ill patients.

2.4.3.3 Age

Age may influence amino acid levels owing to changes in body composition with increasing age, and the accompanying metabolic alterations. Growth has been shown to influence plasma glutamine levels, where the total amino acid level increases significantly in growth of boys and girls, but more rapidly in boys (Armstrong & Stave, 1973). Armstrong and Stave (1973) also reported a significant correlation between glutamine and age in adults of certain age groups. Plasma glutamine levels are, however, the most closely regulated of all the amino acids, with a low variability among individuals (Armstrong & Stave, 1973). Furthermore, Jackson *et al.* (1999) reported similar plasma glutamine levels in elderly (older than 60 years) and younger (younger than 35 years) healthy subjects. It is therefore unclear whether age is an influential factor when interpreting plasma glutamine levels in a healthy population group.

Nevertheless, Oudemans-van Straaten *et al.* (2001) reported that lower plasma glutamine levels (less than 420 μmol/L) were associated with a higher age in ICU patients. The glutamine appearance rate was also found to be lower in elderly subjects (Oudemans-van Straaten *et al*., 2001). Hack *et al.* (1997) concur, reporting lower glutamine exchange rates in older subjects when compared with younger subjects. This may be due to the fact that skeletal tissue takes a larger share of glutamine produced by endogenous synthesis in addition to lower hepatic glutamine synthesis (Hack *et al*., 1997). Moreover, the decreased glutamine levels present in older subjects might be related to their recognised lower protein stores (lean body mass (LBM)) and increased body fat stores with increasing age (Hack *et al*., 1997). It can thus be stated that changes in glutamine kinetics with age may be related to body composition and to changes in glutamine metabolism. Further clarification is, however, required as to the extent of this influence on the plasma glutamine levels of ICU patients.

2.4.3.4 Daily rhythms and hormones

Amino acid concentrations have been reported to vary during the day, rising and falling at different times (Wurtman *et al*., 1968). The reason for these variations is not yet fully known, but may be a consequence of the postprandial overflow of dietary amino acids (Wurtman *et al*., 1968). Another possible cause could be the variation in hydrocortisone and insulin in the blood during the day. However, amino acids present in larger quantities in the blood have a tendency to vary to a lesser extent than those presented in smaller concentrations. This is also true for glutamine, which comprises the majority of the amino acid pool, and therefore plasma glutamine levels are, to a lesser extent, subject to circadian fluctuations (Wurtman *et al*., 1968).

It has been reported that glucagon and adrenaline infusion decrease skeletal muscle glutamine levels in healthy volunteers (Hammarqvist *et al.,* 2001). This phenomenon is similar to that found in trauma. Muscle glutamine levels have been shown to continue to decline even after stress hormone infusion has been discontinued (Hammarqvist *et al*., 2001). These stress hormones can influence protein synthesis in the skeletal muscles and are associated with proteolysis, causing low muscle glutamine levels (Hammarqvist *et al*., 2001).

Other studies have shown, however, that an increase in cortisol concentrations can lead to increased glutamine production, which is described as an important influential factor in glutamine flux (Matthews & Campbell, 1992). Darmaun *et al.* (1988) also reported that cortisol infusion led to an increase in glutamine appearance rate and plasma glutamine levels, which remained elevated even after the infusion was stopped. This is attributed to the fact that cortisol increases endogenous glutamine synthesis (Darmaun e*t al*., 1988). In the afternoon, plasma cortisol levels are low and this may then lead to lower glutamine production at this time of day (Darmaun *et al.*, 1994). Furthermore, increased cortisol levels have been reported when infusing glutamine (Blomqvist *et al*., 1995).

The effect of another hormone, epinephrine, on glutamine metabolism has been deemed to be only modest (Matthews & Campbell, 1992). The influence of growth hormone has also been investigated and researchers found that its administration decreased the glutamine flux from muscle, but had a non-significant effect on plasma glutamine levels (Mjaaland *et al*., 1993). The relevance of these findings to the interpreting of plasma glutamine levels in critical illness is still uncertain. Thus circadian fluctuations and hormones may have a limited effect on glutamine status, but the extent to which this influence will be reflected in the plasma glutamine levels of ICU patients is not clear. More research is therefore required in this population group.

2.4.3.5 Gender

Gender may influence plasma glutamine levels of healthy individuals, as males are generally known to have larger skeletal muscle stores than women, skeletal muscle being the main producer of glutamine. Even though the evidence is not consistent it seems that men generally have higher plasma amino acid concentrations than females do (Munro, 1970:364). The plasma levels in healthy men at an average age were previously found to be 645 µmol/L, compared with 578 µmol/L in females (Armstrong & Stave, 1973). Pregnant females tend to have even lower plasma amino acid concentrations (Munro, 1970:364). The findings of Armstrong & Stave (1973) are compatible with this; they found that plasma glutamine levels are significantly associated with gender in certain age categories and also that there is a more rapid increase in plasma glutamine levels with age in growing boys as compared with girls. This is possibly related to the faster increase in muscle mass in males (Armstrong & Stave, 1973). There is a

dearth of studies investigating the effect of gender on plasma glutamine levels in critically ill patients. However, Viggiano *et al.* (2011) reported that gender did not influence plasma glutamine levels post-operatively in surgical patients, but more research is needed to draw definite conclusions.

2.4.3.6 Health status and medication use

Certain health conditions are known to influence plasma glutamine levels. Noninsulin-dependent diabetes mellitus (NIDDM) would be expected to alter glutamine metabolism, owing to the higher conversion rates of glutamine to glucose and alanine, and therefore these patients may present with lower plasma glutamine levels (Stumvoll *et al*., 1996). However, studies have confirmed that plasma glutamine levels are not influenced by the presence or absence of diabetes (Felig *et al*., 1973; Stumvoll *et al*., 1996). This is due to the fact that glutamine turnover is unaffected, as its oxidation decreases in the muscles of NIDDM patients (Stumvoll *et al*., 1996). Moreover, glutamine release from other tissues is thought to increase in these cases, therefore compensating for the higher conversion rates (Stumvoll *et al*., 1996). Consequently the effect of diabetes on plasma glutamine levels seems to be limited.

A condition that has been reported to influence plasma glutamine levels is depression. Patients suffering from depressive disorders present with increased glutamine and glutamate levels related to compensatory changes in these patients in order to offer a protective effect (Mitani *et al.,* 2006). Patients suffering from chronic obstructive pulmonary disease with mild or no emphysema were also found to have higher muscle glutamine levels than healthy controls, which may be due to increased muscle protein turnover in these patients (Engelen *et al.*, 2000). However, the specific effect on plasma glutamine levels still needs to be clarified.

Patients with human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), frequently present with complications such as infections, diarrhoea and weight loss. Glutamine deficiency has been proven in HIV patients in the absence of critical illness. Thus decreased glutamine levels may be an important contributor to their known suppressed immune systems and may precede the loss of CD4+ T cells (Hack *et al*., 1997). Furthermore, Yarasheki e*t al.* (1998) also proposed that glutamine is a conditionally essential amino acid in patients with HIV and AIDS wasting, as it serves as a primary substrate for T cells. However, the authors reported an increased glutamine appearance rate in AIDS-wasting subjects, possibly due to increased muscle injury or protein degradation, as well as elevated glutamine synthesis (Yarasheki e*t al*., 1998). Therefore higher plasma glutamine levels in these patients would be expected, but this is not the case (Yarasheki e*t al*., 1998). Possible reasons for the continued deficient levels could be that higher amounts of available glutamine are used to provide fuel for the cells of the intestinal tract, to transfer nitrogen to the liver for urea synthesis, to provide a

gluconeogenic substrate for the kidneys and for glutathione synthesis by the liver (Yarasheski *et al*., 1998).

With regards to medical treatment, it has also been reported that the administration of corticosteroids may possibly influence intracellular and plasma glutamine levels (Vinnars *et al*., 1975). In contrast, another study found that glucocorticoids had a neutral effect on plasma glutamine levels in ICU patients (Oudemans-van Straaten *et al*., 2001), which renders their influence questionable. Lastly, the presence and severity of inflammation is reported to be inversely associated with plasma glutamine levels (Hulsewé *et al*., 2004). In this regard, critical illness is seen as a condition with a major influence on plasma glutamine levels. In Sections 2.5.1 and 2.5.2 the available literature on this topic will be discussed in further detail. The plasma glutamine changes found in frequent complications related to critical illness, such as liver and renal failure, as well as MOF, will also be discussed in these sections.

2.5 Glutamine in critical illness

Patients suffering critical illness can be defined as those who are facing the danger of actual or possible life-threatening health problems (ACCN, 2014). Critical illness can be caused by trauma, sepsis, or other medical complications, which are known for over-amplification of the inflammatory response, causing immune dysfunction (Avenell, 2006). Surgery patients can also become critically ill, but these patients usually undergo less cytokine activation and further present with immune suppression, in turn predisposing them to infection (Avenell, 2006). Critical illness can result in metabolic alterations, including hyper-catabolism, leading to a negative nitrogen balance, wasting, immunosuppression and reduced wound healing (Biolo *et al*., 2000; Jackson *et al*., 1999). The main tissues, which are affected in catabolic states, are the muscles (Vinnars *et al*., 1975). A decrease in muscle protein synthesis has been reported in these patients, contributing to a loss of 0.5% to 1% LBM per day or a muscle protein content decrease of up to 10% per five days of ICU stay, corresponding to 2% daily (Finn *et al*., 1996; Gamrin *et al*., 1997; Hammarqvist *et al*., 2001; Vesali *et al*., 2002). Furthermore, a constant, heightened protein degradation rate has been found in ICU patients for at least two weeks (Vesali *et al*., 2002). According to Wilmore (2001), a more severe injury causes a greater extent of catabolism, with higher nitrogen losses. For this reason nitrogen losses are reportedly greater in patients with burns and multiple trauma than in surgery patients (Askanazi, Furst, *et al*., 1980). Nitrogen loss is also related to a patient's LBM stores and overall nutritional status, where undernourished patients will have lower nitrogen losses than those that are well nourished (Wilmore, 2001).

The specific mechanism behind the loss of protein from muscle is still not clear, but the inability of protein synthesis to meet the catabolic demands has been deemed a possible explanation

(Gamrin *et al*., 1997; Soeters & Grecu, 2012). Muscle amino acids are thought to be translocated to the plasma to be utilised by vital and splanchnic organs such as the liver and spleen, as well as the immune system and wounds for gluconeogenesis, oxidation, ureagenesis and to serve as building blocks in protein synthesis (Askanazi, Furst, *et al.,* 1980; Gamrin *et al.,* 1997; Van Acker *et al.,* 2000). Furthermore, increased nitrogen excretion due to muscle breakdown has been reported in these patients (Askanazi *et al.,* 1978). The intensified muscle breakdown can also be related to starvation and the inactivity that is associated with critical illness (Askanazi *et al*., 1978). With reduced muscle usage, an adaptive remodelling of skeletal muscles can occur, which, in turn, will lead to lower muscle strength and will ultimately negatively affect recovery and length of hospital stay (LOHS) (Gamrin *et al*., 1997; Lightfoot *et al*., 2009). As the muscles are the main producers of glutamine, it can then be anticipated that there will also be changes in the glutamine kinetics of these patients.

2.5.1 Plasma and muscle glutamine levels in critical illness

When considering the profound changes in protein and nitrogen metabolism during critical illness, it is not surprising that glutamine metabolism should also be affected. The decrease in the muscle and plasma amino acid pool can be largely attributed to decreased glutamine levels (Vinnars *et al*., 1975). In critical illness and post-surgery the skeletal muscles release glutamine to maintain nitrogen homeostasis, which is thought to be one of the greatest effects of trauma (Askanazi, Carpentier, *et al*., 1980; Van Acker *et al*., 2000; Yarasheski *et al*., 1998).

Earlier studies have proved that there is a reduction in plasma and muscle glutamine levels in selected general ICU, critically ill, post-surgical, multiple trauma, burns and septic patients (Askanazi, Carpentier, *et al*., 1980; Askanazi, Furst *et al*., 1980; Bergström *et al*., 1974*;* Biolo *et al*., 2000; Blomqvist *et al*., 1995; Déchelotte *et al*., 2006; Essen *et al*., 1992; Fläring *et al*., 2003; Gamrin *et al*., 1997; Gottschalk *et al*., 2013; Griffiths *et al*., 1997; Heyland *et al*., 2013; Houdjik *et al*., 1998; Palmer *et al*., 1996; Van Acker *et al*., 2000; Vente *et al*., 1989; Viggiano *et al*., 2012; Vinnars *et al*., 1975). This is why glutamine is currently regarded as a “conditionally essential” amino acid under certain circumstances (Gamrin *et al*., 1997; Houdjik *et al*., 1998; Jackson *et al*., 1999; Oudemans-van Straaten *et al*., 2001). However, not all of the above-mentioned studies clarified the extent to which the glutamine levels were reduced and how many of the patients actually presented with a glutamine deficiency (< 420 µmol/L).

The studies that specified the number of patients presenting with deficient plasma glutamine levels are presented in Table 2.1. Oudemans-van Straaten *et al.* (2001) and Rodas *et al.* (2012) demonstrated that only 31.3% and 43.7% of mixed ICU patients had deficient values (< 420 µmol/L) (Table 2.1). This was also the case for ICU patients admitted with MOF (Heyland *et al*., 2013). It seems, however, that among trauma ICU patients the greater majority (60%) of

patients may present with baseline deficient plasma glutamine levels (< 420 µmol/L), but more study is needed to confirm this (Pérez-Bárcena *et al*., 2014). It is clear that more research should be aimed at investigating the number of ICU patients with deficient plasma glutamine levels, in other words, those requiring glutamine supplementation.

Table 2.1 The number of patients classified with deficient or high plasma glutamine levels in previous research

Medical condition category	Plasma glutamine values		Reference
	Deficient* (% of patients)	High (% of patients)	
ICU patients with multiple-organ failure (*n* = 66)	31.0	15.0 (> 930 µmol/L)	Heyland *et al.* (2013)
Seriously ill, non-electively admitted ICU patients (*n* = 80)	31.3	Not reported	Oudemans-van Straaten *et al.* (2001)
Trauma ICU patients (*n* = 142)	60.0	Not reported	Pérez-Bárcena *et al.* (2014)
Mixed ICU patients, excluding thoracic, neurosurgery and trauma cases (*n* = 174)	43.7	11.5 (> 675 µmol/L)	Rodas *et al.* (2012)

*Levels below 420 µmol/L.
ICU: intensive care unit; *n:* number of patients

Reduced plasma glutamine levels have been reported to occur within 24 to 48 hours after initial injury (Blomqvist *et al*., 1995; Déchelotte *et al*., 2006; Essen *et al.,* 1992; Pérez-Bárcena *et al*., 2014; Van Acker *et al*., 2000; Viggiano *et al*., 2012). In the days following surgery or an insult, these levels may then be subjected to more marked reductions (Askanazi *et al*., 1978). Vesali *et al.* (2002), however, found no change in the plasma glutamine levels of ICU patients when measured on different occasions three to four days apart for the first two weeks of ICU stay. The export of glutamine from muscle was also reported to be unaltered during the first three weeks since ICU admission (Vesali *et al*., 2002). This possibly indicates a mechanism of depletion that is initiated at full strength from the start of critical illness (Gamrin *et al*., 1997). Admission plasma glutamine values can thus be recognised as representative of a patient's glutamine status in the absence of glutamine supplementation (Rodas *et al*., 2012).

Although other amino acids are restored or tend towards restoration during late convalescence, glutamine levels have been found to remain low (Askanazi, Carpentier, *et al*., 1980). It has even been shown to remain decreased for longer than 21 days (Wischmeyer, 2003). The low glutamine concentrations found in the anabolic phase can possibly be related to poor nutrition, together with the metabolic effects of the injury (Askanazi, Carpentier, *et al*., 1980).

Rodas *et al.* (2012) first raised awareness that some patients might present with supra-normal plasma glutamine levels, which may, in turn, be linked to poor outcomes. The authors of this

article determined cut-off points for normal plasma glutamine levels between 400 µmol/L and 930 µmol/L (Rodas *et al*., 2012). It was found that glutamine levels higher than 675 µmol/L were associated with an incrementally higher mortality risk and that those above 928 µmol/L (rounded of to 930 µmol/L) were especially vulnerable (Rodas *et al*., 2012). Therefore very high plasma glutamine levels may be detrimental to health outcome. The discovery of this phenomenon alerted researchers and clinicians to the importance of identifying, prior to supplementation, which patients present with hypoglutaminemia. This was thus a shortcoming of some of the earlier studies listed above, as they reported only on the average glutamine levels to determine whether the population group was deficient, as opposed to the number of patients with deficient or supra-normal levels and the causative factors.

Very high plasma glutamine levels have previously been reported in individual critically ill ICU patients at baseline and after glutamine supplementation (Table 2.1) (Berg *et al*., 2005; Oudemans-van Straaten *et al*., 2001; Rodas *et al*., 2012). Recently it was found that 15% of a sub-study of 66 ICU patients with MOF presented with supra-normal values (> 930 µmol/L) on admission to the ICU (Table 2.1) (Heyland & Dhaliwal, 2013; Heyland *et al.*, 2013). Oudemans-van Straaten *et al.* (2001) also reported "remarkably high" plasma glutamine levels in some patients, but as the cut-off points for supra-normal levels were not defined at the stage that this study was conducted, this was not one of their outcome measures. Rodas *et al.* (2012) also did not specify the total number of patients with plasma glutamine levels above 930 µmol/L, but only those with values above 675 µmol/L, in whom they found an incrementally higher mortality risk, as mentioned above (Table 2.1).

