Contribution of fluids and electrolyte management to lung injury

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Summary

Fluid administration and electrolyte management is perhaps the most common intervention in hospital. Acute lung injury is common in critically ill patients and is associated with increased morbidity and mortality. The current literature suggests that administration of fluids is not straightforward and there are concerns of potential harm with fluid administration. Similarly electrolyte abnormalities are known to have adverse effects in critically ill patients. This work focuses on the effects of fluid and electrolyte management on lung injury.

Effects of sodium, fluid boluses and osmolality on lung injury were separately investigated. I utilised animal models, healthy human volunteers, clinical and epidemiological studies to investigate the effects of fluid and electrolyte management on lung injury

Sodium: Current levels of sodium administration are more than three times the NHMRC recommendations in both adult and paediatric patients, most of which is derived from inadvertent sources such as drug infusions, drug boluses and flushes. Such high levels of administration lead to positive sodium balance which

in turn causes an expansion of the extracellular fluid compartment and is associated with respiratory dysfunction as evidenced by decreased oxygen levels and prolong length of invasive mechanical ventilation.

Fluid boluses: Utilising both basic sciences and clinical studies I found that bolus administration of intravenous fluids had minimal physiological benefit in the circumstances investigated and tended to be harmful. In patients with severe sepsis there was a decrease in oxygen levels after their administration, in healthy subjects after administration of 0.9% saline there was evidence of interstitial oedema, and in animal studies bolus i.v. fluids resulted in permeability pulmonary oedema despite a “safe” (non-hydrostatic) left heart pressure. Such lung injury after administration of fluid boluses is possibly through activation of endothelial calcium ion channels (transient receptor potential vanilloid 4 (TRPV4) channels. This fluid induced lung injury was prevented by administration of a relatively specific TRPV4 blocker (ruthenium red).

Hyperosmolality: Using animal studies I found that induced hypernatremia was lung protective in acute lung injury, independent of fluid or sodium load. Based on my animal work, I hypothesised that lung-protective effects of hypernatremia would reduce its general adverse effects, leading to amelioration of the increase in mortality risk in patients with lung injury. To examine

this we utilised a large administrative database (from the Australia New Zealand Intensive Care Society Centre for Outcome and Resource Evaluation (ANZICS CORE)) and found that high admission serum sodium was associated with an increased odds for ICU death, except in respiratory patients.

In critically ill patients (i) inadvertent sodium administration is common which leads to a large positive sodium balance which is associated with adverse respiratory effects (ii) bolus administration of fluid can induce lung injury, and (iii) induced hyperosmolarity may be lung protective.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Shailesh Bihari

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ABBREVIATIONS AND SYMBOLS

ACCP American College of Chest Physicians

AIDS Acquired immunodeficiency syndrome

AKI Acute kidney injury

ALI Acute lung injury

ANG2 Angiopoietin 2

ANOVA Analysis of variance

ANP Atrial naturitic peptide

ANZICS–APD Australian and New Zealand Intensive Care Society Adult Patient Database

ANZPICR Australian and New Zealand Paediatric Intensive Care Registry;

APACHE Acute physiology and chronic health evaluation

ARDS Acute respiratory distress syndrome

ATS American thoracic society

BAL Broncho alveolar Lavage

BiPAP Bi-phasic positive airway pressure

BIVA Bioelectrical impedance vector analysis

BMI Body mass index

BP Blood pressure

Ca^{2+} Calcium

CCI Charlson co-morbidity index age adjusted score.

CHF Chronic heart failure

CI Confidence interval

COPD Chronic obstructive pulmonary disease

CRRT Continuous renal replacement technique

CVP Central venous pressure

CVVH Continuous veno-venous haemofiltration

CVVHDF Continuous veno-venous haemodiafiltration

DLCO Diffusing capacity of the lung for carbon monoxide

dX/dV Delta reactance/delta lung volume

ECF Extracellular fluid

Echo Echocardiography

EDD Extended daily dialysis

EELV End expiratory lung volume

EET Epoxyeicosatrienoic acid

ERS European respiratory society

F female

FB Fluid boluses

FEV_1 Forced expiratory volume in one second

FFP Fresh frozen plasma

FILI Fluid induced lung injury

FiO2 Fraction of inspired oxygen

FRC Functional residual capacity

FVC Forced vital capacity

GCS Glasgow coma scale

GFR Glomerular filtration rate

HDU High dependency unit

HR Heart rate

HTS Hypertonic saline

i.v. Intravenous

IC Inspiratory capacity

ICF Intra-cellular fluid

ICU Intensive care unit

ID Subject identification number.

IFNγ Interferon gamma

IOS Impulse Oscillometry system

IQR Inter-quartile range

ITGV Intra thoracic gas volume

LIS Lung Injury score

LOS Length of stay

LPS Lipopolysaccharide

LVOT Left ventricular outflow tract

M Male

MAP Mean arterial pressure

MPO Myeloperoxidase

MV Mechanical ventilation.

NA Not applicable

NHMRC National Health and Medical Research Council

Na^+ Sodium

NIBP non-invasive blood pressure

OR Odd's ratio

OT Operation theatre

PaO_2 Partial pressure of arterial oxygen

PEEP Positive end expiratory pressure

PEFR Peak expiratory flow rate

PFT Pulmonary function test

PGI_2 Prostacyclin

PICU Paediatric intensive care unit

PIM Paediatric Index of Mortality;

PLA_2 Phospholipase A_2

PLR Passive leg raising

PRC Packed red blood cells;

RAAS Renin angiotensin aldosterone system

ROC Receiver operating characteristic.

RR Respiratory rate

RRT renal replacement technique

RV Residual volume

SAPS Simplified acute physiology score

SBL supine body length.

SCCM Society of Critical Care Medicine Consensus Conference

SD standard deviation.

SD Standard deviation

SEM Standard error of the mean

SOFA Sequential organ failure assessment score.

TBW Total body water

TLC Total lung capacity

TNF-α Tumour necrosis factor alpha

TPN Total parental nutrition

TRP Transient receptor potential

TRPV Transient receptor potential vanilloid

U Unit

USG Ultrasonography

VWF von Willebrand factor

V_T Tidal volume

VTI Velocity time integral

WPBs Weibel–Palade bodies

SYMBOLS

↑	Increased/elevated
↓	Decreased/reduced
<	Less than
≤	Less than or equal to
>	Greater than
≥	Greater than or equal to
#	Number
ρ	rho
°	Degrees
Ω	Ohm
~	Approximately/no substantial change

PREFACE

This preface is to certify that several chapters within this thesis contain content that is substantially unchanged from the content of multi-author papers which have either been published or are being prepared for publication, (**Appendix 5**) which may lead to some repetition of ideas. The following statements outline the contribution of all authors to the content of manuscripts that have been included in this thesis.

Chapter 1 Introduction

Book Chapter : Bihari S and Bersten AD. Sodium loading in critical care - textbook "Diet and Nutrition in Critical Care" under "Specific nutrients" published by Springer 2013

Bihari S: Proposed the design of the literature review, determined the search terms and completed the search strategy, data extraction, data synthesis and analysis and interpretation, before preparation the initial draft and revisions of the manuscript

Bersten AD: Contributed to the literature review design, interpretation of results and revisions of the manuscript

Chapter 3 Sodium administration in ICU patients

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Peake SL: Contributed to the study design and revisions of the manuscript

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Taylor S: Contributed to the study design

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Chapter 4 Sodium balance in ICU patients

Bihari S, Baldwin C and Bersten AD. Fluid balance does not predict estimated sodium balance in critically ill mechanically ventilated patients. Crit Care Resusc 2013; 15: 89-96

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Bersten AD: Contributed to the study design, interpretation of results and revisions of the manuscript

Bihari S, Peake S, Prakash S, Saxena M, Campbell V, Bersten AD. Sodium balance, not fluid balance, is associated with respiratory dysfunction in mechanically ventilated patients: a prospective, multi-centre study. Manuscript accepted in Crit Care Resusc 2015

Bihari S: Proposed the study design, analysis and interpretation, before preparation of the initial draft and revisions of the manuscript .

Peake SL: Contributed to conduct of study at her study centre – The Queen Elizabeth Hospital (South Australia) and revisions of the manuscript

Prakash S: Contributed to the data analysis

Saxena M: Contributed to conduct of study at his study centre – St. George Public Hospital, Sydney NSW

Campbell V: Contributed to conduct of study at his study centre – Nambour Hospital (QLD)

Bersten AD: Contributed to the study design, interpretation of results and revisions of the manuscript

Chapter 5 Fluid bolus

Bihari S, Prakash S, Bersten AD. Post resuscitation fluid boluses in patients with severe sepsis and septic shock (PRICE study). Shock 2013; 40: 28-34

Bihari S, Prakash S, Bersten AD. Early changes in serum electrolytes and acid-base status with administration of 4% albumin. Intensive Care Med. 2014; 40:1392-3.

