Plasma glutamine concentration among critically ill children

by
Joanna Eksteen (Wilson)

Thesis presented in partial fulfilment of the requirements for the degree Master of Nutrition at the University of Stellenbosch

Supervisor: Prof Renee Blaauw
Co-supervisor: Prof Brenda Morrow
Statistician: Prof D.G. Nel

Faculty of Medicine and Health Sciences
Department of Global Health
Division of Human Nutrition

April 2019

Stellenbosch University https://scholar.sun.ac.za

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 28/10/2018

Joanna Eksteen (Wilson)

Copyright © 2019 Stellenbosch University
All rights reserved

Stellenbosch University https://scholar.sun.ac.za

English Abstract

Background: Glutamine is considered conditionally essential during critical illness and supplementation of the nutrient has become commonplace among adult ICU populations. However, recent concern over the safety of this practice has highlighted the need for focused research on plasma glutamine levels in specific patient groups. There is limited evidence for glutamine supplementation in children, with even less data available on plasma concentrations within this group. The aim of this study was to describe plasma glutamine concentration among critically ill children on admission to and on day two of Paediatric Intensive Care Unit (PICU) stay, and to identify associations between plasma glutamine and markers of clinical condition, nutritional status and intake, and clinical outcome.

Methods: This descriptive cross-sectional study investigated the plasma glutamine concentrations of patients admitted to a tertiary PICU in the Western Cape, South Africa, over a period of one month. Plasma glutamine was analysed using blood samples collected on admission, and on day two of PICU stay. Markers of clinical condition on admission (diagnostic profile, severity of disease, presence of infectious disease, and routine biochemistry) were collected. Age-appropriate anthropometry was conducted, and the nutritional status of participants was assessed using World Health Organization Z scores. Nutritional intake was recorded and analysed for the first two days of PICU stay.

Results: Seventy six participants were included in this study, many of whom (47%) were post-operative cardiac patients. Plasma glutamine concentrations were normal for most participants on admission (median 556.5 umol/l, IQR 459- 664.5 umol/l) and on day two of PICU stay (median 529.0 umol/l, IQR 356.0-716.0 umol/l). No obvious change in plasma levels occurred during this period. Significant differences in plasma glutamine were found between medical and elective surgery ($p = 0.007$) and trauma ($p = 0.013$) patients with trauma observed to have the lowest concentrations on admission (mean 450.3 ± 166.7 umol/l). Differences were also observed between cardiology and gastroenterology ($p = 0.018$), and between sepsis and pulmonology ($p = 0.031$), burns ($p = 0.035$), gastroenterology ($p = 0.006$), and 'other' ($p = 0.049$) diagnoses. Participants with sepsis had the highest plasma glutamine on admission (mean 736.3 ± 142.5 umol/l). Participants with higher plasma glutamine on admission tended to have longer hospital stays ($p = 0.067$), and a higher mortality risk ($p = 0.052$). Although no link was made with mortality, those who died had higher plasma glutamine on day two of PICU stay ($p = 0.057$).

Conclusion: This study found normal plasma glutamine concentrations among critically ill children over the first two days of PICU stay. Significant differences were found in plasma glutamine between diagnostic groups, with sepsis and trauma identified as areas for future study. A tendency was demonstrated toward poorer outcome and increased mortality risk among those with high plasma glutamine. Additional exploratory research is required to better understand plasma glutamine in different paediatric subgroups.

Stellenbosch University https://scholar.sun.ac.za

Afrikaanse Opsomming

Agtergrond: Glutamien word beskou as kondisioneel noodsaaklik tydens kritiese siektes en aanvullings van die nutriënt word gereeld in intensiewe sorg eenhede gebruik. Onlangse kommer oor die veiligheid van dié praktyk beklemtoon die behoedfte vir meer gefokusde navorsing oor plasma glutamienvlakke in spesifieke pasiënt populasies. Daar is 'n gebrek aan navorsing oor die rol van glutamien aanvullings in kinders, en data oor plasma konsentrasies in die groep is selfs meer beperk. Die doel van die studie was om plasma glutamien konsentrasies in kinders in die pediatriese intensiewe sorg eenheid (PICU) te beskryf, beide met hospitaal toelating en op dag twee van verblyf; en om assosiasies tussen plasma glutamien en merkers van kliniese toestand, voedingstatus, inname en ook kliniese uitkomste te identifiseer.

Metodes: Hierdie dwarssnit beskrywende studie het die plasma glutamien konsentrasies ondersoek in pasiënte opgeneem tot 'n tersiêre PICU in die Wes-Kaap, Suid Afrika, oor 'n tydperk van een maand. Bloedmonsters is geneem met toelating en op die tweede dag van PICU verblyf en is geanaliseer vir plasma glutamine konsentrasie. Merkers van kliniese toestande met toelating (diagnostiese profiel, erns van siekte, teenwoordigheid van infeksie, en roetine biochemie) is ingesamel. Ouderdoms-toepaslike antropometrie is geneem en voedingstatus is bepaal met die Wêreldgesondheidsorganisasie Z-tellings. Voedingsinname is gedokumenteer en geanaliseer vir die eerste twee dae van PICU verblyf.

Resultate: Die studie het ses-en-sewentig deelnemers ingesluit, die meerderheid (47%) was post-kardiale chirurgie pasiënte. Plasma glutamien konsentrasies was normaal vir meeste van die deelnemers met toelating [mediaan 556.5 umol/l, interkwartielvariasiewydte (IQR) 459- 664.5 umol/l] en op dag twee PICU verblyf (mediaan 529.0 umol/l, IQR 356.0-716.0 umol/l). Geen ooglopende veranderinge in plasma vlakke het tydens die periode plaasgevind nie. Beduidende verskille in plasma glutamien is gevind tussen mediese en elektiewe chirurgie ($p = 0.007$) en ook trauma ($p = 0.013$) pasiënte, met die laagste konsentrasies met toelating in trauma pasiënte (gemiddeld 450.3 ± 166.7 umol/l). Verskille in glutamien konsentrasie is ook waargeneem tussen kardiologie en gastroënterologie ($p = 0.018$), tussen sepsis en pulmonologie ($p = 0.031$), brandwonde ($p = 0.035$), gastroënterologie ($p = 0.006$), en 'ander' ($p = 0.049$) diagnoses. Deelnemers met sepsis het die hoogste glutamien met toelating gehad (gemiddeld 736.3 ± 142.5 umol/l). Deelnemers met hoër plasma glutamien met toelating was meer geneig om langer in die hospitaal te bly ($p = 0.067$), en het 'n hoër sterfterisiko gehad ($p = 0.052$). Alhoewel daar geen verband met mortaliteit was nie, het dié wat oorlede is hoër plasma glutamien vlakke getoon op dag twee van PICU verblyf ($p = 0.057$).

Gevolgtrekking: Die studie het normale plasma glutamien konsentrasies in kritiese siek kinders tydens die eerste twee dae van PICU verblyf gevind. Beduidende verskille is gevind in plasma glutamien tussen diagnostiese groepe, met sepsis en trauma geïdentifiseer as fokus areas vir toekomstige navorsing. 'n Neiging na swakker uitkomste en hoër sterfterisiko in diegene met hoër plasma glutamien is gedemonstreer. Addisionele verkennende navorsing word benodig vir 'n beter begrip van plasma glutamien in verskillende pediatriese subgroepe.

Stellenbosch University https://scholar.sun.ac.za

Acknowledgments

The principle researcher would like to thank all staff members of the Paediatric Intensive Care Unit at Red Cross War Memorial Children's Hospital, for their assistance during this study. Thanks are also extended to Prof. Daan Nel for his statistical support.

The principal researcher (Joanna Eksteen (Wilson)) developed the research idea in collaboration with her supervisor Prof. Renee Blaauw. The principal researcher developed the protocol, planned the study, undertook data collection without assistance, captured the data for analyses, analysed the data with the assistance of a statistician Prof. Daan Nel, interpreted the data, and drafted the thesis. Prof. Renee Blaauw and Prof. Brenda Morrow provided input at all stages and revised the protocol and thesis.

Stellenbosch University https://scholar.sun.ac.za

Table of Contents

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Abbreviations

ANOVA	Analysis of Variance
APACHE	Acute Physiology and Chronic Health Evaluation
ARV	Antiretroviral
ASPEN	American Society of Parenteral and Enteral Nutrition
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CRP	C-Reactive Protein
DNA	Deoxyribonucleic Acid
ESPEN	European Society for Clinical Nutrition and Metabolism
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology, and Nutrition
GALT	Gut-associated Lymphoid Tissue
H^+	Hydrogen
HIV	Human Immunodeficiency Virus
HSP	Heat Shock Protein
ICU	Intensive Care Unit
Il	Interleukin
IQR	Interquartile Range
K+	Potassium
LOS	Length of Stay
MAPK	Mitogen-Activated Protein Kinases
MOF	Multiple Organ Failure
mTOR	Mammalian Target of Rapamycin
MUAC	Mid Upper Arm Circumference
Na^+	Sodium
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NHLS	National Health Laboratory Service
PCT	Procalcitonin
PELOD	Paediatric Logistic Organ Dysfunction
PICU	Paediatric Intensive Care Unit
PIM	Paediatric Index of Mortality
PRISM	Paediatric Risk of Mortality
RCT	Randomised Controlled Trial
REDOXS	Reducing Deaths due to Oxidative Stress
SAMRC	South African Medical Research Council
SBS	Short Bowel Syndrome
SCCM	Society of Critical Care Medicine
SD	Standard Deviation
SIGNET	Scottish Intensive care Glutamine or seleNium Evaluative Trial
SOFA	Sequential Organ Failure Assessment
TB	Tuberculosis
TCA cycle	Tricarboxcylic Acid (Krebs) cycle
TNF ά	Tumour Necrosis Factor ά
USDA	United States Department of Agriculture
WHO	World Health Organization

Stellenbosch University https://scholar.sun.ac.za

List of tables

Stellenbosch University https://scholar.sun.ac.za

List of figures

Stellenbosch University https://scholar.sun.ac.za

1. Introduction

The amino acid glutamine was discovered over a century ago, having first been isolated in 1883 by Schultze and Boshard from beet juice.[1] Later, in 1932, it was identified in abundance in gliadin, a protein found in wheat.[2] It was not until 1935, however, that the enzymatic synthesis of glutamine from glutamate and ammonia was discovered by Krebs using rat and guinea pig kidney tissue.[3] This provided the first evidence that glutamine is likely present in most mammalian tissues. This was a profound discovery- considering the scientific limitations of the time and the innate instability of glutamine in vitro.[2]

Since these early discoveries, research on the amino acid (particularly in rat models) gained traction and generated important functional knowledge on the nutrient. One of the earliest discoveries of glutamine function was its role in maintaining pH balance, which was described by Goodman and colleagues.[4] Shortly thereafter, Pozefsky identified elevated glutamine release from muscle tissue when compared to other amino acids.[5] In their ground-breaking work, Windmeuller and Speath established the uptake of glutamine from the intestine, so opening the door to further research into its role in gut health.[6,7] Knowledge on the amino acid has since grown exponentially. Glutamine is now appreciated as being unique in comparison to other amino acids in terms of its diverse functional capacity and contribution to various biological systems and homeostasis.[2]

Glutamine has been in the spotlight for several decades for its therapeutic potential during critical illness. However, the focus on this amino acid has changed direction several times. Promising results from early intervention studies in the 1980s established a place for glutamine supplementation of the critically ill, seemingly with no risk of harm.[8,9] Much research followed, investigating the effects of glutamine supplementation among various patient groups.[10] Consequently, support grew for routine supplementation during critical illness.[11,12]

There has, however, been a recent shift in opinion. In 2013, a large multicentre trial reported harm following high dose supplementation of glutamine to critically ill adults.[13] This divided the research community into those who believed supplementation should cease altogether,[14] and others advocating a more targeted approach.[15–17] What became resoundingly clear following this controversy, was the need for a deeper understanding of the patient populations who may benefit from glutamine supplementation and those who would not.[18] As such, a 'back to basics' approach seemed appropriate, in which observational research into plasma glutamine concentrations of specific patient subgroups took precedent over intervention trials.[19]

Stellenbosch University https://scholar.sun.ac.za

The current study, which focuses on plasma glutamine concentration among critically ill children, is a reflection of the current move toward observation and understanding within glutamine research. In paediatric populations, intervention studies have yielded no definitive answers regarding glutamine supplementation thus far, with few studies having measured plasma glutamine prior to supplementation.[20] Limited observational research has been done on plasma glutamine concentrations among critically ill children – warranting further study within this subgroup.[21,22]

Stellenbosch University https://scholar.sun.ac.za

2. Literature Review

Glutamine in the human body

Glutamine is the most abundant amino acid in the plasma and muscle tissue of humans. Skeletal muscle is the major site of production and storage of glutamine, accounting for up to 90 % of the body's pool.[23]

Glutamine in the plasma

Normal plasma glutamine in adult populations has been established at concentrations of 420-930 umol/l.[24] Plasma glutamine is derived from several sources, the first being skeletal muscle degradation. During periods of stress, increased demand for glutamine triggers net proteolysis of skeletal muscle.[25] The resulting free glutamine is exported from the muscle cell's cytosol into the plasma in an attempt to maintain physiological concentrations.[26]

De novo synthesis of glutamine by the enzyme glutamine synthase is the second source of plasma glutamine. Although this occurs mainly in skeletal muscle, synthesis has also been shown to occur under certain conditions in organs such as the lungs,[27] brain, liver, and in adipose tissue.[28,29] During periods of stress, human lungs demonstrate the ability to release glutamine as a result of glucocorticoid signalling and glutamine synthase expression. Although a minor contributor to the glutamine pool, adipose tissue has recently been thought to be a potentially important source of the amino acid.[29] Despite the liver's ability to synthesise and catabolise glutamine, it is thought to play an insignificant role in the body's overall glutamine pool. Glutamine flux in the liver is thought to occur as part of regulatory effects during acidosis and hyperammonemia.[30]

The free glutamine pool is the third source of plasma glutamine. During the diseased state, the free glutamine pool is decreased as it donates glutamine to the plasma.[31]

Dietary intake of glutamine in the form of protein is another source of exogenous glutamine which enters the plasma. Together, glutamate and glutamine comprise 5 – 9% of dietary protein.[32]

Glutamine in the tissues

Tissue concentrations of glutamine are higher than in the plasma. The steep uphill gradient of glutamine concentration between the plasma and tissues is maintained by active transport – primarily through the sodium potassium (Na^+/K^+) pump. This transport system requires hydrolysis of Adenosine Triphosphate (ATP) by the enzyme Na^+/K^+ ATPase.[26] Intracellular concentrations of glutamine are also influenced by the

Stellenbosch University https://scholar.sun.ac.za

cellular production and uptake of glutamine from de novo synthesis, as well as from protein breakdown.[2] Concentrations of glutamine in various tissues vary considerably, with enterocyte concentrations at 2-4 mmol/l compared to skeletal muscle and liver concentrations of 5-20 mmol/l. It is therefore suspected that plasma concentration is not the only factor influencing cellular content, and that additional factors play varying roles in determining glutamine concentration in the different tissues.[26]

Functions of glutamine

Like other amino acids, glutamine provides substrate for protein metabolism. It is, however, considered unique in that it plays a central role in numerous biological and homeostatic pathways – including energy metabolism, immune function, tissue protection, antioxidant capacity, pH regulation, and in the protection of the intestinal barrier, as summarised in Figure 1 (below).[20,33]

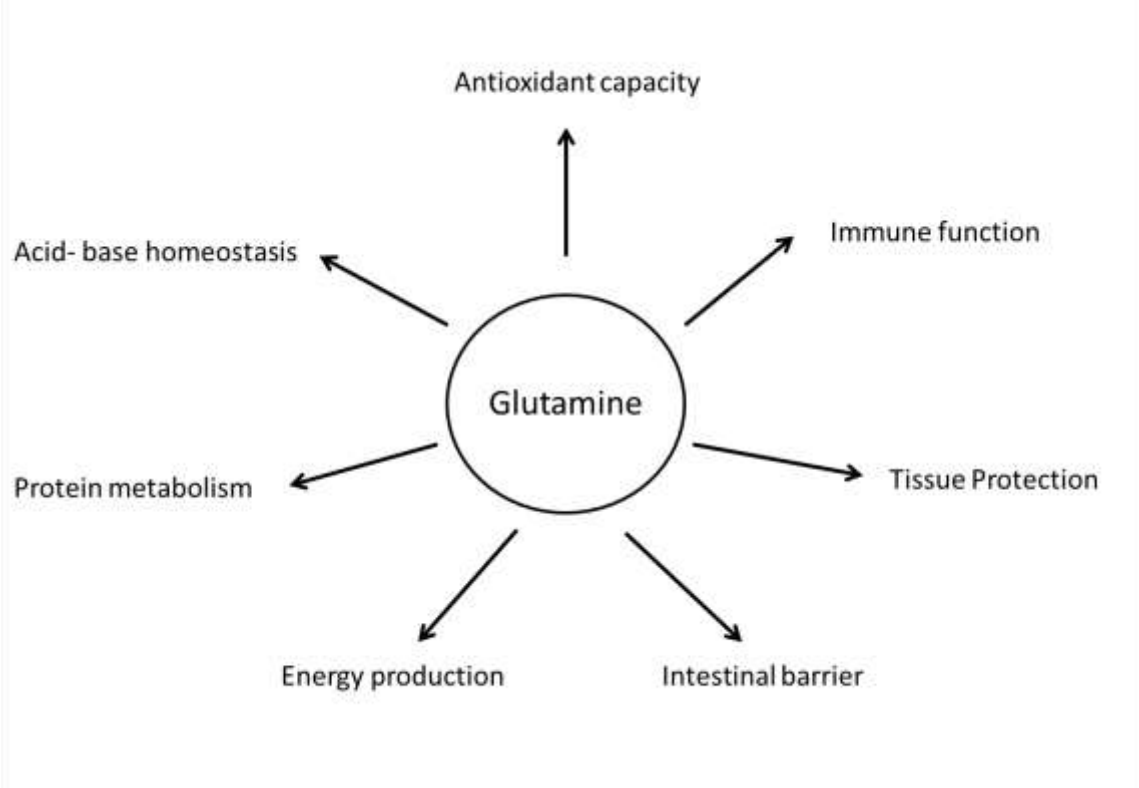


Figure 1: Functions of glutamine (adapted from Mok and Hankard, 2011)[20]

Glutamine is referred to as the universal precursor, in that it is involved in the synthesis not only of peptides and proteins, but also of the neurotransmitter glutamate, hexosamine, purine and pyrimidine, and therefore nucleic acid and nucleotides,[23,26] and the amino acid Arginine.[34]

Protein metabolism

Glutamine is considered an important regulator of protein turnover. In addition to providing a nitrogen source for protein synthesis, glutamine serves as a major transporter of nitrogen between tissues – from

Stellenbosch University https://scholar.sun.ac.za

skeletal muscle in particular to the intestine, kidney, neurons, immune cells, and liver.[35] Unlike other amino acids, glutamine is considered a non-toxic carrier of nitrogen.[2] During periods of stress when glutamine is utilised for purposes other than protein synthesis, a significant amount of ammonia is released during its degradation. This ammonia is, however, immediately scavenged by organs before being able to enter the circulation and cause toxicity.[26]

Glutamine, along with other amino acids, is also thought to inhibit protein degradation in organs such as skeletal muscle, liver, and the intestine by directly influencing the autophagic pathway of proteolysis.[20] Suggested mechanisms for this, as seen in rat models, include intracellular signalling by amino acid receptors, activation of the mTOR (mammalian target of rapamycin) pathway,[36,37] which suppresses autophagic proteolysis through the attenuation of MAPK (Mitogen-Activated Protein Kinases) phosphorylation, and the increase in Heat Shock Protein 70 (HSP70).[20] The inhibitory effect that glutamine has on proteolysis may also be related to its effect on cellular hydration, as protein synthesis is influenced by the degree to which cells shrink or swell. By regulating osmotic swelling in cells, anabolic signals are sent – as opposed to catabolic signals that occur during cell shrinkage.[2,38] In short, glutamine has a protein-sparing effect.

Glucose metabolism

The carbon skeleton of glutamine is an important substrate for gluconeogenesis in the liver, intestine, and kidney. During gluconeogenesis, non-carbohydrate derived carbon substrates are used to produce glucose. This is a major regulator of blood glucose concentration.[2]

During critical illness when hyperglycaemia and insulin resistance are common, glutamine is also thought to influence glucose metabolism by upregulating insulin sensitivity,[39] and by acting as a signalling molecule for insulin secretion.[25,40]

Energy production

Figure 2 (below) illustrates the major pathways of energy production that glutamine is involved in.[41] Once in the mitochondria, glutamine is catabolised by the enzyme glutaminase into glutamate – a reaction that also releases ammonia.[29] Glutamate (and by association glutamine) plays an important role in the Tricarboxcylic Acid (TCA) cycle by serving as the major source of the intermediate ά-ketoglutarate. The TCA or Krebs cycle plays a central role in cellular metabolism, by contributing to energy production.[41] Glutamate is converted into ά-ketoglutarate through transamination or through deamination by glutamate dehydrogenase. Where glucose supply is sufficient, transamination usually occurs. However, during

Stellenbosch University https://scholar.sun.ac.za

physiological conditions in which glucose is limited, the deamination pathway becomes the major route for glutamine to enter the TCA cycle.[33]

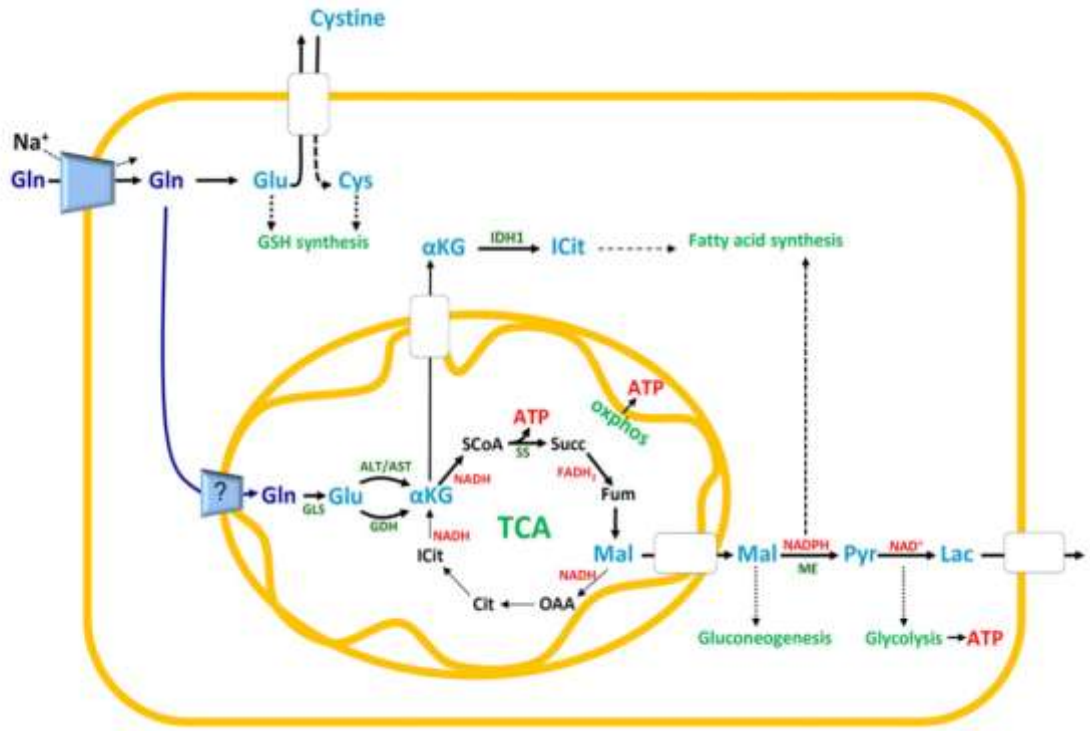


Figure 2: Role of glutamine in energy production[41]

ATP: Adenosine Triphosphate; AST: Alanine Amino Transferase; AST: Aspartate Aminotransferase; Cit: Citrate; Cys: Cysteine; Fum: Fumarate; GLS: Glutaminase; gln: Glutamine; Glu: Glutamate; GDH: Glutamate Dehydrogenase; Icit: Isocitrate; IDH1: Isocitrate Dehydrogenase; KG: Ketoglutarate; Lac: Lactate; Mal: Malate; ME: Malic Enzyme; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; OAA: Oxaloacetate; Pyr: Pyruvate; SCoA: Coenzyme A; Succ: Succinate; SS: SuccinylCoA Synthetase.

Glutamine-derived ά-ketoglutarate serves multiple purposes – first as a major source of energy in the TCA cycle (Figure 2 above). This is primarily achieved by the production of ATP via Succinyl CoA synthetase. In rapidly dividing cells, ATP can also be produced by the export of malate into the cytosol. In a series of reactions, malate is converted into pyruvate and then into lactate with the resulting release of ATP by glycolysis. The entry of glutamine-derived glutamate into the TCA cycle also plays a role in the production of NADPH (Nicotinamide Adenine Dinucleotide Phosphate) from malate, as seen in Figure 2.[41] NADPH increases antioxidant capacity by reducing glutathione from its oxidised state (discussed below), and is necessary for cell proliferation.[35] Thirdly, ά-ketoglutarate from glutamine takes part in anaplerotic reactions that replenish TCA cycle intermediates – particularly in rapidly dividing cells. In such tissues, intermediates exit the TCA cycle at various stages. In combination with amino acids released from peripheral tissues into the circulation, these intermediates produce ATP, nucleotides, phospholipids, and sterols – thereby supporting rapid proliferation of cells and contributing towards metabolic homeostasis.[26]

Stellenbosch University https://scholar.sun.ac.za

Gut health and the intestinal barrier

One of the most well-documented functions of glutamine is its role as a major oxidative fuel and nucleotide substrate for rapidly proliferating cells, particularly those of the gastrointestinal tract and immune system.[42] Enterocytes in the gastrointestinal tract, the small bowel in particular, are the major consumers of glutamine, utilising an estimated 30% of the body's total pool.[43] Most enteral glutamine is metabolised within the intestine itself, with only 25% being transported to other organs.[43,44]

Glutamine is thought to influence enterocyte proliferation through the activation of MAPK[45] and by enhancing the effect of several growth factors.[46] By providing a fuel source for intestinal and gut-associated lymphoid tissue (GALT), glutamine is thought to contribute to the integrity of the intestinal barrier.[42]

Glutamine is also a precursor for hexosamines, which are important in the prevention of bacterial translocation by means of surface mucin and tight intracellular junctions.[42] Tight junctions are comprised of various proteins that act as a barrier, regulating nutrient uptake and preventing harmful pathogens from entering the lumen of the gut.[43] Glutamine is thought to influence the expression of these proteins – thus further maintaining the intestinal barrier.[47,48]

Through its role in antioxidant defence and tissue protection (discussed below), glutamine also offers intestinal cells protection from stress by inhibiting the pro-inflammatory response, reducing apoptosis and by enhancing autophagy.[43]

Antioxidant capacity

Glutamine influences antioxidant defence by serving as a precursor to glutamate, which in turn is used to synthesise the major antioxidant glutathione.[20] Glutathione is present in both reduced and oxidised forms, the ratio of which determines the redox capacity of the cell. In addition to antioxidant functioning, glutathione also regulates immune function, the production of cytokines, cell division and apoptosis, genetic expression, and Deoxyribonucleic Acid (DNA) synthesis.[49]

In vivo experiments have demonstrated a protective effect of the reduced form of glutathione against oxidative stress in the heart,[50] lung, and gut tissues of rats.[51] In humans, glutathione depletion in the skeletal muscle of surgical patients has been prevented by glutamine supplementation. In addition, liver damage during neonatal sepsis has shown to be attenuated by the synthesis of glutathione.[20] These, along

Stellenbosch University https://scholar.sun.ac.za

with other short-term studies, have demonstrated the potential role of glutamine in ameliorating oxidative stress - making this an important avenue for future study.[2]

Immune function

Glutamine is an important substrate for macrophages and lymphocytes. By providing fuel for these cells, glutamine influences immune function.[33] Glutamine is also required for purine and pyrimidine synthesis, providing important building blocks once immune cells are activated.[25] In addition, glutamine is also required for the expression of certain cell surface markers, which are also necessary for the activation of the immune response.[52]

The amino acid has also been shown to modulate cytokine production, thereby proffering anti-inflammatory effects. The mechanism for this is thought to be due to reduced activation of Nuclear Factor-$_{k}$B.[53] This transcription factor is involved in both innate and adaptive immunity and the inflammatory response through expression of genes that encode cytokines.[54]

Tissue protection

The anti-inflammatory effects of glutamine may also be the result of elevated expression of Heat Shock Proteins (HSPs). HSPs or stress proteins are produced by cells in response to physiological stress.[25] Certain HSPs act as molecular chaperones that work to refold or repair damaged proteins or to denature proteins that are irreparably damaged – thereby maintaining cell function.[20] HSPs have been classified into groups: HSP27, HSP40, HSP60, HSP70, HSP90, and HSP110. Glutamine is thought to enhance the expression of HSP25, HSP70, and HSP72.[21] Of these, HSP70 has been most studied and is thought to influence the role that glutamine plays in tissue protection and in the mitigation of pro-inflammatory cytokine release. HSP70 is also thought to supress apoptosis.[53] During states of glutamine depletion, such as during critical illness, the expression of HSP70 is impaired. Glutamine-depleted cells with reduced HSP70 and antioxidant activity have been shown to be more susceptible to apoptosis.[20] Conversely, up-regulation of HSP70 in rat models[21] and adult burn victims has also been linked to reduced mortality.[55] Thus, interest in the role of HSP70 during critical illness has grown.

In animal models, glutamine has been shown to further diminish the inflammatory response by controlling the activity of nitric oxide synthase in organs such as the heart and lungs.[53] One hypothesis for this mechanism is in glutamine's capacity as precursor to Arginine, which is able to enhance nitric acid production.[20,34] However, in human models, the increase in nitric oxide following glutamine administration during pro-inflammatory states has not been consistently demonstrated.[56]

Stellenbosch University https://scholar.sun.ac.za

Acid-base homeostasis

Because glutamine is a non-toxic transporter of ammonia, it plays a fundamental role in maintaining pH homeostasis through its metabolism in the kidney.[20,33] During periods of acidosis (either metabolic or respiratory), glutamine uptake by the kidney increases. Enzymatic cleaving of glutamine by glutaminases releases ammonia from the amide and amino nitrogens.[2] Ammonia enters the renal tubule lumen where it combines with free hydrogen (H^+) ions to form ammonium, which is then excreted in the urine. The acid-base balance is therefore maintained.[33]

Glutamine in adult critical illness

Glutamine plays an essential role in producing the substrates necessary for cellular homeostasis and survival.[26] As such, it has been widely identified as a key nutrient in the physiological response to injury.[57] The amino acid is thought to attenuate inflammation, offer protection from tissue injury, and preserve metabolic function during periods of stress.[25] A shortage of glutamine would imply that the host response to critical illness may be compromised.[26]

Glutamine: conditionally essential during critical illness

In light of the body's ability to produce glutamine, it has traditionally been regarded as a non-essential amino acid. During critical illness however, it is considered conditionally essential - as endogenous consumption by the gut, immune cells, kidney, and liver may exceed its rate of synthesis.[25]

Glutamine in the plasma and muscle during critical illness

— *Prevalence of glutamine depletion*

Both plasma and tissue glutamine concentrations have been shown to decrease during critical illness.[26] In mixed intensive care unit (ICU) populations, plasma glutamine depletion has been observed on admission, with a prevalence of 31%,[24] 38%,[58] 44%,[59] and as high as 65%[60] reported among individual study cohorts. More specifically, reduced plasma glutamine among surgical patients has been reported by several authors. A study on well-nourished patients undergoing colorectal surgery demonstrated a reduction of plasma glutamine from 625 ± 22 umol/l prior to surgery to 431 ± 17 umol/l post-operatively, and of muscle glutamine from 13.2 ± 1.4 mmol/l to 9.6 ± 2.0 mmol/l.[61] Glutamine concentration in muscle tissue has also been shown to reduce significantly within the first 24 hours after elective abdominal surgery.[62] More recently, Viggiano and colleagues confirmed previous findings by showing post-operative reductions in plasma glutamine concentration - more so after major surgery.[63,64]

Stellenbosch University https://scholar.sun.ac.za

Recent data suggest that plasma glutamine depletion is more commonplace among non-elective medical admissions.[58,60] Patients with severe pancreatitis showed a drop in plasma glutamine to less than half that of control subjects, and a reduction in muscle glutamine to less than 20% when compared to controls.[65] Low plasma glutamine has also been demonstrated among patients with trauma,[66] burns,[67] and malnutrition,[68] and has been shown to remain low for several days following major surgery[64] and burns.[67] Reduced glutamine concentration in skeletal muscle[69] and plasma[24,58] have also been reported among septic patients.