Since the ICU population is a heterogeneous group it is impossible to generalise individual metabolic alterations and therefore it is important to identify the specific type of patient that may be hypoglutaminemic. As septic patients can be described as some of the "sickest" ICU patients, one would anticipate that they might have lower glutamine levels than their other ICU counterparts. Interestingly, it has been found that intracellular levels of glutamine do not decrease more after the initial injury, when sepsis develops (Askanazi, Carpentier, *et al*., 1980). Another study also found no difference in glutamine levels between stressed and septic patients (Vente *et al*., 1989). Moreover, Planas *et al.* (1993) reported higher plasma glutamine levels in septic patients, which could possibly be ascribed to the lack of balance between glutamine release and uptake.

This then raises the question as to which patients are actually glutamine deficient and which have high plasma levels, indicating that a one-size-fits-all approach can definitely not be followed with regard to glutamine supplementation. In a letter to the editor, Gottschalk *et al.* (2013) clarified this by dividing glutamine supplementation into two approaches. One describes

glutamine as a pharmaconutrient to be given to stressed patients in order to provide its important functions. The other recommends supplementation only to those patients with a deficiency in order to correct glutamine levels (Gottschalk *et al*., 2013). Cynober and De Bandt (2014) recently reviewed the literature, summarising it well by stating that glutamine is no magic bullet and should be provided only to those patients actually presenting with low glutamine availability. Furthermore, Soeters & Grecu (2012) suggested that more evidence is needed that glutamine deficiency is present in certain disease states. Therefore, investigating plasma glutamine levels prior to supplementation is very important in order to prevent the provision of glutamine to a patient with a normal or already high concentration, but not very practical in most ICU settings. Other options for identifying those patients should therefore be explored, such as specific medical conditions and other biomarkers e.g. inflammatory cytokines. In order to better understand this, the mechanisms responsible for glutamine deficiency or excess will now be discussed.

2.5.2 Mechanisms responsible for glutamine deficiency or excess

The reason for decreased plasma and tissue glutamine levels is not yet fully understood, but is possibly related to a patient's catabolic state, nutritional status prior to an insult, dietary composition, current nutritional depletion and the severity of the underlying illness (Karinch *et al*., 2001; Oudemans-van Straaten *et al*., 2001).

The first cause of decreased plasma glutamine levels may be related to an increase in the distribution space, usually found in severe illness, thereby diluting the extra- and intracellular spaces (Soeters & Grecu, 2012). Soeters & Grecu (2012) warned that, for this reason, plasma glutamine levels should be interpreted with caution in inflammatory situations. However, the authors also advised that tissue glutamine concentrations be interpreted with care, as inflammation may alter the transport of glutamine and cause a drop in tissue concentrations (Soeters & Grecu, 2012). It seems that, in critical illness, the safest method of determining the adequacy of glutamine availability to central organs may be to interpret the release of glutamine from peripheral tissue (glutamine flux). Sufficient data for this is still lacking and its physiological significance has not yet been ascertained (Soeters & Grecu, 2012). Glutamine flux is influenced by proteolysis (particularly from the muscles and liver), *de novo* synthesis from amino acid precursors and losses from the amino acid pool (Van Acker *et a*l., 2000). The whole-body glutamine flux was previously reported to be unchanged in post-operative and critically ill patients (Jackson *et al*., 1999; Van Acker *et al*., 2000). Furthermore, Vesali *et al.* (2002) could not find any correlation between muscle glutamine release and plasma glutamine levels in critically ill patients. Despite a significant increase in glutamine release, plasma levels have been found to decrease in critical illness and post-operative patients as a result of heightened glutamine demands (Jackson *et al*., 1999; Lightfoot *et al*., 2009; Wischmeyer*,* 2003). Thus the

practicality and usefulness of using glutamine flux to determine deficiency is questionable and the best measure currently available for the determination of glutamine status in critical illness is plasma levels.

The different organs in the body respond to an insult in different ways, contributing to changes in glutamine metabolism, partly initiated by the release of stress hormones, e.g. cortisol (Darmaun *et al*., 1988; Hammarqvist *et al.,* 2001; Karinch *et al*., 2001). Initially the body responds to stress by exporting glutamine from the muscle to the splanchnic bed as well as immune cells via changes in amino acid transport pump settings (Askanazi, Carpentier, *et al*., 1980; Blomqvist *et al*., 1995; Lightfoot *et al*., 2009). Thereafter, proteolysis, transamination and increased synthesis follow (Figure 2.3) (Blomqvist *et al*., 1995; Lightfoot *et al*., 2009). These changes are related to the increased activity of GS, which nearly doubles, however, in the absence of changes in glutaminase activity. Increased GS activity has even been reported in the lungs under these conditions (Karinch *et al*., 2001). Reduced plasma glutamine levels increases GS activity in a dose-dependent manner, with the greatest increase at those levels that one can expect to find in critically ill patients (Labow *et al*., 2001). This is the body's way of restoring glutamine homeostasis following an insult. Conversely, the presence of increased glutamine will then facilitate the breakdown of GS (Labow *et al*., 2001).

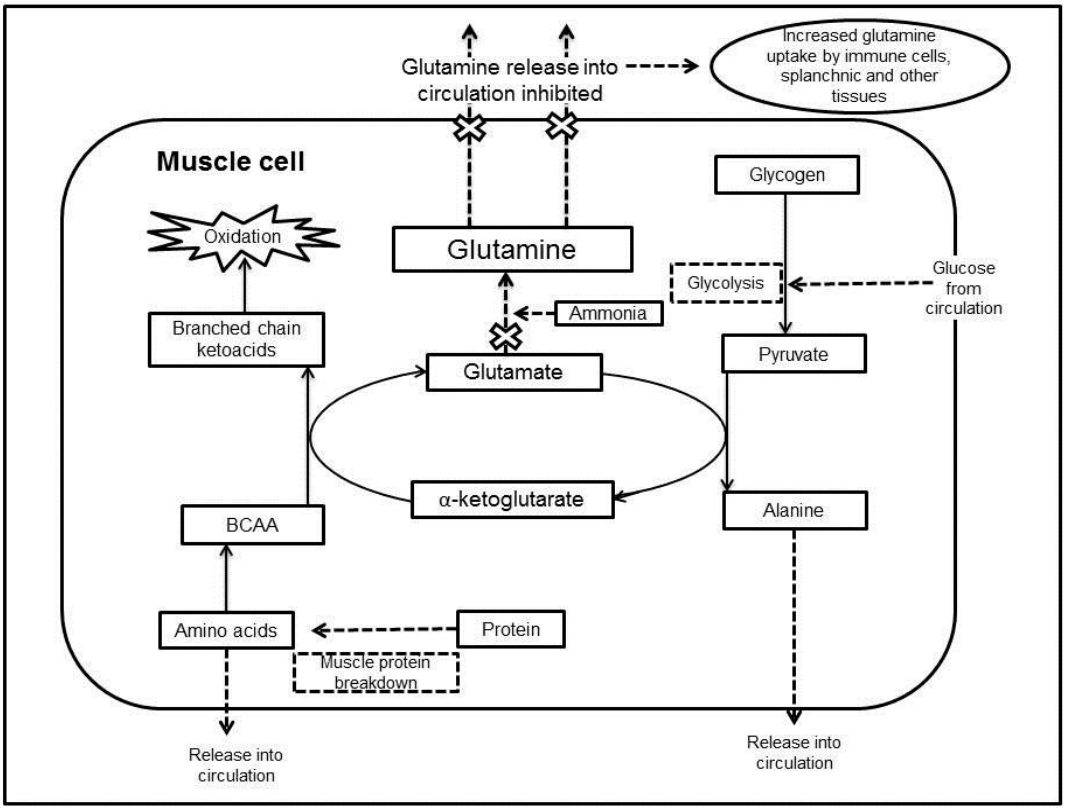

Figure 2.3 The mechanisms behind glutamine depletion in a stressed state (adapted from Biolo *et al.* (2000))

BCAA: Branched-chain amino acids; **X:** pathway inhibited

Oudemans-van Straaten *et al.* (2001) speculated that muscle release (from proteolysis and enhanced synthesis) might lead to an initial increase in plasma glutamine levels, resulting in normal plasma glutamine levels early after the insult, but not necessarily confirming the absence of a glutamine deficiency (Oudemans-van Straaten *et al.*, 2001). However, this is not to be the case. Even though there is an increased glutamine synthesis rate, as explained above, it is still insufficient to compensate for the increased export from muscles, ultimately leading to muscle glutamine depletion (Austgen *et al*., 1992).

Proteolysis and export of amino acids from muscles supply amino acids for the systemic circulation. As already mentioned, this heightened muscle protein breakdown following injury or illness does not lead to increased plasma glutamine levels (Askanazi, Carpentier, *et al*., 1980; Askanazi, Furst, *et al*., 1980). This is largely due to the drainage of plasma glutamine at the splanchnic area, as hepatocytes, lymphocytes and endothelial cells extract and utilise large amounts of glutamine as substrate during critical illness (Figure 2.3) (Blomqvist *et al*., 1995; Karinch *et al*., 2001).

The increase in glutamine clearance is attributed to changes in transport processes, resulting in an increased uptake and utilisation by rapidly proliferating immune cells (Jackson *et al*., 1999; Newsholme, 2001; Van Acker *et al*., 2000). Moreover, the skeletal muscle release and metabolism of glutamine is linked to the response of the immune cells and their use of glutamine as fuel in states of injury (Curi *et al*., 1999). Additionally, there might be an increased consumption of glutamine by other tissues such as the liver, kidneys and gut, which can actively remove amino acids (Askanazi, Carpentier, *et al*., 1980; Van Acker *et al.,* 2000). The liver extracts higher amounts (up to tenfold) of glutamine for gluconeogenesis and ureagenesis, via the maximised amino acid pump settings (Askanazi, Carpentier, *et al*., 1980; Karinch *et al.,* 2001). A portion of the amino acids is also converted to glucose by the kidneys, which then supply a carbon skeleton for glutamine synthesis in peripheral tissues (Soeters & Grecu, 2012). Furthermore, glutamine can be utilised for the production of acute-phase proteins (Askanazi, Carpentier, *et al*., 1980; Van Acker *et al.,* 2000). The depletion of plasma glutamine levels is therefore primarily attributed to the increased metabolic clearance rate of glutamine from the blood for the proliferation, functioning and differentiation of cells (Jackson *et al*., 1999; Oudemans-van Straaten *et al*., 2001). This is further accentuated by an insufficient glutamine synthesis rate to meet the increased demands, contributing to the decreased plasma glutamine levels found in metabolic stress (Jackson *et al*., 1999).

Irrespective of the increased glutamine synthesis at the onset of an injury or illness, the rate of production will decline with the progression of illness (Figure 2.3). This is related to the progressive depletion of LBM as a result of proteolysis, ending in decreased *de novo* glutamine

synthesis and release from skeletal muscles (Biolo *et al*., 2000; Labow *et al*., 2001; Van Acker *et al*., 2000; Vesali *et al*., 2002). In other words, there may be an inadequate substrate in the muscle in the form of other amino acids such as BCAA, cysteine, methionine, serine, threonine, glycine and glutamate for glutamine synthesis (Askanazi, Furst, *et al*., 1980; Van Acker *et al*., 2000; Vente *et al*., 1989). Increased glucose uptake, glycolysis, oxidation of the BCAA and the preferential use of glutamate for alanine synthesis may then accentuate the unavailability of substrate (Figure 2.3) (Biolo *et al*., 2000). It is not only the unavailability of substrate that causes a decline in glutamine production, but also partly the incapacity to use available amino groups (Askanazi, Furst, *et al*., 1980; Van Acker *et al*., 2000). Furthermore, Biolo *et al.* (2000) reported an association between decreased muscle glutamine concentrations and the suppression of the rate of glutamine synthesis in burns patients. In this way muscle glutamine concentrations may regulate its synthesis.

There are also other factors that lead to glutamine deficiency. Inflammatory processes are thought to make a significant contribution to glutamine depletion in critical illness. This is mediated by inflammatory cytokines, which have a major effect on glutamine utilisation in the liver (Karinch *et al*., 2001). In support of this, an independent inverse association between amino acid concentrations and inflammatory markers (including CRP) has been demonstrated in chronic kidney disease patients (Suliman *et al*., 2005). Furthermore, it has been suggested that an increased IL-6 release during inflammation contributes to the reduction in glutamine concentrations (Andreasen *et al*., 2009). As early as 1992, Parry-Billings *et al.* (1992) reported a negative association between plasma IL-6 and glutamine levels in patients undergoing major, but not in minor surgical procedures. Tumour necrosis factor-alpha has further been demonstrated to regulate GS by increasing its expression (Chakrabarti, 1998). This proves the significant effect of inflammation on plasma glutamine levels. Starvation and inactivity, frequently evident in critically ill patients, are also possible contributors to diminished glutamine levels (Askanazi *et al*., 1978). However, these will not be the main causal factors of glutamine deficiency as it has been reported that deficient glutamine levels occur irrespective of nutrition or the degree of trauma (Askanazi, Furst, *et al*., 1980).

One explanation for the high plasma glutamine levels found in individual patients can be related to an overweight nutritional status. The authors in one study recommended that the dosage of glutamine supplementation must be adjusted to a patient's LBM (Berg *et al*., 2005). However, this is still to be confirmed by more research and is not yet practicable in all clinical settings, as LBM is not frequently measured in ICUs. Another cause of high plasma glutamine levels can be the dysfunction of organs that play a role in glutamine's physiology, e.g. liver dysfunction, renal failure and MOF syndrome (Berg *et al*., 2005; Rodas *et al*., 2012).

Vente *et al.* (1989) reported significantly increased plasma glutamine levels in mild renal failure patients. This is due to the reduced utilisation of glutamine by the kidneys in these patients (Cynober & De Bandt, 2013). Supplementing a conservatively treated renal failure patient with glutamine can then cause or worsen azotemia (Vanek *et al*., 2011). Critically ill renal failure patients receiving dialysis will in turn lose amino acids via the dialysate, and it is recommended that glutamine be supplemented at 25 to 35 g or 0.5 g/kg/d per day to compensate for these losses (Wernerman, 2008; Wernerman, 2011). The loss of glutamine, specifically, in the dialysate could not be demonstrated, however, in a study conducted by Berg *et al.* (2007). More research is, therefore, still required in this field.

Furthermore, higher plasma glutamine levels can be detected in some liver patients as a result of hyperammonemia (Engelen *et al.,* 2000). In an animal study, Ytrebø *et al.* (2006) reported modestly high plasma glutamine levels in acute cases of liver failure. In the study conducted by Rodas *et al.* (2012), most of the patients who presented with high glutamine levels also had acute liver failure, confirming this. Glutamine administration can cause increased serum ammonia levels, as well as other altered liver parameters (Roth, 2008). The American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) position paper advised the monitoring of liver function tests when supplementing glutamine and to exercise caution with regards to the supplementation thereof in patients with liver dysfunction (Vanek *et al*., 2011). However, it should be kept in mind that chronic liver failure patients might be malnourished, possibly leading to lower glutamine levels. Glutamine therapy should therefore not be totally excluded in these patients, but they should rather be treated like any other ICU patient (Wernerman, 2008). Nevertheless, the glutamine kinetics in this patient population sub-group requires further investigation. With regard to MOF patients, it is likely that they may present with one of these organ failures and therefore the same considerations apply as discussed above. Heyland *et al.* (2013) found that 15% of MOF patients admitted to the ICU had baseline supra-normal plasma glutamine levels (> 930 µmol/L). The authors further reported harm with early glutamine supplementation in these patients (Heyland *et al.*, 2013).

In head trauma and neurosurgery patients there is also a possibility of the accumulation of glutamate, an excitatory neurotransmitter, in the brain when administering supplemental glutamine (Berg *et al.*, 2006; Vanek *et al*., 2011; Wernerman, 2008). This may then lead to neurotoxicity. Facilitated transport systems exist for glutamine and glutamate transport over the blood brain barrier and high glutaminase activity has been found in brain cells (Lee, Hawkins *et al*., 1998). The brain is also seen as an exporter of glutamine, while glutamate is reabsorbed by nerve endings to be metabolised into glutamine (Berg *et al*., 2006; Wernerman, 2011). This suggests the exercise of caution when supplementing neuro-trauma patients. However, after studying the effect of glutamine supplementation on glutamate concentrations in the brain

tissue, it was found that glutamine supplementation is safe in neuro-ICU patients (Berg *et al*., 2006). The reason for this is that the blood–brain barrier can inhibit entry of glutamine and glutamate into the brain and also expel these amino acids, thereby regulating the brain's glutamine metabolism (Lee, Hawkins *et al*., 1998). This in turn will prevent the accumulation of amino acids in the brain tissue and avoid neurotoxicity (Lee, Hawkins *et al*., 1998). Therefore a diagnosis of head injury is not seen as a contraindication for glutamine supplementation and higher plasma glutamine levels are not expected in this patient group (Wernerman, 2008).

