Micronutrient losses during Renal Replacement Therapy for Acute Kidney Injury

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Thesis submitted to the University of Nottingham

for the degree of Doctor of Medicine

July 2016

Abstract

Micronutrient loss in renal replacement therapy for acute kidney injury.

Malnutrition is common in acute kidney injury (AKI) patients, particularly on the ICU, where they often receive renal replacement therapy (RRT). RRT may exacerbate loss of water-soluble micronutrients (e.g. trace elements, amino acids and B-vitamins). No clinical study has quantified these losses and contrasted between types of RRT that use different methods to remove solutes i.e. by diffusion (intermittent haemodialysis, IHD) by convection (continuous veno-venous haemofiltration, CVVH) or by a combination of both (sustained low-efficiency diafiltration, SLEDf).

Using a prospective, observational design patients (n=24 per modality) were consented before their first treatment session. Blood and RRT effluent (dialysate or filtrate) were sampled at baseline (pre-RRT), mid and end-RRT. Amino acids were measured by HPLC, trace elements by ICP-MS and B-vitamins (B1, B3, B6, B9, B12) by LC-MS. Plasma concentrations were corrected for dialysis dose using the urea reduction ratio (for IHD & SLEDf, but not CVVH). Micronutrient losses (mass-corrected) were calculated as concentration × RRT effluent volume, corrected for plasma concentration and RRT dose (i.e. solute removal index). Data were analysed by restricted maximum likelihood estimating equations (Genstat v16, VSNi Ltd, UK).

Patients receiving CVVH had significantly higher plasma amino acids, but not plasma trace elements, at baseline (amino acids: CVVH, 3762 ± 357; IHD, 2039 ± 337; SLEDf, 2505 ± 423 µmol/L; trace elements: IHD, 4156 ± 465; SLEDf 3732 ± 521; CVVH 3982 ± 465 µg/L). At RRT end, plasma amino acids and trace elements had significantly reduced (429 ± 223 µmol/L; 600 ± 400 µg/L, respectively). No trace element was lost to a greater extent between types of RRT, but many (>10) individual amino acids declined to a much greater extent with SLEDf vs. HD or CVVH (e.g. effect size for lysine was -64 ± 23 µmol/L). Two significant sources of micronutrient loss were noted: to effluent and through dialyser adsorption. The latter contributed <1g amino acids but in effluent recorded losses of up to 25g were noted with CVVH (5-10g for IHD and SLEDf, respectively). Effluent losses of trace elements varied significantly, but in all cases were greater for CVVH (e.g. effect size for copper was +850 ± 475 µg vs. HD or SLED-F). B-vitamins were not detectable in effluent.

Significant loss of micronutrients during RRT, particularly for patients in ICU, is a possible aggravating factor for patients known to be at high risk of malnutrition. The type of RRT used

influences the pattern of loss but not consistently for all nutrients. Adsorption of amino acids to dialysers adds a small, but cumulative loss that may become important if further treatment sessions are indicated.

Publications and Presentations

Publications:

Oh, W.C., Gardner, D.S., Devonald, M.A.J. (2015). Micronutrient and amino acid losses in acute renal replacement therapy. Current Opinion in Clinical Nutrition and Metabolic Care Nov: 18 (6): 593-598.

Oh, W.C., Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J. (2016). Micronutrient and amino acid loss during renal replacement therapy for acute kidney injury. (In preparation)

Oral presentations

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. World Congress in Nephrology 2017, Mexico City, Mexico (Accepted for oral presentation April 2017)

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. American Society of Nephrology Kidney Week 2015, San Diego, USA. November 2015

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. West Midlands Regional Renal Meeting, Birmingham. November 2014

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. Sue Watson Oral Presentation Event, School of Medicine, University of Nottingham. May 2014

Poster presentations

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. British Renal Society, Leeds July 2015

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury ERA EDTA Congress, London May 2015

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J. Micronutrient loss during renal replacement therapy for acute kidney injury. ISICEM Brussels March 2015

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. ESPEN Congress Geneva September 2014

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J Micronutrient loss during renal replacement therapy for acute kidney injury. ERA EDTA Congress Amsterdam June 2014

Oh, W.C, Gardner, D.S., Rigby, M., Mafrici, B., Sharman, A., Harvey, D., Mahajan, R. and Devonald, M.A.J. Micronutrient loss during renal replacement therapy for acute kidney injury UK Renal Association – British Renal Society Joint Congress, Glasgow April 2014

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the test.

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Signed …….

Date ……

Acknowledgements

First and foremost I would like to thank God who has sustained and strengthened me in this journey of doing research in Acute Kidney Injury (AKI). Without Him, I would not have been able to complete what has been a wonderful and fruitful period of learning.

Next I would like to thank my wife Joanne for her support, prayers and patience over the last 3 years. I am especially grateful for her willingness to drive me to the hospital whenever there was a patient to recruit.

I would like to thank my supervisors, Dr Mark Devonald, Prof David Gardner and Prof Ravi Mahajan for their guidance and supervision of my research project. I would especially like to thank Dr Devonald for giving me the opportunity to undertake a period of research in AKI. This period of research has ignited my interest in AKI and I hope to take this interest forward as I start work as a consultant nephrologist.

Special thanks must also go to the medical and nursing staff in the Department of Nephrology and Critical Care at Nottingham University Hospitals (NUH) NHS Trust. This includes the consultant nephrologists and intensivists who allowed me to approach and recruit patients under their care for my research project. I am also grateful to the nursing staff in the renal unit and in the ICU who had assisted me in collecting the relevant patient samples during my recruitment.

Thank you also to the patients who had given their consent to participate in this study. I am grateful for their willingness to contribute towards research in renal medicine.

I would like to thank Bruno Mafrici and his colleagues from the department of dietetics in NUH NHS Trust for their contribution towards my research project. I am grateful for their advice, time and efforts in assisting me in contributing my data. Thanks also to Mark Rigby who is the lead dialysis nurse for his input and advice in my project. I am also grateful to the Biobank department at the David Evans Research Centre for teaching me some essential laboratory techniques as well as for allowing me to use their laboratory to process my research samples. I would also like to thank the senior research scientists in the department, Ian Daniels and Christine Porter for their advice and support whilst working as a clinical researcher.

I would also like to express my sincere thanks and gratitude to the Nottingham University Hospitals Charity Body for their contribution towards funding my research work and postgraduate studies. Special mention must also go towards the National Institute for Health Research body for awarding our research group the Research for Patient Benefit grant which also helped fund my research work and postgraduate studies. I would also like to thank the renal Patient and Public Involvement group for supporting my research project.

I would like to thank the School of Medicine at the University of Nottingham for their support during my period of research. I am especially grateful for having period of extension during my thesis pending period as I had to be away from my work due to family bereavement.

Finally I would like to thank my parents for their love and support throughout my life. I am grateful to them for their patience, interest and support in my studies and career as a doctor.

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Abbreviations

A, B, C

AIN	Acute Interstitial Nephritis
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ANOVA	Analysis of Variance
AT	Anti-thrombin
ATP	Adenosine Triphosphate
BCAA	Branched Chain Amino Acids
BMI	Body Mass Index
CKD	Chronic Kidney Disease
CRP	C Reactive Protein
CRRT	Continuous Renal Replacement Therapy
Cu	Copper
CVVH	Continuous Veno-venous Haemofiltration
CVVHDF	Continuous Veno-venous Haemodiafiltration

D, E, F

DNA	Deoxyribonucleic Acid
DOPPS	Dialysis Outcomes Practice and Pattern Study
ED	Extended Dialysis
EGRAC	Erythrocyte Glutathione Reductase Activity Coefficient
ESRD	End Stage Renal Disease

FAD	Flavin Adenine Dinucleotide
FMN	Flavin Mononucleotide
G, H, I	
GFR	Glomerular Filtration Rate
GSHPx	Glutathione Peroxidase
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICU	Intensive Care Unit
IHD	Intermittent Haemodialysis
IL-6	Interleukin-6
J, K, L,	
KDIGO	Kidney Disease: Improving Global Outcomes
K	Potassium
LDL	Low Density Lipoprotein
M, N, O	
MIS	Malnutrition Inflammation Score
MUST	Malnutrition Universal Screening Tool
Na	Sodium
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NGAL	Neutrophil Gelatinase Associated Lipocalin
NRS	Nutrition Risk Screening

O, P, Q

R, S, T

RRT	Renal Replacement Therapy
SGA	Subjective Global Assessment
SIRS	Systemic Inflammatory Response Syndrome
SLED-F	Sustained low efficiency diafiltration
SLED	Sustained low efficiency dialysis
SRI	Solute Removal Index
TNF	Tumour Necrosis Factor

U, V, W,

URR	Urea Reduction Ratio

X, Y, Z

Zn	Zinc

1. Introduction

1.1 Acute Kidney Injury

1.1.1 Definition and Staging

Acute kidney injury (AKI) is characterised by an abrupt decline in kidney function occurring over hours or days, resulting in the retention of metabolic waste products and dysregulation of fluid, electrolyte and acid-base homeostasis (Palevsky et al., 2013). AKI is a common complication of critical illness and is associated with a high mortality rate, ranging from 50 to 70% (Uchino et al., 2005, Nash et al., 2002, Liano and Pascual, 1998, Lameire et al., 2005, Mehta et al., 2004). In addition, AKI is also associated with other adverse patient outcomes, such as the development of *de novo* chronic kidney disease (Bucaloiu et al., 2012) and progression to end stage renal disease (Wald et al., 2009).

There has been a variety of definitions used for AKI worldwide. Consequently, this has been an obstacle to conducting valid and robust epidemiological studies in this important field of nephrology. It is now acknowledged that a standard definition of AKI is required in order to establish effective clinical management guidelines.

In 2004, the first consensus definition was published by the Acute Dialysis Quality Initiative group (Bellomo et al., 2004). This definition is known as RIFLE which is an acronym describing Risk of renal dysfunction; Injury to the kidney; Failure of kidney function, Loss of kidney function, and End stage kidney disease. Risk, Injury and Failure define the different levels of severity in AKI while persistent Loss and End stage kidney disease are outcomes of AKI. RIFLE was designed to establish the presence of AKI and to determine its severity. In order to achieve these objectives, two important criteria are used.

Firstly, the change in serum creatinine from baseline and secondly the urine output per body weight over a defined period. The classification system used by RIFLE was intended to enable the clinician to distinguish mild and severe AKI, to allow early and appropriate management of this condition. The change in kidney function in AKI must be both abrupt (occurring within 1-7 days) and sustained (more than 24 hours).

Serum creatinine is commonly used to measure kidney function because it is easily and readily measured and is reasonably specific to the kidney. However there are several disadvantages to measuring serum creatinine. Creatinine is a late marker of kidney damage and levels are

influenced by various factors including catabolic state, muscle mass, volume of distribution and use of medications.

The introduction of RIFLE to define and stage AKI led to many published epidemiological and outcome studies. A systematic review of 24 studies which used the RIFLE classification highlighted several important conclusions (Ricci et al., 2008). All these studies concluded that RIFLE is an uncomplicated tool that can be applied in different populations to classify AKI. In addition, RIFLE appears to be a reliable and consistent outcome predictor. All studies demonstrate a stepwise increase in mortality with worsening RIFLE class. Finally there is a suggestion that mild degrees of kidney dysfunction may have an adverse impact on clinical outcomes (Chertow et al., 2005).

However, the RIFLE classification has limitations. Firstly, it is based on changes in serum creatinine, which can occur relatively late after kidney injury. AKI is usually diagnosed when there has already been a decline in glomerular filtration rate (GFR). In contrast, there is growing interest in developing new biomarkers which can detect kidney injury at an earlier stage, even before GFR falls significantly. For example, tubular damage can be identified early and this precedes alterations in GFR. There is now evidence of emerging biomarkers such as cystatin C (Herget-Rosenthal et al., 2004) and neutrophil gelatinase associated lipocalin (Mishra et al., 2005). Secondly, a baseline or reference creatinine level is required in RIFLE and this result may not be available. The third limitation is the use of urine output as a criterion. Outside the ICU and renal unit, accurate hourly urine output is rarely documented. Furthermore, urine output might be confounded by factors such as the use of diuretics. Moreover, the use of urine output alone in the RIFLE criteria in comparison to serum creatinine yielded a different outcome (Hoste et al., 2006, Cruz et al., 2007).

In 2007, a new classification known as the Acute Kidney Injury Network (AKIN) classification was introduced. The AKIN classification is a refined version of the RIFLE classification. Five changes were made. First, stage 1 - 3 have replaced Risk, Injury and Failure. Second, an absolute increase of 0.3 mg/dL or 26.5 µmol/L was added to the criteria for stage 1. Third, the definition of stage 3 included patients requiring renal replacement therapy regardless of their serum creatinine and urine output. Fourth, the stages of Loss and End stage kidney disease have been removed in the AKIN classification. Fifth, the increase in creatinine must occur in < 48 hours. In 2012, KDIGO (Kidney Disease: Improve Global Outcomes), an international nephrology body aimed at developing and implementing guidelines for the management of kidney disease, published its

clinical guideline on the management of AKI. According to the guidelines published by KDIGO (2012), AKI is thus defined as

- Increase in serum creatinine by > 0.3 mg/dl (> 26.5µmol/L) within 48 hours; or
- Increase in serum creatinine to 1.5 times baseline, which is known or presumed to have occurred within the prior seven days; or Urine volume <0.5ml/kg/hr for 6 hours.

1.1.2 Epidemiology

Both RIFLE and AKIN classifications have been used to investigate the epidemiology of AKI in various settings. In the intensive care unit, AKI is a common complication of critically ill patients. A prospective study conducted in 19 Italian ICUs showed that the incidence of AKI was 10.8% with 3.3% requiring RRT (Cruz et al., 2007). Another multicentre study in Australia demonstrated that the incidence of AKI in the ICU was 36% (Bagshaw et al., 2008). Both studies used the RIFLE criteria. Outside the ICU, a retrospective cohort study in Scotland (Ali et al., 2007) showed that the incidence of AKI and acute on chronic renal failure was 1811 and 336 per million respectively. This incidence is higher than that reported in a prospective study in Madrid, Spain where neither the RIFLE nor the AKIN criteria was used to define AKI (Liano and Pascual, 1996). Large epidemiological studies in North America have also shown that the incidence of AKI is progressively increasing (Hsu et al., 2007). The elderly population represent a subgroup that is susceptible to developing AKI. The elderly population is rapidly growing in the Western world and this may explain the progressive rise in the incidence of AKI. Various studies across Europe and North America have shown that the elderly have a higher incidence of AKI compared to the younger population (Baraldi et al., 1998) (Pascual and Liano, 1998, Pascual et al., 1990) (Xue et al., 2006). In the United Kingdom, the characteristics and outcomes of patients with severe AKI (defined as a serum creatinine of >300umol/L and/or urea >40 mmol/L) in all adult ICU has been described (Kolhe et al., 2008). This cohort of patients which includes both RRT and non-RRT dependent AKI patients is the largest ever described. This study reported several important findings. Firstly, the prevalence of severe AKI in all ICU admissions was 6.3%. Second, among AKI patients, oliguria was associated with a higher mortality compared to non-oliguric patients. Third, sepsis appears to be the predominant cause of AKI with 47.3% of cases caused by this insult. Fourth, mortality of AKI in ICU was 43.3%, increasing to 58.6% at time of hospital discharge. One shortcoming of this study is that the definition of severe AKI did not follow the current definition

from KDIGO. Also, the data did not distinguish patients who receive RRT from those who did not. Thus, subgroup analyses could not be performed to evaluate the effect of RRT in the outcomes of these patients. In patients with AKI outside ICU, several important findings have also been reported in recently studies. A study in a large teaching hospital in UK (Porter et al., 2014) showed that with electronic-alert reporting, the epidemiology and outcomes of AKI can be described. This study showed that incidence of AKI was 10.7%. Secondly, community acquired AKI is more common than hospital acquired AKI. Third, the in hospital mortality of AKI irrespective of its severity was 18.5 %. Fourth, worsening stages of AKI also portend a higher mortality.

1.1.3 Aetiology of AKI

The aetiology of AKI is wide and frequently multi-factorial. A thorough clinical assessment of the patient with AKI is crucial to identify the cause, evaluate the severity and to initiate the appropriate treatment. The table below summarises the main categories of AKI.

Site of Injury	Insult
Prerenal	Sepsis, hypovolaemia, haemorrhage, renovascular disease, cardiac failure, liver disease
Intrinsic	Glomerulonephritis, acute interstitial nephritis
Postrenal	Urinary tract neoplasms, renal calculi

Table 1 - Aetiology of AKI. The aetiology of AKI is classed into three categories based on the site of injury. There is a wide range of insults to the site of injury and this is described in the second column of the table.

1.1.3.1 Pre-renal AKI

The hallmark of pre-renal AKI is inadequate renal perfusion. There are a variety of causes that precipitate a reduction in renal blood flow, described in table 1. Patients who are elderly are at

risk of developing hypovolaemia, polypharmacy and high prevalence of atherosclerotic renovascular disease. Non-steroidal anti-inflammatory drugs can cause pre-renal AKI in the setting of renal hypoperfusion (Abuelo, 1995, Shankel et al., 1992). In addition, drugs such as angiotensin-converting-enzyme inhibitors and diuretics can cause pre-renal AKI in patients who have pre-existing renovascular disease (Bridoux et al., 1992, Hricik et al., 1983). Patients who are hospitalised develop AKI frequently due to sepsis, cardiac failure and liver impairment (Hou et al., 1983, Nash et al., 2002, Shusterman et al., 1987).

1.1.3.2 Intrinsic AKI

Intrinsic causes of AKI are categorised by the site of injury. There are four different locations where the insult can occur. These sites include the glomerulus, the vasculature, the tubules and the interstitium. Ischaemic acute tubular necrosis can be a consequence of non-resolving pre-renal AKI. Toxins also represent a significant cause of intrinsic AKI. Hospitalised patients commonly receive radiocontrast agents and aminoglycoside antibiotics. Moreover, patients with malignant disease are commonly treated with chemotherapeutic agents such as cisplatin which is nephrotoxic. The mechanisms by which drugs can cause AKI are described in the table below. Intrinsic AKI can also be caused by an allergic reaction to a drug. This phenomenon is known as Acute Interstitial Nephritis (AIN). Drug-induced AIN is frequently reversible once the offending drug is removed. Corticosteroids may accelerate renal recovery in AIN but this remains a controversial issue due to a lack of controlled studies (Galpin et al., 1978). AKI can also, though relatively infrequently, be caused by a rapidly progressive glomerulonephritis. This requires a rapid assessment and diagnosis as prompt treatment with immunosuppressive agents and plasma exchange can lead to renal recovery (Jennette and Falk, 1990, Mason and Pusey, 1994).

1.1.3.3 Post-renal AKI

Post renal AKI is defined as AKI due to obstruction of the urinary tract. Common causes of post renal AKI include prostatic hypertrophy, prostate and cervical cancer and retroperitoneal diseases. Other important causes of obstructive AKI can be broadly classified as intraluminal, extra-luminal or intra-tubular obstruction.

1.1.4 Pathophysiology of AKI

1.1.4.1 Ischaemic AKI

Ischaemic AKI is defined as an abrupt loss of kidney function due to inadequate renal blood flow. Renal hypo-perfusion is often due to hypovolaemia, a reduction in cardiac output or renal artery stenosis. It has been well established that restoration of renal blood flow does not lead to an immediate improvement in renal function (Basile et al., 2012). Renal ischaemia triggers a sequence of events leading to further tissue damage and functional defects, which are independent of renal blood flow. In ischaemic AKI, damage to the endothelium leads to an imbalance between production of endogenous vasoconstrictive factors and vasodilatory factors. The endogenous production of nitric oxide is decreased in the setting of endothelial damage. Experimental studies have shown that the heterogeneity in intrarenal blood flow contributes to the pathophysiology of ischaemic AKI (Bonventre, 1993) (Mason et al., 1989) (Mason et al., 1984) (Vetterlein et al., 1986). These studies show reduction in blood flow to the outer medulla which leads to swelling of the tubular and endothelial cells as well as promoting neutrophil adhesion to capillaries and venules. These changes ultimately cause vascular congestion and consequently impede blood flow. Thus, the nutrient and oxygen supply to the tubules in this region is compromised, leading to tubular cell injury.

Ischaemic AKI involves tubular cell injury and death. In the setting of ischaemia, cellular debris is released into the tubular lumen as a consequence of tubular cell injury. This process leads to the formation of casts, tubular lumen obstruction and raised tubular pressures resulting in reduced glomerular filtration rate. Early structural and morphological changes seen in ischaemic AKI include the formation of blebs in the apical membranes of the proximal tubular cells, with loss of the brush border. There is a loss of polarity in the proximal tubule cells and disturbance in the integrity of their tight junctions (Molitoris, 1991) (Molitoris et al., 1989) (Molitoris et al., 1988). This is due to changes in the actin and microtubule cytoskeletal networks. A study by Molitoris et al showed that Na/K ATPase activity was redistributed from the basolateral membrane to the apical membrane, leading to a reduction in sodium and sodium coupling proximal transport (Molitoris, 1991, Molitoris et al., 1989) (Molitoris et al., 1988).

1.1.4.2 Septic AKI

1.1.4.2.1 Introduction

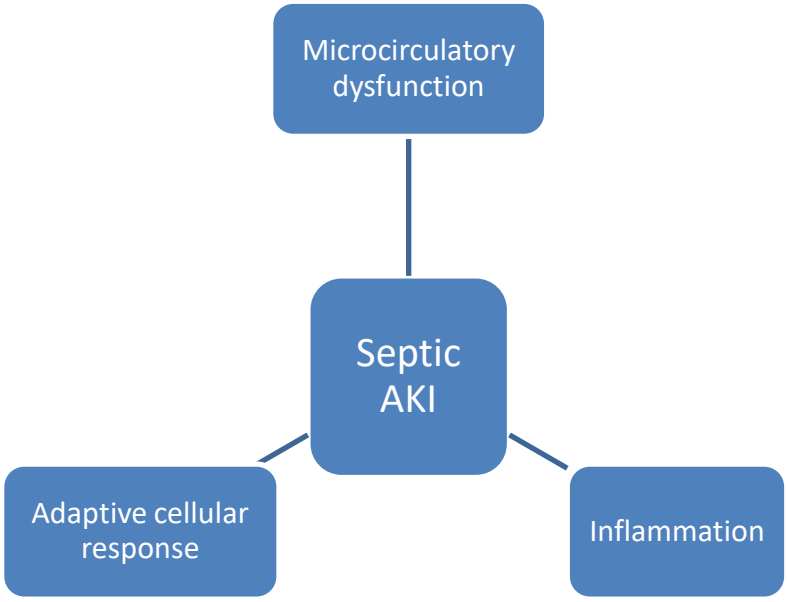
Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). AKI is a frequent and serious complication in septic patients admitted to the ICU (Lafrance and Miller, 2010). Patients who are elderly are also at high risk of developing septic AKI (Ishani et al., 2009). Sepsis is a common cause of AKI, accounting for more than 50% of cases with AKI in ICUs and is associated with increased mortality (Uchino et al., 2005). However, AKI is rarely the cause of death in these patients. This is evident not only among the AKI cohort in the ICU but also outside the ICU(Schneider et al., 2012) (Selby et al., 2012). A study by Selby et al showed that in a general hospital population, the main causes of death in patients with AKI stages 1 to 3 were sepsis, cardiovascular disease and malignancy (Selby et al., 2012). Despite the significant rise in mortality, progress and research in the field of septic AKI is limited. This is due to several factors. First, there is a lack of information pertaining to the histopathology of septic AKI. Second, there is a dependence on using serum creatinine in the assessment of kidney function. Third, there is a reliance on animal models to explain the pathogenesis of septic AKI due to the lack of human studies and difficulties in obtaining samples for histological analysis, as a renal biopsy would usually not be indicated clinically.

1.1.4.2.2 Pathophysiology of septic AKI

The pathophysiology of septic AKI is distinct from non-septic AKI. Previous literature has emphasised that septic AKI is caused by a disruption in the renal macrocirculation (Schrier and Wang, 2004). However, there is now an increasing body of evidence which shows that septic AKI can develop in the absence of renal hypoperfusion. Several clinical studies have demonstrated that a significant number of patients with septic AKI did not have reduced renal blood flow (Gomez et al., 2014) (Le Dorze et al., 2009) (Prowle et al., 2009). In addition, a study by Murugan et al (Murugan et al., 2010) showed that among patients with community acquired pneumonia, AKI still occurred in the absence of severe sepsis and haemodynamic instability. Moreover, higher cytokine levels were observed in patients with AKI. Another important finding is the histopathology of septic AKI. Post mortem kidney biopsies of patients with septic shock have shown that the majority of renal tubular cells are normal (Takasu et al., 2013, Lerolle et al., 2010).

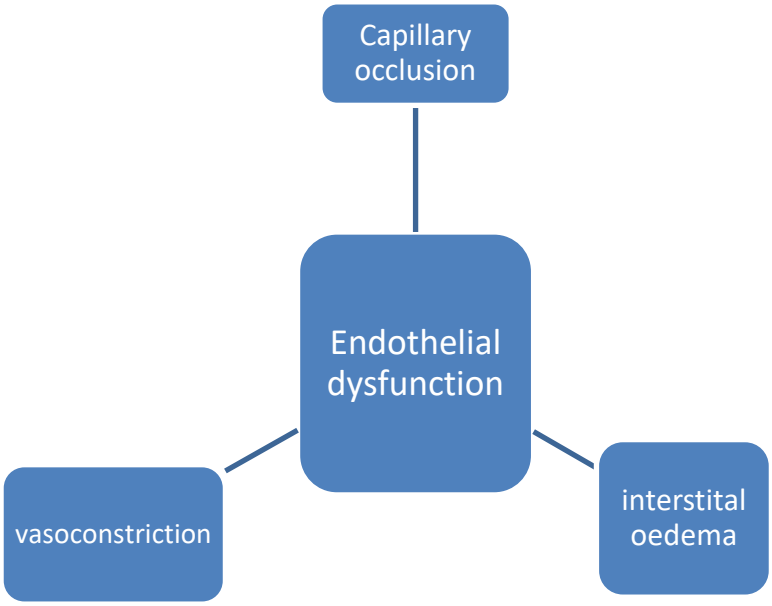
Although there is evidence of renal tubular injury, the severity of the injury do not seem to correlate with the clinical phenotype.

The absence of hypoperfusion and significant histological abnormalities suggests that there are other mechanisms which are involved in the pathogenesis of AKI. There is evidence that there are multiple mechanisms which explain the development of septic AKI. These mechanisms interact and potentiate each other within the renal microcirculation. These mechanisms include endothelial dysfunction, inflammation, coagulation disturbance and an adaptive cell response to injury (Le Dorze et al., 2009).



Endothelial cells are important regulators of vascular tone, leukocyte recruitment and function. They also regulate the responsiveness of the smooth muscle tone (Sprague and Khalil, 2009). Therefore, injury to the endothelial cell during sepsis results in a decreased production of vasodilators (such as nitric oxide) and a more pronounced effect of vasoconstriction. During endothelial cell injury, the interaction between platelets, fibrin, leukocytes and the swollen endothelial cells leads to capillary occlusion (De Backer et al., 2011). In addition, there is increased vascular permeability in sepsis and this leads to interstitial oedema and fluid retention (Prowle and Bellomo, 2010b) (Bagshaw et al., 2012). The presence of interstitial oedema increases the diffusion distance for oxygen to target cells (Hollenberg et al., 2004) (Holthoff et

al., 2012). As a consequence, tissue hypoxia in the kidney occurs, leading to the development of AKI.



Inflammation plays an important role in the pathogenesis of septic AKI. Clinical studies have shown a strong association between cytokine levels and development of septic AKI (Murugan et al., 2010) (Payen et al., 2012). During sepsis, a host response is triggered, where mechanisms of inflammation not only contribute to clearance of infection and tissue recovery, but also organ injury. During the initial phase of severe sepsis, the cytokine storm triggers a variety of responses including leukocyte and endothelial cell activation, microvascular dysfunction, hypoxia and tissue damage (Angus and van der Poll, 2013). Pro-inflammatory mediators activate endothelial cells and increase vascular permeability, leading to significant injury in the kidney via expression of adhesion molecules and release of additional pro-inflammatory mediators. During sepsis, pro-inflammatory cytokines are released leading to increased recruitment of leukocytes. Leukocytes leaving the peritubular capillaries are in close proximity to the tubular epithelial cells and thus can directly activate these cells by releasing pro-inflammatory mediators and DAMPS. The tubular epithelial cells can also be activated from the tubular side (El-Achkar et al., 2008). DAMPS, PAMPS and pro-inflammatory cytokines are filtered in the glomerulus, enter the proximal tubules and can directly activate tubular epithelial cells resulting in a change of the metabolic and functional state of these cells. Renal tubular cells which are exposed to inflammation and microcirculatory dysfunction adapt to the altered tubular environment by shutting down other

tubular cells in a paracrine manner. Several studies have also shown that apoptosis of tubular cells is uncommon during sepsis induced AKI. This implies that tubular epithelial cells which are exposed to hypoxia and inflammation limit processes which lead to apoptosis or necrosis. In the setting of hypoxia and inflammation, the tubular cells adapt downregulating metabolism and undergoing cell cycle arrest. This response is mediated by mitochondria which attenuates further damage and provide cells with the opportunity to recover function. Swollen mitochondria, which can be found in septic AKI, reduced tubular cell function by prioritizing the existing energy to functions that are required for cell survival.

1.1.4.2.3 Potential for diagnostic and therapeutic targets in septic AKI

The knowledge that inflammation, microvascular dysfunction and adaptive responses of tubular cells are involved in the development of septic AKI provides opportunities to identify diagnostic and therapeutic targets. With respect to inflammation, this has led to further research into therapeutic strategies to reduce inflammation in the treatment of AKI. One potential strategy is to remove pro-inflammatory mediators such as IL-6 because they are associated with the development of septic AKI (Payen et al., 2012). In addition, elimination of cytokines and endotoxin using haemoperfusion has been shown to protect against septic AKI (Cantaluppi et al., 2008). Another novel therapeutic strategy is the application of exogenous alkaline phosphatase in septic patients. Several clinical studies have shown that alkaline phosphatase reduced the requirement and duration of renal replacement therapy (Heemskerk et al., 2009a) (Pickkers et al., 2012). The renal microcirculation is also another potential therapeutic target in septic AKI. Use of vasodilators to improve microcirculatory perfusion such as nitroglycerin and nitric oxide are currently under investigation (Trzeciak et al., 2008) (Heemskerk et al., 2009b) (Spronk et al., 2002). However, due to the variety of mechanisms involved in the pathophysiology of septic AKI, it is currently unlikely that a single therapeutic option alone would be effective in treating septic AKI.