— *Elevations in plasma glutamine*

Although to a far lesser degree, elevated plasma glutamine concentrations have been demonstrated among certain critically ill patients. The prevalence of this is not consistently reported on, however, one study found that 7% of patients had raised plasma glutamine levels, defined as >930 umol/l.[58] Elevated plasma glutamine with associated increases in ammonia and hepatic encephalopathy have been well documented among patients with acute fulminant liver failure, but are less well described among those with chronic or acute-on-chronic liver disease.[70] More recently, Helling and colleagues studied a cohort of 100 patients with liver failure. The group demonstrated that all subgroups of liver failure, including chronic, acute-on-chronic, acute fulminant, and post-hepatectomy, may be associated with raised plasma glutamine levels.[71]

Patients with chronic renal failure have also been shown to have elevated plasma glutamine levels, as uptake of glutamine by the kidneys is markedly reduced.[72,73] In this instance, raised plasma glutamine has been shown to positively correlate with blood levels of the waste products urea and ammonia. Such alterations in amino acid metabolism during chronic renal disease are not thought to be entirely resolved by dialysis.[72]

Lastly, single case reports of terminal patients with multiple organ failure (MOF) have also documented extremely elevated plasma glutamine levels. Although the mechanism behind this is unclear, reduced cellular integrity is suggested as a contributor.[18] Recently, however, Nienaber and colleagues demonstrated low plasma glutamine concentrations in 75% of patients with MOF.[58] Thus, both low and high concentrations have been observed in this group.

Plasma glutamine and outcome during critical illness

Several observational studies have provided valuable data on plasma glutamine concentration during critical illness and have established a relationship between plasma glutamine, markers of clinical condition, and clinical outcome. Table 1 (below) provides a summary of this research.

Stellenbosch University https://scholar.sun.ac.za

In their prospective cohort study, Oudemans-van Straaten and colleagues made the first link between plasma glutamine levels on admission to ICU and clinical outcome. Plasma glutamine concentrations among non-elective adult patients on admission to the ICU were measured on admission, and were subsequently categorised as low (<420 umol/l) and normal to high (≥420 umol/l). Measures of illness severity, predicted mortality, and hospital mortality were compared between groups. Low plasma glutamine was demonstrated in approximately one-third of critically ill patients, and a significant association between low plasma glutamine and hospital mortality was found. Age and shock as the primary diagnosis were shown to be predictors of low plasma glutamine. Trends toward associations between low plasma glutamine and low albumin, higher severity of illness scores and mortality predictions, were shown. However, these were non-significant. Importantly, this study was the first to demonstrate an unfavourable outcome associated to low plasma glutamine.[24]

An observational study conducted in 2012 by Rodas et al. went on to describe plasma glutamine in a mixed cohort of critically ill adult patients on admission to ICU, in relation to clinical outcome. Plasma glutamine concentrations were analysed in relation to predictors of mortality and actual mortality. Forty four percent of patients were reported to have low plasma glutamine on admission. The study corroborated Oudemans-van Straaten's findings by identifying plasma glutamine of <420µmol/l as an independent risk factor for post-ICU mortality. Increased mortality was also demonstrated among patients with circulating glutamine of >930µmol/l - suggesting that both low and high concentrations are associated with adverse effect. Rodas described this relationship between plasma glutamine and mortality as a U-shaped curve.[59]

In 2016, Nienaber and colleagues, in two mixed ICU settings, explored plasma glutamine concentration on admission in relation to markers of infection, gender, and diagnosis. This study was the first of its kind to take place in South Africa, and sought to identify biomarkers that could be used as a proxy for plasma glutamine depletion. Plasma glutamine was shown to be normal among most patients. Depletion was reported among 38% of cases - a figure comparable to previous findings.[24,58] This study was useful in that it reported on elevated plasma concentrations, which were found in 7% of patients. In addition, an important distinction was made between medical and surgical patients; the former demonstrating significantly lower plasma glutamine concentrations. A relationship with the infectious marker C-Reactive Protein (CRP) was established, and a critical CRP cut-off was identified, above which plasma glutamine was low. It was suggested that this be investigated further as a potential proxy for plasma glutamine, to be used in combination with a holistic assessment of individual patients.[58]

In the same year, Buter and colleagues investigated differences in plasma glutamine concentrations between elective and non-elective admissions from a cohort of mixed ICU patients. The study was unique in that plasma glutamine was measured on admission and daily throughout ICU stay. Sixty five percent of

Stellenbosch University https://scholar.sun.ac.za

patients admitted to the ICU had low plasma concentrations, which is the highest reported prevalence of glutamine depletion to date. Plasma glutamine concentrations among non-elective patients on admission and on day one were found to be significantly lower when compared to elective patients, and appeared to remain lower throughout the course of ICU stay. Links between plasma glutamine, severity of disease, and the presence of infection were also made.[60]

Another study worth mentioning (not included in Table 1, below) is that of Perez-Barcena and colleagues. This randomised, double-blinded, multi-centre trial investigated the efficacy of supplemental parenteral infusions among trauma patients, but was unique in that it measured plasma glutamine on admission, on day six of ICU stay, and also investigated plasma glutamine in relation to clinical outcome. The study found that 60% of trauma patients were glutamine deplete on presentation to ICU, and 48% remained so six days later, despite supplementation. Low plasma glutamine on day six was associated with increased infection, and longer ICU and hospital stay.[66]

The early observational work by Oudemans-van Straaten et al.[24] and Rodas et al.,[59] which identified low plasma glutamine as an independent risk factor for mortality, led to the growing premise that many critically ill patients are glutamine deplete and require supplementation. In essence, these studies built the rationale for glutamine supplementation during critical illness.[18]

Stellenbosch University https://scholar.sun.ac.za

Table 1: Observational research on plasma glutamine in critically ill adults

Reference	Design	Subjects	Observation	Outcomes	Plasma glutamine- related results
Oudemans-van Straaten et al. (2001) PG depletion and patient outcome in acute ICU admissions	Single centre prospective cohort study	80 non-elective ICU patients	PG* concentration was measured within 24 hours of admission and was dichotomised as low (<420umol/l) and normal to high (≥420umol/l)	PG in relation to hospital mortality, severity of disease, predicted mortality, and standardised mortality ratio (using APACHE II, SAPS II, MPM 0 and 24)	1. Mean PG was 523umol/l (range 220-1780umol/l) 2. Low plasma glutamine was identified in 31% of patients and was associated with higher hospital mortality (p = 0.01), age (p = 0.03), and the diagnosis of shock 3. Trends existed for associations between low PG and lower albumin and higher severity of disease and predicted mortality 4. High PG was associated with increased CK
Rodas et al. (2012) Glutamine and glutathione at ICU admission related to outcome	Single centre prospective cohort study	174 mixed ICU patients, excluding thoracic, neurosurgery, and trauma	PG and glutathione concentration were measured within 48 hours of admission. PG was dichotomised as <420umol/l and ≥420umol/l	PG and glutathione in relation to ICU and post ICU all-cause 6-month mortality and predictors of mortality (APACHE II, SOFA)	1. 44% of patients had low PG on admission 2. Both low (<420umol/l) and high (>930umol/l) PG were found to be independent risk factors for post ICU all-cause 6-month mortality (p = 0.029 and p = 0.043 respectively) 3. Patients with PG <420umol/l had higher mortality compared to those with PG > 420umol/l (p = 0.037) 4. PG was unrelated to mortality risk scoring and LOS
Buter et al. (2016) Plasma glutamine levels in patients after non-elective or elective ICU admission: an observational study	Single centre observational study	178 mixed ICU patients, including elective (n = 88) and non-elective (n = 90) admissions	PG concentration was measured on admission and daily throughout ICU stay	PG in elective versus non-elective patients, PG change over time, PG in relation to severity of disease (APACHE IV) and prevalence of ICU infections	1. 65% of patients were glutamine deplete on admission (34% of elective and 74% of non-elective admissions) 2. PG on admission was significantly different between elective (median 430umol/l [IQR 330-550umol/l]) and non-elective admissions (median 250umol/l [IQR 90-370umol/l]), p <0.001 3. PG remained higher in elective patients over the course of ICU stay 4. A positive correlation between PG and APACHE IV was shown (p <0.001) 5. PG was significantly lower among those with infection (p <0.001)

Stellenbosch University https://scholar.sun.ac.za

Nienaber et al. (2016) Prevalence of glutamine deficiency in ICU patients: a cross-sectional analytical study	Multi-centre cross-sectional analytical study	60 mixed ICU patients	PG concentration was measured on admission. Low PG defined as <420umol/l, and high PG as >930umol/l	PG in relation to infectious markers (Il-6 and CRP), gender, and diagnosis	1. Median PG on admission was normal at 497umol/l (IQR 387-644umol/l) 2. 38% of patients had low PG and 7% had high PG on admission 3. PG was unrelated to gender 4. A significant difference in PG between medical (median 475umol/l [IQR 372-627umol/l]) and surgical patients (median 515umol/l [IQR 468-782umol/l]) was shown (p = 0.042) 5. PG was inversely correlated to IL-6 (p <0.05) and CRP (p = 0.08) 6. A CRP cut off of 95.5mg/l was identified, above which PG was low

APACHE II: Acute Physiology and Chronic Health Evaluation score; CK: Creatine Phosphokinase; CRP: C Reactive Protein; ICU: Intensive Care Unit; Il-6: Interleukin-6; LOS: Length of Stay; MPM: Mortality Probability Model; PG: Plasma Glutamine; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment
** PG measured as the sum of plasma glutamine and glutamic acid*

Stellenbosch University https://scholar.sun.ac.za

Glutamine supplementation

History

Glutamine supplementation was first studied in the 1980s. Several randomised controlled trials (RCTs) administered parenteral glutamine as a single amino acid or as the more stable dipeptide at doses of 10-12 g/day, with the goal of establishing if this prevented depletion during critical illness, and/or had any bearing on clinical outcome.[8,9,74] These studies precipitated many more, which seemed to confirm a beneficial effect of supplemental glutamine via the parenteral route and indicated amelioration of plasma glutamine depletion during the diseased state.[75] Improvements in intestinal barrier function and GALT, as well as enhanced production of short life proteins required during metabolic stress were shown.[15] Furthermore, reductions in infectious morbidity,[10,75,76] mortality,[10,25,76] and ICU[10] and hospital stay[76] were demonstrated by meta-analyses among patients who received glutamine supplementation.

Enteral versus parenteral glutamine

Clinical benefit has been attributed largely to parenteral provision of glutamine.[25,57,77] Although l- glutamine is omitted from standard parenteral solutions due to poor solubility and degradation during heating (affecting the stability of the solution), dipeptides containing glutamine offer a more soluble and stable form of the amino acid, which can be administered parenterally.[11]

Administration of glutamine via the enteral route has produced less convincing results in most patient groups, with meta-analyses showing no obvious clinical advantage.[75,78] Some evidence does, however, support enteral supplementation. Reduced ICU and hospital length of stay (LOS) have been shown among burns and mixed ICU patients receiving glutamine via the enteral route, and reduced mortality has also been shown among burns patients.[12] This may be explained by the fact that enteral functioning is to some degree preserved in these patients when compared to other critically ill patients with sepsis or shock, so allowing for absorption of the amino acid.[26] The notion that gastrointestinal availability of glutamine influences efficacy of supplementation is further supported by the fact that enteral administration of glutamine has been shown to only marginally increase plasma glutamine levels in most critically ill patients, whereas parenteral supply results in an immediate increase in plasma concentration.[18]

International guidelines and dosage

On the back of such promising clinical results, glutamine supplementation among critically ill adults was until recently considered safe and prudent practice - used as a means to meet increased demand for the amino acid during hyper-catabolism. This was reflected in several international guidelines recommending

Stellenbosch University https://scholar.sun.ac.za

routine supplementation. In 2009, the American Society of Parenteral and Enteral Nutrition (ASPEN) recommended a routine enteral dose of 0.3-0.5 g/kg/day of glutamine for all burns, trauma, and mixed ICU patients. This was largely based on the nutrient's trophic effect on the gut and the consequent maintenance of the intestinal barrier.[12]

In the same year, the European Society for Clinical Nutrition and Metabolism (ESPEN) recommended intravenous doses of 0.2-0.4 g/kg/day l-glutamine (0.3-0.6 g/kg/day of glutamine containing dipeptide) for all critically ill adults receiving parenteral nutrition - suggesting that this become the standard of care. This guideline made reference to extensive evidence garnered over the previous decade demonstrating clinical benefit from parenteral provision of glutamine, and noted that no harmful effect had been shown to date.[11]

Based on data demonstrating elevations in plasma glutamine among certain subgroups of the critically ill, both international guidelines[11,12] and the manufacturer of parenteral glutamine dipeptides do not recommend supplementation for patients with renal or hepatic insufficiency.[18]

In their review of 36 clinical trials, Kim and Wischmeyer also provided guidance on the dosage of glutamine, suggesting that optimal clinical benefit is associated with doses of 0.35-0.5 g/kg. In addition, the pair recommended that glutamine be supplemented within the first 48 hours of ICU admission, and continued for a minimum of five days.[25]

SIGNET (Scottish Intensive care Glutamine or seleNium Evaluative Trial)

A study conducted in 2011 by Andrews and colleagues cast some doubt on the routine use of parenteral glutamine supplementation. This double-blinded RCT aimed to investigate the effect of parenteral glutamine and selenium supplementation on infection and mortality among critically ill adult patients. Ten critical care units in Scotland participated in the study, including a total of 502 patients, all whom required parenteral nutrition. Participants were randomised to receive 20.2 g glutamine per day or 500 ug selenium per day, or both, for a period of up to seven days. The study showed no significant effect of glutamine supplementation on infection rate and on six-month mortality. In addition, no effect was demonstrated on ICU or hospital LOS, duration of antibiotic use, and on the modified SOFA (Sequential Organ Failure Assessment) scores.[79] This study was, however, subsequently criticised for the low dose of glutamine prescribed, and for not reporting on the dosage actually received by participants. The short duration of the treatment period was also seen as a shortcoming of the study.[18]

Stellenbosch University https://scholar.sun.ac.za

MetaPlus study

In their double-blinded RCT, van Zanten et al. went on to investigate the effect of immune modulating nutrients among a group of ventilated critically ill adults. Three hundred and one patients across 14 centres were randomised to receive a high protein enteral feed enriched with glutamine, Omega 3 fatty acids, and antioxidants, or an isocaloric high protein feed. Baseline plasma glutamine was measured and was found to be low among both the intervention (365 ± 161 umol/l) and control groups (365 ± 136 umol/l), with no between group differences identified. Among the intervention group, average glutamine intake was 0.28 g/kg, lower than the recommended dose. The study found no significant differences between the incidence of infection, infectious subtypes, SOFA scores, ICU and hospital LOS, and the duration of mechanical ventilation. A significant increase in six-month mortality was, however, shown in the medical subgroup of the intervention arm, inferring a harmful effect of high protein immune-modulating enteral nutrition containing a relatively low dose of glutamine. These findings once again suggested a cautious approach to glutamine supplementation.[80] However, in their post-hoc safety analysis, Hofman et al. concluded that the harmful effect observed in the MetaPlus study was mediated by an increase in the ratio of Omega 3 to long chain fatty acids, and was unrelated to glutamine.[81]

REDOXS (Reducing Deaths due to Oxidative Stress) trial

Routine glutamine supplementation was further challenged by the REDOXS trial. This multi-centre, double-blinded RCT reported increased in-hospital and six-month mortality among 1218 critically ill adult patients receiving supra-physiological doses of glutamine. Patients were randomised on admission to receive enteral and parenteral doses of glutamine (0.6-0.8 g/kg/day), antioxidant supplementation, a combination of both, or a placebo.[13]

This trial differed from previous research by administering the highest dose of glutamine (>0.5 g/kg/day) to date to a particularly inclusive group of patients. Usually excluded from glutamine trials for concern over toxicity, patients with acute liver and renal failure, many of whom were in shock, were included in this cohort.[19] In fact, one-third of study participants had renal dysfunction. This contravened current clinical guidelines on the use of glutamine as a supplement in terms of both dosage and patient profile.[15] The intervention was also the first to supplement enteral and parenteral glutamine simultaneously.[17] Based on the blinding and randomisation procedures, the international, multi-centre nature of the trial, large sample size, and the intention-to-treat analysis, the external validity of these findings are considered high.[13] Because of its novel approach, however, the trial could not negate previous findings of benefit associated with glutamine supplementation, but did raise questions over the safety of this practice.[15]

Stellenbosch University https://scholar.sun.ac.za

— *Criticism of the REDOXS study*

The REDOXS trial was based on the assumption that all critically ill patients are glutamine deplete.[14] In reality, plasma glutamine was not consistently low in a small subset of patients (n = 66) at baseline and at days four and seven. In fact, 15% of these patients demonstrated high plasma glutamine concentrations.[13] Furthermore, no information regarding clinical outcome was published for this subgroup for whom plasma glutamine was measured.[18] Arguably, the exploratory dose-finding study conducted prior to the trial would have been an opportune time to investigate plasma glutamine in this population to inform the subsequent intervention.

— *Post REDOXS uncertainty*

Post REDOXS, significant uncertainty over the use of routine supplementation arose among clinicians worldwide.[15] Questions surrounding pharmacological versus nutritional dosage of glutamine, enteral versus parenteral administration versus both, and over the supplementation of patients with MOF versus those with moderate risk of mortality, have been asked.[18]

Post REDOXS meta-analyses

In order to clarify the sudden controversy surrounding glutamine supplementation, three noteworthy meta-analyses were conducted between 2013 and 2014. In 2013, Bollhalder and colleagues[77] included 40 RCTs in their analysis, which involved 3107 critically ill and post-surgical patients. The analysis included the SIGNET study of Andrews et al.,[79] but excluded the REDOXS study of Heyland and colleagues.[13] The analysis found a significantly lower infection rate and hospital LOS among patients supplemented with parenteral glutamine. When studies which supplemented >0.2 g/kg/day glutamine (in keeping with clinical guidelines on the use of intravenous glutamine) were extracted, the authors also found a significant reduction in short-term mortality among all patient groups.[77]

In 2014, Wischmeyer et al. performed a meta-analysis of RCTs conducted between 1997 and 2013, which focused on parenteral glutamine supplementation as part of nutritional support. Twenty six trials (excluding the REDOXS study), equating to 2484 critically ill patients, were included. Meta-analysis found significant improvements in hospital mortality and LOS, with a strong trend towards reduced infectious complications, length of ICU stay, and overall mortality among patients receiving parenteral glutamine supplementation. The group concluded that parenteral glutamine supplementation, when given alongside adequate nutrition support, is associated with significant clinical benefit.[17]

Stellenbosch University https://scholar.sun.ac.za

In their Cochrane review, Tao and colleagues sought to assess the effect of glutamine supplementation among critically ill adult patients. Data from 53 trials including 4671 patients were analysed. The group concluded that there is moderate evidence to support reduced infection and length of time spent on mechanical ventilation as a result of glutamine supplementation. Low quality evidence for glutamine supplementation reducing length of hospital stay was reported, and no conclusive evidence was found to suggest that glutamine supplementation influences mortality or ICU LOS.[82]

More recently, in 2017, Stehle and colleagues conducted a meta-analysis with the goal of resolving the controversy surrounding glutamine supplementation. The authors criticised previous meta-analyses given their inclusion criteria, arguing that studies using free glutamine and those administering the more stable dipeptide should not be included in the same analysis due to discrepancies in kinetics, and therefore in dosage. In addition, the heterogeneity of patients included in two of the most recent meta-analyses was also questioned.[15] Bollhalder et al.[77] and Tao et al.[82] included both critically ill and post-surgical patients. This, Stehle and colleagues argued, may have introduced bias into their results regarding clinical outcome. In order to overcome these shortfalls, the group conducted an analysis using strict eligibility criteria for RCT inclusion. Only studies testing clinical outcome among patients without renal or hepatic failure, who were haemodynamically and metabolically stable, were included. In addition, only studies using supplemental glutamine dipeptide at doses based on current guidelines (0.3-0.5 g/kg/day, at less than 30% of prescribed nitrogen intake) via the parenteral route, in combination with the provision of adequate nutrition, were considered. Thus, both the REDOXS and SIGNET RCTs were excluded from this meta-analysis.[15]

Fifteen RCTs met the above inclusion criteria - involving a total of 842 patients. Meta-analysis revealed significant reductions in infectious complications, duration of mechanical ventilation, hospital and ICU LOS, and in-hospital mortality among patients supplemented with glutamine dipeptide via the parenteral route. No difference in ICU mortality was demonstrated between groups.[15]

Despite these meta-analyses, questions still remain over the use of glutamine supplementation in critically ill adult patients. In his 2015 commentary, Wernerman pointed out several reasons why meta-analyses have been unable to provide clarity on the subject. The first problem is that patients who are glutamine deplete have not been fully investigated. As such, the mechanism of hypoglutaminaemia and its association with mortality is not properly understood. Without a clear picture of this mechanism, meta-analyses cannot provide definitive answers. Heterogeneous patient groups and between study differences in dosage, supplementation period, route of administration, and the combination of nutrients supplemented, also make comparison very difficult.[16]

Stellenbosch University https://scholar.sun.ac.za

Werneman also made the point that a clear distinction should be made between supplementation and repletion in intervention studies. The former implies that patients with normal plasma concentrations are supplemented with glutamine, and the latter that patients who are glutamine deplete receive the nutrient.[16] Failure to take heed of this important distinction may account for the adverse findings of recent interventions[16] - particularly given the link that established between elevated plasma glutamine and mortality.[59] In short: a lack of understanding of the mechanisms of potential harm and benefit of glutamine, and of which patients require it and why, have made interventions up until this point potentially hazardous.

Understanding plasma glutamine

Wernerman argued that post REDOXS, it is clear that when given in pharmacological doses to a heterogeneous group of patients with a high risk of mortality, who are receiving inadequate nutrition, glutamine supplementation may well be harmful. However, he suggested that a targeted approach to supplementation may still be warranted given the wealth of evidence to support this, but stressed that basic research to gain a deeper understanding of patients' plasma glutamine concentration is required, before supplementation can be considered.[18]

Surprisingly few studies have investigated plasma glutamine as an indicator for supplementation, with only two interventions having measured plasma glutamine at baseline.[66,80] The unexpected finding by Rodas and colleagues[59] that both low and high plasma glutamine concentrations may be associated with increased mortality prompted calls to review current supplementation practices, and highlighted the need for a greater understanding of glutamine status within specific patient groups.[19] As has rightly been pointed out, it would not be ethical to conduct intervention studies going forward without first monitoring and understanding plasma concentrations.[18]

Glutamine in children

There is limited evidence for the use of glutamine supplementation in paediatrics. Even less data is available on plasma glutamine concentrations within this group. As such, the role of glutamine in children requires further scrutiny.[20] In addition to the physical demands of growth, development, and illness, hospitalised children are at increased risk of infection and malnutrition.[83] Focused research within this vulnerable group is therefore important.

Stellenbosch University https://scholar.sun.ac.za

Glutamine supplementation in children

The systematic review conducted in 2011 by Mok and Hankard provides the most comprehensive overview of clinical studies examining the effects of enteral and parenteral glutamine supplementation among infants and children.[20] Although clinical benefits have been reported in certain subgroups of children receiving glutamine, results have thus far been inconsistent.[84]

Several RCTs investigating glutamine supplementation in premature or low birth weight infants have reported reduced incidence of hospital-acquired sepsis and atopic dermatitis.[20] However, repeated meta-analyses have, to date, failed to demonstrate consistent clinical benefit within this population.[85–88] More recently, the effect of enteral glutamine supplementation on brain growth and outcome within a cohort of 102 preterm infants (<32 weeks gestation) was studied. The group found significantly fewer neonatal infections, as well as increased head circumference in the first year of life among those who received glutamine.[89] Within the same cohort, longitudinal benefit was also demonstrated among infants receiving glutamine. Significant increases in structural volume of the brain stem, white matter, and the hippocampus were shown at eight years of age; an effect which was thought to be mediated by fewer infections during the neonatal period. Interestingly, no differences in cognitive and motor functioning or behavoiur were demontrated between groups at school age.[90] Because glutamine has the potential to benefit many facets of neonatal care - including growth and development, sepsis and atopy prevention, and gastrointestinal integrity - its role in preterm nutrition requires further attention.

Few high-quality trials exist for specific groups of older infants and children. The use of glutamine following gastrointestinal surgery has garnered interest as a means to promote intestinal adaptation. However, meta-analysis of two small RCTs involving infants with gastro-intestinal disease found insufficient evidence to confirm clinical benefit.[91] Although several case-series reports have indicated improvements in growth,[92] intestinal absorption,[92,93] and stool frequency[92] among patients with short bowel syndrome (SBS), firm conclusions cannot be drawn without more rigorous research in this area. Glutamine supplementation among paediatric patients with Crohn's Disease has also been investigated as a means to attenuate catabolism and induce remission.[20] However, reports from an RCT conducted on 15 children with active Crohn's disease found no benefit in remission rates, intestinal permeability, and micronutrient profiles. In fact, patients who were supplemented with glutamine demonstrated poorer outcomes with regards to the Crohn's disease activity index[94] and nutritional status.[94–96] This RCT was one of two studies included in a recent Cochrane review, which concluded that insufficient evidence exists to support glutamine supplementation as a means to induce remission among patients with Crohn's disease.[97]

Stellenbosch University https://scholar.sun.ac.za

Results from RCTs are also conflicting with regard to enteral glutamine supplementation during diarrhoeal disease and malnutrition, with some trials demonstrating shorter duration of diarrhoea[98] and improvements in intestinal barrier function.[99] More recently, oral glutamine supplementation during diarrhoeal disease was re-examined in an RCT of 138 Ugandan children aged two to 60 months admitted to hospital for persistent diarrhoea. No improvement in outcome was demonstrated among participants who received supplementation.[100]

Although an *in vitro* study has demonstrated improved immune capability (bactericidal function) of glutamine-exposed neutrophils isolated from paediatric patients post burn injury,[101] conclusive evidence from human trials for the use of glutamine in burns is lacking.[102] In a small double-blinded, randomised crossover trial, Sheridan and colleagues measured plasma glutamine in children (n = 7) with severe burns, following 48-72 hours of enteral glutamine supplementation. Plasma glutamine increased moderately but non-significantly with glutamine supplementation (600 ± 0.0 umol/l) when compared to an isonitrogenous enteral control (400 ± 0.0 umol/l), and whole body protein sparing was not demonstrated.[103] This is the only RCT to have assessed glutamine supplementation in children with burn injuries to date.[104]

Promising results have been shown for oral glutamine supplementation in oncology, with several RCTs reporting reduced severity and duration of oral mucositis in children undergoing chemotherapy,[105] bone marrow,[106] or stem cell transplantation.[107] Other benefits attributed to glutamine supplementation include reductions in parenteral nutrition usage and associated costs, and also in opiate use.[20] Although significant benefit has not been consistently demonstrated across all available studies, enteral glutamine doses of up to 0.65 g/kg/day have been reported to be safe and well tolerated among paediatric oncology patients.[108]

— *Limitations of existing research*

Definitive answers regarding the use of glutamine in children have been undermined by a simple lack of research as well as by methodological limitations within existing studies.[84] For example, despite adopting rigorous experimental designs, most trials - by means of sample size - were underpowered to detect subtle differences in clinical outcome.[20] In trials where clinical outcome was the primary endpoint, this may have compromised internal validity. In addition, limited sample size would have threatened the external validity of this research.[109]

Reduced statistical power would also have increased the likelihood of Type II error, which raises concerns over the safety and therefore the ethics of these interventions.[110] As yet, no adverse effects on morbidity or mortality have been associated with glutamine supplementation in children; however, these may have been overlooked by Type II error.[111] This issue of safety was raised by Heyland and Dhaliwal, who

Stellenbosch University https://scholar.sun.ac.za

acknowledged that the dose-finding trial conducted prior to the REDOXS study was underpowered to detect changes in mortality.[14] This held serious consequences for the intervention which followed.

Meta-analysis of RCTs is made difficult by heterogeneous cohorts and practical differences in the use of glutamine. Studies on glutamine supplementation in trauma[112] and burns[113] patients included mixed samples of children and adults. In addition, those involving infants undergoing gastrointestinal surgery included a variety of diagnoses - thereby limiting the application of findings to specific age or patient groups.[114] The route of administration and the relative dosages of glutamine have varied markedly between interventions, with the duration of supplementation ranging from two[103] to seven days[113] among burn and trauma patients, to one to two years in SBS.[93] This makes it difficult to separate the effects of dose and time on clinical outcome. In many cases, glutamine was administered in combination with other immune-modulating nutrients, making it difficult to isolate the effects of the single nutrient.[112,113]

As a result of such disparity, the role of glutamine in paediatrics remains undefined. This, combined with newfound concerns over safety, suggest that the glutamine status of children must be further understood before large-scale interventions can be ethically and safely undertaken.[20]

Plasma glutamine in children

— *Data from intervention studies*

Data on circulating levels of plasma glutamine in paediatric populations is scarce, with few interventions having described plasma glutamine levels prior to supplementation.

While the work done in 2004 by Sheridan and colleagues provided useful provisional data on plasma glutamine levels in children with burns, patients were recruited well into their hospital stay - meaning that plasma levels were not reflective of glutamine status during acute injury.[103] Although crossover designs (in which patients form their own control) reduce the effect of confounding variables, this study's external validity was limited by its small sample size.[109] The crossover design also raises the possibility of a carry-over effect, whereby glutamine administered in the intervention phase may have exerted an effect in the control phase. Conversely, the short intervention phase may have been insufficient to prompt a significant plasma glutamine response.[20] Both possibilities question the internal validity of the study.

In their intervention study, Chaloupecky et al. investigated the plasma amino acid profiles of infants following cardiac surgery. Patients were randomised to receive glucose intravenous support, or parenteral nutrition containing amino acids. Plasma glutamine was low (287 ± 86.4 umol/l and 278.3 ± 95.5 umol/l) in

Stellenbosch University https://scholar.sun.ac.za

both intravenous fluid and parenteral groups respectively on day two post-surgery. Interestingly, plasma glutamine concentration had normalised by day seven post-operatively in patients from both groups.[115]

— *Data from observational studies*

Observational studies, although few in number, have provided valuable information on plasma glutamine concentrations among critically ill children. A summary of the observational studies in paediatrics is provided in Table 2 (below).