When considering all the above evidence, it is clear that a glutamine deficiency is possibly caused by increased glutamine demands, accentuated by the eventual insufficient synthesis to meet them (especially at later stages during critical illness) and that high levels are possibly caused by the dysfunction of organs that utilise glutamine.

2.5.3 Implications of changes in plasma glutamine levels

Deficient plasma glutamine levels can lead to decreased tissue glutamine availability, which may impair the execution of its important functions, as discussed in Section 2.3 (Oudemans-van Straaten *et al*., 2001). The first and major implication of glutamine deficiency is that it can pose a serious limitation to protein synthesis (Askanazi, Carpentier, *et al.,* 1980). Secondly, it can negatively influence the functioning of the immune system and the gut barrier, possibly impairing the body's capacity to counter on-going illness and injury (Oudemans-van Straaten *et al*., 2001; Wischmeyer, Lynch, *et al*., 2001). It can further lead to the compromised activation of genes that serve an important role in immune responses and cell protection. Here glutamine deficiency has been shown to lead to the reduction of HSP70 expression and glutathione levels, which then further contribute to immunosuppression and may predispose a patient to organ failure (Eliasen *et al*., 2006; Oliveira *et al*., 2010, Roth *et al*., 2002; Wischmeyer, 2006). Decreased glutathione levels have been reported after surgical trauma, which is partly attributed to low glutamate (and therefore glutamine) availability for its synthesis and which can cause an increased risk of oxidative injury (Fläring *et al*., 2003). Furthermore, an amino acid shortage may lead to an adaptive response causing decreased glucose and urea production from amino acids, affecting a patient's nitrogen and glucose metabolism (Hack *et al*., 1997).

Taking the above into consideration, it is not surprising that a baseline glutamine deficiency has also been shown to correlate with increased mortality rates and is an important independent predictor of mortality in ICU patients (Oudemans-van Straaten *et al*., 2001; Rodas *et al*., 2012). Moreover, plasma glutamine levels have been found to predict outcome even better than other mortality prediction methods and clinical variables used in the hospital setting (Oudemans-van Straaten *et al*., 2001). Additionally, post-ICU mortality is associated with admission plasma glutamine levels (Rodas *et al*., 2012). Pérez-Bárcena *et al.* (2014), however, recently found that

low admission glutamine levels were not associated with any outcomes measured, but that low levels later on during ICU stay (specifically on day six) correlated with infection rates, length of ICU stay and LOHS. It seems, therefore, that the emphasis is now shifting from the determination of baseline glutamine status towards the importance of glutamine levels during the course of ICU stay. Clearly more research is required to confirm this. In an earlier study by Parry-Billings *et al.* (1992), the authors proposed that glutamine could be a useful marker to determine the severity of illness and major complications. However, more recently, no association between glutamine levels and the severity of illness could be found (Rodas *et al*., 2012).

Rodas *et al.* (2012) reported a U-shaped curve association between plasma glutamine levels and mortality. The team of researchers found an increased mortality rate with both low (< 400 µmol/L), and high (> 930 µmol/L) plasma glutamine levels, which indicates the importance of a balance in glutamine metabolism (Rodas *et al.*, 2012). The reason for increased mortality rates being associated with high plasma glutamine levels could be higher glutathione production from glutamine, altering the redox balance of the patient (Fläring *et al*., 2003). It could also be related to the increased nitrogen excretion rate and ammonia levels resulting from high glutamine levels and leading to organ damage (renal and liver failure). Nevertheless, these are only potential causes and the exact mechanism behind this is still to be investigated by further research. Just as the imbalance in glutamine levels has been proved to have detrimental health effects, its supplementation presented various beneficial outcomes in a variety of patient groups. The most recent literature available on glutamine supplementation in critically ill patients will be discussed in the next section (Section 2.6).

2.6 Glutamine supplementation in critical illness

Since the recognition of the significance of glutamine in critical illness, it has been extensively researched in patients from various clinical settings. Supplementation studies have demonstrated improved outcomes in cancer, critical illness, trauma, burns, severe acute pancreatitis, and surgical patients (Bollhalder *et al*., 2013; Déchelotte *et al.*, 2006; Fuentes-Orozco *et al.,* 2008; Lin *et al*., 2013; Wang *et al*., 2010; Wischmeyer, 2003; Wischmeyer *et al.,* 2014; Yue *et al*., 2013). However, there is still a debate on the clinical relevance of supplementation because of the heterogeneity of the patient populations, dosages, durations, and route of administration in the different trials. The beneficial effects of glutamine are reported to be largely dependent on dose and the route of administration (Bollhalder *et al*., 2013; Wischmeyer, 2003). Glutamine can be administered via the oral, enteral or parenteral route (Roth, 2008). It has also been reported that supplementing glutamine in the form of ALA-GLN dipeptide provides results superior to those of glycyl-glutamine (GLY-GLN) supplementation

(Wang *et al*., 2010; Yue *et al*., 2013). This literature review will summarise the large body of evidence available on glutamine supplementation by including the largest, most recent and most relevant randomised controlled trials, as well as the most recent systematic reviews and meta-analyses.

2.6.1 Parenteral glutamine supplementation

Glutamine supplementation, via the parenteral route, is thought to lead to the maintenance of the glutamine pool (Blomqvist *et al*., 1995; Novak *et al*., 2002; Wilmore, 2001; Yue *et al*., 2013). In support of this, various studies have found statistically significant increases in plasma glutamine levels after administering IV glutamine (Andreasen *et al.*, 2009; Berg *et al*., 2005; Goeters *et al*., 2002; Hammarqvist *et al*., 1989; Jiang *et al*., 1991). Tjäder *et al.* (2004) found that 24 hours of 0.28 g/kg parenteral glutamine supplementation normalised plasma glutamine levels in ICU patients. However, Pérez-Bárcena *et al.* (2014) reported that 39% of trauma ICU patients still had low plasma glutamine levels after six days of IV glutamine treatment. Therefore more research is required to shed light on the extent to which glutamine supplementation will restore glutamine balance in patients.

Griffiths e*t al.* (1997) conducted the first trial on IV-administered glutamine in critically ill ICU patients and reported reduced hospital costs and reduced six-month mortality rates, concluding that glutamine should form part of the constituents of total parenteral nutrition (TPN) formulas. Following this, Goeters *et al.* (2002) first administered glutamine in the form of ALA-GLN dipeptide and reported that it improved the six-month survival rate, but could not find any benefit in hospital or ICU mortality, LOHS, or any nutritional and inflammatory parameters. These early trials were the reason that glutamine was labelled a ”life-saving nutrient” (Preiser & Wernerman, 2003). This was then followed by numerous publications investigating parenteral glutamine supplementation in further detail. They were conducted on heterogeneous patient groups, but all had certain aspects in common.

Some of these aspects were: they were comprised of patients that required TPN; glutamine was provided as supplement to nutrition support; glutamine was administered only at the initiation of TPN and not typically on ICU admission; they included dosages of 0.3 to 0.5 g/kg/day; excluded those patients with renal or liver failure; and never combined enteral and parenteral glutamine administration routes (Wischmeyer *et al.*, 2014). To mention all these studies to date would be appropriate but impossible, as 11 clinical trials have been conducted on parenteral glutamine supplementation in the short period since 2009, not to mention all the trials prior to this date. Some of the most recent systematic reviews and meta-analyses are presented in Table 2.2 in order of the publication date of the research.

Table 2.2 Systematic reviews and meta-analyses of studies of glutamine supplementation

Year	Study reference	Research design	Number of trials included	Characteristics of trials included		Outcomes of GLN supplementation			
				Patient population	Route of GLN delivery	Mortality	Infectious complications	Length of stay	Other findings
2002	Novak *et al.* (2002)	Systematic review	14	Elective surgery	PN or EN	No effect	↓	↓	PN GLN & dose > 0.2 g /kg/d: ↓mortality & LOHS.
				Critically ill adults		Trend ↓*	Trend ↓ *	No effect	EN GLN & low dose: no effect on mortality & LOHS.
2005	Avenell (2005)	Meta-analysis	15	Critically ill adults	PN or EN	PN GLN: trend ↓* EN GLN: No effect	PN GLN: trend ↓* EN GLN: ↓	Not reported	GLN not harmful in MOF or in general.
				Post-surgery		No effect	PN GLN: ↓	Not reported	
2010	Wang *et al.* (2010)	Meta-analysis	14	Surgical adults	PN	No effect	Trend ↓* ALA-GLN: ↓ GLY-GLN: no effect	ALA-GLN & GLY-GLN: ↓	–
2013	Asrani *et al.* (2013)	Meta-analysis	12	Acute pancreatitis	PN or EN	↓	↓	PN GLN: ↓ EN: No effect	–
2013	Bollhalder *et al.* (2013)	Meta-analysis	40	Critically ill or major surgery	PN	Trend ↓ short-term * ↓ in critically ill	Surgical patient:↓ Critically ill: no effect	↓ LOHS No effect on length of ICU stay	Dose > 0.2 g/kg/d & duration ≥ 9 days: ↓ mortality, infectious complications, & LOHS.

*Non-significant; ↓reduced; ↑increased; > greater than; < less than

ALA-GLN: alanyl-glutamine; EN: enteral; GLN: glutamine; GLY-GLN: glycyl-glutamine; g/kg/d: gram per kilogram bodyweight per day; ICU: intensive care unit; LOHS: length of hospital stay; MOF: multiple organ failure; PN: parenteral

Table 2.2 Systematic reviews and meta-analyses of studies of glutamine supplementation *(cont.)*

Year	Study reference	Research design	Number of trials included	Characteristics of trials included		Outcomes of GLN supplementation			
				Patient population	Route of GLN delivery	Mortality	Infectious complications	Length of stay	Other findings
2013	Lin *et al.* (2013)	Meta-analysis	4	Burns adults	PN or EN	↓	↓	No effect	–
2013	Yue *et al.* (2013)	Meta-analysis	16	Abdominal surgery adults	PN	No effect	Overall: ↓ ALA-GLN: ↓ GLY-GLN: no effect	ALA-GLN & GLY-GLN: ↓	–
2014	Chen *et al.* (2014)	Meta-analysis	18	Adult ICU patients	PN or EN	Medical & Mixed ICU: No effect Surgical ICU: trend ↓*	Medical & Mixed ICU: no effect Surgical ICU or with PN GLN: ↓	No effect	Dose > 0.5 g/kg/d ↑ mortality.
2014	Tao *et al.* (2014)	Systematic review	53	Critically ill or major elective surgery	PN or EN	PN GLN: ↓short –term EN GLN: No effect	PN GLN: ↓ EN GLN: no effect	↓ LOHS No effect on length of ICU stay	–
2014	Wischmeyer *et al.* (2014)	Systematic review	26	ICU excluded: renal and liver failure patients	PN with: complete nutritional support, dose 0.3-0.5 g/kg/d, not on ICU admission	↓	Trend ↓*	↓ LOHS Trend ↓ length of ICU stay	–

*Non-significant; ↓reduced; ↑increased; > greater than; < less than

ALA-GLN: alanyl-glutamine; EN: enteral; GLN: glutamine; GLY-GLN: glycyl-glutamine; g/kg/d: gram per kilogram bodyweight per day; ICU: intensive care unit; LOHS: length of hospital stay; MOF: multiple organ failure; PN: parenteral

Some individual randomised controlled trials will also be included in the discussion below in order to highlight and elaborate on research findings. Firstly, the research regarding IV glutamine supplementation in surgical patients will be discussed. Yeh *et al.* (2008) found no effect on morbidity or LOHS when supplementing gastrointestinal surgery patients with parenteral glutamine. Nevertheless, most other trials conveyed good results, as reviewed by Novak *et al*. (2002). This review, together with recent meta-analyses, investigated the effect of IV glutamine supplementation on post-operative, abdominal and elective surgery patients, and reported that it reduced LOHS and improved nitrogen balance (Table 2.2) (Bollhalder *et al.*, 2013; Novak *et al*., 2002; Wang *et al*., 2010; Yue *et al*., 2013). Owing to the low death rate of surgery patients, it is difficult to draw conclusions regarding its effect on mortality, specifically (Avenell, 2006; Bollhalder *et al*., 2013; Novak *et al.,* 2002; Yue *et al*., 2013). Results regarding the effect of IV glutamine supplementation on infectious complications in this patient group seem promising, but a significant benefit has not been consistently found (Avenell, 2006; Bollhalder *et al.,* 2013; Novak *et al*., 2002; Wang *et al.,* 2010; Yue *et al.,* 2013). Therefore it seems that parenteral glutamine supplementation may be beneficial in the surgical population with regard to the reduction of LOHS and possibly infectious complications.

Another population group that may form part of an ICU setup is burns patients. In a trial conducted by Wischmeyer, Lynch, *et al.* (2001), it was found that when administering IV glutamine in addition to enteral nutrition in burns patients, a reduced incidence of gram-negative bacteraemia and overall inflammation, as well as an improved nutritional status, were evident. More recently, a small meta-analysis on the effect of glutamine supplementation in burns patients reported an inverse association between glutamine and mortality, as well as complications associated with bacteraemia (Table 2.2) (Lin *et al*., 2013). Parenteral glutamine supplementation is therefore beneficial in this patient group.

The effect of IV supplementation has also been investigated in acute pancreatitis patients (Fuentes-Orozco *et al.*, 2008; Ockenga *et al.*, 2002). These trials found that glutamine administration improved nutritional status, including an improved nitrogen balance and anabolic response to feeding. Furthermore, a decrease in infectious complications, length of TPN requirement and CRP levels was also evident in the glutamine-supplemented group (Fuentes-Orozco *et al*., 2008; Ockenga *et al*., 2002). A 2013 meta-analysis that included studies supplementing severe acute pancreatitis patients with IV glutamine also reported beneficial outcomes and therefore IV glutamine supplementation can be recommended in this patient group (Table 2.2) (Arsani *et al*., 2013).

The A.S.P.E.N. issued a position paper in 2011, reviewing the available scientific data on parenteral glutamine supplementation. They stated that it decreases infectious complications, LOHS and mortality in critically ill or ventilated patients (Vanek *et al*., 2011). A review of those

trials that specifically investigated critically ill or general ICU patients once again reveals an abundance of available literature. However trials conducted in this population group are usually subjected to the inclusion of small patient groups in order to ensure homogenous patient characteristics (Cynober & De Bandt, 2013). One such study that did not have this problem was the Scottish Intensive care Glutamine or SeleNium Evaluative Trial (SIGNET). This study was large and multi-centred, including 502 patients with GIT failure from 10 centres in Scotland. It was, furthermore, representative of a "real life" situation, as the study group included mixed patients, as would be expected in the ICU setting. Disappointingly, the authors reported no effect on mortality, LOHS or morbidity in IV glutamine-supplemented patients, contradicting those results found in earlier smaller studies (Andrews *et al*., 2011). This study was, however, criticised for its shortcomings, including the poorly defined, low dosages (20.2 g/d, not expressed as gram per kilogram per day); late implementation; short administration periods; poor reporting of missing values; protocol violations; and no follow up on drop-outs (Bollhalder *et al*., 2013; Cynober & De Bandt, 2013). Furthermore, the study also included older and younger patients with varying nutritional statuses, some receiving nutritional support and others not (Cynober & De Bandt, 2013).

Conversely, the Scandinavian trial on IV glutamine supplementation, another multi-centre trial published in the same year, depicted a lower ICU mortality, which was not maintained at six months in combined parenterally and enterally fed ICU patients (Wernerman *et al*., 2011). This study was, however, far from perfect as it lacked statistical power, did not determine plasma glutamine values, had missing values for important parameters and did not define the gender of all patients (Cynober & De Bandt, 2013).

The outcomes of reviews and meta-analyses in the ICU and critically ill population group that are presented in Table 2.2, reflects the results of the two trials mentioned above and other randomised controlled trials in this patient group (Bollhalder *et al*., 2013; Chen *et al*., 2014; Tao *et al*., 2014; Wischmeyer *et al*., 2014). A consistent mortality benefit could not be found (Table 2.2); however, in two very recent articles, a reduction in mortality rates was linked to IV glutamine supplementation (Tao *et al.*, 2014; Wischmeyer *et al*., 2014). As the results from the SIGNET had a large influence on the findings of meta-analyses, it was excluded in the one conducted by Bollhalder *et al.* (2013), where after the effect on mortality became significant. Findings regarding the effect of parenteral glutamine supplementation on infection risk and LOHS or length of ICU stay are also inconsistent and therefore require further research (Table 2.2). Bollhalder *et al.* (2013) concluded that large, high-quality randomised controlled trials were needed to justify parenteral glutamine supplementation in critical illness. Such a study was to be published later that same year that would change the way glutamine supplementation is viewed (Heyland *et al*., 2013). This study will be discussed in Section 2.6.3.

2.6.2 Enteral glutamine supplementation

The evidence on enteral glutamine supplementation is less conclusive. Providing enteral glutamine might have a direct benefit for the cells of the GIT, aiding in cell proliferation (Fish *et al*., 1997; Jones *et al*., 1999). Only a fraction of glutamine, supplemented via the enteral route, is found in the blood, causing a slight increase in plasma glutamine levels (Schroeder *et al*., 2005; Wernerman, 2011). This is due to the fact that the majority of enteral glutamine is eliminated and metabolised in the upper part of the jejunum by enterocytes and immune cells, as a source of energy and nitrogen (Darmaun *et al*., 1994; Déchelotte *et al*., 1991; Haisch *et al*., 2000; Melis *et al*., 2005; Wernerman, 2011). This then also leaves the rest of the GIT unsupported. Another part is absorbed and used by the liver, before it can influence and correct plasma glutamine levels (Wernerman, 2011).