1.1.5 Outcomes of AKI

1.1.5.1 Short-term outcomes

It is now widely recognised that AKI is an independent risk factor for mortality (Coca et al., 2009). A meta-analysis performed by Coca et al highlighted that even small increments in serum

creatinine were associated with increased mortality (Coca et al., 2007). Other studies (Chertow et al., 2005) have also shown that patients with mild AKI have longer hospital stay and that AKI is associated with higher economic costs. Not surprisingly, studies have also shown that there is a progressive increase in mortality associated with worsening degrees of AKI (Hoste et al., 2006). This relationship is demonstrated across various hospital settings (Chertow et al., 2005). The effect of baseline renal function also contributes to the relationship with AKI and mortality. Patients with acute on chronic kidney disease have higher short-term mortality compared with patients with normal renal function who develop AKI (Thakar et al., 2005). Fluid overload is a serious complication in AKI. Among patients with stage 3 AKI requiring RRT, 90 day mortality for those who were hypervolaemic was twice as high as in those without hypervolaemia (Vaara et al., 2012). A large European multicentre study showed that positive fluid balance was associated with increased mortality in patients with AKI in the critical care unit (Payen et al., 2008). The positive correlation between fluid overload in AKI patients and mortality has also been demonstrated in other large studies (Bellomo et al., 2012) (Bouchard et al., 2009). Presence of hypervolaemia in AKI is thus a bad prognostic factor.

1.1.5.2 Long-term outcomes

Even mild forms of AKI are associated with long-term adverse clinical outcomes (Coca et al., 2009). A retrospective study demonstrated that AKI is associated with increased long-term mortality which increases with the severity of AKI (Lafrance and Miller, 2010). Lo et al (Lo et al., 2009) demonstrated that an episode of AKI requiring dialysis is independently associated with a twofold increase in death. The high long-term mortality rate also extends to the population of AKI patients who recovered their renal function. A study by Bucaloiu et al (Bucaloiu et al., 2012) demonstrated that in a retrospective cohort study with median follow-up of 3 years, patients with normal renal function who subsequently developed AKI had increased risk of death. The duration of AKI is also a contributing factor to the long-term mortality risk in this patient population. A retrospective study of 1,324 patients undergoing aortic surgery highlighted that even transient worsening of renal function in the post-operative period was associated with increased long-term mortality (Welten et al., 2007). The study also showed unsurprisingly that persistent AKI was associated with the highest long-term mortality. As discussed previously, patients who develop AKI after cardiac surgery are at increased risk of in-hospital death (Lassnigg et al., 2004). In addition, there is evidence of increased long-term mortality risk, independent of

whether kidney function had recovered at discharge from hospital (Loef et al., 2005). The elevated risk in long-term mortality cannot be fully explained by severity of the AKI alone. One study showed that patients with AKI had higher additional mortality in the long term which was unrelated to the severity of illness or the treatment modality offered during hospitalization (Van Berendoncks et al., 2010). Among the AKI survivors, quality of life was reported to be lower compared with age-matched patients (Hoste et al., 2006).

CKD is now increasingly recognised as a long-term adverse outcome in patients who survive an episode of AKI. A large study from North America has shown that an episode of AKI requiring RRT increases the risk of developing progressive CKD (Lo et al., 2009). There is accumulating evidence that patients who recover renal function following AKI have increased risk of developing *de novo* chronic kidney disease (Bucaloiu et al., 2012). Patients with AKI have increased risk of progressing to end stage kidney disease requiring long-term RRT (Wald et al., 2009). This risk is significantly higher in elderly patients (Pascual et al., 1990) (Schmitt et al., 2008) and in those with pre-existing chronic kidney disease (Hsu et al., 2009). Presence of CKD prior to AKI is associated with a lower probability of renal recovery (Schiffl, 2006). Severity of AKI can also predict progression of CKD (Chawla et al., 2011). In addition, multiple episodes of AKI increase risk of CKD progression as highlighted by Thakar et al (Thakar et al., 2011).

1.2 Renal Replacement Therapy in Acute Kidney Injury

1.2.1 Introduction

The kidney is a vital organ which serves to remove nitrogenous waste products, maintain acid-base and electrolyte balance and regulate blood pressure and volume. In AKI, these functions are compromised but can often be restored rapidly if correct treatment is administered early and appropriately. In severe AKI, the kidney will cease to function adequately, which can lead to life-threatening consequences such as cardiac arrhythmias and pulmonary oedema. Under such circumstances, renal replacement therapy (RRT) is provided as an emergency and life-saving treatment. RRT is a supportive treatment which performs the essential functions of the kidney. These include removing nitrogenous waste products and excess fluid, correcting metabolic acidosis and electrolyte abnormalities.

1.2.2 Intermittent haemodialysis

Haemodialysis is an extracorporeal treatment which removes solutes from blood using the process of diffusion. Diffusion of solutes occurs across a semi-permeable membrane which separates two compartments within the dialyser. These two compartments comprise a blood based compartment where blood from the patient is delivered to the dialysers and secondly a compartment containing dialysis fluid. Diffusion of solutes occurs across the dialysis membrane in the direction of the dialysis fluid down the concentration gradient of the solute. The blood and dialysis fluid flow counter-current in order to maximise the concentration gradient and thereby increase efficiency of the process.

Haemodialysis requires a dialyser, adequate flow of dialysate and blood, the latter being dependent on satisfactory vascular access. Reliable safety and monitoring systems are also essential. The goal of dialysis is to restore the composition of the body's electrolytes and fluid status towards normal or safe levels. To achieve this, the dialysate has to contain appropriate and precise concentrations of electrolytes. Different concentrations of dialysate concentrate are available, the choice of which can be tailored to individual requirements. For example, different potassium concentrations are available. The aim would be to reduce serum potassium concentration to a safe level but to avoid unnecessarily large and abrupt changes in serum concentration, as this would increase the risk of cardiac arrhythmia.

The dialysis fluid consists of an acid component, a bicarbonate component and purified water. These three components are mixed in the dialysis machine before delivery to the patients. The acid component consists of sodium, potassium, calcium, magnesium and chloride at specific concentrations. The bicarbonate component contains sodium bicarbonate and sodium chloride. Purified water is delivered from the water purification system located in the dialysis unit to prepare the dialysate in accordance to strict regulations

Figure 1 - The major components of dialysate

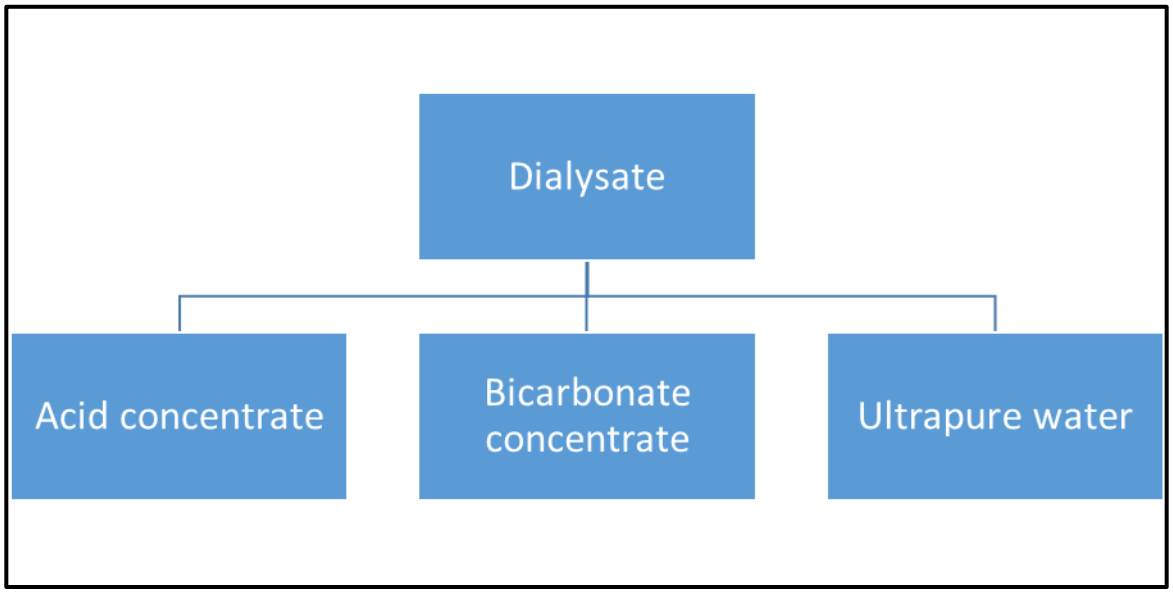


Figure 1 The major components of dialysate. The dialysis fluid consists of an acid component, bicarbonate component and purified water. These components are mixed in the dialysis machine to constitute the dialysis fluid before delivery to the patient. The acid component consists of sodium, magnesium, potassium, calcium and chloride at specific concentrations. The bicarbonate component contains sodium bicarbonate and sodium chloride. Purified water is prepared using the process of reverse osmosis from the water purification system located in the dialysis unit.

Table 2 - The electrolyte composition in dialysate

Electrolyte	Concentration (mmol/L)
Sodium	137
Chloride	105
Calcium	3.0
Acetate	4.0
Potassium	2.0
Bicarbonate	33
Magnesium	0.75
Dextrose	200mg/dl

Table 2 - The electrolyte composition in the dialysate. The concentration of the electrolytes are approximately similar to the physiological concentrations in plasma. In the presence of an abnormal plasma electrolyte concentration, the dialysate concentration of that electrolyte is altered to a non-physiological level to allow correction to be achieved via dialysis.

Water used in dialysate is purified using a process known as reverse osmosis, which removes organic molecules and salt ions. Reverse osmosis requires a membrane which sieves organic molecules and repels salt ions while passing pure water through micropores at its surface. Pressure is applied across the membrane to overcome the inherent osmotic pressure of the solution.

1.2.3 The Dialyser

The properties of the dialyser membrane are crucial to enable safe and efficient dialysis treatment. There are three important characteristics of the membrane to consider. Firstly, the dialyser membrane is made from biocompatible materials which in modern treatments are usually synthetic compounds. Biocompatibility implies minimal inflammatory response created by the dialyser, in effect a foreign body, being in contact with the blood compartment. Lack of biocompatibility can result in an inflammatory response which can occasionally lead to serious clinical consequences such as hypotension, hypoxia, pyrexia and leucopenia. This reaction is mediated by release of pro-inflammatory molecules, complement activation and oxidative stress (Canivet et al., 1994, Marchant et al., 1996, Patarca et al., 1992, Puentes et al., 1994, Schaefer et al., 1989, Walker et al., 2004). Secondly, the surface area of the dialysis membrane needs to be considered. A dialysis membrane with a high surface area irrespective of pore size will achieve a higher clearance of small molecules such as urea. However larger molecules will not be adequately removed if the membrane consists of small pores. Flux is thus the third characteristic of the membrane and this is defined as the pore size of the membrane. High flux membranes have larger pores and can deliver high clearance of middle and large sized molecules. Low flux membranes with relatively small pores can clear only molecules with relatively low molecular weight.

1.2.4 Vascular access in AKI:

Placement of suitable vascular access is crucial to enable adequate provision of RRT. Dialysis catheters in patients with AKI are usually placed in one of three major veins: the internal jugular, femoral or (less commonly) subclavian vein. Continued functioning of the dialysis catheter is influenced by location of its placement. Although placement in the subclavian vein is associated with the lowest rate of infectious complications, this site is not recommended because it increases risk of developing central vein stenosis which amongst other problems, would jeopardise subsequent vascular access if the patient were to require long-term RRT. Evidence for this comes from observational studies in patients with ESRD on long-term haemodialysis (Cimochowski et al., 1990) (Schillinger et al., 1991). Use of femoral catheters is associated with the highest risk of infection and thus is best avoided to minimize incidence of catheter-related blood stream infection (Pronovost, 2008). Other disadvantages of femoral vein

placement include higher incidence of catheter malfunction (Hryszko et al., 2004) (Liangos et al., 2004, Naumovic et al., 2004). Catheter malfunction is also observed more frequently with left-sided internal jugular catheters compared with right-sided catheters (Oliver et al., 2002). Access recirculation is also a recognised complication in RRT and this occurs more frequently with femoral catheters compared with subclavian and internal jugular catheters (Leblanc et al., 1996) (Little et al., 2000). Another factor to consider is the increased risk of iliac vein stenosis following use of femoral catheters. This complication could cause problems if renal transplantation were required, as the iliac vein is used for anastomosis with the renal vein of the implant. Presence of a femoral dialysis catheter will also reduce the patient's mobility. Patients with AKI often require RRT in an emergency setting and inserting a dialysis catheter has to be performed without delay. Thus the majority of patients with AKI receive RRT via a non-tunnelled dialysis catheter. Few studies have evaluated use of different types of dialysis catheters and sites in AKI.

1.2.5 Continuous Renal Replacement Therapy (CRRT)

Patients with stage 3 AKI are often critically ill and are managed in the intensive care unit (ICU). These patients are frequently haemodynamically unstable and many require vasopressor support to maintain their mean arterial pressure. Commencing RRT in these patients can be challenging as RRT can aggravate their haemodynamic instability. Risk of RRT-induced hypotension can be reduced by using continuous renal replacement therapy (CRRT), which is the most widely used type of RRT in ICUs worldwide (Prowle and Bellomo, 2010a). Two main types of CRRT are used in clinical practice: continuous veno-venous haemofiltration (CVVH) and continuous veno-venous haemodiafiltration (CVVHDF).

1.2.5.1 Principles of CRRT

Convection is the predominant process which drives removal of solutes in CRRT. Movement of plasma water and molecules is driven by a hydrostatic pressure gradient applied across the semipermeable membrane of the haemofilter. This forces plasma water from the blood compartment into the effluent compartment, dragging molecules smaller than the pore size with it. Convective therapies are effective at removing middle and large sized molecules, because the

haemofilter pore size is relatively large. This solute clearance mechanism contrasts with that of intermittent haemodialysis (IHD), in which solutes are removed by diffusion. Here, clearance of solutes depends upon maintenance of a concentration gradient rather than a pressure gradient. Diffusion is an efficient mechanism for removal of small molecules (such as potassium) but is less efficient for clearance of middle and large sized molecules. It is not yet clear whether greater clearance of larger 'middle molecules' using convective therapies leads to better clinical outcomes in patients with AKI; this remains a topical and controversial debate internationally. Convection is the predominant component driving solute clearance in CVVH. In contrast, CVVHDF combines diffusion and convection. Again there is so far no convincing evidence that one modality achieves better clinical outcomes than the other, but various studies have been published. A prospective randomised trial (Saudan et al., 2006) demonstrated that survival of patients receiving CVVHDF was significantly higher than patients receiving CVVH, implying that small molecule clearance is important in determining the outcomes for patients with AKI requiring RRT, but larger prospective studies are required to answer this question.

1.2.5.2 The CRRT circuit

Components of CRRT include a central venous catheter, haemofilter, replacement fluid, anticoagulation in addition to the machine which contains umps for blood and replacement fluid as well as sophisticated monitoring and safety mechanisms. In the CRRT circuit, hydrostatic pressure forces a portion of plasma water across the haemofilter membrane, taking solutes with it by convection. The resulting filtrate produced by this process is often termed effluent. Physiologically balanced replacement fluid is added to the circuit either (or both) before or/and after the haemofilter so that the patient does not become volume depleted.

1.2.5.3 Choice of replacement solution in CRRT

Replacement fluid consists of a buffer solution and physiological concentrations of electrolytes. Phosphate is not included in most commercially available solutions and must often be supplemented during extended periods of CRRT. Replacement solutions require a buffer to correct the metabolic acidosis associated with AKI. Both lactate and bicarbonate are commonly used. Lactate is metabolised to bicarbonate in the liver. Studies have demonstrated that both lactate and bicarbonate replacement solutions have similar efficacy in correcting metabolic acidosis during CRRT (Heering et al., 1999) (Kierdorf et al., 1995, Leblanc et al., 1995) (Morgera

et al., 1997) (Thomas et al., 1997). Plasma lactate levels are generally higher when lactate-based solutions are used. Potential complications of this include haemodynamic intolerance (Leblanc et al., 1995), cerebral dysfunction (Veech, 1988) and increase in urea generation (Kierdorf et al., 1995) (Veech, 1988). Lactate-based replacement solutions are contraindicated in patients with lactic acidosis or lactate intolerance, defined as a rise of 5 mmol/L or more during CRRT with lactate-based solutions (Barton et al., 1991) (Hilton et al., 1998). Patients with hepatic failure have developed worsening acidosis when lactate-based solutions are used in both CRRT and intermittent haemofiltration (Davenport et al., 1991, Davenport et al., 1990a, Davenport et al., 1990b).

1.2.5.4 Anticoagulation in CRRT

Exposure of blood in the extracorporeal circuit to a variety of foreign surfaces can lead to thrombogenicity and clotting of the circuit. This disrupts the treatment schedule and reduced dose of delivered CRRT as well as wasting expensive consumable components. Anticoagulation in CRRT is crucial for maintaining function of the extracorporeal circuit but also has potential adverse effects, most important of which is haemorrhage. Two types of anticoagulation used in CRRT: heparin and citrate. Heparin remains the most commonly prescribed anticoagulant in CRRT (Uchino et al., 2007b). It works by changing the conformation of antithrombin (AT). This results in the inactivation of coagulation factors, in particular factor Xa. The main disadvantage of heparin is the increased risk of bleeding because it causes systemic anticoagulation rather than acting only in the extracorporeal circuit. Critically ill patients with AKI already have relatively high risk of bleeding due to recent surgery, trauma, mucosal lesions and coagulopathy.

1.2.6 Extended Hybrid Therapies

Hybrid therapies combine features of CRRT and IHD and are being used increasingly as an alternative to CRRT or IHD. These therapies are essentially dialysis (+/- filtration) treatments which remove solutes at a lower rate over a longer period of time compared than in conventional haemodialysis. The two main hybrid modalities are Sustained Low-Efficiency Dialysis (SLED) and Sustained Low-Efficiency Diafiltration (SLEDf). In 1998, SLED was introduced as an alternative to IHD or CRRT in North America (Marshall et al., 2001). This study suggested that SLED was well-tolerated in critically ill patients with AKI requiring RRT. Another cohort study comprising patients from four critical care units also showed that patients receiving SLED had comparable

haemodynamic stability to patients receiving CRRT (Fieghen et al., 2010). In addition, adequate small solute clearances were also achieved with use of SLED. SLED has been shown to deliver a high dose of dialysis while avoiding the significant risk of urea disequilibrium (Marshall et al., 2002).

1.2.6.1 Principle of Hybrid Therapies

The process of solute transport in SLED is diffusion, as is the case with IHD. The same dialysis machine can be used to deliver IHD and SLED, as long as the blood and dialysate pump speeds can be reduced sufficiently for SLED. Duration of treatment with SLED is 8 to 12 hours, which is significantly longer than the duration of each IHD session. The effect is a slower rate of solute clearance over a longer time, which is less haemodynamically stressful. In SLEDf, diffusive and convective clearance is used in combination to achieve better removal of middle and large sized molecules. The convective component of this treatment requires availability of ultrapure online replacement fluid. The online replacement fluid is similar in composition to that used in CVVH. The type of vascular access used is similar to that used in CRRT and IHD. Anticoagulation with heparin is also prescribed and delivered to maintain the patency of the extracorporeal circuit. However, in contrast to CRRT, hybrid therapies such as SLED can be performed safely and successfully without the use of anticoagulation. This has been demonstrated in a prospective observational study comparing SLED and CRRT (Berbece and Richardson, 2006). Treatment schedules for SLED or SLEDf will vary. Typically the treatment is used daily for 6-12 hours. Blood flow rate is typically 200ml/min with dialysate flow rate of 200 - 300ml/min. In SLEDf, typical fluid replacement rate of 100ml/min.

1.2.7 Comparison of RRT modalities in AKI

There is currently no consensus about which RRT modality is superior for management of AKI. Each modality has strengths and weaknesses and should be chosen to best suit the individual patient's requirements. In practice local facilities and expertise are obviously influential. Severe AKI often occurs in the setting of multi-organ failure. Patients who develop AKI may also have co-morbidities which influence treatment choice. There is considerable variation in the use of RRT for AKI worldwide. In addition to modality choice, dose, timing of initiation, duration of RRT and standards of monitoring differ significantly.

CRRT is widely used by nephrologists and intensivists in managing patients with AKI who are haemodynamically unstable. A recent survey of general adult critical care units in the United Kingdom showed that CRRT is the most commonly used modality in this setting (Jones and Devonald, 2013). CRRT appears to confer physiological advantages over IHD (Ronco and Bellomo, 2007). These include greater azotemic control (Clark et al., 1994), improved phosphate control (Tan et al., 2001), haemodynamic stability (Paganini et al., 1984), prevention of cerebral disturbance (Ronco et al., 1999) and enhanced renal recovery (Manns et al., 1997). CRRT removes fluid at a slower rate over a prolonged period of time. Risk of hypotension is reduced and vasopressor requirement reduced (Rabindranath et al., 2007). CRRT possibly is superior for control of fluid balance (Ronco and Bellomo, 2007) (Bouchard and Mehta, 2009). Bouchard et al showed that CRRT optimised fluid balance more effectively compared with IHD (Bouchard et al., 2009). IHD was associated with a more positive fluid balance which is an adverse prognostic factor in critically ill patients with AKI (Payen et al., 2008) (Bouchard et al., 2009). Some studies have suggested that CRRT is associated with higher rate of renal recovery than IHD. This is attributed to the propensity of IHD to cause intradialytic hypotension which can delay renal recovery (Uchino et al., 2007a, Mehta et al., 2001, Jacka et al., 2005, Bell et al., 2007).

CRRT is the modality of choice in patients with AKI and acute brain injury (Ronco et al., 1999) (Davenport, 2001). In patients with intracranial pathology, two dialysis-related complications can exacerbate the neurological status. Firstly, dialysis-induced hypotension can lead to reduction in cerebral perfusion pressure. Second, rapid fall in plasma osmolality during dialysis can precipitate cerebral oedema and raised intracranial pressure, known as dialysis disequilibrium. CRRT has its disadvantages. Patency of the extracorporeal circuit is usually maintained by anticoagulation with heparin. Continuous anticoagulation increases the risk of haemorrhage. Anticoagulants such as heparin can cause thrombocytopenia. Low molecular weight heparin is now increasingly used in critically ill patients with low bleeding risk (Joannidis et al., 2007). However, the effects of systemic anticoagulation and prolonged half-life and difficulties in reversal remain significant challenges. Use of regional citrate anticoagulation is increasing. Citrate has been shown to lower the rate of bleeding complications and increase survival of the extracorporeal circuit, compared with heparin (Kutsogiannis et al., 2005, Kutsogiannis et al., 2000, Monchi et al., 2004, Wu et al., 2012). Regional citrate anticoagulation might improve patient and renal survival comparison to low molecular weight heparin (Oudemans-van Straaten et al., 2009).

Metabolic abnormalities such as hypophosphataemia occur frequently in patients receiving CRRT. Phosphate has low molecular weight and is easily removed during RRT. Low serum phosphate is associated with adverse effects in critically ill patients. Effects include impaired cardiac function, reduced respiratory muscle function and increased mortality (Tan et al., 2001) (Morimatsu et al., 2002) (Zazzo et al., 1995). CRRT increases the risk of hypophosphataemia compared with non-continuous modalities such as IHD and SLEDf.

Hybrid therapies have become an attractive alternative to CRRT in management of stage 3 AKI. These therapies not only combine the strengths of both diffusive and convective treatments but also reduce their weaknesses. Several randomised controlled trials have compared use of hybrid treatments with CRRT in critically ill patients with AKI (Baldwin et al., 2007) (Kielstein et al., 2004) (Gedmintas et al., 2010) (Kumar et al., 2004).

These studies demonstrated that adequate fluid removal is feasible and well-tolerated using extended low-efficiency dialysis. Kielstein et al (Kielstein et al., 2004) showed that there was no significant difference in mean arterial pressure, heart rate, cardiac output, systemic vascular resistance and dose of catecholamines between extended dialysis and CRRT. Patients treated with extended dialysis achieved more rapid correction of metabolic acidosis and required lower dose of heparin compared with CRRT. The non-continuous nature of hybrid treatments facilitates arrangement of patients to have access to investigations and procedures during the day. In addition, patients can recover their mobility far quicker with the aid of more frequent physiotherapy if they have a RRT-free period.

Hybrid treatments such as SLED and SLEDf may have advantages over IHD in achieving effective solute removal. These hybrid therapies not only enable a higher clearance of small solutes, but also minimise solute disequilibrium and provide greater clearance of middle and large sized molecules (Kielstein et al., 2004). Although SLED is inferior to CRRT in terms of large solute clearance, the combination of diffusion and convection in SLEDf can lead to much higher solute clearance of some solutes compared with diffusion alone (Marshall et al., 2004). Hybrid therapies are cheaper than CRRT as the purchase of expensive replacement fluid is not required and they do not require one-to-one nursing care. Some studies have suggested that cost of CRRT is eight times higher than hybrid therapies (Berbece and Richardson, 2006).

Overall, SLED and SLEDf are safe and effective alternatives to CRRT and IHD. Although no large

randomised controlled trials have compared mortality and renal recovery in these three modalities, hybrid therapies provide further options in the management of critically ill patients with AKI.

1.2.8 Timing of RRT in AKI

Optimal timing of initiation of RRT in patients with AKI is not known. Whilst there are some definite indications for urgent commencement of RRT in AKI such as severe hyperkalaemia, severe acidosis or pulmonary oedema not responding to diuretics, it is otherwise currently unclear whether relatively early or late initiation is beneficial with respect to clinical outcomes such as mortality and renal recovery. There are difficulties in conducting studies evaluating timing of RRT in AKI. Firstly, the definition of 'early' or 'late' varies from centre to centre (Clark et al., 2012). Secondly, there is a lack of uniform clinical practice among nephrologists concerning the initiation of RRT. Variables such as serum creatinine, serum urea, urine output, fluid balance, time from admission to ICU or start of RRT (Bagshaw et al., 2009). A meta-analysis of 15 studies concluded that earlier institution of RRT is associated with an improvement in patient survival (Karvellas et al., 2011). However, this must be interpreted with caution because most of the studies included in this study were retrospective, of poor quality and heterogenous. In contrast, a well-designed randomised controlled trial of 106 patients did not demonstrate any difference in mortality or renal recovery between early and late initiation of RRT in AKI (Bouman et al., 2002). In this study early initiation was defined as after 12 hours of oliguria or creatinine clearance of less than 20ml/min. Late initiation was defined as the usual pre-existing indications for initiation. Starting RRT in critically ill patients with AKI has complications and must be carefully weighed up against the possible benefits of RRT. Potential complications include risk of infection from indwelling dialysis catheter (Palevsky et al., 2005), RRT-induced hypotension and its consequences and leukocyte activation from contact with dialysis membranes (Teehan et al., 2003).

The aetiology of AKI may influence timing of RRT initiation. Patients with septic AKI might benefit from earlier initiation (Lameire et al., 2011). The reason is that early RRT in sepsis can decrease circulating levels of inflammatory cytokines and alter underlying pathophysiological mechanisms of the disease (Grootendorst, 1994). Sepsis is a syndrome which leads to a release of excessive amounts of pro-inflammatory cytokines such as tumour necrosis factor α (TNF α), interleukin 1β

and IL-6 in the circulation (Bone et al., 1997). A prospective observational study evaluating the removal of cytokines during CRRT in septic patients with AKI highlighted two important findings (De Vriese et al., 1999). Firstly, pro-inflammatory cytokines were removed during CVVH but this did not improve the haemodynamic and respiratory parameters of these patients. Secondly, the naturally-occurring inhibitors of inflammation were removed to the same extent as the inflammatory cytokines. Removal of these inflammatory inhibitors may explain lack of improvement in the clinical parameters of the patients. Extracorporeal removal of inflammatory cytokines did not confer survival benefit, arguing against early RRT in septic AKI.

1.2.9 RRT dose in AKI

Measurement of RRT dose in AKI has generated significant interest. Previous studies have demonstrated an inverse relationship between dialysis adequacy and mortality in patients with ESRD (Collins et al., 1994, Lowrie et al., 1981, Owen et al., 1993).For patients with AKI, there is evidence that increasing the dose of RRT does not improve outcomes (Palevsky et al., 2008, Bellomo et al., 2009). However, the method of quantifying dose in RRT in patients with AKI remains controversial. Definitions of dose include different functions of renal replacement and may include:

- Clearance of small solutes
- Clearance of middle molecules
- Volume of fluid removal
- Duration of therapy

1.2.9.1 Kt/V

Measuring clearance of urea as a surrogate for RRT dose is attractive because urea is a low molecular weight solute abundant in plasma, has uncharged chemical structure with low protein binding capabilities and has the same volume of distribution as total body water (Basile and Lomonte, 2012). Urea levels reflect dietary protein intake and clearance of urea can indicate efficiency of small solute removal (Gotch and Sargent, 1985). Kt/V, an index of urea clearance, is

defined as the dialyser urea clearance (K in mls/min) multiplied by the duration of dialysis treatment (t, in minutes) and divided by volume of distribution of urea in the body (V in millilitres), which is approximately equal to the total body water (Gotch and Sargent, 1985).

Using Kt/V to measure dialysis dose has disadvantages. Firstly Kt/V is an unreliable marker of urea clearance in AKI because this group of patients exhibit large variation in urea generation rates (Ricci et al., 2006). Kt/V will become a reliable marker of urea clearance only if production of urea is equal to removal of urea (Sargent, 1983). Secondly, Kt/V assumes that the volume of distribution of urea is equal to the total body water (50-60% of body weight) (Friedman and Jaber, 1999). Whilst this assumption may be applied in a steady state condition such as ESRD, it is not valid in patients with AKI because these patients can become hypervolaemic with increased total body water. Thus measurement of V is difficult. Factors influencing the rate of urea generation in AKI include patient specific factors, disease specific factors and medical therapy (Jorres et al., 2013). In addition, patients with ESRD and AKI differ in many other ways. One of the main differences is in discrepancy between prescribed and delivered dose of dialysis (Evanson et al., 1998, Evanson et al., 1999). In ESRD, delivered Kt/V has been reported to be 10% lower than the prescribed dose. In contrast, in AKI delivered Kt/V values are reported to be 33% lower than those prescribed (Venkataraman et al., 2002). Another study demonstrated that 70% of RRT sessions delivered a Kt/V of less than 1.2 (Evanson et al., 1998). Many factors contribute to underdelivery of dialysis in AKI. These include minimal use of anticoagulation and the presence of access recirculation, both of which are recognised complications of venous catheter use (Ricci et al., 2006, Evanson et al., 1998, Evanson et al., 1999). It is important to acknowledge that urea merely estimates clearance of small solutes. It is not necessarily representative of all small solutes and certainly does not provide accurate quantitative information on removal of larger or protein-bound solutes. Kt/V is merely a tool to measure one aspect of kidney function. It does not give any useful measure of other important parameters of RRT such as volume management, acid base correction and middle molecule removal (Jorres et al., 2013).