The early observational study by Villares et al. investigated the amino acid profile (inclusive of glutamine) of post-operative patients with congenital heart disease. Plasma aminogram analyses were conducted prior to surgery, and on day one, three, and seven post-operatively. On average, plasma amino acids were found to be normal at baseline, significantly reduced after surgery, and slowly normalised over time. Plasma glutamine was found to be low on day one and three post-operatively. Glutamine was, however, unique in that it was the only amino acid to remain low on day seven - demonstrating a much slower recovery.[116]

The clinical audit conducted by Marino and colleagues was one of the few studies to have described plasma glutamine (in relation to HSP70) in a specific group of critically ill children, without the provision of glutamine. Data on disease severity and clinical outcome were collected retrospectively from the records of children admitted to intensive care with acute meningococcal disease, and from patients post-discharge. Glutamine concentrations were determined using stored plasma samples. The audit demonstrated significantly lower plasma glutamine during the acute phase of illness when compared to convalescence. Inverse relationships were found between plasma glutamine and markers of inflammation, LOS, and clinical outcome, suggesting, as in adult studies, an association between low plasma glutamine and disease severity.[21]

Ekmark and colleagues went on to investigate plasma glutamine concentrations in patients admitted to a Paediatric Intensive Care Unit (PICU) in relation to clinical outcome. This study found that 40% of patients were glutamine deplete when compared to healthy controls. In addition, low plasma glutamine on admission to PICU was significantly associated with the development of MOF. Because mortality in the paediatric population is low, MOF was used in this study as a proxy for clinical outcome using the PELOD (Paediatric Logistic Organ Dysfunction) score - calculated on admission and again on day five of PICU stay. Interestingly, plasma glutamine concentrations were found to normalise in most patients who stayed longer than five days in the intensive care unit.[22]

These studies established an important precedent for future research by describing plasma glutamine in paediatric patients without intervention. Their demonstration of significant associations between plasma

Stellenbosch University https://scholar.sun.ac.za

glutamine and clinical outcome has highlighted the need for similar observational research among children in different contexts.

Motivation for the current study

Glutamine is considered a conditionally essential amino acid during periods of critical illness. As such, supplementation of the nutrient has become commonplace among adult ICU populations. Recent concern over the safety of glutamine supplementation has, however, highlighted the need for focused research on plasma glutamine levels in specific patient groups.

There is limited evidence for the use of glutamine supplementation in children, with even less data available on plasma concentrations within this group. Two observational studies have, however, demonstrated associations between low plasma glutamine and increased inflammatory markers, length of hospital stay,[21] and incidence of MOF, suggesting that, as in adults, plasma glutamine may be a useful marker of clinical outcome among critically ill children.[22] A limitation within existing research is the different time points at which plasma glutamine has been measured. While most studies have investigated plasma glutamine on admission to PICU,[21,22] repeat measurements have been conducted on day three[116] or five[22] of hospital stay, or later during convalescence[21]. As a result very little is known about the degree to which plasma glutamine changes, particularly within the acute stage of critical illness. It is clear that the glutamine status of critically ill children must be further understood - in terms of plasma concentration and the degree of change which occurs within a specific time period - before intervention studies can be undertaken. Exploratory studies such as this one provide the unique opportunity to generate new knowledge within a field in which very little is known.

Stellenbosch University https://scholar.sun.ac.za

Table 2: Observational research on plasma glutamine in critically ill children

Reference	Design	Subjects	Observation	Outcomes	Plasma glutamine-related results
Villares et al. (2008) Plasma aminogram in infants operated on complex congenital heart disease	Prospective study	55 children < 3 years old with CHD admitted for elective surgery	Amino acid concentrations were measured at baseline, on day 1, 3, and 7 post- operatively	Plasma amino acid concentration at each time point in relation to nutritional status and biochemical indices	1. Plasma amino acid concentrations were normal at baseline 2. Concentrations of all amino acids decreased significantly after surgery ($p < 0.05$) with a slow trend towards normalisation 3. On day 3, glutamine, isoleucine, alanine, arginine, and threonine remained low ($p < 0.05$) 4. Glutamine was the only amino acid to remain significantly low a week post-surgery 5. Nutritional status and amino acid concentration were unrelated
Marino et al. (2014) Glutamine depletion and heat shock protein 70 in children with meningococcal disease	Clinical audit	143 infants and children diagnosed with meningococcal disease on admission to PICU, 73 convalescent children post discharge, 35 healthy controls	Stored plasma was used to measure PG*, HSP70, and the inflammatory mediators Il-6, Il-10, and TNF ά	PG and HSP70 at each time point and in relation to each other, clinical indices, inflammatory mediators, mortality risk (PRISM), glutamine intake, clinical outcome	1. PG was significantly lower on admission (mean 310umol/l ± 130umol/l) compared to convalescence (mean 400umol/l ± 140umol/l), $p < 0.001$ 2. PG was 52 % and 26% below the normal range on admission and during convalescence respectively 3. PG was inversely related to PICU LOS ($p < 0.001$), duration of ventilation ($p < 0.001$), lactate ($p < 0.001$), and CRP ($p < 0.001$) 4. PG was positively correlated to glutamine intake ($p = 0.023$)
Ekmark et al. (2015) Plasma glutamine deficiency is associated with multiple organ failure in critically ill children	Observational study	149 infants and children admitted consecutively to a mixed medical and surgical (excluding cardiac) PICU, 60 healthy controls	PG and PELOD were measured within 48 hours of admission and on day 5 of PICU stay	PG concentration at each time point and in relation to PELOD score	1. Median PG on admission was 446umol/l (IQR 323-556umol/l) which was lower than healthy controls ($p < 0.001$) 2. 40% of patients were glutamine deplete on admission 3. Low PG was associated to the development of MOF ($p = 0.001$) 4. For most children PG normalised by day 5 5. Patients with low PG tended to have a longer PICU stay

*HSP70: Heat Shock Protein 70; Il-6: Interleukin-6; Il-10: Interleukin-10; LOS: Length of Stay; MOF: Multiple Organ Failure; PG: Plasma Glutamine; PRISM: Paediatric Risk of Mortality; TNF ά: Tumour Necrosis Factor ά *PG was reported as the sum of PG and glutamic acid with a normal cut off of ≥600umol/l*

Stellenbosch University https://scholar.sun.ac.za

3. Methods

Research question

In light of clear gaps in existing knowledge regarding plasma glutamine in children, and considering the need for exploratory research within this group, the following question was raised:

What is the plasma glutamine concentration of children on admission to PICU and during the first two days of PICU stay?

Aim

To investigate plasma glutamine concentration among patients on admission to PICU and on day two of PICU stay - in relation to clinical condition and outcome.

Objectives

1. Describe plasma amino acid concentrations, inclusive of glutamine, on admission to PICU and on day two of PICU stay.
2. Identify associations between plasma glutamine concentration and clinical condition on admission to PICU, nutritional status, and nutritional intake.
3. Identify associations between admission plasma glutamine concentration and PICU and hospital LOS.
4. Identify associations between plasma glutamine concentration and mortality.

Null hypotheses

H_{01} = Plasma amino acid concentrations do not change during the first two days of PICU stay.

H_{02} = No relationship exists between plasma glutamine concentration and clinical condition on admission to PICU.

H_{03} = No relationship exists between plasma glutamine concentration and the nutritional status (determined by age-related Z scores) of children in PICU

H_{04} = No relationship exists between plasma glutamine concentration and dietary protein or energy intake during the first two days of PICU stay.

H_{05} = No relationship exists between plasma glutamine concentration and PICU or hospital LOS.

H_{06} = No relationship exists between plasma glutamine and mortality.

Stellenbosch University https://scholar.sun.ac.za

Study design and method

A descriptive, cross-sectional study was carried out.

Target population

Due to the cross-sectional nature of the study, all patients admitted to the Red Cross War Memorial Children's Hospital (RCWMCH) PICU between 06 November and 05 December 2016, were eligible for inclusion in the study.

RCWMCH is a tertiary centre that provides intensive care to infants and children from within the public healthcare sector in Western Cape Province, South Africa. It also accepts patients requiring specialist services from further afield in South Africa and neighbouring African countries.[117]

RCWMCH's PICU admits medical and surgical patients aged 0-18 years, most of whom are under the age of two years. At the time of study, the unit had a capacity of 22 beds, and admitted an average of 80-100 patients per month, with an average LOS of three and a half days.

Selection criteria

Inclusion criteria

All patients admitted to RCWMCH PICU between 06 November and 05 December 2016 were recruited, provided that:

- they were below the age of 18 years
- written informed consent was obtained from a parent or legal guardian within 48 hours of admission
- blood sampling was possible within 24 hours of admission

Exclusion criteria

Patients were excluded from the study if:

- they were 18 years or above
- written informed consent was not obtained from a parent or legal guardian within 48 hours of admission
- routine phlebotomy was not required during PICU admission
- blood sampling was not possible within 24 hours of admission
- they had a known haemoglobin level of below 7 g/dl at the time of blood sampling

Stellenbosch University https://scholar.sun.ac.za

- they were deemed too unstable for additional blood sampling by the medical team (any patient could be excluded from the study at the discretion of the attending clinician)

Sampling strategy

Due to the cross-sectional nature of the study, consecutive sampling was used to recruit participants over a one-month period. During the planning phase of the study, sample size was pragmatically estimated at 75-100 participants. This decision was based on logistical considerations such as the data collection period, unit occupancy, and on cost – with the expense of plasma amino acid analysis proving to be a limiting factor.

Data collection

A standard data collection sheet (Addenda C) was used to collect data. Data were collected on admission to PICU (referred to for the purposes of this study as day zero) and again 48 hours after admission (during day two of PICU stay), as well as at PICU and hospital discharge. Table 3 (below) indicates the data that were collected at each time point:

Table 3: Data collection at each time point

	Data Collected	PICU Day 0	PICU Day 2	PICU Discharge	Hospital Discharge	Data Source
Admission data	PICU admission date	✓				Medical file
Demographics	Age (years, months)	✓				Medical file
	Gender	✓				
Diagnosis and illness severity	Diagnostic category	✓				Medical file
	PIM 3 score	✓				
	HIV/TB status	✓				
Anthropometry	Weight (kg)	first	available	opportunity		Clinical examination by dietitian
	Length/ height (cm)					
	MUAC (cm)					
Biochemistry	Plasma aminogram	✓	✓			Laboratory
	Albumin (g/l)	✓				Medical file
	CRP (mg/l)	✓				
	PCT (ng/ml)	✓				
	Lactate (mmol/l)	✓				
	Glucose (mmol/l)	✓				
Nutrition	Type and route of feed		✓			Medical file
	Volume of feed per day		✓			
Clinical outcome	Ventilation (days)			✓		Medical file
	Length of PICU stay (hours)			✓		
	Length of hospital stay (days)				✓	
	Mortality			✓	✓	

CRP: C Reactive Protein; HIV: Human Immunodeficiency Virus; MUAC: Mid Upper Arm Circumference; PIM3: Paediatric Index of Mortality; PCT: Procalcitonin; TB: Tuberculosis

Stellenbosch University https://scholar.sun.ac.za

Measurements

— *Clinical condition on admission*

Diagnosis, disease severity, presence of infectious disease, and routine biochemistry were used as measures of clinical condition on admission to PICU. In order to strengthen the data analysis, diagnoses were categorised as medical, surgical (elective or emergency), or trauma – and further sub-categorised according to primary diagnosis. This was based on a combination of criteria outlined by Heyland and colleagues[13] in 2013, and on unit-specific patient profiles (as seen in Addenda C).

Human immunodeficiency virus (HIV) and tuberculosis (TB) status were noted due to the region's high prevalence of infectious disease among young children.[118,119] Paediatric Index of Mortality 3 (PIM 3) is a widely accepted indicator of disease severity and mortality risk, and is part of routine practice in the unit.[120] A previous version of this tool had previously been validated in the RCWMCH setting.[117] As such, PIM 3 scores were used as a marker for illness severity in this study, from which the probability of mortality was calculated.

— *Anthropometry*

Anthropometry was, where possible, conducted prior to PICU discharge. When this was not feasible, convalescent measurements were taken at a ward level before hospital discharge. Where oedema was present, anthropometry measurements were delayed until this had resolved.

Weight, length or height, and mid upper arm circumference (MUAC) were used to assess nutritional status. All anthropometry was conducted according to international standards using calibrated equipment.[121] A non-stretch measuring tape was used to determine MUAC in infants and children aged six months or above. Naked weight was measured using an electronic baby scale for patients younger than two years, and an electronic scale was used for older children in light clothing. Recumbent length was measured for patients less than two years old using a portable length mat, and where possible standing heights were measured for older children using a stadiometer.

Weight, length, and height measurements were plotted on World Health Organization (WHO) growth charts and interpreted according to standard deviation (Z) scores. Scores of <-2 or >+2 were used to indicate under- or over-nutrition respectively.[122] Preterm infants <37 weeks gestation were excluded from nutritional status analyses, so as not to skew the data. Proportionality was assessed using weight-for-length and body mass index (BMI)-for-age charts for those aged five years or below and those older than five years respectively.

Stellenbosch University https://scholar.sun.ac.za

For infants and children aged six months to five years, MUAC was interpreted using WHO standards, with measurements of <11.5 cm indicating severe acute malnutrition, and ≥11.5 - <12.5 cm indicating moderate acute malnutrition.[122] For older children, MUAC was classified according to Mramba and colleagues' growth curves, which were developed in accordance with WHO standards.[123] When both were available, MUAC and weight-for-length or BMI-for-age categories were compared, and the most unfavourable classification chosen to describe each participant's acute nutritional status.

— *Routine biochemistry*

According to PICU protocol, routine biochemical analyses are, for most patients, performed on admission. Following this, daily samples are collected at 05:00 – and for those who require it, additional samples are drawn later in the day. All phlebotomy is conducted by medical doctors.

For this study, biochemical results from admission and on day two of PICU stay were collected. Where available, albumin, Procalcitonin (PCT) and CRP were recorded as markers of inflammation,[124] and lactate as an indicator of inadequate tissue oxygenation and damage.[125]

— *Plasma amino acid analysis*

At the time of routine phlebotomy, on admission to PICU, an additional 500 ul (0.5 ml) of whole blood was drawn for amino acid analysis. A second blood sample of 500 ul (0.5 ml) was drawn for amino acid analysis during the first set of routine bloods conducted 48 hours after admission. This took place on day two of PICU stay. A total volume of 1000 ul (1.0 ml) additional blood was therefore required per patient for research purposes. This volume complied with the Stellenbosch University Health Research Ethics Committee guidelines for paediatric blood-draw volumes for research purposes at a single time point.[126] Patients were excluded from further blood sampling if their haemoglobin was known to be below 7.0 g/dl at the time of blood draw. In addition, the medical team was encouraged to exclude patients deemed to be clinically unfit for additional blood sampling.

Where possible, samples were drawn using an existing cannula, and were collected in heparinised vials, centrifuged within 30-60 minutes, and stored in the laboratory at -80°C, pending batch analysis. Once all samples had been collected, blood was analysed by the National Health Laboratory Service (NHLS) at RCWMCH Hospital, using gas chromatography.

Total plasma glutamine was expressed independently, as well as by the sum of plasma glutamine and glutamic acid, in order to account for the breakdown of glutamine to glutamic acid during processing.[21]

Stellenbosch University https://scholar.sun.ac.za

Plasma glutamine of <420 umol/l was categorised as low, and >930 umol/l as high, as referenced in the current literature.[24,59] Age-dependent reference ranges described by Blau et al. were also used to categorise plasma glutamine concentrations.[127] Combined values of plasma glutamine and glutamic acid were not categorised due to the absence of established reference ranges.

In order for plasma glutamine to be analysed, a full plasma aminogram was conducted. As a result, baseline and day two values of all amino acids were reported in this study as a matter of interest.

As per laboratory protocol, blood samples were stored on site at -20°C for two months following analysis. At this time, the samples were dispatched to and destroyed by the external contractor - BCI Medical Waste Management.

— *Nutritional intake*

Feed type, route, and volume were recorded for the first 48 hours of PICU stay (from admission until the end of day one). This was done in order to reflect the intake of participants in the period leading up to day two plasma glutamine sampling. From this data the average daily energy and protein intake were calculated, compared to individual nutritional requirements, and expressed as a percentage thereof.

Breastfeeding infants were excluded from dietary analysis, as daily feed volume was unquantifiable. For participants who received food, meal content was analysed using RCWMCH food service menus, as well as food composition databases from the United States Department of Agriculture (USDA)[128] and the South African Medical Research Council (SAMRC).[129]

Nutritional requirements for participants, from birth at term to 18 years old, were calculated using ASPEN guidelines on the provision of nutritional support to critically ill infants and children.[130] Energy and protein requirements for preterm infants were calculated using recommendations established by the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)[131] and by Uauy and Koletzko.[132] The energy requirements for participants admitted with burns were calculated using an average of two well-established diagnosis-specific formulae from Mayes and the WHO (the latter is routinely doubled in the case of burns).[133,134] Estimated protein requirements for these participants were based on the work of McCarthy.[135]

— *Measures of clinical outcome*

Common indicators of clinical outcome in critically ill patients were collected, including information on the duration of ventilation, length of PICU and hospital stay, and both in-PICU and in-hospital mortality. For the

Stellenbosch University https://scholar.sun.ac.za

purposes of this study, mechanical ventilation included all modes of respiratory support – excluding high flow and nasal prong oxygen. Participants who died in the PICU or on a ward level were excluded from PICU and hospital LOS analyses respectively.

Data analysis

For the purposes of this study, plasma glutamine on admission and on day two of PICU stay were described and compared. Associations between plasma glutamine and various indicators of clinical condition, nutritional status, nutritional intake, and clinical outcome were investigated. Where applicable, day 0 (admission) and/or day two plasma glutamine concentrations were used for the analysis. When appropriate, the change in plasma concentration that occurred between day 0 and day two (referred for the purposes of this study as 'glutamine change') was also used for analysis.

It has been suggested that plasma glutamine concentration should be reported as the sum of plasma glutamine and glutamic acid.[21,24] For the purposes of this study, all statistical analyses were repeated using day 0 and/or day two plasma glutamine and glutamic acid, and, where appropriate, the change which occurred between admission and day two. The results in this dissertation focus primarily on plasma glutamine, but do report any significant results found using the sum of plasma glutamine and glutamic acid.

Data were entered onto a prepared spread sheet in Microsoft Office Excel 2014. Data analysis was planned by the researcher and was performed with the assistance of a statistician using STATISTICA version 12 software (StatSoft Inc, Tulsa, USA). Counts and percentages were used to describe categorical and ordinal data – and were, where appropriate, represented visually using histograms. Shapiro Wilk or Kolmogrov-Smirnov tests were used to test for normality. Depending on the distribution of numerical data, means ± standard deviation (SD) and medians with interquartile range (IQR) were used as measures of central tendency and spread. Parametric inferential analyses were used when numerical data were normally distributed.

Relationships between continuous variables were investigated using Pearson's or Spearman's correlation, and significant results were represented using scatterplots. Analysis of variance (ANOVA), t-tests or their non-parametric alternatives, were used to compare groups. A p value of <0.05 was used to represent statistical significance and 95% confidence levels were used.

Stellenbosch University https://scholar.sun.ac.za

Ethical considerations

This study adhered to the ethical guidelines outlined by the Declaration on Helsinki.[136] Prior to commencement, ethical approval was granted by the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, University of Stellenbosch (reference S12/05/085), as well as by the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (reference 681/2016). Permission was also sought and obtained from RCWMCH management.

Informed consent and assent

Written informed consent was obtained by the researcher from each participant's parent or legal guardian within 48 hours of admission to PICU. The information discussed with caregivers included details of the study - including deferred consent for blood sampling conducted on admission, assurance of anonymity, expected risk and benefit, and of their right to withdraw at any time. If absent from the ward, caregivers were telephoned for verbal consent, and relevant paperwork was completed on presentation to hospital. If no informed consent had been obtained within the first 48 hours of admission, the patient was excluded from the study – with no further blood samples drawn and no amino acid analyses conducted.

Written informed consent was obtained from caregivers in their choice of English, Afrikaans, or isiXhosa, and a signed copy was provided in this language (Addenda A). Where isiXhosa was preferred, a registered nurse fluent in the language was used as an interpreter. Wherever possible, assent was obtained from children above the age of six years when they were well enough, and this was taken in the language of their choice (Addenda B).

Confidentiality and anonymity

Confidentiality and anonymity were ensured by allocating each patient a unique identification code which was used throughout the study, and also by entering data into a spread sheet accessible to only three members of the research team.

Risk and benefit

Participants and their families did not benefit directly from the study. In order to reduce the risk of discomfort, anthropometrical measures were delayed if the patient was experiencing distress. Where malnutrition was identified, patients were referred to a dietitian for nutritional support.

Drawing blood is an invasive procedure and is therefore an ethical concern. Logistical and ethical details regarding the blood sampling that took place during this study have been discussed above.

Stellenbosch University https://scholar.sun.ac.za

Conflict of interest

Partial external funding was secured from Fresenius Kabi for the biochemical analysis of data. Funds were accepted with the agreement to full disclosure of all research findings. In order to maintain transparency, all sources of funding have been declared.

Stellenbosch University https://scholar.sun.ac.za

4. Results

Demographics

Of the 86 eligible patients, 76 were included in the final study. The reasons for exclusion are indicated in Figure 3 (below). No patient was deemed clinically unfit for inclusion in the study by the attending medical team.

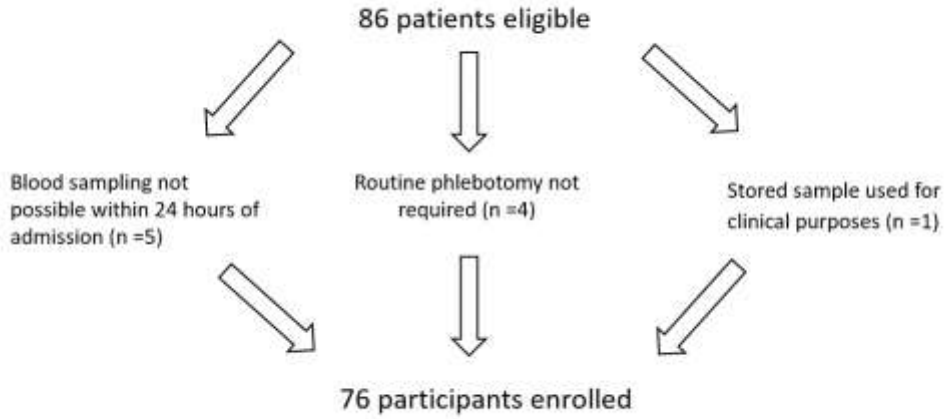


Figure 3: Recruitment of participants

Study participants had a median age of 19 months, ranging from 33 weeks gestational age to 12 years and eight months old. As seen in Table 4 (below), 72% (n = 55) of participants were below the age of five years. Of the eight (10.5%) premature infants included, most were still <37 weeks gestation at the time of study. The sample consisted predominantly of males (n = 54, 71.1%), and most participants were admitted to the PICU from other wards (Table 4).

Stellenbosch University https://scholar.sun.ac.za

Table 4: Demographic information (n = 76)			
		n	%
Gender	Male	54	71.1
	Female	22	28.9
Age	< 1 year	33	43.4
	1–5 years	22	28.9
Preterm	Total	8	10.5
	CGA <37 weeks	5	6.6
	CGA >37 weeks	3	3.9
Admitted from	Ward	45	59.2
	Medical emergency	20	26.3
	Other hospital	11	14.5
Diagnostic category	Surgery-elective	41	53.9
	Surgery-emergency	5	6.6
	Medical	24	31.6
	Trauma	6	7.9
CGA: Corrected Gestational Age			

Clinical information

Diagnostic profile

The diagnostic profile of participants was to a large extent comprised of elective surgery (n =41, 53.9%), followed by medical, trauma, and emergency surgery cases (Table 4).

The most common primary diagnosis was cardiology (n = 36, 47.4%), followed by pulmonology (n = 11, 14.5%), gastroenterology (n = 8, 10.5%), sepsis (n = 4, 5.3%), and burns (n = 3, 3.9%). For statistical purposes, participants admitted for orthopaedic, maxillofacial, oncology, nephrology, malnutrition, neurology, and other reasons, were grouped as 'other' (n = 14, 18.4%), as indicated in Figure 4 (below).

Stellenbosch University https://scholar.sun.ac.za

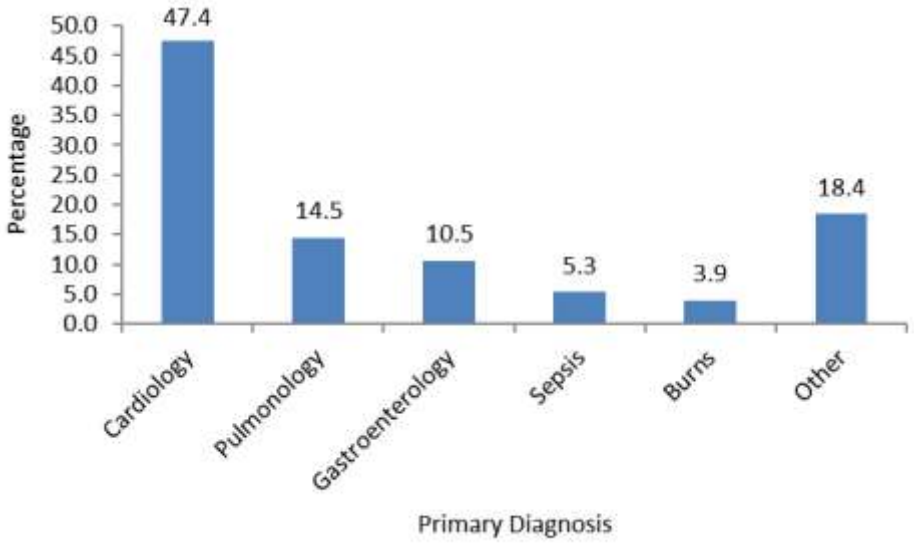


Figure 4: Primary diagnosis of study participants

Severity of disease

Severity of disease and risk of mortality was determined using PIM 3 scores for each patient. The median PIM 3 score was -4.23 (IQR -4.74 - -3.21), equating to a median risk of mortality of 1.4% (IQR 0.8-3.9%).

Infectious disease

Table 5 (below) illustrates the burden of infectious disease among study participants at the start of the study – most of whom were TB- and HIV-negative and HIV unexposed.

Table 5: Burden of infectious disease (n = 76)

		n	%
HIV status	Positive	3	3.9
	Negative	72	94.7
	Unknown	1	1.3
HIV exposure	Exposed	10	13.2
	Unexposed	64	84.2
	Unknown	2	2.6
TB status	Positive	5	6.6
	Negative	71	93.4
	Unknown	0	0.0

HIV: Human Immunodeficiency Virus; TB: Tuberculosis

Stellenbosch University https://scholar.sun.ac.za

Biochemistry

Routine biochemical parameters of participants on admission are presented in Table 6 (below).

Table 6: Routine biochemistry on admission

	n	Mean	± SD	Min- Max
CRP (mg/l)	17	38.6	± 85.1	0-332.0
PCT (ug/l)	14	14.2	± 17.1	0-61.0
Albumin (g/l)	28	29.3	± 9.0	9.0-44.0
	n	Median	IQR	Min- Max
Lactate (mmol/l)	73	1.6	1.1-3.0	0.7-16.0
Glucose (mmol/l)	73	7.0	5.6-8.4	0.9-20.6
Urea (mmol/l)	76	4.2	3.3-5.7	1.1-34.4
Creatinine (umol/l)	75	31.0	22-43.5	8.0-1039.0
Bilirubin (umol/l)	16	10.5	5.8-45.3	2.0-145.0
Conjugated bilirubin (umol/l)	16	3.5	1.0-11.8	1.0-30.0
ALT (U/l)	16	25.0	17.8-48.0	1.0-30.0
AST (U/l)	16	57.0	37.8-239.3	29.0-562.0
ALP (U/l)	12	187.5	137.3-304	54.0-718.0
GGT (U/l)	10	20.0	17.0-46.5	11.0-168.0
Haemoglobin (g/dl)	73	11.4	9.9-12.7	4.9-20.8
White cell count (x109)	73	13.3	10.4-18.8	2.0-43.5
Platelets (x 109)	73	256.0	164.0-344.0	14.1-80.0

ALT: Alanine Transferase; AST: Aspartate aminotransferase; CRP: C- Reactive Protein; GGT: Gamma-glutamyl transferase; IQR: Interquartile range; PCT: Procalcitonin; Min: Minimum; Max: Maximum; SD: Standard Deviation

Of the 76 participants for whom blood was taken on admission to PICU, 17 had CRP and 14 had PCT measured as markers of infection. Of these, six (35.3%) and 10 (71.4%) had raised CRP and PCT levels respectively. The albumin of 28 participants was measured on admission. Because reference ranges vary per age group, a cut off of ≤20 g/l was used to demonstrate obviously low albumin levels. Five (17.9%) of these participants were shown to have low albumin.

Clinical outcome

Mechanical ventilation

Fifty six participants (74.7%) received ventilation during PICU stay, for a median duration of 23.7 hours (IQR 0-78 hours). One patient required 514 hours of ventilatory support.

Length of stay

Mean LOS in the PICU was 144.3 ± 239.7 hours, equating to six days. Several patients who spent less than 24 hours in the PICU were, however, excluded from the study as blood sampling was not possible during

Stellenbosch University https://scholar.sun.ac.za

this time. Median length of hospital stay was 10 days (IQR 7-20 days), ranging from a minimum of two to a maximum of 88 days (n = 68). Data from deceased participants (n = 8, 10.5%) were excluded from LOS calculations.

Mortality

Eight deaths (10.5%) occurred over the duration of the study, seven of which took place in PICU and one in another ward.

Nutritional status

Most participants fell within normal parameters for weight-for-age, length/height-for-age, and weight-for-length/height or BMI, as indicated in Table 7 (below). Thirty seven percent (n = 23) of children were underweight for age, and a further 24.2% (n = 15) were found to be stunted. For infants and children above six months of age, MUAC was also measured, which mostly fell within normal ranges for age (n = 33, 73.3%). According to MUAC measurement alone, 17.8% (n = 8) of participants were moderately wasted, and 8.9% (n = 4) were severely wasted. However, when data on MUAC and weight-for-height were combined, 10 participants (16.1%) were moderately wasted and a further 10 participants (16.1%) were severely wasted – as seen in Table 7. One participant (1.3%) was admitted solely for the treatment of severe acute malnutrition.

Stellenbosch University https://scholar.sun.ac.za

Table 7: Nutritional status of participants on admission		
	n	%
Weight-for-age		
Normal	39	62.9
Moderately underweight	15	24.2
Severely underweight	8	12.9
Length/height-for-age		
Normal	47	75.8
Moderate stunting	8	12.9
Severe stunting	7	11.3
Weight-for-height/ BMI		
Normal	42	67.8
Moderate wasting	10	16.1
Severe wasting	8	12.9
Obese	2	3.2
MUAC		
Normal	33	73.3
Moderate wasting	8	17.8
Severe wasting	4	8.9
Weight-for-height +MUAC		
Normal	40	64.5
Moderate wasting	10	16.1
Severe wasting	10	16.1
Obese	2	3.3
BMI: Body Mass Index; MUAC: Mid Upper Arm Circumference		

Nutritional intake

The nutritional intake of participants was recorded for the first two days of PICU stay, from admission to the end of day one. Data on the nutritional intake of participants on day 0 and day one of PICU stay were available for 75 and 72 participants respectively – the detail of which is presented in Table 8 (below). One third of participants remained nil per mouth during the first 24 hours of admission, with 1.3% (n = 1) having received exclusive parenteral nutrition. During the course of day one, 55% (n = 40) had been transitioned onto exclusive enteral tube feeding, and 34.7% (n = 25) onto oral feeds. Only a small number (n = 4, 5.5%) remained nil per mouth, with three (4.1%) receiving parenteral nutrition. Only one (1.4%) participant received no feeds at all. By the end of day one, 68 participants (94.3%) had received some enteral nutrition. Breast milk in the form of breastfeeding or expressed breast milk was received by 18.1% (n = 13) by the end of day one.