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Wiersema U: Contributed to conduct of study with echocardiographic measurements
Schembri D: Contributed to conduct of study with respiratory measurements
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Lawrence M: Contributed to the study with PCR and ELISA measurements (results not included in the PhD)
Bersten AD: Contributed to the study design, interpretation of results and revisions of the manuscript

Chapter 6 Effect of serum sodium and osmolality on lung injury

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Pilcher D: Contributed to the study design

Prakash S: Contributed to the data analysis

Bersten AD: Contributed to the study design, interpretation of results and revisions of the manuscript

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Dixon D: Contributed to the animal study design, data interpretation and manuscript preparation

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CHAPTER 1: INTRODUCTION

Fluid administration and electrolyte management is a common intervention in hospital and disturbances in fluid and electrolytes are among the most common clinical problems encountered in the intensive care unit (ICU) (Bellomo 2014; Lee 2010; Sedlacek *et al.* 2006).

Acute lung injury is common in critically ill patients and is associated with increased morbidity and mortality. The current literature suggests that administration of fluids is not innocuous and there are concerns of potential harm with fluid administration (Bellomo 2014). Similarly electrolyte abnormalities are known to have adverse effects in critically ill patients (Rosner *et al.* 2010; Sedlacek *et al.* 2006).

1.1 Intravenous fluid administration, and fluid boluses, are common practice

Administration of intravenous (i.v.) fluid for the purpose of either fluid resuscitation or for maintenance fluid requirements is ubiquitous in pre-hospital and hospital practice, particularly in critically ill patients. 0.9% saline, the most commonly used i.v. crystalloid, has annual sales of 10 million and 200 million litres in the UK and USA, respectively (data from Baxter Healthcare) (Award *et al.* 2008). During fluid resuscitation, fluid boluses, the rapid administration of a set volume of i.v. fluid, are usually administered. The speed, amount, timing, and physiological targets for such fluid delivery are determined by clinicians on the basis of physiological

reasoning, observational evidence, personal preference, local culture, mentorship, marketing forces, heuristic bias, guidelines, and expert opinion (Bellomo 2014).

Uncertainty regarding timely administration of an appropriate volume and type of i.v. fluid underpins many significant studies of fluid therapy over the last 10 years (Finfer *et al.* 2004; Myburgh *et al.* 2012; Perner *et al.* 2012; Delaney *et al.* 2013), many supported by the National Health and Medical Research Council (NHMRC), where large volumes of fluid were administered. For example, in patients receiving early goal direct therapy 3499±2438 ml of fluid was administered, while patients in the control arm received 4981±2984 ml of fluids in the first 24 hours (Rivers *et al.* 2001), and in the 6S study (Perner *et al.* 2012), based on a 72 kg person, patients on day 1 in the hydroxyethyl starch group received a median 5825 ml (trial plus other fluid).

1.2 Acute Lung injury

Acute lung injury and its more severe form acute respiratory distress syndrome (ARDS) has a substantial impact on public health (Rubenfeld *et al.* 2005). The incidence of ARDS varies considerably across the world (Rubenfeld *et al.* 2005; Luhr *et al.* 1999; Bersten *et al.* 2002), in part relating to availability of intensive care services (Linde-Zwirble *et al.* 2004). Recent data suggest an incidence of ARDS, ranging from 15.3–58.7 cases per 100,000 person-years (Rubenfeld *et al.* 2005; Arroliga *et al.* 2002). Attributable mortality also varies with reported ranges of 41–58% (Rubenfeld *et al.* 2005;

The ANZIC Influenza Investigators 2009; Zilberberg *et al.* 1998). The problem of ARDS has been highlighted by the recent H1N1 influenza pandemic where intensive care units (ICU) treated an unprecedented number of cases of ARDS (The ANZIC Influenza Investigators 2009). Since the original description of ARDS (Ashbaugh *et al.* 1967), substantial progress has been made in understanding the natural history and pathogenesis of this lethal syndrome but it still remains a major cause of admission, morbidity and mortality in ICU patients. At Flinders Medical Centre ICU more than sixty percent of patients require mechanical ventilation during their stay in the ICU and lung protective mechanical ventilation (The Acute Respiratory Distress Syndrome Network 2000) remains the only current treatment strategy for ARDS.

1.3 Is intravenous fluid administration safe?

A general adverse effect associated with i.v. fluids is positive fluid balance which has been associated with poorer kidney function (Bouchard *et al.* 2009), delayed return of gastrointestinal function after surgery (Lobo *et al.* 2002), diminished lung function and longer duration of mechanical ventilation (Wiedemann *et al.* 2006). In the CHEST study, which compared fluid boluses with either hydroxyethyl starch or 0.9% saline, 25.6% of patients developed new onset respiratory failure (Myburgh *et al.* 2012). Positive fluid balance, a common effect of fluid administration has also been associated with increased mortality in both patients with lung injury

(Sakr *et al.* 2005) and sepsis (Simmons *et al.* 1987; Murphy *et al.* 2009).

The FEAST randomized trial (Maitland *et al.* 2011) compared no fluid resuscitation with 0.9% saline (crystalloid) or 5% albumin (colloid) resuscitation in African children (n=3170) with severe infection. Unexpectedly the control group, no fluid boluses, had the least mortality at both 48 hours and at four weeks when compared with groups receiving fluid boluses of either 0.9% saline or 5% albumin. While there may be have been specific issues related to the study population and associated management, this well conducted trial challenges current concepts regarding fluid resuscitation for patients with severe infection. However, possible mechanisms for the increase in mortality remain speculative.

1. 4 Electrolyte abnormality in ICU

Electrolyte abnormalities are known to have adverse effects in critically ill patients (Rosner *et al.* 2010; Sedlacek *et al.* 2006). Hypernatremia, which generally reflects inadequate total body water and can be exacerbated by sodium overloading, contributes to high serum osmolality and is associated with increased mortality risk in critically ill patients, independent of age and severity of disease (Linder *et al.* 2007; Hoorn *et al.* 2008).

1. 5 Fluids and their component- electrolytes and resultant osmolality

Commonly administered fluids have different constituents in terms of their electrolytes and the resultant osmolality. For example 0.9%

saline contains sodium 150 mmol/l, chloride 150 mmol/l with an osmolality of 300 mOsm/l (Baxter Sodium Chloride 0.9%) where as 4% albumin contains sodium 140 mmo/l, chloride 128 mmol/l and albumin 40 g/l with an resultant osmolality 270 mOsm/l (CSL Biotherapies).

1.6 Why is sodium important - physiologic considerations

Sodium (Na^+) is a major extracellular ion and can affect the distribution of water in intra- and extracellular spaces, and their balance determines the serum osmolality. Multiple studies have shown that fluid (water and sodium) overload may contribute to poor outcomes. Sodium administration in ICU can lead to Na^+ repletion (in the presence of ongoing losses) or loading.

Total body sodium is about 60 mmol/kg; about 4000 to 4200 mmol in an adult male. Sodium distributes into the extracellular fluid (50%), bone (45%) and intracellular fluid (5%). Exchangeable Na^+ is 70% of the total Na^+ (measured using sodium^{24}). The non-exchangeable Na^+ is mostly in bone crystal.

Intracellular sodium is low - about 12 mmol/l for muscle cells and 20 mmol/l for red blood cells. Intracellular levels are kept low by both Na^+-K^+ ATPase pumps which exchange intracellular sodium for extracellular potassium and the low sodium permeability of the membrane. Interstitial and intravascular compartments have

similar, sodium levels, close to 140 mmol/l. There are two factors responsible for this phenomenon

1) Gibbs-Donnan effect- this causes the sodium concentration [Na^+] in plasma to be higher than [Na^+] in interstitial fluid by about 6 or 7 mmol/l due to the presence of plasma proteins leading to non-diffusible Na^+ in plasma which results in an increase in [Na^+] by 6 or 7 mmol/l.
2) Plasma solid effects: plasma consists of plasma water (93%) and plasma solids (7%). Plasma solids are mostly plasma proteins. Although Na^+ is present only in the plasma water component, it is measured as though it was present in whole plasma. This is a problem in the common laboratory methods for measuring [Na^+] such as flame emission spectrophotometry, and the indirect ion-selective electrode.

The decrease in measured plasma [Na^+] due to the plasma solids effect is about the same magnitude as the increase in [Na^+] in plasma water due to the Gibbs-Donnan effect. The result is that the measured [Na^+] is about same as the [Na^+] in interstitial fluid.

It is noteworthy that not only does the Gibbs- Donnan equilibrium result in an increase in the [Na^+] in the plasma; it also contributes to the actual oncotic pressure which is higher than predicted by the Van't Hoff equation for the actual protein concentration (0.9 mOsm/l) that is present. Hence, the Gibbs- Donnan equilibrium contributes to the fluid equilibrium dynamics between the

intravascular and interstitial spaces, although it is measured equally in both spaces.

1.7 Control of sodium and its applications to intensive care

The extracellular volume is primarily regulated through control of Na^+ balance, which is, in turn, regulated through control of effective plasma volume and its composition. Although the standard Western diet contains approximately 150 mmol of Na^+ per 24 hours, this varies widely and urinary Na^+ excretion varies between 0.2 and 242 mmol per 24 hours, (Intersalt, 1988) reflecting a balance between Na^+ input and output.

1.7.1 RENAL HANDLING OF SODIUM

99.4% of the filtered Na^+ is reabsorbed by the kidney. Sodium crosses the luminal membrane to enter the proximal tubule. About 65% of the filtered load of Na^+ is reabsorbed in the proximal tubule. This is about 16,400 mmol/day (0.65 x 140mmol/l x 180 l/day) and occurs via:

- diffusion through sodium channels
- carrier mediated facilitated diffusion through the luminal membrane
- co-transport (with glucose and amino acids)
- counter transport (against H^+ secretion)

The rest of the Na^+ is reabsorbed through the $Na^+K^+Cl^-$ ATPase pump (a metabolically active pump) in the thick ascending limb of the loop of Henle and the Na^+Cl^- symporter in the distal convoluted

tubules in the kidneys (site of action of loop and thiazide diuretics respectively).