1.2.9.2 Single pool versus double pool urea kinetics

Patients with end stage renal disease experience significant rise in plasma urea levels immediately following the cessation of a dialysis session. This rise in urea is known as the rebound phenomenon and occurs over 30-60 minutes (Daugirdas et al., 2004). Rebound occurs due to the

effect of dialysis in causing a concentration gradient between intracellular and extracellular levels of urea. In order to adjust for this phenomenon when assessing delivered dose of dialysis, plasma urea concentration is measured 30-60 minutes after the end of dialysis. This disequilibrium in body pools can also be exacerbated by differences in regional blood flow (Daugirdas and Schneditz, 1995).

1.2.9.3 Solute reduction index

Blood-based kinetics is an indirect method of measuring dialysis dose in AKI. This method may be flawed and inaccurate as plasma concentrations of urea are influenced by various factors including volume of distribution, regional differences in body pools and access recirculation (Friedman and Jaber, 1999) (Himmelfarb et al., 2002). The solute reduction index (SRI) is another tool to measure delivered dose of dialysis and has been considered gold standard for measuring dialysis dose (Evanson et al., 1999). SRI measures the amount of urea removed during a dialysis session whilst Kt/V or URR measures the fractional change in blood urea concentration. SRI utilises dialysate based kinetics and provides a more accurate measurement of RRT dose. SRI is not influenced by blood based kinetics and is not affected by intercompartmental distribution, disequilibrium between different pools and mode of therapy (Cheng et al., 1998).

1.2.9.4 CRRT dose in AKI

Clearance of small solutes with CRRT is a function of effluent flow (Lyndon et al., 2012). In CRRT effluent consists of the ultrafiltrate in continuous veno-venous haemofiltration as well as spent dialysate in haemodiafiltration (Lyndon et al., 2012). Dose of CRRT is quantified using effluent flow rate measured in millilitres per kilogram of body weight per hour. Two highly cited randomised controlled trials suggested that increasing dose of CRRT confers a survival benefit in patients with AKI (Saudan et al., 2006) (Ronco et al., 2000). However results from these studies have to be interpreted with caution. Firstly, these were single centre studies conducted by physicians committed to the practice of CRRT. This increases likelihood of a Hawthorne effect (the additional beneficial effect of an overall treatment independent increase in attention and care given to recruited patients). Secondly, the study sample was small with an imbalance of patient characteristics at randomisation. One study (Ronco et al., 2000) had a higher number of

sicker patients in the low dose arm despite randomisation. Two landmark multicentre randomised controlled trials have since addressed the dose of CRRT and mortality (Palevsky et al., 2008) (Bellomo et al., 2009) . These two studies, RENAL and ATN, concluded that increasing effluent flow rate above 25ml/kg/hour in CRRT does not improve outcome in patients with AKI. In addition, a dose response relationship exists at an effluent flow rate of less than 20ml/kg/hour and therefore prescribing CRRT below this level should be avoided. Thus, for patients receiving CRRT, a dose of 20-25mls/kg/hour is commonly prescribed currently.

1.2.9.5 Adsorption of solutes in RRT

Adsorption is removal of molecules from blood or plasma by attachment to a surface incorporated in a module within an extracorporeal circuit. Sorbents are substances with unique physical and chemical characteristics which have the ability to adsorb on their surface other elements in dissolution. Sorbents can be divided into two categories:

1. Those that have hydrophobic properties and therefore adsorb the molecules dissolved in the solution in contact with the sorbent.
2. Those that eliminate solutes by chemical affinity

Role of adsorption in small and middle molecule removal in RRT. Two types of membranes are commonly used in RRT in patients with AKI. They are:

a. Polysulfone
b. Polymethylmethacrylate

The polysulfone membrane is asymmetric in structure. This means that for a membrane thickness of 30 microns, just a 1 micron layer is responsible for the separation process, while 29 microns have structural functions alone. Polymethylmethacrylate is characterised by a symmetric structure. The pores in this structure are larger, longer and winding, designed to trap different substances. Middle molecules such as beta-2 microglobulin are removed significantly via adsorption to this membrane, whereas with high flux polysulfone membranes these middle molecules are removed predominantly by filtration.

1.3 Malnutrition in AKI

AKI is common in critically ill patients and is associated with high mortality and morbidity. There is a high prevalence of malnutrition (42%) in AKI (Fiaccadori et al., 1999). Malnutrition is an independent predictor of mortality in patients with AKI (Fiaccadori et al., 1999). Presence of co-morbidities, prolonged hospital stay and use of RRT also exacerbate malnutrition in these patients. RRT, when used in the management of patients with severe AKI, not only removes ureamic toxins and corrects electrolyte abnormalities, but can potentially remove essential water-soluble micronutrients as well. This has been shown in small studies using CRRT and losses have also been observed in patients with ESRD requiring regular haemodialysis (Fissell et al., 2004). Micronutrients essential for health include trace elements, water-soluble vitamins and amino acids (Fissell et al., 2004).

1.3.1 Measuring nutritional status in AKI

Evaluation of nutritional status in patients with AKI represents a significant challenge. Use of conventional nutritional tools such as body mass index (BMI), body weight, anthropometric measurements and, serum protein levels in the evaluation of these patients can be misleading in critical illness and where there are changes in distribution of total body water (Fouque et al., 2008). For example, patients with severe AKI can be hypervolaemic, making determination of dry body weight difficult. In this setting, evaluation of nutritional status using body weight would be inaccurate. A definition of nutritional insufficiency has now been established by the International Society of Renal Nutrition and Metabolism. Protein-energy wasting is defined as a condition of decreased body stores or protein and energy fuel stores, which can occur in either AKI or CKD, regardless of the cause, and is associated with diminished functional capacity related to metabolic stresses (Fouque et al., 2008). In order to diagnose protein-energy wasting in kidney disease, the following criteria must be met.

1. Low serum albumin, cholesterol and transthyretin
2. Reduced body mass
3. Decreased muscle mass

4. Reduced protein and energy intake

1.3.1.1 Subjective Global Assessment:

Subjective Global Assessment (SGA) is a clinical tool used to evaluate the nutritional status of a patient. SGA is a simple multifactorial method consisting of thorough medical and dietary history, physical examination and functional assessment (Baker et al., 1982, Detsky et al., 1987).The tool has not only been used in patients with chronic kidney disease (Cianciaruso et al., 1995, Enia et al., 1993, Kalantar-Zadeh et al., 1998, Young et al., 1991) but also in other patient subgroups, such as liver (Hasse et al., 1993) and lung transplant candidates (Madill et al., 1993). SGA is a practical and inexpensive tool which utilises the clinical judgement of the assessor. Another advantage is that SGA can be used by the other members of the healthcare team such as dietitians and nurses. SGA correlates well with other laboratory parameters such as serum albumin and total iron binding capacity (Kalantar-Zadeh et al., 1998). There is an inverse correlation between acute phase reactants such as serum ferritin and SGA scores (Kalantar-Zadeh et al., 1998). SGA assesses the following features in the history and physical examination of the patient:

a. weight change
b. dietary intake
c. gastrointestinal symptoms persisting for more than 2 weeks.
d. functional capacity
e. underlying disease
f. effect of metabolic stress
g. loss of subcutaneous fat
h. muscle wasting
i. sacral oedema
j. ascites

Patients assessed using SGA are divided into three categories based on their nutritional status:

a. SGA class A: well-nourished

b. SGA class B: moderately malnourished or at risk of malnutrition

c. SGA class C: severely malnourished.

Assessment of nutritional status with SGA using just 3 levels of severity limits the tool's reliability and accuracy. Most of the components used in SGA do not have specific definitions and there is lack of guidance on its use (Kalantar-Zadeh et al., 1998). Assessment of each component is subjective (Kalantar-Zadeh et al., 1999). A modified malnutrition score by Kalantar-Zadeh et al (Kalantar-Zadeh et al., 1999) objectively quantified nutritional status of patients with ESRD requiring regular haemodialysis. In addition, a significant correlation was demonstrated between the malnutrition score and important anthropometric and biochemical parameters.

1.3.1.2 Malnutrition Inflammation Score

In order to establish a more objective and quantitative nutritional assessment tool in patients with ESRD, a Malnutrition Inflammation Score (MIS) was developed. This score incorporates subjective components from SGA where each component is given a score from 0 (normal) to 3 (severely abnormal). Three new variables were added: body mass index (BMI), serum albumin and total iron binding capacity (Kalantar-Zadeh et al., 2001). This study showed that malnutrition and inflammation based on worsening MIS scores were associated with increased mortality and hospitalisation rates.

1.3.1.3 SGA in AKI

A prospective observational study in Italy used SGA to describe nutritional status of patients with AKI (Fiaccadori et al., 1999). This study demonstrated increasing in-hospital mortality of patients with AKI with worsening SGA class. Traditional nutritional parameters were also assessed and the worst values were found in patients who were severely malnourished as defined by SGA. Patients with SGA class C were also found to have the highest prevalence of complications, including sepsis and septic shock. Therefore, nutritional assessment of patients with AKI should be performed routinely, as this will identify patien

ts who are severely malnourished and require nutritional support.

1.4 Nutritional support in AKI

Oral intake is often inadequate in patients with AKI due to anorexia associated with uraemia, increased nutrient requirement in catabolic illness and potentially significant nutrient losses during RRT. There is need for an alternative route of nutrient administration in order to optimise the patient's nutritional status. Two routes of nutrient provision are commonly used in critically ill patients: enteral and parenteral. In patients with AKI, studies have shown that enteral feeding offers several advantages over parenteral. Firstly, the volume of feed is smaller with the enteral route, reducing risk of hypervolaemia. Second, cost of enteral feeding is lower and third, survival rate is higher (Metnitz et al., 2002). However, patients with AKI might not tolerate enteral feeding as uraemia can adversely affect motility of the gastrointestinal tract. One study (Fiaccadori et al., 2004) demonstrated that enteral feeding is safe and effective in patients with AKI. This study also showed no significant difference in gastrointestinal or mechanical complications between patients with AKI and patients with normal kidney function.

1.5 Metabolic derangements in AKI

AKI is a syndrome that does not occur in isolation but is frequently a complication of severe illness such as sepsis and multi-organ failure. Metabolic changes in AKI are not just due to derangement in kidney function, but also to underlying disease processes. The modality and intensity of RRT can influence nutrient balance in a patient with AKI. A key feature of the metabolic response in AKI is acceleration of protein catabolism. Several factors contribute to the hypercatabolic process in AKI. A major stimulus is increase in hepatic gluconeogenesis from amino acids (Druml, 1998). Another factor is development of insulin resistance in AKI (Druml, 2001). It is now recognised that insulin resistance is common in AKI and that severity of hyperglycaemia correlates with mortality (Basi et al., 2005). In the presence of insulin resistance, protein synthesis is reduced and protein degradation enhanced (Druml, 2001). Metabolic acidosis is another factor contributing to excessive protein breakdown in AKI (Druml, 1998). Secretion of catabolic hormones, release of proteases and inflammatory mediators such as tumour necrosis factor alpha and interleukins also play a crucial role in driving this catabolic process in AKI (Druml, 1998). In addition to abnormalities in carbohydrate and protein metabolism, patients with AKI also exhibit significant

alterations in lipid metabolism. The key lipid abnormality in AKI is impairment of lipolysis. There is reduction in enzymatic activity of peripheral lipoprotein lipase and hepatic triglyceride lipase. Total triglyceride and triglyceride fraction of plasma lipoproteins are increased and plasma cholesterol, especially HDL cholesterol is decreased (Druml et al., 1983).

1.6 AKI and micronutrients

Micronutrients are essential for health as they play a central role in metabolism and contribute significantly to maintenance of tissue function. They are involved in key biochemical, metabolic and immunological processes. Trace elements acts as co-factors in metabolism and are involved in modulation of enzyme activity. Vitamins play important roles as coenzymes in metabolism. Micronutrients also possess antioxidant properties. Oxidative metabolism inevitably leads to generation of harmful free radicals. An adequate supply of micronutrients can prevent oxidative damage caused by free radicals. In disease states such as AKI and critical illness, high prevalence of malnutrition can adversely affect micronutrient status. Micronutrient levels can be depleted in three distinct ways:

a. Reduced intake
b. Increasing demands
c. Excessive losses

In the setting of critical illness and AKI, anorexia is common and usually occurs as a consequence of uraemia and metabolic acidosis. Anorexia is also prevalent in older people, who are at higher risk of malnutrition and micronutrient depletion compared with the general population. However, giving older patients micronutrient supplements to improve clinical outcomes remains controversial. A meta-analysis highlighted lack of benefit of multivitamin and mineral supplements in reducing the incidence of infection in the elderly population (El-Kadiki and Sutton, 2005). Multiple co-morbidities in critical illness will also exacerbate the compromised nutritional status of these patients and increase risk of micronutrient depletion. Patients with AKI and critical illness are in a catabolic state. Consequently, there is net increase in protein breakdown and energy expenditure (Schneeweiss et al., 1990). This increases demand on supply of micronutrients such as trace elements and vitamins. Increased losses of micronutrients can

also occur as a consequence of diarrhoea, vomiting or blood loss. Several small studies have also demonstrated that trace elements and water-soluble vitamins are lost during CRRT in patients with AKI (Story et al., 1999, Berger et al., 2004, Fortin et al., 1999, Klein et al., 2002, Klein et al., 2008). This is unsurprising due to the water-soluble nature of the micronutrients. It is becoming evident that micronutrient losses can occur during RRT and that this phenomenon requires further investigation. It is currently not known whether RRT associated micronutrient losses lead to adverse clinical outcomes such as increased risk of mortality, delayed wound healing, decreased renal survival and prolonged recovery time with sepsis. Loss of micronutrients has not previously been reported in patients with AKI treated with haemodialysis or extended hybrid therapies. In addition, the effect of nutritional supplementation on micronutrient status in AKI has not previously been reported. Critically ill patients are characterised by inadequate pre-admission micronutrient status (Preiser et al., 2015). The trace elements which are affected include selenium, iron and zinc (Hercberg et al., 2004) (Rayman, 2012). In a study of critically patients who have suffered major burns, supplementation of trace elements led to an improvement in trace elements status (Berger and Shenkin, 2007). The improvement in trace element status was also associated with important clinical benefits such as a reduction in infectious complications and improvement in wound healing. Another study (Berger et al., 1998) showed that an improvement in trace element levels following supplementation reduced the rates of pneumonia in patients with major burns and decreased length of stay in ICU. The provision of parenteral nutrition containing amino acids has also led to a rise in plasma amino acid levels in critically ill septic patients (Garcia-de-Lorenzo et al., 1997). Among patients with sepsis, plasma levels of branched chain amino acids which includes arginine valine, leucine and isoleucine increased significantly following administration of total parenteral nutrition (Garcia-de-Lorenzo et al., 1997). In addition, the rise in plasma levels of these amino acids was associated with a reduction in mortality in septic patients. In contrast, there is substantial evidence (Tonelli et al., 2009) that patients with ESRD requiring long-term haemodialysis have altered levels of micronutrients. A meta-analysis of 128 studies showed that levels of selenium, zinc and manganese were significantly lower compared with healthy controls (Tonelli et al., 2009). Vitamin deficiency is frequently found in patients with advanced chronic kidney disease who are not taking nutritional supplements (Piper, 1985). Suboptimal vitamin status in this cohort of patients can lead to adverse outcomes. An international multi-centre study known as the Dialysis Outcomes Practice Pattern Study (DOPPS) demonstrated that patients with ESRD on long-term

haemodialysis have higher mortality rate if they do not receive water-soluble vitamin supplements (Andreucci et al., 2004). Analysis was adjusted for age, gender, race, comorbidity, haemoglobin, serum albumin and BMI. Few published studies have investigated losses of these important substances during different modalities of RRT in patients with AKI. Effects of RRT on micronutrient status in AKI require further investigation.

1.7 Trace Elements in AKI

1.7.1 Zinc

Zinc is the most abundant intracellular trace element. Diet is the main source of zinc with foods such as meat, fish, legumes, nuts and wholegrain cereals being rich sources. In humans, approximately 95% of zinc is stored intracellularly. Approximately 60% of total body zinc is located in skeletal muscle and another 30% stored in bone. The remaining 5% is present in skin. An adult human contains about 2g of zinc. Less than 0.1% of zinc is in plasma. Although zinc is predominantly stored intracellularly, turnover is slow, making it less accessible during periods of deficiency.

1.7.1.1 Absorption, transport and metabolism of zinc.

Dietary zinc is absorbed via a carrier-mediated transport process throughout the small intestine. Zinc is then transported in plasma by albumin and alpha macroglobulin. Bioavailability of zinc depends on external and host-related factors. Dietary enhancers and inhibitors play a significant role in influencing bioavailability of zinc. Zinc is predominantly excreted in faeces, with a small amount lost in sweat. Changes in zinc intake are regulated by an efficient homeostatic mechanism. With reduced intake, intestinal absorption is upregulated and excretion via the kidney and gastrointestinal tract reduced.

1.7.1.2 Physiological role of zinc

Zinc has several key physiological roles, including regulation of immune system (Rink and Gabriel, 2000, Rink and Kirchner, 2000) and of metabolic process and maintenance of antioxidant defence

mechanisms. At a cellular level, zinc is essential for protein synthesis, signal transduction and gene transcription.

1.7.1.3 Assessment of zinc status

There are various methods of measuring levels of zinc in the human body. These include measuring concentrations in plasma, hair, erythrocyte, leukocyte and platelets. At an individual patient level, none of these markers is sufficiently specific and sensitive to diagnose zinc deficiency. On the other hand, at a population level, measuring serum or plasma zinc is useful in identifying subgroups at high risk of zinc deficiency (Hotz & Brown, Food and Nutritional Bulletin 25 (supp2): S94-S204). In order to evaluate zinc status, a combination of dietary, biochemical and functional physiological indices is recommended. Serum zinc is the most commonly used biomarker of zinc status. The usefulness of this biomarker to assess zinc status at a population level has been confirmed by several studies (Brown et al., 2002) (Donovan and Gibson, 1995). Concentration of serum zinc is maintained by homeostatic mechanisms when dietary intake is limited. Serum zinc levels may remain within the normal range despite presence of clinical features of zinc deficiency (Fickel et al., 1986) (Milne et al., 1987, Ruz et al., 1991). Twelve to 22% of zinc is in the serum, with the remainder inside erythrocytes. Zinc is transported in serum mainly bound to albumin, so conditions that affect albumin concentration will influence serum zinc concentrations.

Concentration of zinc in erythrocytes is approximately ten times higher than in plasma. Erythrocytes have a lifespan of 120 days and thus erythrocyte zinc concentrations do not reflect short-term changes in dietary zinc intake or recent changes in body zinc stores (Neggers et al., 1997). There are several problems with using erythrocyte zinc to measure zinc status. Firstly, there are no established interpretive criteria for erythrocyte zinc level. Second, there are no standardized units for expression of zinc concentrations in erythrocytes, making comparison among studies difficult. Third, results for erythrocyte zinc have been conflicting in conditions with features of tissue zinc depletion (Vitoux et al., 1999).

1.7.1.4 Zinc in critical illness and renal disease

Plasma zinc levels are also altered in critical illness. A prospective study (Besecker et al., 2011) showed that critically ill patients without sepsis had low levels of plasma zinc. In the critically ill septic cohort, plasma zinc levels were further depressed. There is also an inverse relationship between plasma zinc levels and cytokine production. There is now substantial evidence to suggest that zinc is redistributed from the vascular compartment into the tissue compartment during sepsis (Besecker et al., 2011, Brown, 1998). A low plasma zinc level therefore does not necessarily imply low total body zinc. There is also an association between zinc deficiency and renal disease. A recent meta-analysis showed that patients with ESRD on long-term haemodialysis have lower zinc levels compared with the general population (Tonelli et al., 2009). In patients with AKI requiring RRT, zinc has been found in effluent, suggesting that RRT aggravates losses of zinc (Berger et al., 2004, Klein et al., 2008).

1.7.2 Copper

Copper is an essential micronutrient with an important role in cellular metabolism. It is a component of several cuproenzymes, an integral structural element and a regulatory agent

1.7.2.1 Absorption, transport and metabolism of copper

Absorption of copper occurs primarily in the stomach and small intestine. Copper is transported across the intestinal mucosal wall into the portal circulation where it is bound to albumin and histidine and is deposited in the liver. Caeruloplasmin is the major copper-containing protein in plasma and is synthesized in the liver. Homeostasis of copper is achieved by a balance of absorption and distribution as well as biliary and urinary excretion. The liver is the site of copper metabolism.

1.7.2.2 Physiological role of copper

The antioxidant activity of copper has been attributed to increased activity of zinc copper oxide dismutase. In healthy volunteers, an antioxidant effect was demonstrated following

supplementation of copper. However, copper also has pro-oxidative effects. Copper ions contribute to various oxidative reactions, such as

a. Conversion of superoxide to hydrogen peroxide and hydroxyl radicals.
b. Oxidative modification of LDL in the arterial wall
c. Oxidative damage to DNA

1.7.2.3 Assessment of copper status

Several biomarkers are available to assess copper status, including plasma copper, caeruloplasmin and Cu/Zn superoxide dismutase (Harvey et al., 2009). However, these biomarkers have poor sensitivity and specificity (Harvey and McArdle, 2008). In the setting of high copper exposure, there is downregulation of copper uptake in the duodenum and increase in biliary copper excretion (Turnlund et al., 1989, Turnlund et al., 1998, Harvey et al., 2003, Araya et al., 2005a). Therefore, high copper intake does not necessarily lead to a proportionate rise in copper stores. The most accurate assessment of copper status to date is measurement of copper content in the liver (Araya et al., 2003, Hambidge, 2003) but there are currently no practical methods of measuring this.

One approach to assessment of copper status is measurement of ceruloplasmin. Ninety-five percent of copper is bound to this protein (Harris and Gitlin, 1996). It is a copper transport protein synthesized primarily in liver and binds six to eight copper ions per molecule (Bielli and Calabrese, 2002) (Holmberg and Laurell, 1948). Men have lower ceruloplasmin levels compared with women (Mendez et al., 2004). Ceruloplasmin levels are increased by oestrogen, pregnancy and the contraceptive pill (Ganaraja et al., 2004).

Ceruloplasmin has several limitations as a marker of copper excess (Araya et al., 2005b). Firstly, it is an acute phase reactant, and so levels rise in inflammation and infection(Gruys et al., 2005). Second, elevated ceruloplasmin levels have been observed in patients with malignancy and myocardial complications (Senra Varela et al., 1997, Stassar et al., 2001, Wang et al., 2002). Third, ceruloplasmin is age-dependent (Montagna et al., 1994)and subject to seasonal variations (Kanikowska et al., 2005). Serum copper has similar limitations as a biomarker of copper status

because it reflects ceruloplasmin levels. In contrast, ceruloplasmin levels decrease in severe copper deficiency and therefore can indicate copper deficiency in humans (Feillet-Coudray et al., 2000).

1.7.2.4 Copper in critical illness and renal disease

Ceruloplasmin is an acute phase response protein in critical illness. Increased serum copper concentration has been observed in response to infection, inflammation and various chronic diseases. A recent study showed a direct association between dietary or serum copper and C reactive protein (CRP) (Bui et al., 2012). The authors concluded that oxidative stress induced by copper can lead to a significant inflammatory response.

1.7.3 Iron

Dietary iron is derived mainly from meat and cereal products.

1.7.3.1 Absorption, transport and metabolism of iron

Haem iron is well-absorbed in the small intestine. Various factors affect absorption of intestinal iron. An acidic environment and reducing agents such as ascorbic acid enhance absorption of iron. In contrast, dietary fibre, phytate and tannins impair its absorption. Once absorbed, iron is bound to the carrier protein transferrin and is transported to bone marrow where it is used to synthesize haemoglobin. Red blood cells have a life span of approximately 120 days. They are taken up by macrophages in the reticuloendothelial system where the iron is released and bound to transferrin in plasma for recirculation. Excess iron is stored within hepatocytes. Hepcidin is a peptide hormone that has recently been discovered to be a key regulator of iron stores. Hepcidin is primarily secreted by liver and acts to restrict the amount of iron that enters the blood stream. Expression of hepcidin is promoted by various factors including presence of adequate levels of iron and by inflammation. Hepcidin accumulates in chronic kidney disease due to reduced renal clearance (van der Weerd et al., 2015). Other factors which increase hepcidin production in renal disease include the inflammatory effect of dialysis and administration of intravenous iron.

Conversely, administration of erythropoetin has an inhibitory effect on hepcidin expression. Iron is lost minimaly in urine and is significantly depleted with blood loss. More than half of the body's iron is present in red blood cells as haemoglobin, which transport oxygen throughout the body. Iron is stored in ferritin which is predominantly found in liver.

1.7.3.2 Assessment of iron status

Laboratory methods for assessing iron status include serum ferritin, transferrin saturations, erythrocyte protoporphyrin, mean corpuscular volume, serum transferrin receptor, haemoglobin and packed cell volume.

1.7.3.3 Physiological role of iron

Iron plays an important role in many metabolic functions. Iron present in haemoglobin is used to transport oxygen, which is essential for cell respiration. Iron is utilised in myoglobin to store oxygen in muscle. In addition, iron plays an important role in DNA synthesis. However, an excess amount of iron can be harmful as it can lead to production of toxic free radicals and cell death. The amount of iron in the body is tightly regulated at a cellular and systemic level by a network of proteins involved in its distribution and transport.

1.7.3.4 Iron in critical illness and renal disease

In patients with chronic kidney disease, iron homeostasis is impaired. There are multiple causes of iron deficiency in chronic kidney disease. Patients can have true iron deficiency or functional iron deficiency. In the former, both circulating iron levels and total body iron stores are reduced. In functional iron deficiency, circulating levels of iron are low even in presence of normal body iron stores. Factors which predispose patients with chronic kidney disease to iron deficiency include increased blood loss, increased iron utilization from erythropoietin therapy, impaired dietary iron absorption and impaired iron release from body storage sites.

1.7.4 Selenium

Selenium is an important trace element with both antioxidant and anti-inflammatory effects (Alhazzani et al., 2013). Main dietary sources of selenium include meat and fish. Fruit and vegetables contain low levels of selenium. Recommended dietary daily requirement for a healthy adult ranges between 60 and 100 micrograms /day(Rayman, 2000). In addition to normal dietary sources, selenium can be supplemented via enteral and parenteral routes (Angstwurm and Gaertner, 2006). Selenium toxicity has been widely reported in the literature and is associated with symptoms of nausea, vomiting, loss of hair and nails, rash, tooth decay, skin lesions, fatigue and peripheral neuropathy (Burk, Levander : Selenium in Modern Nutrition in Health and Disease; Philadelphia PLWW 2006; pp312-325). The optimal safe dose of selenium has not yet been established. Nevertheless, a dose of 5 microgram/kg/day does not appear to cause adverse events and up to 1000 micrograms/day can be used for short-term supplementation (Angstwurm and Gaertner, 2006, Heyland, 2007).

1.7.4.1 Absorption, transport and metabolism of Selenium

About 50-80% of dietary selenium is absorbed in the small intestine. Dietary forms of selenium consist of selenocysteine and selenomethionine. Selenocysteine is the active form of selenium in selenoproteins while selenomethionine contributes to tissue selenium but is not available for synthesis of functional forms of selenoproteins until it is catabolised and converted into selenocysteine. Selenium is predominantly excreted in urine, faeces and breath. Homeostatic regulation of selenium is influenced mainly by urinary excretion rather than absorption (Levander and Burk, 1994). The body responds to low selenium levels by reducing urinary excretion (Robinson et al., 1985).

1.7.4.2 Physiological role of selenium

Selenium is involved in antioxidant defence systems, thyroid hormone metabolism and redox control of enzymes and proteins (McKenzie et al., 2002). Its role in development of the acquired immune system is also well-recognised (Arthur et al., 2003). Selenium exerts its effects on important metabolic pathways through a number of selenoproteins including gluthathione

peroxidases, iodothyronine deiodinases, thioredoxin reductases, selenoprotein P, selenoprotein W and selenophosphate synthetase 2.

The antioxidant properties of selenium is mediated through effects of gluthathione peroxidase which removes lipid hydroperoxide and hydrogen peroxide. These gluthathione peroxidases are located in different tissue compartments. Therefore selenium can function as antioxidant in both the intracellular and extracellular space (Arthur, 2000, Pfeifer et al., 2001). Iodothyronine deiodinase is another major class of selenoproteins. They are involved in catalysing conversion of thyroxine (T4) to biologically active triiodothyronine (T3). Deficiency in selenium will result in elevated plasma levels of T4 and reduction in active T3. Assessment of plasma T4:T3 ratio may indicate activity of these selenoproteins in inaccessible tissues (Olivieri et al., 1995). The other selenoproteins are thioredoxin reductase, selenoprotein P, selenoprotein W and selenophosphate synthetase 2. The first three of these possess antioxidant properties (Brown and Arthur, 2001, Behne and Kyriakopoulos, 2001). Selenoprotein P functions as a transport protein for selenium to the brain (Hill et al., 2004). Selenoprotein is present in muscle and may have a role in muscle metabolism (Burk et al., 1997). Selenophosphate synthetase 2 is involved in selenium homeostasis (Thomson 2003, selenium physiology). In addition, this selenophosphate is an essential inorganic precursor for synthesis of selenocysteine from serine during selenoprotein synthesis (Arthur et al., 2003).

1.7.4.3 Assessment of selenium status

At present there is no single functional marker that can reliably assess selenium status. It is currently recommended that a series of markers is used, the choice depending on which specific function of selenium is under investigation. Biomarkers currently used include plasma or whole blood selenium, plasma glutathione peroxidase (GSHPx-3), erythrocyte glutathione peroxidase, selenoperoxidase activities in red cells, plasma selenoprotein P and thyroid hormone levels (Arthur, 1999). Selenium status can be assessed by measuring concentrations in plasma, whole blood, hair and toenail. Plasma and serum concentrations of selenium change rapidly in response to intake of selenium. Dietary selenium intake is the primary determinant of plasma selenium concentrations (Robberecht and Deelstra, 1994). Whole blood and erythrocyte selenium on the other hand, reflect long-term selenium status. The clinical significance of hair selenium was first

noted by Chinese workers studying Keshan disease. A close relationship between hair and whole blood selenium concentrations was observed (Chen et al., 1980). Concentration of selenium in toenails is a good reflection of long-term retrospective selenium status because selenium is incorporated as nails grow. The advantage of using toe nails to measure selenium levels is that it is simple, non-invasive and selenium levels have been noted to be relatively high (Hadjimarkos and Shearer, 1973). In addition, there appears to be good correlation between toenail selenium and geographical location (Steven Morris et al., 1983) as well as with selenium supplementation (Hunter et al., 1990).