Stellenbosch University https://scholar.sun.ac.za

Table 8: Mode of feeding				
	Day 0 (n = 75)		Day 1 (n = 72)	
	n	%	n	%
Nil per mouth	24	32.0	4	5.5
- NPM on exclusive PN	1	1.3	3	4.1
PN + NGT/NJT	0	0.0	3	4.1
Exclusive NGT/NJT	33	44.0	40	55.5
Oral	18	24.0	25	34.7
Received breast milk	11	14.6	13	18.1

NGT: Nasogastric Tube; NJT: Nasojejunal Tube; NPM: Nil per mouth; PN: Parenteral Nutrition

Of the 60 participants for whom nutritional requirements could be calculated, only 5% (n = 3) met 100% caloric requirements over the first 48 hours of PICU stay (day 0 to end day one), and only 6.7% (n = 4) met protein requirements during this time. Although 16.7% (n = 10) of participants met 66% of energy targets, the vast majority did not come close, with a median achievement of 30.8% (IQR 17.1-53.4%) and 36.2% (IQR 18.1-16.1%) of caloric and protein requirements respectively – as seen in Table 9 (below).

Table 9: Nutritional intake (n = 60)			
		n	%
100% Requirements Met	Energy	3	5
	Protein	3	6.7
66% Requirements Met	Energy	10	16.7
	Protein	14	23.3
		Median	IQR
Average Intake per Day	Energy (kcal)	202.3	67.3 - 378.8
	Protein (g)	5.2	1.7 - 13.7
Average Intake per Kilogram	Energy (kcal/kg)	18.2	9.7 - 31.1
	Protein (g/kg)	0.5	0.3 - 0.9
% Requirements Met	Energy	30.8	17.7 - 53.4
	Protein	36.2	18.1 - 61.1

Plasma glutamine

Baseline blood samples for amino acid analysis were taken from all 76 participants on admission (day 0), and day two samples were drawn from those participants who were still in PICU at this time (n = 40, 52%). Median plasma glutamine on day 0 was 556.5 umol/l (IQR 459.5–664.5 umol/l) and on day two was 529.0 umol/l (IQR 356.0–716.0 umol/l). The medians of the combined concentration of plasma glutamine and glutamic acid were 632 umol/l (IQR 544–784 umol/l) on day 0 and 649 umol/l (IQR 456–831 umol/l) on day two.

Stellenbosch University https://scholar.sun.ac.za

Based on reference ranges used in current literature,[24,59] most participants demonstrated normal plasma glutamine on day 0 (n = 59, 77.6%), with this proportion reducing slightly by day two (n = 27, 67.5%) – as indicated in Figure 5 (below). The percentage of participants demonstrating low plasma glutamine increased over time from day 0 (n =13, 17.1%) to day two (n = 11, 27.5%). However, none of these between-group differences were significant (p = 0.205).

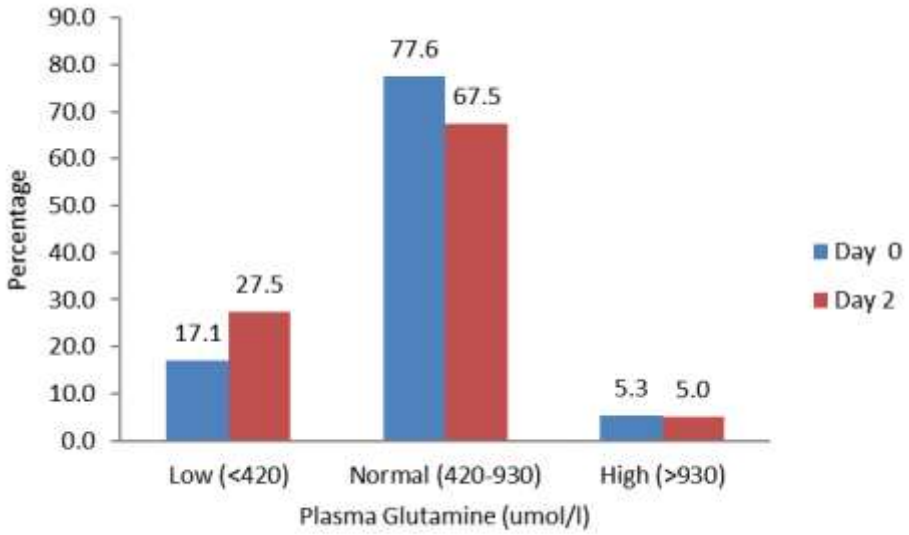


Figure 5: Plasma glutamine on day 0 and day two

When categorised according to NHLS reference ranges,[127] plasma glutamine was again shown to be predominantly normal on day 0 (n = 53, 69.7%) and day two (n = 25, 62.7%) – as seen in Figure 6 (below). Again, no significant differences were shown within the categories of low, normal and high (p = 0.764). A higher proportion of participants was however shown to have high plasma glutamine on both day 0 (n = 12, 15.8%) and day two (n = 8, 20%), when compared to the international reference ranges demonstrated in Figure 5.

Stellenbosch University https://scholar.sun.ac.za

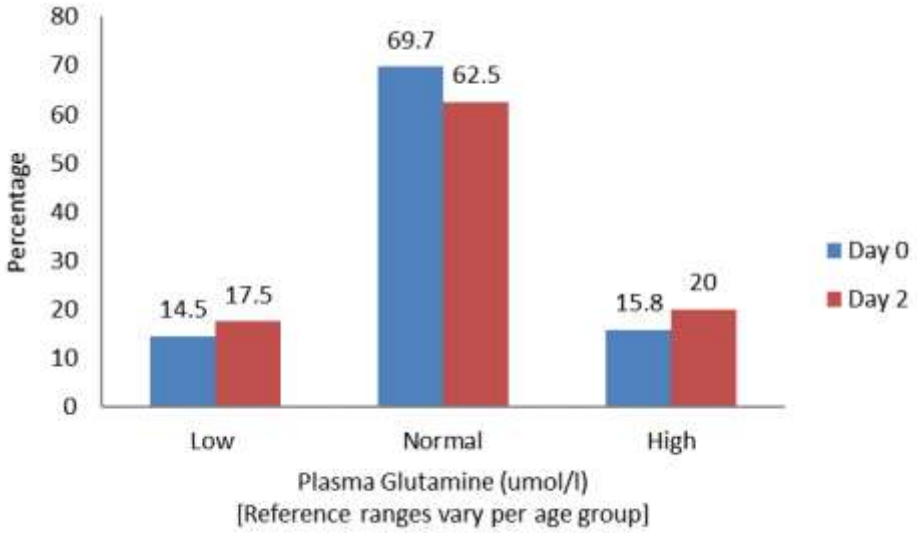


Figure 6: Plasma glutamine on day 0 and day two using laboratory reference ranges

Plasma glutamine over time

ANOVA testing showed no significant difference between plasma glutamine on day 0 and day two of PICU stay ($F(1,39) = 0.21$, $p = 0.886$). A positive relationship was, however, demonstrated between plasma glutamine on day 0 and day two (Spearman $r = 0.38$, $p = 0.001$), indicating an increased likelihood of lower plasma levels on day two if levels were low on admission (Figure 7, below). With outliers removed, this relationship was, however, not significant.

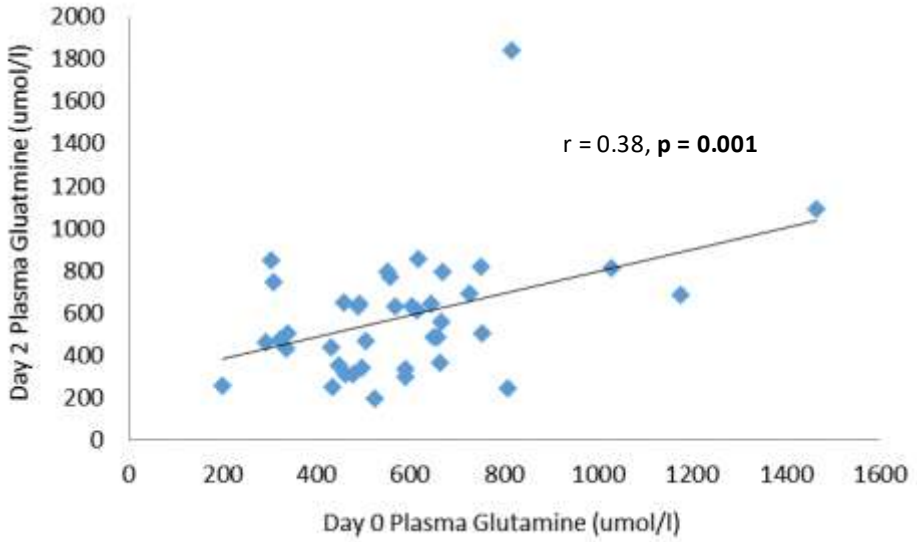


Figure 7: Relationship between day 0 and day two plasma glutamine

Stellenbosch University https://scholar.sun.ac.za

Aminogram results

In order to analyse plasma glutamine concentration, a full aminogram was conducted on each blood sample. Table 10 (below) illustrates the measures of central tendency and spread for each of the remaining amino acids and describes their classification according to NHLS reference values. Significant differences in the plasma concentrations of alanine ($p = 0.011$), cysteine ($p = 0.000$), serine ($p = 0.027$), and tryptophan ($p = 0.009$) were shown between days 0 and two (Table 10, below).

Stellenbosch University https://scholar.sun.ac.za

Table 10: Aminogram description						
Amino acid (umol/l)	Day	Mean/Median	SD/ IQR	Low (n, %)	Normal (n, %)	High (n, %)
Alanine*	0	210.5	150.0 - 308.0	20 (26.3)	45 (59.2)	11 (14.5)
	2	168	122.0 - 220.0	16 (40.0)	21 (52.5)	3 (7.5)
α Aminobutyrate	0	12	9.5 -16.5	21 (27.6)	54 (71.1)	1 (1.3)
	2	13.5	8.5 - 17.5	12 (30.0)	28 (70.0)	0 (0.0)
Asparagine	0	36.5	29.0 - 46.5	14 (18.4)	59 (77.6)	3 (3.9)
	2	44	27.0 - 48.0	10 (25.0)	28 (70.0)	2 (5.0)
Aspartic acid	0	7	4.5 - 8.0	7 (9.2)	66 (86.8)	3 (3.9)
	2	7	5.0 - 9.0	2 (5.0)	36 (90.0)	2 (5.0)
Cysteine*	0	40	± 15.6	23 (30.3)	43 (55.3)	11 (14.5)
	2	25	16.5 - 31.0	22 (55.0)	15 (37.5)	3 (7.5)
Glutamic acid	0	78.5	49.5 - 127.0	2 (2.6)	31 (40.8)	43 (56.6)
	2	78	48.0 - 125.5	3 (7.5)	15 (37.5)	22 (55.0)
Glycine	0	183	139.5 - 240.0	7 (9.2)	59 (77.6)	10 (13.2)
	2	179	134.5 - 256.0	4 (10.0)	30 (75.0)	6 (15.0)
Histidine	0	59	46.0 - 72.5	15 (19.7)	57 (75.0)	4 (5.3)
	2	49.5	37.0 - 60.5	15 (37.5)	21 (52.5)	4 (10.0)
Isoleucine	0	48.5	± 22.8	12 (15.8)	59 (77.6)	5 (6.6)
	2	45	30.5 - 67.5	10 (25.0)	27 (67.5)	3 (7.5)
Leucine	0	102.7	± 42.1	10 (13.2)	59 (77.6)	7 (9.2)
	2	85.5	59.5 - 118.0	5 (12.5)	33 (82.5)	2 (5.0)
Lysine	0	155.5	115.0 - 198.5	5 (6.6)	45 (59.2)	26 (34.2)
	2	197.5	120.0 - 253.5	0 (0.0)	22 (55.0)	18 (45.0)
Methionine	0	11	8.0 - 17.0	35 (46.1)	37 (48.7)	4 (5.3)
	2	14	8.5 - 20.0	14 (35.0)	25 (62.5)	1 (2.5)
Ornithine	0	45	32.0 - 61.5	9 (11.8)	61 (80.3)	6 (7.9)
	2	53	37.0 - 76.0	0 (0.0)	37 (92.5)	3 (7.5)
Phenylalanine	0	73.5	62.5 - 88.0	0 (0.0)	38 (50.0)	38 (50.0)
	2	77	63.0 - 90.0	0 (0.0)	21 (52.5)	19 (47.5)
Proline	0	128	89.0 - 172.0	14 (18.4)	54 (71.1)	8 (10.5)
	2	134	95.5 - 194.0	7 (17.5)	29 (72.5)	4 (10.0)
Serine*	0	98	75.5 - 137.5	15 (19.7)	58 (76.3)	3 (3.9)
	2	129.5	77.5 - 175.5	5 (12.5)	29 (72.5)	6 (15.0)
Threonine	0	73	52.5 - 97.5	16 (21.1)	55 (72.4)	5 (6.6)
	2	86.5	65.5 - 143.5	5 (12.5)	27 (67.5)	8 (20.0)
Tryptophan*	0	29.5	23.0 - 42.0	17 (22.4)	56 (73.7)	3 (3.9)
	2	37	± 18.3	10 (25.0)	26 (65.0)	4 (10.0)
Tyrosine	0	63	49.0 - 83.0	7 (22.4)	58 (76.3)	11 (14.5)
	2	60.5	42.5 - 82.0	3 (7.5)	33 (82.5)	4 (10.0)
Valine	0	140.7	± 47.7	17 (22.4)	58 (76.3)	1 (1.3)
	2	119	87.0 - 117.0	9 (22.5)	28 (70.0)	3 (7.5)

IQR: Interquartile range; SD: Standard deviation

**Significant differences found between day 0 and day two concentrations (alanine: p = 0.011; cysteine: p = 0.000; serine: p = 0.027; tryptophan: p = 0.009).*

Stellenbosch University https://scholar.sun.ac.za

Associations between plasma glutamine and clinical condition on admission

Statistical analyses of the association between clinical condition and plasma glutamine were conducted using both day 0 plasma glutamine levels and the change in plasma glutamine that occurred between admission and day two.

Plasma glutamine and diagnosis

Plasma glutamine levels on admission were shown to differ significantly among diagnostic categories (p = 0.020), as presented in Figure 8 (below). Post hoc testing revealed significant differences between medical and elective surgical participants (p = 0.007) and between medical and trauma participants (p = 0.013).

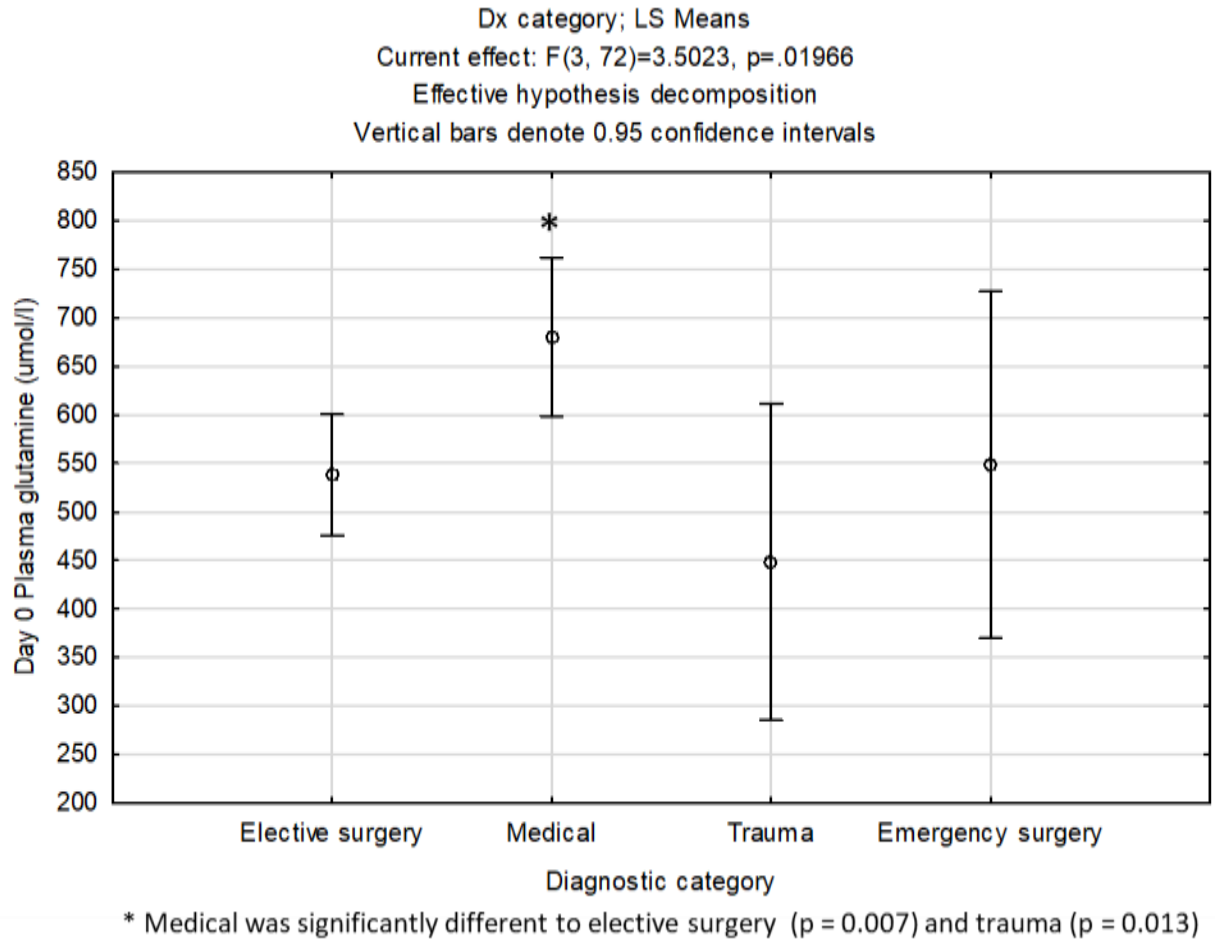


Figure 8: Day 0 plasma glutamine and diagnostic category

Fifty percent of trauma participants (n = 3) presented with a low plasma glutamine concentration on admission. Proportionately, this was followed by surgical (13.7%, n = 7), and medical (12.5%, n = 3) diagnoses. Conversely, 3.9% (n = 2) of surgical patients were admitted with high plasma glutamine,

Stellenbosch University https://scholar.sun.ac.za

compared to 3.7% (n = 1) of medical participants. No high plasma glutamine concentrations were found among participants admitted for trauma.

No significant difference was found between plasma glutamine on day 0 and day two within each individual diagnostic category - as seen in Table 11 (below).

Table 11: Change in plasma glutamine and diagnostic category

	Plasma Glutamine	Mean ± SD (umol/l)	t (df)	p
Elective Surgery	Day 0	529.1 ± 163.8	0.30 (22)	0.766
	Day 2	518.3 ± 194.1		
Medical	Day 0	800.1 ± 317.6	0.31 (9)	0.763
	Day 2	753.3 ± 449.6		
Trauma	Day 0	450.3 ± 166.7	-0.27 (3)	0.814
	Day 2	500.0 ± 269.9		
Emergency Surgery	Day 0	503.7 ± 165.5	-1.36 (2)	0.307
	Day 2	588.0 ± 181.0		

Df: Degrees of freedom; SD: Standard deviation

Plasma glutamine on admission was also not shown to differ significantly between participants admitted under cardiology, pulmonology, gastroenterology and other disciplines, nor did plasma glutamine change significantly over time from day 0 to day two between these groups (Table 12, below). Participants with sepsis demonstrated the highest plasma glutamine on admission, with a mean of 736.3 ± 142.5 umol/l, as well as the most variation in plasma glutamine (mean 284.5 ± 399.5 umol/l) between admission and day two of PICU stay.

Table 12: Plasma glutamine and primary diagnosis

	Day 0 Plasma Glutamine		Plasma Glutamine Change*	
	Mean (umol/l) ± SD	ANOVA	Mean (umol/l) ± SD	ANOVA
Cardiology	621.9 ± 204.5	p = 0.096	38.3 ± 188.6	p = 0.547
Pulmonology	516.7 ± 205.2		24.7 ± 396.4	
Gastroenterology	432.4 ± 149.2		-49.0 ± 138.5	
Sepsis	736.3 ± 142.5		284.5 ± 399.5	
Burns	492.7 ± 175.7		-16.7 ± 467.6	
Other	561.4 ± 238.5		-168.0 ± 545.6	

ANOVA: Analysis of variance; SD: Standard deviation
**Refers to the difference in concentration between day 0 and day 2*

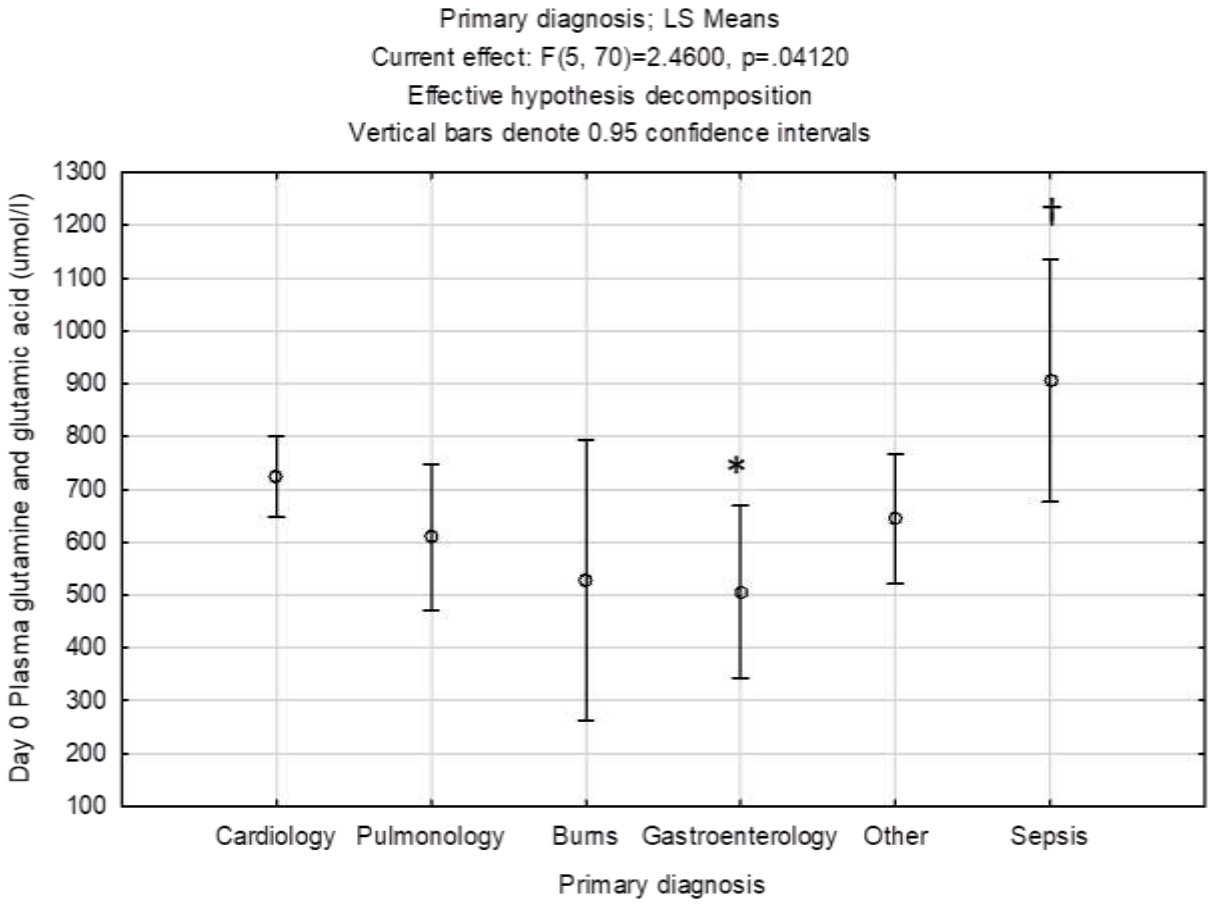
Significant differences were noted between primary diagnoses when day 0 plasma glutamine and glutamic acid values were combined ($F(5,70) = 2.46$, $p = 0.041$). No difference in the degree of change of plasma glutamine and glutamic acid was demonstrated between groups (Table 13, below).

Stellenbosch University https://scholar.sun.ac.za

Table 13: Plasma glutamine and glutamic acid and primary diagnosis				
	Day 0 Plasma Glutamine and Glutamic Acid		Plasma Glutamine and Glutamic Acid Change *	
	Mean (umol/l) ± SD	ANOVA	Mean (umol/l) ± SD	ANOVA
Cardiology	724.4 ± 236.6	**p = 0.041**	-27.6 ± 202.8	p = 0.444
Pulmonology	609.5 ± 222.5		-8.0 ± 430.6	
Gastroenterology	506.2 ± 173.2		55.7 ± 154.6	
Sepsis	906.0 ± 196.5		18.3 ± 506.4	
Burns	528.0 ± 184.0		18.3 ± 506.4	
Other	644.0 ± 259.2		151.0 ± 516.0	

ANOVA: Analysis of variance; SD: Standard deviation
**Refers to the difference in concentration between day 0 and day 2*

Significant differences in day 0 plasma glutamine and glutamic acid were found between cardiology and gastroenterology (p = 0.018), and between sepsis and the following: pulmonology (p = 0.031), burns (p = 0.035), gastroenterology (p = 0.006), and other (p = 0.049) - as shown in Figure 9 (below).



* Gastroenterology was significantly different to cardiology (p = 0.018)
† Sepsis was significantly different to pulmonology (p = 0.031), burns (p = 0.035), gastroenterology (p = 0.006), and other (p = 0.049)

Figure 9: Day 0 plasma glutamine and glutamic acid and primary diagnosis

Stellenbosch University https://scholar.sun.ac.za

Plasma glutamine and severity of disease

As demonstrated in Figure 10 (below), Spearman's correlation showed a trend toward a positive relationship between plasma glutamine on admission and the probability of death – as determined by the PIM 3 score.

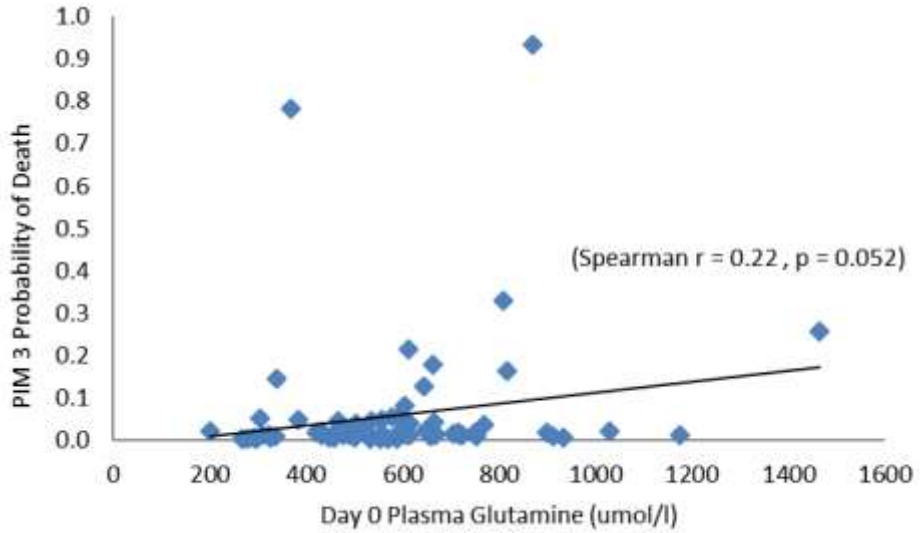


Figure 10: Plasma glutamine on admission and the probability of mortality

The relationship between the probability of death and a combined value of day 0 plasma glutamine and glutamic acid reached significance (r = 0.23, p = 0.046), as demonstrated in Figure 11 (below). The relationship was, however, not significant when outliers were removed.

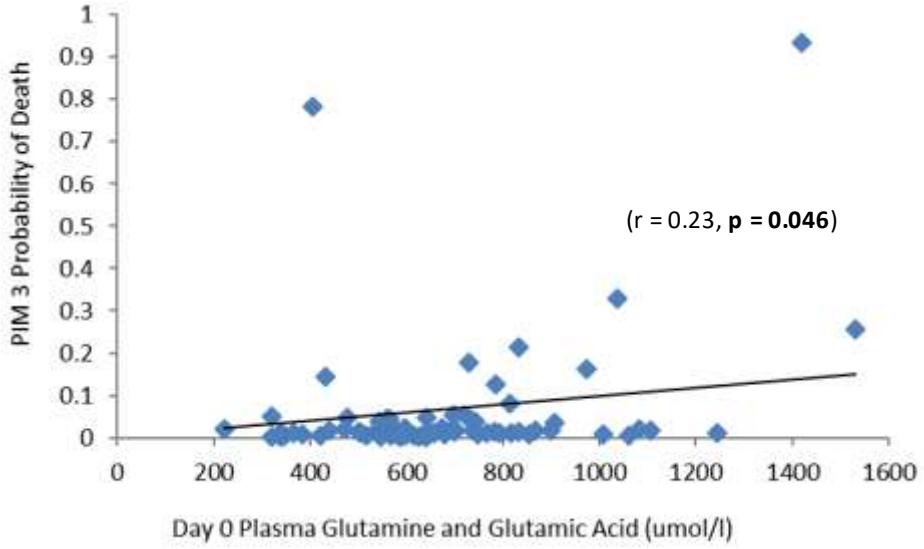


Figure 11: Plasma glutamine and glutamic acid on admission and the probability of mortality

Stellenbosch University https://scholar.sun.ac.za

Plasma glutamine and infectious disease

ANOVA testing was done to investigate associations between plasma glutamine on admission and participants' infectious disease status (Table 14, below). No difference was demonstrated between plasma glutamine on admission among participants who were HIV exposed versus those who were not exposed (p = 0.340), nor between participants who were HIV positive or negative (p = 0.329). Similarly, no significant difference in day 0 plasma glutamine was shown between TB positive or negative participants (p = 0.627). The degree to which plasma glutamine changed from day 0 to day two was also not significantly different among any of the above groups.

Table 14: Plasma glutamine and infectious disease

		Day 0 Plasma Glutamine		Plasma Glutamine Change*	
		Mean (umol/l) ± SD	ANOVA	Mean (umol/l) ± SD	ANOVA
HIV exposure	Yes	516.9 ± 165.3	p = 0.340	60.7 ± 179.0	p = 0.579
	No	586.4 ± 218.9		-11.8 ± 305.6	
HIV status	Positive	473.3 ± 112.0	p = 0.329	203.5 ± 77.1	p = 0.329
	Negative	581.5 ± 213.9		-3.8 ± 292.7	
TB	Positive	532.0 ± 265.4	p = 0.627	-11.0 ± 19.8	p = 0.931
	Negative	579.6 ± 207.6		7.53 ± 296.7	

ANOVA: Analysis of variance; HIV: Human Immunodeficiency Virus; TB: Tuberculosis; SD: Standard deviation
**Refers to the difference in concentration between day 0 and day 2*

Plasma glutamine and routine biochemistry

As demonstrated in Table 15 (below), Spearman's correlation showed no significant relationships between day 0 plasma glutamine and any of the biochemical tests conducted on admission. A trend toward a positive association was, however, demonstrated between plasma glutamine on admission and platelet count (Spearman r = 0.22, p = 0.058). No associations were demonstrated between plasma glutamine and lactate, or markers of renal (urea and creatinine) and hepatic (bilirubin and liver enzymes) function. Several markers of infection – including CRP, PCT, and white cell count – were negatively correlated to plasma glutamine on admission, although none of these relationships were significant.