The daily solute loss in a healthy kidney is about 700 mOsmol/day. As the maximum urinary osmolality under extreme condition is 1400 mOsmol/kg, to excrete the solute load, the obligatory or minimum water loss as urine is about 500 ml/day (i.e. 1400/700). Solutes that contribute to the majority of the daily solute load are : Na^+ : 100-150 mmol/day, K^+ : 70-100 mmol/day, Cl^- : 150 mmol/day, urea : 400 mmol/day and creatinine : 12 mmol/day

If the daily intake of Na^+ of a healthy individual is decreased to low amounts (say 10 mmol/day) then the kidney, over a few days is able to decrease the Na^+ excretion to comparable low levels which forms the rationale of decreasing the Na^+ load in patients with chronic kidney disease.

Hormones that act on the kidney to regulate salt and water together with their site of action are shown in Table 1.1. Other source of water and Na^+ loss from the body are summarized in Table 1.2.

Table 1.1: Hormonal control of sodium and water in the kidney

Hormones	**Site of action**	**Effect**
Aldosterone	Collecting duct	Increase sodium reabsorption Increase water reabsorption
Angiotensin II	Proximal tubule, thick ascending loop of Henle/ distal tubule, collecting tubule	Increase sodium reabsorption Increase water reabsorption
Antidiuretic hormone	Distal tubule, collecting tubule	Increase water reabsorption
Atrial natriuretic peptide	Distal tubule, collecting tubule	Increase sodium reabsorption

Table 1.2: 24 hour losses of sodium and water from extra renal sites

Site	**Volume (ml)**	**Na^+ (mmol/l) losses**
Salivary	500 to 1000	50
Gastric	1500	60 to 100
Pancreatic	400 to 1000	140
Bile	400 to 1000	140
Small Intestine	1000 to 3000	140
Large Intestine		60

1.7.2 CRITICALLY ILL PATIENTS HANDLING OF SODIUM

Critically ill patients are different from the healthy population in multiple ways and are at risk of sodium retention

- Most of them cannot control their own dietary salt intake
- Serum albumin levels are low hence affects the Gibbs-Donnan equilibrium thereby altering the distribution of water.
- Fluid overload is commonly present and a positive fluid balance is associated with poor lung (Wiedemann *et al.* 2006) and kidney function (Bouchard *et al.* 2009), delayed return of gastrointestinal function after surgery (Lobo *et al.* 2002) and an increased risk of mortality (Boyd *et al.* 2011).
- Stress and the resultant effect on the plasma volume leads to the activation of the renin angiotensin aldosterone axis causing Na^+ retention.
- Acute kidney injury is common in critically ill patients and many of them require renal support (5% of patients admitted to the ICU).

Although a moderate range of total body sodium content is well tolerated, once effective plasma volume is significantly affected, short-term and longer-term homeostatic responses are initiated. A fall in effective plasma volume leads to activation of baroreceptors with augmentation of myocardial performance and peripheral vascular tone, and maintenance of plasma volume through shift of fluid from the interstitium. Longer term responses include reduced Na^+ loss by the kidney and sweat glands, through a direct effect of

aldosterone. When the baroreceptors are stimulated the increase in sympathetic tone reduces Na^+ loss through reduced glomerular filtration rate, and through increased tubular Na^+ reabsorption, both through a direct effect and through the actions of increased renin, angiotensin II, and aldosterone. Dopamine is produced in the kidney following conversion from l-dopa under the action of the cytosolic enzyme l-amino acid decarboxylase present in the proximal tubules (Seri *et al.* 1988). This is upregulated following a high-salt administration, leading to increased urinary sodium loss as dopamine acts to inhibit sodium reabsorption in the proximal tubule, (Seri *et al.* 1990) and contributes to the increase in urine output sometimes seen following administration of low-dose dopamine. The renal synthesis of prostaglandins, such as prostaglandin E_2 and prostacyclin (PGI_2), tends to maintain renal blood flow and glomerular filtration rate through vasodilation, and directly increase water and sodium excretion. In critical illness, decreased glomerular filtration rate and impaired activity of dopamine in the proximal tubule of the kidney (Seri *et al.* 1988), where dopamine normally inhibits Na^+ reabsorption (Seri *et al.* 1990), lead to Na^+ retention. As dopaminergic renal vasodilation in part acts through release of PGI_2, critically ill patients already have a prostaglandin-driven kidney rendering it ineffective. Moreover, baroreflex activation which is mediated by several systems (renin angiotensin aldosterone system (RAAS), sympathetic activity, aldosterone, endothelin and vasopressin) have hydro-saline

retention activity. Vasopressin mediates its effects via adenylcyclase-dependent signalling in the renal collecting ducts and is accomplished by upregulation of the aquaporin-2 water channels (Goldsmith 2006). This up regulation results in increased movement of water from the collecting ducts back into the plasma, increasing free water reabsorption, which leads to a further increase in water retention. Furthermore reduction in intrarenal perfusion and the consequent fall in GFR creates a viscous cycle leading to further reflex activation of RAAS with tubular reclamation of salt and water.

Positive-pressure ventilation and positive end-expiratory pressure (PEEP) raise intrathoracic pressure, resulting in reduced venous return and transmural pressure, with consequent complex neurohumoral responses leading to Na^+ and water retention (Bersten 2006). Because assisted, supported, and spontaneous modes of ventilation progressively ameliorate the elevation of intrathoracic pressure and its consequences, different ventilator modes variably reduce venous return. Reductions in stroke volume, cardiac output, and BP then lead to stimulation of high-pressure baroreceptors, and altered regional blood flow. Both low and high-pressure baroreceptor stimulation lead to increased sympathetic outflow, and release of renin, aldosterone, and ANP. Renal denervation does not prevent sodium and water retention (Boemke *et al.* 1998). Angiotensin-converting enzyme inhibitors (Kaczmarczyk *et al.* 1992) and deliberate hypervolemia, (Boemke *et al.* 1998)

however, reduce sodium and water retention during positive-pressure ventilation.

Right atrial transmural pressure and stretch are also reduced by PEEP and positive-pressure ventilation, and this leads to reduced secretion of ANP, (Wilkins *et al.* 1995; Andrivet *et al.* 1988) with consequent reduction in water and sodium excretion reversed by restoration of venous return with lower body positive pressure. PEEP levels above 10 cm H_2O may lead to an increase in central venous pressure (CVP), and regional venous pressures, which in the kidney contribute to reduced sodium and water excretion, independent of neurohumoral effects (Rossaint *et al.* 1993). In summary, various neurohumoral responses to positive pressure ventilation lead to retention of sodium and water, as a homeostatic response to raised intrathoracic pressure. A major consequence of this response is expanded plasma volume, and a tendency toward systemic and pulmonary oedema.

Although Kotchen and coworkers (Kotchen *et al.* 2013) have summarised the problems of high chronic salt intake. Chronic high salt intake is associated with high blood pressure and increased rates of cardiovascular disease. Recent clinical trials have shown that reduced chronic salt intake is associated with decreased risks of cardiovascular events and death (Kotchen *et al.* 2013). From a

more acute medical perspective problems of high salt administration have not received much attention.

1.7.3 CURRENT NHMRC RECOMMENDATIONS

Recommended dietary daily intake of sodium in a healthy person according to age and sex has been published by the Australian National Health and Medical Research Council (NHMRC) and the New Zealand Ministry of Health (MoH) (Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes, 2013) but there are no such recommendations for critically ill patients.

1.8 Distribution and control of water

In the normal adult male, total body water accounts for approximately 60% of body weight. In turn, approximately 40% of body weight is intracellular water and approximately 20% is distributed into the extracellular fluid volume, made up of interstitial fluid (approximately 16%), plasma volume (approximately 4%), and usually negligible volumes of lymph and transcellular fluid (cerebrospinal fluid and pericardial, intrapleural, and peritoneal fluid). The extracellular volume is distributed in interstitial fluid and plasma volume, and consists of two compartments. Seventy percent of the volume is rapidly equilibrating (approximately 20 minutes), and the remainder slowly equilibrates (approximately 24 hours) in dense connective tissue and bone. Sodium balance regulates the

extracellular volume, whereas water balance regulates the intracellular volume (Bersten 2006).

Water balance is primarily determined by thirst and the renal action of arginine vasopressin, also termed *antidiuretic hormone,* which is secreted from the posterior pituitary following synthesis in the hypothalamus, in response to a wide variety of stimuli, particularly plasma osmolality.