There are several disorders which are associated with low plasma or serum selenium concentrations. These include disorders of the gastrointestinal tract, muscle, neurological diseases, inflammatory diseases, chronic renal failure, cancer and cardiovascular diseases (Neve, 1991). There are currently no universal normal reference levels for plasma or serum selenium (Alfthan and Neve, 1996). Values for healthy adults can vary from 0.5 to 2.5 µmol/L, depending on geographic region. In patients with clinical features of severe selenium deficiency, plasma selenium levels below 0.1 µmol/L have been observed and they respond promptly to selenium repletion (Terada et al., 1996, van Rij et al., 1979).

1.7.4.4 Selenium in critical illness and renal disease.

Selenium deficiency can lead to adverse effects in human health. Low levels of selenium have been observed in patients with malignancy (Schrauzer, 2009), cardiovascular disease(Flores-Mateo et al., 2006) and viral infection (Beck et al., 2003). Current evidence also suggests that selenium deficiency is associated with higher risk of death, multiple organ failure and higher markers of oxidative stress (Heyland et al., 2005). A prospective cohort study of critically ill patients demonstrated that patients with systemic inflammatory response syndrome (SIRS) and severe sepsis had 40% lower selenium levels than patients without SIRS (Forceville et al., 1998). Several studies have also shown that low selenium levels observed in septic patients are associated with increased morbidity and mortality (Sakr et al., 2007, Angstwurm et al., 2007, Valenta et al., 2011). There is increasing evidence to suggest that selenium levels are altered in both acute and chronic renal failure (Metnitz et al., 2000, Girelli et al., 1993). In ESRD, selenium levels have been shown to be depressed in renal replacement therapy (Fujishima et al., 2011b,

Dworkin et al., 1987, Saint-Georges et al., 1989, Tonelli et al., 2009, Kallistratos et al., 1985, Hampel et al., 1985, Foote et al., 1987). Causes of selenium deficiency in this group of patients are inadequate intake of dietary selenium and significant losses during haemodialysis (Saint-Georges et al., 1989). The relationship between selenium deficiency in renal disease and mortality has been investigated. A prospective cohort study from Japan has shown that serum selenium levels are inversely related to the risk of death. An important observation was the high infectious disease-related mortality among patients with low levels of selenium (Fujishima et al., 2011a). Does supplementation with selenium lead to an improvement in the morbidity and mortality of these patients? It is currently unclear whether mortality can be reduced with supplementation. However, other outcomes such as nutritional status and immune parameters have been evaluated in patients with ESRD. A randomised controlled trial has shown that supplementing haemodialysis patients with selenium led to improvement in nutritional status and that this improvement was possibly mediated by reducing oxidative stress and inflammation (Salehi et al., 2013). In AKI, there have been a few uncontrolled small studies which have evaluated selenium losses during CRRT(Berger et al., 2004).

The immunoregulatory function of selenium is an essential component of the patients defence mechanism in critical illness (Rayman, 2000). Selenium is a cofactor of various selenoenzymes, of which gluthathione peroxidase is the most important. Gluthathione peroxidase removes free radicals in the body. There is a correlation between plasma selenium levels and the activity of glutathione peroxidase. The levels of this antioxidant have been shown to be depressed in patients with AKI and ESRD requiring long-term haemodialysis (El-Far et al., 2005).

1.8 Water-soluble vitamins

Vitamins are organic substances required by the human body in small amounts for key metabolic processes to occur. Vitamins are not usually synthesized by the human body and are thus an essential component of a healthy diet. Vitamins are classed as either lipid soluble or water-soluble.

1.8.1 Thiamine (Vitamin B1)

Thiamine is a water-soluble vitamin and abundant in meat, potatoes, cereal and fish. The thiamine molecule is not biologically active but is activated when converted to a coenzyme which is its pyrophosphate form.

1.8.1.1 Absorption, transport and metabolism of Thiamine

Thiamine is absorbed via passive diffusion predominantly in the jejunum and ileum. The vitamin is then transferred to the portal circulation via an active transport process. In the tissues, free thiamine and thiamine monophosphate are taken up. These two molecules are phosphorylated further to form thiamine diphosphate and in the nervous system, thiamine triphosphate. Free thiamine is excreted in urine and sweat.

1.8.1.2 Physiological role of thiamine

Thiamine has a major metabolic role. Thiamine diphosphate, which is the active coenzyme of thiamine, is involved in three oxidative decarboxylation reactions:

a) pyruvate dehydrogenase in carbohydrate metabolism
b) alpha-ketoglutarate dehydrogenase in the citric acid cycle
c) branched chain keto-acid dehydrogenase in metabolism of leucine, isoleucine and valine.

Thiamine diphosphate is the coenzyme for transketolase which is involved in the pentose phosphate pathway of carbohydrate metabolism. This is an important pathway for carbohydrate metabolism, and an alternative to glycolysis in all tissues. Thiamine triphosphate also plays a significant role in nerve conduction.

1.8.1.3 Assessment of thiamine status

Thiamine status can be measured by assessing the activity of the transketolase enzyme.

1.8.1.4 Thiamine in critical illness and renal disease.

Thiamine deficiency is recognised in critical illness. Important features of deficiency include Wernicke Korsakoff syndrome, acute cardiac failure and lactic acidosis. Depleted levels of thiamine can lead to a poor clinical outcome. Thiamine levels are likely to be further reduced in renal replacement therapy as thiamine is water-soluble and thus significant levels can be lost in the effluent. In a study by Berger et al (Berger et al., 2004), thiamine was detected in the effluent of patients with AKI undergoing CRRT.

1.8.2 Vitamin B2 (Riboflavin)

Riboflavin, is found in milk and dairy products, which is unusual among water-soluble vitamins. Riboflavin is also found in cereal, meat, oily fish and green vegetables.

1.8.2.1 Absorption, transport and metabolism of riboflavin

Riboflavin is absorbed in the free form by a specific active process in the proximal small intestine. Riboflavin occurs in most foods as protein complexes of the coenzyme forms flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Absorption is dependent on hydrolysis of these complexes to produce the free vitamin. Two steps are required in the hydrolysis process. Firstly the riboflavin coenzymes are released from protein complexes by proteolytic activity of the intestinal enzymes. Then the coenzymes are hydrolysed by brush border phosphatases to liberate free riboflavin. These phosphatases include non-specific alkaline phosphatase, FAD pyrophosphatase, and FMN phosphatase. FAD pyrophosphatase converts FAD to FMN, and FMN phosphatase converts FMN to free riboflavin.

Riboflavin is transported in plasma as free riboflavin and FMN. Fifty percent of free riboflavin and 80% of FMN are bound to plasma proteins. Riboflavin enters tissue in its free form and is converted to FMN and FAD. High concentrations of this vitamin are found in kidney, liver and

heart. Riboflavin is primarily excreted in urine. A normal human adult excretes 200µg in 24 hours. Faecal excretion also occurs but only in small amounts.

Figure 2 - Riboflavin - absorption, transport, storage, excretion

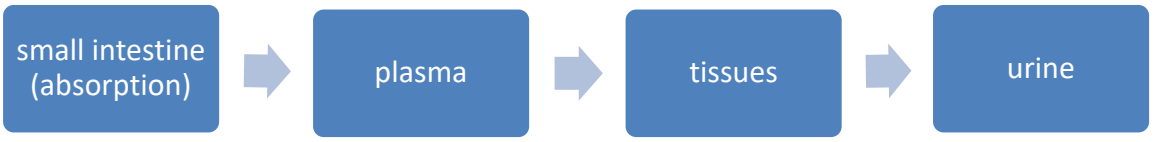


Figure 2: Riboflavin is absorbed in the small intestine. During absorption, riboflavin is converted to FMN and free riboflavin and is then transported in plasma to tissues such as kidney, liver and heart. Riboflavin is predominantly excreted in the urine.

1.8.2.2 Physiological role of riboflavin

Riboflavin is the main component of FMD and FAD. Both these coenzymes interact with over 100 enzymes known as flavoproteins. Flavoproteins play an important role in metabolism of carbohydrates, amino acids and lipids. Flavoproteins are also involved in conversion of other vitamins such as pyridoxine and folate to their coenzyme forms. These enzymes also have a protective role against oxidative stress. Glutathione reductase is an important flavoprotein which maintains glutathione in its reduced form to protect cells against oxidative damage.

1.8.2.3 Assessment of riboflavin status

Vitamin B2 exists in blood as riboflavin, flavin mononucleotide (FMD) and flavin adenine dinucleotide (FAD). A variety of methods can be used to determine the status of vitamin B2. Measurement of plasma riboflavin offers several advantages. Plasma riboflavin is less extensively

bound to protein and thus less likely to be influenced by the systemic inflammatory response. In addition plasma riboflavin is more responsive to supplementation. Disadvantages to use of plasma riboflavin also exist. Firstly, plasma riboflavin does not correlate well with functional tests in healthy subjects (Hustad et al., 2002). Second, riboflavin is not the physiologically active form of vitamin B2 and so the clinical relevance of measuring plasma riboflavin is uncertain (Massey, 2000). In contrast, FAD is the physiologically active form of vitamin B2 and red cell FAD correlates well with functional tests (Hustad et al., 2002). The biological variation of red cell FAD is small and is less likely to be volatile in response to supplementation. Thus, these factors support the idea that red cell FAD is an accurate reflection of vitamin B2 status, especially in critical illness (Vasilaki et al., 2010). In addition to directly measuring the FAD levels in red cells, a functional test of vitamin B2 status would be useful. The functional test most frequently used is the erythrocyte glutathione reductase activity coefficient (EGRAC). This is a measure of riboflavin tissue saturation and is inversely proportional to riboflavin status (Gariballa et al., 2009). Inadequate riboflavin status is reflected by decreased activity of flavin-dependent enzymes, including erythrocyte gluthathione reductase with corresponding decrease in consumption of oxygen.

1.8.2.4 Riboflavin in critical illness and renal disease

No published studies have evaluated riboflavin status in acute kidney injury. Studies have shown that a high proportion of critically ill patients have suboptimal riboflavin status (Gariballa et al., 2009). Deficiency of this vitamin in this setting is important as riboflavin is required for normal immune function, by maintenance of normal glutathione status (Grimble, 1997). It has been reported that during critical illness, suboptimal levels of riboflavin are associated with adverse clinical outcomes (Shenkin et al., 1989). Patients who are critically ill exhibit significantly high levels of inflammatory markers such as C-reactive protein (CRP) and cytokines. Rise in these inflammatory markers results in a reduction in food intake and deficiency in protein and vitamin status (Gariballa and Forster, 2006). Riboflavin supplementation in critically ill patients restores levels of this vitamin (Gariballa et al., 2009), and may have a beneficial effect on immune responses (Shenkin et al., 1989) (Grimble, 1997).

1.8.3 Niacin (Vitamin B3)

Niacin is known as nicotinic acid. The active form is its amide, nicotinamide. Niacin is water-soluble and is synthesized from the essential amino acid tryptophan. Niacin is found in foods such as yeast, milk, eggs, meat, green vegetables, beans and cereal grains. It differs from other water soluble vitamins in that it can be synthesized endogenously. However, the precursor of niacin, tryptophan, is an essential amino acid which can be supplied only by dietary intake. Niacin is converted to two forms in the body: nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP)**.**

1.8.3.1 Absorption, transport and metabolism of Niacin

Niacin is present in food as NADH and NADPH. Both these substances are released by hydrolysis in the intestinal lumen by pyrophosphatase to nicotinamide ribonucleotide and riboside. Absorption of nicotinamide mainly occurs in stomach and small intestine. Niacin is transported in plasma as unbound forms of both the acid and amide. Nicotinic acid and nicotinamide both enter peripheral tissues by passive diffusion. Niacin is metabolised in most tissues and its metabolites are excreted in urine.

Figure 3: Niacin - absorption, circulation, storage and excretion

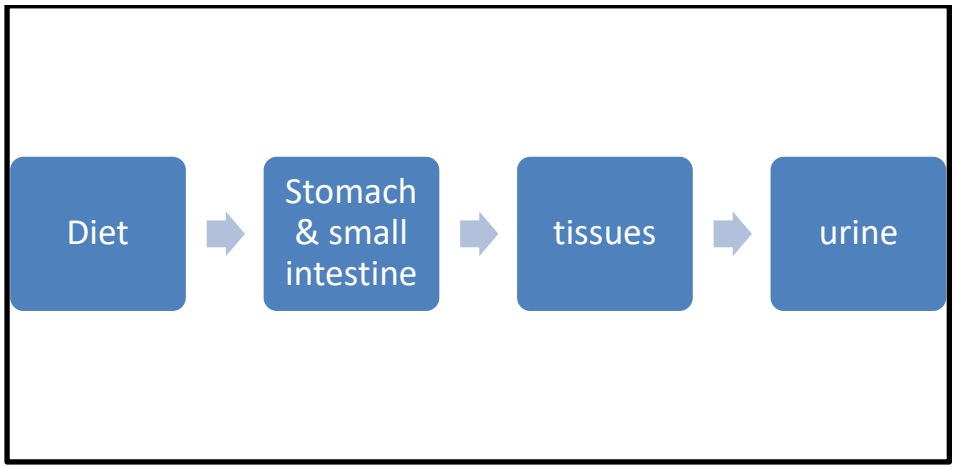


Figure 3: Niacin is absorbed mainly in the stomach and small intestine. Niacin is then transported in the plasma to the peripheral tissues. Niacin is metabolised in most of the tissues and its metabolites are then excreted in the urine.

1.8.3.2 Physiological role of niacin

Nicotinamide is the active form of niacin and the main constituent of co-enzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate). Both these enzymes are involved in numerous oxidation and reduction reactions. NAD is involved in oxidation of metabolic fuels body such as carbohydrates and fatty acids. NADP exists mainly in the reduced form and plays an active role in fatty acid synthesis. It interacts with gluthathione reductase to maintain glutathione in its reduced form.

In lipid metabolism, niacin lowers plasma low density lipoprotein and triglyceride levels while increasing plasma levels of high density lipoproteins. Several studies have shown that treatment with niacin reduces the risk of fatal coronary events, mainly by attenuating coronary atherosclerosis.

Figure 4: Physiological role of niacin

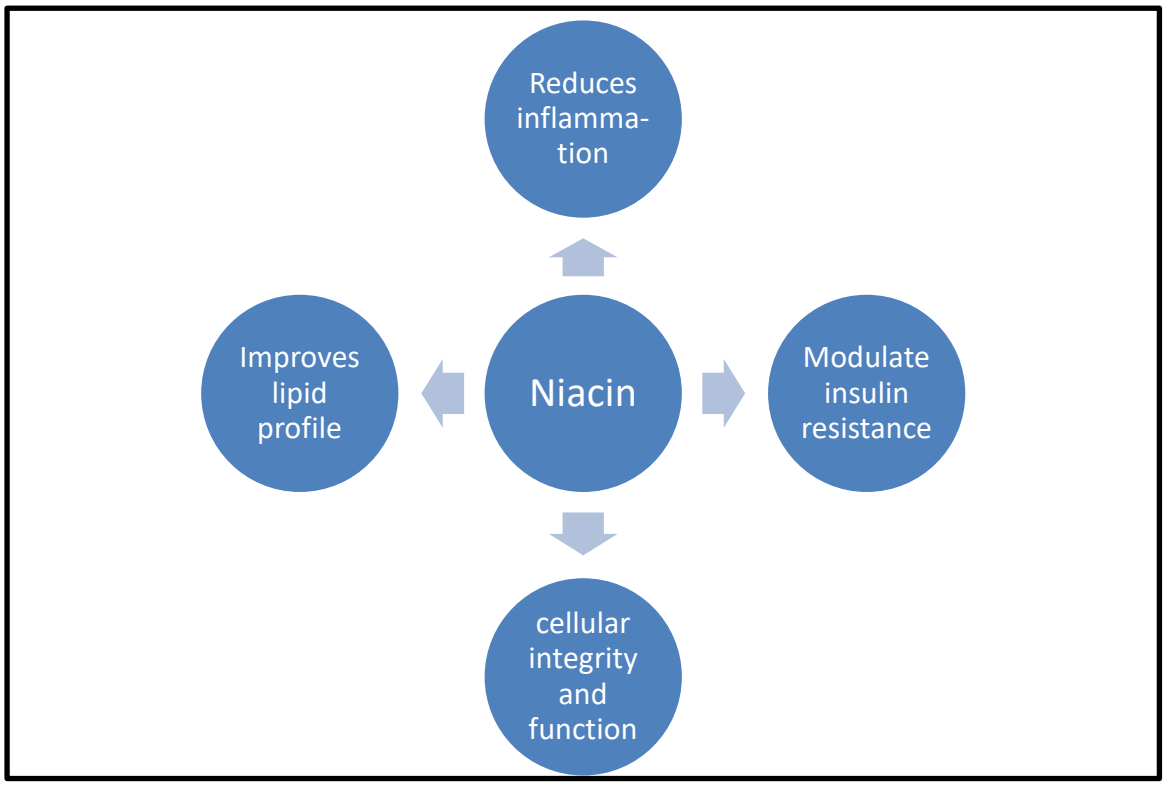


Figure 4: Niacin possesses lipid-lowering and anti-inflammatory properties. In addition, niacin maintains cellular integrity and function and reduces insulin resistance.

1.8.3.3 Assessment of niacin status

Various methods have been used to evaluate niacin status in the human body. There is currently no consensus as to the best biochemical indicator of niacin status. In 1989, Fu et al demonstrated that erythrocyte NAD level appears to be the most sensitive, reliable and convenient indicator for the assessment of niacin status in human subjects. In addition, there is an association between plasma tryptophan and erythrocyte NAD levels. Low levels of plasma tryptophan associated with low levels of erythrocyte NAD may indicate a more severe deficiency in niacin than low erythrocyte NAD and normal plasma tryptophan levels. Another marker for niacin status is the ratio of erythrocyte NAD and NADP. Under physiological conditions concentration of erythrocyte NAD is greater than NADP. Concentration of NADP is usually constant, so a reduction in erythrocyte NAD will reduce the NAD: NADP ratio. Where this ratio, known as the erythrocyte niacin index, falls below 1.0, the individual may be at risk of niacin deficiency.

Figure 5: Indicators of niacin status

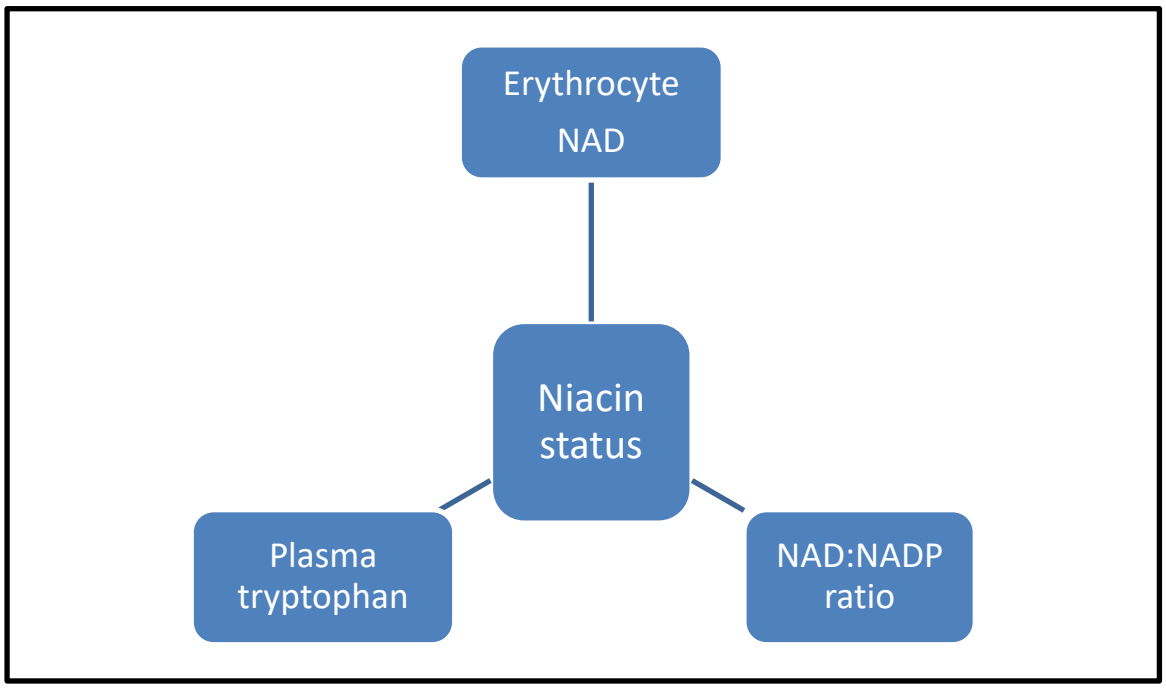


Figure 5: There are various methods of assessing niacin status but currently no consensus as which is the best indicator

1.8.3.4 Niacin in critical illness and renal disease

Progressive decline in kidney function in humans is mediated by both haemodynamic and non-haemodynamic factors. Haemodynamic factors include systemic and glomerular hypertension and hyperfiltration while non-haemodynamic factors include oxidative stress, inflammation and lipid disorders. In animal studies, niacin has been shown to slow progression of chronic kidney disease due to its ability to attenuate oxidative stress, inflammation, hypertension and proteinuria. In human studies, low dose niacin supplementation has been shown to increase HDL cholesterol, lower triglyceride levels, reduce serum phosphate levels and even improve GFR in patients with CKD. There is currently no published studies to evaluate the role of niacin in acute kidney injury. Given that AKI is associated with increased oxidative stress and is a pro-inflammatory state, niacin may have a protective role in patients who develop AKI.

1.8.4 Pyridoxine (vitamin B6)

Vitamin B6 occurs naturally in three forms or vitamers: pyridoxine, pyridoxal and pyridoxamine. All three forms are precursors of pyridoxal phosphate, which is a coenzyme with numerous metabolic functions. Dietary sources of vitamin B6 include meat and cereals.

1.8.4.1 Absorption, transport and metabolism of Pyridoxine

All three vitamers of vitamin B6 are rapidly absorbed via passive diffusion in the jejunum and ileum. Ingested pyridoxine is dephosphorylated in the intestinal mucosa and is released into the portal circulation as pyridoxal. Pyridoxal is taken up in liver and converted to pyridoxal phosphate. Pyridoxal phosphate is then bound to albumin in the circulation and transported to extrahepatic tissues. Muscle is the storage site for 80% of the body's total vitamin B6. Pyridoxal phosphate is the vitamer stored in muscle.

1.8.4.2 Physiological role of pyrodoxine

Pyridoxal phosphate acts as a coenzyme in three main areas of metabolism:

a. transamination of amino acids
b. cofactor of glycogen phosphorylase in muscle and liver
c. regulation of steroid hormones

1.8.4.3 Assessment of pyridoxine status

Vitamin B6 status can be assessed by measuring plasma levels of pyridoxal phosphate and urinary excretion of 4-pyridoxic acid.

1.8.4.4 Pyridoxine in critical illness and renal disease

Low vitamin B6 levels are associated with impaired immune response in critical illness (Cheng et al., 2006). Low vitamin B6 level is likely to be caused by consumption as a result of severe

metabolic demands in critical illness. During the systemic inflammatory response, albumin, which is the main binding protein of pyridoxal phosphate in plasma, is redistributed from plasma. The resulting reduction in plasma albumin depletes circulating levels of pyridoxal phosphate. Supplementation of B6 in critically ill patients might therefore not increase plasma levels of pyridoxal phosphate (Quasim et al., 2005, Louw et al., 1992, Huang et al., 2002). In patients with AKI treated with continuous convective therapies, losses of pyridoxal-5-phosphate have been quantified (Fortin et al., 1999). It is not known whether losses occur during IHD and SLEDf when these modalities are used in the management of AKI.

1.8.5 Folate

Folic acid in food is derived from polyglutamates. A healthy adult human should receive dietary folate intake of 50-100µ/day in order to maintain adequate folate stores. In the body, there are naturally occurring forms of folate. These are tetrahydrofolate, 5-methyhl THF and 10 formyltetrahydrofolate. Folic acid is the synthetic form of this vitamin and is converted to THF following absorption. Dietary sources include yeast extracts, dark green vegetables and wholegrain cereals.

1.8.5.1 Absorption, transport and metabolism

Dietary folate in the form of polyglutamates is cleaved to monoglutamate in the jejunum where it is absorbed. Folate enters plasma and is rapidly cleared by hepatocytes. Folate at physiological levels enters cells by binding to a folate receptor.

1.8.5.2 Physiological role of folate

Folic acid is essential for cell development, specific biochemical reactions such as conversion of homocysteine to methionine, and for metabolism of some anticonvulsant drugs. Folate also has several important functions. Folate together with vitamin B12 is important for production of red blood cells. Deficiency in these two micronutrients leads to macrocytic anaemia. The abnormally enlarged red blood cells have reduced capacity to carry oxygen. Secondly, folic acid is vital for nerve function. Dietary folate deficiency can have serious consequences. In pregnant women for

example, lack of folate in the diet can lead to neural tube defects, spinal bifida and anencephaly in the fetus. Thirdly, folate is essential for formation of DNA and cell division. Biochemically folate act as one carbon transfer reactions. These are important for formation of purines and pyrimidines which are constituents of DNA.

1.8.5.3 Assessment of folate status

Serum folate is used to assess status but red cell folate is also used, including for estimation of hepatic stores.

1.8.5.4 Folate in critical illness and renal disease

Folic acid can be rapidly removed during RRT due to its water-solubility. Folate losses in patients with AKI requiring CRRT have been reported (Fortin et al., 1999).

1.8.6 Vitamin B12

Vitamin B12 is unique among vitamins for two reasons. Firstly, it is the only vitamin to contain a mineral, cobalt. Secondly, vitamin B12 can be synthesized only by microorganisms. Higher plants are unable to synthesize this vitamin so it is absent in a vegan diet. Vitamin B12 is present in animal products such as meat, poultry, fish and to a certain extent dairy products and eggs. Due to its cobalt content, the term cobalamin is used for compounds with vitamin B12 activity. Methylcobalamin and 5-deoxyadenosylcobalamin are forms of vitamin B12 present in the human body. The form of cobalamin used in most nutritional supplements and fortified foods, cyanocobalamin, is readily converted to 5-deoxyadenosylcobalamin and methylcobalamin in the body.

1.8.6.1 Absorption, transport and metabolism of Vitamin B12

Although small amounts of vitamin B12 can be absorbed via diffusion across the intestinal mucosa, the vast majority of vitamin B12 is absorbed by binding to a specific protein in the intestinal lumen. This protein is known as intrinsic factor and is a glycoprotein secreted by the

parietal cells of gastric mucosa, which also secrete hydrochloric acid. Intrinsic factor binds vitamin B12 in the intestinal lumen and is absorbed predominantly in the terminal ileum. In the circulation, vitamin B12 binds with transcobalamin I, which is required for tissue uptake of vitamin B12 and transcobalamin II, which is the storage form of the vitamin. Vitamin B12 is excreted predominantly in bile and faeces.

1.8.6.2 Physiological role of vitamin B12

In mammals, cobalamin is a cofactor for two enzymes: methionine synthase and L methylmalonyl-coenzyme A mutase. Methylcobalamin is required for the function the folate dependent enzyme, methionine synthase. This enzyme is required for synthesis of the amino acid, methionine from homocysteine. 5 deoxyadenyl is required by the enzyme that catalyses the conversion of L methylmalonyl A to succinyl coenzyme A (Succinyl CoA) which then enters the citric acid cycle. Succinyl CoA is important in the production of energy from lipids and proteins and is required for synthesis of haemoglobin.

1.8.6.3 Assessment of vitamin B12 status

Measuring plasma levels of vitamin B12 is the method of choice.

1.8.6.4 Vitamin B12 in critical illness and renal disease

Altered levels of vitamin B12 are associated with high morbidity and mortality. Pernicious anaemia is a consequence of vitamin B12 deficiency. The underlying pathogenesis is impaired secretion of intrinsic factor. Vitamin B12 deficiency can also lead to severe neurological disorders such as peripheral neuropathy, cognitive impairment and subacute combined degeneration of the spinal cord. There have been conflicting reports with regards to the effect of vitamin B12 in critical illness. Manzanares and Hardy (Manzanares and Hardy, 2010) reported that vitamin B12 can be a novel treatment in management of patients with systemic inflammatory response. This is due to its antioxidant properties and its ability to modulate systemic inflammation occurring in critical illness. However, an observational study (Sviri et al., 2012) showed that elevated serum vitamin B12 levels are associated with increased mortality in critically ill patients. In renal disease,

the water-solubility of vitamin B12 suggests that it might be lost during RRT. Chandna et al (Chandna et al., 1997) demonstrated that in patients with ESRD requiring regular haemodialysis, serum vitamin B12 levels are low and that this is due to losses on dialysis as well as poor dietary intake. Another study (Cross and Davenport, 2011) showed that no additional losses occurred when patients were treated with haemodiafiltration compared with haemodialysis. No study has compared clearances of vitamin B12 in patients with AKI treated with RRT.

1.9 Amino Acids

Amino acids are the building blocks of protein and are required in three metabolic processes in the human body: protein synthesis, oxidation and metabolism to other molecules. Amino acids consist of a carbon skeleton and an amino group. The carbon skeleton group undergoes oxidation to produce urea in the liver. Urea is transported to kidney for excretion in the urine. The carbon skeleton of the amino acid releases energy in the form of adenosine triphosphate (ATP) when it is oxidised to carbon dioxide and water.

1.9.1 Essential and non-essential amino acids

Amino acids can be classified as essential or non-essential. Essential amino acids cannot be synthesized by the human body and must be acquired from diet. Non-essential amino acids are synthesized from metabolic compounds derived from the human body such as fat, carbohydrate and other amino acids.

1.9.2 Sources of amino acids

Amino acids are derived from proteins which are macromolecules found in external dietary sources as well as in human tissue. The most common sources of dietary protein are meat, fish, milk, cheese, eggs, cereals and legumes.

1.9.3 Digestion, absorption, transport and metabolism of amino acids.

As food containing protein is consumed, dietary protein undergoes hydrolysis in the stomach and the small intestine to produce amino acids. These amino acids are absorbed from the small intestine and enter the circulation where they are transported to various organs and tissues. Protein hydrolysis to amino acids is catalysed by enzymes secreted by the pancreas. Amino acids then enter enterocytes of the small intestine by various transport mechanisms including passive diffusion and active transport. Once in the enterocyte, most amino acids diffuse down a concentration gradient to enter the portal circulation.