Mann Whitney U tests were conducted to compare plasma glutamine between patients with raised, and those with normal, markers of infection on admission. No significant differences were found between participants with elevated CRP (p = 0.097, n = 17) or PCT (p = 0.621, n = 14), when compared with those with normal infectious parameters. The plasma glutamine concentration of participants with obviously low albumin (≤20 g/l) at admission was also not significantly different to those with normal albumin levels (p = 0.631, n = 28). Similarly, no difference was demonstrated between groups when the sum of glutamine and glutamic acid was used.

Stellenbosch University https://scholar.sun.ac.za

Table 15: Day 0 plasma glutamine and biochemistry			
	n	Spearman r	p
Lactate (mmol/l)	73	0.04	0.759
Glucose (mmol/l)	73	0.00	0.997
Urea (mmol/l)	76	0.01	0.963
Creatinine (umol/l)	75	0.03	0.815
CRP (mg/l)	17	-0.18	0.497
PCT (ug/l)	14	-0.25	0.391
Albumin (g/l)	28	0.26	0.180
Bilirubin (umol/l)	16	0.31	0.247
Conjugated bilirubin (umol/l)	16	0.36	0.166
ALT (U/l)	16	0.01	0.965
AST (U/l)	16	0.01	0.974
ALP (U/l)	12	0.38	0.217
GGT (U/l)	10	0.41	0.238
Haemoglobin (g/dl)	73	-0.12	0.313
White cell count ($x10^9$)	73	-0.05	0.670
Platelets ($x\ 10^9$)	73	0.22	0.058

ALT: Alanine Transferase; AST: Aspartate Aminotransferase; CRP: C- Reactive Protein; GGT: Gamma-Glutamyl Transferase; PCT: Procalcitonin

Associations between plasma glutamine and nutritional status

Statistical analyses of the association between nutritional status and plasma glutamine were conducted using both day 0 plasma glutamine levels and the change that occurred in glutamine concentration between days 0 and two.

ANOVA testing did not reveal any significant differences in day 0 plasma glutamine between any of the anthropometrical categories (Table 16, below). In addition, no notable difference was shown in the degree to which glutamine changed over time among any of these categories.

Stellenbosch University https://scholar.sun.ac.za

Table 16: Plasma glutamine and nutritional status				
	Day 0 Plasma Glutamine		Plasma Glutamine Change*	
	Mean (umol/l) ± SD	ANOVA	Mean (umol/l) ± SD	ANOVA
Weight-for-age				
Normal	554.9 ± 236.0		20.4 ± 286.7	
Moderately underweight	546.9 ± 131.4	p = 0.895	-158 ± 517.5	p = 0.532
Severely underweight	588.8 ± 184.2		-21.6 ± 184.7	
Length/height-for-age				
Normal	558.8 ± 188.3		62.4 ± 249.2	
Moderate stunting	638.9 ± 339.9	p = 0.500	0.3 ± 264.6	p = 0.817
Severe stunting	522.0 ± 54.8		-3.8 ± 176.4	
Weight- for- height/ BMI				
Normal	562.1 ± 219.1		58.3 ± 256.9	
Moderate wasting	609.6 ± 225.2	p = 0.866	35.0 ± 210.0	p = 0.523
Severe wasting	529.0 ± 92.2		-51.2 ± 183.5	
Obese	547.5 ± 31.8		332.0± -	
Weight-for-height +MUAC				
Normal	565.9 ± 224.2		58.3 ± 256.9	
Moderate wasting	560.3 ± 201.0	p = 1.00	35 ± 183.5	p = 0.523
Severe wasting	569.7 ± 145.4		-51.2 ± 183.5	
Obese	547.5 ± 31.8		332.0± -	

ANOVA: Analysis of variance; BMI: Body Mass Index; MUAC: Mid Upper Arm Circumference; SD: Standard deviation

**Refers to the difference in concentration between day 0 and day 2*

A significant inverse relationship was demonstrated between day 0 plasma glutamine and participant weight (Spearman r = -0.24, p = 0.046, n = 72) - as seen in Figure 12 (below). Significant inverse relationships were also shown between plasma glutamine on admission and participant length/height (Spearman r = -0.26, p = 0.035, n = 64), and MUAC (Spearman r = -0.27, p = 0.044, n = 57).

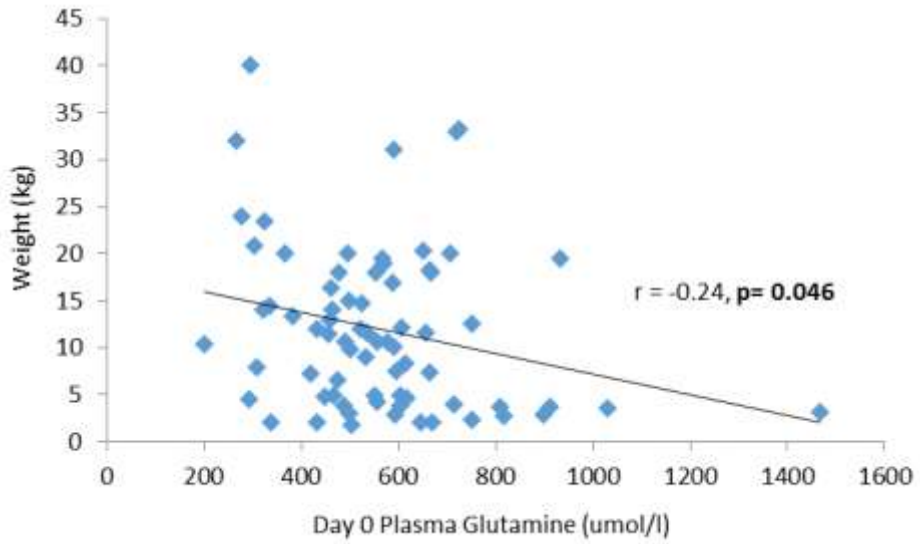


Figure 12: Plasma glutamine on admission and participant weight

Stellenbosch University https://scholar.sun.ac.za

No significant relationship was found between plasma glutamine on admission and weight-for-height/BMI, nor between the degree of change in plasma glutamine over time and any of the above anthropometrical parameters.

Associations between plasma glutamine and nutritional intake

Associations between nutritional intake during the first two days of PICU stay and day two plasma glutamine were investigated using Spearman's correlation. No significant associations were demonstrated - as indicated in Table 17 (below). Similarly, no significant associations were found between nutritional intake and the degree to which plasma glutamine changed over the first 48 hours of PICU stay.

Table 17: Day two plasma glutamine and nutritional intake

	n	Spearman r	P
Average energy (kcal/day)	38	-0.14	0.413
Average protein (g/day)	38	-0.15	0.367
Energy requirements met (%)	36	-0.12	0.488
Protein requirements met (%)	36	-0.20	0.248

ANOVA testing was conducted to identify differences in day two plasma glutamine among participants who had met 66% of energy requirements and those who had not. No significant difference was noted (p = 0.730). Although plasma glutamine increased more from day 0 to day two among participants who met 66% of requirements (mean 107 ± 137.3 umol/l) compared to those who did not (mean 20.8 ± 239.2 umol/l), this difference was not significant (p = 0.550).

Associations between plasma glutamine and clinical outcome

Spearman's correlations were run to investigate associations between clinical outcome and plasma glutamine on day 0 and day two of PICU stay, as well as the change that occurred over this time (Table 18, below). No significant associations were demonstrated between plasma glutamine and the length of time spent on mechanical ventilation or PICU LOS. A positive relationship was, however, found between day 0 plasma glutamine and hospital LOS (Spearman r = 0.26, p = 0.032), indicating that higher plasma glutamine on admission is associated with longer time spent in hospital (Figure 13, below). Outlier sweeps were conducted for each of the clinical outcome parameters, the results of which are indicated, in parentheses, in Table 18. With outliers excluded, a significant relationship was not demonstrated between plasma glutamine on admission and hospital LOS. However, a trend toward a positive association was shown (Spearman r = 0.23, p = 0.067).

Stellenbosch University https://scholar.sun.ac.za

Table 18: Plasma glutamine and clinical outcome			
	n	Spearman r	p
Mechanical ventilation (hr)			
Day 0 Glutamine	75 (70)	0.14 (0.13)	0.227 (0.303)
Day 2 Glutamine	40 (35)	-0.13 (-0.10)	0.420 (0.552)
Change in Glutamine*	40 (35)	0.18 (0.10)	0.272 (0.570)
PICU LOS (hr)			
Day 0 Glutamine	69 (64)	0.07 (0.081)	0.558 (0.523)
Day 2 Glutamine	36 (32)	-0.28 (-0.19)	0.097 (0.285)
Change in Glutamine	36 (32)	0.29 (0.30)	0.088 (0.100)
Hospital LOS (days)			
Day 0 Glutamine	68 (65)	0.26 (0.23)	**0.031** (0.067)
Day 2 Glutamine	35 (33)	-0.13 (-0.14)	0.446 (0.440)
Change in Glutamine	35 (33)	0.31 (0.31)	0.074 (0.079)

LOS: Length of stay
Results following the removal of statistical outliers are presented in parentheses
**Refers to the difference in concentration between day 0 and day 2*

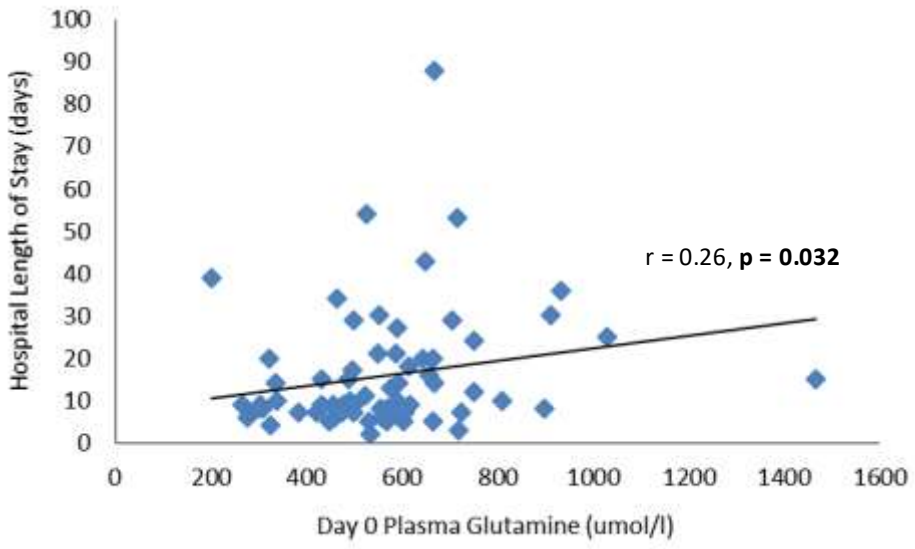


Figure 13: Day 0 plasma glutamine and hospital length of stay

Interestingly, the change that occurred between plasma glutamine and glutamic acid (combined) from admission to day two was negatively correlated with hospital LOS (Spearman r = -0.34, p = 0.046, n = 35) – as demonstrated in Figure 14 (below). This result was further confirmed when outliers were excluded (Spearman r = -0.36, p = 0.043, n = 33).

Stellenbosch University https://scholar.sun.ac.za

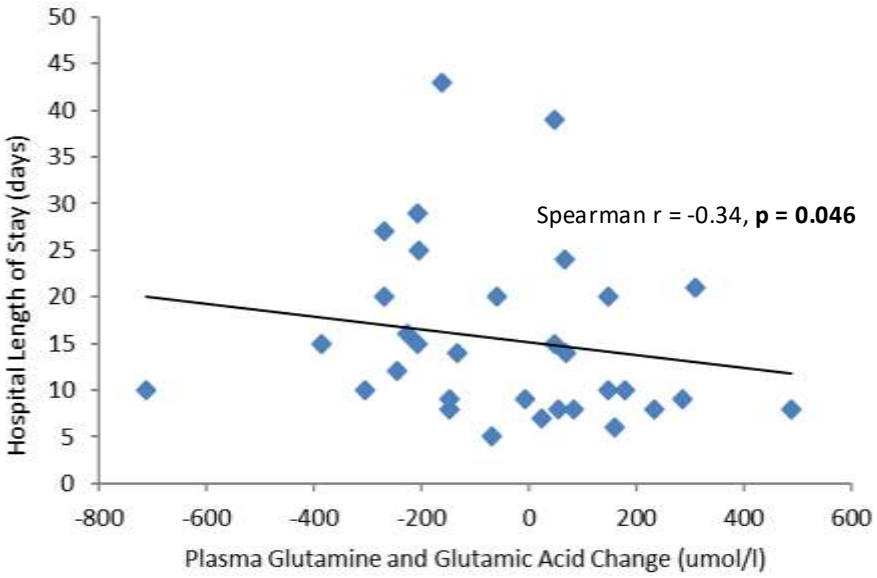


Figure 14: Plasma glutamine and glutamic acid change and hospital length of stay

ANOVA testing was done to further investigate the relationship between plasma glutamine on admission and the length of hospital stay. Plasma glutamine was categorised as low (<420 umol/l), normal (420-930 umol/l), and high (>930 umol/l), and the means were compared between groups. No significant difference in hospital LOS was noted between groups.

ANOVA was used to compare plasma glutamine among participants requiring ventilation with those who did not. No significant differences were found in day 0 or in day two plasma glutamine between groups. Participants requiring ventilation demonstrated a slight reduction in plasma glutamine over the first 48 hours of PICU stay (mean -1.36 ± 302.6 umol/l), whereas those who were not ventilated showed an increase in plasma glutamine over this time (mean 78.25 ± 101.2 umol/l). This difference was not, however, significant (p = 0.610).

Differences in plasma glutamine were also investigated among participants who died versus those who survived. No significant difference was noted in plasma glutamine on admission between participants who died, compared to those who survived (p = 0.158). Higher plasma glutamine on day two (mean 812.4 ± 583.2 umol/l, n = 5) was demonstrated in participants who died, when compared to surviving participants (mean 547.3 ± 222.1 umol/l, n = 35), a difference which approached significance (p = 0.057).

Plasma glutamine was shown to increase from day 0 to day two among participants who survived (mean 26.4 ±236.9 umol/l, n = 35) and to reduce among those who died (mean -132.2 ± 556.7 umol/l, n = 5). Again, this was not a significant result (p = 0.256). From the data, it did appear as if there was greater

Stellenbosch University https://scholar.sun.ac.za

variability in plasma glutamine on day 0 and day two, and a greater margin of change during this time among those who died.

Stellenbosch University https://scholar.sun.ac.za

5. Discussion

Introduction

It is clear that before large-scale interventions are conducted, a more thorough understanding of plasma glutamine concentration among specific patient subgroups is needed.[18] One group for whom data is lacking is children - particularly the critically ill.[20] This exploratory study investigated the plasma glutamine status of critically ill children on admission, and during the first two days of PICU stay. Furthermore, associations between plasma glutamine and clinical condition on admission, nutritional status, nutritional intake, and clinical outcome were explored. Data were collected from consecutive patients admitted to the RCWMCH PICU over a one-month period, making the sample largely representative of the unit's typical patient profile.

The study found that plasma glutamine concentrations on admission to and on day two of PICU stay were, for the majority of participants, normal. No notable change in plasma glutamine status took place over this period. Significant differences existed in plasma glutamine between diagnostic groups. However, no associations were demonstrated with regard to other markers of clinical condition on admission. Both nutritional status and intake appeared to be largely unrelated to plasma glutamine, as were most markers of clinical outcome. A positive trend was, however, demonstrated between plasma glutamine on admission and hospital LOS. In addition, significantly more change in plasma glutamine and glutamic acid occurred over the first two days in PICU in those who spent less time in hospital. Although no link was made between plasma glutamine and mortality, higher plasma glutamine was demonstrated on day two of PICU stay among those who died - a result which approached significance.

When comparing these results to the available literature, a distinction must be made between observational studies (as detailed in Tables 1 and 2 in the literature review) and interventions in which glutamine was administered as a supplement - the latter of which are not directly comparable to the current study.

Plasma glutamine during the first two days of PICU stay

Plasma glutamine on admission to PICU

In their work with children diagnosed with meningococcal disease, Marino et al. demonstrated low plasma glutamine on admission to the PICU and concluded that, as with many adults, paediatric patients experience plasma glutamine depletion during the acute phase of critical illness.[21] This was in direct

Stellenbosch University https://scholar.sun.ac.za

contrast to this study, which observed mostly normal plasma glutamine concentrations on admission to PICU.

Other studies have also shown normal plasma glutamine concentrations among critically ill patients on admission, but reported a higher prevalence of glutamine depletion compared to this study.[22,24,59,60] Ekmark and colleagues reported mean plasma glutamine concentrations comparable to this study, but found that 40% of patients within their paediatric cohort were glutamine deplete.[22] This is markedly higher than the 17.1% of participants presenting with low plasma glutamine in this study. In adult literature, the reported prevalence of glutamine depletion has varied among mixed and specific subgroups of critically ill patents.[24,58–60,66] Both Oudemans-van Straaten et al.[24] and Nienaber and colleagues[58] reported normal plasma glutamine on presentation to the ICU (at similar concentrations to this study) - but identified depletion among 31% and 38% of patients respectively. Higher proportions of up to 65% of patients with glutamine depletion have been noted by others.[60] Thus, there is no clear picture regarding the prevalence of glutamine depletion among critically ill children or adults. This study diverges from what has been demonstrated in the paediatric and adult literature, by showing normal plasma glutamine concentrations and proportionately fewer patients with glutamine depletion on admission to PICU.

Healthy controls have been used in some studies for comparative purposes. Both Ekmark et al.[22] and Marino and colleagues[21] reported significantly lower plasma glutamine concentrations among critically ill children when compared to healthy subjects. This distinction in plasma glutamine concentrations between healthy and critically ill individuals has also been shown in the literature on adults.[65] The current study did not, however, include a control group. In addition, data on normal plasma glutamine concentration among healthy South African children does not exist, making it difficult to contextualise these results.

Despite a well-established link between mortality and plasma glutamine concentrations >930 umol/l,[59] very few studies have reported on the prevalence of plasma glutamine above this cut off. In the controversial REDOXS study, 15% of the small subset of patients for whom glutamine concentration was measured had raised plasma levels, which arguably was sufficient reason to avoid the high dose blanket supplementation to follow.[13] Closer to home in South Africa, Nienaber and colleagues found that 7% of critically ill adult patients had elevated glutamine concentrations on admission to ICU.[58] Similarly, this study found that 5% of participants had plasma concentrations >930 umol/l.

Plasma glutamine on day two of PICU stay

This study showed that, on average, plasma glutamine concentrations on day two of PICU stay remained normal. Although not statistically significant, the percentage of patients with low plasma glutamine

Stellenbosch University https://scholar.sun.ac.za

increased from admission (17%) to day two (27%) of PICU stay - suggesting that plasma glutamine status worsened fractionally during this period.

Exploratory research among critically ill paediatric patients has demonstrated some improvement in glutamine status over time; however, the time-frames used to obtain repeat plasma samples were vastly different between studies.[21,22] Marino and colleagues reported plasma glutamine on admission and again during convalescence (a measure which took place, on average, 55 days post PICU discharge), and found an improvement in mean plasma glutamine from 52% to 26% below the normal range respectively.[21] In their study, Ekmark et al. found that plasma glutamine concentrations among a mixed cohort of critically ill children had normalised by day five of PICU stay, without exogenous supplementation.[22] Similarly, slow improvement in plasma glutamine was demonstrated from admission to day six of ICU stay among adult trauma patients.[66] All three of these studies could not, however, describe the changes that occurred in plasma glutamine during the acute phase of critical illness.

The work done in 2008 by Villares et al. among children with congenital heart disease is perhaps more comparable to the current study, in that plasma glutamine was measured early on in the post-operative period on day one, three, and then later on day seven. Although slow correction was seen in the concentrations of all amino acids over time, glutamine recovery was the most delayed - remaining significantly lower than pre-operative concentrations seven days after surgery.[116] This delayed recovery by up to one week is in contrast to other paediatric findings, but corroborates what had previously been found in adult patients following major surgery.[64]

In the current study, participants presenting to the PICU with glutamine depletion were more likely to have reduced concentrations 48 hours later - an association that lost statistical significance after the removal of outliers. In addition, the prevalence of low plasma glutamine increased (not significantly) from admission to day two of PICU stay. This could infer that in these patients, plasma glutamine correction had not yet occurred during the first 48 hours of PICU stay.

In their observations of critically ill adults, Buter and colleagues measured plasma glutamine concentrations daily throughout the course of ICU stay. It is unfortunate that a detailed description of the changes that occurred in plasma glutamine over time was not included in their final report.[60] This would have provided useful information about the glutamine response during and beyond the acute phase of critical illness.

The purpose of observational research is to increase knowledge in a particular field, and to inform future interventions. The results of this study have challenged the notion that critically ill children are glutamine deplete during the acute phase of illness – and do not support blanket supplementation with the nutrient.

Stellenbosch University https://scholar.sun.ac.za

Reasons for the discrepancy between this study and others (within paediatric critical care), and the differences in the reported prevalence of glutamine depletion, need to be considered.

Reference ranges

It must be noted that the reference ranges used for plasma glutamine vary between studies. In 1992, Laposta established the lower limit of plasma glutamine at 420 umol/l.[137] Based on mortality outcomes in critically ill adults, both Oudemans-van Straaten[24] and Rodas and colleagues[59] confirmed this cut off and dichotomised their data accordingly. Ekmark et al. corroborated this in paediatrics, by reporting that 420 umol/l was -2SD below that of healthy controls and could therefore be considered low.[22] The group subsequently established a link between low plasma glutamine and increased morbidity. Thus mortality data in adults and morbidity data in children have confirmed this lower limit.[18]

Less is known about what constitutes high levels of plasma glutamine. Concentrations of ≥930 umol/l in adult patients have been associated with increased mortality. However, this result was based on only a few patients demonstrating high glutamine concentrations.[59] Ekmark and colleagues suggested an upper limit of 760 umol/l; a value +2SD higher than that seen in healthy controls.[22] Paediatric age-specific reference ranges for plasma glutamine have also been described by Blau et al. - ranges that were used by the laboratory employed in this study.[127]

The study by Marino and colleagues was unique in that a combined value for glutamine and glutamic acid was calculated, in order to account for the spontaneous conversion of glutamine to glutamic acid during processing. A reference range of >600 umol/l was used to denote normal levels - a cut off that has not been described elsewhere.[21] Oudemans-van Straaten et al. acknowledged this loss of plasma glutamine to glutamate by analysing both, and found a 6.5% loss of glutamine to glutamic acid. As such, the authors chose to analyse their data using the combined value of plasma glutamine and glutamic acid.[24] It is clear that there are differences in reference ranges and in the measurement of plasma glutamine among available observational studies, making comparison difficult and firm conclusions nearly impossible to draw.

For the purposes of this study, plasma glutamine was investigated independently as well as in combination with plasma glutamic acid. This was done to allow for comparison between studies and to exclude the possibility of falsely low plasma glutamine concentrations. In addition, plasma glutamine concentration was analysed using reference ranges established by clinical outcome data[24,59] as well as the paediatric ranges specified by Blau et al.[127] In both cases, low proportions of patients were shown to be glutamine deplete on admission to PICU; numbers which elevated slightly but non- significantly by day two.

Stellenbosch University https://scholar.sun.ac.za

Differences between the reference ranges were more apparent when looking at participants with high plasma glutamine. According to the reference ranges of Blau et al.,[127] 15.8% of patients had elevated plasma glutamine on admission, which increased to 20% by day two. This contrasts with the 5.3% and 5% of participants shown to have raised plasma concentrations on admission and day two when using the more popular reference range. These disparities raise questions over which reference ranges should be used in paediatrics, and how this choice may have influenced the results of existing studies.

Plasma glutamine: a proxy for whole body glutamine?

Increased consumption of glutamine during critical illness is thought to precipitate tremendous flux of the amino acid between organs. It is unclear to what extent plasma glutamine levels represent this mobilisation.[24] As a result, there is controversy over the use of plasma concentration as a proxy for whole body glutamine during periods of metabolic stress. Questions have been asked about whether we are measuring glutamine status correctly. What is also unknown at this point is if, at certain stages of critical illness, plasma glutamine concentration reflects whole body glutamine better than others. This would have a major bearing on how existing studies are interpreted and compared.

A reduction of up to 72% in skeletal muscle glutamine concentration has been reported among several critically ill adult populations.[61,62,65,138,139] As a result, glutamine concentration in skeletal muscle has been studied as a means to evaluate whole body glutamine during critical illness.[24] This was, however, challenged in 2004 by Tjader and colleagues who demonstrated that muscle glutamine concentrations remained persistently low in patients with MOF - despite escalating doses of intravenous supplementation. The group hypothesised that low concentration of glutamine in the muscle may reflect a general state of inflammation rather than whole body depletion.[140] Soeters et al. also suggested that tissue glutamine concentrations be interpreted with caution, as alterations in membrane integrity and transport systems could mean that the steep uphill gradient between plasma and tissue cannot be maintained.[26] Questions, therefore, still remain over the use of skeletal muscle concentration as a proxy for glutamine status during critical illness.

Plasma glutamine response to critical illness is thought to be less distinct than that of skeletal muscle, and may vary over time.[24] As Oudemans-van Straaten and colleagues pointed out - the release of glutamine from muscle tissue may initially increase or maintain plasma glutamine concentration. As such, normal plasma glutamine concentrations may not exclude whole body glutamine depletion; however, low plasma glutamine is likely to reflect depletion. Low plasma glutamine would therefore be considered a relevant marker of whole body glutamine depletion.[24] In contrast, others postulate that low plasma glutamine may

Stellenbosch University https://scholar.sun.ac.za

not be due to an overall shortage, but due to fluid shifts that occur during metabolic stress. According to this theory, increases in intravascular and extravascular spaces during critical illness may have a dilutional effect on amino acids in the plasma.[26]

Despite this uncertainty, the finding that low plasma glutamine is an independent risk factor for increased mortality among adult patients,[24,59] and is linked to MOF among critically ill children,[22] indicates that plasma glutamine is a useful clinical biomarker. Furthermore, plasma glutamine is currently the most cost-effective, accessible, and least invasive proxy. What would be useful is consensus about the reference ranges used and the measurement of plasma glutamine independently or in conjunction with glutamic acid. This would allow for comparison between studies, which would add to the limited knowledge base in this field in a significant way.

Clinical condition on admission

Diagnostic profile

Worldwide, PICUs are home to a heterogeneous mix of conditions, ranging from acute or chronic illness, to post-operative care, injury, and others.[141] The diagnostic profile of the current cohort was heavily weighted by elective surgical - in particular, post-operative cardiac patients, who comprised just less than half of the total participants.

Solomon and colleagues conducted a retrospective audit of patients admitted to the PICU of RCWMCH between the years 2000 and 2006. A notable difference between studies in RCWMCH was the rise in post-operative cardiac patients over time - with 11.8% of bed spaces occupied by this subgroup in 2000, 17.8% in 2006, and 47% over a one-month period in 2016.[117] The current cohort appears to represent the changing diagnostic profiles seen within the unit.

— *Plasma glutamine and diagnostic profile*

Observational studies on plasma glutamine in paediatrics have differed in terms of the diagnostic profile of participants. Marino et al. focused solely on patients with severe meningococcal disease - a diagnosis frequently associated with sepsis and MOF.[21] On the other hand, Ekmark and colleagues included a more diverse PICU population of surgical and medical patients; a cohort that included neurosurgical and thoracic, but excluded cardiac surgery patients.[22] Similarly, in their work with critically ill adults, Oudemans-van Straaten et al. included seriously ill patients admitted non-electively to ICU, thus excluding cardiac and other elective surgical patients.[24] Rodas et al. also excluded thoracic, neurosurgical, and trauma patients.[59] In contrast to these studies, post-operative cardiac patients comprised a large proportion of the current

Stellenbosch University https://scholar.sun.ac.za

cohort. Given such variation between studies, consideration must be given to diagnostic profile as a potential reason for the differences in reported prevalence of plasma glutamine depletion duri ng critical illness.

The question must also be asked if the inclusion of so many post-operative cardiac patients may have inclined this study to show more favourable glutamine concentrations compared to others. Thi s is particularly relevant as plasma glutamine concentrations among cardiac patients in this study were shown to be normal in the immediate post-operative period, and on day two of PICU stay. This finding is not, however, corroborated by other literature - further insulating this study from others. Plasma glutamine concentrations of paediatric post-operative cardiac patients have been shown to decrease significantly following surgery (on day one, two, and three),[115,116] and to remain low for up to one week.[116]

Plasma glutamine concentrations between different diagnostic groups were compared in order to ascertain if any one group in particular influenced the results of this study. Significant differences were demonstrated in combined concentrations of plasma glutamine and glutamic acid between cardiology and gastroenterology diagnoses, and between sepsis and pulmonology, burns, gastroenterology, and 'other'. This suggests that from a diagnostic perspective, sepsis, more than cardiology, stood out. Only 5% of participants in this study presented to the PICU with a primary diagnosis of sepsis, making firm conclusions difficult to draw. This subgroup of septic patients was, however, shown to have the highest plasma glutamine and combined values of glutamine and glutamic acid, as well as the widest range of concentrations.

The relationship between plasma glutamine and sepsis has been investigated before, with rather interesting results. In their work with adults, Oudemans-van Straaten and colleagues identified septic shock as the primary diagnosis most frequently related to low plasma glutamine.[24] Reduced concentrations of glutamine have also been described among other populations of septic adult patients.[58,142,143] In paediatrics, little is known about plasma glutamine during sepsis. In their study on infants and children with meningitis, a diagnosis frequently associated with sepsis, Marino et al. reported low plasma glutamine during the acute phase of illness.[21] This is in sharp contrast to the findings of this study.

Alterations in glutamine metabolism have also been identified during sepsis. In 2013, Kao and colleagues described increased hepatic clearance of glutamine and reduced uptake by enterocytes among septic patients.[142] In sepsis, therefore, it appears as though immune cells and the liver are the primary consumers of glutamine - compared to non-septic patients whose immune and gastrointestinal systems utilise most glutamine during critical illness.[143]

Stellenbosch University https://scholar.sun.ac.za

Glutamine supplementation has been increasingly discouraged among septic patients on the back of the 'inflammation hypothesis'.[143] In situations of increased glutamine consumption, proteolysis of skeletal muscle occurs in order to provide the glutamine required.[26] As a major fuel source for immune cells, glutamine consumption increases once lymphocytes are activated. It has been suggested that the release of interleukin 1 (Il-1) and interleukin 2 (Il-2) from lymphocytes and macrophages depends on plasma glutamine concentration.[144,145] Instead of enhancing immune function, large infusions of glutamine given to patients with sepsis or organ failure could, in fact, prompt an exaggerated inflammatory response which may have a negative impact on outcome.[143] This hypothesis could explain the contradicting results between studies, which have been conducted on patients with varying degrees of inflammation and sepsis.[13,15,79]

Interestingly, 68% of patients enrolled in the REDOXS study had septic shock. In their dose finding study prior to the intervention, plasma glutamine was normal among most of the 66 patients included.[13] Although no information was provided regarding the diagnostic profile of this subgroup of patients, it would have been interesting to see what proportion was septic.

It appears as if both plasma glutamine concentration and the role of exogenous glutamine during sepsis are areas that require more scrutiny. Increased mortality has been demonstrated among patients with sepsis who received glutamine supplementation.[13] As such, an in-depth understanding of plasma glutamine concentration in this group is now clearly required. Although the numbers of septic participants in the current study were low, the finding that plasma glutamine was highest among this group contradicts what has been demonstrated in adult patients thus far. The question now is whether this suggests a different glutamine response to sepsis in paediatrics, or whether this was unique to the mixed cohort included in this study.