The finding that enteral glutamine is used mostly in the splanchnic area, at the intestinal tract and surrounding immune cells, possibly indicates that enteral supplementation might need to be combined with parenteral supplementation to have a significant systemic effect in glutamine-deficient patients (Melis *et al.*, 2005). However, other articles reported significant dose-dependent increases in plasma levels with enteral glutamine supplementation in patients, as well as in healthy adults (Castell & Newsholme, 1997; Déchelotte *et al*., 1991; Houdjik *et al*., 1998; Melis *et al*., 2005; Peng *et al*., 2006). Nevertheless, parenteral glutamine supplementation will have a more marked effect than enterally provided glutamine on plasma glutamine levels (Melis *et al.*, 2005). This may be the reason behind findings of Novak *et al.* (2002), who reported that parenteral glutamine supplementation was associated with a significant reduction in mortality, LOHS and infectious complications in surgery patients, while enteral supplementation was not (Novak *et al.,* 2002). Enteral glutamine supplementation has, however, been found to be effective in especially burns and trauma patients, decreasing the incidence of sepsis, pneumonia, bacteraemia, infectious morbidity and mortality (Garrel *et al*., 2003; Houdjik *et al*., 1998; Wischmeyer, Lynch, *et al.*, 2001).

Solid evidence is available to support the administration of enteral glutamine in burns patients. A recent small meta-analysis, including four studies administering parenteral or enteral glutamine supplementation in burns patients, reported reductions in hospital mortality (odds ratio (OR) = 0.13; 95% confidence interval (CI) 0.03-0.51) and complications due to gram-negative bacteraemia (OR = 0.27; 95%CI 0.08-0.92), with no effect on LOHS in glutamine-supplemented groups (Lin *et al*., 2013). In contrast, Peng *et al.* (2006) also investigated the effect of enteral glutamine supplementation in burns patients and found that it decreased the patients' LOHS. Additionally, enteral glutamine administration has been reported to reduce and normalise intestinal permeability and improve wound healing in this patient group (Novak *et al*., 2002; Zhou *et al*., 2003).

When investigating the effects of enteral glutamine with specific reference to ICU or critically ill patients, there is available literature supporting its use. Outcome benefits found in an earlier study with enteral supplementation in ICU patients included: reduced TPN requirement, a shorter ICU and LOHS and an overall reduction in costs (Jones *et al*., 1999). In 2001, Wilmore (2001) reported that enteral glutamine administered to trauma patients significantly reduced the incidence of infection. Additionally, Avenell (2006) and Conejero *et al.* (2002) also found a reduced infection rate with enteral glutamine supplementation in critically ill patients with SIRS. This was opposed by findings of two of the most recent articles published, reporting that enteral glutamine supplementation had no effect on the infectious complications of critically ill ICU patients (Table 2.2) (Chen *et al*., 2014; Tao *et al*., 2014). Furthermore a recent smaller study, demonstrated that enterally supplemented ICU patients with SIRS did not benefit with regard to oxidative stress markers, cell counts or pro-inflammatory cytokines when compared with a control group (Cavalcante *et al*., 2012). Providing glutamine via the enteral route to critically ill or ICU patients also has no effect on mortality risk (Table 2.2) (Avenell, 2005; Chen *et al*., 2014; Novak *et al*., 2002; Tao *et al*., 2014).

Therefore it is clear that the administration of enteral glutamine supplementation is supported especially in trauma and burns patients and may be beneficial in general ICU patients. However, these findings are based on a small amount of literature and more research is needed to ascertain the role of enterally administered glutamine in the ICU setting.

2.6.3 The Reducing Deaths due to Oxidative Stress (REDOXS) study

The year 2013 marked the start of a new era for glutamine supplementation as the publication of the REDOXS study cast doubt on the safety and efficacy of glutamine supplementation in all patient groups. Heyland *et al.* (2013) conducted the largest glutamine supplementation trial thus far, with a randomised, blinded design, which had an intention-to-treat analysis, and included 1223 critically ill adults with MOF from 40 ICUs. The patients were supplemented with combined enteral and parenteral glutamine at high dosages of 0.5 g/kg/d ALA-GLN IV, based on ideal body weight, combined with 30 g of enteral glutamine, which was started within 24 hours following admission to the ICU (Heyland *et al*., 2013). Anti-oxidants were also supplemented, either in combination with glutamine or without. Surprisingly, the results of the study showed an increase in hospital and six-month mortality with glutamine supplementation, with no effect on rate of organ failure or infectious complications (Heyland *et al*., 2013). The authors concluded that high dosages of glutamine should not be provided to MOF patients (Heyland *et al*., 2013).

However, there were some discrepancies seen in this trial when it was compared with previous trials that reported glutamine supplementation to be harmless. The REDOXS study included patients with MOF, including liver and renal failure cases, which are usually contra-indications for glutamine supplementation (Cynober & De Bandt, 2013; Heyland *et al*., 2013). In liver and

renal failure extra glutamine supplementation may lead to the accumulation of ammonia, although this was not one of the outcomes measured (Heyland *et al*., 2013). In a post-hoc analysis of this trial, it was reported that glutamine supplementation increased mortality in those patients with baseline renal dysfunction, while this was not the case in those without it (Heyland *et al*., 2014). Higher urea levels were also found in patients that were supplemented with glutamine (Heyland *et al*., 2014). Therefore renal dysfunction could definitely have contributed to the outcomes of this study.

Secondly, glutamine was administered at the highest dosage (two to three times higher than in other studies) prescribed both enterally and parenterally, which was not the case in prior trials (Heyland & Dhaliwal, 2013; Heyland *et al*., 2013). These high dosages were chosen because the authors predicted that patients with MOF would present with a greater depletion of nutrients and therefore have a greater benefit from a higher dose (Heyland & Dhaliwal, 2013; Heyland *et al.,* 2014). This was not the case, as the plasma glutamine levels of a sub-study of 66 patients revealed that only 31% were deficient (Heyland & Dhaliwal, 2013; Heyland *et al*., 2014). However, in the post-hoc analysis it was reported that the adjustment for the baseline covariates did not change the higher mortality outcome (Heyland *et al*., 2014). Consequently, a higher mortality rate occurred irrespective of baseline glutamine levels.

Another difference in this trial, compared with previous trials, was the fact that glutamine supplementation was initiated early, within 24 hours after admission and that the nutritional requirements of these patients were not yet met at this time (fed less than 50% of target) (Heyland *et al*., 2013; Preiser & Wernerman, 2013). It could thus be that glutamine deficiency might occur only later during the progression of illness and that early supplementation provided toxic levels (Preiser & Wernerman, 2013). Lastly, the type of admission could also have affected the outcomes of the REDOXS study (Preiser & Wernerman, 2013). The patients enrolled in the study were predominantly medical patients, where the benefit of glutamine supplementation is not as firmly established as in trauma and burns patients (Preiser & Wernerman, 2013). To what extent this could be an indication that this group should be differentially excluded from glutamine supplementation is, however, uncertain.

As result of this study, Preiser & Wernerman (2013) posed the following question: “Glutamine, a potentially toxic nutrient, but why, when and in which patients?” Two recent meta-analyses were published in order to address this question. Wischmeyer *et al.* (2014) conducted a systematic review of all studies on parenteral glutamine supplementation in critically ill patients, including the results of the REDOXS trial. The authors concluded that parenteral glutamine supplementation, provided in conjunction with sufficient nutritional support, is still significantly associated with reduced hospital mortality, LOHS and a trend towards reduced infectious complications (Table 2.2) (Wischmeyer *et al*., 2014). The main recommendation made was to

use glutamine as part of nutritional support in order to improve outcomes of critically ill patients (Wischmeyer *et al.*, 2014). However, the meta-analysis by Chen *et al.* (2014) comprised of all the randomised controlled trials of glutamine supplementation in critically ill patients, including the REDOXS study, did not support the findings mentioned above. These results indicated that glutamine supplementation does not have any significant mortality or LOHS benefit, but that high dosages are significantly associated with an increased mortality risk. There was, however, a significantly reduced rate of nosocomial infections found in glutamine-supplemented groups, which was evident in both parenteral nutrition and surgical patients in a sub-group analysis (Chen *et al*., 2014).

When summarising this part of the literature, it is clear that outcomes from trials investigating glutamine supplementation varied. This may be related to the dosages and duration used, the patient groups selected and the route of administration. It is important to distinguish between supplementing glutamine in order to correct deficient values or to increase glutamine concentrations to supra-physiological levels. From of the above literature there are a few key messages that can be applied in clinical practice:

- The first is that glutamine supplementation is not suitable for all patient groups, as a deficiency may not be present (Cynober & De Bandt, 2013; Heyland *et al.,* 2013).
- Secondly, the dosage of glutamine administration is important and high dosages (> 0.5 g/kg/d) might increase mortality risk and are therefore not recommended (Chen *et al.*, 2014; Heyland *et al.*, 2013). Furthermore, supplementation should be initiated only after the initial acute phase and resuscitation period (Cynober & De Bandt, 2013; Heyland & Dhaliwal, 2013).
- Thirdly, the route of administration is important, as parenteral glutamine supplementation seems to provide superior benefits in comparison with glutamine administered via the enteral route. This is especially true in critically ill and surgical patients, where it may be of aid in decreasing nosocomial infection rates (Chen *et al.,* 2014; Wischmeyer *et al.,* 2014). Enteral glutamine has been shown to be beneficial in trauma and burns patients and may be of value to other critically ill patients, but more studies are required.
- Lastly, there are certain patient groups who should not receive glutamine, including patients with MOF, shock, acute liver failure, acute right heart failure, renal failure and patients with insufficient nutritional support (Cynober & De Bandt, 2013; Heyland & Dhaliwal, 2013).

2.6.4 Current recommendations in the literature for glutamine supplementation

Glutamine is an unstable amino acid in aqueous solutions and cannot be heat sterilised. It must therefore be supplemented as heat-stable and water-soluble dipeptides, such as ALA-GLN or GLY-GLN, in parenteral and enteral formulas (Amores-Sánchez & Medina, 1999; Wang *et al*., 2010; Yue *et al*., 2013). Berg *et al.* (2002) reported that glutamine-containing dipeptides are safely tolerated by peripheral veins and can therefore be administered via this route when the central route is unavailable. Several recommendations have been published for glutamine supplementation and different organisations recommend glutamine supplementation as part of their clinical guidelines.

The A.S.P.E.N., the European Society for Clinical Nutrition and Metabolism (ESPEN) and the Canadian Critical Care Clinical Practice Guidelines (CCPG) committee recommend supplemental enteral and IV glutamine in certain patient groups (Dhaliwal *et al*., 2014; Kreymann, 2010; McClave *et al*., 2009; Singer *et al*., 2009). The glutamine supplementation recommendations of these different societies are summarised in Table 2.3.

Table 2.3 Clinical practice glutamine supplementation guidelines from major societies

	A.S.P.E.N.	**ESPEN**	**CCPG**
Enteral glutamine	Trauma, burns and mixed ICU patients (Grade B)	Burns and trauma patients (Grade A)	Burns and trauma patients
Parenteral glutamine	All patients on TPN (Grade C)	ICU patients on TPN (Grade A)	Critically ill patients on TPN (considered)
Reference	McClave *et al.* (2009)	Singer *et al.* (2009)	Dhaliwal *et al.* (2014)

A.S.P.E.N.: American Society for Parenteral and Enteral Nutrition; CCPG: Canadian Critical Care Clinical Practice Guidelines; ESPEN: European Society for Clinical Nutrition and Metabolism; ICU: intensive care unit; TPN: total parenteral nutrition

The effectiveness of glutamine supplementation is dependent largely on the route as well as the dose administered (Wischmeyer, 2003). As already mentioned, the parenteral route of glutamine supplementation seems to be preferable to the enteral route (Novak *et al*., 2002). The superiority of parenteral glutamine can possibly be ascribed to the typical difficulties in the delivery of enteral nutrition to patients, as well as the better availability of parenteral glutamine for target organs, irrespective of GIT function. It can also be related to the dosages and the usual delay in the initiation of enteral diets in critically ill patients (De-Souza & Greene, 2005; Novak *et al*., 2002; Soeters & Grecu, 2012). Furthermore, IV glutamine administration is similar to endogenous production in that it is uniformly distributed and taken up in the splanchnic area

(Wernerman, 2011). The different societies recommend glutamine supplementation as standard care in patients receiving TPN (Table 2.3) (Dhaliwal *et al*., 2014; McClave *et al*., 2009; Singer *et al*., 2009). However, in the latest update from the CCPG, glutamine has now been downgraded to "it should be considered" as part of TPN, as opposed to the earlier recommendation stating that it was "strongly recommended". The CCPG also now recommend that it is not provided to patients in shock and that high dosages of both parenteral and enteral glutamine should not be administered (Dhaliwal *et al*., 2014).

Glutamine seems to have a dose-response effect, where a higher dosage leads to higher plasma glutamine levels and is therefore more effective (Heyland *et al*., 2007). Low dosages will thus not be sufficient to normalise plasma glutamine levels (Vesali *et al*., 2002). Differing recommendations regarding glutamine supplementation dosages have been published. Specifically referring to enteral supplementation, a dosage of 0.4-0.5 g/kg/d has been recommended in burns and trauma patients (Cynober & De Bandt, 2013; Wischmeyer, 2011).

For parenteral glutamine supplementation, it seems that exogenous IV glutamine supplementation of greater than 0.2 g/kg/d (0.3-0.5 g/kg/d) has been uniformly found to normalise plasma glutamine status in a dose-dependent manner (Bollhalder *et al*., 2013; Griffiths *et al.,* 1997; Heyland *et al*., 2007; Novak *et al*., 2002; Tjäder *et al.,* 2004; Vanek *et al*., 2011; Wernerman *et al.,* 2011; Wischmeyer *et al.,* 2014). The upper range should, however, not be exceeded (Wischmeyer *et al.,* 2014). The ESPEN, A.S.P.E.N. and CCPG recommend 0.2-0.4 g L-glutamine/kg/d (0.3-0.6 g/kg/d ALA-GLN) to be administered in all patients receiving parenteral nutrition (Dhaliwal *et al*., 2014; Kreymann, 2010; McClave *et al*., 2009; Singer *et al*., 2009). In patients on continuous renal replacement therapy, higher dosages of 25-35 g/d may be required (Wernerman, 2008).

The exact time when glutamine supplementation should be initiated is still debatable, as early glutamine administration may be harmful (Heyland *et al*., 2013). Nevertheless, it is of greater importance that it is supplemented together with nutritional support that meets a patient's energy and protein requirements and comprises not more than 20% of protein recommendations (Cynober and De Bandt, 2013). Wischmeyer *et al.* (2014) recently reported that parenteral glutamine supplementation should not take place early in the acute phase of critical illness in patients with MOF or unresuscitated shock requiring significant vasopressor support.

Concerning the length of administration, Bollhalder *et al.* (2013) found that supplementation continuing over nine days is required to exert beneficial effects in critically ill patients (Table 2.2). Recently, Cynober & De Bandt (2013) reviewed the available literature and

concluded that glutamine should be supplemented for five to ten days, but that in burn injuries it can be supplemented until wound closure.

Following the publishing of the results from the REDOXS study, a commentary was issued with an updated glutamine supplementation algorithm (Figure 2.4) (Heyland & Dhaliwal, 2013). In Figure 2.4 it is clear that glutamine supplementation should be reserved for the correct patients and administered accordingly. It should also be initiated only after the resolution of shock and MOF, as mentioned earlier (Wischmeyer *et al*., 2014). Furthermore, it can be recommended that those patients with acute renal failure not on dialysis or patients with acute liver failure should also not be supplemented (Cynober and De Bandt, 2013; Gottschalk, 2013; Heyland & Dhaliwal, 2013; Wischmeyer *et al*., 2014).