1.9.4 Physicochemical properties of amino acids

All 20 amino acids contain a primary amino group and a carboxylic acid group. They differ from each other in the nature and type of side chain, which determines their physicochemical properties.

1.9.5 Role of essential amino acids

Essential amino acids are used as a source of energy when carbohydrate and fatty acid levels are depleted (Newsholme and Blomstrand, 1996). Studies on young healthy adults have described that essential amino acids play an important role in stimulation of muscle protein synthesis (Smith et al., 1992, Smith et al., 1998) A study by Volpi et al (Volpi et al., 2003) compared the effect of essential amino acids on muscle protein synthesis with non-essential amino acids in the elderly. The study demonstrated that in an elderly person essential amino acids are predominantly responsible for the amino acid stimulation of muscle protein synthesis, whereas non-essential amino acids did not provide any additional significant stimulation of muscle anabolism above that reached with essential amino acids alone. There are nine essential amino acids: phenylalanine, valine, threonine, tryptophan, leucine, isoleucine, methionine, lysine and histidine.

1.9.6 Role of non-essential amino acids

There are eleven non-essential amino acids: alanine, asparagine, aspartic acid, cysteine, glycine, glutamine, serine, proline, tyrosine, glutamic acid and arginine. Non-essential amino acids play important roles in regulating gene expression, cell signalling, antioxidative responses, neurotransmission and immunity (Wu et al., 2013). Several of these amino acids become conditionally essential under specific circumstances. These conditionally essential amino acids include arginine, cysteine, glutamine, tyrosine, glycine, proline and serine. Under conditions of stress such as surgery, physical trauma and sepsis, non-essential amino acids such as glutamine and arginine become conditionally essential. Glutamine acts as an important fuel for lymphocytes and macrophages. Studies have shown that plasma and muscle glutamine concentrations decrease during these stressful settings and this may reflect the reduced effectiveness of the immune system (Castell et al., 1994). In sepsis, most studies have shown that plasma glutamine levels are decreased. This is due to increased utilization of glutamine by lymphocytes and macrophages during proliferation or phagocytosis. During sepsis, endogenous production of arginine is reduced and intake from diet is diminished. In addition, catabolism of arginine is increased by enhanced utilization of arginine in the arginase and nitric oxide pathways (Luiking et al., 2004) (Luiking et al., 2005).

1.9.7 Amino acid metabolism in critical illness and renal disease

Metabolism of amino acids is significantly altered in critical illness and renal disease. Several important metabolic disturbances occur during sepsis. First, development of insulin resistance leads to impaired uptake of glucose into tissues. Second, utilization of fat as an alternative source of energy is also disrupted by circulating insulin. Therefore lipolysis is inhibited and other ways of restoring the energy deficit are required. Muscle is broken down as an alternative source of energy which leads to release of large amounts of amino acids into the circulation. These amino acids are oxidised to provide energy. Previous studies have attempted to characterise the amino acid profile in septic patients. A study of 15 patients with sepsis showed that the aromatic and sulphur containing amino acids were significantly elevated in this group of patients (Freund et al., 1978). Another study demonstrated an inverse relationship between levels these amino acids

and survival. In contrast, there appears to be a positive correlation between arginine and branched chain amino acids and survival in sepsis (Freund et al., 1979). In AKI, the key metabolic abnormality is the accelerated catabolic process leading to release of large amounts of amino acids from skeletal muscle into the circulation.

1.9.8 Amino acid losses in RRT

There have been small uncontrolled studies which investigated the losses of amino acids in RRT. Amino acids have a low molecular weight ranging from 60 to 220 KDa. Therefore RRT should theoretically remove these substances leading to clinically significant losses. Two previous studies demonstrated significant amino acid losses in patients undergoing CRRT (Davenport and Roberts, 1989) (Davies et al., 1991). Losses were higher if diffusion was used in addition to convection in CRRT. Interestingly, aetiology of AKI predicted serum amino acid levels. AKI patients with sepsis had lower serum amino acid level than patients with cardiogenic shock. A study by Chua et al (Chua et al., 2012) demonstrated that amino acid losses in SLEDf were lower than CRRT. Other factors such as the degree of protein binding and electrical charge of amino acids will also determine their clearances during RRT.

1.10 Aims and Hypotheses

1.10.1 Rationale

Patients with AKI are often critically ill and have high risk of morbidity and mortality. In addition, malnutrition is common in this group of patients. Malnutrition itself has been identified as a predictor of in-hospital mortality for patients with AKI independent of complications and co-morbidities. In severe AKI, renal replacement therapy may be used to remove toxins and waste products by diffusion, convection or a combination of these, depending on the type of RRT. It is inevitable that these same mechanisms will also remove some useful or essential substances, depending on their size and other physical properties. Loss of nutrients such as trace elements, water-soluble vitamins and amino acids could conceivably aggravate the existing malnutrition of

patients with severe AKI. Some studies have been undertaken in patients with ESRD requiring long term haemodialysis, showing that nutrients such as water-soluble vitamins are lost during dialysis treatment. Giving supplements water-soluble vitamins in long term haemodialysis has been standard practice for many years and has improved outcomes. However very few studies have addressed the question of nutrient loss in RRT for AKI. Even fewer have compared those losses between the various types of RRT that have become available to treat AKI over recent years. These range from intermittent diffusion-based treatments like intermittent haemodialysis (IHD), to hybrid techniques combining diffusion and convection such as sustained low-efficiency diafiltration (SLEDf), through to continuous convection-based treatments like continuous veno-venous haemofiltration (CVVH).

1.10.2 Hypotheses and research question

The hypotheses are firstly that patients with AKI will lose micronutrients during treatment with RRT. These micronutrients include vitamins, trace elements and amino acids. Secondly, micronutrient losses will differ between RRT modalities, because of the different clearance mechanisms employed by these modalities. This project addresses the following primary questions:

1. Does RRT for AKI lead to significant losses of micronutrients?
2. Do these losses differ qualitatively and quantitatively between IHD, SLEDf and CVVH?

The trace elements of interest are zinc, selenium, copper and iron. The losses of these 4 trace elements are described in chapter 4. We hypothesize that patients with AKI stage 3 will lose each of these elements during RRT. In addition, the second hypothesis is that the losses of these trace elements will differ depending on which RRT modality is used. We anticipate that the losses will be different because the mechanism of solute clearance used in each RRT modality are distinct from each other.

The vitamins of interest in this study are the B vitamins. Due to their water-solubility characteristics, we hypothesize that patients with AKI stage 3 will experience significant losses of these micronutrients during RRT. We hypothesize also that the losses of these vitamins will vary depending on which RRT modality is used. The results of these losses are described in chapter 5.

The amino acids of interest in this study are glutamine, arginine and the branched chain amino acids. The branched chain amino acids which are of interest in this study are leucine, isoleucine and valine. Due to their low molecular weight and water-solubility, we hypothesize that patients with AKI stag will lose amino acids during RRT. We anticipate that these losses will differ depending on which RRT modality is used. In this study, 3 RRT modalities are used and each modality employs a different mechanism of solute transport: convection in CVVH, diffusion in IHD and a combination of diffusion and convection in SLEDf. The results of the amino acid losses are described in chapter 6.

2. Methods and Materials

2.1 Study Design

A prospective observational study was conducted at the adult renal unit and the adult general intensive care units at Nottingham University Hospitals NHS Trust (NUH). The study protocol, consent forms and participant information sheets were approved by the Research Ethics Committee and NUH Research and Innovation Department. The study was conducted in accordance with regulations of the Declaration of Helsinki.

2.2 Participants

Patients with Acute Kidney Injury stage 3 who required renal replacement therapy (RRT) were recruited into the study. The RRT modalities used in these patients included intermittent haemodialysis (IHD), continuous veno-venous haemofiltration (CVVH) and sustained low-efficiency diafiltration (SLEDf). Exclusion criteria were: age below 17 years, end stage renal disease on maintenance dialysis, a functioning kidney transplant, chronic kidney disease stage 5 and haemoglobin level of less than 80 g/L not requiring blood transfusion. Patients who were unable to consent were considered for the study if a consultee was available to provide an opinion. This is in accordance to the Mental Health Act 1983.

2.3 Sampling

Participants were recruited into the study prior to their first RRT session. The first venous blood sample was drawn from the dialysis catheter prior to RRT commencement. Subsequent venous samples were obtained hourly during IHD, every 1.5 to 2.0 hours during SLEDf session and every 6 hours during CVVH. The first two RRT sessions were included in the study. If the participant required only a single session, no further samples were taken upon completion of the study. Samples of effluent or dialysate were obtained at regular intervals during the first two RRT sessions from the effluent port of the dialysis machine. The first dialysate sample was taken at the start of the RRT session. The second dialysate sample was taken midway through the session and the third at end of the session. In patients treated with CVVH, a sample of the effluent was taken from each discarded bag. Thus the number of samples obtained depended on the number of effluent bags that were changed in one treatment session. Spot urine samples x3 were

obtained during the treatment session at the start, midway and end of the treatment. This was repeated for the second RRT session. The volume of urine over 24 hours was recorded from the start of the RRT session. The definitions of dialysate, replacement fluid and effluent are described below.

Dialysate is the fluid used in the process of removing solutes during IHD and SLEDf. The direction of the dialysate is counter-current to the flow of blood in the dialyser. Please refer to table 1 and figure 2 for the composition of dialysate. The dialysate sample obtained at baseline (prior to commencement of RRT) is pure dialysate that has not been exposed to IHD or SLEDf.

Replacement fluid (please refer to chapter 1.2.5.3 on page 39) is the fluid used to replace the volume of fluid that would be removed via convection during CVVH. The composition and volume of replacement fluid is unaffected by the CVVH.

Effluent is the filtrate or waste fluid that is produced by the effect of convection during CVVH

2.4 Dietetic assessment

Formal dietetic assessment was undertaken by the renal dietitian from the research group. The following clinical parameters were obtained from each patient and recorded on a dietetic assessment form.

a) Weight
b) Height
c) Body mass index (BMI)
d) History of weight loss
e) Presence of gastrointestinal symptoms
f) Evidence of muscle depletion

In addition, route of feeding was noted and the protein and energy requirements calculated for each patient using the Henry equation and PENG equation.

2.5 RRT prescription

The modality and dose of RRT were prescribed by the nephrologist or intensivist directly responsible for the participants care. IHD and SLEDf were performed using the Fresenius FX 5008 machine with a Biorema 13H dialyser. For patients requiring IHD, a two hour haemodialysis session with minimal or no anticoagulation was the standard prescription for the first treatment session. This was followed by a second session within the next two days which was usually 3-4 hours duration. For patients requiring SLEDf, the duration of the first treatment session is six hours. The duration of the second and subsequent sessions last eight hours. For patients requiring CVVH, the Prismaflex machine with AN69 membrane filter was used. A dual lumen venous catheter was inserted into the internal jugular or femoral vein for vascular access. The procedure is performed by a trained physician from the renal or intensive care team. Anticoagulation with enoxaparin, a low molecular weight heparin was used in the extracorporeal circuit. The standard dialysate used in patients receiving IHD or SLEDf is described below.

Replacement fluids used for CVVH are:

a) Haemolactol
b) Potassium-free lactosol
c) Prismasol

The prescription parameters for IHD and SLEDf are described below:

Parameters	**IHD**	**SLEDF**
Blood flow rate (mls/min)	200	250-350
Dialysate flow rate (mls/min)	500-1000	200
Dialysate temperature (C)	37	37
Duration of session (hrs)	2-4	6-8

Table 3: Prescription parameters for IHD and SLEDf. The characteristics for prescribing IHD and SLEDf include the blood flow rates, the dialysate low rates and the duration of the session. The first RRT session for IHD and SLEDf is usually lower compared to subsequent sessions. The dose of CVVH is calculated based on the estimated or actual body weight of the patient. The dose of CVVH (mls/hr) = estimated/actual body weight x 35

2.6 Clinical data

Baseline demographic data were recorded for each participant, including age, sex and ethnicity of the participant. Cause of AKI and presence of chronic kidney disease (CKD) were also noted. Presence of co-morbidities such as diabetes mellitus, hypertension, cardiovascular disease, cerebrovascular disease, peripheral vascular disease and malignancy were noted. Site of vascular access and clinical parameters of the RRT session were recorded. Any nutritional supplement or feed given via oral, enteral or parenteral route was recorded. Baseline renal and haematological parameters were recorded and markers of inflammation such as CRP and albumin were also obtained. Baseline concentrations of the micronutrients of interest (trace elements, amino acids and water soluble vitamins) were measured. Subsequent concentrations of these substances were obtained at regular intervals during and after each of the two RRT sessions.

2.7 Sample processing and analysis

All blood, effluent and urine samples were sent to the laboratory at David Evans Medical Research Centre. Blood samples were obtained in lithium heparin tubes and were centrifuged at 1000 revolutions per minute (r.p.m) for 10 minutes.

2.8 Amino acid analysis

Plasma obtained from whole blood anticoagulated with heparin has been reported to be the best material for investigation of the steady state of amino acid concentration. The relative proportion, although not the actual concentrations of amino acids in plasma, are similar to those in skeletal muscle with the exception of valine. Tryptophan is the only free amino acid which is bound to protein and 50-70% of the circulating tryptophan may be bound and thus lost in deproteinisation. Whole blood was collected directly into heparin-containing tubes, centrifuged for 10 minutes and plasma separated, ensuring that the cellular buffy-coat was completely avoided. Separated plasma was cooled to 4C. For each plasma to be deproteinised, a conical centrifuge tube containing 60mg of solid 5-sulhosalicylic acid (SSA) was used. The tubes are cooled to 4° C. 980 µl of plasma sample and 20 µl internal standard norleucine was added to the conical centrifuge tube. The sample and SSA were mixed immediately and the resultant mixture

allowed to stand for 1 hour at 4°C. The mixture was centrifuged for 15 minutes at 4°C in a high speed centrifuge. The calibration standard was treated in the same way as the samples to ensure that both were loaded for analysis at the same pH and gave identical retention times for corresponding peaks. The supernatant was removed and filtered through a 0.2µm filter. 20 µl of treated standards or sample was injected into Biochrom 20 plus for analysis.

2.9 Calculating dialysis dose

Dialysis dose was calculated using three different formulas: urea reduction ratio, Kt/V and solute removal index.

2.10 Analysis of trace elements

Blood samples were obtained from the arterial port of the RRT circuit at the start of RRT, midway through and at the end. A blood sample was also taken one hour after discontinuation of RRT. In patients treated with CVVH, blood samples were obtained from the peripheral arterial cannula sited in the patient's radial artery. Blood samples were collected using 4ml lithium heparin tubes. They were sent immediately to the laboratory and centrifuged for 10 minutes. Plasma was then pipetted into cryovials in 2ml aliquots and stored -80°C. The RRT effluent was placed in 2ml aliquots into similar cryovials and stored at -80C as well. At the end of the 1st RRT session, the dialyser was kept to measure the amount of trace element adsorption. Trace element analysis were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the School of Veterinary Sciences, University of Nottingham.

2.10.1 Analysis of major and trace elements in plasma and effluent:

Elemental analysis was conducted on 500 µl of plasma or effluent after nitric-acid digestion (i.e. biofluid added to 3.0 mL of 70% Trace Analysis Grade (TAG) HNO_3, 2.0 mL H_2O_2 and 3.0 mL milli-Q water [18.2 MΩ cm]; Fisher Scientific UK Ltd, Loughborough, UK). Parameters for inductively coupled plasma-mass spectrometry (ICP-MS; iCAP^{TM} Q, Thermo Fisher Scientific Inc., Waltham, MA, USA) were: using a He collision cell with 'kinetic energy discrimination' to reduce polyatomic interference, we were able to discriminate Ag, Al, As, B, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, S, Sr, Tl, U, V and Zn. Lithium, Be and P were determined in standard (vacuum) mode and Se in 'hydrogen-cell' mode, with in-sample switching. Internal standards were Ge, Rh and Ir. Certified reference materials (CRMs) were included in duplicate for each ICP-MS run and were SeroNorm^{TM} L-2 (REF203105, LOT0903107) and SeroNorm^{TM} L-2 (REF210705, LOT1011645; LGC, Middlesex, UK) for plasma and urine, respectively. Recovery was >93% for the majority of major and trace elements. Operational variation was <10% after n=12 independent analyses and blanks (n = 10) were run to determine Limits of Detection (LOD; 3*SD) and Quantification (LOQ, 10*SD). Intra-assay variability for all elements was <2%. For reference, limits of detection and quantification, together with measured values of ultrapure water, tap-water and filtrate (PrismaSol) are presented in **Table 4.**

2.11 Analysis of B-vitamins

2.11.1 Analysis of B-vitamins in effluent:

Samples of effluent were analysed for B-vitamin concentration using liquid chromatography-mass spectrometry (LC-MS). Unknown samples were interpolated from standard curves of each B-vitamin (0.25 to 10 ppm [0.25-10 mg/L). Vitamin B9 (folic acid) was first solubilized in 0.1% NaOH. All B-vitamins were purchased from Sigma-Aldrich (Cheshire, UK). ***Chromatography***: Conditions for liquid chromatography were; using an Agilent 1100 series fitted with a Phenomenex Luna 5U C18 (2) 100A (250 x 3 mm ID; particle size-5 µm) column. Mobile phase A was (H_2O plus 0.1% Formic acid) and mobile phase B was (Acetonitrile plus 0.1% Formic acid). Sample injection volume was 10µl, with a mobile phase flowrate of 0.5 ml/min. Chromatography

was run using a pre-programmed linear gradient of mobile phase A:mobile phase B i.e. from 0-10 min, A: B 100:0 (v/v) to A: B 50:50 (v/v); Linear gradient from 10-12.5 min, A: B 50:50 (v/v) to A: B 0:100 (v/v); the gradient was kept constant from 12.5-15 min, at A: B 0:100 (v/v); linear gradient from 15-15.4 min, A: B 0:100 (v/v) to A: B 100:0 (v/v); kept constant from 15.4 min to 22 min, at A: B 100:0 (v/v). This was to equilibrate the column between samples. ***Mass spectrometry:*** Samples were analyzed using a Micromass Ultima, (Manchester, UK). Conditions were operated in positive ion electrospray mode for all B-vitamins with the exception of B9, which was run in negative ion mode. The capillary voltage was set to 3.5 kV, cone voltage was 50V and multiplier at 660V. The source temperature and dissolvation gas temperature was set to 100°C and 450°C, respectively. Single-ion recording (SIR) of mass-to-charge ratio (m/z) were obtained for B1 (thiamine hydrochloride, m/z 265.25, molecular weight 337.27 g/mol), B2 (riboflavin, m/z 377.28, molecular weight 376.36 g/mol), B3 (nicotinic acid, m/z 123.10, molecular weight 123.109 g/mol), B6 (pyridoxal hydrochloride, m/z 170.15, molecular weight 203.62 g/mol, B9 (folic acid, m/z 440.45, molecular weight 441.4 g/mol) and B12 (cyanocobalamin, m/z 679.72, molecular weight 1356.123 g/mol). Dwell time for each ion was 0.20 s.

Table 4: Limits of detection, quantification and baseline reference samples for ICP-MS

Major element	CRM Seronorm L2	CRM Seronorm	LOD (ppb)	LOQ (ppb)	Ultrapure Water	Tap Water	Filtration fluid
Sulphur	1335 (105)	671 (101)	7.87	26.2	Nd	37.4±2.1	Nd
Calcium	119 (102)	119 (94)	0.075	0.25	Nd	52.0±1.5	48.5±4.4
Phosphorus	110 (113)	807 (102)	0.082	0.27	Nd	0.95±0.1	Nd
Sodium	3531 (102)	2815 (89)	0.079	0.26	0.60±0.42	35.8±1.9	2804±16
Magnesium	33.9 (96)	78 (89)	0.017	0.05	0.05±0.00	10.4±0.5	13.8±1.3
Potassium	221 (104)	1921 (92)	0.067	0.22	0.38±0.07	6.76±0.3	127±13
Trace element (all µg L^{-1})							
Zinc	1532 (115)	1281 (104)	10.17	33.9	Nd	489±273	94.8±27.
Iron	2150 (95)	13.9 (147)	0.64	2.16	11.5±2.5	17.7±2.5	15.0±5.5
Aluminium	120 (138)	107 (108)	6.98	23.2	Nd	7.40±2.1	Nd
Copper	1925 (91)	56.3 (108)	0.11	0.36	1.64±0.23	100±32	4.5±2.5
Manganese	14.5 (106)	9.3 (109)	0.087	0.29	2.69±0.71	1.12±0.1	Nd
Strontium	110 (107)	120 (97)	0.08	0.27	0.52±0.11	247±2	22.4±2.0
Selenium	136 (106)	71.7 (117)	0.31	1.06	Nd	10.0±3.4	2.0±0.5
Barium	-	50 (84)	0.64	2.15	Nd	70.7±7.6	5.1±12.3
Chromium	5.7 (101)	30.1 (95)	0.14	0.48	Nd	Nd	Nd
Lead	0.66 (159)	73.9 (98)	0.19	0.64	Nd	Nd	Nd
Boron	82.1 (102)	-	0.026	0.088	Nd	0.10±0.0	0.10±0.0
Vanadium	1.1 (149)	26 (99)	0.048	0.16	Nd	Nd	0.68±0.0
Molybdenu	1.21 (149)	48 (92)	0.19	0.64	Nd	Nd	0.86±0.4
Rubidium	8.7 (109)	1150 (113)	0.093	0.31	Nd	4.95±0.6	4.23±12.
Lithium	9689 (116)	100 (98)	0.049	0.16	Nd	7.27±0.5	0.26±0.8
Arsenic	0.38 (164)	261 (107)	0.33	1.12	Nd	Nd	Nd
Cadmium	0.14 (136)	4.9 (111)	0.062	0.20	Nd	0.16±0.0	Nd
Cobalt	3.05 (98)	10.1 (102)	0.019	0.06	Nd	0.09±0.0	Nd
Caesium	0.02 (233)	6.6 (110)	0.021	0.072	Nd	0.15±0.0	Nd

Table 4: Certified reference materials (CRM) were obtained from LGC Standards, Bury, UK. Limits of detection (LOD) and quantification (LOQ) were calculated from calculating standard deviation (SD) of 10 operational blank samples as LOD = 3 × SD and LOQ = 10 × SD. Elemental composition of ultrapure water (n=6), tap water (n=6) or PrismaSol filtration fluid (n=18) was determined by ICP-MS. Percentage recovery is mean recovery from 12 independent runs. nd, not detectable in

sample above LOQ. Beryllium, Nickel, Silver, Titanium, Thallium and Uranium were measureable but values were not consistently above limits of detection/quantification and are therefore not reported.

2.12 Statistical analysis

Spot comparisons between patients receiving different types of dialysis were by analysis of variance (ANOVA) for normally distributed continuous data (e.g. plasma creatinine at admission) or by Kruskall-Wallis ANOVA for skewed data (e.g. C-reactive protein at admission). Continuous data are presented as means with standard deviation (1 SD) or standard error of the mean (1 SE) or estimated standard error of the differences between means (s.e.d.) where appropriate, as indicated in the text. 95% confidence intervals may be approximated from the data as the mean ± 2 s.e.d. Comparison of categorical or binomial data between treatment groups (e.g. proportion of patients with diabetes at admission) was made by using chi-squared test. Categorical data are presented as mean proportions (i.e. number [percent of total]). Chi-squared test was also used in the comparison of nutritional status between treatment groups where categorical data was presented (e.g. measures of nutritional status; mild-moderate-severe). Repeated measurements from the same individual (e.g. plasma or effluent amino acids at the start and end of RRT) were analyzed with time as a within-subject fixed effect by RM-ANOVA, including appropriate correction (i.e. included as a co-variate in the analysis) for dialysis dose (e.g. URR or SRI) and baseline plasma concentration of micronutrient. As appropriate, un-balanced or skewed data were $\log_{10}$ transformed prior to analysis. Any time-series analyses in which there were a few missing data points were analyzed by restricted maximum likelihood (REML) with the subject as a nested, random effect. To explore any potential relationships between fixed effects and ordinal categorical data (e.g. nutritional status) logistic generalized estimating equations were used, adjusted for potential covariates (e.g. body weight, initial blood pressure and/or serum creatinine). Appropriateness of each statistical comparison was assessed by visualising histograms of residuals and further residual (on y-axis) plots of 1) fitted-values and 2) expected normal quantiles. We considered $P \leq 0.050$ as indicating statistical significance. The appropriate experimental *n* for each comparison and statistical method used is indicated in each table and figure legend. All data were analyzed using Genstat v17 (VSNi, Rothampsted, UK). In the analysis of changes in plasma and effluent levels of amino acids, multiple comparisons and co-morbidities

were accounted for by applying Bonferroni correction. When applying Bonferroni correction, statistical significance is accepted at P=0.0025 or below. As 20 amino acids are analysed and compared, P value is calculated as P=0.05 divided by 20= 0.0025).

2.13 Power calculation for sample size

The clinical study was powered using data from a pilot study involving n=5 patients per type of RRT, measuring 21 different amino acids in plasma and effluent. Corrected for baseline plasma concentration (IHD, 1513 ± 403; SLEDf, 1894 ± 471; CVVH, 3141 ± 267 μmol/L; P=0.06) and RRT dose using SRI (IHD, 0.28 ± 0.04; SLEDf, 0.30 ± 0.04; CVVH, 0.45 ± 0.09 units; P=0.06) we calculated mean amino acid losses (IHD, 6746; SLEDf, 9420; CVVH, 10792 mgs), between-individual S.E.M. = 3848; within-individual S.E.M. = 861. Setting α at 0.05 and experimental power at 90% required n=9 patients per treatment (size of the difference to be detected, 2674 [9420-6746], between subject variance = 107900000; within subject variance = 3227000). However, accounting for multiple comparisons (e.g. separate analysis of 20 amino acids) and patients with varied co-morbidities, we adjusted α to 0.0025. This increased our sample size to n=17 patients per treatment. Allowing for unforeseen increased variance in outcomes and possible attrition we aimed to recruit n=24 patients per group (n=72 patients in total).

3. Descriptive data on study population

3.1 Patient characteristics

All suitable patients who were approached gave consent prior to participation in the study. Study participants were predominantly male, elderly and had multiple co-morbidities. Baseline characteristics of patients in the 3 RRT groups are described in table 4. Demographic features (age and sex) of the study population were similar across the 3 RRT groups. Patients receiving CVVH had a higher CRP at baseline, which is consistent with this group having a higher proportion of sepsis-associated AKI. Patients receiving SLEDf had lower blood pressures and plasma bicarbonate compared with the IHD and CVVH groups. Overall, patients with AKI stage 3 are at high risk of malnutrition as assessed by the MUST screening tool (MUST score of 2) (table 3). Seventy-seven percent of the study population were at least moderately malnourished based on SGA (SGA score of B and C) (table 3). Using the NRS tool, 71% of the study population were malnourished and required nutritional support. In the first RRT session, patients undergoing SLEDF had higher URR and Kt/V compared with patients on CVVH and IHD. On the other hand, patients receiving CVVH had higher SRI compared with IHD and SLEDf groups.

Table 5. Patient characteristics at admission stratified by type of renal replacement therapy

Characteristic	All (n=72)	HD (n=33)	SLEDf (n=15)	CVVH (n=24)	P-value*
Age, yr	65 ± 13	66 ± 14	70 ± 11	62 ± 12	0.17
Weight, kg	84.9 ± 21.8	91.4 ± 24.2	77.4 ± 20.2	80.6 ± 16.9	0.057
BMI	26.8 ± 6.1	27.6 ± 6.3	25.7 ± 7.6	26.4 ± 4.8	0.47
Male	54 (75)	25 (75)	10 (66)	19 (79)	0.43
Systolic blood pressure, mm Hg	128 ± 27	140 ± 25	99 ± 20	121 ± 23	<.001
Diastolic blood pressure, mm Hg	68 ± 12	75 ± 11	59 ± 9	62 ± 9	<.001
Ethnicity (% Caucasian)	71 (99)	33 (100)	14 (93)	24 (100)	-
Inotropic support (%) inotropes)	24 (33)	0	0	24 (100)	-
Co-Morbidities					
Septic AKI	38 (52)	10 (30)	8 (53)	20 (83)	<.001
Multiple AKI causes	25 (35)	8 (24)	6 (40)	11 (45)	0.21
CKD	31 (43)	16 (48)	9 (60)	6 (25)	0.06
Diabetes	35 (43)	18 (54)	8 (53)	9 (37)	0.40
Hypertension	41 (56)	22 (66)	8 (53)	11 (45)	0.27
Serum parameters					
Creatinine, µmol/L	575 ± 313	692 ± 307	592 ± 388	404 ± 175	<.001
Urea, mmol/L	34.3 ± 15.5	36.7 ± 9.6	30.5 ± 12.5	29.8 ± 15.2	0.08
Sodium, mmol/L	133 ± 7	132 ± 7	133 ± 7	135 ± 7	0.28
Potassium, mmol/L	5.24 ± 1.00	5.15 ± 1.0	5.57 ± 0.95	5.15 ± 1.03	0.25
Bicarbonate, mmol/L	17.8 ± 5.9	17.7 ± 5.5	14.8 ± 5.7	19.9 ± 5.9	0.019
Phosphate, mmol/L	2.37 ± 1.05	2.56 ± 1.02	2.59 ± 1.19	1.99 ± 0.96	0.08
Albumin, g/L	22.5 ± 7.7	23.8 ± 6.9	22.8 ± 8.9	20.4 ± 7.8	0.21
C-reactive protein, mg/L	140 ± 118	114 ± 112	112 ± 99	196 ± 122	0.01

Table 5. Data are Mean ± 1SD for continuous variables and number of patients (% of group total) positive for each category. *Statistical differences between groups of patients on admission were assessed by Kruskall-Wallis One-Way ANOVA for continuous variables and chi-squared test for categorical data. All data analysis was conducted using Genstat v17 (VSNi, UK). Statistical significance was accepted at $P < 0.05$.