Aside from primary diagnoses, this study also showed differences in plasma glutamine across diagnostic categories - with concentrations on admission differing significantly between medical and trauma, and between medical and elective surgical patients. Trauma patients presented with proportionately more glutamine depletion on admission, followed by surgical and then by medical patients. In their intervention, Perez-Barcena et al. reported glutamine depletion among 60% of trauma cases on presentation to the ICU.[66] This, in some way, supports the finding of this study with regard to trauma. However, these results are in contrast to what has been found in mixed cohorts of critically ill adults. In their study of ICU patients, Oudemans-van Straaten and colleagues reported a normal to high plasma glutamine among patients admitted with trauma, and showed that 37% and 21% of medical and surgical patients had low plasma glutamine on admission to ICU respectively.[24] This suggests that proportionately more medical patients experience glutamine depletion when compared to surgical patients. Along similar lines, Buter et al. reported significantly lower plasma glutamine concentrations among non-elective (medical, trauma and

Stellenbosch University https://scholar.sun.ac.za

emergency surgery), when compared to elective (primarily surgical) patients on presentation to the ICU.[60] This was further confirmed in 2016 by Nienaber and colleagues, who showed lower plasma glutamine among medical versus surgical patients on admission to the ICU.[58]

Potential reasons for these findings have been put forward. Buter et al. suggested that increased infection in non-elective, when compared to post-operative elective surgical patients, may have played a role. In addition, non-elective patients generally experience an extended period of time from the onset of illness to ICU admission. The authors suggested that metabolic stress over a longer period may have resulted in more glutamine depletion among this group of patients.[60] Of course, this rationale cannot explain the results of the current study.

In several studies on plasma glutamine concentration among mixed critically ill populations, patients were not stratified according to diagnosis, but rather by severity of disease and clinical outcome.[22,59] Observational studies of specific diagnostic groups such as those with meningococcal disease and post-operative cardiac surgery, have proven valuable in describing low plasma glutamine among particular patients. Additional data on the diagnostic profile of patients would be a useful addition to future studies, in order to identify which, if any, patients are consistently glutamine deplete.

The current study has highlighted the importance of diagnosis when studying plasma glutamine in critically ill children. Sepsis, in particular, emerged as a diagnosis that requires further investigation with regard to plasma glutamine. In addition, the study's novel findings with regard to diagnostic groups certainly warrant closer scrutiny.

Severity of illness and mortality risk

In this study, a positive correlation was shown between the combined concentrations of plasma glutamine and glutamic acid on admission and the probability of mortality. Similarly, participants with higher plasma glutamine (independent of glutamic acid) on admission tended to be at increased risk of death. Both associations were, however, weak and significance was lost once outliers were removed. These results are not definitive, but provide some suggestion of a potential relationship between plasma glutamine and mortality risk.

The relationship between plasma glutamine, severity of disease, and mortality risk has been studied among critically ill adult cohorts. Only one study to date has established a link between plasma glutamine and severity of disease.[60] However, most have shown low plasma glutamine concentration on admission to ICU as being independent of disease severity and mortality risk profiling.[146] Rodas et al. found no significant

Stellenbosch University https://scholar.sun.ac.za

difference in APACHE II (Acute Physiology and Chronic Health Evaluation) scores between patients with plasma glutamine concentrations of <420 umol/l when compared to those with normal glutamine status.[59] In their study, Oudemans-van Straaten and colleagues used four scoring systems to assess severity of illness and predicted mortality. Although a trend was noted toward lower plasma glutamine being associated with higher severity of illness and mortality predictions, no significant link was found between plasma glutamine and patient risk scoring.[24] From these results, it appears as if low plasma glutamine is not always a characteristic of the sickest of patients in an ICU setting.[146] This concept among adult populations is an important one, and one which was largely ignored by Heyland et al. when recruiting septic patients with MOF into the REDOXS study.[13] The results to follow could perhaps have been avoided, had glutamine depletion not been presumed among the sickest of ICU patients.

Because of the paucity of data on plasma glutamine levels in critically ill children, little is known about the relationship between severity of disease scoring and plasma glutamine. Both PRISM (Paediatric Risk of Mortality) and PIM 2 scores were used by Marino et al. to predict mortality - neither of which were associated with plasma glutamine concentration. The authors mentioned however, that glutamine status was in some way associated with disease severity, in that significant inverse relationships were shown between plasma glutamine and PICU LOS, duration of mechanical ventilation and the biochemical measures lactate, and CRP.[21] This could highlight a difference in the relationship between plasma glutamine and severity of disease, as determined using a scoring tool versus clinical presentation and outcome.

In addition to the lack of data in paediatric patients, comparison between existing studies is made difficult by the use of different scoring tools. In saying that, neither the available data from adult nor paediatric cohorts support the finding of this study - which suggests that the higher the plasma glutamine on admission, the higher the risk of mortality.

This result could theoretically coincide with the U-shaped curve discussed by Rodas and colleagues, in which both low and high plasma glutamine concentrations were associated with increased mortality.[59] It must be noted that predicted and actual mortality are not directly comparable. Because of the limited sample size of this study, demonstrating an association between actual mortality and plasma glutamine was unlikely to occur. Despite this - could a positive association (albeit a weak trend) between plasma glutamine and mortality risk scoring suggest that as in adults, elevated plasma glutamine places children at risk of potential harm? This question certainly warrants further study.

Another question more specific to this study, is whether statistical outliers should have been excluded from the data analysis. It could be argued that in a setting where data were collected from live subjects, both high and low plasma glutamine concentrations could have been clinically relevant and should not have

Stellenbosch University https://scholar.sun.ac.za

been disregarded. Inclusion of statistical outliers would have strengthened the argument put forward by this study that higher plasma glutamine and glutamic acid on admission to PICU is associated with an increased risk of mortality.

Infectious disease status

Like many low-resourced countries, South Africa faces a significant burden of infectious disease. At 12.6% of the population and 19% of the global estimate, the country is thought to have the highest prevalence of HIV in the world in adults over the age of 15 years.[118,147] Although the epidemic remains at record levels, new infections are gradually declining.[147] Almost 30% of South Africans are below the age of 15 years. An estimated 280 000 children aged 0-14 years were living with HIV in 2017, equating to 1.7% of the population within this age bracket.[118] In this study, 3.9% of participants were infected with HIV - more than double the national prevalence.

TB is another major challenge within the South African health sector, and is thought to be closely related to HIV.[148] An estimated 0.8% of the population suffer from active disease every year, 13% of which are children.[119] Six percent of participants included in this study tested positive for TB, a figure which is significantly higher than the domestic estimate.

In light of the above context, it made sense for this study to explore potential associations between plasma glutamine and infectious disease. In their early work on protein metabolism in conditions associated with cachexia, Hack et al. suggested that HIV infection was associated with low plasma glutamine concentration - likely the result of concomitant infections and increased burden on the immune system.[149] More recently, this was challenged by Nienaber and colleagues who reported normal plasma glutamine concentrations among HIV-positive adults on admission to two South African ICUs. The group demonstrated comparable inflammatory markers between HIV-positive and HIV-negative patients, and argued that less pronounced inflammation in HIV-infected patients than expected, could account for normal plasma glutamine concentrations in this group.[58]

Most studies on plasma glutamine status do not mention infectious disease - likely because the disease burden is far lighter elsewhere. However, the therapeutic role of glutamine supplementation in infectious disease has, to some degree, been considered. People living with HIV who received exogenous glutamine demonstrated reduced duration and severity of diarrhoea associated with both infection[150] and antiretroviral (ARV) therapy.[151] In addition, glutamine has been studied as a potential means to reduce the oxidative burden of HIV. Supplementation of the amino acid to HIV-infected patients was shown to increase glutathione levels, but has not yet been shown to directly impact antioxidant capacity.[152]

Stellenbosch University https://scholar.sun.ac.za

This study is, to our knowledge, the first to investigate associations between plasma glutamine and infectious disease status among critically ill children - in the absence of glutamine supplementation. No significant differences were found in plasma glutamine among those infected with HIV or TB and those who were not, implying that infectious disease has no bearing on glutamine status. Because the numbers of participants with infectious disease were low, these results must however be interpreted with caution. Despite this, the findings of this study enhance knowledge in an area in which research is lacking. In the case of HIV infection, and perhaps in the context of a larger study, it would have been interesting to explore the relationship between ARV use and plasma glutamine, as this association has not yet been investigated elsewhere.

Biochemistry

Biochemical indicators of clinical condition, including markers of infection, albumin, lactate, and measures of hepatic and renal function, have been investigated in relation to plasma glutamine concentration.[21,143] This study found no significant associations between plasma glutamine and any biochemical measure, which is surprising given what the literature has demonstrated thus far. Albumin was one of two biochemical indices measured by Oudemans-van Straaten and colleagues, and was reported to be significantly lower in patients who were glutamine deplete compared to those with normal plasma concentrations.[24] A positive association between plasma glutamine and albumin was recently confirmed in 2015 by Pan et al., in their work with adult colorectal cancer patients.[153] In the paediatric cohort of Marino et al., albumin was reported to be normal on admission to PICU, and no association with plasma glutamine was found.[21] Combined with the results of this study, it appears as if the relationship between plasma glutamine and albumin that has been demonstrated in adults, has not yet been confirmed in children.

Marino and colleagues did, however, describe inverse relationships between plasma glutamine and lactate, as well as CRP, but found no relationship with Il-6, Il-10, and tumour necrosis factor (TNF) ά.[21] Inverse associations between plasma glutamine and markers of infection and inflammation (particularly Il-6[64] and CRP[154]) have been well established in adult literature. This was recently confirmed in a mixed cohort of critically ill adult patients by Neinaber et al., who demonstrated an inverse relationship between plasma glutamine and CRP, and a trend toward the same with the inflammatory marker Il-6.[58] Interestingly in the current study, PCT, CRP, and white cell count were inversely correlated to plasma glutamine - but not significantly so. The direction of these correlations is in keeping with existing literature, and could reflect raised glutamine utilisation by immune cells during periods of physiological stress. In this study, however, plasma glutamine concentrations were comparable among participants with normal and high markers of infection.

Stellenbosch University https://scholar.sun.ac.za

Although not consistently the case, elevated plasma glutamine concentrations have been identified in adult patients with liver failure[71] and chronic renal dysfunction.[73] Raised plasma glutamine concentrations were also recently demonstrated by Fadel et al. in children with chronic kidney disease, when compared to healthy controls.[72] No associations between plasma glutamine and urea, creatinine, or any of the liver enzymes, were demonstrated in this study. In the study by Marino et al., 81% of the paediatric patients included required renal support. However, no association was demonstrated between plasma glutamine and urea or creatinine.[21] Although Ekmark and colleagues focused on the development of MOF in relation to plasma glutamine, they did not mention the effect that specific organ failure had on plasma glutamine concentration.[22] Thus, the plasma glutamine response to liver failure seen in adults is yet to be corroborated in the paediatric population. In addition, observational studies have thus far not confirmed Fadel et al.'s[72] finding of elevated plasma glutamine among children with kidney disease. This may be because acute, not chronic, kidney injury occurs more frequently in a mixed PICU setting.

The work done by Marino et al. provides the most comprehensive analysis of plasma glutamine in relation to biochemical indices in paediatric patients.[21] Reasons for the differing results seen in this study could include cohort differences such as disease severity. At baseline, participants in this study demonstrated lower markers of infection and lactate, as well as more favourable markers of renal function, when compared with those in Marino et al.[21] This could suggest that the current cohort of mixed PICU patients were not, on average, as ill as the group with meningococcal disease. In addition, only routine biochemistry was available for analysis in this study - meaning that more specialist measures were limited. It is unclear if the results of this study would have been different with a larger sample of all biochemical indices.

Interestingly, this study demonstrated a trend toward a positive correlation with platelet count. In a series of biochemical papers, Vasta et al. described the active metabolism of glutamine by platelets.[155] Platelets are known to have high energy requirements, and the role of glucose as a fuel source for these cells is well established.[155,156] Glutamine has, however, also been identified as a source of energy for platelets - both in the presence or absence of glucose - and is also thought to play a role in the activation of these cells.[156] Most studies report on platelet count as a baseline measure of clinical condition rather than in relation to plasma glutamine concentration.[13,21] In light of the active oxidation of glutamine by platelets, and based on the trend found in this study, perhaps this biochemical parameter warrants further study.

Stellenbosch University https://scholar.sun.ac.za

Nutritional status

Prevalence of malnutrition

South Africa continues to face a high prevalence of childhood malnutrition. Based on the recent South Africa Demographic and Health Survey, an estimated 27% of children under the age of five years are stunted (10% of whom are severely stunted), 3% are wasted, 6% are underweight-for-age, and 13% are overweight.[157] This illustrates the double burden of nutrition South Africa; one of chronic under-, as well as over-nutrition.

Considering the prevalence of malnutrition among children in South Africa, this study sought to describe the anthropometrical parameters of study participants, and to identify potential associations between nutritional status and plasma glutamine. Twenty four percent of participants were found to be stunted. This was marginally lower than the national estimate, and was therefore largely expected. Interestingly, underweight-for-age and wasting were much higher among study participants at 37% and 32% respectively - despite only one patient having been admitted for malnutrition alone. Overweight and obesity in this cohort were only identified in 3% of participants, which is half the global estimate.

There is extensive literature which suggests that, worldwide, hospitalised children are at increased risk of malnutrition.[83,158] Research suggests that 6-14% of children in hospital are malnourished, and experience lengthier hospital stays and increased mortality as a result.[158] It makes sense that in a setting like South Africa where the national prevalence of malnutrition is high, sick and/or hospitalised children would be at even greater risk. In 2014, Brink et al. investigated the prevalence of malnutrition in 222 infants and children admitted to the medical paediatric ward of a tertiary hospital in South Africa. Patient age was comparable to the current study, ranging from one day to 14 years, comprising mainly of children below the age of 18 months (63%). The study found that 40.5% of participants were stunted, 33.3% were underweight for age, and 23.4% were wasted. Again, these findings were much higher than national estimates, and in the case of underweight-for-age and wasting, were more comparable to what was found in this study. No overweight or obese children were identified by Brink et al., which was in keeping with the low figures shown by this study.[159]

Interestingly, Brink and colleagues reported that HIV-infected children, who comprised 10.8% of study participants, had poorer nutritional status compared to non-exposed individuals.[159] In this study, 3.9% of participants were HIV-infected - a proportion unlikely to have been the sole cause for such high figures of underweight and wasting. Instead, both studies seem to reflect the growing problem of malnutrition

Stellenbosch University https://scholar.sun.ac.za

among African nations, from which similar figures of under-nutrition have been reported.[160,161] These far exceed estimates of malnutrition during hospitalisation in developed settings.[158]

Plasma glutamine and nutritional status

Some work has been done on the effect of glutamine supplementation on nutritional status.[85,162] However, far less attention has been paid to the interplay between nutritional status and plasma glutamine in patients not receiving exogenous glutamine. During critical illness it is generally accepted that glutamine depletion, rather than reflecting malnutrition, is likely the result of increased consumption of the nutrient in response to metabolic stress.[26] In this context, and likely also due to the difficulties faced in measuring anthropometry accurately in an ICU setting, many studies on plasma glutamine in paediatric[21,22] and adult[59] populations have not included nutritional status as part of their analysis.

Despite this, the question remains: what is the plasma glutamine status of malnourished individuals? Based on the prevalence of malnutrition found in the current study, it was an important question to ask. In this study, significant inverse relationships were demonstrated between day 0 plasma glutamine and weight, length/height, and MUAC. These relationships did not, however, account for age - meaning that the practical relevance of these findings is limited. When anthropometry was categorised into more meaningful data using Z scores, no significant differences in plasma glutamine were shown between participants who were underweight-for-age, stunted, or wasted compared to those who were not. Based on the results of this study, plasma glutamine appears unrelated to nutritional status.

This result has been corroborated by most of the adult literature. In their study on adult patients with gastrointestinal cancer, Hulst et al. made an early link between plasma glutamine and nutritional status. Glutamine extraction from different intestinal sites was investigated in relation to plasma glutamine concentration and nutritional status. Plasma glutamine was found to be positively associated with the percentage ideal body weight achieved, and to glutamine extraction from the small bowel. These results may have been influenced by the diagnostic group studied, in which increased consumption of glutamine by tumours and chronic malnutrition were likely.[68] This link has not however been demonstrated since. Instead, comparable BMI measurements have been shown among critically ill adults with low and normal plasma glutamine concentrations.[24] In their study of adult patients with colorectal cancer, Pan et al. also reported no significant associations between plasma glutamine and body weight, height, or BMI.[153] Similarly, in a cohort of patients with intestinal disease, plasma glutamine was shown to be unrelated to the participants' percentage weight loss or ideal body weight.[26]

Stellenbosch University https://scholar.sun.ac.za

Data on nutritional status and plasma glutamine in paediatrics are extremely scarce. In their work with infants and children with congenital heart disease, Villares and colleagues reported that preoperative plasma glutamine concentrations were independent of nutritional status. This result, among a population notorious for diminished nutritional status, is telling.[116] It appears, therefore, that most adult and paediatric (albeit limited) data support the findings of this study.

Nutritional intake

Nutritional requirements and intake

There has been a recent shift in the direction of nutrition support among critically ill children - with the focus of care being on the avoidance of both under- and over-feeding. The goal of nutrition support during acute illness is to provide sufficient nutrition, which supports the catabolic state and preserves lean body mass.[163] It remains unclear as to exactly how much substrate critically ill patients require, particularly as both hypo- and hyper-metabolism have been identified in this extremely heterogeneous group of patients.[130]

The collaborative document published by ASPEN and the Society of Critical Care Medicine (SCCM) in 2017 provides what is currently considered the gold standard for nutritional support in the PICU setting for infants and children aged one month to 18 years. According to these recommendations, energy requirements are most accurately determined using indirect calorimetry.[130] In the absence of this, energy requirements are admittedly an inexact science - with low-grade evidence to support specific predictive equations.[164] This paper recommends using the Schofield equation without the addition of stress factors to determine energy requirements, as alternative formulae are known to either over- or under-estimate energy needs.[130] In terms of protein requirements, an estimated 1.5 g/kg is thought to be sufficient to avoid a negative balance among most critically ill children.[130,163] This may, however, be significantly higher in infants.[163] Nutrient requirements for preterm infants and patients with burn injury are, however, known to be high. As such, these groups are excluded from the above recommendations. The nutritional requirements for preterm infants have been well documented.[131,132] In the case of patients with burns, predictive equations specific to this diagnosis are encouraged.[133,134]

Despite conservative estimates of nutrient requirements in this study, only 5% and 6.7% of participants reached full energy and protein requirements respectively. In addition, median achievement of caloric and protein needs was low at 30.8% and 36.2%. This phenomenon of inadequate nutrient provision is not unique to this study, or to this particular PICU. Optimal feeding practices are made difficult in the critical-care setting due to several factors. Haemodynamic instability, fluid restrictions, feed intolerance, and

Stellenbosch University https://scholar.sun.ac.za

obligatory periods of pre-operative fasting are some of the barriers faced.[165] Mehta et al. suggest that in light of these difficulties, the goal of 66% of energy requirements should be met by the end of the first week in PICU. Energy provision within this target is thought to reduce cumulative deficits and to improve clinical outcome.[130] Although the duration of this study did not allow for a full week's nutrition assessment, 16.7% of participants met this target within 48 hours of PICU admission.

In both adult[166] and paediatric[130] critical-care settings, enteral nutrition is well established as the preferred mode of feeding, and should be initiated within 24-48 hours of admission. In fact, a delayed approach to parenteral nutrition is advised in the well-nourished critically ill child by up to one week, as a means to prioritise enteral feeding and to avoid PN-related complications.[130] In this study, early initiation of enteral feeding was achieved for most (94.3%) patients, with only one patient receiving no enteral or parenteral nutrition in the first 48 hours of PICU stay. After 24 hours of admission, exclusive PN had been initiated in 4.1% of participants. Because this study included preterm infants and neonates, delayed initiation of PN was not applicable for all patients. In short, this study found that enteral feeding was initiated early, but that nutrient provision was suboptimal over the first 48 hours of PICU admission – despite the conservative estimation of requirements.

Plasma glutamine and nutritional intake

In this study, it made sense to evaluate potential associations between day two plasma glutamine and the nutritional intake of participants in the 48 hours prior to this. No associations were identified between plasma glutamine and nutritional intake. Based on Mehta et al.'s target of achieving 66% of energy requirements, differences in plasma glutamine were investigated between those who met this energy goal and those who did not.[130] Apart from a slight increase in plasma glutamine from admission to day two in those who achieved this target, no significant differences were found.

Most data relating to nutritional intake and glutamine stem from intervention studies in which glutamine was administered as a supplement.[79,80] As a result, the focus has been on the relationship between glutamine dosage and the subsequent plasma glutamine response. Very few studies have considered how energy and protein intake contribute to plasma glutamine concentration. In a small intervention among post-operative cardiac infants, Chaloupecky et al. investigated the effect of protein containing parenteral nutrition on plasma amino acid concentrations, when compared to intravenous glucose infusion. Despite the provision of 0.8 g/kg/day of amino acids to the intervention group on day one post-operatively, plasma glutamine concentrations were shown to significantly decline in both groups - suggesting that either nutritional intake is unrelated to plasma glutamine, or that the dose of protein administered was ineffective in maintaining plasma levels.[115] Based on the recommendations of Mehta et al., it can be argued

Stellenbosch University https://scholar.sun.ac.za

that the protein dose administered to these infants was suboptimal.[130] In this study, the average protein intake of participants was 0.5 g/kg/day. Despite this inadequate intake, normal plasma glutamine concentrations were maintained from admission to day two of PICU stay. This serves to confirm that in this study, plasma glutamine and nutritional intake were not associated.

Nutritional data from observational studies are also limited. In their iconic studies on plasma glutamine in adults, neither Oudemans-van Straaten et al.[24] nor Rodas et al.[59] included data on nutritional intake. In paediatrics, Emark and colleagues described their staged approach to nutrition - from hypocaloric feeding on day one of PICU stay to optimal nutrition support by day five, based on the patients' stress response.[22] Despite this background information, no nutritional data were analysed in relation to plasma glutamine. It would have been interesting to compare such data with the results of this study, as the approach to nutritional support was vastly different between cohorts.

Also in paediatrics, Marino et al. reported a total provision of 0.9 g/kg/day of protein, and found that total glutamine intake was significantly correlated with the time taken to reach full feeds. However, neither protein nor glutamine intake were analysed in relation to plasma glutamine.[21] This is likely because enteral glutamine has been reported to be an ineffective method of influencing plasma glutamine concentrations.[21,167] Therefore, the total intake of enteral glutamine was not calculated in the current study. Glutamine intake would also have been difficult to assess due to the variable dietary intake of participants. Most paediatric feeds have an l- glutamine content of 0.3 g/100ml, whereas standard infant formulae contain minimal amounts, mainly in the form of glutamate.[21] In this study, many older infants and children received a mixed hospital diet within 48 hours of PICU stay, which would have made the calculation of total glutamine intake challenging.

Clinical outcome

Markers of clinical outcome

In this study, as in many critical-care settings, the duration of mechanical ventilation, length of PICU and hospital stay, and mortality were used as markers of clinical outcome. This cohort was comparable to other observational studies on plasma glutamine in critically ill children, in that most participants were mechanically ventilated. PICU and hospital LOS, at an average of six and 10 days respectively, were comparatively shorter. However, at 10.8%, mortality was noticeably higher in this patient group when compared to the 4.7[21] to 5%[22] reported by other international cohorts.

Stellenbosch University https://scholar.sun.ac.za

Plasma glutamine and clinical outcome

Surprisingly few intervention studies on plasma glutamine supplementation among critically ill patients have measured plasma glutamine.[66,80] As a result, outcome measures are reported in relation to the efficacy of supplementation, rather than in relation to the plasma glutamine response.

There is compelling evidence from observational studies in adult patients that both low and high plasma glutamine concentrations on admission to ICU are independent risk factors for mortality.[24,59] There is limited information on what plasma glutamine concentrations during ICU stay mean, in terms of outcome.[146]

Far less is known about plasma glutamine as a predictor of clinical outcome among critically ill infants and children.[22] The observational studies by Marino et al.[21] and Ekmark et al.[22] provide the most robust data on clinical outcome and plasma glutamine in paediatric populations.

In this study, relationships were explored between clinical outcome and plasma glutamine on admission, day two, and the degree of change that occurred during this time. This was done to address the general gap in paediatric knowledge, but also to investigate if plasma glutamine during PICU stay is associated with clinical outcome.

— *Mechanical ventilation*

No association was found between plasma glutamine and the duration of mechanical ventilation. This is in contrast to an inverse relationship that was found between plasma glutamine and the length of mechanical ventilation in paediatric patients with meningococcal disease.[21] The relationship between mechanical ventilation dependency and glutamine has only been consistently explored post-supplementation of the nutrient. While some interventions have reported reduced dependency, others have shown a significantly increased duration of mechanical ventilation in those receiving glutamine.[77] It seems there is inconsistency in the reported relationship between plasma or supplemental glutamine and this outcome measure.

— *Length of Stay*

Observational studies have failed to show an association between plasma glutamine and LOS among critically ill adult patients who have not received exogenous glutamine.[24,59] In their intervention study of trauma patients, Perez-Barcena et al. were the first to identify an inverse relationship between plasma glutamine concentration and ICU and hospital LOS.[66] This relationship has also been identified in paediatrics. Plasma glutamine was found to be inversely correlated to PICU LOS,[21] and children with a low

Stellenbosch University https://scholar.sun.ac.za

plasma concentration of <420 umol/l tended to spend an excess of five days in the PICU.[22] These results suggest that the lower the plasma glutamine, the longer the PICU stay. In this study, plasma glutamine and PICU LOS appeared unrelated. However, a positive relationship was demonstrated between plasma glutamine on admission and hospital LOS. Although the significance of this relationship was lost when statistical outliers were removed (again raising questions over the validity of excluding outliers from a clinical setting), a trend was still evident. This finding is unlike others in adult and paediatric literature in its suggestion that the higher the plasma glutamine on presentation to the PICU, the longer the hospital stay.

This study also found that the less variability in plasma glutamine and glutamic acid during the first two days spent in PICU, the longer the hospital stay. This result has not been described before, but offers a glimpse into the potential importance of plasma glutamine change over time in relation to clinical outcome. Interestingly, the data from participants who died were excluded from LOS analyses. Most patients who died spent longer in PICU and in hospital compared to those who survived, and had the greatest variability in plasma glutamine between day 0 and day two. Therefore, the exclusion of this data may have skewed the above result. At face value, however, the results of this study suggest that a high plasma glutamine on admission, with little variation in plasma glutamine and glutamic acid concentrations over the first two days of PICU stay, place patients most at risk of increased hospital stay.

Participants in this study spent less time in the PICU and/or hospital compared to other study cohorts.[21,22] Reasons for this could include between-cohort differences in diagnostic profile and disease severity (as with meningococcal disease compared to a mixed PICU group) or due to differences in bed availability between individual units. In settings such as RCWMCH where resources are limited, demand for critical-care beds is high - often resulting in delayed admissions and early discharges. According to Murthy and Wunsch, differences in patient turnover between units have the ability to skew data on clinical outcome.[168] Whether this could have influenced the results of this study is unknown.

— *Mortality*

This study showed a tendency toward higher plasma glutamine concentration on day two among those who died. Elevated plasma glutamine has previously been reported in children with malaria who have died, when compared to those who survived.[169] One would assume that this result is in keeping with the positive associations found in this study between plasma glutamine, mortality risk, and hospital LOS. Because mortality and LOS stay data were kept independent from one another it is interesting that both results suggest poorer clinical outcome among those with higher plasma glutamine on admission.

Stellenbosch University https://scholar.sun.ac.za

There has been a suggestion that the association between mortality and plasma glutamine may not be unique to glutamine itself - but may reflect general changes in amino acid concentrations in critically ill patients.[146] In their work with septic patients, Hirose et al. found that an altered amino acid balance was associated with increased mortality. Maximum concentrations of glutamine, glutamate, phenylalanine, and histidine were shown to be significantly different between those who died compared to those who survived.[170] Interestingly, in this study, 57% and 50% of participants were shown to have high plasma glutamate and phenylalanine, respectively, on admission. This could, given more research, suggest a similar trend in paediatric populations as in adults. Most studies on plasma glutamine have traditionally focused on the amino acid independently, and/or in relation to glutamic acid. This finding may however prompt further studies on the interplay between different amino acids and clinical outcome.

Although no significant association was found between plasma glutamine concentration and mortality, this study was likely underpowered to detect one - had it existed. Mortality among paediatric cohorts is lower than among adults, making this a difficult outcome measure to use in research. Instead, some researchers have used alternatives such as the PELOD score as a proxy for mortality.[171] According to this score, low plasma glutamine on admission to PICU, as well as reduced concentrations over time, were associated with the development of MOF.[22] This reinforces the point that a potential link between plasma glutamine status and mortality could, up until this point, have been overlooked by underpowered studies. In addition, Rodas and colleagues reported a link between plasma glutamine and six-month mortality in adults.[59] The only study in paediatrics to have extended beyond the hospital period was Marino et al.[21] Therefore, a potential association between plasma glutamine and mortality post-discharge has not been sufficiently investigated.

Summary of plasma glutamine and clinical outcome

Seen together, the results of this study, as well as those by Marino et al.[21] and Ekmark et al.,[22] imply that paediatric patients with both low and high plasma glutamine on presentation to PICU may be more prone to increased morbidity and poorer outcome. This potential U-shaped curve in morbidity is how Rodas and colleagues described the relationship between plasma glutamine and mortality in adults.[59] There are still many gaps in this argument, one of which is the low number of participants actually presenting with high plasma glutamine in this study and in others[22,59] - which makes firm conclusions hard to draw. Apart from plasma glutamine concentration on admission to the PICU, it appears as if the degree to which plasma levels change over time may be significant in terms of outcome. Rigorous research will be required to confirm the exact relationship between plasma glutamine and clinical outcome in critically ill infants and children.

Stellenbosch University https://scholar.sun.ac.za

Strengths and limitations of the study

Strengths

1. This study was exploratory, and provided a novel opportunity to investigate plasma glutamine concentrations among critically ill infants and children in RCWMCH. No study of this type has been conducted in South Africa or in Africa.
2. Concentrations of plasma glutamine were measured on admission and on day two of PICU stay. This allowed for investigation into the change that occurred in plasma glutamine over the first 48 hours of critical illness – something which has not been reported on before in a mixed population of critically ill children.
3. Both plasma glutamine as an independent measure, as well as the combined concentration of plasma glutamine and glutamic acid, were included in all statistical analyses. This allowed for comparison between studies which focused on the amino acid independently, as well as those reporting on both amino acids. This also ensured that any spontaneous conversion of plasma glutamine to glutamic acid was accounted for, thus avoiding falsely low plasma glutamine concentrations.
4. In order to maintain the integrity of the blood samples collected for study purposes, a rigorous, standardised protocol was followed with regard to collection, storage, and analysis. Once collected, samples were sent (time sensitively) to the laboratory, where they were stored at -80°C, and were later batch analysed – thereby avoiding undue degradation of the specimens, as well as significant differences in storage time.
5. Accurate anthropometry was conducted on participants by a registered dietitian as soon as was clinically possible, and once oedema had resolved. As a result, estimated measurements of weight and length or height were not used. This strengthens the study's findings with regard to the nutritional status of participants.
6. The PIM 3 scores of each participant were cross checked by a consultant paediatrician before being recorded for study purposes. In addition, a previous version of the same tool has been validated in the RCWMCH setting. Again, this strengthens the accuracy of the study's findings with regard to severity of disease and mortality risk.

Limitations

1. As with all observational work, the findings of this study are merely a representation of the cohort that was observed and cannot be extrapolated further.
2. Sample size was largely governed by the availability of study funds, with aminogram analysis proving costly. As in most research, additional study participants would have been of statistical benefit – by

Stellenbosch University https://scholar.sun.ac.za

increasing power and avoiding Type II error. In addition, the heterogeneity of diagnoses and ages of participants further reduced the power of the study to detect between-group differences.