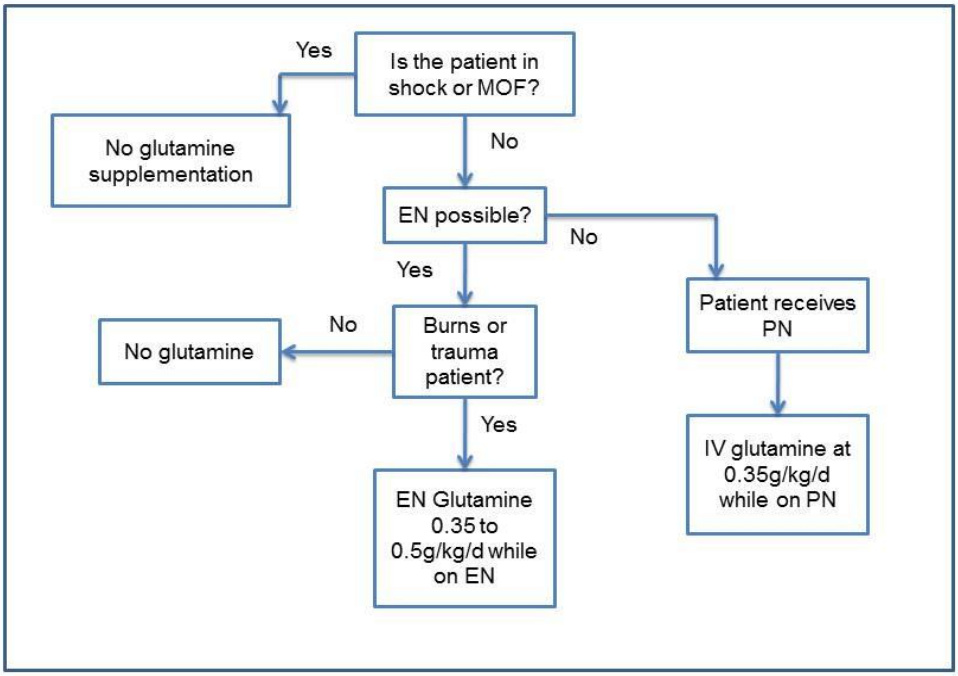

Figure 2.4 Glutamine supplementation algorithm (adapted from Heyland & Dhaliwal (2013))

EN: enteral nutrition; IV: intravenous; g/kg/d: gram per kilogram bodyweight per day; MOF: multiple organ failure; PN: parenteral nutrition

2.7 Summary of the literature and future research

It is clear from the available literature that glutamine serves important functions in multiple organs and tissues in the human body. However, in critical illness glutamine becomes a "conditionally essential amino acid" and a deficiency may occur, thereby leading to an inability to exert its key roles in the body. Measuring plasma glutamine levels as marker of glutamine status can identify this deficiency. Deficient levels may then have further detrimental effects on outcome, as well as an increased mortality risk.

Ample evidence exists on glutamine supplementation both enterally and parenterally, providing quite substantial proof of its benefits in mediating improved outcomes. These studies have found glutamine supplementation to be safe and effective for use. This belief has been challenged, however, by recent evidence, as it has become clear that not all critically ill patients are necessarily glutamine deficient. Moreover, supra-normal glutamine levels can also be found in certain patients, and these levels correlate with an increased mortality risk. The question as to which patients will present with an abnormal glutamine status remains, as well as how these patients can be identified in the ICU setting, by e.g. using other proxy biomarkers.

Several societies recommend glutamine use in ICU populations as part of their clinical guidelines. Yet there are specific patient groups where glutamine supplementation should be administered with caution; these include acute renal failure patients not on dialysis, acute liver failure patients, as well as those with right heart failure, MOF or patients in shock. Furthermore, the recommended dosages should not be exceeded (> 0.5 g/kg/d) and administration should be initiated only after the acute phase of injury or illness when the patient is stable.

With regard to supplementation studies, future research can be aimed at the further examination of the mechanism of action of glutamine supplementation. In this respect, the molecular mechanism of glutamine in the intestine, to maintain the intestinal integrity and its relationship with the development of SIRS and MOF, requires additional investigation. To ascertain good supplementation practice, studies on the timing of administration, with reference to when to initiate glutamine therapy and for how long it should be administered before discontinuation, are still required to confirm recommendations. Furthermore, studies administering glutamine beyond ICU stay can also be conducted, as it has been shown that supplementation is associated with reduced post-ICU mortality (Rodas *et al*., 2012). A South African-based cost-benefit study needs to be conducted to establish whether practical application in the South African setting is financially efficient. Moreover, further investigation is required on whether the rise in plasma glutamine levels following supplementation has an association with improved outcomes.

Concerning plasma glutamine levels, more research is also needed on the epidemiology of glutamine depletion. Further investigation is further required to explain why supra-normal plasma glutamine levels and high dosages of glutamine are related to increased mortality risks in patients. Heyland & Dhaliwal (2013) mentioned in their commentary on the REDOXS study that future studies should be aimed at the measurement of baseline plasma glutamine levels of ICU patients, to guide the necessity of supplementation in certain patient groups. This also relevant to the South African population, as local research on glutamine in the critical care setting is still lacking. This will be addressed and focused on in this research project and mini-dissertation.

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CHAPTER 3 – ARTICLE

PLASMA GLUTAMINE LEVELS IN ADULT ICU PATIENTS: A CROSS-SECTIONAL STUDY

3 CHAPTER THREE – ARTICLE

3.1 Title page

Plasma glutamine levels in adult ICU patients: a cross-sectional study

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Introduction

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Book chapter, or article within a book
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Abstract

Introduction: Not only is glutamine deficiency an independent predictor of mortality in intensive care unit (ICU) patients, but glutamine supplementation is also recommended for its proven outcome benefits. However, recent data suggest that early glutamine supplementation in certain patient groups increase mortality. The aim of this study was to investigate the plasma glutamine levels of adult ICU patients on ICU admission. In addition, the possible relationship between plasma glutamine levels, gender, diagnosis and selected inflammatory markers was investigated.

Methods: This observational, cross-sectional study included 60 mixed adult ICU patients who met the inclusion criteria. Blood samples were collected within 24 hours of ICU admission for plasma glutamine, interleukin-6 (IL-6), and C-reactive protein (CRP) analysis. Plasma glutamine levels were compared with those of paired healthy individuals, while gender- and diagnosis-related differences in plasma glutamine levels were investigated within the study population, using the Mann-Whitney and Kruskal-Wallis analysis of variance tests. The relationship between plasma glutamine levels and IL-6 or CRP was examined using the Spearman rank test. A non-parametric receiver operating characteristic curve was computed to determine the CRP concentration cut-off point above which glutamine becomes deficient.

Results: Intensive care unit patients had significantly lower plasma glutamine levels than the healthy control group (496 µmol/L vs 718 µmol/L, $p < 0.0001$). Of the patients, 38.3% ($n = 23$) had deficient (< 420 µmol/L) and 6.7% ($n = 4$) had supra-normal levels (> 930 µmol/L). No significant difference could be detected between the plasma glutamine levels of different genders ($p = 0.116$) or different diagnosis categories ($p = 0.325$). There was a significant inverse association between plasma glutamine and CRP levels ($r = -0.44$, $p < 0.05$), but only a trend towards an inverse association with IL-6 concentrations ($r = -0.23$, $p = 0.08$). A CRP cut-off value of 95.5 mg/L was determined as the point above which glutamine levels became deficient.

Conclusions: ICU patients had lower plasma glutamine levels than their healthy counterparts. However, not all were found to be glutamine deficient. Some presented with normal and supra-normal levels, therefore, suggesting that only selected patients be considered for glutamine supplementation. Plasma glutamine levels are inversely associated with CRP and a CRP cut-off value may be of use when predicting which patients presents with a glutamine deficiency in the clinical setting.

Keywords: Glutamine, intensive care unit, C-reactive protein, interleukin-6, gender

Introduction

Pharmaconutrition is a concept referring to nutrients that are administered as pharmacological agents and which form part of the medical treatment plan. It is currently applied in various clinical settings to improve patient outcomes [1]. Glutamine, the most abundant non-essential amino acid, is the most-researched pharmaconutrient to date [2, 3]. This amino acid is endogenously synthesised mainly by the skeletal muscle, but can also be exported by various other organs in order to maintain the amino acid pool [2, 4-7]. However, under circumstances of stress and catabolism, a glutamine-deficient state is thought to occur, and is attributed to increased glutamine demands together with insufficient endogenous synthesis to meet these demands [8, 9]. Previous studies have shown decreased levels of plasma and muscle glutamine in selected critically ill, post-surgical, multiple trauma, burns, septic and general intensive care unit (ICU) patients [7, 8, 10-13], resulting in the classification of glutamine as a "conditionally essential amino acid" under circumstances of stress and injury.

Under these circumstances, glutamine deficiency contributes to an inappropriate response to stress and injury, ultimately leading to an increased mortality risk [14, 15]. This is ascribed to the unavailability of glutamine to perform its beneficial functions in the body, which include providing cellular protection against stress and injury, serving as a fuel for immune cells and enterocytes, having anti-inflammatory effects, serving as a precursor of glutathione synthesis and inducing heat-shock protein synthesis [16-19]. In addition, it is important in various anabolic activities, including the non-toxic transfer of nitrogen, replenishing the citric cycle, nucleic acid synthesis and the maintenance of the acid-base homeostasis of the kidneys [20-23].

Glutamine supplementation has been extensively studied for its contribution to the improvement of patient outcomes, mediated by the refilling of the deficient glutamine pool and the execution of its beneficial functions. Until recently, glutamine supplementation has been deemed safe and effective in a variety of patient groups, including the severely ill, burns, pancreatitis and surgical patients [24-29]. Outcome benefits such as reductions in length of hospital stay (LOHS), length of ICU stay, mortality risk and infectious complications, as well as an improvement in nitrogen balance, have previously been reported, depending on the specific patient group as well as the dose and route of administration [24-29].

Recently, however, the Reducing Deaths due to Oxidative Stress (REDOXS) study questioned the safety and applicability of the supplementation of glutamine in all patient groups. In the REDOXS study an increased mortality risk was found when supplementing multiple organ failure (MOF) patients with high dosages of enteral and parenteral glutamine within 24 hours after ICU admission [30]. Furthermore, when a sub-sample of this study population was analysed, it was found that only 31% of patients were glutamine deficient (< 420 µmol/L) while

15% had supra-normal glutamine levels (> 930 µmol/L). Moreover, Rodas *et al.* [15] found that a U-shaped curve represents the association between glutamine and mortality, where both low (< 420 µmol/L) and high (> 930 µmol/L) glutamine levels were associated with a higher mortality risk.

It is, therefore, important to distinguish which patients are in fact glutamine deficient in order to determine those to be supplemented or those to whom supplementation may cause harm. In order to do this, it would be ideal to measure plasma glutamine levels of all patients prior to supplementation, but this is not a routine practice in the clinical setting. Consequently, it is important to identify other markers, which could aid in detecting patients presenting with a deficiency or an excess of glutamine. The current study was undertaken in order to address this. The main aim of this study, was to examine plasma glutamine levels of adult ICU patients on day one of admission in an attempt to establish if they had a deficiency (< 420 µmol/L), and to compare these glutamine levels with those of a healthy control group. In addition, the influence of gender and different diagnoses on glutamine status and the relationship between glutamine levels and selected inflammatory markers, C-reactive protein (CRP) and interleukin-6 (IL-6), were investigated.

Methods

Study design and setting

This observational cross-sectional study, which included an analytical component, was performed at two ICU settings in the Tshepong and Potchefstroom General Hospitals in the North West province, in South Africa. The units are both mixed ICUs, consisting predominantly of medical patients, but also admitting surgery and trauma patients. Recruitment and blood sampling of the healthy control participants were conducted at the Metabolic Unit of the North-West University, Potchefstroom Campus, South Africa. The study was carried out in accordance with the declaration of Helsinki and ethical approval was obtained from the North-West University ethics committee (ethics number NWU-00186-13-S1), the Policy, planning, research monitoring and evaluation committee of the North West Department of Health, and the Patient Safety Groups of both hospitals (see Annexures A and B). Informed, voluntary consent to participate in the study was obtained from all participants or their legal proxy (see Annexures C to E for consent forms).

Patients and control group participants

All adult patients (> 18 years) admitted to the ICUs of the two participating hospitals during the study period (March to August, 2014) were eligible for inclusion, provided they met the following inclusion criteria: informed consent obtained and blood sampling conducted within 24 hours

post-ICU admission. Patients excluded were those receiving total parenteral nutrition with added glutamine prior to blood sampling; those receiving glutamine-enriched enteral feeding, high protein or amino acid-based oral supplements prior to blood sampling; and those who were pregnant or lactating.

A power calculation was done using the main aim of the study as reference. The number calculated to achieve a power of 80%, with a significance of less than 0.05 for a large to medium effect, was 26 to 65 per group (ICU patient and healthy control group). From the admission records of the hospital, a dropout analysis was performed to identify the characteristics of the patients admitted to the ICU during the study period, but not included in the study. Secondary information, including the diagnosis of the patient, human-immunodeficiency virus (HIV) status (if known and noted in the patient file), gender, age, health status prior to ICU admission, medication, race and feeding regime were obtained from either the patient file or the attending medical officer (see Annexure F).

A healthy control group was included in the study in order to compare the plasma glutamine levels of critically ill and healthy individuals. The healthy control group was enrolled in the study from March to September 2014, in the North West province. They were recruited so as to match ICU patients in gender, race and age (within five years). The participants had to be older than 18 years, with no self-reported acute or chronic illnesses and taking no acute or chronic medication. C-reactive protein concentrations were measured to control for the presence of active inflammation in these individuals.

Blood sampling and laboratory analysis

Venous blood samples were taken from the patients by a registered nurse within 24 hours following acute ICU admission, as well as from the healthy participants, from the antecubital vein or dorsal area of the hand, using a 21-gauge Venofix butterfly needle (B/Braun), and transferred to ethylene diamine tetra-acetic acid (EDTA) and serum gel tubes. Tubes were inverted gently and kept on ice until centrifugation. Blood samples for glutamine and IL-6 analysis were collected in EDTA tubes and centrifuged within 30 minutes at 3000 x gravitational force for 15 minutes (4°C). Plasma was then transferred to collection tubes and stored at -80°C, pending analysis. Plasma samples were stored within one hour post-sampling and for no longer than 30 days. Plasma glutamine levels were determined by an adapted method of the EZ:faast amino acid analysis procedure (Phenomenex, Torrance, CA, USA) for the analysis of free physiological amino acids, using gas chromatography-mass spectrometry (Hewlett Packard HP 6890 series Gas Chromatography system, Palo Alto, CA, USA and Agilent 5973N Mass Selective Detector, Santa Clara, CA, USA). Plasma glutamine levels of below 420 µmol/L were classified as deficient and above 930 µmol/L as supra-normal [14, 15].

Interleukin-6 was measured by means of the IL-6 Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN, USA), using the Thermofisher Scientific Multiskan FC (Vanta, Finland). The minimum sensitivity of the assay was < 0.70 pg/mL. Normal levels of circulating IL-6 are more or less 1 pg/mL in healthy individuals, but for the purpose of this research, IL-6 levels below 10 pg/mL were regarded as normal [31, 32].

For the determination of CRP concentrations, serum gel tubes were prepared for analysis by centrifuging samples at 4000 revolutions per minute for 20 minutes. Analysis was conducted by means of the Sequential Multiple Analyser Computer, using the Beckman Coulter DxC880i (Beckman Coulter, Ireland). A reference CRP concentration of below 5 mg/L was regarded as normal, whereas mild inflammation, active and severe infection were classified according to 10 - 50 mg/L, 50 - 200 mg/L and > 200 mg/L respectively [33, 34].

Statistical analysis

The computer software package IBM SPSS® Statistics 22 (Statistical Package for Social Sciences, IBM, NY, USA) was used for statistical analysis. A p-value of ≤ 0.05 was considered as statistically significant. The normality of the data was determined by the Shapiro-Wilks test and histograms. Non-parametric data were log-transformed to improve normality and reported as geometric means and 95% confidence interval (CI). Normally distributed data are reported as means (95% CI), and data that are still non-parametric following log transformation, as medians [25^{th} - 75^{th} percentiles]. The independent T-test was used to determine the difference between the baseline characteristics of the ICU patients and the healthy control group. The Mann-Whitney U test was used to determine the differences between median plasma glutamine levels of ICU patients and the healthy control group, as well as between genders.

The influence of different diagnoses on plasma glutamine levels was examined by using the Kruskal-Wallis analysis of variance (ANOVA) with the Bonferroni adjustment. In order to determine the relationship between plasma glutamine levels and inflammatory markers, the Spearman rank test was used. Other variables, such as age, which could influence the levels of plasma glutamine, were included in the statistical models using analysis of covariance (ANCOVA). A receiver operating characteristic (ROC) curve was computed to determine the maximum CRP cut-off value at which glutamine becomes deficient.

Results

During the study period 159 patients were admitted to the ICUs of the participating hospitals, of wich 60 were included in the ICU patient study group. Some reasons why patients were not included were: informed consent was not obtained within 24 hours, either as a result of the legal proxy not being available or a patient unwilling to participate; blood sampling was not conducted

within 24 hours post-ICU admission; the patient died prior to blood sampling; patients were transferred from another hospital's ICU; and patients were below 18 years of age. Of these patients excluded from the study group, 32 were surgery patients, 49 were medical and 12, neurosurgery patients. The baseline characteristics of the ICU patient group are presented in Table 1.

The healthy control group was comprised of 34 participants, based on a post-hoc power analysis indicating that this group was large enough to compare to the ICU patient group with regard to their plasma glutamine levels. The aim was to match the healthy control group with the ICU patient group in respect of gender, age and race. The healthy control group consisted of 20 females and 14 males, which was not significantly different from the gender distribution of the ICU patient group (p = 0.11). Also, no difference was observed in the race distribution of the two groups.