Vasopressin activates V 2 receptors on the basolateral surface of the distal renal tubule and collecting duct, leading to an increase in water permeability, and reabsorption of filtrate, through fusion of aquaporin-2 with the luminal membrane (Table 1.1). Vasopressin also reduces water clearance by decreasing renal medullary blood flow, and independently increases the renal medullary concentration gradient by stimulating a urea transporter (Holmes *et al.* 2001). Under normal circumstances, a plasma osmolality of 280 mOsm/kg suppresses vasopressin secretion allowing maximal urinary dilution. As osmolality progressively rises to 295 mOsm/kg, so does the secretion of vasopressin, with an associated reduction in free water clearance. The kidney can normally concentrate filtrate up to 1200 mOsm/kg under the influence of vasopressin, although this tends to deteriorate with age and renal dysfunction. High-pressure stretch receptors in the aortic arch and carotid sinus sense a significant (>10%) fall in blood pressure (BP), leading to an increase in vasopressin release. As vasopressin also causes vasoconstriction through stimulation of V1 receptors, this is an important

homeostatic response in shock, but appears to be reset within 32 hours of sustained hypovolemia (Iwasaki *et al.* 1995). Stimulation of low pressure stretch receptors in the atria primarily results in an increase in both sympathetic tone and renin, and a decrease in atrial natriuretic peptide (ANP), with vasopressin release unaffected until the systemic BP falls. Hence from an evolutionary point of view the human body, when under stress, is designed to conserve water and sodium.

Administration of fluids together with the propensity to conserve water and sodium can lead to the development of both systemic and pulmonary oedema.

1.9 Fluid and lung injury

Radiologic evidence of left ventricular failure is found when the pulmonary artery occlusion pressure exceeds 17 mmHg (Forrester *et al.* 1976). Initially this is seen as upper lobe venous engorgement; higher hydrostatic pressures are progressively associated with interstitial and then alveolar oedema (hydrostatic lung injury) (Forrester *et al.* 1976). In comparison acute lung injury (permeability lung injury) is traditionally characterised by protein rich pulmonary oedema occurring at lower hydrostatic pressures together with a complex inflammatory infiltrate. However, over the last 20 years or so concepts regarding the development and clearance of pulmonary oedema have changed dramatically (Londino

et al. 2013). For example Kaestle and co-workers (Kaestle *et al.* 2007) estimated that only 30% of alveolar fluid influx at an elevated pulmonary capillary pressure was due to direct hydrostatic effects with the remaining 70% due to active changes in bidirectional fluid flux. This fundamental conceptual change is consistent with clinical and chest radiographic improvement in critically ill patients following negative fluid balance despite the absence of an elevated hydrostatic pressure (Bersten 2006).

Fluid boluses are often administered with the aim of improving tissue perfusion (Hollenberg *et al.* 2001; Zanotti-Cavazzoni *et al.* 2009), are a common practice in medical and surgical ICUs (Axler *et al.* 1997) and are a key component in the effective management of such patients. However, it is also becoming increasingly evident that excessive volume administration can worsen outcome (Durairaj *et al.* 2008; Vincent *et al.* 2006; Wiedemann *et al.* 2006). Recent evidence from the Feast study (Maitland *et al.* 2011) which showed an increase in mortality in children with severe infection with fluid boluses, raises further questions about the safety of these fluid boluses.

1.10 Role of TRPV4 channels

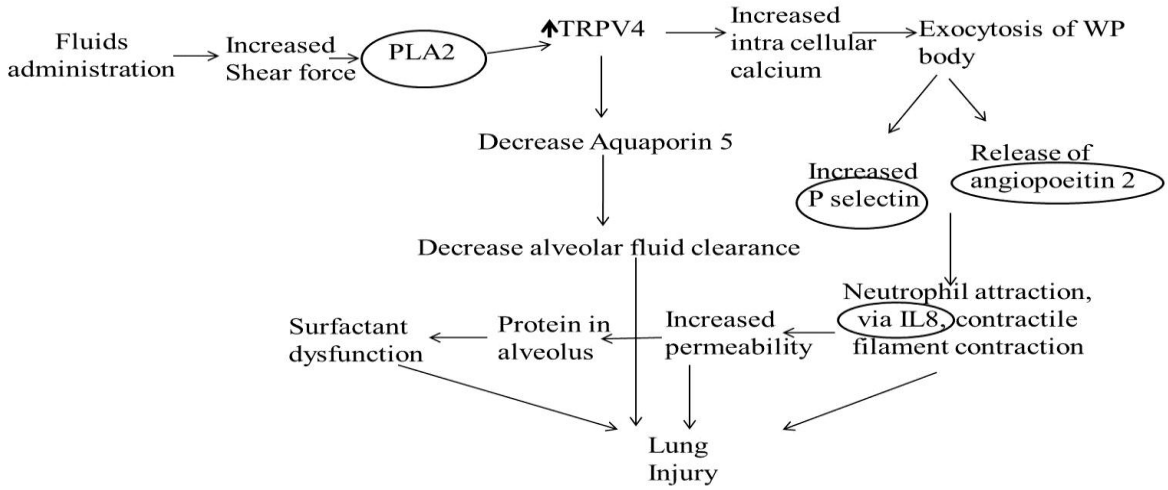
The transient receptor potential (TRP) ion channel superfamily is involved in sensing and transmission of a broad variety of external or internal stimuli, including mechanical stress (Yin *et al.* 2010). TRP vanilloid (TRPV) 4 has mechanotransductive properties and is

abundantly expressed in pulmonary blood vessels (Yin *et al.* 2010). Activation of these channels by shear forces (Troidl *et al.* 2009), stretch (Mochizuki et a 2009), over-inflation (Hamanaka *et al.* 2007; Jurek *et al.* 2014), hypothermia (Hamanaka *et al.* 2007), increased hydrostatic pressure (Yin *et al.* 2008) and hypotonicity (Liedtke *et al.* 2006) leads to the rapid intracellular influx of calcium ions. Calcium influx is doubled after activation of TRPV4 channels in less than 40 seconds and lasts up to 8 minutes (Parker *et al.* 2013). *In vitro* activation of TRPV4 requires hydrolysis of membrane phospholipids via phospholipase A_2 (PLA_2) (Figure 1.1) and subsequent arachidonic acid metabolism by cytochrome P450 epoxygenases to form epoxyeicosatrienoic acids (EET) (Jian *et al.* 2008). EET activation of TRPV4 channels and the resultant Ca^{2+} influx leads to increased permeability of the alveolocapillary barrier, and reduction in alveolar fluid clearance via down regulation of fluid channels, including aquaporin (AQP)5 (Sidhaye *et al.* 2008), manifesting as lung injury (Alvarez *et al.* 2006). In addition, TRPV4 activation and elevated cytosolic Ca^{2+} concentration may further contribute to acute lung injury due to effects on endothelial permeability and function via the exocytosis of Weibel–Palade bodies (endothelial storage vesicles) (Lowenstein *et al.* 2005) containing stored proteins including P-selectin and angiopoietin-2 (Ang-2) (Romani *et al.* 2003; Fiedler *et al.* 2004). High pulmonary vascular pressures lead to de-granulation of Weibel-Palade bodies and increased expression of P-selectin in lung capillaries which can be blocked with gadolinium, a non-specific

physiological blocker of TRPV induced calcium influx (Kuebler *et al.* 1999). P-selectin expression on the endothelium in the presence of chemotactic mediators including IL-8, is integral to neutrophil recruitment at sites of tissue inflammation and has been associated with various forms of acute lung injury in animal models (Mulligan *et al.* 1993) and clinically in ARDS (Sakamaki *et al.* 1995). Ang-2, a biomarker of lung injury (Terpstra *et al.* 2013), is low with conservative fluid therapy which in turn is associated with better outcomes in acute lung injury (Agrawal *et al.* 2013; Calfee *et al.* 2012).

It can therefore be hypothesised that administration of bolus intravenous fluids leads to a transient rise in the shear forces across the pulmonary endothelium leading to activation of the TRPV4 channels and which manifests via secondary processes as lung injury (Figure 1.1), and may explain the increase in mortality in the FEAST study (Maitland *et al.* 2011).

Figure 1.1: Schematic suggesting pathways involved in fluid induced lung injury



1.11 Effects of osmolality and its relationship with sodium

Serum osmolality is one of the chief determinants of movement of water between the extracellular and intracellular spaces. It is determined mainly by serum [Na^+]. Serum [urea] and [glucose] also make a small contribution to the serum osmolarity, but the effective osmolality (tonicity) is largely the result of serum sodium as both urea and glucose can move freely to intracellular spaces.

Serum osmolarity is expressed per litre (calculated) while osmolality is per kg (measured). Of the multiple methods available to calculate the serum osmolarity, probably the simplest formula is the best (Worthley *et al.*, 1987): 2 X serum sodium (135-140mmol/l) + serum glucose (4 to 6 mmol/L) + serum urea (3 to 4 mmol/l) = 280 to 290 mOsmol/L. Serum osmolality is measured by utilizing the colligative property of a solution – freezing point depression; others being vapour pressure elevation, boiling point elevation and osmotic pressure.

There is a small difference between the calculated and measured values accounting for the unmeasured osmotic active ions and molecules dissolved in the plasma which can lead to spurious low [Na^+] (due the plasma solid effects) and unexplained water movement.

Since the measured and calculated are reported units of osmolality and osmolarity respectively there is often confusion as how to

address the difference between the calculated and measured values (osmol gap). While it is possible to convert between osmolality and osmolarity, thereby deriving a more mathematically correct osmol gap calculation, in actual clinical practice this is not done. This is because the difference in absolute value of these two measurements that can be attributed to the difference in units will be negligible in a clinical setting. For this reason, the terms are often used interchangeably. A normal osmol gap is < 10 mOsm/kg.