3. Consecutive sampling has the potential for bias.[172] By recruiting participants over one month during summer time, the diagnostic profile of the study sample may have reflected seasonal differences. For example, from anecdotal evidence, RCWMH PICU admits more pulmonology (medical) cases over the winter months when compared to the summer months.
4. Patients who were transferred to other wards before blood sampling was possible were excluded, as were those who did not require routine phlebotomy during their PICU stay. It can therefore be argued that the patients who were less ill were omitted from the study, which could potentially have skewed the results.
5. Biochemistry was included in this study as a marker of clinical condition on admission. In order to minimise additional blood sampling for participants, only routine biochemistry was available and specific biochemical values could not be requested. This reduced the study's ability to investigate the relationship between plasma glutamine and specific markers of infection and of renal and hepatic function more thoroughly.
6. Unlike other observational studies on plasma glutamine among critically ill children, this study did not include a group of healthy controls. This could be considered a limitation, as comparison between healthy and acutely ill children in this particular setting could not be made. However, because most study participants demonstrated normal plasma glutamine concentrations, this argument could be negated.
7. Ideally, this study would have included additional plasma glutamine measurements – both later in the course of PICU stay and also during convalescence. This would have allowed for comparison between other studies with regard to plasma glutamine response beyond the stage of acute illness. This would also have enabled some investigation into the possibility that the release of glutamine from skeletal muscle during critical illness may initially increase or maintain plasma levels – thus not providing an accurate representation of whole body stores. Again, the number of aminogram analyses was dictated by funding limitations and ethical considerations with regard to additional blood sampling.
8. Because mortality is comparatively low among critically ill paediatric populations versus adults, it would have been useful to include an alternative outcome measure (such as the development of MOF), which could have served as a proxy for mortality. Because of a small sample size, significant associations between plasma glutamine and mortality could have been missed. As a result, this study allowed only for commentary on what was observed between those who died versus those who survived.

Stellenbosch University https://scholar.sun.ac.za

9. No outcome data were collected with regard to the development of organ failure. This prevented further investigation into the elevations in plasma glutamine seen in patients with renal and liver failure.

Rejection or failure to reject null hypotheses

Based on the results of this study, the following null hypotheses were accepted:

1. Plasma amino acid concentrations do not change during the first two days of PICU stay.
2. No relationship exists between plasma glutamine concentration and the nutritional status (determined by age-related Z scores) of children in PICU.
3. No relationship exists between plasma glutamine concentration and dietary protein or energy intake during the first two days of PICU stay.
4. No relationship exists between plasma glutamine and mortality.

The remaining null hypotheses were rejected:

5. No relationship exists between plasma glutamine concentration and clinical condition on admission to PICU.
6. No relationship exists between plasma glutamine concentration and PICU or hospital LOS.

Stellenbosch University https://scholar.sun.ac.za

6. Conclusion

The aim of this cross-sectional study was to describe plasma glutamine concentrations of critically ill infants and children on admission to and during the first two days of PICU stay. Furthermore, the study sought to identify associations between plasma glutamine and markers of clinical condition on admission (diagnosis, presence or absence of infectious disease, severity of disease, and biochemistry profile), nutritional status, dietary intake, and indicators of clinical outcome (duration of mechanical ventilation, PICU and hospital LOS, and mortality). Plasma glutamine was expressed both as an independent measure, and as the combination of plasma glutamine and glutamic acid, to account for the conversion of glutamine to glutamate during processing.

For most participants, plasma glutamine concentration fell within normal limits on both admission (median 556.5 umol/l, IQR 459-664.5 umol/l) and on day two of PICU stay (median 529.0 umol/l, IQR 356.0-716.0 umol/l). Relatively few (n = 13, 17.1%) participants presented with low plasma glutamine on admission, and even fewer demonstrated elevated concentrations (n = 4, 5.3%). No significant changes in plasma glutamine occurred over the first 48 hours of PICU stay. This suggests that participants who were glutamine deplete on admission were more likely to have low concentrations 48 hours into their PICU stay. Based on these results, the null hypothesis regarding plasma glutamine concentration was accepted. The findings of this study were unexpected, as previous observational research found glutamine depletion among greater proportions of children on admission to PICU. In addition, previous studies showed some improvement in plasma glutamine over time.

Plasma glutamine on presentation to the PICU was investigated in relation to the diagnostic profile of participants. The cohort was largely made up of elective surgical patients (n = 41, 53.9%), and was heavily weighted by cardiology as a primary diagnosis (n = 37, 47%). Some of the most significant findings of this study were the differences shown in plasma glutamine concentrations between diagnostic categories: medical and elective surgical (p = 0.007) and medical and trauma diagnoses (p = 0.013), in particular. On presentation to the PICU, participants admitted for trauma demonstrated the lowest plasma glutamine (mean 450.3 ± 166.7 umol/l), and medical diagnoses the highest (800.1 ± 317.6 umol/l). Low plasma glutamine was demonstrated in 50% (n = 3) of trauma admissions – highlighting this subgroup as the most vulnerable to glutamine depletion. Again, these findings are not corroborated by existing literature on adults, which demonstrates medical patients to be the most susceptible to glutamine depletion on presentation to the ICU.

Differences in plasma glutamine and glutamic acid were also evident between the primary diagnoses cardiology and gastroenterology (p = 0.018), and between sepsis and pulmonology (p = 0.031), burns (p =

Stellenbosch University https://scholar.sun.ac.za

0.035), gastroenterology (p = 0.006), and 'other' (p = 0.049). Participants admitted for sepsis were observed to have the highest plasma glutamine (736.3 ± 142.5 umol/l), whereas gastroenterology diagnoses had the lowest (432.4 ± 149.2 umol/l). These findings deviate from existing observational research, which demonstrates low plasma glutamine among septic adult patients. Although less is known in paediatrics, there has been a similar suggestion of glutamine depletion among septic patients. In this study, post-operative cardiology cases, which comprised nearly half the participants, had normal plasma glutamine concentrations on day 0 and day two. Again, this is a novel finding, as existing literature documents low plasma glutamine in the post-operative period. Based on the finding that plasma glutamine concentration differs according to diagnostic profile, the null hypothesis with respect to clinical condition on admission was rejected.

Based on severity of disease scoring, this study reported a median risk of participant mortality of 1.4% (IQR 0.8-3.9%). Plasma glutamine tended to be lower among those with reduced risk of mortality (Spearman r = 0.22, p = 0.052). Similarly, a positive association was found between a combined value of plasma glutamine and glutamic acid and mortality risk (Spearman r = 0.23, p = 0.046). Both correlations were, however, weak and significance was lost with the removal of outliers. Although these findings provide a mere suggestion of an association between mortality risk and plasma glutamine – no such relationship has been identified in adult or paediatric literature.

No further associations were demonstrated between plasma glutamine and other markers of clinical condition. In terms of infectious disease, most participants were HIV (n = 72, 94.7%) and TB (n = 71, 93.4%) negative - although at 3.9% (n= 3) the prevalence of HIV was double the national estimate. Plasma glutamine on admission was comparable between those who were infected and those who were not. This was the first study to investigate the relationship between plasma glutamine concentration and infectious disease in critically ill children.

This study relied on routine biochemistry as a measure of clinical condition on admission. No associations were demonstrated between plasma glutamine and any biochemical indices – including markers of infection, albumin, lactate, and measures of renal and hepatic functioning. However, participants with lower platelet counts tended to have lower plasma glutamine on admission (Spearman r = 0.22, p = 0.058). Although the link between glutamine as a fuel source for platelets has been established, no association between plasma glutamine and platelets among critically ill patients has been reported to date. Glutamine depletion has previously been associated with low albumin, and with elevated lactate, Il-6, and CRP.

Under-nutrition of participants in this study exceeded national estimates, with 37% (n = 23) of children presenting underweight-for-age, 24.2% (n = 15) stunted, and 32.2% (n = 20) wasted. Over-nutrition was

Stellenbosch University https://scholar.sun.ac.za

significantly lower at 3.2% (n = 2). No association was found between plasma glutamine and nutritional status – a finding that has been supported by existing research in adults and children. Instead, plasma glutamine depletion during critical illness is thought to be the result of increased demand for the nutrient during metabolic stress. In light of these results, the null hypothesis regarding nutritional status and plasma glutamine was accepted.

Although early enteral feeding was established for most (n = 68, 94.3%) participants in this study, energy and protein requirements were met by only 5% (n = 3) and 6.7% (n = 4) respectively in the first 48 hours of PICU stay. Inadequate nutritional intake is well documented in PICU settings and was not unique to this study. No relationship between plasma glutamine and nutritional intake was observed. As a result, the relevant null hypothesis was accepted. Limited research exists on nutritional intake and plasma glutamine concentration, with most data stemming from interventions that administered glutamine as a supplement. In children, plasma glutamine has been shown to reduce significantly despite the administration of 0.8 g/kg/day of protein. In contrast, with a lower protein intake of 0.5 g/kg/day in this study, plasma glutamine remained on average, normal – thereby confirming that plasma glutamine status and nutritional intake appear to be unrelated.

Participants in this study spent on average 6 (±10) and 10 days (IQR 7-20 days) in the PICU and in hospital respectively, and most (n = 56, 74.7%) required ventilatory support. Eight children (10.5%) died during the course of this study. No associations were demonstrated between plasma glutamine and the duration of mechanical ventilation or PICU LOS. A positive association was identified between plasma glutamine on admission and hospital LOS (Spearman r = 0.26, p = 0.032), although only a trend existed when statistical outliers were removed (Spearman r = 0.23, p = 0.067). These findings differ from existing research in children, which has shown a link between glutamine depletion and PICU, not hospital, LOS. An interesting and inexplicable finding of this study was the inverse relationship between the change that occurred in plasma glutamine and glutamic acid and hospital LOS (Spearman r = -0.34, p = 0.046). This has not been described elsewhere but suggests that the change which occurs in plasma concentrations over time may have some significance in terms of outcome. Based on these results, the null hypothesis regarding PICU and hospital LOS was rejected.

Although no association was found between plasma glutamine and mortality, this study was likely underpowered to detect one – had it existed. Adult studies have established an association between plasma glutamine on admission to ICU and mortality. Conclusions regarding mortality are more difficult to achieve in paediatric cohorts, as mortality rates tend to be lower and research studies smaller. A notable observation in this study, however, was that participants who died tended to have higher plasma glutamine

Stellenbosch University https://scholar.sun.ac.za

on day two of PICU stay (p = 0.057). Despite this, the null hypothesis relating to plasma glutamine and mortality was accepted.

This study was strengthened by rigorous laboratory protocols, measurement of plasma glutamine both independently and in combination with glutamic acid, and through accurate anthropometry and severity of disease scoring. The exploratory nature of the study also meant that it could expand on the limited knowledge base within this field. It was weakened, however, by the small, heterogeneous sample size, consecutive sampling method, limited number of plasma glutamine measures per participant, the reliance on routine biochemistry, and on the absence of a healthy control group. In addition, as with all observational work, the findings of this study cannot be extrapolated further.

In summary, this study demonstrates normal plasma glutamine among the majority of infants and children admitted to the RCWMCH PICU over a one-month period, with no significant concentration changes occurring over the first 48 hours of PICU stay. The findings of this study do not, therefore, support routine supplementation among critically ill children. The difference in plasma glutamine concentration shown between diagnostic groups is an important finding, with trauma and sepsis diagnoses emerging most susceptible to lower and higher concentrations respectively. This study also provides some suggestion that high plasma glutamine concentrations during the acute phase of critical illness may be associated with increased mortality risk and poorer outcome. When combined with existing data in paediatrics, this could suggest that – as in adult populations – both low and high plasma glutamine concentrations are associated with adverse effect. Extensive research is, however, needed to provide clarity on the relationship between plasma glutamine and clinical outcome.

In their review article, Smedberg and Werneman asked the question – "Is the glutamine story over?"[146] In paediatrics, with such limited data available, and in light of the findings of this study, it appears as if the story has barely begun.

Stellenbosch University https://scholar.sun.ac.za

7. Recommendations

1. Clinical recommendation

This study found that plasma glutamine concentrations were normal on admission and on day two of PICU stay. This result does not support routine supplementation of glutamine to critically ill children.

2. Research recommendations

Most data on glutamine in paediatric populations stem from intervention studies, with surprisingly few having measured plasma concentration prior to supplementation. The safety of blanket supplementation of glutamine has, however, recently been questioned in adult literature. As a result, there is a need for increased understanding of plasma glutamine concentrations in specific populations. There is a paucity of data from observational studies on plasma glutamine concentration, particularly among critically ill children. As such, more observational research in this group is required before further interventions can take place.

2.1 Based on the findings of this study, the following areas require further research in relation to plasma glutamine:

2.1.1. Diagnostic profile

Observational studies on plasma glutamine in critically ill children have, to date, included participants with different diagnostic profiles – making comparison difficult. Studies conducted in mixed PICU settings (such as this one) include heterogeneous patient populations, making it challenging to identify between-group differences. Research into specific diagnostic groups will allow for firmer conclusions to be drawn. The findings of this study would suggest that trauma and sepsis diagnoses warrant further study to confirm whether these groups are susceptible to more extreme plasma concentrations, and if the glutamine status of children within these diagnostic groups is consistently different to adult populations.

2.1.2. Severity of disease and mortality risk

Only one previous observational study in paediatrics included severity of disease scoring – using a different tool to the PIM 3 applied in this study. Because scoring systems are not directly equivalent, the standardised use of a scoring system across studies would allow for meaningful comparison. This study suggested a potential association between plasma glutamine and mortality risk. Because so few have investigated this relationship, more attention is certainly warranted.

Stellenbosch University https://scholar.sun.ac.za

2.1.3. Markers of renal and hepatic functioning

Evidence from adult populations suggests that plasma glutamine is elevated during liver and chronic renal failure, however limited evidence for this exists in children. This study found no association between plasma glutamine and biochemical markers of renal and hepatic functioning – but was limited by the fact that many patients did not have these indices measured during their stay. A combination of biochemical testing and the diagnosis of renal or hepatic failure would be useful to include in future studies on plasma glutamine in children.

2.1.4. Markers of infection

The relationship between plasma glutamine and biochemical markers of infection needs further study, with only one observational study in children having demonstrated an inverse relationship between CRP and plasma glutamine. This study observed inverse but non-significant associations between markers of infection and plasma glutamine – but again, data were limited.

2.1.5. Clinical outcome

Because death rates are lower among children compared to adults, mortality is a difficult outcome measure to use in paediatric research. In fact, many studies, including this one, are underpowered to detect significant changes in mortality. Large, sufficiently powered observational studies are required to establish if, as in adults, a link between plasma glutamine and mortality exists. Failing this, more attention needs to be given to outcome measures such as the PELOD score, which could be used as a proxy for mortality.

Observational studies in children have, to date, only established an inverse relationship between plasma glutamine and PICU LOS. This study suggested that higher plasma glutamine resulted in a longer hospital stay. Both these relationships require further study.

2.2 With regard to plasma glutamine concentration, the results of this study suggest that more attention be given to:

2.2.1 Both low and high plasma glutamine concentrations

Unlike other research, this study did not identify plasma glutamine depletion as a significant problem among critically ill children. It did, however, suggest that higher plasma glutamine may be associated with mortality risk, hospital LOS, and could be more prevalent in those who die. A link between mortality and low and high plasma glutamine has been established in adults, and the association between glutamine depletion and morbidity has been identified in children. This, in addition to what has been demonstrated in this study, suggests that attention needs to be paid to

Stellenbosch University https://scholar.sun.ac.za

both low and high plasma glutamine concentration – particularly with regard to mortality risk scoring and clinical outcome. This will provide more definitive answers regarding the need for, and safety of, glutamine supplementation in paediatric populations.

2.2.2 Changes in plasma glutamine over time

The clinical significance of the direction and degree of plasma glutamine change over time should also be explored in more detail. There is a discrepancy in the existing literature over the direction of plasma glutamine change over time. In certain adult populations, plasma glutamine has been shown to remain low for some time, whereas gradual improvement has been demonstrated in children. Direct comparison is, however, made difficult by the different time-frames used by each study. This study found an association between plasma glutamine (in combination with glutamic acid) change and hospital LOS – suggesting that the degree of change may be clinically relevant. In short, more information is required regarding how plasma glutamine changes over time, and if this is clinically important.

2.2.3 Measurement of plasma glutamine

Because glutamine spontaneously converts to glutamate, there remains uncertainty about whether to measure plasma glutamine independently or in combination with glutamic acid. Existing observational and intervention studies have employed different approaches. While a combined measure of plasma glutamine and glutamic acid may avoid falsely low concentrations, there are, as yet, no defined reference ranges for the sum of these two amino acids. This study used both methods to measure plasma glutamine and found different results between the two. It is therefore extremely important that consensus is achieved over how best to measure plasma glutamine, and that studies employ a standardised approach going forward. Only then can meaningful evaluation of the literature begin.

2.2.4 Reference ranges for plasma glutamine

Similarly, there is no unanimity over the reference ranges for plasma glutamine in children. Age-specific reference ranges exist, but are not reported on in the paediatric literature. Instead, some have utilised cut offs established in adult studies based on clinical outcome – cut offs that have been confirmed by only one paediatric study. As a result, plasma glutamine concentrations are categorised differently across the literature, making conclusions hard to draw. Consensus is again required, and a uniform approach is needed.

Stellenbosch University https://scholar.sun.ac.za

8. Reference List

1. Meister A. Metabolism of glutamine. Physiol Rev. 1956;36:103–27.
2. Meynial-Denis D. Glutamine: biochemistry, physiology, and clinical applications. Boca Raton: CRC Press; 2017.
3. Krebs HA. Metabolism of amino acids: deamination of amino acids. Biochem J. 1935;29(7):1620–44.
4. Goodman AD, Fuisz RE, Cahill GF. Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. J Clin Invest. 1966;45(4):612–9.
5. Pozefsky T, Felig P, Tobin JD, Soeldner JS, Cahill GF. Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. J Clin Invest. 1969;48(12):2273–82.
6. Windmueller HG, Spaeth AE. Uptake and metabolism of plasma glutamine by the small intestine. J Biol Chem. 1974;249(16):5070–9.
7. Windmueller HG, Spaeth AE. Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. Arch Biochem Biophys. 1975;171(2):662–72.
8. Hammarquist F, Werneman J, Ali R, Von Der Decken A, Vinnars E. Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. Ann Surg. 1989;209(4):455–61.
9. Stehle P, Mertes N, Puchstein C, Zander J, Albers S, Lawin P, et al. Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. Lancet. 1989;333(8632):231–3.
10. Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: A systematic review of the evidence. Crit Care Med. 2002;30(9):2022–9.
11. Singer P, Berger MM, Van den Berghe G, Biolo G, Calder P, Forbes A, et al. ESPEN Guidelines on parenteral nutrition: intensive care. Clin Nutr. 2009;28(4):387–400.
12. McClave SA, Martindale RG, Vanek VW, McCarthy M, Roberts P, Taylor B, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). J Parenter Enter Nutr. 2009;33(3):277–316.
13. Heyland D, Muscedere J, Wischmeyer PE, Cook D, Jones G, Albert M, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med. 2013;368(16):1489–97.
14. Heyland DK, Dhaliwal R. Role of glutamine supplementation in critical illness given the results of the REDOXS study. J Parenter Enter Nutr. 2013;37(4):442–3.
15. Stehle P, Ellger B, Kojic D, Feuersenger A, Schneid C, Stover J, et al. Glutamine dipeptide-supplemented parenteral nutrition improves the clinical outcomes of critically ill patients: A systematic evaluation of randomised controlled trials. Clin Nutr. 2017;17:75–85.
16. Wernerman J. How to understand the results of studies of glutamine supplementation. Crit Care. 2015;19:385–8.
17. Wischmeyer PE, Dhaliwal R, McCall M, Ziegler TR, Heyland DK. Parenteral glutamine

Stellenbosch University https://scholar.sun.ac.za

supplementation in critical illness: A systematic review. Crit care. 2014;18(2):76–93.

18. Wernerman J. Glutamine supplementation to critically ill patients? Crit Care. 2014;18(2):214–20.

19. Oldani M, Sandini M, Nespoli L, Coppola S, Bernasconi DP, Gianotti L. Glutamine supplementation in intensive care patients. Medicine. 2015;94(31):E1319-32.

20. Mok E, Hankard R. Glutamine supplementation in sick children: Is it beneficial? J Nutr Metab. 2011;2011:617597.

21. Marino L V, Pathan N, Meyer R, Wright V, Habibi P. Glutamine depletion and heat shock protein 70 (HSP70) in children with meningococcal disease. Clin Nutr. 2014;33(5):915–21.

22. Ekmark L, Rooyackers O, Wernerman J, Fläring U. Plasma glutamine deficiency is associated with multiple organ failure in critically ill children. Amino Acids. 2015;47(3):535–42.

23. Newsholme P, Lima MMR, Procopio J, Pithon-Curi TC, Doi SQ, Bazotte RB, et al. Glutamine and glutamate as vital metabolites. Brazilian J Med Biol Res. 2003;36(2):153–63.

24. Oudemans-van Straaten HM, Bosman RJ, Treskes M, van der Spoel HJ, Zandstra DF. Plasma glutamine depletion and patient outcome in acute ICU admissions. Intensive Care Med. 2001;27(1):84–90.

25. Kim M, Wischmeyer PE. Glutamine. In: Singer P, editor. Nutrition in intensive care medicine: Beyond physiology. Basel: Karger; 2012. p. 90–6.

26. Soeters PB, Grecu I. Have we enough glutamine and how does it work? A clinician's view. Ann Nutr Metab. 2012;60(1):17–26.

27. Labow BI, Abcouwer SF, Lin CM, Souba WW. Glutamine synthetase expression in rat lung is regulated by protein stability. Am J Physiol. 1998;275(5 Pt 1):877–86.

28. Labow BI, Souba WW, Abcouwer SF. Mechanisms governing the expression of the enzymes of glutamine metabolism—glutaminase and glutamine synthetase. J Nutr. 2001;131(9 Suppl):S2467-2474.

29. Stipanuk M, Caudill M. Biochemical, physiological, and molecular aspects of human nutrition. 3rd ed. St. Louis: Elsevier; 2013.

30. Häussinger D, Graf D, Weiergräber OH. Glutamine and cell signaling in liver. J Nutr. 2001;131(9 Suppl):S2509-2514.

31. Watford M. Glutamine and glutamate: Nonessential or essential amino acids? Anim Nutr. 2015;1(3):119–22.

32. Lenders CM, Liu S, Wilmore DW, Sampson L, Dougherty LW, Spiegelman D, et al. Evaluation of a novel food composition database that includes glutamine and other amino acids derived from gene sequencing data. Eur J Clin Nutr. 2009;63(12):1433–9.

33. Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: Cell biology, physiology, and clinical opportunities. J Clin Invest. 2013;123(9):3678–84.

34. Ligthart-Melis GC, van de Poll MC, Boelens PG, Dejong CH, Deutz NE, van Leeuwen PA. Glutamine is an important precursor for de novo synthesis of arginine in humans. Am J Clin Nutr. 2008;87(5):1282–9.

35. Curi R, Lagranha CJ, Doi SQ, Sellitti DF, Procopio J, Pithon-Curi TC, et al. Molecular mechanisms of glutamine action. J Cell Physiol. 2005;204(2):392–401.

Stellenbosch University https://scholar.sun.ac.za

36. Kadowaki M, Kanazawa T. Amino acids as regulators of proteolysis. J Nutr. 2003;133(6 Suppl 1):S2052-2056.

37. Kalhan SC, Edmison JM. Effect of intravenous amino acids on protein kinetics in preterm infants. Curr Opin Clin Nutr Metab Care. 2007;10(1):69–74.

38. Lavoinne A, Husson A, Quillard M, Chédeville A, Fairand A. Glutamine inhibits the lowering effect of glucose on the level of phosphoenolpyruvate carboxykinase mRNA in isolated rat hepatocytes. Eur J Biochem. 1996;242(3):537–43.

39. Bakalar B, Duška F, Pachl J, Frič M, Otahal M, Pažout J, et al. Parenterally administered dipeptide alanyl-glutamine prevents worsening of insulin sensitivity in multiple-trauma patients. Crit Care Med. 2006;34:381–6.

40. Déchelotte P, Hasselmann M, Cynober L, Allaouchiche B, Coëffier M, Hecketsweiler B, et al. L-alanyl-L-glutamine dipeptide–supplemented total parenteral nutrition reduces infectious complications and glucose intolerance in critically ill patients: The French controlled, randomized, double-blind, multicenter study. Crit Care Med. 2006;34(3):598–604.

41. Scalise M, Pochini L, Galluccio M, Indiveri C. Glutamine transport. From energy supply to sensing and beyond. Biochim Biophys Acta. 2016;1857(8):1147–57.

42. Stehle P, Kuhn KS. Glutamine: An obligatory parenteral nutrition substrate in critical care therapy. Biomed Res Int. 2015;2015:545467.

43. Kim MH, Kim H. The roles of glutamine in the intestine and its implication in intestinal diseases. Int J Mol Sci. 2017;18(5):1051–66.

44. Newsholme E, Cari A. Quantitative aspects of glucose and glutamine metabolism by intestinal cells. Gut. 1994;35(1 Suppl):S13-17.

45. Rhoads JM, Argenzio RA, Chen W, Rippe RA, Westwick JK, Cox AD, et al. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. Am J Physiol. 1997;272(5 Pt 1):943–53.

46. Ko T, Beauchamp R, Townsend C, Thompson J. Glutamine is essential for epidermal growth factor-stimulated intestinal cell proliferation. Surgery. 1993;114:147–53.

47. Li N, Lewis P, Samuelson D, Liboni K, Neu J. Glutamine regulates Caco-2 cell tight junction proteins. Am J Physiol. 2004;287(3):G726-733.

48. DeMarco VG, Li N, Thomas J, West CM, Neu J. Glutamine and barrier function in cultured Caco-2 epithelial cell monolayers. J Nutr. 2003;133(7):2176–9.

49. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004;134(3):489–92.

50. Wischmeyer P, Jayakar D, Williams U, Singleton K, Riehm J, Bacha E, et al. Single dose of glutamine enhances myocardial tissue metabolism, glutathione content, and improves myocardial function after ischemia-reperfusion injury. J Parenter Enter Nutr. 2003;27(6):396–403.

51. Thomas S, Prabhu R, Balasubramanian KA. Surgical manipulation of the intestine and distant organ damage—protection by oral glutamine supplementation. Surgery. 2005;137(1):48–55.

52. Roth E. Nonnutritive effects of glutamine. J Nutr. 2008;138(10 Suppl):S2025-2031.

53. Wischmeyer PE. Glutamine: Mode of action in critical illness. Crit Care Med. 2007;35(9 Suppl):S541-544.

Stellenbosch University https://scholar.sun.ac.za

54. Liu G, Ren W, Fang J, Hu CAA, Guan G, Al-Dhabi NA, et al. l-Glutamine and l-arginine protect against enterotoxigenic Escherichia Coli infection via intestinal innate immunity in mice. Amino Acids. 2017;49(12):1945–54.

55. Ogura H, Hashiguchi N, Tanaka H, Koh T, Noborio M, Nakamori Y, et al. Long-term enhanced expression of heat shock proteins and decelerated apoptosis in polymorphonuclear leukocytes from major burn patients. J Burn Care Rehabil. 2002;23(2):103–9.

56. Duggan C, Gannon J, Walker WA. Protective nutrients and functional foods for the gastrointestinal tract. Am J Clin Nutr. 2002;75(5):789–808.

57. Weitzel L, Wischmeyer PE. Glutamine in critical illness: The time has come, the time is now. Crit Care Clin. 2010;26(3):515–25.

58. Nienaber A, Dolman RC, van Graan AE, Blaauw R. Prevalence of glutamine deficiency in ICU patients: A cross-sectional analytical study. Nutr J. 2015;15(1):73–82.

59. Rodas PC, Rooyackers O, Hebert C, Norberg Å, Werne rman J. Glutamine and glutathione at ICU admission in relation to outcome. Clin Sci. 2012;122(12):591–7.

60. Buter H, Bakker AJ, Kingma WP, Koopmans M, Boerma EC. Plasma glutamine levels in patients after non-elective or elective ICU admission: An observational study. BMC Anesthesiol. 2016;16:15–20.

61. van Acker B, Hulsewé K, Wagenmakers A, Soeters P, von Meyenfeldt M. Glutamine appearance rate in plasma is not increased after gastrointestinal surgery in humans. J Nutr. 2000;130(6):1566–71.

62. Essen P, Wernerman J, Sonnenfeld T, Thunell S, Vinnars E. Free amino acids in plasma and muscle during 24 hours post-operatively--a descriptive study. Clin Physiol. 1992;12(2):163–77.

63. Viggiano E, Passavanti MB, Pace MC, Sansone P, Spaziano G, Viggiano A, et al. Plasma glutamine decreases immediately after surgery and is related to incisiveness. J Cell Physiol. 2012;227(5):1988–91.

64. Parry-Billings M, Baigrie RJ, Lamont PM, Morris PJ, Newsholme EA. Effects of major and minor surgery on plasma glutamine and cytokine levels. Arch Surg. 1992;127(10):1237–40.

65. Roth E, Zöch G, Schulz F, Karner J, Mühlbacher F, Hamilton G, et al. Amino acid concentrations in plasma and skeletal muscle of patients with acute hemorrhagic necrotizing pancreatitis. Clin Chem. 1985;31(8):1305–9.

66. Pérez-Bárcena J, Marsé P, Zabalegui-Pérez A, Corral E, Herrán-Monge R, Gero-Escapa M, et al. A randomized trial of intravenous glutamine supplementation in trauma ICU patients. Intensive Care Med. 2014;40(4):539–47.

67. Parry-Billings M, Evans J, Calder PC, Newsholme EA. Does glutamine contribute to immunosuppression after major burns? Lancet. 1990;336(8714):523–5.

68. van der Hulst RR, von Meyenfeldt MF, Deutz NE, Soeters PB. Glutamine extraction by the gut is reduced in depleted patients with gastrointestinal cancer. Ann Surg. 1997;225(1):112–21.

69. Roth E, Funovics J, Mühlbacher F, Schemper M, Mauritz W, Sporn P, et al. Metabolic disorders in severe abdominal sepsis: Glutamine deficiency in skeletal muscle. Clin Nutr. 1982;1(1):25–41.

70. Clemmensen J, Kondrup J. Splanchnic and leg exchange of amino acids and ammonia in acute liver failure. Gastroenterology. 2000;118(6):1131–9.

71. Helling G, Wahlin S, Smedberg M, Pettersson L, Tjäder I, Norberg Å, et al. Plasma glutamine concentrations in liver failure. PLoS One. 2016;11(3):E0150440.

Stellenbosch University https://scholar.sun.ac.za

72. Fadel FI, Elshamaa MF, Essam RG, Elghoroury EA, El-Saeed GSM, El-Toukhy SE, et al. Some amino acids levels: Glutamine,glutamate, and homocysteine, in plasma of children with chronic kidney disease. Int J Biomed Sci. 2014;10(1):36–42.

73. Tizianello A, De Ferrari G, Garibotto G, Gurreri G, Robaudo C. Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. J Clin Invest. 1980;65(5):1162–73.

74. Souba WW. Total parenteral nutrition with glutamine in bone marrow transplantation and other clinical applications. J Parenter Enter Nutr. 1993;17(5):403.

75. Oliveira GP, Dias CM, Pelosi P, Rocco PRM. Understanding the mechanisms of glutamine action in critically ill patients. An Acad Bras Cienc. 2010;82(2):417–30.

76. Melis GC, ter Wengel N, Boelens PG, van Leeuwen PAM. Glutamine: Recent developments in research on the clinical significance of glutamine. Curr Opin Clin Nutr Metab Care. 2004;7(1):59–70.

77. Bollhalder L, Pfeil AM, Tomonaga Y, Schwenkglenks M. A systematic literature review and meta-analysis of randomized clinical trials of parenteral glutamine supplementation. Clin Nutr. 2013;32(2):213–23.