Table 1 Baseline characteristics of the ICU patient group

Characteristic	**ICU patients**	
Age, year*	46 (32.3; 57.8)	
	n	**%**
Gender:		
Female	25	41.7
Male	35	58.3
ICU admission category:		
Medical	43	71.7
Surgery**	15	25.0
Trauma	2	3.3
Reason for ICU admission:		
Cardiovascular/vascular	10	16.7
DKA/HONK	13	21.7
Gastrointestinal disorder	5	8.3
Liver failure	1	1.7
Neurological disorder	2	3.3
Post-surgery	9	15.0
Respiratory disorder	9	15.0
Renal failure	2	3.3
Septic shock	3	5.0
Trauma	2	3.3
Other	4	6.7

*Age reported as mean (95% CI).;

** Surgery patients include: neurosurgery (n = 3), elective surgery (n = 4) and emergency surgery (n = 8) patients.

DKA, diabetic ketoacidosis; HONK, hyperosmolar non-ketotic coma; ICU, intensive care unit; n, number/ frequency

The ages among groups were normally distributed and therefore the data was reported as mean (95% CI) (Table 1). The mean age of the healthy control group [39 (95% CI 30.8; 48.5)]

was found to be significantly lower than that of the ICU patient group [46 (95% CI 32.3; 57.8), p = 0.002]. The age difference was related to elderly patients being admitted to the ICUs during the study period (10 patients over the age of 60), coupled by the difficulty of finding eligible age match healthy control participants, without any acute or chronic illnesses (inclusion criteria). This was corrected for, however, in the statistical comparison between the two groups.

The plasma glutamine levels of the study population were found to have a non-normal distribution and were therefore reported as medians [25th - 75th percentiles]. In Table 2, the median plasma glutamine levels of different medical condition categories are presented, together with the distribution of patients among deficient (< 420 µmol/L), normal (420 - 930 µmol/L), or supra-normal (> 930 µmol/L) plasma glutamine levels in each category. The median plasma glutamine level of ICU patients was significantly lower than that of the participants in the healthy control group (496 µmol/L [386 - 644 µmol/L] vs 718 µmol/L [628 - 801 µmol/L], p < 0.0001). After correcting for age, the difference remained statistically significant (p = 0.001).

Table 2 Glutamine status of the study population

Group	**Median plasma GLN level (µmol/L)** medians [25th **–** 75th percentiles]	**P-value**	**Deficient: < 420 µmol/L**	**Normal: 420 – 930 µmol/L**	**Supra-normal: > 930 µmol/L**
			n (%)		
ICU patients (*n* = 60)	497 [387 **–** 644]	0.001	23 (38.3)	33 (55.0)	4 (6.70)
Healthy controls (*n* = 34)	718 [628 **–** 801]		**–**	34 (100)	**–**
ICU men (*n* = 35)	558 [395 **–** 697]	0.116	12 (34.3)	19 (54.3)	4 (11.4)
ICU women (*n* = 25)	466 [380 **–** 543]		11 (44.0)	14 (56.0)	**–**
Admission categories:					
Medical (*n* = 42)	475 [372 **–** 627]	0.325	18 (42.8)	23 (54.8)	1 (2.40)
Surgical (*n* = 16)	515 [468 **–** 782]		4 (25.0)	9 (56.2)	3 (18.8)
Trauma (*n* = 2)	432 [381 **–** 432]		1 (50.0)	1 (50.0)	**–**
Specific conditions:[*]					
HIV+ (*n* = 8)	562 [375 **–** 1062]		2 (25.0)	5 (62.5)	1 (12.5)
Liver failure (*n* = 4)	497 [389 **–** 643]		2 (50.0)	1 (25.0)	1 (25.0)
Renal failure[#] (*n* = 7)	575 [388 **–** 714]		3 (42.9)	4 (57.1)	**–**
MOF (*n* = 4)	355 [310 **–** 689]		3 (75.0)	1 (25.0)	**–**
Sepsis (*n* = 4)	355 [310 **–** 689]		3 (75.0)	1 (25.0)	**–**
DM (*n* = 19)	380 [273 **–** 500]		10 (52.6)	9 (47.4)	**–**

Non-parametric data reported as median [25th **–** 75th percentile].
[#]Patients not dialysed; [*]Median plasma glutamine levels and distribution for certain conditions, that are expected to alter glutamine status.

DM, diabetes mellitus; GLN, glutamine; HIV+, human immunodeficiency virus seroreactive; ICU, intensive care unit; MOF, multiple-organ failure; n, number/ frequency

In the ICU patient group, 38.3% of patients were glutamine deficient, while 6.7% had supra-normal levels (> 930 µmol/L). All participants in the healthy control group presented with

normal plasma glutamine levels (Table 2). A non-significant difference was found when comparing the median plasma glutamine levels of female and male ICU patients (p = 0.116). No significant difference could be detected between the median plasma glutamine levels of the different admission categories (medical, surgery, trauma) (Table 2). The inability to demonstrate a difference between the groups was possibly related to the statistical power, as very few trauma patients (only two) were admitted to the ICUs during the study period. However when comparing the median plasma glutamine levels of medical and surgical patient groups, a significantly lower level was found in the medical patient group, after adjusting for age (p = 0.042). Of the 42 medical patients included, 42.8% had deficient plasma glutamine levels, whereas this was true for only 25% of the surgical group (Table 2). One patient in the medical group and three of the patients admitted for surgical reasons had supra-normal plasma glutamine levels. Specific conditions that have previously been described as altering plasma glutamine levels were included in Table 2 to enable easy interpretation.

Additionally, the association between plasma glutamine levels and inflammatory markers (CRP and/or IL-6 levels) was investigated. A trend towards an inverse association could be found between plasma glutamine levels and IL-6, but this was not significant (r = −0.23, p = 0.08) (Figure 1). After adjusting for age by means of a partial correlation, the correlation remained insignificant. However, a significant inverse association was demonstrated between CRP and plasma glutamine levels, after the same adjustments were made (r = -0.44, p < 0.05) (Figure 2).

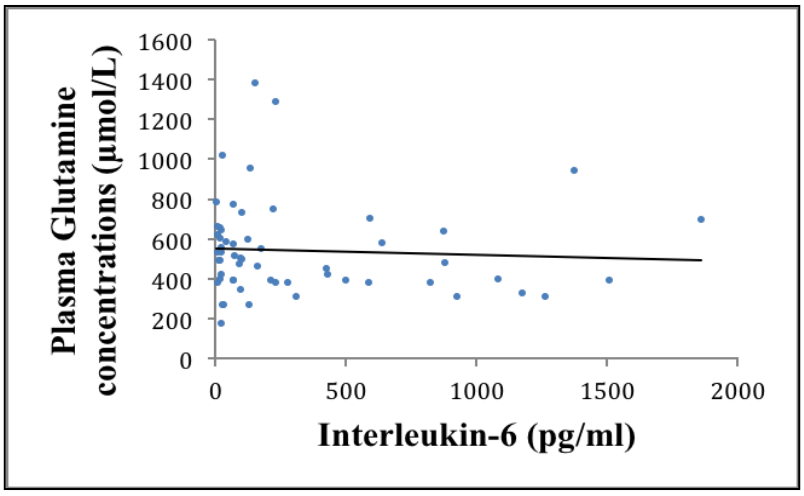

Figure 1 Relationship between plasma glutamine and interleukin-6 levels (r = -0.23, p = 0.08)

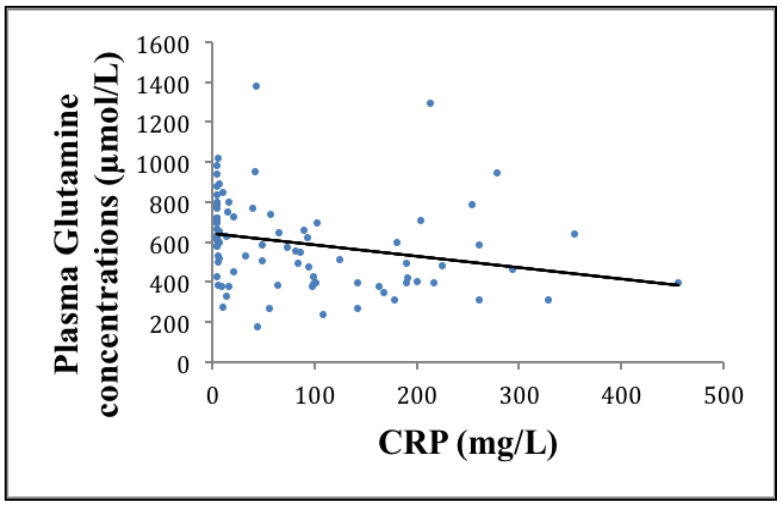

Figure 2 Relationship between plasma glutamine and CRP levels ($r = -0.44$, $p < 0.05$)
CRP, C-reactive protein

A non-parametric ROC curve was computed to determine the CRP level cut-off point at which plasma glutamine levels became deficient (Figure 3). The optimal cut-off was obtained from the Youden index value at 95.5 mg/L [Sp (Specificity) = 69%, Sn (Sensitivity) = 62.2%, AUC (area under the curve): 0.759 (95% CI 0.653; 0.865), $p < 0.0001$]. Of the patients that had CRP values above 95.5 mg/L, 74% had plasma glutamine levels below 420 µmol/L.

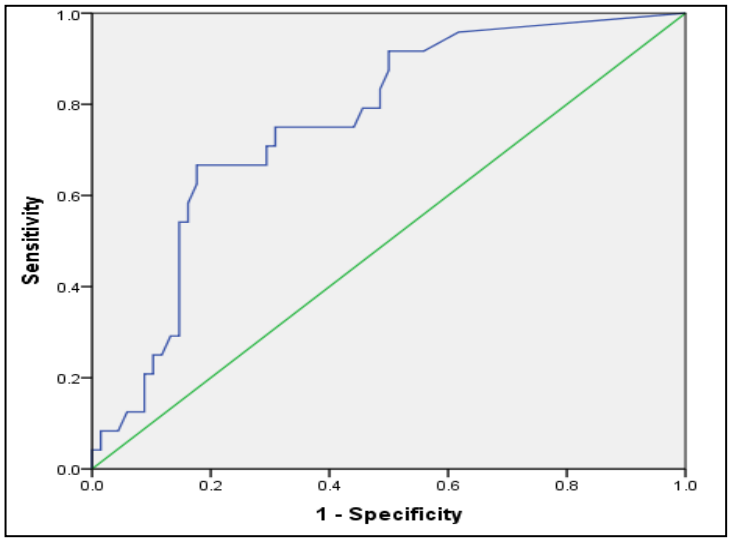

Figure 3 The receiver operating characteristic curve, computing the C-reactive protein level above which glutamine becomes deficient
Area under curve (AUC): 0.759 (95% CI 0.653; 0.865, $p < 0.0001$).

The IL-6 and CRP values were also categorised to present the plasma glutamine levels visually in different categories of inflammation (Figure 4 and 5). It was evident that the majority of patients with normal IL-6 levels (< 10 pg/mL), i.e. without inflammation, also had normal plasma glutamine levels. On the other hand, those with inflammation presented with lower plasma

glutamine levels, especially those in the 150 – 500 pg/mL IL-6 and 50 – 200 mg/L CRP groups. In the hyper-inflammatory groups (IL-6 > 500 pg/mL and CRP > 200 mg/L) the glutamine levels were scattered, ranging from very high to very low plasma glutamine levels.

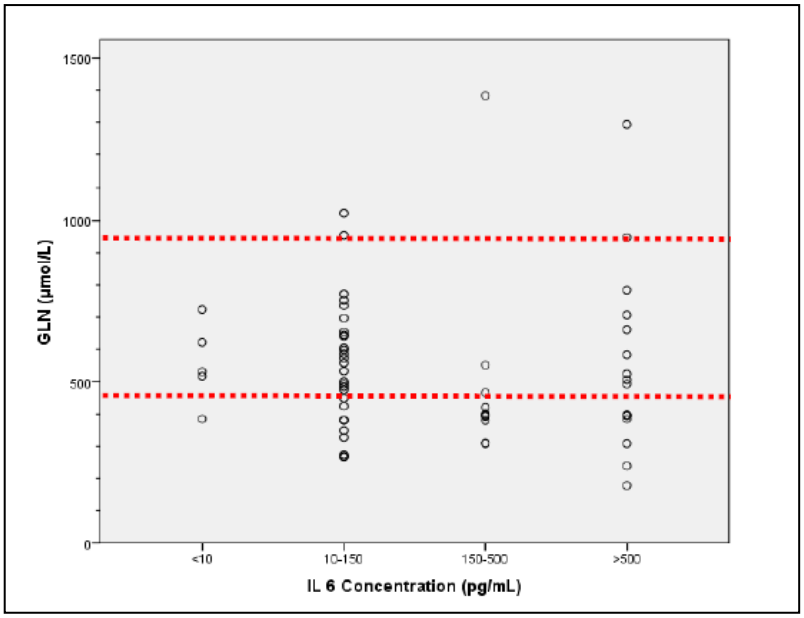

Figure 4 Plasma glutamine levels presented per interleukin-6 category

The area between the dashed lines represents normal plasma glutamine reference ranges.
GLN, glutamine; IL-6, interleukin-6

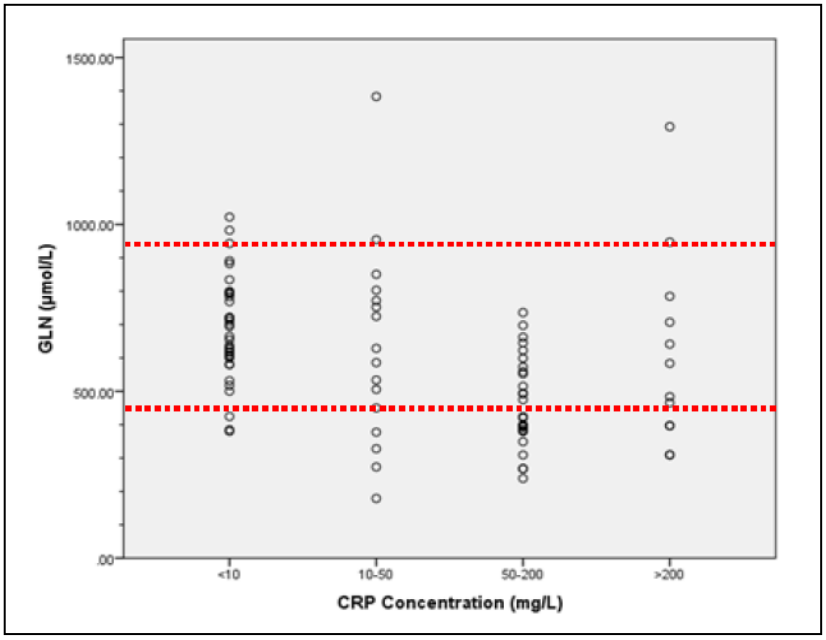

Figure 5 Plasma glutamine levels presented per C-reactive protein category

The area between the dashed lines represents normal plasma glutamine reference ranges.
GLN, glutamine; CRP, C-reactive protein

Discussion

This study aimed to establish whether mixed ICU patients present with a glutamine deficiency. This is important as the measurement of plasma glutamine levels is currently not readily available and is expensive and impractical in most hospital settings. In order to obtain a truly representative sample of a real-life ICU population, all patients admitted to the chosen ICUs during the study period were eligible for inclusion.

One of the strengths of this study was that it compared the plasma glutamine levels of ICU patients with those of a healthy control participant group. The findings indicated that the ICU patients had a significantly lower median plasma glutamine level in comparison with their healthy counterparts ($p < 0.05$). In most other studies, a healthy control group was not included, and plasma glutamine values were compared with normal reference values that would be expected in a healthy population. However, in previous studies post-operative plasma glutamine levels have been compared with pre-operative values in the same individuals, and lower levels have also consistently been reported post-operatively [7, 35]. The current study, therefore, adds to the literature in that patients admitted to ICU had lower plasma glutamine levels, on the first day of admission, than those of healthy individuals.

The median plasma glutamine level of this ICU population (497 µmol/L [387 **–** 644 µmol/L]) was, however, not below the 420 µmol/L threshold and could therefore not be described as deficient. This is comparable with findings by Oudemans-van Straaten *et al.* [14], indicating a median plasma glutamine level of 495 µmol/L (interquartile range 350 **–** 600 µmol/L) in ICU patients on day one of ICU admission. Furthermore, when investigating the frequency of patients who presented with deficient, normal or supra-normal plasma glutamine levels in the current ICU patient group, it was found that only 38.2% had deficient and more than half of the patients (55%) in fact had normal levels on ICU admission. This supports the results of other studies, which reported that approximately 31 to 43% of ICU patients had deficient glutamine levels on day one of admission to the ICU [14, 15, 36].

The mechanism whereby glutamine becomes depleted during inflammatory and stressed situations is attributed to its translocation from the muscle to supply the increased utilisation rates for proliferation, differentiation and functioning of vital splanchnic organs, such as the liver and immune system [37, 38]. Initially, an increased glutamine synthesis rate to compensate for losses may be evident, but ultimately the *de novo* synthesis may decrease by up to 48% in catabolic situations. Finally, insufficient to meet increased demands, it will result in glutamine depletion [6, 10, 37-39]. Low plasma glutamine levels are possibly life threatening, as they reflect the unavailability of glutamine to fulfil its important functions in cells [14, 40]. In addition,

deficient plasma glutamine levels on admission are an independent predictor of increased ICU and post-ICU mortality rates in mixed ICU patients [14, 15].