Table 5: RRT characteristics of study participants

Characteristic	HD (n=33)	SLEDf (n=15)	CVVH (n=24)	P-value*
RRT (First session, T1)				
Prescribed RRT time, mins	120 ± 5	344 ± 42	1440 ± 0	-
Actual RRT time, mins	122 ± 22	282 ± 105	1225 ± 341	-
Blood flow rate, ml/min	207 ± 17	214 ± 29	234 ± 25	<.001
Plasma flow rate, ml/min	145 ± 13	148 ± 21	169 ± 25	<.001
Effluent flow rate, ml/min	462 ± 121	209 ± 26	†35 ± 0	-
Serum urea pre-RRT, mmol/L	36.7 ± 9.6	35.9 ± 24.1	29.8 ± 15.2	0.03
Serum urea post-RRT , mmol/L	28.5 ± 8.4	22.7 ± 24.3	18.7 ± 9.0	<.001
Urea reduction ratio (URR)	0.31 ± 0.14	0.54 ± 0.18	0.38 ± 0.16	<.001
Kt/v	0.41 ± 0.21	0.94 ± 0.42	0.85 ± 0.41	<.001
Solute removal index (SRI)	0.28 ± 0.14	0.45 ± 0.51	0.51 ± 0.21	<.001
RRT (Second session, T2)	**(n=11)**	**(n=4)**	**(n=15)**	
Prescribed RRT time, mins	180 ± 26	360 ± 0	1440 ± 0	-
Actual RRT time, mins	185 ± 30	323 ± 56	1244 ± 319	-
Blood flow rate, ml/min	225 ± 25	203 ± 5	234 ± 28	-
Plasma flow rate, ml/min	159 ± 24	140 ± 7	140 ± 58	-
Effluent flow rate, ml/min	516 ± 65	200 ± 0	†35 ± 0	-
Serum urea pre-RRT, mmol/L	25.8 ± 11.6	25.8 ± 19.5	13.6 ± 5.1	-
Serum urea post-RRT , mmol/L	15.7 ± 8.0	10.0 ± 6.4	10.1 ± 4.1	-
Urea reduction ratio (URR)	0.17 ± 0.09	0.35 ± 0.17	0.38 ± 0.16	-
Kt/v	0.21 ± 0.11	0.51 ± 0.25	0.83 ± 0.34	-
Solute removal index (SRI)	0.40 ± 0.11	0.26 ± 0.01	1.40 ± 0.93	-

Table 6. Values are Mean ± 1SD for continuous data and number (% of group total) for categorical data. Only a proportion of study participants received a second RRT session (IHD, n=11, SLED-F, n=4, CVVH, n=15) in this cohort. Hence information is included for comparison (to first session, T1) but was not formally compared. For T1, *statistical differences were assessed by Kruskall-Wallis One-Way ANOVA for continuous data. All data analysis was conducted using Genstat v17 (VSNi, UK). †, fixed filtration fluid rate (mls/kg min^{-1})

3.2 Nutritional Status of study Cohort

Table 6: Nutritional status of study participants stratified by RRT

Characteristic	All (n=73)	HD (n=33)	SLEDf (n=15)	CVVH (n=24)	P-value*
Dietetic assessment					
MUST 0	11 (15)	7 (21)	2 (13)	2 (8)	0.06
1	2 (3)	2 (6)	0 (0)	0 (0)	-
2	60 (82)	25 (73)	13 (87)	22 (92)	0.13
NRS $\leq$2	21 (29)	16 (47)	2 (13)	3 (12)	<0.001
NRS $\geq$3	52 (71)	18 (53)	13 (87)	21 (88)	0.55
SGA A	17 (23)	10 (29)	3 (20)	4 (17)	0.09
B	36 (49)	20 (59)	7 (47)	9 (38)	0.03
C	20 (28)	4 (12)	5 (33)	11 (46)	0.21
Serum parameters					
Plasma glucose, mmol/L	6.97 $\pm$ 3.58	7.13 $\pm$ 3.87	7.54 $\pm$ 4.02	6.55 $\pm$ 3.06	0.78

Table 7. Values are Mean $\pm$ 1SD for continuous data and number (% of group total) for categorical data. *Statistical differences between groups of patients on admission were assessed by Kruskall-Wallis One-Way ANOVA for continuous data and chi-squared test for categories of nutritional assessment tools. All data analysis was conducted using Genstat v17 (VSNi, UK). Statistical significance was accepted at $P < 0.05$. Key: Subjective Global Assessment (SGA) tool assessed using a 7-point scale: A (6-7) = well nourished, SGA B (3-5) = mild-moderately malnourished, SGA C (1-2) = severely malnourished; Malnutrition Universal Screening Tool (MUST) on a 3-point scale: MUST 0 = low risk, MUST 1 = medium risk, MUST 2 = high risk of malnutrition; Nutritional Risk Screening (NRS) was assessed using a 6-point scale with $\leq$2 points = nutritional support not indicated and NRS $\geq$3 = nutritional support indicated.

3.3 Discussion

The vast majority of patients with AKI in ICU had sepsis. Overall compared with patients receiving IHD and SLEDf, patients receiving CVVH had higher CRP. More patients in the CVVH group had multiple causes of AKI.

3.3.1 Elderly patients with AKI

Most patients in this study were elderly. This group has high risk for developing AKI. There are various reasons why older patients are susceptible to developing AKI. Firstly, the increasing number of comorbidities with age contributes to the high incidence of AKI (Coca, 2010). Secondly, greater need for procedures, surgery and drugs. Third, the kidney becomes more vulnerable with age because of age-dependent structural and functional alterations (Lindeman et al., 1985, Lindeman and Goldman, 1986, McLachlan et al., 1977, Darmady et al., 1973, Tauchi et al., 1971, Hollenberg et al., 1974, Davies and Shock, 1950, Rowe et al., 1976a, Rowe et al., 1976b, Reckelhoff and Manning, 1993, Qiao et al., 2005, Chou et al., 1997). As a consequence, older patients are likely to have a reduced glomerular filtration rate at baseline and diminished renal reserve.

The following points summarises changes in the ageing kidney

a) Reduction in total renal mass (Lindeman and Goldman, 1986)
b) Glomerulosclerosis (McLachlan et al., 1977)
c) Reduction in active cortical parenchyma
d) Thickening of the glomerular basement membrane (Darmady et al., 1973)
e) Mesangial expansion (Tauchi et al., 1971)
f) Reduction in amount and length of tubules (Lindeman and Goldman, 1986)
g) Thickening of large vessel walls (Tauchi et al., 1971)
h) Reduction in renal blood flow (10% per decade above age 40) (Hollenberg et al., 1974)
i) Reduction in GFR (approximately 1ml/min/year above the age of 45) (Davies and Shock, 1950, Lindeman et al., 1985, Rowe et al., 1976a)
j) Blunted nitric oxide production (Reckelhoff and Manning, 1993)

k) Decreased maximum osmolality (Rowe et al., 1976b)

l) Increased susceptibility to apoptosis (Qiao et al., 2005)

m) Decrease in renal growth factors (Chou et al., 1997)

3.3.2 Sepsis-associated AKI

Fifty percent of the patients in this study had sepsis as the presumed cause of AKI. Over 80% of patients with AKI in ICU had sepsis as the main insult. This result is consistent with published studies where 45 - 75% of AKI cases were associated with sepsis (Uchino et al., 2005, Angus et al., 2001, Bagshaw et al., 2005, Silvester et al., 2001, Neveu et al., 1996). More than half of patients with sepsis-associated AKI required admission to ICU and received CVVH. The patients in ICU could not tolerate other forms of RRT due to their haemodynamic instability and hence were treated with CVVH. The patients with sepsis-associated AKI in ICU all had concomitant non renal organ failure. Previous studies have also demonstrated that sepsis-associated AKI is an independent predictor of hospital death (Neveu et al., 1996, Brivet et al., 1996).

3.3.3 CKD

In this study, 43% of patients who developed AKI had pre-existing CKD. Most of these patients also had co-morbidities such as diabetes mellitus and hypertension. Previous studies have shown that the association between pre-existing CKD and AKI could be confounded by co-morbid disease and exposure to nephrotoxic insults (Seliger et al., 2008, Chapin et al., 2010). Nevertheless CKD has been identified as an important risk factor for AKI after multivariate adjustment for comorbidities in the setting of radiocontrast administration, sepsis and cardiac surgery (Waikar et al., 2008).

3.3.4 Multi-factorial AKI

This study demonstrated that AKI frequently has a multifactorial aetiology. This is common especially in the elderly population and has been described in previous studies (Davidman et al., 1991, Kohli et al., 2000).

3.3.5 AKI and RRT characteristics

Duration of the first RRT session for each patient differed depending on RRT modality. In patients treated with IHD, duration of the first treatment session is usually no more than 2 hours to reduce risk of developing dialysis disequilibrium syndrome. This syndrome is characterised by neurological symptoms caused by rapid removal of urea during haemodialysis (Patel et al., 2008). When dialysis disequilibrium occurs, an osmotic gradient occurs between the brain and the plasma due to rapid haemodialysis. This results in cerebral oedema and the affected patient can present with neurological symptoms such as headache, nausea, convulsions, vomiting, muscle cramps, tremors and altered conscious level. In severe cases, the patient can die from advanced cerebral oedema. Patients with pre-existing neurological disease such as stroke, head trauma and malignant hypertension are at an increased risk of developing dialysis disequilibrium syndrome (Yoshida et al., 1987, Peterson and Swanson, 1964). In addition to reducing duration of the first dialysis session, lowering the blood flow rates of the initial session can also reduce risk of dialysis disequilibrium syndrome (Arieff, 1994). A dialyser with a smaller surface area is also used to reduce this risk (Arieff, 1994). In patients receiving SLEDf, duration of the first RRT session was also shorter at 6 hours compared with 8 hours. Effluent flow rate in SLEDf was also lower than in IHD. Both these features of RRT prescription are intended to reduce the risk of dialysis disequilibrium in patients receiving SLEDf (Marshall et al., 2004). In comparison to IHD and SLEDf, CVVH is less efficient at achieving adequate solute clearance. CVVH removes uraemic toxins at a slow rate and theoretically reduces the risk of dialysis disequilibrium syndrome (Bagshaw et al., 2004).

3.3.6 RRT dose

In patients receiving IHD and SLEDf, urea reduction ratio (URR), Kt/V and solute removal index (SRI) were used as determinants of RRT dose. Dose of dialysis in IHD and SLEDf can be assessed using blood based kinetics and dialysate based kinetics. Using blood based kinetics, dose of dialysis is measured using URR and Kt/V (Clark et al., 1998) (Himmelfarb and Ikizler, 2000, Kanagasundaram et al., 2003) . Overall, the delivered dose of RRT is lower compared with patients with ESRD. There is currently a lack of prospective studies which evaluate the minimum dose of RRT required in the management of AKI. One prospective study demonstrated an

improvement in survival when intermittent haemodialysis was delivered at a URR of greater than 58% (Kanagasundaram et al., 2003). This cut-off value is lower than that recommended for patients with ESRD. Due to a paucity of studies addressing the issue of minimum RRT dose in AKI, a consensus statement was made by the Acute Dialysis Quality Initiative that patients with AKI should receive the minimum dose that is recommended for patients with ESRD (Kellum et al., 2002).

In this study, the URR was low in all three modalities for a number of reasons. Firstly, URR was calculated from the first dialysis session. Duration of the first dialysis session is always lower to reduce risk of dialysis disequilibrium. Second, the actual duration of RRT delivered to patients was lower than what was prescribed in the SLEDf and CVVH group. Third, the delivered blood flow rates in all three RRT groups were much lower than prescribed. This is due to the vast majority of patients in this study having dialysis delivered via a non-tunnelled femoral dialysis catheter which provide lower blood flow rates than internal jugular dialysis catheters.

3.3.7 Nutritional status in AKI

In this study, the majority of patients with AKI stage 3 were malnourished. Using the Subjective Global Assessment tool (SGA), 77% of patients were malnourished. There were no significance difference in the number of malnourished patients across the three RRT groups. The finding of malnutrition in AKI is consistent with a study published by Fiaccadori et al. (Fiaccadori et al., 1999) in which the prevalence of malnutrition in AKI was 40%. However, that study included patients with less severe degrees of AKI. Another important finding by Fiaccadori et al is that mortality of the patients with AKI increases with severity of the malnutrition (Fiaccadori et al., 1999). Thus nutritional status does play a significant role as malnutrition is independently associated with altered immune response and increased risk of infection (Chandra, 1972).

There is currently no gold standard tool to diagnose malnutrition in hospitalised patients (Souba, 1997, Jeejeebhoy et al., 1990). Traditional methods using anthropometric and biochemical parameters are often inaccurate and flawed due to presence of several non-nutritional factors, especially in patients in renal disease (Bergstrom, 1995, Ikizler and Hakim, 1996). SGA is a simple, multifactorial assessment tool used to evaluate nutritional status. SGA is based on the experience of the clinician and is described by taking a careful medical and dietary history, performing a physical examination and functional assessment of the patient. SGA has been demonstrated to

be a valuable and inexpensive tool in assessing the nutritional status of surgical patients and patients with end stage renal disease. Good inter-observer reproducibility was also evident in patients with chronic kidney disease and AKI.

Although previous studies have suggested that malnutrition is an independent risk factor for morbidity and mortality in patients with AKI, it would not be possible to prove causality without intervention studies. This would require an interventional study to demonstrate that improvement in nutritional status is associated with decrease in mortality, complications and use of healthcare resources. Previous published randomised controlled trials involving parenteral administration of essential amino acids in patients with AKI have not shown any improvement in mortality rates. However, aside from the presence of malnutrition on admission, other nutritional factors do need to be considered. These factors include extent of catabolism, nitrogen depletion, calorie deficit and nutritional management during hospital stay. Studies have shown that nutritional status does decline during inpatient hospital stay. Contributing factors include inadequate utilization of artificial nutrition and lack of attention to nutrient intake. In patients with AKI, hyper-catabolism is also important.

In addition this finding remained when two other nutritional assessment tools, NRS and MUST were used. BMI was not significantly different across the 3 RRT groups and was not below the ideal range. This is interesting as it appears to contrast with findings of malnutrition as described using the 3 nutritional assessment tools. Published literature have suggested that BMI is an inaccurate and unreliable marker of malnutrition in critical illness. This is attributable to confounding factors such as oedema and ascites which are frequently present in severe AKI. A 'normal' BMI can therefore potentially be misleading and can mask the presence of malnutrition. MUST is utilised as a tool to identify adult patients who are at risk of malnutrition. Therefore MUST is different from SGA because MUST does not evaluate the severity of malnutrition. The NRS tool combines elements of both SGA and MUST. NRS is used to screen for malnutrition risk and then a more detailed evaluation is undertaken to grade severity of malnutrition and to identify patients who require nutritional support.

4. Trace Element loss during RRT

4.1 Introduction

Trace elements are water-soluble micronutrients essential for health. Their levels are frequently altered in the setting of critical illness (Heyland et al., 2005). Trace elements can be depleted in critical illness due to combination of factors including decreased oral intake, increase in requirements for these micronutrients and through losses from renal replacement therapy. Previous small studies have shown that patients who develop AKI and require RRT can lose trace elements (Berger et al., 2004, Klein et al., 2008). Few studies have evaluated trace element status in AKI. No studies have compared losses of trace elements in different RRT modalities in AKI. Trace elements have numerous important functions. They are involved in regulation of the immune system and in key metabolic processes. In this study we are interested predominantly in four trace elements: zinc, selenium, copper and iron. These four play important roles in strengthening the immune system in response the critical illness. They also have antioxidant properties which are crucial in protection against oxidative stress. AKI is a pro-inflammatory state and is associated with increased oxidative stress. Patients with severe AKI are also immunocompromised and at increased risk of infection.

4.2 Methods

Please refer to Chapter 2.10 in Materials and Methods section

4.3 Trace elements analysis in AKI patients

Table 7: The change in selected plasma trace elements during RRT

Trace element (µgs/L)	Mode of RRT			s.e.d.	Time on RRT			s.e.d.	P-values		
	IHD	SLED-F	CVVH		Base	Mid	End		Mode	Time	M × T
Zinc	678	526	525	65	630	560	570	23	0.01	0.009	0.76
Iron	1475	972	1495	372	1582	1279	1201	139	0.36	0.01	0.88
Copper	1158	834	923	130	980	1009	1003	27	0.05	0.50	0.45
Selenium	51.5	40.7	37.5	5.9	44.6	43.4	43.5	0.9	0.02	0.21	0.57

Table 8: ***Select plasma trace elements during RRT***: Data are pooled means ± s.e.d. (standard error of the differences between means) for the main fixed effects of RRT mode and time on RRT. The first three columns (IHD, SLED-F and CVVH) represent the pooled means for RRT mode. For example, there is higher copper levels in IHD group compared to the SLED-F group as the mean difference is more than double the s.e.d. The next 3 columns (base, mid, end) represent the pooled means for time on RRT. Data were analysed using Restricted Maximum Likelihood (REML) as the main statistical test with Mode of RRT and Time on RRT as the fixed effects (time included as a repeated measure) and individual Patient ID as a random term in the model. REML was also used to analyse data with interaction between Mode of RRT and Time on RRT as fixed effect and patient ID as the random term. Means were analysed after correction for dose of dialysis (i.e. urea reduction ratio included as a co-variate fitted first in the model). 95% confidence intervals around each mean may be estimated as 1.96 × s.e.d. P-values were adjusted to account for the number of comparisons (x4; 0.05/4 = 0.012).

Figure 6: Zinc losses in effluent during RRT

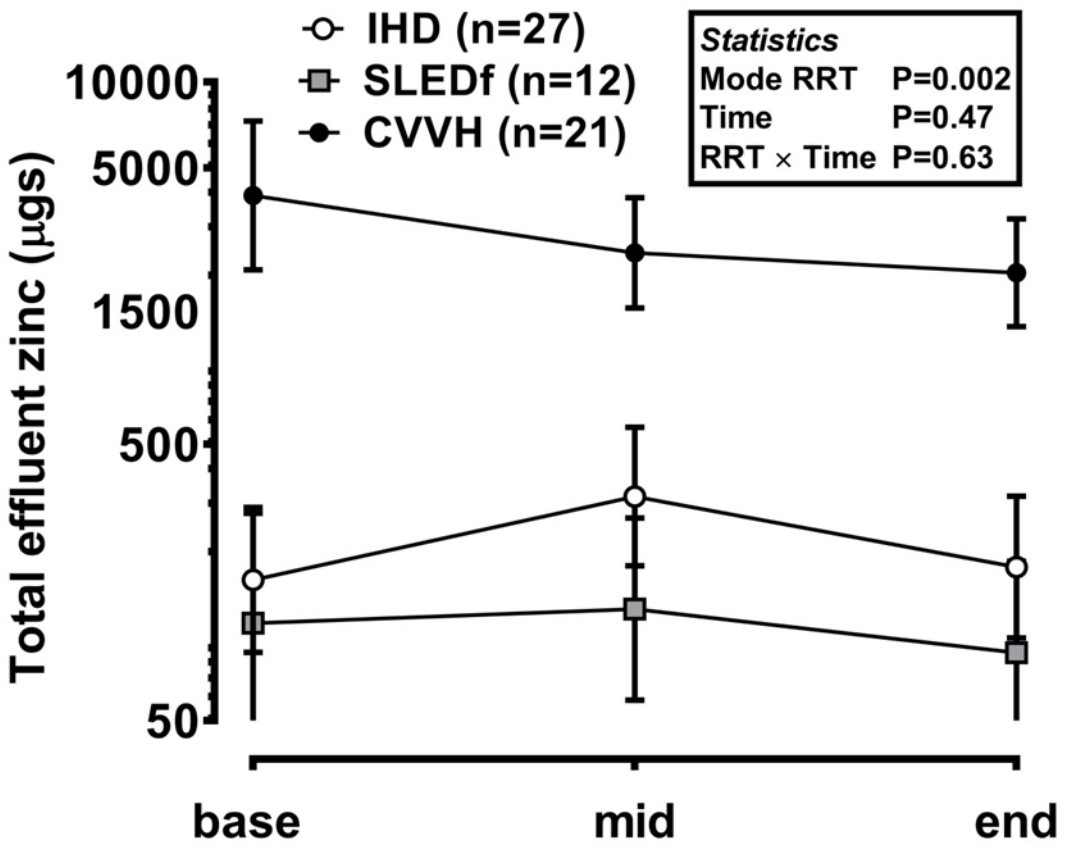


Figure 6: Total amount of effluent Zinc during different modes of RRT. Effluent was sampled after priming of each RRT machine but before a patient was connected (i.e. 'base'), half-way through each RRT session (i.e. 'mid) and at the end of each RRT session ('end'). Zinc concentration (µg/L effluent) was measured in spot samples at each time point and estimated total amounts calculated by multiplying measured spot concentration (µgs/L) by the total volume of effluent produced for each patient during that RRT session. Data (means ±S.E.) are presented corrected (i.e. included as co-variates in the statistical model) for dose-of-dialysis (solute removal index for effluent losses) and plasma concentration of zinc at baseline. If necessary, to normalise residual error before statistical analysis, data were $\log_{10}$ transformed. Graphs were generated in Graphpad Prism 6 (Graphpad Software Inc, Ca, USA). Analysis was by mixed effect models with RRT mode and time as fixed effects and Patient ID as a nested

random effect, using Genstat v18 (VSNi, Rothampsted, UK). Statistical significance was accepted at P=0.012 (adjusted for the number of comparisons).

Figure 7: Copper losses in effluent during RRT

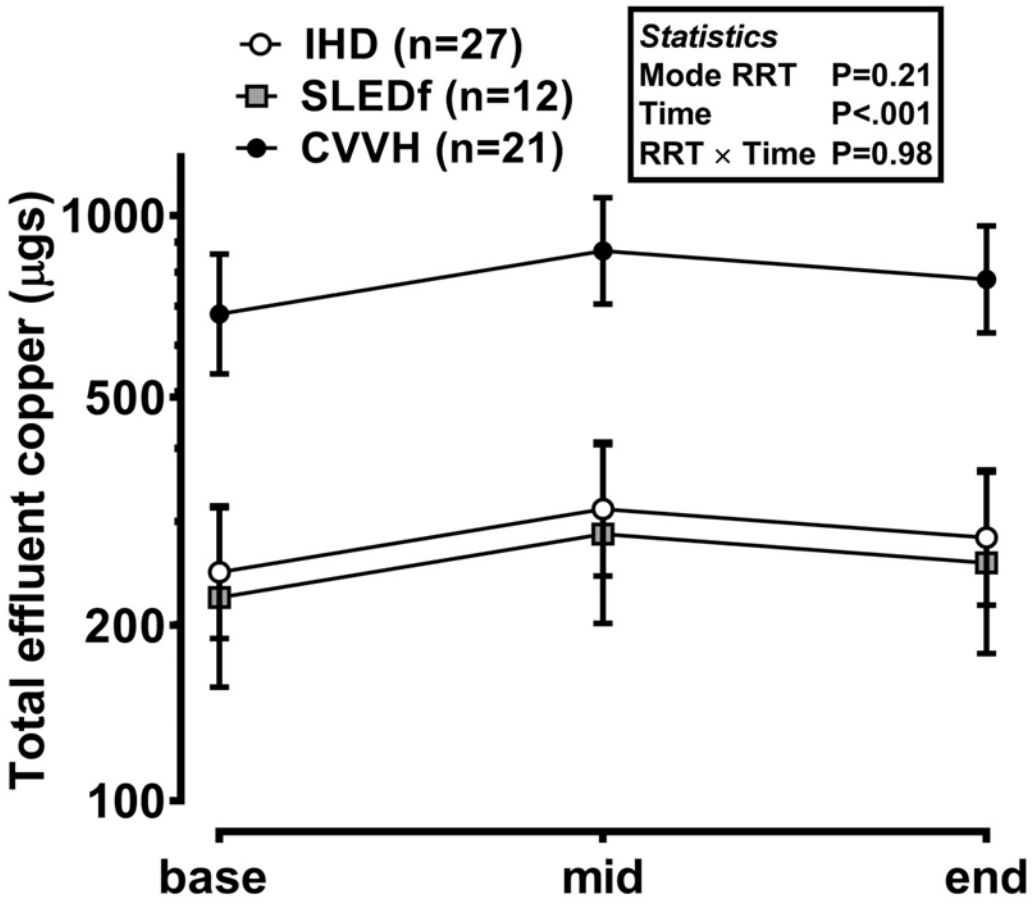


Figure 7: Total amount of effluent copper during different modes of RRT. Effluent was sampled after priming of each RRT machine but before a patient was connected (i.e. 'base'), half-way through each RRT session (i.e. 'mid) and at the end of each RRT session ('end'). Copper concentration (µg/L effluent) was measured in spot samples at each time point and estimated total amounts calculated by multiplying measured spot concentration (µgs/L) by the total volume of effluent produced for each patient during that RRT session. Data (means ±S.E.) are presented corrected (i.e. included as co-variates in the statistical model) for dose-of-dialysis (solute removal index for effluent losses) and plasma concentration of copper at baseline. If necessary, to normalise residual error before statistical analysis, data were $\log_{10}$ transformed. Graphs were generated in Graphpad Prism 6 (Graphpad Software Inc, Ca, USA). Analysis was by mixed effect models with RRT mode and time as fixed effects and Patient ID as a nested

random effect, using Genstat v18 (VSNi, Rothampsted, UK). Statistical significance was accepted at P=0.012 (adjusted for the number of comparisons).

Figure 8: Selenium losses in effluent during RRT

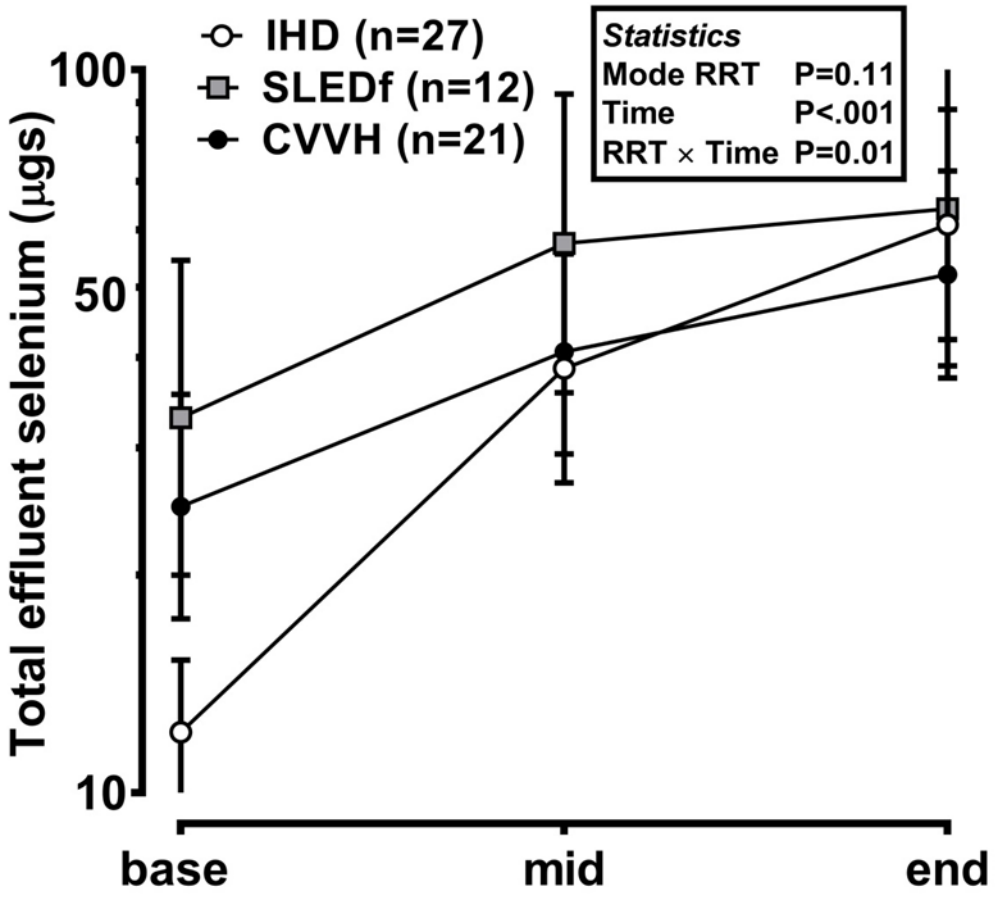


Figure 8: Total amount of effluent selenium during different modes of RRT. Effluent was sampled after priming of each RRT machine but before a patient was connected (i.e. 'base'), half-way through each RRT session (i.e. 'mid) and at the end of each RRT session ('end'). Selenium concentration (µg/L effluent) was measured in spot samples at each time point and estimated total amounts calculated by multiplying measured spot concentration (µgs/L) by the total volume of effluent produced for each patient during that RRT session. Data (means ±S.E.) are presented corrected (i.e. included as co-variates in the statistical model) for dose-of-dialysis (solute removal index for effluent losses) and plasma concentration of selenium at baseline. If necessary, to normalise residual error before statistical analysis, data were $\log_{10}$ transformed. Graphs were generated in Graphpad Prism 6 (Graphpad Software Inc, Ca, USA). Analysis was by

mixed effect models with RRT mode and time as fixed effects and Patient ID as a nested random effect, using Genstat v18 (VSNi, Rothampsted, UK). Statistical significance was accepted at P=0.012 (adjusted for the number of comparisons).

Figure 9: Iron losses in effluent during RRT

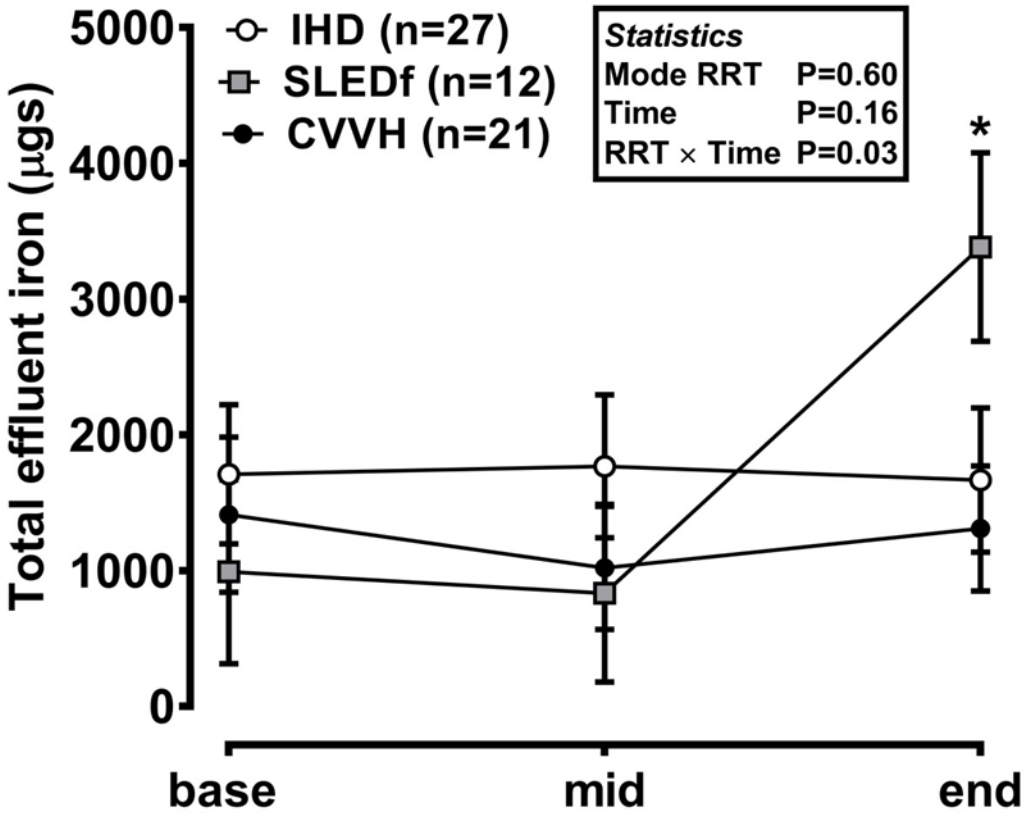


Figure 9: Total amount of effluent iron during different modes of RRT. Effluent was sampled after priming of each RRT machine but before a patient was connected (i.e. 'base'), half-way through each RRT session (i.e. 'mid) and at the end of each RRT session ('end'). Iron concentration (µg/L effluent) was measured in spot samples at each time point and estimated total amounts calculated by multiplying measured spot concentration (µgs/L) by the total volume of effluent produced for each patient during that RRT session. Data (means ±S.E.) are presented corrected (i.e. included as co-variates in the statistical model) for dose-of-dialysis (solute removal index for effluent losses) and plasma concentration of iron at baseline. If necessary, to normalise residual error before statistical analysis, data were $\log_{10}$ transformed. Graphs were generated in Graphpad Prism 6 (Graphpad Software Inc, Ca, USA). Analysis was by mixed effect models with RRT mode and time as fixed effects and Patient ID as a nested random effect, using Genstat v18 (VSNi, Rothampsted, UK). Statistical significance was accepted at P=0.012 (adjusted for the number of comparisons).