1.12 Effects of high serum sodium (high serum osmolality)

Hypernatremia is associated with increased mortality risk in critically ill patients (Linder *et al.*, 2007; Hoorn *et al.* 2008). Avoidance of such a state has therefore been considered important. There have been reports of increased mortality risk with high serum [Na^+] (Darmon *et al.* 2013) within the first 24 hours of ICU admission. In addition to causing intracellular dehydration, high serum osmolarity aggravates peripheral insulin resistance (Bratusch-Marrain, DeFronzo 1983), leading to hyperglycemia. It also impairs hepatic gluconeogenesis and lactate clearance and is associated with neurological impairment that might lead to prolonged duration of mechanical ventilation and delayed weaning (Adrogué, Madias 2000; Druml *et al.* 1986). It can impair cardiac function, decrease left ventricular contractility (Lenz *et al.* 1986; Kozeny *et al.* 1985) and cause rhabdomyolysis (Acquarone *et al.* 1989; Opas *et al.* 1977).

On the contrary, hyperosmolality (effectively hypertonicity), has been shown to rescue T cells from suppression by trauma-induced anti-inflammatory mediators (Loomis *et al.* 2001), suppresses neutrophil activation (Angle *et al.*, 1998; Junger *et al.* 2012; Deith *et al.* 2003) and affects macrophage migration (Kim *et al.* 2013); all of which can mitigate lung injury (Angle *et al.* 1998).

Serum tonicity can up- or down-regulate the transient receptor potential (TRP)4 ion channel which plays a critical role in lung vascular mechanotransduction (Yin *et al.* 2010). Specifically, hypotonicity can activate these channels (Chen *et al.* 2009a; Chen *et al.* 2009b; Becker *et al.*, 2009; Garcia-Elias *et al.*, 2008; Wegiersk *et al.* 2009; Mizuno *et al.*, 2003; Liedtke *et al.* 2003) leading to endothelial calcium influx, and a rise in pulmonary vascular permeability (Yin *et al.* 2010). Alternatively, hypertonicity can suppress these channels leading to a decrease in pulmonary vascular permeability, which may be beneficial in patients with lung injury.

Hyperosmolarity increases type 1 alveolar epithelial cell repair (Wang *et al.*, 2011) and augments actin filament formation and E-cadherin expression at the endothelial cell periphery (Safder *et al.* 2003). Moreover it blocks TNF-α–induced P-selectin expression in an actin-dependent manner which helps in remodeling of the endothelial barrier (Safder *et al.* 2003).

Hyperosmolar solutions have ameliorated pulmonary injury after hemorrhagic shock in experimental models with improved

splanchnic blood flow and reduced adhesion and cytotoxicity of neutrophils compared with the use of isotonic solutions (Angle *et al.*, 1998; Shi *et al.*, 2002). A 15-min infusion of hyperosmolar sucrose, which increases vascular osmolarity by approximately 50 mOsm, strengthens the lung endothelial barrier, and enhances actin polymerization in the endothelial periphery (Safder *et al.*, 2003; Quadri *et al.* 2003). Similarly, a brief period of vascular hyperosmolarity protects against acid-induced lung injury when the infusion is administered shortly before, or shortly after, acid instillation in the airway (Safder *et al.* 2005).

In summary although high serum sodium (high serum osmolality) has been associated with deleterious systemic effects, induced high serum sodium leading to high serum osmolality may have lung protective effects.

CHAPTER 2: PRIMARY RESEARCH OUTLINE

2.1 What are the current levels of sodium administration and sodium balance in ICU patients? Is there any relationship between sodium balance and respiratory dysfunction? Clinical studies were used to examine this question.

2.2 Why are fluid boluses utilised in ICU patients? Do they lead to lung injury? If so, what are the mechanisms. Clinical studies, studies involving healthy volunteers and animal models were used to explore this question.

2.3 Does high serum osmolality ameliorates lung injury? Animal and epidemiological studies were used to explore this question.

CHAPTER 3: SODIUM ADMINISTRATION IN ICU PATIENTS

3.1 Sodium administration in a single centre tertiary level ICU

Positive fluid balance is associated with worse outcomes in critically ill patients (Boyd *et al.* 2011), probably due to extracellular fluid expansion. Therefore, both water and sodium may be important because water distributes to both intra- and extracellular spaces and sodium distributes into the extracellular spaces and may exacerbate interstitial oedema both in the lungs and the systemic circulation.

This effect might be more relevant in mechanically ventilated patients. Positive pressure ventilation and positive end expired pressure (PEEP) both raise intra-thoracic pressure. This results in reduced venous return, and consequent complex neurohumoral responses in turn lead to sodium and water retention (Bersten 2006).

As potential sources of sodium in critically ill patients include resuscitation fluids, maintenance fluids, enteral and parental feeds, venous and arterial line flushes, transfusions, replacement fluid and many medications, my hypothesis was that critically ill patients inadvertently receive excess amounts of sodium during their stay in intensive care.

To examine this, a retrospective sodium administration audit was performed on twenty consecutive patients receiving prolonged mechanical ventilation during their stay in single centre tertiary level Intensive Care Unit (ICU).

The primary objective was to estimate the amount of sodium administered to patients who were invasively ventilated for more than 5 days in the ICU, and secondary objectives were to investigate whether sodium administration had any association with oxygenation, length of stay in ICU and serum sodium level.

3.1.1 METHOD

A retrospective analysis of the amount of sodium administered per day (in mmol) from all sources was performed on 20 consecutive patients who required mechanical ventilation for more than five days (120 hours) at the Flinders Medical Centre (South Australia) ICU. This is a 32 bed, tertiary level adult general ICU.

Patients were excluded if they were aged ≤ 18 years, were pregnant, required chronic haemodialysis, had an admission diagnosis of traumatic brain injury, or had diabetic ketoacidosis or a hyperosmolar hyperglycaemic state.

Patient case notes, data sheets, operation notes, and total parental nutrition (TPN) data sheets were retrieved and reviewed. The

following patient baseline data were recorded: demographics, diagnosis, APACHE II score at admission, duration of invasive mechanical ventilation, length of stay in ICU, ICU outcome, requirement for diuretics, requirement for dialysis, daily administered fluid and fluid balance (taking account of all recorded sources including urine, drains, gastrointestinal) .

Total daily administered sodium (from all sources) was recorded in the following categories:

1. Resuscitation sodium - total sodium administered as fluid boluses during either initial management or ongoing care.
2. Maintenance sodium - sodium administered (including given as a replacement for renal and/or gastrointestinal loss) as a constant infusion.
3. Infusion sodium - sodium which was used a vehicle for other drugs, such as sedatives, vasopressor agents, antimicrobials and insulin.
4. Enteral feed sodium.
5. Total parentral nutrition sodium.
6. Transfusion sodium - sodium from packed red blood cells, platelets, fresh frozen plasma and cryoprecipitate administration.
7. Drugs sodium - sodium present in the administered drugs themselves.
8. Flushes sodium - sodium given as flushes with each central venous and arterial access. The flush used in the current

study was heparinised (2 U/ml) 0.9% saline, administered at a rate of 4mls per hour for every flush-based catheter present.

A master table (Appendix 1) was constructed with the sodium content of all drugs/ fluids administered to these patients. The sodium content of the blood products was estimated by taking the average of sodium contained in ten samples of each of the different blood products.

Also recorded from each patient's daily data for the time period 8am to 9am each day were: daily renal function, serum sodium, daily PaO_2/FiO_2 ratio, $PaCO_2$, minute ventilation, PEEP and central venous pressure.

Data were recorded from 24 hours prior to intubation, or from the time of admission to hospital (if less than 24 hours) until 24 hours after extubation or death (if the patient remained intubated and ventilated at the time of death).

3.1.2 STATISTICAL ANALYSES

Data were analysed using SPSS (version 19.0, Chicago, IL, USA). Data was not normally distributed and are reported as median and range. The Pearson correlation was used to correlate the daily administered sodium with: APACHE II score, total ICU stay, PaO_2/FiO_2 ratio (on the following morning), net fluid balance and

serum sodium. For all analyses, a p value of less than 0.05 was considered significant.