In this study, only 6.7% of patients were found to have supra-normal plasma glutamine levels (> 930 µmol/L) on admission to the ICU. Similarly, Heyland *et al.* [36] reported supra-normal admission glutamine levels in 15% of ICU patients with multiple organ failure (MOF). Other studies also found such high levels in selected patients, but did not report on the exact frequency and, therefore, a clear comparison cannot be made with findings of this research [14, 15]. Supra-normal plasma glutamine levels may be related to an increased release of amino acids from damaged muscle cells, as such levels were previously found to be associated with high creatinine kinase levels [14]. High glutamine levels are also attributed to organ failures, such as renal and liver failure, as the liver consumes almost half of the exported glutamine and plays an important role in the preservation of glutamine balance [41, 42]. Likewise, in conservatively treated renal failure patients, there may be reduced glutamine utilisation by the kidneys, causing increased plasma glutamine levels [42]. Supra-normal plasma glutamine levels in this study group could possibly be ascribed to acute liver failure as part of the diagnosis, although this was not evident in all liver failure cases. Both modest and very high plasma glutamine levels have been previously found in acute liver failure cases [15, 43-45]. In the current study, only one of the four patients with liver failure had a plasma glutamine level above 930 µmol/L. This patient had acute liver failure, while two of the others were diagnosed with chronic liver failure, which is thought to rather lead to glutamine deficiency, possibly related to malnutrition, that is frequently found in these patients [44]. Another reason for high plasma glutamine levels may have been related to glucocorticoid administration, however, the definite effect of this on plasma glutamine levels have not yet been established.

Supplementing patients who already have high plasma glutamine levels or those with normal glutamine levels may pose a risk. Rodas *et al.* [15] recently reported a U-shaped curve association between plasma glutamine levels and mortality, where both low and very high plasma glutamine levels were associated with an increased mortality [15]. This increased mortality risk may be due to higher glutathione production as a result of high levels of plasma glutamine, a precursor of glutathione, thereby altering the redox balance [46]. It can also be related to an increased nitrogen excretion rate as well as increased ammonia levels associated with high glutamine levels, leading to renal or liver failure [22]. Nevertheless, these are only potential causes and the exact mechanism behind this is still to be elucidated. The current research indicates that it may be harmful to supplement all ICU patients, as the majority of the patients in the current ICU study group had levels above the deficient 420 µmol/L reference.

When considering diagnosis categories, it was difficult to distinguish differences between the glutamine status of trauma, surgery and medical conditions, as patient numbers in some of the categories were very small. In a very recent study it was found that 60% of trauma ICU patients presented with deficient plasma glutamine levels prior to glutamine supplementation [13, 47]. In the current study it was difficult to draw any conclusions as to how trauma, as a primary diagnosis, influenced plasma glutamine levels, due to the low number of trauma patients included in the study group.

Almost half of the patients admitted for medical reasons had deficient levels on admission to the ICU, suggesting that these patients should not be excluded from glutamine supplementation. Of the medical patients included, 19 had diabetes, which was previously thought not to influence glutamine status [48, 49], interestingly, however, in the current study the majority of the diabetic patients presented with deficient glutamine levels on admission. This may have been related to additional conditions, other than diabetes, causing glutamine imbalances. Some of the included patients with renal, liver, MOF and sepsis, as determined by clinical history, physical findings and blood cultures, had deficient and normal glutamine levels, contrary to previous reports of elevated levels to be expected in these patient categories [36, 50, 51]. This again stresses the fact that ICU or critically ill patients are highly variable and that it is difficult to make assumptions on glutamine levels based on previous findings.

Earlier research suggested that a positive HIV status caused reduced plasma glutamine levels as a result of complications, such as infections and an increased need to maintain immune balance [52, 53]. Findings in the current study showed that HIV reactive patients predominantly had normal plasma glutamine levels. It should be mentioned, however, that HIV status was not tested in the patients, but only recorded if already diagnosed, and that the progression of the disease was not documented. Nevertheless, this was not the typical glutamine profile that one would expect in this patient group.

Regarding post-surgery patients, reduced plasma glutamine levels have been well established as a characteristic of their amino acid profile [7, 12, 35, 54]. In the current study, more than half of the surgery patients presented with normal plasma glutamine levels. Nevertheless, the degree to which levels are affected may be related to the severity, complexity, and duration of the surgery, which was not within the scope of this study [35, 54, 55]. It is, therefore, difficult to draw firm conclusions regarding the plasma glutamine levels in different diagnoses, owing to the rather small sample size of each medical condition group. The present study, however, supports the literature suggesting that a one-size-fits-all approach cannot be followed in identifying those patients who require glutamine supplementation.

As great variation in plasma glutamine levels was evident within medical condition groups, other factors that may have influenced plasma glutamine levels were also considered. In earlier studies, plasma glutamine levels were found to be significantly associated with gender in certain age categories of healthy individuals [56]. Whether this is also true for critically ill patients still has to be investigated, as no significant gender-related difference could be detected among the plasma glutamine levels of ICU patients in the current research population. This confirms findings by Viggiano *et al.* [35], who also reported that gender did not influence plasma glutamine levels post-operatively.

The results of the current research further indicate, in support of previous findings, that a definite relationship between plasma glutamine levels and inflammation exists. Two well-known inflammatory markers, IL-6 and CRP, were measured to determine their association with plasma glutamine levels. Interleukin-6, together with tumour-necrosis factor, drives inflammation, causing the release of CRP and other markers of the acute-phase response from the liver [57]. It is also an early marker of inflammation and a predictor of the severity of the injury and major complications [57]. A trend towards an inverse association was found between plasma glutamine and IL-6 levels ($p = 0.08$). This is supported by earlier studies reporting a negative association between IL-6 and glutamine and establishing IL-6 levels as a contributing factor to glutamine deficiency in infection [54, 58]. Glutamine supplementation has also been shown to modulate IL-6 production in human monocytes and animal studies [59, 60].

C-reactive protein is an inflammatory marker frequently measured in the hospital setting. Suliman *et al.* [61] reported an inverse association between CRP and amino acids in chronic kidney disease patients. The parenteral supplementation of glutamine has further been found to significantly reduce CRP concentrations [62-64]. In the current study an inverse association was also found between CRP and plasma glutamine levels ($p < 0.05$). A cut-off point of 95.5 mg/L was determined as the CRP value above which glutamine levels can potentially be expected to become deficient (< 420 µmol/L). This is a novel and important finding of this research and can be used in the clinical setting as a guide in the identification of patients with a possible glutamine deficiency. It can be viewed as an additional factor together with others when selecting which patients should be supplemented or not. However, further investigation is needed to confirm this.

This study had certain limitations. As this was an observational study, its results reflect mainly the population included in the study group and cannot be extrapolated to all ICU settings. As previously mentioned, the study group included mostly medical and surgical patients, with very few trauma admissions. Burns, oncology and dialysed renal failure patients are admitted to other ICUs in the hospitals where the study was conducted, and were also not included in the

study sample. Body composition measurements were not taken into account in the interpretation of data. Another limitation was that glutamine was measured only once, at ICU admission. Recently, Pérez-Bárcena *et al.* [13] found that low glutamine levels later during ICU stay (specifically on day six) correlated with infection rates, length of ICU stay and LOHS. More studies are required to address the time course of the depletion and restoration of plasma glutamine levels. It should also be kept in mind that IL-6 and CRP levels are highly variable and that peak concentrations of both these markers may not have been reached at the time of blood sampling. Lastly, the power of the study was possibly insufficient to differentiate between plasma glutamine levels in different diagnoses. It does, however, add valuable evidence to the current body of literature on plasma glutamine levels.

Conclusion

This study demonstrated that ICU patients presented with significantly lower plasma glutamine levels in comparison with healthy individuals on day one of ICU admission. Fifty-five percent of patients presented with normal plasma glutamine levels, while only 38.3% were glutamine deficient and 6.7% had supra-normal levels. These results suggest caution in the identification of patients suitable for glutamine supplementation. It is difficult to identify which patients require glutamine supplementation based on their medical diagnosis and gender. A significant inverse association was found between plasma glutamine and CRP levels, with a cut-off CRP value of 95.5 mg/L above which glutamine levels can be predicted to become deficient. This will aid in identifying those patients who could be expected to have a glutamine deficiency, when CRP values are available, as is the case in most clinical settings. A trend towards a negative relationship between plasma glutamine levels and IL-6 was also evident. These results, therefore, support earlier findings, which refuted a one-size-fits-all approach in glutamine supplementation of ICU patients. Based on the current literature and the findings of this study clinicians should consider the holistic profile of the patient, including the patient condition and other biomarkers e.g. CRP, when selecting patients for glutamine supplementation. Future research should be aimed at the determination of fluctuations in glutamine status during ICU stay and further elucidation of the patient profile that would benefit from glutamine supplementation.

Key messages

- Mixed ICU patients had lower plasma glutamine levels in comparison with healthy individuals on day one of admission to the ICU; however, only 38.3% were glutamine deficient and 6.7% presented with very high plasma glutamine levels.

- It is difficult to predict which patients will be glutamine deficient when considering only their medical diagnosis, as values vary within diagnosis categories.
- There was no significant gender-related difference in the plasma glutamine levels of ICU patients.
- There was a significant inverse relationship between plasma glutamine and CRP levels and a trend towards an inverse association between plasma glutamine and IL-6.
- A cut-off CRP value of 95.5 mg/L was identified as the point beyond which glutamine levels are expected to become deficient.

List of abbreviations

ANCOVA: analysis of covariance; ANOVA: analysis of variance; AUC: area under the curve; CI: confidence interval; CRP: C-reactive protein; EDTA: ethylene diamine tetra-acetic acid; ELISA: enzyme-linked immunosorbent assay; HIV: human immunodeficiency virus; ICU: intensive care unit; IL-6: interleukin-6; LOHS: length of hospital stay; MOF: multiple organ failure; REDOXS: Reducing Deaths due to Oxidative Stress; ROC: receiver operating characteristic; Sn: Sensitivity; Sp: specificity.

Competing interests

The authors declare that they have no competing interests.

Authors contributions

AN was involved in the conception and design of the project and the acquisition, analysis and interpretation of data, as well as the drafting, editing and submission of the manuscript. RCD was involved in the conception and design of the project, the interpretation of the data and the critical revision of the draft of this manuscript. RB and AEvG were involved in the conception and design of the project, as well as in critically revising the draft of the manuscript. All the authors approved the final version to be published.

Acknowledgements

The authors would like to thank all the participants in the study as well as the dietitians and nursing staff of both ICUs. Special thanks are due to Prof Variava, Dr Radebe, G. Witkoei, M. de Koker and D. Hartmann, whom assisted to establish and complete the study in the separate hospitals, as well as to the staff of the Wits Perinatal HIV Research Unit for assistance with blood preparation and storage and Mrs E. Rossouw, who assisted with the biochemical analysis. This study was funded by the Nutricia Research Foundation, the National Research

Foundation and the North-West University. We would also like to thank Marike Cockeran for the revision of the statistical analysis and Mary Hoffman for the language editing of this article.

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CHAPTER 4 – GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4 CHAPTER 4 – GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Introduction

This final chapter will provide a summary of the main findings of the research project as well as general conclusions and recommendations. The aim and objectives are repeated below, to provide an easy reference as to what was to be addressed in this research project and mini-dissertation.

Research aim

The aim of this cross-sectional, observational study was to examine the plasma glutamine levels of adult intensive care unit (ICU) patients in order to establish whether a deficiency exists (plasma levels less than 420 µmol/L), as well as to investigate possible associations between selected inflammatory markers (C-reactive protein (CRP) and interleukin-6 (IL-6)) and low plasma glutamine levels. In addition, the influence of gender and different diagnoses on plasma glutamine levels was examined.

Research objectives

The objectives of this research were to:

I. measure the plasma glutamine levels of adult ICU patients on admission to the ICU to determine whether they were deficient (< 420 µmol/L) and to compare these levels with those of a healthy control group;

II. determine whether there was an association between glutamine and selected inflammatory markers, namely CRP and IL-6;

III. establish whether there was a difference between the plasma glutamine levels of medical, trauma and surgical ICU patients; and

IV. determine whether gender influenced plasma glutamine levels of ICU patients.

4.2 Plasma glutamine levels of adult ICU patients on admission

The presence of glutamine deficiency in ICU patients has been reported previously and is recognised as an independent predictor of mortality (Gotschalk *et al*., 2013; Oudemans-van Straaten *et al*., 2001; Rodas *et al*., 2012). The current study indicated that mixed ICU patients had significantly lower plasma glutamine levels in comparison with healthy individuals, within 24 hours post-ICU admission. However, it cannot be assumed that all ICU patients will have a

glutamine deficiency (levels < 420 µmol/L), as these results showed that only 38.3% of patients presented with deficient levels, while 55% had normal plasma glutamine levels within the first 24 hours of admission. Furthermore 6.7% presented with supra-normal glutamine levels, which is alarming, as glutamine levels above 930 µmol/L are also associated with an increased mortality risk (Rodas *et al*., 2012). Patients presenting with normal glutamine levels, who are supplemented, are at risk of developing very high levels owing to an imbalance between glutamine provision and utilisation. Moreover, supplementation of patients presenting with baseline supra-normal plasma glutamine levels could result in the exacerbation of the already high glutamine status. The consequence may be an increased mortality risk, as was evident in a recent trial by Heyland *et al*. (2013). Supplementation should therefore be reserved for selected patients who are at risk of becoming glutamine deficient. This study further aimed to provide some clarification in the selection of patients requiring supplementation from a mixed general ICU group.

4.3 Plasma glutamine status among gender and diagnosis categories

It was found that there were no significant gender-related differences in plasma glutamine levels of the ICU patient group, which was also supported by earlier findings of Viggiano *et al*. (2011). When considering diagnosis categories, it was difficult to distinguish differences between the glutamine status of trauma, surgery and medical conditions, as patient numbers in some of the categories were very small. Nevertheless, almost half of the patients admitted for medical reasons had deficient levels on admission to the ICU, suggesting that these patients should not be excluded from glutamine supplementation. Of the medical patients included, 19 had diabetes, which was previously thought not to influence glutamine status (Felig *et al*., 1973; Stumvoll *et al*., 1996); interestingly, however, in the current study the majority of the diabetic patients presented with deficient glutamine levels on admission. This may have been related to additional conditions, other than diabetes, causing glutamine imbalances. A significant number of renal, liver and multiple organ failure (MOF) patients had deficient glutamine levels, contrary to previous reports of elevated levels to be expected in these patient categories. This again stresses the fact that ICU or critically ill patients are highly variable and that it is difficult to make assumptions on glutamine levels based on previous findings.

In surgical patients it was also established that not all presented with a glutamine deficiency on admission to the ICU. Most of these patients had normal plasma glutamine levels. However, the degree to which levels are affected by surgery may be related to the severity (major or minor procedures), complexity and duration of the surgery, which were not measured in this study (Parry-Billings *et al*., 1992; Viggiano *et al*., 2012; Vinnars *et al*., 1975).

Supra-normal plasma glutamine levels in this study group could possibly be ascribed to acute liver failure as part of the diagnosis, although this was not evident in all liver failure cases. Another reason for high plasma glutamine levels may have been related to glucocorticoid administration, however, the definite effect of this on plasma glutamine levels have not yet been established.

These results cannot provide definite conclusions with regard to which patients will be glutamine deficient or have supra-normal levels, but should serve as a warning to clinicians about the variability of glutamine status within patient groups. As it is difficult to use the medical condition or gender as independent predictive factors of a patient's glutamine status, other biochemical markers were also investigated.

4.4 The relationship between plasma glutamine and inflammatory markers

First, the association between IL-6 and plasma glutamine levels was tested. This marker was used as it has been reported to drive inflammation and to be a predictor of the severity of injury, as well as of patient outcome (Mihara *et al*., 2012). In the current study, only a trend towards an inverse association between IL-6 and plasma glutamine levels was found. However, in previous studies a negative correlation was proved and the lack of statistical significance found in the current study could possibly be ascribed to an insufficient power to detect an association (Andreasen *et al*., 2009; Parry-Billings *et al*., 1992).

A statistically significant inverse association was found between CRP and plasma glutamine levels. This may be a practical marker that can be used to predict a glutamine deficiency, as it is frequently measured in the ICU setting. To make a useful recommendation regarding the utilisation of CRP levels as predictor of plasma glutamine status, a cut-off of 95.5 mg/L was established. The cut-off indicates that one can expect glutamine to become deficient at CRP values above 95.5 mg/L. This is a novel finding and will aid in bringing research to practice in the selection of which patients to supplement or not. However, more research is needed to confirm these results. This approach should also be viewed as an additional factor to take into account, together with other factors such as patient condition and feeding regime, when deciding whether to supplement glutamine in the clinical setting. The results of the current research project further indicate, in support of previous findings, that a definite relationship between plasma glutamine levels and inflammation exists, as was expected.