4.4 Discussion

4.4.1 Trace elements in RRT effluent in AKI

Trace elements were detected in RRT effluent in all 3 modalities in AKI. The amount detected in RRT effluent was dependent on which modality was used. This study has shown that trace elements are removed using both diffusion and convection in RRT. This is a novel finding, as no previous studies have compared trace element losses in 3 different types of RRT modality. In this study we focused on 4 trace elements: zinc, copper, selenium and iron.

The amount of zinc and copper lost in patients receiving CVVH is significantly higher compared to IHD and SLEDf. There are several possible contributing factors. Firstly, all patients on CVVH received artificial nutrition, including trace elements, via parenteral or enteral routes, leading to higher plasma levels and greater losses via filtration. Trace elements are an important part of nutrition supplementation in critical illness. Secondly, zinc, copper and the other trace elements were detected in significant quantities in the CVVH replacement fluid. Again this would lead to greater losses in filtrate. Thirdly, zinc and copper are predominantly bound to albumin in plasma. Dialysis would not remove them in significant amounts. Convection –based RRT is able to remove solutes of larger molecular weight. This would not usually include much albumin, but losses of protein-bound elements are likely to be greater than in pure diffusion.

During RRT, the amount of trace element losses did not change with time. This finding was observed in zinc and copper where the rate of loss was similar at mid –session and end session. This was demonstrated in all three RRT groups. RRT effluent was sampled at more frequent time points in a subgroup of patients to determine more accurately whether the losses were similar throughout the duration of treatment.

Losses were again found to be constant throughout the treatments, thus justifying that the rate of loss did not change from mid-session to end –session.

The amount of iron lost was highest in patients receiving SLEDf compared with those receiving IHD and CVVH. This has not previously been demonstrated in previous studies. This finding suggests that the combination of diffusion and convection leads to greater losses of iron compared to either process alone.

Losses of selenium during RRT was similar across the three RRT modalities. However, the amount of selenium lost was significantly higher at the end of the RRT sessions compared with mid-session.

4.4.2 Plasma trace element levels in AKI

In this study, plasma levels of zinc, selenium and copper were lower in patients with AKI (in all RRT groups) than levels reported for healthy controls (Hurst et al., 2010). Plasma levels of iron in these patients were similar to those reported in healthy adults (LG, 2007). These findings are of clinical importance as trace elements play vital roles in the endogenous antioxidant defence system. Trace elements such as copper, selenium, zinc and iron are required for activity of antioxidants such as glutathione peroxidase, catalase and superoxide dismutase. There are several reasons why plasma trace elements levels are decreased in AKI. Firstly, the majority of patients in this study have the systemic inflammatory response syndrome (SIRS). In SIRS, the vascular permeability increases leading to capillary leakage of micronutrients into the interstitial space. Some trace elements such as zinc are bound to plasma albumin. In patients who are hypoalbuminaemic, there is redistribution of the micronutrient from the intravascular to extravascular space. Second, there can be haemodilution in AKI and third, reduced oral intake of trace elements contributes to deficiency even prior to the start of RRT. Fourth, AKI is associated with increased oxidative stress (Himmelfarb et al., 2004), which leads to higher demand for the trace elements which are involved in the antioxidant defence system.

4.4.3 Changes in plasma trace element levels during RRT in AKI

The changes in plasma trace element levels observed varied depending on the trace element studied. There was significant reduction in plasma zinc and iron levels during the RRT session, observed in all three RRT groups. In contrast, there was no significant reductions in plasma copper and selenium in all RRT groups. Several factors could explain the lack of reduction in plasma trace element levels in all 3 RRT groups. Firstly, in patients receiving CVVH, nutritional supplementation containing most trace elements are administered via enteral or parenteral routes. Losses due to CVVH are therefore offset by supplementation. Second, in all 3 RRT modalities, redistribution of trace elements from intracellular to extracellular compartment can

attenuate reductions in plasma levels caused by RRT losses. Third, the dose of RRT is relatively low so trace element losses might not be great enough to cause net reduction in plasma levels, especially given the likely redistribution between intra- and extracellular levels. The low dose of RRT is explained by two reasons. Firstly, delivered RRT dose is less than the prescribed dose. Second, prescribed dose for the first RRT session (with reference to IHD and SLEDf) is deliberately lowered to reduce risk of dialysis disequilibrium. The maintenance of plasma trace element concentrations were not reported in previous studies (Bhogade R, 2013). One study compared effect of dialysis on zinc and copper levels in AKI patients and demonstrated a significant reduction in these elements during a dialysis session (Bhogade R, 2013). Hosokawa et al actually reported an increase in serum zinc and copper concentrations during dialysis (Hosokawa et al., 1985).

5. B-vitamin losses during RRT

5.1 Introduction

B vitamins are water-soluble substances with low molecular weight. They are thus easily removed during RRT. Whilst there are several studies which have evaluated the status of B vitamins in chronic dialysis, few studies have involved AKI and none has compared the effect of different RRT modalities on B vitamin loss in AKI. These vitamins act as cofactors for many enzymatic processes. Increased amounts of B vitamins are required in critically ill patients in order to support the augmented enzymatic processes associated with hypermetabolism (Fortin et al., 1999). B vitamins also play an important role in regulation of the immune system. Inadequate vitamin status can lead to a compromise of immune function. For example, deficiency in folate reduces immune competence and hence decreases resistance to infections (Dhur et al., 1991). Folic acid supplementation has been associated with reduction in reported infections, thus suggesting that folate strengthens the innate immunity and provided protection against infections (Bunout et al., 2004). Deficiency in vitamins B6 and B12 has been associated with impaired immune response (Miller and Kerkvliet, 1990) (Tamura et al., 1999). In this study we investigated the effect of RRT on a range of B vitamins, including thiamine, riboflavin, niacin, pyridoxine, folate and vitamin B12.

5.2 Methods

Please refer to chapter 2.12 in Materials and Methods section

5.3 Results

All water-soluble B vitamins were undetectable in effluent in all 3 RRT groups. Plasma levels of B vitamins were not measured for regulatory reasons in the laboratory undertaking the assays.

5.4 Discussion

5.4.1 Vitamin losses during RRT in AKI

B vitamins were undetectable in RRT effluent from all 3 modalities in this study. These results were not consistent with data published by Berger et al (Berger et al., 2004), who reported that thiamine was detected in the effluent using HPLC. However, whilst plasma levels of B vitamins in our study could not be analysed, the effect of adsorption on B vitamins was studied using a separate *in vitro* experiment. In this experiment, the same RRT dialyser was used as in the clinical study. The experiment showed that significant amounts of B vitamins were adsorbed, implying that this might be a more significant route of B vitamin loss than diffusion or convection. No previous studies have reported the effect of adsorption on vitamin losses in RRT.

Although we do not have data on plasma B vitamin levels in patients with AKI, previous studies have indicated that patients with AKI requiring RRT have depleted plasma B vitamin levels (Story et al., 1999) (Fortin et al., 1999). There are several reasons why patients with AKI are likely to have deficiency in water-soluble vitamins. Firstly, prevalence of malnutrition in patients with AKI is high (Fiaccadori et al., 1999). Dietary intake is the predominant source of water-soluble vitamins as the body is unable to synthesize the compounds in sufficient quantities. Secondly, there is an increase in demand of water-soluble vitamins during critical illness. Water-soluble vitamins act as important anti-oxidants and patients with AKI have been shown to have increased oxidative stress (Himmelfarb et al., 2004). Thirdly, patients with AKI often have several co-morbidities and are prescribed medicines which can interfere with absorption of vitamins (Leblanc et al., 2000). Fourth, metabolism of B vitamins is impaired in uraemia (Stein et al., 1985) (Kopple and Swendseid, 1975). Several studies have demonstrated that whilst vitamin status was normal in patients with renal impairment, the principal active form was deficient. This finding suggests that there is impaired metabolism of B vitamins in uraemia (Ross et al., 1989) (Spannuth et al., 1977).

There are important clinical implications for patients with AKI who have lost water-soluble vitamins during RRT. In patients undergoing regular RRT, deficiencies in B vitamins have been associated with depression of the immune response (Dobbelstein et al., 1974) (Casciato et al., 1984), neuropathy (Rostand, 1976) (Teehan et al., 1978) (Taniguchi et al., 1987), impaired amino

acid and lipid metabolism (Kleiner et al., 1980) and haematological abnormalities (Siddiqui et al., 1970) (Hampers et al., 1967).

6. Amino acid losses during RRT

6.1 Introduction

Amino acids can be classed as essential or non-essential amino acids depending on whether they can be synthesized endogenously in the human body. Essential amino acids can be acquired only exogenously, from diet.

Amino acids are directly involved in protein synthesis and play a key role in regulation of the immune system (Li et al., 2007). In this study, branched chain amino acids (BCAAs) are of interest as they have important and diverse physiological and metabolic roles. BCAAS include leucine, isoleucine and valine. BCAAs promote protein synthesis and reduce breakdown. In addition, BCAAs may have therapeutic benefits in management of patients with liver disease, renal failure, sepsis and surgical injury (Skeie et al., 1990) (Blackburn et al., 1979) (Mori et al., 1988). They are also required for lymphocyte growth and proliferation as well as cytotoxic T lymphocyte and natural killer cell activity (Calder, 2006)

Glutamine is the most abundant free amino acid in the human body (Brosnan, 2003). It is predominantly produced in skeletal muscle (Lacey and Wilmore, 1990). Although glutamine is considered a non-essential amino acid as it can be synthesized endogenously, it is conditionally essential during critical illness i.e. in critical illness, endogenous production of glutamine is insufficient to meet metabolic demands and thus supplementation is required. Glutamine is involved in protein and lipid synthesis (Corbet and Feron, 2015) and helps to regulate acid-balance in kidney by producing ammonium (Lacey and Wilmore, 1990). Glutamine is an important source of cellular energy (Aledo, 2004). It acts as an energy source for rapidly dividing cells in the gastrointestinal tract (enterocytes, colonocytes) and immune system (lymphocytes, macrophages) (Ziegler et al., 2000) (Newsholme, 2001). During critical illness, glutamine plays a crucial role in inducing cellular protection pathways, modulating the inflammatory response and preventing organ injury (Wischmeyer, 2008). Studies have shown that glutamine is frequently depleted in patients who are admitted to the intensive care unit (Heyland et al., 2013) (Oudemans-van Straaten et al., 2001) (Rodas et al., 2012). Deficiency in glutamine has also been associated with a high mortality (Oudemans-van Straaten et al., 2001, Rodas et al., 2012). There is evidence of benefit of glutamine supplements in these patients

including reduction in mortality, infectious complications and hospital length of stay (Wischmeyer, 2008).

Few studies have looked at the losses of amino acids during RRT in AKI. They have been small and focused on use of CRRT (Davenport and Roberts, 1989). One recent study has evaluated losses of amino acids in SLEDf (Chua et al., 2012). No study has compared losses of amino acids in 3 different RRT modalities

6.2 Methods in brief

Please refer to Chapter 2.8 in Materials and Methods Sections

6.3 Results

The total plasma concentration of all standard amino acids (n=20) at baseline was similar between patients receiving IHD *vs.* SLED-f (1812 ± 517 *vs.* 2675 ± 527 µmol/L, respectively) but were considerably higher in patients receiving CVVH (3194 ± 564 µmol/L). RRT reduced the plasma concentration of amino acids in patients receiving SLED-F (to 1732 ± 529 µmol/L; P=0.02), but had no effect in those receiving IHD or CVVH (IHD; 1853 ± 523, CVVH; 2845 ± 512 µmol/L). The average, unadjusted loss of amino acids was significantly influenced by mode of RRT (IHD, 5.13 ± 3.1 *vs.* SLED-F, 8.21 ± 4.07 vs. CVVH, 18.69 ± 3.04 gms; P<0.01).

6.4 Plasma and effluent levels of amino acids

Table 8: The change in plasma and effluent amino acids during RRT

	Δ PLASMA (µmol/L)				EFFLUENT (mgs)				*P*-value	
Mode of RRT…	**IHD**	**SLED-F**	**CVVH**	**s.e.d**	**IHD**	**SLED-F**	**CVVH**	**s.e.d**	**Plasma**	**Effluent**
Alanine	-37	-129	-34	70	788	998	1586	374	0.002	0.06
Arginine	-3.2	-14.2	7.7	11.0	242	288	723	164	0.66	0.006
Asparagine	-1.5	-18.9	-13.2	14.1	249	368	571	138	0.03	0.047
Aspartic acid	-2.1	-11.4	-19.7	7.8	51	88	97	27	0.003	0.15
Cysteine	-19.4	-33.1	-44.4	14	192	235	227	39	<.001	0.43
Glutamine	-17.7	-152	-84.5	83.0	127	179	191	42	0.005	0.23
Glycine	-17.1	-54	-36.2	47	436	616	811	175	0.008	0.07
Histadine	-5.2	-22.6	-26.2	13	237	302	454	101	<.001	0.07
Isoleucine	1.9	-17.3	8.2	10.8	164	270	473	85	0.96	0.001
Leucine	6.2	-31.1	-11.0	19.1	397	645	876	152	0.96	0.005
Lysine	-11.7	-64.7	12.6	29.9	569	738	1463	312	0.18	0.009
Methionine	-4.8	-13.1	-8.3	11.9	172	228	351	80	0.02	0.058
Phenylalanine	2.2	-19.2	-20.5	16.8	363	540	752	165	0.03	0.056
Proline	-16.2	-67.3	-15.8	65.4	705	839	1419	300	0.07	0.029
Serine	-6.6	-21.5	-6.7	18.0	194	303	452	95	0.03	0.016
Threonine	-9.9	-26.7	8.1	21.4	339	508	757	185	0.32	0.057
Tryptophan	0.57	-1.08	0.38	1.4	12	30	35	10	0.74	0.058
Tyrosine	0.48	-18.2	1.28	8.1	305	401	573	128	0.27	0.099
Valine	-16	-71	-9	36	433	616	1010	174	0.001	0.003
GABA	-4.7	-12.9	-7.6	5.7	70	95	111	24	<.001	0.19
Aminoadipicacid	-0.10	-3.2	-5.7	2.4	61	81	85	26	0.005	0.60
aminoisobutyricacid	-18.0	-42.2	-63.6	25.1	86	160	213	66	<.001	0.12
Citruline	-8.6	-13.3	-5.4	5.2	77	112	175	32	<.001	0.006
Cysthionine	-3.9	-8.8	-19.4	7.1	57	65	85	33	<.001	0.60
Hydroxyproline	-4.4	-10.8	-16.3	18.7	90	75	110	38	0.003	0.66
methylhistidine_1	-4.7	-14.8	-7.8	8.2	72	107	83	18	0.001	0.17
methylhistidine_3	-6.0	-14.8	-11.4	3.9	61	88	107	15	0.001	0.009
Taurine	-36.3	-40.9	-54.4	26.9	202	352	261	79	0.001	0.19

Table 9: *Δ Plasma losses*: Data reflect average decrease over the course of each RRT session (concentration at End minus baseline). Statistics (*P*-value, *plasma*) are for the effect of time on

plasma concentration. ***Effluent losses***: Since there was no significant difference between losses estimated at mid or end RRT session, data reflect pooled average losses (for Mid and End samples). Statistics (*P*-value, *effluent*) are for the effect of RRT on net loss in effluent. 95% confidence interval for the data may be estimated as 1.96 × s.e.d. Some α-amino acids were measureable but at limits of quantification, and unreliable (anserine, betalanine, carnosine, ethanolamine, gammaaminobutyricacid, homocysteine, hydroxylysine, phosphoserine, phosphoethanolamine, sarcosine). Key: 's.e.d', standard error of the differences between means. *P*-value testing for effect of RRT mode only (as timepoints are pooled estimates). Accounting for multiple comparisons (separate analysis of 20 amino acids) and patients with multiple co-morbidities, Bonferroni correction was applied. Statistical significance is accepted at P=0.0025 or below (P=0.05 divided by 20 = 0.0025).

Figure 10: Changes in plasma levels of amino acids during RRT

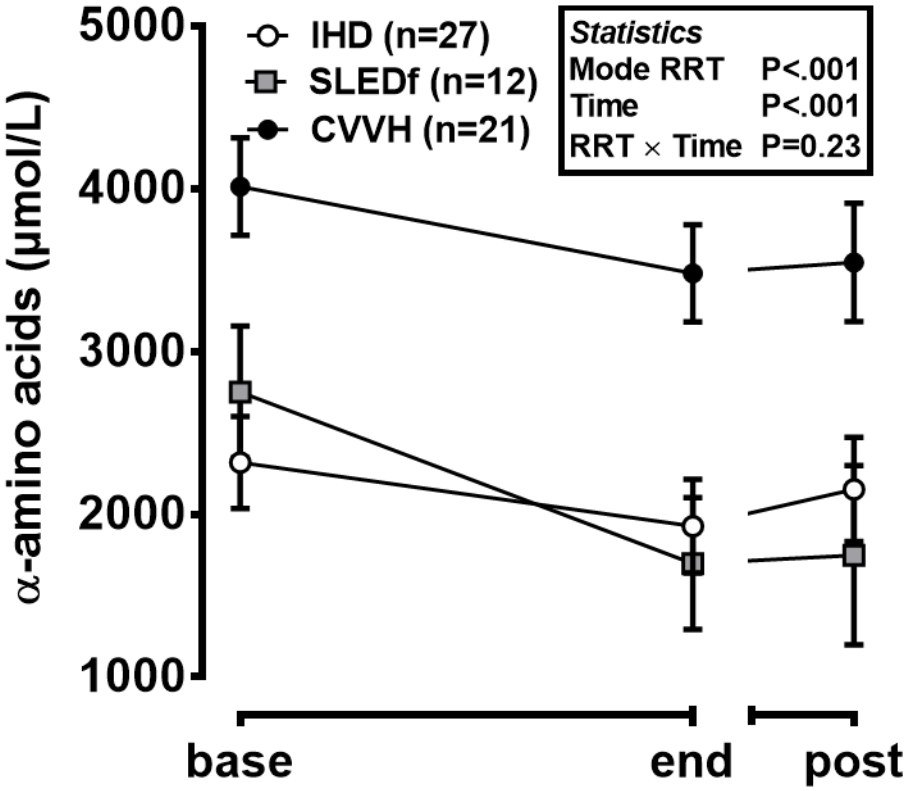


Figure 10. Plasma amino acids during RRT. Concentrations of amino acids were measured in spot samples of plasma. All alpha amino acids (x20 standard proteogenic plus a further 18 amino acids) were measured after deproteinisation and derivatization using a Biochrom 20 (Biochrom, Cambridge, UK). Data (means ±S.E.) are presented corrected (i.e. included as co-variates in the statistical model) for dose-of-dialysis (urea reduction ratio). If necessary, to normalise residual error before statistical analysis, data were $\log_{10}$ transformed. Graphs were generated in Graphpad Prism 6 (Graphpad Software Inc, Ca, USA). Analysis was by RM-ANOVA or mixed effect models, as appropriate, with RRT mode and time as fixed effects and patient ID as a nested random effect, using Genstat v18 (VSNi, Rothampsted, UK). Statistical significance was accepted at $P<0.05$. Statistical significance was accepted at $P=0.012$ (adjusted for the number of comparisons).

Figure 11: Arginine losses in effluent during RRT

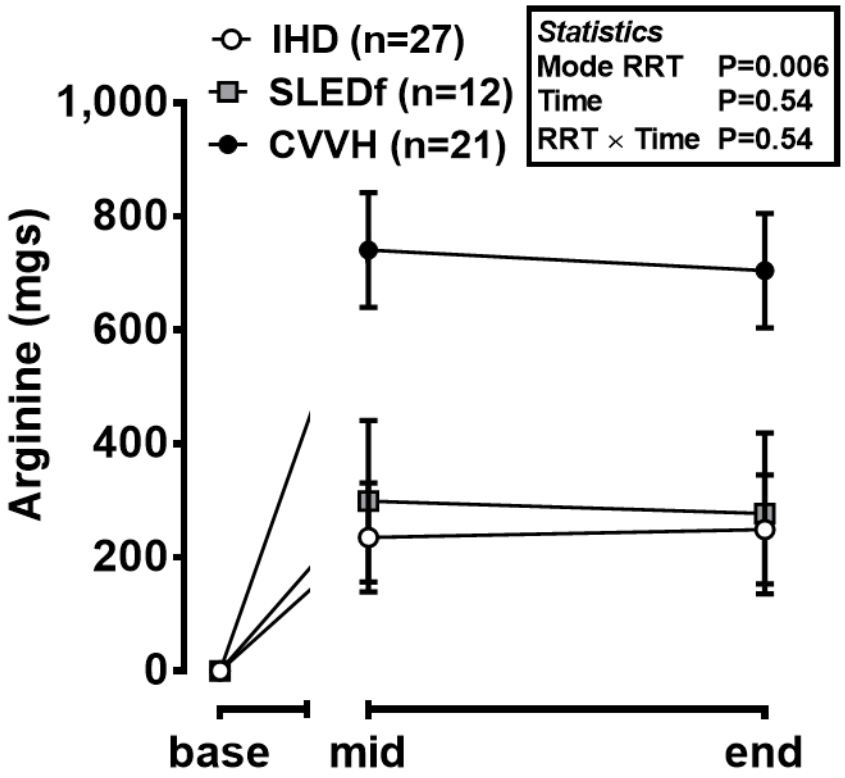


Figure 11. Arginine losses in effluent during RRT. Concentrations of amino acids were measured in spot samples of baseline effluent (i.e. after 'priming' each dialyser; IHD, SLED-F) or in replacement solution (e.g. PrismaSol for CVVH). No amino acids were present at baseline (n=10 replicates) and thus 'base' is set to zero. Total losses were estimated by multiplying all mass-corrected concentrations (i.e. µmoles/L to µgs/L) by the total volume of effluent produced for each patient. Effluent was also sampled during ('mid', half-way through for each mode), and at the end of each RRT session. All alpha amino acids (x20 standard proteogenic plus a further 18 amino acids) were measured after deproteinisation and derivatization using a Biochrom 20 (Biochrom, Cambridge, UK). Data (means ±S.E.) for arginine are presented corrected (i.e. included as co-variates in the statistical model) for dose-of-dialysis (solute removal index) and baseline plasma concentration. If necessary, to normalise residual error before statistical analysis, data were $\log_{10}$ transformed. Graphs were generated in Graphpad Prism 6 (Graphpad Software Inc, Ca, USA). Analysis was by RM-ANOVA or mixed effect models, as appropriate, with RRT mode and time as fixed effects and patient ID as a nested random

effect, using Genstat v18 (VSNi, Rothampsted, UK). Statistical significance was accepted at $P<0.05$. Statistical significance was accepted at $P=0.012$ (adjusted for the number of comparisons).

Figure 12: Glutamine losses in effluent during RRT

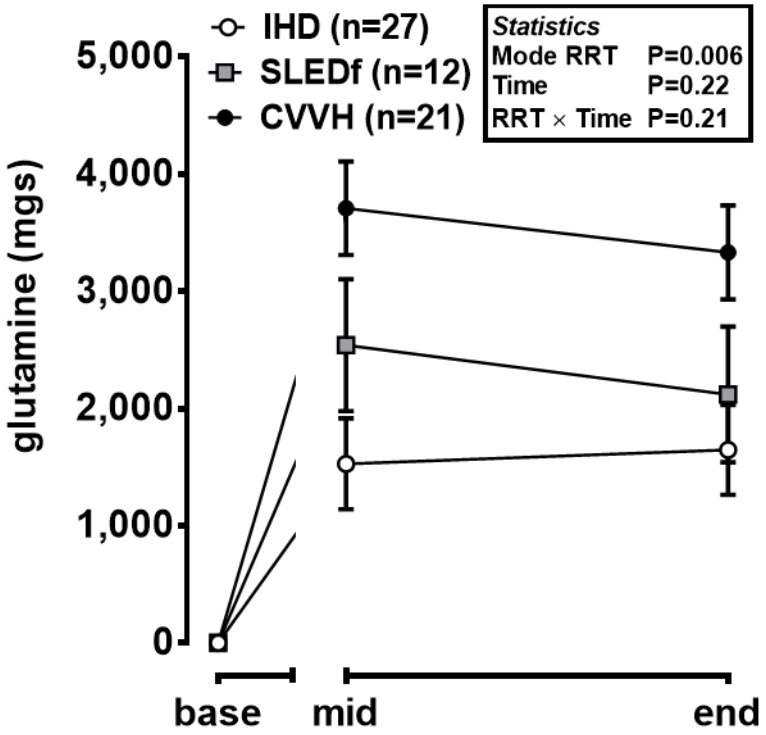


Figure 12. Glutamine losses in effluent during RRT. Measurements and analysis similar to that used for arginine in Figure 11

Figure 13: Isoleucine losses in effluent during RRT

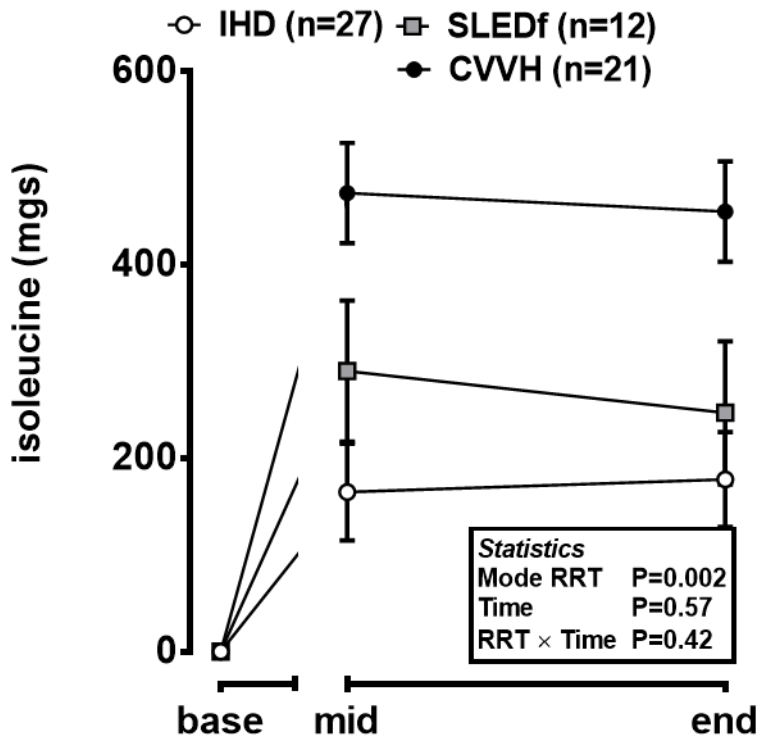


Figure 13. Isoleucine losses in effluent during RRT. Measurements and analysis similar to that used for arginine in Figure 11

Figure 14: Leucine losses in effluent during RRT

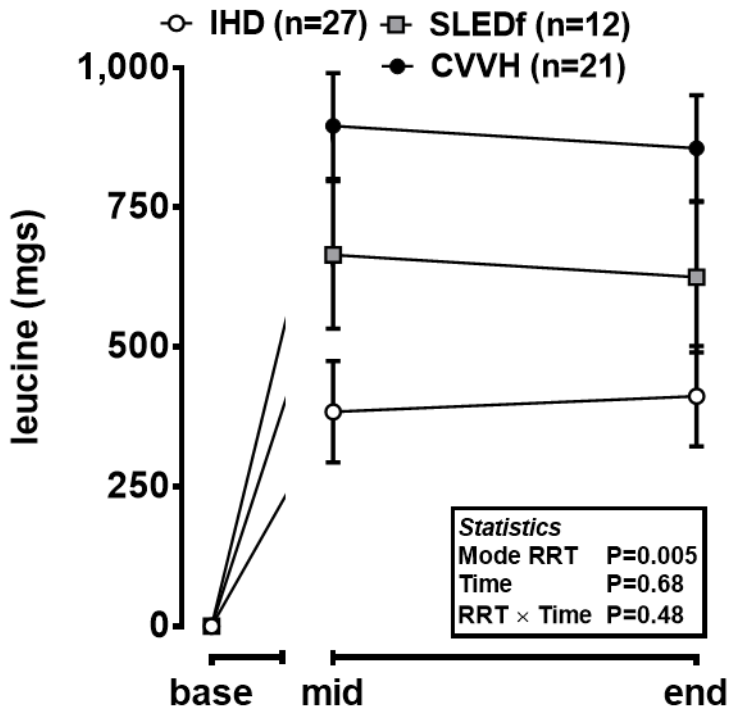


Figure 14. Leucine losses in effluent during RRT. Measurements and analysis similar to that used for arginine in Figure 11

Figure 15: Valine losses in effluent during RRT

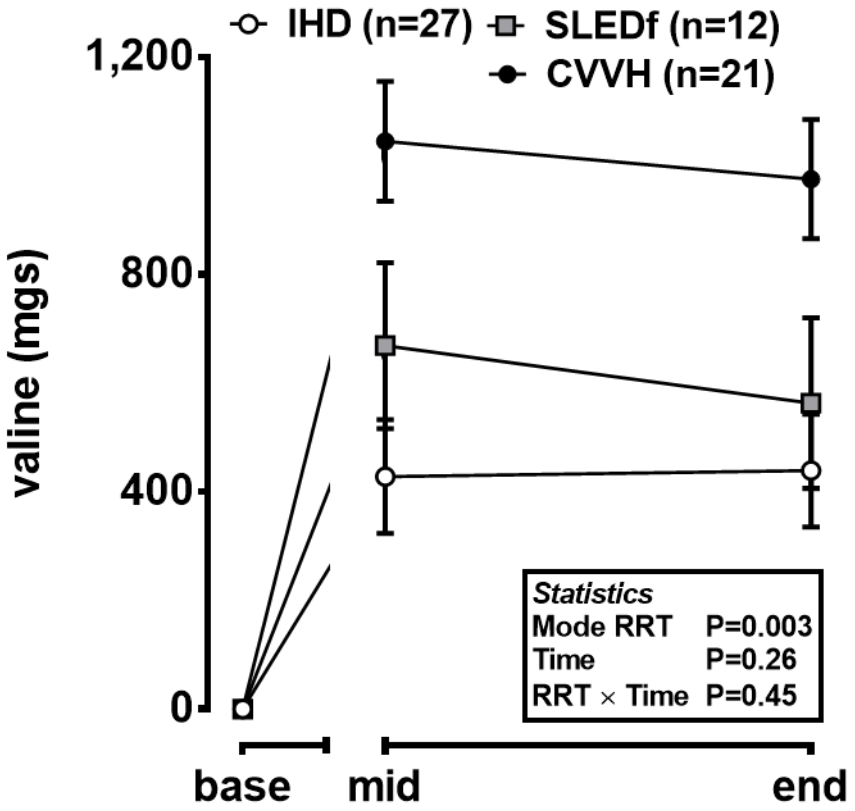


Figure 15: Valine losses in effluent during RRT. Measurements and analysis similar to that used for arginine in Figure 11

6.5 Discussion

6.5.1 Amino acid losses during RRT in AKI

In this study we have demonstrated two important findings. Firstly, median unadjusted amino acid losses were highest in patients receiving CVVH. Although these losses were attenuated after adjusting for RRT dose and baseline amino acid concentration, they still remain higher than losses in patients treated with IHD and SLEDf. Two previous studies have shown that (Davenport and Roberts, 1989, Chua et al., 2012) amino acid losses are significant in patients in CVVH. However, comparison of amino acid losses across IHD, SLEDf and CVVH has not been previously reported, and this study has shown that there is a significant difference between them. This suggests that a purely convection-based treatment leads to greater loss of amino acids in patients with AKI compared with diffusion and hybrid treatments. Losses by diffusion have been reported in patients with ESRD receiving thrice weekly haemodialysis (Navarro et al., 1998). RRT membranes used in AKI may differ in their physical properties which could influence extent and type of amino acid losses. RRT membranes which are bioincompatible can increase protein catabolism and lead to higher losses of amino acids into the dialysate. This effect was first shown using sham dialysis in healthy subjects (Gutierrez et al., 1990). Physical properties such as flux or pore size of the RRT membrane are crucial as more amino acids could theoretically be lost in the dialysate using high flux membranes. The higher amino acid losses in CVVH observed in this study may be due to use of polyacrylnitrile membrane (AN69). For IHD and SLEDf treatments, polysulfone membranes were used which was associated with lower amino acid losses. This finding has also been reported in patients with ESRD undergoing thrice weekly haemodialysis(Navarro et al., 1998). All patients treated with CVVH were given enteral or parenteral supplementation, both containing relatively high concentrations of amino acids. This is an important confounding factor leading to the increased measured losses in this group. This has previously been described in patients with ESRD receiving regular haemodialysis (Wolfson et al., 1982).In the study by Wolfson et al (Wolfson et al., 1982), measured amino acids losses in dialysate increased when patients received an infusion of amino acids. Moreover, the type of membrane used in previous studies did not appear to alter the effect of intradialytic supplementation on amino acid losses (Navarro et al., 2000).