3.1.3 RESULTS

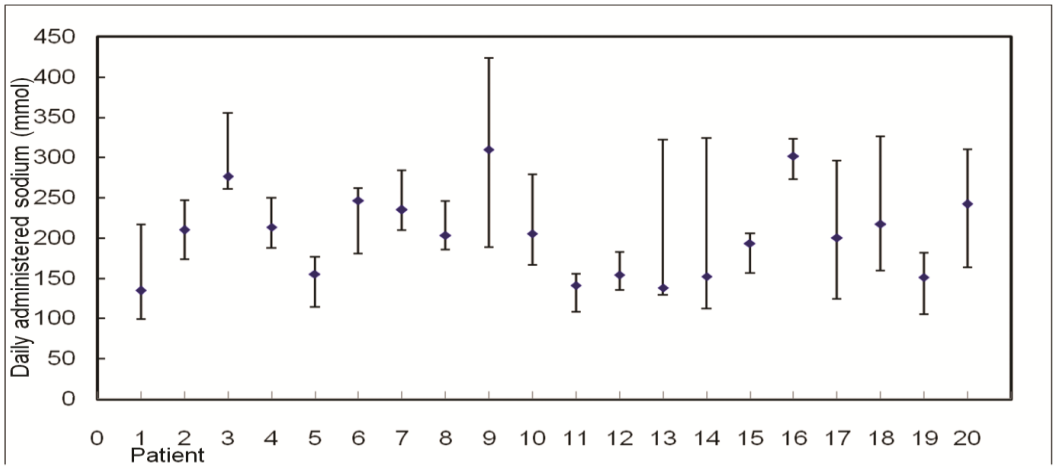
3.1.3.1 Patient Characteristics

There were 13 male and 7 female patients with median age 71.9 (range 19.8 to 89.2) years. The median duration of mechanical ventilation was 9 (6, 20) days, with a median ICU stay of 11.6 (6, 21) days. Of the 20 patients studied, 12 died, 5 were discharged home, 2 were readmitted to ICU and 1 was transferred to another hospital. The median APACHE II score at admission was 29 (18–41). Seven of the 20 patients required dialysis. Twelve out of 20 patients had diuretics administered at some stage during their course of ICU stay

3.1.3.2 Sodium and fluid

The median average daily sodium administration was 225.5 (151, 355) mmol (Figure 3.1). The median daily net fluid balance was positive at 351 ml (-759 ml to +1125 ml) and median daily fluid intake was 2352 ml (1437 ml to 3798 ml).

Figure 3.1: Daily sodium intake (mmol) in the single centre study



Data are expressed as median and interquartile range

The median and range of contributions to the total sodium administered, from each source were: Infusions: 22.2% (1.2 – 39.9); Drugs 21.6% (0 – 35.5); Flushes 17.4% (9.3 – 24.5); Enteral feeds 17% (0- 39.5); Resuscitation 16% (2.5 – 36.9); Maintenance fluids 5.8% (0 – 24); Transfusions 3.9% (0 –9.5) and Total Parental Nutrition 0.1% (0- 2.6) (Table 3.1).

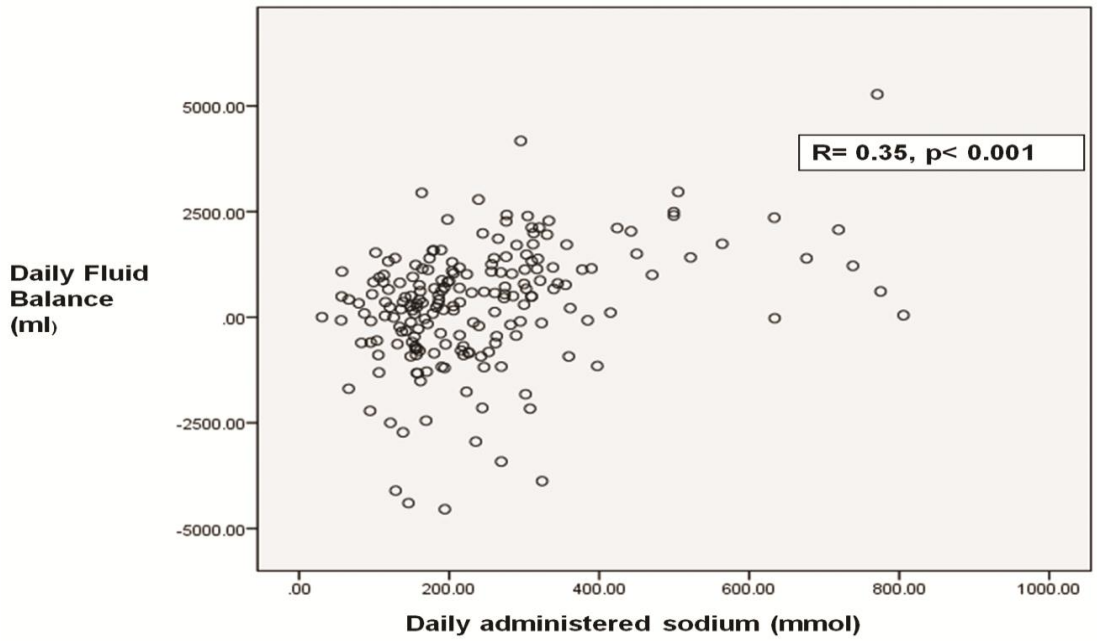
Table 3.1: Contributions of each source to the total sodium administered in the single centre study

Sodium source	Median % (range)
Resuscitation	16% (2.5%–36.9%)
Maintenance fluids	5.8% (0–24.0%)
Infusions	22.2% (1.2%–39.9%)
Flushes	17.4% (9.3%–24.5%)
Medicines	21.6% (0–35.5%)
Transfusions	3.9% (0–9.5%)
Enteral feeds	17.0% (0–39.5%)
Total parental nutrition	0.1% (0–2.6%)

The mean daily serum sodium was 141 ± 5 mmol/l (mean±SD). Fourteen of the twenty patients had serum sodium greater than 145 mmol/L and three of the twenty patients developed hypernatremia, defined as levels greater than or equal to 150 mmol/L at some stage during their stay in ICU.

Daily sodium administered was correlated with net daily fluid balance ($p<0.001$, $r = 0.35$) (Figure 3.2).

Figure 3.2: Correlation of daily administered sodium and daily fluid balance in the single centre study



However, there was no correlation between daily administered sodium and: APACHE II score (p = 0.1); total ICU stay (p = 0.48); PaO_2/FiO_2 ratio next morning (p=0.62); or daily plasma sodium (p=0.67).

3.1.4 DISCUSSION

In this cohort of ICU patients receiving prolonged mechanical ventilation more than 220 mmol of sodium was administered daily. The average daily net fluid balance here was comparable to the conservative group of the FACTT study (Wiedemann *et al.* 2006), and the average daily fluid intake was less than the conservative group of FACTT, suggesting we achieved good fluid balance in our cohort of patients. However, the average daily sodium here was >220 mmol daily, indicating that critically ill mechanically ventilated patients inadvertently receive a high load of sodium, despite

achieving neutral fluid balance. A similarly high level of non-dietary sodium administration has also been recently reported in cardiac patients [Tafreshi *et al.* 2011].

The cause of this high sodium administration unexpectedly was not due to resuscitation as generally expected; but was from infusions, flushes and drugs. The amount of sodium administered as infusions is most surprising. Similarly, the amount of sodium administered as flushes was also very high and may still be under-recorded. Some of the drugs (especially antibiotics) contain significant amount of sodium. It is noteworthy that the usual maintenance fluid in our unit is 4% dextrose and 1/5 saline, which has a relatively low sodium content. Many units around the world administer 0.9% saline as the usual maintenance fluid, which can lead to a large increase in sodium intake.

Fourteen of the twenty patients had serum sodium ≥ 145 mmol/L and three patients reached level ≥ 150 mmol/L at some stage during their ICU stay. Hypernatraemia is recognised to be a common and important electrolyte disorder in critically ill patients and is an independent predictor for mortality [Palevsky *et al.* 1996]. Most cases of hypernatremia in the ICU developed after admission, suggesting an iatrogenic component. The strategy of aiming to achieve a negative fluid balance as evidenced by use of diuretics in our study and combined with a high sodium load and balance,

predisposes to hypernatremia, as has been previously described (Hoorn *et al.* 2008; Lindner *et al.* 2009).

The high level of daily sodium administered to mechanically ventilated patients was correlated with net positive fluid balance. This has been associated with poorer lung function (Wiedemann *et al.* 2006), poorer kidney function (Bouchard *et al.* 2009), delayed return of gastrointestinal function after surgery (Lobo *et al.* 2002) and an increased risk of mortality (Boyd *et al.* 2011). This association with worse outcome is probably due to extracellular fluid expansion, which can affect these organ systems.

3.1.5 STUDY LIMITATIONS

This was a small (n = 20) retrospective observational study with all of the limitations inherent to this study design. This audit was not powered to study the correlation of daily administered sodium and APACHE II score, total ICU stay and oxygenation, therefore a larger study is required to examine these variables.

Although these data are consistent with inadvertent sodium loading in critically ill patients, it is from a single centre and sodium balance was not estimated. It remains to be seen whether these observations are applicable to other units.

3.1.6 SUMMARY

Sodium administration to critically ill patients requiring prolonged mechanical ventilation at a single centre was high. This finding from a single centre needs to be confirmed in other centres.

3.2 Sodium administration in critically ill patients in Australia and New Zealand: a multi-centre point prevalence study

In a single-centre study, I demonstrated that the amount of sodium administered to ICU patients receiving invasive mechanical ventilation for more than 5 days was over twice (Section 3.1) the recommended daily intake of 100 mmol (Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes, 2013). Moreover, the main sources of sodium administration were intravenous (i.v.) maintenance fluids, flushes and drugs (Section 3.1).

This is particularly important in ICU patients as they are at risk of sodium retention due to activation of the renin-angiotensin-aldosterone system (Jungmann *et al.* 1987) and impaired activity of dopamine in the proximal tubule of the kidney (Seri *et al.* 1988) where dopamine normally inhibits sodium reabsorption (Seri *et al.* 1990). Depending upon concomitant water balance, the administration of large amounts of sodium, combined with the propensity for sodium retention may have important clinical implications such as hypernatremia (Palevsky *et al.* 1996), which has been associated with poor outcomes (Hoorn *et al.* 2008; Lindner *et al.* 2009), and changes in intra- and extracellular fluid volumes.