78. Kreymann KG, Berger MM, Deutz NEP, Hiesmayr M, Jolliet P, Kazandjiev G, et al. ESPEN guidelines on enteral nutrition: Intensive care. Clin Nutr. 2006;25(2):210–23.

79. Andrews PJD, Avenell A, Noble DW, Campbell MK, Croal BL, Simpson WG, et al. Randomised trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. BMJ. 2011;342:D1542.

80. van Zanten ARH, Sztark F, Kaisers UX, Zielmann S, Felbinger TW, Sablotzki AR, et al. High-protein enteral nutrition enriched with immune-modulating nutrients vs standard high-protein enteral nutrition and nosocomial infections in the ICU. JAMA. 2014;312(5):514–25.

81. Hofman Z, Swinkels S, van Zanten ARH. Glutamine, fish oil and antioxidants in critical illness: MetaPlus trial post hoc safety analysis. Ann Intensive Care. 2016;6(1):119–31.

82. Tao KM, Li XQ, Yang LQ, Yu WF, Lu ZJ, Sun YM, et al. Glutamine supplementation for critically ill adults. Cochrane Database Syst Rev 2014, Issue 9.Art. No.: CD010050. DOI: 10.1002/14651858.CD010050

83. White M, Lawson K, Ramsey R, Dennis N, Hutchinson Z, Soh XY, et al. A simple nutrition screening tool for pediatric inpatients. J Parenter Enter Nutr. 2014;40(3):392–8.

84. Meyer R, Marino L. Nutrition in critically ill children. In: Shaw V, editor. Clinical Paediatric Dietetics. 4th ed. Oxford: Wiley-Blackwell; 2014. p. 77–9.

85. Neu J, Roig JC, Meetze WH, Veerman M, Carter C, Millsaps M, et al. Enteral glutamine supplementation for very low birth weight infants decreases morbidity. J Pediatr. 1997;131(5):691–9.

86. van den Berg A, van Zwol A, Moll HA, Fetter WPF, van Elburg RM. Glutamine-enriched enteral nutrition in very low-birth-weight infants. Arch Pediatr Adolesc Med. 2007;161(11):1095–101.

87. Moe-Byrne T, Wagner JV, McGuire W. Glutamine supplementation to prevent morbidity and mortality in preterm infants. Cochrane Database Syst Rev 2012, Issue 3:Art. No.:CD001457. DOI: 10.1002/14651858.CD001457.pub4

88. Moe-Byrne T, Brown JV, McGuire W. Glutamine supplementation to prevent morbidity and

Stellenbosch University https://scholar.sun.ac.za

mortality in preterm infants. Cochrane Database Syst Rev 2016, Issue 4.Art. No.: CD001457. DOI: 10.1002/14651858.CD001457.pub.6

89. de Kieviet JF, Vuijk PJ, van den Berg A, Lafeber HN, Oosterlaan J, van Elburg RM. Glutamine effects on brain growth in very preterm children in the first year of life. Clin Nutr. 2014;33(1):69–74.

90. de Kieviet JF, Oosterlaan J, Vermeulen RJ, Pouwels PJW, Lafeber HN, van Elburg RM. Effects of glutamine on brain development in very preterm children at school age. Pediatrics. 2012;130(5):E1121-7.

91. Brown J, Moe-Byrne T, McGuire W. Glutamine supplementation for young infants with severe gastrointestinal disease. Cochrane Database Syst Rev 2014, Issue 12:Art. NO.:CD005947. DOI:10.1002/14651858.CD005947.pub4

92. Zhu W, Li N, Ren J, Gu J, Jiang J, Li J. Rehabilitation therapy for short bowel syndrome. Chin Med J. 2002;115(5):776–8.

93. Weiming Z, Ning L, Jieshou L. Effect of recombinant human growth hormone and enteral nutrition on short bowel syndrome. J Parenter Enter Nutr. 2004;28(6):377–81.

94. Akobeng AK, Miller V, Stanton J, Elbadri AM, Thomas AG. Double-blind randomized controlled trial of glutamine-enriched polymeric diet in the treatment of active crohn's disease. J Pediatr Gastroenterol Nutr. 2000;30(1):78–84.

95. Akobeng AK, Clayton PE, Miller V, Hall CM, Thomas AG. Low serum concentrations of insulin-like growth factor-I in children with active crohn disease: Effect of enteral nutritional support and glutamine supplementation. Scand J Gastroenterol. 2002;37(12):1422–7.

96. Akobeng AK, Richmond K, Miller V, Thomas AG. Effect of exclusive enteral nutritional treatment on plasma antioxidant concentrations in childhood Crohn's disease. Clin Nutr . 2007;26(1):51–6.

97. Akobeng AK, Elawad M, Gordon M. Glutamine for induction of remission in Crohn's disease. Cochrane Database Syst Rev 2016, Issue 2:Art. No.:CD007348. DOI: 10.1002/14651858.CD007348.pub2

98. Yalçin SS, Yurdakök K, Tezcan I, Oner L. Effect of glutamine supplementation on diarrhea, interleukin-8 and secretory immunoglobulin A in children with acute diarrhea. J Pediatr Gastroenterol Nutr. 2004;38(5):494–501.

99. Lima AA, Brito LF, Ribeiro H, Martins MC, Lustosa A, Rocha E, et al. Intestinal barrier function and weight gain in malnourished children taking glutamine supplemented enteral formula. J Pediatr Gastroenterol Nutr. 2005;40(1):28–35.

100. Kamuchaki JM, Wobudeya E, Kiguli S, Bortolussi R. Efficacy of glutamine supplementation on the outcome of children admitted with persistent diarrhea in Uganda: A randomized controlled study. Paediatr Child Health. 2013;18(1):E1.

101. Ogle CK, Ogle JD, Mao JX, Simon J, Noel JG, Li BG, et al. Effect of glutamine on phagocytosis and bacterial killing by normal and pediatric burn patient neutrophils. J Parenter Enter Nutr. 1994;18(2):128–33.

102. Rousseau AF, Losser MR, Ichai C, Berger MM. ESPEN endorsed recommendations: Nutritional therapy in major burns. Clin Nutr. 2013;32(4):497–502.

103. Sheridan RL, Prelack K, Yu Y-M, Lydon M, Petras L, Young VR, et al. Short-term enteral glutamine does not enhance protein accretion in burned children: A stable isotope study. Surgery. 2004;135(6):671–8.

Stellenbosch University https://scholar.sun.ac.za

104. Jeschke MG, Herndon DN. Burns in children: Standard and new treatments. Lancet. 2014;383(9923):1168–78.

105. Anderson PM, Schroeder G, Skubitz KM. Oral glutamine reduces the duration and severity of stomatitis after cytotoxic cancer chemotherapy. Cancer. 1998;83(7):1433–9.

106. Anderson PM, Ramsay NK, Shu XO, Rydholm N, Rogosheske J, Nicklow R, et al. Effect of low-dose oral glutamine on painful stomatitis during bone marrow transplantation. Bone Marrow Transplant. 1998;22(4):339–44.

107. Miller MM, Donald D V, Hagemann TM. Prevention and treatment of oral mucositis in children with cancer. J Pediatr Pharmacol Ther. 2012;17(4):340–50.

108. Ward E, Smith M, Henderson M, Reid U, Lewis I, Kinsey S, et al. The effect of high-dose enteral glutamine on the incidence and severity of mucositis in paediatric oncology patients. Eur J Clin Nutr. 2009;63(1):134–40.

109. David M, Sutton C. Social research: The basics. Oxford: SAGE Publications Ltd; 2004.

110. Polgar S, Thomas S. Introduction to research in the health sciences. Sydney: Elsevier; 2013.

111. Tsang R, Colley L, Lynd LD. Inadequate statistical power to detect clinically significant differences in adverse event rates in randomized controlled trials. J Clin Epidemiol. 2009;62(6):609–16.

112. Yang D, Xu J. Effect of dipeptide of glutamine and alanine on severe traumatic brain injury. Chin J Traumatol. 2007;10(3):145–9.

113. Chuntrasakul C, Siltham S, Sarasombath S, Sittapairochana C, Leowattana W, Chockvivatanavanit S, et al. Comparison of a immunonutrition formula enriched arginine, glutamine and omega-3 fatty acid, with a currently high-enriched enteral nutrition for trauma patients. J Med Assoc Thai. 2003;86:552–61.

114. Albers MJIJ, Steyerberg EW, Hazebroek FWJ, Mourik M, Borsboom GJJM, Rietveld T, et al. Glutamine supplementation of parenteral nutrition does not improve intestinal permeability, nitrogen balance, or outcome in newborns and infants undergoing digestive-tract surgery: Results from a double-blind, randomized, controlled trial. Ann Surg. 2005;241(4):599–606.

115. Chaloupecký V, Hučín B, Tláskal T, Kostelka M, Kučera V, Janoušek J, et al. Nitrogen balance, 3-methylhistidine excretion, and plasma amino acid profile in infants after cardiac operations for congenital heart defects: The effect of early nutritional support. J Thorac Cardiovasc Surg. 1997;114(6):1053–60.

116. Villares JMM, Leal LO, Díaz IS, Gonzalez PG. Plasma aminogram in infants operated on complex congenital heart disease. Nutr Hosp. 2008;23(3):283–7.

117. Solomon LJ, Morrow BM, Argent AC. Paediatric Index of Mortality scores: An evaluation of function in the paediatric intensive care unit of the Red Cross War Memorial Children's Hospital. South African J Crit Care. 2014;30(1):8–13.

118. UNAIDS. South Africa [Internet]. 2017 [cited 2018 Oct 13]. Available from: http://www.unaids.org/en/regionscountries/countries/southafrica/

119. World Health Organization. Global tuberculosis report 2016. Geneva: World Health Organization; 2016.

120. Straney L, Clements A, Parslow RC, Pearson G, Shann F, Alexander J, et al. Paediatric Index of Mortality 3. Pediatr Crit Care Med. 2013;14(7):673–81.

Stellenbosch University https://scholar.sun.ac.za

121. World Health Organization. Training course on child growth assessment: Measuring a child's growth. Geneva: World Health Organization; 2008.

122. World Health Organization. Child growth standards. Geneva: World Health Organization; 2006.

123. Mramba L, Ngari M, Mwangome M, Muchai L, Bauni E, Walker AS, et al. A growth reference for mid upper arm circumference for age among school age children and adolescents, and validation for mortality: Growth curve construction and longitudinal cohort study. BMJ. 2017;358:3423.

124. Marshall JC. Measurements in the intensive care unit: What do they mean? Crit care. 2003;7(6):415–6.

125. Bakker J, Nijsten MW, Jansen TC. Clinical use of lactate monitoring in critically ill patients. Ann Intensive Care. 2013;3(1):12–20.

126. Stellenbosch University Health Research Ethics Committee. Guideline for paediatric blood volume for research purposes. Stellenbosch: Stellenbosch University; 2016.

127. Blau N, Duran M, Gibson KM, editors. Laboratory guide to the methods in biochemical genetics. Berlin: Springer; 2008.

128. United States Department of Agriculture. Food Composition Databases [Internet]. 2018 [cited 2018 Oct 12]. Available from: https://ndb.nal.usda.gov/ndb/

129. South African Medical Research Council. SAFOODS: South African Food Data System [Internet]. 2018 [cited 2018 Oct 12]. Available from: http://safoods.mrc.ac.za/

130. Mehta NM, Skillman HE, Irving SY, Coss-Bu JA, Vermilyea S, Farrington EA, et al. Guidelines for the provision and assessment of nutrition support therapy in the pediatric critically ill patient: Society of Critical Care Medicine and American Society for Parenteral and Enteral Nutrition. J Parenter Enter Nutr. 2017;41(5):706–42.

131. Agostoni C, Buonocore G, Carnielli V, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: Commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50(1):85–91.

132. Uauy R, Koletzko B. Defining the nutritional needs of preterm infants. In: Koletzko B, Poindexter B, Uauy R, editors. Nutritional care of preterm infants: Scientific basis and practical guidelines. Basel: Karger Publishers; 2014. p. 4–10.

133. Gottschlich M, Mayes T. Burns. In: Merritt R, editor. The ASPEN nutrition support practice manual. 2nd ed. Silver Spring: ASPEN; 2005. p. 296–300.

134. Chan MM, Chan GM. Nutritional therapy for burns in children and adults. Nutrition. 2009;25(3):261–9.

135. McCarthy H. Burns. In: Shaw V, editor. Clinical paediatric dietetics. 6th ed. Oxford: John Wiley & Sons; 2015. p. 707–14.

136. World Medical Association. Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4.

137. Laposata M. The New England Journal of Medicine SI unit conversion guide. Boston: NEJM Books; 1992.

138. Gamrin L, Essén P, Forsberg AM, Hultman E, Wernerman J. A descriptive study of skeletal muscle metabolism in critically ill patients: Free amino acids, energy-rich phosphates, protein, nucleic acids, fat, water, and electrolytes. Crit Care Med. 1996;24(4):575–83.

Stellenbosch University https://scholar.sun.ac.za

139. Hammarqvist F, Luo JL, Cotgreave IA, Andersson K, Wernerman J. Skeletal muscle glutathione is depleted in critically ill patients. Crit Care Med. 1997;25(1):78–84.

140. Tjäder I, Rooyackers O, Forsberg AM, Vesali RF, Garlick PJ, Wernerman J. Effects on skeletal muscle of intravenous glutamine supplementation to ICU patients. Intensive Care Med. 2004; 30(2):266–75.

141. Ibiebele I, Algert CS, Bowen JR, Roberts CL. Pediatric admissions that include intensive care: A population-based study. BMC Health Serv Res. 2018;18(1):264–72.

142. Kao C, Hsu J, Bandi V, Jahoor F. Alterations in glutamine metabolism and its conversion to citrulline in sepsis. Am J Physiol Metab. 2013;304(12):E1359-64.

143. Oudemans-van Straaten HM, van Zanten AR. Glutamine supplementation in the critically ill: Friend or foe? Crit Care. 2014;18(3):143–6.

144. Ardawi MS. Glutamine and glucose metabolism in human peripheral lymphocytes. Metabolism. 1988;37(1):99–103.

145. Calder PC. Glutamine and the immune system. Clin Nutr. 1994;13(1):2–8.

146. Smedberg M, Wernerman J. Is the glutamine story over? Crit Care. 2016;20(1):361–6.

147. Statistics South Africa. Mid-year population estimates 2017. Pretoria: South African National Government; 2017.

148. Churchyard GJ, Mametja LD, Mvusi L, Ndjeka N, Hesseling AC, Reid A, et al. Tuberculosis control in South Africa: Successes, challenges and recommendations. South African Med J. 2014;104(3):244–8.

149. Hack V, Schmid D, Breitkreutz R, Stahl-Henning C, Drings P, Kinscherf R, et al. Cystine levels, cystine flux, and protein catabolism in cancer cachexia, HIV/SIV infection, and senescence. FASEB J. 1997;11(1):84–92.

150. Moling O, Avi A, Rimenti G, Mian P. Glutamine supplementation for patients with severe cryptosporidiosis. Clin Infect Dis. 2005;40(5):773–4.

151. Huffman FG, Walgren ME. L-glutamine supplementation improves nelfinavir-associated diarrhea in HIV-infected individuals. HIV Clin Trials. 2003;4(5):324–9.

152. Burini RC, Moreto F, Yu Y-M. HIV-positive patients respond to dietary supplementation with cysteine or glutamine. In: Watson R, editor. Health of HIV infected people. Oxford: Academic Press; 2015. p. 245–69.

153. Pan YP, Chang PH, Fan CW, Tseng WK, Huang JS, Chen CH, et al. Relationship between pre-treatment nutritional status, serum glutamine, arginine levels and clinicopathological features in Taiwan colorectal cancer patients. Asia Pac J Clin Nutr. 2015;24(4):598–604.

154. Suliman ME, Qureshi AR, Stenvinkel P, Pecoits-Filho R, Bárány P, Heimbürger O, et al. Inflammation contributes to low plasma amino acid concentrations in patients with chronic kidney disease. Am J Clin Nutr. 2005;82(2):342–9.

155. Vasta V. Glutamine transport and enzymatic activities involved in glutaminolysis in human platelets. Biochim Biophys Acta. 1995;1243(1):43–8.

156. Vasta V, Meacci E, Farnararo M, Bruni P. Glutamine utilization in resting and stimulated platelets. J Biochem. 1993;114(2):163–6.

157. South African National Department of Health, Statistics South Africa, South African Medical Research Council, ICF. South Africa demographic and healthy survey 2016: Key indicator report.

Stellenbosch University https://scholar.sun.ac.za

Pretoria (South Africa) and Rockville (USA); 2017.

158. Joosten KFM, Hulst JM. Malnutrition in pediatric hospital patients: Current issues. Nutrition. 2011;27(2):133–7.

159. Brink J, Pettifor JM, Lala SG. The prevalence of malnutrition in children admitted to a general paediatric ward at the Chris Hani Baragwanath Academic Hospital: A cross-sectional survey. SAJCH. 2014;8(3):112–6.

160. de Onis M, Blössner M. The World Health Organization global database on child growth and malnutrition: Methodology and applications. Int J Epidemiol. 2003;32(4):518–26.

161. Muller O, Krawinkle M. Malnutrition and health in developing countries. Can Med Assoc J. 2005;173(3):279–86.

162. Coghlin Dickson TM, Wong RM, Negrin RS, Shizuru JA, Johnston LJ, Hu WW, et al. Effect of oral glutamine supplementation during bone marrow transplantation. J Parenter Enter Nutr. 2000;24(2):61–6.

163. Coss-Bu JA, Hamilton-Reeves J, Patel JJ, Morris CR, Hurt RT. Protein requirements of the critically ill pediatric patient. Nutr Clin Pract. 2017;3 Suppl 1:S128-141.

164. Hardy CM, Dwyer J, Snelling LK, Dallal GE, Adelson JW. Pitfalls in predicting resting energy requirements in critically ill children: A comparison of predictive methods to indirect calorimetry. Nutr Clin Pract. 2002;17(3):182–9.

165. Canarie MF, Barry S, Carroll CL, Hassinger A, Kandil S, Li S, et al. Risk factors for delayed enteral nutrition in critically ill children. Pediatr Crit Care Med. 2015;16(8):E283-289.

166. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient. J Parenter Enter Nutr. 2016;40(2):159–211.

167. Melis GC, Boelens PG, van der Sijp JRM, Popovici T, De Bandt JP, Cynober L, et al. The feeding route (enteral or parenteral) affects the plasma response of the dipetide Ala-Gln and the amino acids glutamine, citrulline and arginine, with the administration of Ala-Gln in preoperative patients. Br J Nutr. 2005;94(1):19–26.

168. Murthy S, Wunsch H. Clinical review: International comparisons in critical care - lessons learned. Crit Care. 2012;16(2):218–25.

169. Planche T, Dzeing A, Emmerson AC, Onanga M, Kremsner PG, Engel K, et al. Plasma glutamine and glutamate concentrations in Gabonese children with Plasmodium falciparum infection. QJM. 2002;95(2):89–97.

170. Hirose T, Shimizu K, Ogura H, Tasaki O, Hamasaki T, Yamano S, et al. Altered balance of the aminogram in patients with sepsis - the relation to mortality. Clin Nutr. 2014;33(1):179–82.

171. Leteurtre S, Duhamel A, Deken V, Lacroix J, Leclerc F. Daily estimation of the severity of organ dysfunctions in critically ill children by using the PELOD-2 score. Crit Care. 2015;19(1):324–30.

172. Crookes P, Davies P. Research into practice: Essential skills for reading and applying research in nursing and health Care. 2nd ed. London: Bailliere Tindall; 2004.

Stellenbosch University https://scholar.sun.ac.za

9. Addenda

Addenda A: Participant information leaflet and consent form

TITLE OF THE RESEARCH PROJECT:

Plasma glutamine concentration among critically ill children

REFERENCE NUMBER*:* S16/05/085 (Stellenbosch University) 681/2016 (UCT)

PRINCIPAL RESEARCHER: Mrs Joanna Eksteen (Wilson)

ADDRESS:
Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University

CONTACT NUMBER:

Your child is being invited to take part in a research study. It is very important that you understand what the study is about before you agree to participate. The study will be explained to you in person and written information will be given to you to keep. Please tell the researcher if you do not understand any part of what is being explained.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University and the University of Cape Town and** will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

Do you have to participate in the study?

You do not have to take part if you do not wish to. Participation in the study is voluntary. It is also very important for you to know the following:

- You can withdraw from the study at any time without giving a reason
- Your child's medical care will not change in any way if you take part or do not take part in the study

What is the research about?

This research is about glutamine, which is a type of amino acid. Amino acids are the smallest building blocks of protein in the body. When people are very ill or injured, their bodies use more glutamine for healing. This means that their body's stores of glutamine may be low. Until now, most research has been done in adult patients and very little has been done in children. It is important for us to understand if children's glutamine stores are low when they are ill.

This research will investigate the blood levels of glutamine in children who are very ill. We will also investigate differences in levels according to the type of illnesses children have and how ill they

Stellenbosch University https://scholar.sun.ac.za

are. We also want to see if glutamine levels affect children's outcome, for example, how long they stay in hospital. This will help us to better understand glutamine levels in sick children.

Why has your child been invited to participate?

This study aims to gather information about plasma glutamine from all patients admitted to the Paediatric Intensive Care Unit during the month of August 2016. Your child has been invited to participate in this study because he or she has been admitted during this time.

What will be expected of you and your child?

Two additional blood samples will be taken from your child: one on admission to the Intensive Care Unit and another on day 2 of their stay. Please note that:

- This will be taken at the same time as other routine blood tests
- Blood will be collected safely by a health care professional

During their stay in hospital your child will be weighed and measured. This will take about 10 minutes and will involve:

- Standing on or being placed on a scale
- Standing or lying down for a height measurement
- Having a tape measure placed around the arm

Researchers will also collect information from your child's medical notes. This will include information on:

- Personal details
- Diagnosis
- HIV/TB status
- Blood test results

What are the risks and benefits involved in taking part?

- The study has been approved by the Health Research Ethics Committee. This means that the researchers have to adhere to certain strict standards to ensure that the study is safe and ethical.
- Blood will be drawn in a safe and clean way by a health care professional to reduce the risk to your child. This will not require a new needle to be placed, so your child will not feel pain.
- If your child becomes distressed during weighing or measuring, we will stop and try again later. If the researcher identifies your child to be malnourished, he/ she will be referred to a dietitian.
- By participating in the study, you and your child will not benefit directly. We hope that what we learn from the study will help us to better understand how to care for sick children in the future.

How will your child's information be used?

- Only one member of the research team will have access to your child's medical file
- Any information recorded will be labelled using a special code
- In this way your child's name will not be used

Stellenbosch University https://scholar.sun.ac.za

- All information will be stored by the research team for 5 years before being destroyed
- Information will be entered into a computer database which will only be viewed by three members of the research team
- However, sponsors of the study, study monitors, auditors or Human Research Ethics Committee members may in some circumstances need to inspect research records
- If the results of this study are shared, no names will be used
- Blood samples will be stored in the hospital's laboratory until they are analysed. They are then stored for a further 2 months until they are destroyed.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to participate in the study, and there will be no costs involved if you do take part.

Who can you contact for more information?

If you have any questions or concerns, please contact:

- Joanna Eksteen
  - Telephone:
- Should you have concerns or complaints which cannot be addressed by the principle investigator you are welcome to contact:
- The Health Research Ethics Committee (University of Stellenbosch)
  - Telephone:
- The University of Cape Town Health Research Ethics Committee Chair
  - Professor Marc Blockman
  - Telephone:

<u>Declaration by parent/ legal guardian</u>

I……parent/legal guardian of

……(Child name and surname)
agree that my child can take part in the research study '*Plasma glutamine concentration among critically ill children'.*

I declare that:

- I have read and understood the consent form (or had it explained to me)
- The consent form is written in a language which I understand
- I have had a chance to ask questions and all my questions have been answered
- I have not been pressurised to take part in this study
- I understand that participation in this study is voluntary
- I understand that I may choose to leave the study at any time

Signed at (*place*) ..... on (*date*) ……..2016

…… ……

Signature of parent/ legal guardian **Signature of witness**

Stellenbosch University https://scholar.sun.ac.za

Declaration by investigator

I (Name of researcher) …… declare that:

- I have explained the information in this document to:
  ……(Parent/ legal guardian)
- I encouraged him/her to ask questions and took time to answer each one
- I am satisfied that he/she understands all aspects of the research

Signed at (*place*) .....on(*date*)……2016

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I (name) …… declare that:

- I assisted the investigator (name) …… to explain the information in this document to (name of participant) …….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (place) ..... on (date) ……2016.

....

Signature of interpreter **Signature of witness**

Stellenbosch University https://scholar.sun.ac.za

Addenda B: Participant information leaflet and assent form

RESEARCH PROJECT TITLE: Blood levels of glutamine in very sick children

REFERENCE NUMBER*:* S16/05/085 (Stellenbosch University) 681/2016 (UCT)

RESEARCHER: Mrs Joanna Eksteen (Wilson)

ADDRESS:
Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University

CONTACT NUMBER:

***What is RESEARCH*?**

Research is something we do to find new knowledge about the way things (and people) work. We use research projects or studies to help us find out more about disease or illness. Research also helps us to find better ways of helping or treating children who are sick.

What is this research project all about?

This research is about glutamine which is a type of protein. When people are sick their bodies use more glutamine for healing. This can mean that the body's stores of glutamine are low. This research project aims to measure the amount of glutamine there is in the blood of children who are very sick. This will help us to understand how to treat children who are sick.

Why have I been invited to take part in this research project?

All children who are in the intensive care unit during the month of November are being asked to take part in this research. You have been invited to take part because you are in the intensive care unit during this time.

Stellenbosch University https://scholar.sun.ac.za

Who is doing the research?

The main researcher is Joanna Eksteen (Wilson). She is a dietitian who works for Red Cross Children's Hospital. She is also a student at the University of Stellenbosch. She will be asking you if you want to take part and will be collecting most of the information for the study.

What will happen to me in this study?

During the first few days in the intensive care unit the doctors will take some blood from you every day. This helps them to decide how to treat your illness. For this study we will take a little bit of extra blood from you on the day you are brought in and again on your second day in the intensive care unit. You will not have to be pricked again for this, as the doctors will do this all at the same time. This is done in a safe and clean way.

The researcher will measure your weight, and height. When you are well enough and are not in any pain, you will be asked to stand on a scale to be weighed and for your height to be measured.

The researcher will also look at your medical information and will record information about why you were brought into the hospital, what happened to you in hospital, and how long you stayed in the hospital.

Can anything bad happen to me?

This study is designed not to harm you or place you at any risk. The blood tests and measurements will not cause you pain and will be done in a safe and clean way. If you feel upset at any time please let your parents and the researcher know.

Can anything good happen to me?

You will not be given anything for taking part in this research. You will be treated the same as all the other patients.

Will anyone know I am in the study?

The researcher will give every child who takes part in the study a special number. This number will be used instead of your name. In this way only the researcher will know that the information collected is yours. Your number and information will be stored on the computer and will be seen by only 3 people on the research team. If we write about what we found in this study, we will not use your name.

Who can I talk to about the study?

If you have any questions about the research or if you are worried about anything you can telephone:

- Joanna Eksteen (Main Researcher)
  - Telephone:

Stellenbosch University https://scholar.sun.ac.za

If your questions have not been properly answered you can also contact:

- The Health Research Ethics Committee (University of Stellenbosch)
  - Telephone:

- The University of Cape Town Health Research Ethics Committee Chair
  - Professor Marc Blockman
  - Telephone:

What if I do not want to do this?

You do not have to take part in this research if you do not want to. You can say no at any time, even if your parents have agreed for you to take part. Nothing bad will happen to you and you will not be in trouble.

Do you understand this research study and are you willing to take part in it?

YES	NO

Has the researcher answered all your questions?

YES	NO

Do you understand that you can pull out of the study at any time?

YES	NO

_____ _____

Signature of Child Date

Stellenbosch University https://scholar.sun.ac.za

Addenda C: Data Collection Sheet

DATA COLLECTION SHEET

Participant number	

DATA TO COLLECT AT PICU ADMISSION

DEMOGRAPHIC INFORMATION

1. Gender

Male		Female	

2. Date of birth of patient

Day		Month		Year			

ADMISSION INFORMATION

3.	**Date of admission data collection**				
4.	**Date of hospital admission**				
5.	**Date of PICU admission**				
6.	**Admitted from (X)**	Home		Ward	

MEDICAL INFORMATION

7. Diagnostic Category	**(X)**
7.1 Medical	
7.2 Surgical	
• Elective surgery	
• Emergency surgery	
7.3 Trauma	

Stellenbosch University https://scholar.sun.ac.za

8.1 Primary PICU Diagnosis	(X)	Specify
Abdominal/ Gastroenterology		
Cardiology/ vascular		
Respiratory		
Nephrology		
Hepatology		
Endocrine		
Oncology		
Neurology		
Haematology		
Orthopaedic		
Head/ maxillofacial		
Sepsis		
Malnutrition		
Other		

9. Infectious Disease		(X)
9.1 HIV Positive	Yes	
	No	
	Exposed	
	Unknown	
9.2 TB Positive	Yes	
	No	
	Unknown	

Stellenbosch University https://scholar.sun.ac.za

10. Risk of mortality: PIM 3 Score	

BIOCHEMISTRY

11. Biochemical measure		**Date of analysis**	**Value**
11.1	**CRP** (mg/l)		
11.2	**PCT** (ng/ml)		
11.3	**Albumin** (g/l)		
11.4	**Lactate** (mmol/l)		
11.5	**Blood glucose** (mmol/l)		Highest:

DATA TO COLLECT AT PICU ADMISSION AND DAY 2

12.1 Plasma glutamine on admission		
NOTE: If Hb is known to be <7 g/dl on admission the patient must be excluded from the study		
Date of sample	**Time of sample**	**Value**
12.2 Plasma glutamine at 48 hours		
NOTE: if Hb is <7 g/dl do not take the second blood sample		
Date of sample	**Time of sample**	**Value**

Stellenbosch University https://scholar.sun.ac.za

DATA TO COLLECT ON DAY 2 OF PICU STAY

FEED INFORMATION

13. Feed	Day 0	Day 1	Day 2
13.1 TPN			
Bag type			
Rate/hr			
Hours ran			
13.2 Enteral			
Feed type			
Total volume prescribed			
Total volume achieved			
13.3Oral			
Feed type			
Total volume prescribed			
Total volume achieved			

DATA TO COLLECT AT PICU DISCHARGE

ANTHROPOMETRY

14. Measuring technique		(X)
14.1 Weight	Infant scale	
	Electric scale	
	Measurement not possible	
14.2 Length/ height	Recumbent length	
	Standing height	
	Measurement not possible	

15. Measurements

Stellenbosch University https://scholar.sun.ac.za

15.1 Weight (kg)	
15.2 Length/ height (cm)	
15.3 MUAC (cm)	

16.1 Presence of ascites (X)	**Yes**		**No**	
16.2 Presence of oedema				
Location of oedema	**Category**		**Indicate Option (X)**	
No visible oedema	None			
Both feet/ankles	Mild (+)			
Both feet plus lower legs, hands/ lower arms	Moderate (++)			
Generalised oedema	Severe (+++)			

PICU DISCHARGE INFORMATION

17.	**Date of PICU discharge data collection**	
18.	**Date of PICU discharge**	

19. Discharge Options	**(X)**	
9.1 Transferred to another hospital		
19.2 Transferred to another ward		**i. Ward and date**
19.3 Deceased in PICU		**i. Date**

Stellenbosch University https://scholar.sun.ac.za

MEASURES OF CLINICAL OUTCOME

20. Ventilation Requirements	
20.1 Length of time on mechanical ventilation (days)	

DATA TO COLLECT AT HOSPITAL DISCHARGE

21. Date of hospital discharge	
22. If deceased, date	