The second main finding is that the amount of amino acid loss does not change with time during the RRT session. This finding was observed in all three RRT modalities. We have demonstrated that for each modality, the amount lost at mid-session is similar to that lost at the end of the RRT

session. Effluent concentration of amino acids appears to be constant throughout a single RRT session. We provided further evidence for this by sampling effluent at more frequent time points between mid and end session in a subset of patients.

6.5.2 Baseline Amino acid levels in AKI

This study demonstrated that patients treated with CVVH have significantly higher plasma amino acid levels compared with patients treated with IHD and SLEDf. There are several explanations for this. Firstly, patients receiving CVVH represented a sicker group of patients who had multi-organ failure. These patients have higher catabolic rate with increased protein breakdown and release of amino acids into the circulation. Secondly, these patients were treated with enteral or total parenteral nutrition which included amino acids, which would have raised plasma levels.

6.5.3 Changes in plasma levels of amino acids during RRT

In this study, plasma amino acid levels decreased during RRT in patients receiving CVVH and SLEDf but not in those receiving IHD. This finding does not explain the pattern of amino acid losses into the dialysate in this group. However, maintenance of plasma amino acids during RRT has previously been reported (Kihara et al., 1997) (Schmidt et al., 2014). These studies also showed substantial losses of amino acids in dialysate. A likely explanation for the stable plasma amino acid levels is production of amino acids from protein catabolism.

The effect of protein catabolism during haemodialysis has been observed in several early studies. Borah et al (Borah et al., 1978) showed that the nitrogen balance of patients receiving haemodialysis was strongly negative for dialysis days, which was independent of dietary protein intake. Urea generation rate was reported to be 30% greater during dialysis than between dialysis sessions.

Several mechanisms could potentially explain the higher catabolic rate seen during haemodialysis. Interaction between the dialyser and blood could lead to an enhanced catabolic effect. Bio-incompatible membranes are more likely to cause greater muscle proteolysis leading to higher levels of plasma amino acids. In contrast, use of biocompatible membranes such as

polysulfone and polyacrylnitrile appear to cause a much lower catabolic effect (Gutierrez et al., 1992). In our study polysulfone and polyacrylnitrile membranes were used so these may have had a lesser effect on maintenance of plasma amino acid levels.

Secondly, losses of glucose in dialysate could lead to increased demand for glucose, especially during critical illness. In healthy individuals, glucose is generated from dietary intake of carbohydrate and from hepatic glycogenolysis. During starvation in critical illness, amino acids are a source of glucose and are released via muscle catabolism for hepatic gluconeogenesis (Gutierrez et al., 1994).Therefore, preventing dialysate losses of glucose might reduce protein catabolism. However a study by Gutierrez (Gutierrez et al., 1994) has shown that adding glucose into the dialysate would not lead to a decrease in amino acids losses in the dialysate. Thus this suggests that other mechanisms contribute to maintenance of plasma amino acid levels.

A significant proportion of patients in this study had sepsis as a cause of AKI. The combined catabolic effect of sepsis and AKI provide a major stimulus for release of amino acids into the circulation, thus potentially compensating for RRT-related losses.

7. Final Discussion

This thesis has demonstrated firstly that micronutrients are lost during RRT for AKI and secondly that these losses vary between RRT modalities. The micronutrients of interest included amino acids, trace elements and water-soluble vitamins.

This study also confirms that the prevalence of malnutrition is high in AKI. Presence of malnutrition is evident in these patients before commencement of RRT. Use of malnutrition screening and assessment tools is therefore important to identify patients who are at risk of having or developing micronutrient deficiency.

This study showed that patients with stage 3 AKI have significantly lower plasma levels of zinc, selenium, copper and iron compared with healthy volunteers. These levels were measured prior to commencement of renal replacement therapy, providing further evidence that patients with severe AKI are malnourished. Zinc, copper, selenium and iron are lost in RRT effluent and these losses could aggravate the pre-existing micronutrient deficiencies. The extent of trace element loss is influenced by the modality of RRT. Protein-bound trace elements such as zinc and copper are less easily removed by RRT modalities reliant upon significant degree of diffusion (IHD and SLEDf) compared with purely convection-based therapies (CVVH). In this study, losses of micronutrients were measured from a single RRT session. Patients requiring multiple RRT sessions will presumably have a high risk of greater cumulative micronutrient losses. Moreover, in the IHD and SLEDf group, duration of the first RRT session is shorter than subsequent sessions, which presumably influences the extent of micronutrient losses.

It is also possible that trace elements could be lost in urine during an episode of AKI. However, in this study urinary losses of trace elements were not measured because most patients were anuric. In those patients were not anuric, measured dialysate losses may have underestimated the total loss. Although this study demonstrated loss of trace elements during RRT for AKI, it is important to acknowledge that we cannot draw any conclusions about the clinical consequences of these losses. To investigate clinical outcomes such as mortality, renal recovery or even clinical nutritional status would require a far bigger and more complex study.

This study demonstrated that B-vitamins were undetectable in RRT effluent during an episode of AKI. This was to some extent unexpected, as one hypothesis was that water- soluble vitamins would be removed during RRT. We were unable to measure plasma levels of B vitamins because of laboratory regulatory restrictions. There are three plausible reasons for absence of B vitamins

in RRT effluent. Firstly the low dietary intake in these patients could lead to deficiency. Second, the high metabolic demand and oxidative stress in these patients could further diminish their vitamin status such that losses to RRT effluent might be so low as to be undetectable. Third, these water-soluble vitamins could be adsorbed onto the RRT membrane and/or the extracorporeal circuit, leaving little to reach the effluent. A separate *in vitro* study performed by our research group demonstrated that adsorption of B vitamins to the RRT membrane occurs. This is a novel finding and is a possible cause of vitamin losses in patient treated with RRT. Future *in vivo* studies should include investigation of micronutrient losses by adsorption of these micronutrients during RRT for AKI. In addition, the measurement of plasma B vitamin levels before, during and after RRT must be measured in future studies.

Amino acids are lost in all 3 RRT modalities. Patients treated with CVVH lost more amino acids compared with patients treated with SLEDf and IHD. We concluded that purely convective therapies result in greater total amino acid losses than other RRT modalities. In addition, we have shown that adsorption of amino acids onto the RRT membrane can potentially cause significant losses. We have also provided some evidence that adsorption of amino acids to the RRT membrane could potentially cause significant amino acid losses. Again, the research group's separate *in vitro* study demonstrated the novel finding that amino acids are adsorbed to the RRT membrane. Future studies would include performing an in vivo study evaluating adsorption of amino acids in greater detail. Comparing the adsorption of essential amino acids versus non-essential amino acids in the RRT membrane would be of interest.

Although we have measured losses from only a single RRT session and did not measure urinary losses, we assume that patients who require further RRT sessions will lose proportionately more amino acids. When amino acids are analysed individually, a similar pattern emerges with highest losses in the CVVH group. We have shown large amounts of the BCAAs, arginine and glutamine are lost in CVVH in comparison to the other two RRT modalities. This might be significant clinically as the BCAAs and arginine are essential amino acids. In critical illness, glutamine becomes conditionally essential as demands exceed the endogenous supply. As described for trace elements, this study is not able to provide evidence that patients would benefit from amino acid supplementation. A prospective randomised controlled trial would be required to determine whether micronutrient supplementation confers clinical benefit.

To our knowledge, this is the largest study to date evaluating micronutrient losses during RRT for AKI. No published study has compared losses of micronutrients in 3 different RRT modalities.

However, this study does have several limitations. Firstly, micronutrient losses were quantified only in the first RRT session. The first RRT session for patients receiving IHD and SLEDf is shorter in duration than subsequent sessions. Many patients in the study required two or more RRT sessions, but the cumulative losses from all RRT sessions for each patient were not quantified. Second, plasma levels of B vitamins could not be measured in the study population due to the regulatory restrictions in the laboratory. Third, urinary losses of the micronutrients were not measured and thus measured losses in the plasma and effluent could be an underestimate of total losses.

The results of this study have implications for future research in this area of AKI and RRT. Published studies evaluating micronutrient losses in AKI have so far been small and underpowered. It is becoming increasingly clear that prevalence of malnutrition in AKI is high and has an adverse impact on mortality in this patient population. This study has provided data to justify further larger prospective clinical studies to investigate:

1. Whether RRT-associated micronutrient losses lead to worse clinical outcomes.
2. Whether specific micronutrient supplements would be of clinical benefit to patients with AKI requiring RRT.
3. Whether specific micronutrient supplements should be different for different RRT modalities.

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9. Appendices

9.1 Case Report Form (CRF) 1: Dietetic assessment form

Version 1

Date: 10.10.2012

MUST score

SGA score (7 scale)

SGA (ABC)

NRS score

Study title: Micronutrient loss in acute renal replacement therapy

Principal Investigator: Dr Mark Devonald

Weight 3-6 months before admission ___ kg

History of weight loss: Yes / No

Current weight (specify Estimated or accurate) ___ kg

Dry (oedema-free) weight ____ kg

Height (specify estimated or accurate) ___ m

BMI ___ kg/m2

Presence of : (tick as many)

- Nausea ☐
- Vomiting ☐
- Diarrhoea ☐
- Constipation ☐
- None ☐

Is the patient at risk of refeeding syndrome?

- Yes ☐

- No ☐

Is the patient on propofol?

- Yes (specify total volume in 24 hours) ☐ _____
- No ☐

Is the patient on dextrose? ☐

- Yes (specify total volume in 24 hours) _____
- No ☐

Is the patient on glutamine ☐

- Yes (specify daily dose) _____
- No ☐

Is the patient on any water soluble vitamin preparation (i.e. thiamine, pabrinex, others)?

- Yes (specify dose and name) ☐ _____
- No ☐

Is the AKI either? ☐

- Catabolic (sepsis, MOF,Multi-system disease, Rhabdomyolysis)
- Non catabolic (single organ failure, hypovolaemia, obstruction) ☐

Is there any evidence of muscle depletion (subjective)

- None ☐
- Moderate ☐
- Severe ☐

Nutritional requirements Energy

- (Henry equation 2005) _____
- 25-35kcal body weight (RA 2011) _____

Nutritional requirements Protein

- PENG equation _____

How the patient is currently fed? Tick as many as appropriate

- Orally ☐
- Gastric (NG, PEG) ☐
- Jejunal (NJ, Jejunostomyes) ☐
- Parenteral ☐

Estimated provision of nutrients:

- Per oral: request recall 24 hours _____
- Enteral : record enteral feeding regime (type of feed and rate) _____
- Parenteral: record parenteral regime (regime and rate) _____

Was the patient with AKI receiving RRT reviewed by the dietitian within 24 hours?

- Yes ☐
- No ☐

9.2 Case Report Form 2

Version 1

Date: 10.10.2012

Study title: Micronutrient loss in acute renal replacement therapy

Principal Investigator: Dr Mark Devonald

Research Site: Nottingham University Hospitals NHS Trust

Patient Initials:

Patient hospital no: Date of Birth:

Patient study number: A___(HD) B___(SLEDf) C___(CVVH)

I am confident that the information supplied in this case record form is complete and accurate. I confirm that the study was conducted in accordance with the protocol and that written informed consent was obtained prior to the study.

Investigator's signature……..

Date of signature…….

Inclusion criteria

1. Does the participant have AKI requiring RRT? Yes/No
2. Is the participant age of 17 years or older? Yes/No
3. Has the participant willingly given written informed consent? Yes/No

If any of the inclusion criteria is circled no then the patient is not eligible for the study.

Exclusion criteria

1. Does the participant have end stage renal disease? Yes/No
2. Does the participant have a kidney transplant? Yes/No
3. Does the participant have Stage 5 Chronic Kidney Disease? Yes/No
4. Does the patient have a haemoglobin level of <8g/dL and is not receiving a blood transfusion?

If any of the exclusion criteria is circled yes then the patient is not eligible for the study.

Demographic data

Age:

Sex:

Ethnicity:

Date of admission to:

1. Renal unit:

2. Intensive Care Unit:

Site of vascular access

Left/right: _____

Femoral/Jugular/ Subclavian: _____ vascath

AV fistula/graft: _____

RRT Session 1

Please circle which treatment was used:

Treatment 1A / 1B / 1C/ 2A / 2B / 3A Date session commenced: _____

Prescribed duration of RRT:___ hrs Actual duration of RRT: ___hrs

Blood flow rate:_____ mls/min Dialysate flow rate: _____ mls/min

Prescribed dose of CVVH: ____ mls/kg/hr

Prescribed net fluid loss ___ L Actual net fluid loss: ___ L

Anticoagulation:

Pre RRT weight:___ kg

Type of dialysis fluid(please circle): Type of replacement fluid:

A7 / A10 / A3 / A27

24 hour urine volume: ____mls

Session 2

Please circle which treatment was used:

Treatment 1A / 1B / 1C/ 2A / 2B / 3A Date session commenced: _____

Prescribed duration of RRT: ____ hrs Actual duration of RRT: ___ hrs

Blood flow rate:___ mls/min Dialysate flow rate: ____ mls/min

Prescribed Dose of CVVH:____ mls/kg/hr

Prescribed net fluid loss: ___ L Actual net fluid loss: ____ L

Anticoagulation:

Type of dialysis fluid (please circle): Type of replacement fluid:

A7 / A10 / A3 / A27

24 hour urine volume: ____mls

Dietetic assessment (within 24 hrs)

Yes / No (please circle)

Cause of AKI

Diagnosis:

Sepsis / Non-sepsis (please circle)

Pre-existing chronic kidney disease (eGFR < 60 mL/min 7-365 days pre-admission)

Yes / No (please circle)

Baseline GFR (average eGFR 7-365 days pre-admission)_____ mls/min

Lowest GFR in previous 7-365 days _____mls/min

CKD stage 3a/ 3b / 4 / 5 (please circle)

Baseline proteinuria (PCR/ACR) _____ mg/mmol

Co-morbidities (please tick)

1. Diabetes Mellitus ☐
2. Hypertension ☐
3. Cardiovascular disease (IHD, cardiac failure, valvular disease) ☐
4. Cerebrovascular disease ☐
5. Peripheral vascular disease ☐
6. Malignancy ☐

9.3 Case Report Form 3

Version 2

Date: 21.08.2013

Study title: Micronutrient loss in acute renal replacement therapy

Principal Investigator: Dr Mark Devonald

Treatment 1: Haemodialysis

Treatment 1A

Prescribed duration of treatment: 2 hours

Actual duration of treatment:

Reasons for incomplete treatment:

Please follow guidelines for sampling in treatment 1A

Total volume of dialysate collected:

Total duration:

Time (mins)	Actual time	Blood (mL)	Urine (mL)
0		3	10
+ 30		3	
+ 60		3	10
+ 90		3	
+ 120		3	10
60 post dialysis		3	

Time (mins)	Actual time	Dialysate (mL)
0		10
+ 15		10
+ 30		10

+ 45		10
+ 60		10
+ 75		10
+ 90		10
+ 105		10
+ 120		10

Treatment 1B

Prescribed duration of treatment: 3 hours

Actual duration of treatment:

Reasons for incomplete treatment:

Please follow guidelines for sampling in treatment 1B

Total volume of dialysate collected:

Total duration:

Time (mins)	Actual time	Blood (mL)	Urine (mL)
0		3	10
+ 30		3	
+ 60		3	
+ 90		3	10
+ 120		3	
+ 150		3	
+ 180		3	10

60 post dialysis		3	

Time (mins)	Actual Time	Dialysate (mL)
0		10
+ 15		10
+ 30		10
+ 45		10
+ 60		10
+ 75		10
+ 90		10
+ 105		10
+ 120		10
+ 135		10
+ 150		10
+ 165		10
+ 180		10

Treatment 2: SLED-f

Treatment 2A

Prescribed duration of treatment: 6 hours

Actual duration of treatment:

Reasons for incomplete treatment:

Please follow guidelines for sampling in treatment 2A

Total volume of dialysate collected:

Total duration:

Time (mins)	Actual time	Blood (mL)	Urine (mL)
0		3	10
+ 90		3	
+ 180		3	10
+ 270		3	
+ 360		3	10
60 post SLED-f		3	

Time (mins)	Actual time	Effluent (mL)
0		10
+ 30		10
+ 60		10
+ 90		10
+ 120		10
+ 150		10
+ 180		10
+ 210		10
+ 240		10

+ 270		10
+ 300		10
+ 330		10
+ 360		10

Treatment 2B

Duration of treatment: 8 hours

Actual duration of treatment:

Reasons for incomplete treatment:

Please follow guidelines for sampling in treatment 2B

Total volume of dialysate collected:

Total duration:

Time (mins)	Actual time	Blood (mL)	Urine (mL)
0		3	10
+ 120		3	
+ 240		3	10
+ 360		3	
+ 480		3	10
60 post SLED-f		3	

Time (mins)	Actual time	Effluent (mL)
0		10
+ 30		10
+ 60		10
+ 90		10
+ 120		10
+ 150		10
+ 180		10
+ 210		10
+ 240		10

+ 270		10
+ 300		10
+ 330		10
+ 360		10
+ 390		10
+ 420		10
+ 450		10
+ 480		10

Treatment 3A: CVVH

Prescribed duration of a single CVVH treatment: 24 hours

Actual duration of a single CVVH treatment:

Reason for incomplete treatment: clotted filter/ needing investigations outside unit/others

If 'others' chosen, please state the reasons:

Total volume of filtrate collected:

Total duration:

Time (hr)	Actual time	Blood (Green top tube)	Urine (mL)
0		4mL P0	10
+ 3		4mL P1	
+ 6		4mL P2	
+ 12		4mL P3	10
+ 24		4mL P4	10
1 hr after 2nd CVVH session (or 1 hour after filter circuit clots)		4mL P post	

Obtain 10mL effluent from every discarded effluent bag during CVVH

Time effluent bag discarded	Effluent (please take 10ml per bag and place into sterile top container)- please tick

	E1
	E2
	E3
	E4
	E5
	E6
	E7
	E8

9.4 Participant Consent Form

Version: 5.0 Date: 20/08/2013

Micronutrient loss in acute renal replacement therapy

Principal Investigator: Dr Mark Devonald

Patient Study ID: …….. Initials: ……

- Patient initial each box

1. I confirm that I have read and understand the information sheet dated **20/08/2013 (version 6.0)** for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. ☐

3. I understand that my medical records may be looked at by authorised individuals from the Sponsor for the study, the UK Regulatory Authority or the Independent Ethics Committee in order to check that the study is being carried out correctly. ☐

4. I agree that the following samples can be taken and the information gathered about me can be used for this research study, as described in the information sheet. ☐

a. Plasma and Urine

b. Dialysis fluid

5. I understand that even if I withdraw from the above study, the data and samples collected from me will be used in analysing the results of the trial, unless I specifically withdraw consent for this. ☐

6. I consent to the storage including electronic, of personal information for the purposes of this study. I understand that any information that could identify me will be kept strictly confidential and that no personal information will be included in the study report or other publication. ☐

7. I consent to the storage of my samples at the end of this study for future use in other research studies. ☐

8. I agree that my GP, or any other doctor treating me, will be notified of my participation in this study. ☐

9. I agree to take part in the study. ☐

_____ _____

_____

Name of the patient *(Print)* date Patient's signature

_____ _____

_____

Name of person taking consent *(Print)* date Signature

Original to be retained and filed in the site file. 1 copy to patient, 1 copy to be filed in patient's notes.

9.5 Consultee Declaration Form

Version: 4.0 Date: 20/08/2013

Micronutrient loss in acute renal replacement therapy

Principal Investigator: Dr Mark Devonald

Patient name: …….. Date of Birth ……

Patient Study ID: …….. Initials: ……

- Patient initial each box

1. I confirm that I have been consulted about my relative/friend's participation in this research study. I have read and understood the information sheet dated **20/08/2013 (version 6.0)** for this study and have had the opportunity to ask questions. ☐

2. In my opinion my relative/friend would have no objection in taking part in this study. ☐

3. I understand that I can request that my relative/friend be withdrawn from the study at any time, without giving any reason, without their medical care or legal rights being affected. ☐

4. I understand that the following samples will be taken from my relative/friend and the information gathered about them can be used for this research study, as described in the information sheet. a. Blood and Urine ☐

b. Dialysis fluid.

5. I understand that my relative/friend's medical records may be looked at by authorised individuals from the Sponsor for the study, the UK Regulatory Authority or the Independent Ethics Committee in order to check that the study is being carried out correctly ☐

6. I understand that even if my relative/friend is withdrawn from the above study, the data and samples collected from my relative/friend will be used in analysing the results of the trial. ☐

7. I understand that personal information will be stored (including electronic storage) only for the purposes of this study. ☐

8. I understand that any information that could identify my relative/friend will be kept strictly confidential and that no personal information will be included in the study report or other publication ☐

9. I understand that samples collected from my relative/friend at the end of the study will be stored for future use in other research studies ☐

_____ _____ _____

Name of the consultee *(Print)* Date Signature of consultee

_____

Relationship to patient

Are you the patient's next of kin? Yes / No

_____ _____

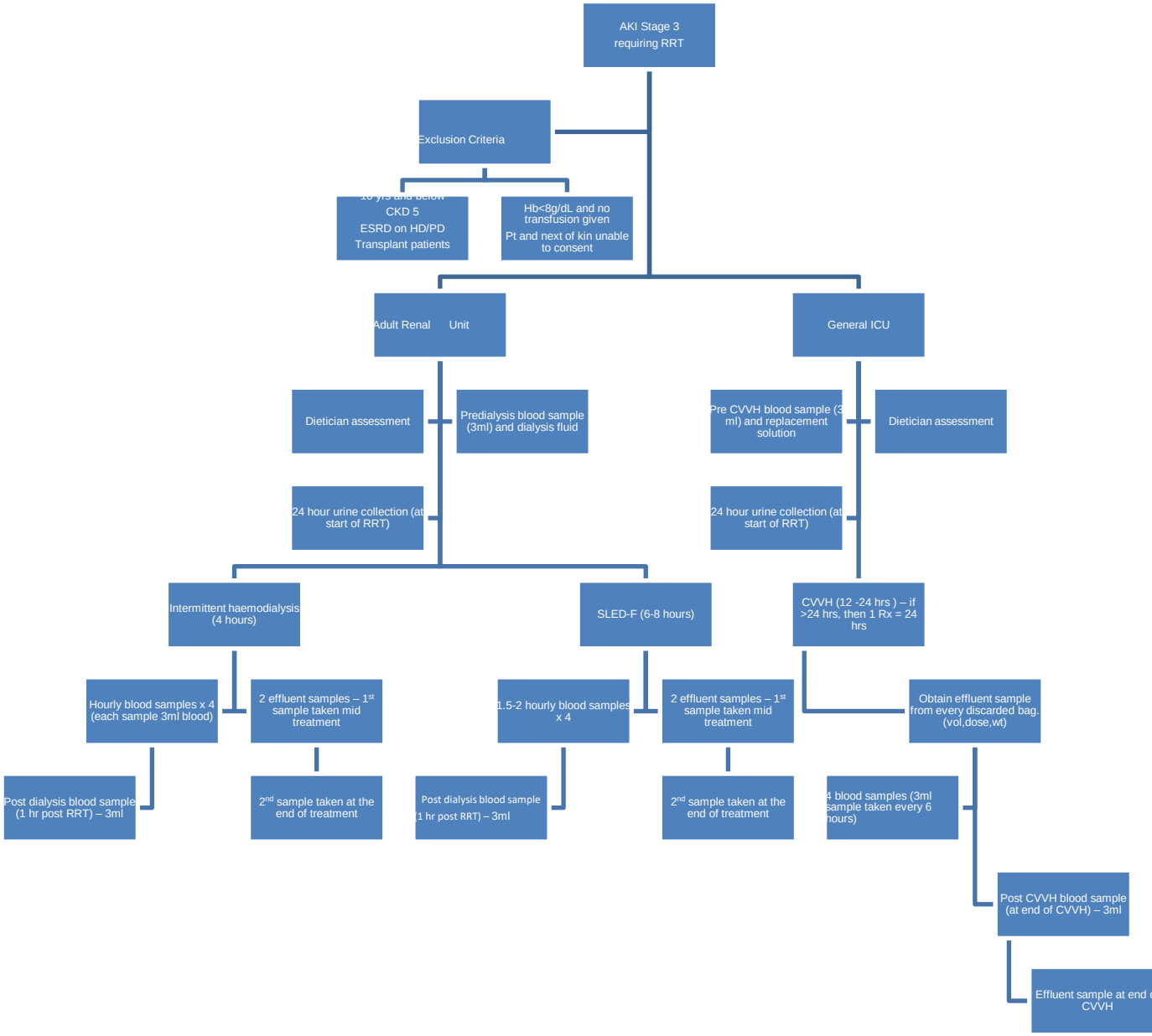
_____

Name of person obtaining opinion*(Print)* date Signature

Original to be retained and filed in the site file. 1 copy to patient, 1 copy to be filed in patient's notes.

Nottingham University Hospitals NHS Trust

Figure 166: Study protocol flowchart



9.6 Dialyser characteristics

Characteristics	FX 60
Qb (mL/min)	200/300/400
Urea clearance (mL/min)	193/261/303
Creatinine clearance (mL/min)	182/230/262
Phosphate clearance (mL/min)	177/220/248
Vitamin B12 clearance (mL/min)	135/155/167
Inulin clearance (mL/min)	95/104/109
K0A urea mL/min	967
UF – coefficient (at Qb max) mL/h/mmHg	46
S (sieving coefficient) Inulin	1
S (sieving coefficient) B2 -M	0.8
S (sieving coefficient) Albumin	0.001
TMP max mmHg	600
Max dialysate flow mL/min	1000
V (blood priming volume) mL	74
Δ P (pressure drop blood, Qb = 300ml/min)	105
Δ P (pressure drop dialysate, Qd = 500ml/min)	40
A (effective surface area) m^2	1.4
Membrane	Helixone
Sterilisation method	INLINE steam

9.10 CVVH membrane characteristics

Characteristics	Prismaflex M60/M100/150
Weight (g)	780/800/860
Overall dimensions (cm)	27x22x9/27x22x9/27x22x9
Blood volume in set (ml)	93/152/189
Minimal patient weight (kg)	11/30/30
Range of blood flow rate (ml/min)	50-180/75-400/100-450
Maximum transmembrane pressure (TMP) (mmHg/kPa)	450/60
Maximum blood pressure (mmHg/kPa)	500/66.6
Effective surface area (m^2)	0.6/0.9/1.5
Fibre internal diameter (wet)	240µm
Fibre wall thickness	50µm
Blood priming volume (ml)	42/66/105
S (sieving coefficient) urea	1
S (sieving coefficient) creatinine	1
S (sieving coefficient) vitamin B12	1
S (sieving coefficient) inulin	0.95
S (sieving coefficient) myoglobin	0.55
S (sieving coefficient) albumin	<0.01
AN69 HF hollow fibre	Acrylonitrile and sodium methallyl sulfonate coploymer
Filter housing and headers	Polycarbonate
Filter potting compound	Polyurethrane
Tubing material	Plasticized polyvinyl chloride (PVC)
Cartridge	PETG
Sterilization mode	EtO (Ethylene Oxide)