However, the results of a single-centre study reflect local practice and are not generalisable to other centres. To confirm these results, a multi-centre, single-day point prevalence study was undertaken in conjugation with the Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group (CTG) and the George Institute for Global Health.

The primary aim of the study was to determine the total amount of sodium administered to critically ill patients in Australian and New Zealand ICUs and to determine the most common sources of administration.

3.2.1 METHODS

All Australian and New Zealand CTG-affiliated ICUs were invited to participate. Approval was obtained, when required, from individual participating site research ethics committees. The study was a prospective, cross-sectional, observational audit and, as such, the requirement for individual subject consent was waived at all sites.

All adult patients (≥ 16 years) present in participating ICUs at 10 a.m. on the study day were enrolled. Routine survey data for all patients included age, sex, weight (estimated/measured), ICU admission Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score within the preceding 24 hours of study day and ICU admission source. Data regarding ICU admission diagnoses

(operative vs. non-operative, burns, and trauma) and specific diagnoses on study day (acute lung injury [ALI], acute respiratory distress syndrome [ARDS], sepsis) were collected. Requirement for renal replacement therapy (RRT) on the study day was also collected. Vital status 28 days after study day was ascertained using hospital administrative databases. Serum sodium (highest) on the study day was also recorded and patients were defined as having hyper- or hypo- natremia if; serum sodium ≥ 150 mmol/L (Lindner *et al.* 2009) or < 130 mmol/L (Asadollahi *et al.* 2006) respectively.

Patients receiving an oral diet where at least 50% of the dietary requirements were met by oral intake and enteral and/or parenteral nutrition was not being administered on the study day were excluded for the purposes of ascertaining the sources of sodium administration.

Data on all remaining patients included:

i) i.v. bolus fluids administered for volume expansion or "fluid resuscitation" i.e. crystalloid infusion ≥ 5 ml/kg/hr or ≥ 400 ml/hr or any colloid bolus or infusion

ii) blood products i.e. red blood cells, platelets, fresh frozen plasma

iii) i.v. maintenance or replacement fluids i.e. crystalloids given by continuous infusion

iv) i.v. drug infusions i.e. drugs administered by continuous infusion together with its vehicle

v) i.v. drug boluses together with its vehicle

vi) i.v. flushes associated with haemodynamic monitoring e.g. intra-arterial or central venous catheter

vii) enteral nutrition

viii) parenteral nutrition.

For all i.v. fluids and blood products, the type and volume administered over the 24-hour study day was recorded and the amount of sodium administered was calculated based on previously described sodium concentrations (Appendix 1). For drug infusions and boluses, sodium content was calculated from both the sodium content of the drug and the type and volume of carrier fluid or diluent. For enteral and parenteral nutrition, information on the type and volume of feed was recorded and the sodium content calculated accordingly. For custom parenteral nutrition, the sodium content was recorded. No data on oral sodium intake was collected.

3.2.2 STATISTICAL ANALYSIS

Variables are reported as mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. Sources of sodium administration are reported as percentages with 95% CI. Antimicrobials were analysed separately to other i.v. drugs due to the high sodium load of some agents (Section 3.1; appendix 1).

Pearson's correlation was used to test for the association between sodium administered (log transformed for normal distribution) and the following factors: age, weight, APACHE II score and day of ICU stay, SOFA score, serum sodium, fluid administered and 24 hour fluid balance on study day. Predictor variables for sodium administration (age, sex, APACHE II score, site, and variables significant at α ≤0.10) were analysed using multiple linear regression (SPSS version 2.0, Chicago, IL, USA). Day 1 data was not included in the model as 24-hour data was incomplete (median[IQR] ICU length of stay 14[11-18] hours). In addition, the amount of sodium administered during routine care as opposed to the initial resuscitation phase that occurs at ICU admission is potentially different.

For all analyses, a P value of less than 0.05 was considered significant.

3.2.3 RESULTS

3.2.3.1 Patient characteristics

Five hundred and eleven patients were enrolled into the point prevalence survey from 46 tertiary referral, metropolitan and rural hospital ICU's (Appendix 2). One hundred and fifty-five patients (30.3%) were excluded because of oral intake (n=148; 28.9%) or missing data (n=7; 1.4%).

Of the remaining 356 patients (40 sites), 64.3% (n=229) were male and the mean(SD) age and estimated body weight (on study day) were 58.5 (18.0) years and 81.6 (24.0) kg, respectively. Twenty-eight day mortality was 12.6%. Other patient characteristics are shown in Table 3.2.

Table 3.2: Patient characteristics in the adult ICUs on the point prevalence study day

Characteristic	**n=356**
Age, years*	58.5 (18)
Male sex, n (%)	229 (64.3)
Weight, kg *#	81.6 (24)
APACHE II score *	20 (8)
ICU admission source, n (%)	
Emergency department	110 (30.8)
Hospital ward	75 (21.1)
Operating theatre	109 (30.6)
Other	62 (17.4)
APACHE III diagnostic categories, n (%)	
Cardiovascular	40/247 (16.2)
Respiratory	65/247 (26.3)
Gastrointestinal	15/247 (6.0)
Neurological	39/247 (15.8)

Sepsis	35/247 (14.2)
Trauma	22/247 (8.9)
Other	29/247 (11.7)
SOFA score on study day**	7(4-11)
Respiratory SOFA score on study day **	2(1-3)
Sepsis on study day, n (%)	127 (35.6)
ALI/ARDS on study day, n (%)	38 (10.6)
Renal replacement therapy, n (%)	41 (11.5)
Hospital length of stay, days **	5.0 (2.0-13.0)
28-day mortality, n (%)	45 (12.6)

**Mean (SD); ** Median (IQR); # Weight estimated or actual*
APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; ALI, acute lung injury; ARDS, acute respiratory distress syndrome

3.2.3.2 Serum sodium

Serum sodium on the study day was 140.5(5.0) mmol/L). Sixty-nine patients (19.3%) had a serum sodium ≥145 mmol/L and 18 (5.1%) were hypernatraemic (≥150 mmol/L). Fifty seven patients (16.0%) had a serum sodium <135 mmol/L and 6 (1.7%) were hyponatraemic (≤130 mmol/L) on the study day.

3.2.3.3 Sodium and fluid administration

The total amount of sodium administered at individual study sites ranged from 90.0 (56.5-243.3) to 500.1(199.5–604.1) mmol. Overall sodium administration across all the sites was 224.5 (144.9–367.6)

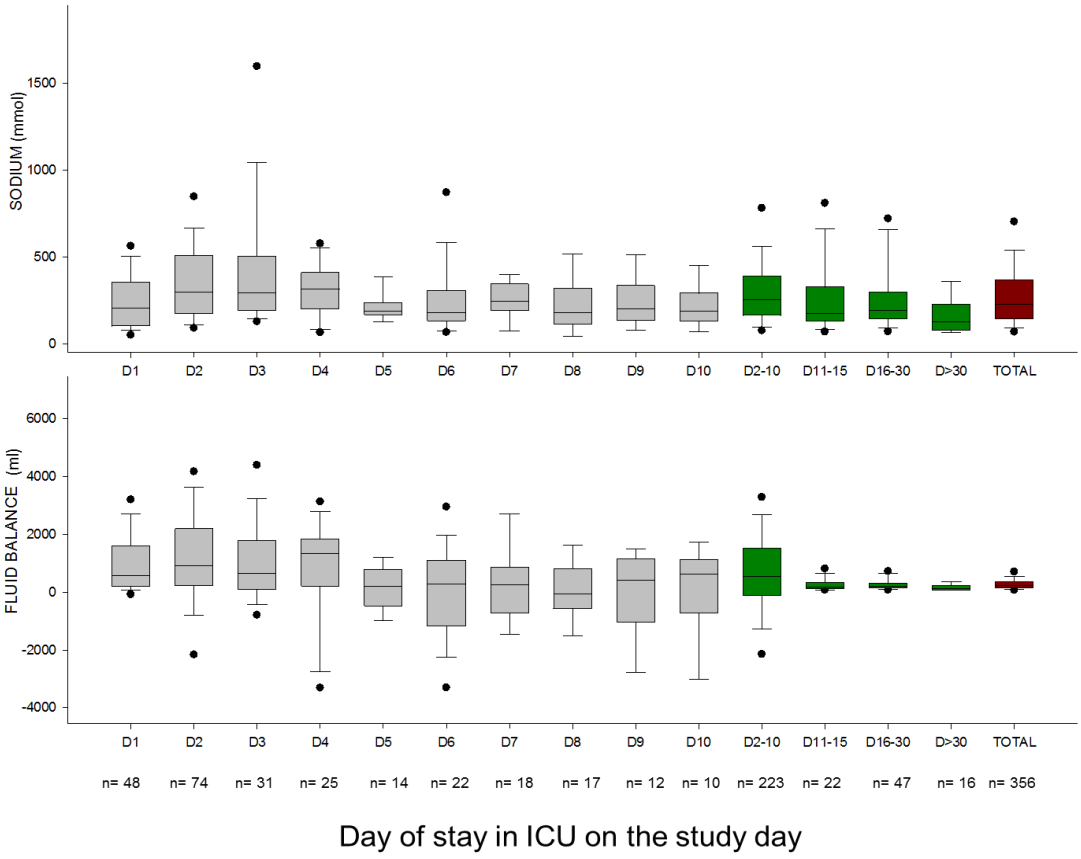
mmol or 2.8 (1.6-4.7) mmol/kg. Twenty-four hour fluid balance was +503.5 (+2.5 to +1345) ml. Sodium administration according to diagnostic category is shown in Table 3.3. Sodium administration and fluid balance according to the day of stay in ICU are shown in Figure 3.3.