4.5 Conclusions and practical recommendations emanating from this study

i. Firstly, lower glutamine levels can be expected in patients, in comparison with a healthy population group, within 24 hours following ICU admission. The necessity of early glutamine supplementation is questionable, however, as the majority of patients in the

current study were not glutamine deficient. It is therefore recommended that patients should be individually assessed and selected to determine their need of glutamine supplementation.

ii. Because of a lack of power in the current study it is difficult to draw conclusions as to which conditions will cause a glutamine deficiency or excess. However, a considerable variability in plasma glutamine levels was established within the category of each diagnosis or condition, owing to the heterogeneity of any ICU patient group. This makes it almost impossible to predict, based exclusively on the diagnosis, which patients require supplementation. Therefore it is recommended that a full assessment, considering all the factors that may influence glutamine status (e.g. the presence or absence of inflammation, organ failure, CRP levels, etc.), should be conducted prior to supplementation.

iii. According to the results of the current study, gender cannot be used to predict the glutamine status of an ICU patient.

iv. Lastly, the presence and severity of inflammation was shown to correlate with glutamine status. C-reactive protein may be a useful marker to identify patients at risk of glutamine deficiency (those with CRP levels above 95.5 mg/L). However, the patient profile should be considered holistically when selecting these patients and should not be based on one predictive factor alone.

4.6 Limitations of the research project

i. The first major limitation of the current, and indeed, of almost all observational ICU studies, is that findings are representative only of the group investigated and cannot be directly extrapolated to other ICU patient groups. This is based on the well-known heterogeneity of ICU populations in general.

ii. In the current study, only one blood sample, on day one of admission to the ICU, was obtained from the patients. It is therefore not reflective of the plasma glutamine status of patients throughout the course of ICU stay. It may have been that those patients who presented with normal plasma glutamine levels on admission would later become glutamine deficient and *vice versa*. This is also true for IL-6 and CRP levels that reach their peak concentrations at different time points during the inflammatory process and may not have been comparable among all patients included. The reason that only one measurement was taken in the current study was related to practical difficulties, as well as to resource and financial constraints.

iii. The third limitation was the power to detect an association between IL-6 and plasma glutamine levels, as well as a difference between the plasma glutamine levels of patients in different condition categories. In this regard, the few trauma admissions provided a significant limitation to any conclusions to be drawn in that specific patient group. A larger sample size may have resulted in more clear-cut and practical findings. The power calculation was based on the main aim of this study, i.e. to compare the plasma glutamine levels of ICU patients and a healthy population group.

iv. A more detailed analysis of patients not included in the study, but admitted to the chosen ICUs during the study period, would have been advantageous. This could then clarify whether the patient group studied was representative of all the patients admitted to the two ICUs during the study period.

v. Body composition measurements were not taken in the current study, and this may have served as a confounding factor in the interpretation of the results. However, in a previous study on surgical patients, no association was reported between plasma glutamine levels and the patients' percentage ideal body weight, fat free mass, or percentage weight loss (Hulsewé *et al*., 2004).

vi. The age difference found between the healthy control group and the ICU patient group was also seen as a limitation. This was due to practical difficulties in identifying older participants without any acute or chronic diseases, who would participate in the study. This was controlled for in statistical analysis.

vii. Lastly, the researchers did not conduct an in-depth assessment of the patients' medical condition, but recorded the diagnosis and clinical evaluation provided in the patients' files or as received from the medical officer. Human immunodeficiency virus (HIV) tests were also not conducted to identify HIV reactive patients, but their status was based only on the information available from their files.

4.7 Future research

In conducting the current project, gaps were found in the available literature that could be addressed by future research. These may include:

i. Further research into the exact profile of a patient expected to have a glutamine deficiency or excess should be conducted, specifically with regard to patient conditions, as well as readily available biomarkers that correlate with glutamine levels. This could be addressed by larger studies, including a more specific patient group, subjected to

restricted inclusion criteria. As there were few trauma patients in the current research project, this is a population group requiring future research;

ii. Plasma glutamine levels should be measured at different time points through the course of ICU and hospital stay, as well as after discharge in the recovery phase. This will give a better indication as to the point at which plasma glutamine levels become deficient and are restored, and therefore when supplementation is required;

iii. Research is also required to confirm the CRP cut-off value at which glutamine levels become deficient, in order to make definite practical recommendations in this regard and to support the findings of the current study; and

iv. The mechanism behind the very high plasma glutamine levels found in selected ICU patients and how these contribute to an increased mortality risk also requires further investigation.

In conclusion, it is clear that the aim and objectives of this study was reached and that the findings of this research, irrespective of its limitations, add to the literature regarding plasma glutamine levels of adult ICU patients.

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ANNEXURES

ANNEXURE A: Ethical approval from the North-West University Ethics Committee

NORTH-WEST UNIVERSITY
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NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

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South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

To whom it may concern

Faculty of Health Sciences
Tel: 018 299-2092
Fax: 018 299-2088
Email: Minrie.Greeff@nwu.ac.za

4 March 2014

Dear Dr. Dolman

Ethics application: NWU-00186-13-S1

"Plasma glutamine levels in critically ill intensive care unit patients"

Thank you for the amendments made to your application. All ethical concerns have been addressed and ethical approval is granted.

Yours sincerely

Prof Minrie Greeff
Ethics Committee - Humans Chair Person

Original details: Prof Minrie Greeff(10187308) C:\Users\13210572\Documents\ETIEK\2013 ETHICS\NWU-00186-13-S1.docm
4 March 2014

File reference: NWU-00186-13-S1

ANNEXURE B: Ethical approval from the North-West Department of Health Ethics Committee

health
Department of
Health
North West Province
REPUBLIC OF SOUTH AFRICA

3801 First Street
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POLICY, PLANNING, RESEARCH, MONITORING AND EVALUATION

To : **Mrs A Nienaber**

From : **Policy, Planning, Research, Monitoring & Evaluation**

Subject : **Approval Letter- Plasma glutamine level in critical ill patients in intensive care**

Purpose

To inform the researcher that permission to undertake the above mentioned study has been granted by the North West Department of Health. The researcher is expected to arrange in advance with the chosen districts or facilities, and issue this letter as prove that permission has been granted by the provincial office.

Upon completion, the department expects to receive a final research report from the researcher.

Kindest regards

Acting Director: PPRM&E
Mr. B Redlinghys

30/6/14
Date

DEPARTMENT OF HEALTH
PRIVATE BAG X2068
2014 -04- 30
SUPERINTENDENT GENERAL

Healthy Living for All

ANNEXURE C: Consent form for intensive care unit patients

Plasma glutamine levels in critically ill patients in intensive care

CONSENT TO BE A RESEARCH PARTICIPANT

We are a team of researchers from the Nutrition department of the North-West University working on plasma glutamine levels of intensive care unit (ICU) patients and we would like to invite you to give consent and participate in our study. To follow is information about the study so that you can make an informed decision whether to participate or not.

1. PURPOSE OF THE STUDY

The purpose of this study is to examine the plasma glutamine levels (a type of protein) of adult ICU patients-, in order establish if they have a shortage in their blood and to determine other markers that also rise and fall in the blood together with plasma glutamine levels. Plasma glutamine levels are the level of a protein in your blood that is very important to your health and may affect your condition as well as the time you spent in hospital. In addition, the influence of gender and different conditions on plasma glutamine levels will be examined. You are being asked to participate in this study because you are an ICU patient older than 18 years and the information/measurements from your blood sample are very valuable to us.

2. PROCEDURE

If you agree to be in this study it will entail:

- That a 9mL blood sample will be taken from you, by a qualified registered nursing sister within 24 hours after admission to the ICU;
- The blood will then be tested by laboratory personnel at the North- West University and hospital laboratory for glutamine, C-reactive protein and Interleuken-6 levels;
- Some background information will be recorded from your file including your diagnosis, medications, age, gender, race and health status.

3. RISKS/DISCOMFORTS

The drawing of blood is part of usual ICU procedures and should not cause any extra discomfort. Blood drawing can however cause some pain, bruising and anxiety. The nurse drawing the blood will be trained and will follow procedures to ensure your safety

and to make the drawing of the blood as pain free as possible as well as to prevent bruising. She will also reassure and support you during the sampling and counselling will be available if you are in any way anxious during the process. We will also record some of the information as already mentioned from your hospital file. Some of your privacy might be lost during this study but your name will never be made known and your data will be handled confidentially. Only the team of researchers will work with the information that you shared. All sensitive information will be protected by locking it up and storing it on a password protected computer at the university.

4. BENEFITS

This study will not hold any direct benefit for you, but will not harm you either. It will however help us to understand and treat ICU patients better in the future. This will make sure that we give the right patients glutamine supplementation and so we may be able to improve their condition and result in them spending less time in the hospital.

5. COSTS

There will be no cost to you as a result of your participation in this study.

6. PAYMENT

You will receive no payment for participation.

7. QUESTIONS

You are welcome to ask any questions to a member of the research team before you decide to give consent. You are also welcome to contact Arista Nienaber if you have any further questions concerning your consent at 0835453763.

8. FEEDBACK OF FINDINGS

The findings of the research will be shared with you if you are interested. You are welcome to contact us regarding the findings of the research. We will be sharing the findings with you as soon as it is available.

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

CONSENT FORM

PARTICIPATION IN THIS RESEARCH IS VOLUNTARY.
You are free to decline to be in this study, or to withdraw at any point even after you have signed the form to give consent without any consequences.

Should you be willing to participate you are requested to sign below:

I _____ hereby voluntarily consent to participate in the above mentioned study. I am not coerced in any way to participate and I understand that I can withdraw at any time should I feel uncomfortable during the study. I also understand that my name will not be disclosed to anybody who is not part of the study and that the information will be kept confidential and not linked to my name at any stage. I also understand what might be the possible risks and should I need further discussions someone will be available.

_____	_____
Date	Signature of the participant
_____	_____
Date	Signature of the person obtaining consent

ANNEXURE D: Consent form for intensive care unit patients' next-of-kin

Plasma glutamine levels in critically ill patients in intensive care

CONSENT TO BE A RESEARCH PARTICIPANT

We are a team of researchers from the Nutrition department of the North-West University working on plasma glutamine levels of intensive care unit (ICU) patients and we would like to invite you to give consent on behalf of your family member to participate in our study. To follow is information about the study so that you can make an informed decision whether to participate or not.

We understand that this is a very difficult time for your family. We would really appreciate it if you would be willing to go through this document.

1. PURPOSE OF THE STUDY

The purpose of this study is to examine the plasma glutamine levels (a type of protein) of adult ICU patients-, in order establish if they have a shortage in their blood and to determine other markers that also rise and fall in the blood together with plasma glutamine levels. Plasma glutamine levels are the level of a protein in your blood that is very important to your health and may affect a patient's condition as well as the time they spent in hospital. In addition, the influence of gender and different conditions on plasma glutamine levels will be examined. Your family member is being asked to participate in this study because they are an ICU patient older than 18 years and their information/ measurements from their blood sample are very valuable to us.

2. PROCEDURE

If you agree that your family member participate in this study it will entail:

- That a 9mL blood sample will be taken from your family member within 24 hours after admission to the ICU, by a qualified nursing sister;
- The blood will then be tested at the North-West University and hospital laboratory for glutamine, C-reactive protein and Interleuken-6 levels;
- Other information will be recorded from their file including diagnosis, medications, age, gender, race and health status.

3. RISKS/DISCOMFORTS

The drawing of blood is part of usual ICU procedures and should not cause any extra discomfort. Blood drawing can however cause some pain, bruising and anxiety. The nurse drawing the blood will be trained and will follow procedures to ensure your family members safety and to make the drawing as pain free as possible as well as to prevent bruising. She will also reassure and support your family member during the sampling and counselling will be available if they are in any way anxious during the process. We will also record some of the information as already mentioned from their file. Some of their privacy might be lost during this study, but their name will never be made known and their data will be handled confidentially. Only the team of researchers will work with the information that you shared. All sensitive information will be protected by locking it up and storing it on a password protected computer at the University.

4. BENEFITS

This study will not hold any direct benefit for you, but will not harm you either. It will however help us to understand and treat ICU patients better in the future. This will make sure that we give the right patients glutamine supplementation and so we may be able to improve their condition and result in them spending less time in the hospital.

5. COSTS

There will be no cost to you or your family member as a result of their participation in this study.

6. PAYMENT

You will receive no payment for their participation.

7. QUESTIONS

You are welcome to ask any questions to a member of the research team before you decide to give consent. You are also welcome to contact Arista Nienaber if you have any further questions concerning you consent at 0835453763.

8. FEEDBACK OF FINDINGS

The findings of the research will be shared with you if you are interested. You are welcome to contact us regarding the findings of the research.

CONSENT FORM

PARTICIPATION IN THIS RESEARCH IS VOLUNTARY.
You are free to decline to be in this study, or to withdraw at any point even after you have signed the form to give consent without any consequences.

Should you be willing to give consent that your family member participates you are requested to sign below:

I _____ hereby voluntarily consent that _____ participate in the above mentioned study. I am not coerced in any way to participate and I understand that the patient can withdraw at any time should they feel uncomfortable during the study. I also understand that their name will not be disclosed to anybody who is not part of the study and that the information will be kept confidential and not linked to their name at any stage. I also understand what might be the possible risks and should I need further discussions someone will be available.

_____ _____

Date Signature of the next-of-kin

_____ _____

Date Signature of the person obtaining consent

ANNEXURE E: Consent form for healthy control group participants

Plasma glutamine levels in critically ill patients in intensive care

CONSENT TO BE A RESEARCH PARTICIPANT

We are a team of researchers from the Nutrition department of the North-West University working on plasma glutamine levels of intensive care unit (ICU) patients and we would like to invite you to give consent and participate in our study. To follow is information about the study so that you can make an informed decision whether to participate or not.

1. PURPOSE OF THE STUDY

The purpose of this study is to examine the plasma glutamine levels (a type of protein) of adult ICU patients-, in order establish if they have a shortage in their blood and to determine other markers that also rise and fall in the blood together with plasma glutamine levels. Plasma glutamine levels are the level of a protein in your blood that is very important to your health and may affect your condition as well as the time spent in hospital. In addition, the influence of gender and different conditions on plasma glutamine levels will be examined. You are being asked to participate in this study as part of the control group so that we can compare your levels with those of ICU patients.

2. PROCEDURE

If you agree to be in this study it will entail:

- That a 9mL blood sample will be taken from you, by a qualified registered nursing sister;
- The blood will then be tested by laboratory personnel at the North- West University and hospital laboratory for glutamine, C-reactive protein and Interleuken-6 levels;
- Additional information will be gathered from you in a structured interview and the questions asked will be limited to your medical history, any medications that you are on as well as your age, gender, race and health status.

3. RISKS/DISCOMFORTS

The drawing of blood is not supposed to cause you any harm and discomfort, although it can cause some pain, bruising and anxiety. The nurse drawing the blood will be trained and will follow procedures to ensure your safety and to make the drawing of the blood as pain free as possible as well as to prevent bruising. She will also reassure and support you during the sampling and counselling will be available if you are in any way anxious during the process. We will also ask you some questions as already mentioned, but this will only take a few minutes of your time. Some of your privacy might be lost during this study but your name will never be made known and your data will be handled as confidential. Only the team of researchers will work with the information that you shared. All sensitive information will be protected by locking it up and storing it on a password protected computer at the university.

4. BENEFITS

This study will not hold any direct benefit for you, but will not harm you either. It will however help us to understand and treat ICU patients better in the future. This will make sure that we give the right patients glutamine supplementation and so we may be able to improve their condition and result in them spending less time in the hospital.

5. COSTS

There will be no cost to you as a result of your participation in this study.

6. PAYMENT

You will receive no payment for participation. A snack will be provided to you after the blood sampling took place.

7. QUESTIONS

You are welcome to ask any questions to a member of the research team before you decide to give consent. You are also welcome to contact Arista Nienaber if you have any further questions concerning your consent at 0835453763.

8. FEEDBACK OF FINDINGS

The findings of the research will be shared with you if you are interested. You are welcome to contact us regarding the findings of the research. We will be sharing the findings with you as soon as it is available.

CONSENT FORM

PARTICIPATION IN THIS RESEARCH IS VOLUNTARY.
You are free to decline to be in this study, or to withdraw at any point even after you have signed the form to give consent without any consequences.

Should you be willing to participate you are requested to sign below:

I _____ hereby voluntarily consent to participate in the above mentioned study. I am not coerced in any way to participate and I understand that I can withdraw at any time should I feel uncomfortable during the study. I also understand that my name will not be disclosed to anybody who is not part of the study and that the information will be kept confidential and not linked to my name at any stage. I also understand what might be the possible risks and should I need further discussions someone will be available.

_____	_____
Date	Signature of the participant
_____	_____
Date	Signature of the person obtaining consent

ANNEXURE F: Secondary information collection form

Information to be obtained from ICU patient

Code: _____

Date & time information recorded: _____

Completed by: _____

Section A: SOCIO-DEMOGRAPHIC INFORMATION

Participant name	
Hospital number	
Date & time of admission to ICU (D/M/Y)	
Date of birth (D/M/Y)	
Age	
Gender	
Race	

Section B: MEDICAL INFORMATION

Diagnosis:

_____

_____

Had the patient been in hospital prior to ICU admission? If yes provide admission date:

_____

Chronic illnesses:

_____

_____

Current medication:

_____

_____

APACHE II score: _____

Section C: Nutritional information

Current feeding regime of the patient:

Feeding route (oral/ enteral/ parenteral/ NPO)	
Diet (hospital code) (if applicable)	
Enteral or parenteral formula (if applicable)	
Feeding rate (if applicable)	
Date and time patient started on feeding regime	
Diet/ formula administered as prescribed	

Any supplements received in the week prior to or since ICU admission?

_____

Any additional information or comments:

_____

_____

_____

_____

Signature of fieldworker obtaining information