Table 3.3: Sodium administration according to diagnostic category on the point prevalence study day

Diagnostic category	n (%)	Sodium administered, mmol
Post-operative	109 (30.6)	224.3 (142.0 – 368.5)
Trauma	61 (17.0)	256.7 (165.1- 445.0)
Burns	3 (0.8)	464.9 (168.6 – 686.5)
Sepsis	127 (35.6)	224.3 (142.0 – 368.5)
ALI/ARDS	38 (10.6)	200.8 (140.9 – 367.7)

Data expressed as median (IQR). Post–operative, trauma (operative and non-operative) and burns diagnostic categories were at ICU admission. Sepsis and ALI/ARDS diagnoses were on study day. ALI, acute lung injury; ARDS, acute respiratory distress syndrome.

Figure 3.3: Sodium and fluid balance on the point prevalence study day according to day of stay in ICU post-ICU admission



Data presented as box plot representing median, upper and lower quartile with the 10th and 90th percentile either side, dots are 95th and 5th percentiles. Day 1 has 14 (11-18) (median and IQR) hours of data. n represent the total number of patients according to the day of stay in ICU on the study day.

For those patients enrolled on day 1 (median [IQR] ICU length of stay 14[11-18] hours) of ICU admission (48/356; 13.4%), 202.9 (101.5-352.9) mmol of sodium was administered on the study day. The main sodium source was i.v. maintenance or replacement fluids (77.5 mmol; 38.2% [95%CI 37.3-39.0] of all sodium administered). Other sources included i.v. fluid boluses (44.4 mmol; 22.0% [95% CI 21.3-22.7]), i.v. drug boluses other than antimicrobials (22.9 mmol;11.3% [95% CI 10.7-11.8]),]), i.v. drug infusions (19.1 mmol;

9.4% [95% CI 8.9-9.9]), i.v. flushes (12.6 mmol; 6.2% [95% CI 5.8-6.6]), blood products (12.4 mmol; 6.1% [95% CI 5.6-6.5]), i.v. antimicrobial (7.2 mmol; 3.5% [95% CI 3.2-3.8]), enteral nutrition (6.2 mmol;3.1% [95% CI 2.7-3.3]), and parenteral nutrition (0.3 mmol; 0.1% [95% CI 0.0-0.1]). The total amount of fluid administered as a bolus on day 1 was 800 (467 -1048) ml (median and IQR)

For patients present between day 2-10 of ICU admission (223/356; 62.6%), 255.1 (163.2–390.5) mmol of sodium was administered on the study day. Twenty-four hour fluid balance was +550 ml (-126 to +1515) ml (Figure 3.3). The main source of sodium administration was i.v. maintenance or replacement fluids (69.3/224.5 mmol; 30.9% (95%CI 30.6-31.2) of all sodium administered). Of the 225 patients receiving i.v. maintenance or replacement fluids, 33.7% (76/225) received 0.9% saline. Hartmann's® solution (55/225; 24.4%), 5% dextrose (34/225; 15.1%) and 4% dextrose and 0.18% saline (21/225; 9.3%) were the next most common fluids infused.

Other sodium sources included i.v. fluid boluses (36.5 mmol; 16.3% [95% CI 16.0-16.4]), i.v. drug boluses other than antimicrobial (27.6 mmol;12.3% [95% CI 12.1-12.5]), enteral nutrition (26.5 mmol;11.8% [95% CI 11.5-12.0]), i.v. drug infusions (19.3 mmol; 8.6% [95% CI 8.4-8.8]), i.v. flushes (16.6 mmol; 7.4% [95% CI 7.2-7.5]), blood products (13.5 mmol; 6% [95% CI 5.8-6.4]),

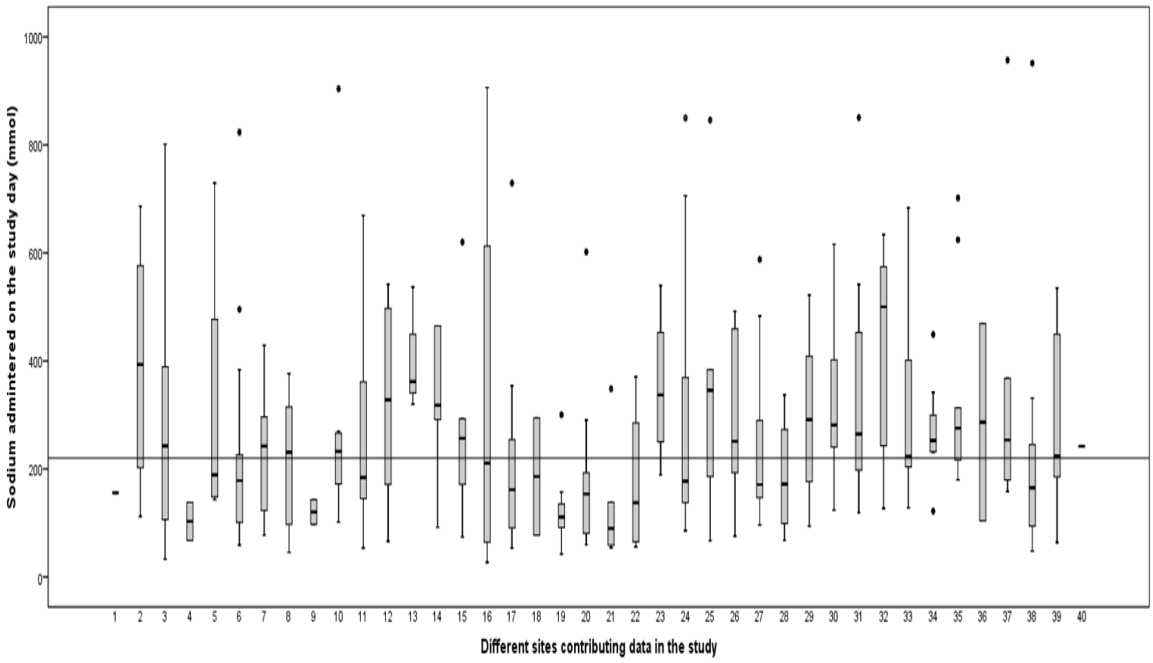
i.v. antimicrobial (11.2 mmol; 5% [95% CI 4.8-5.1]) and parenteral nutrition (4.3 mmol; 1.9% [95% CI 1.8-2.0]). 0.9% saline was the most commonly used vehicle for i.v. drug boluses (177/234; 75.6%) and i.v. drug infusions (152/236; 64.4%). Heparinised saline was the most common i.v. flush fluid (214/ 218; 98.1%).

The highest proportion of sodium administered from i.v. fluid boluses occurred on Days 2 and 3 of ICU admission. Seventy-four patients (20.8%) received a fluid bolus on Day 2 (513 [332 -1637] ml, median [IQR]) and sodium administration was 66.0 mmol (22.3% [95% CI 21.8-22.8]) of all sodium administered). Patients present on Day 3 (31/356; 20.8%), received 467 [257 -1971] ml, median [IQR] as fluid bolus amounting to 55.2 mmol (18.8% [95% CI 18.1-19.4]) of administered sodium. Overall albumin (4 % or 5%) was the most commonly administered bolus fluid (46/125 fluid boluses; 36.8%), followed by 0.9% saline (30/125; 24%) and Hartmann's® solution (16/125; 12.8%).

Sodium administration was weakly correlated with study day total SOFA score (P=0.001, r=0.19), the respiratory component of the SOFA score (P=0.032, r=0.14), 24-hour administered fluid (P=0.038, r=0.11) and fluid balance (P<0.001, r=0.45). Using multiple linear regression modelling (R^2=0.115), factors associated with administered sodium were site (standardized β coefficient 0.105, P =0.044) (Figure 3.4), age (- 0.227, P<0.001), administered fluid on

study day [0.121, P=0.03] and day of stay in ICU [- 0.167, P=0.01] (Figure 3.3).

Figure 3.4: Sodium administration on the point prevalence study day at different sites



Data represented as box plot representing median, upper and lower quartile with the 95th and 5th percentile either side, dots are outliers. Horizontal line represents the median level of administered sodium 224.5 mmol.

3.2.4 DISCUSSION

In this multi-centre point prevalence study of ANZ ICUs, the median sodium administration was greater than 220 mmol on the study day. In contrast, fluid balance on the study day was only 500 ml positive; suggesting that ICU patients, despite a small positive fluid balance, receive a high sodium load in excess of recommended daily requirements for a healthy population (Nutrient Reference Values for Australia and New Zealand including Recommended Dietary

Intakes, 2013); albeit there is no recommended daily intake for critically ill patients in ICU.

The principal source of sodium administration was not i.v. fluid resuscitation as one may have pre-supposed, but instead was primarily due to i.v. infusions, in particular maintenance fluids, as well as i.v. drug infusions, boluses and flushes. These sources of sodium are inadvertent and potentially modifiable, depending on clinician choice for "routine" i.v. fluid administration. These findings are also similar to the results of our single centre study (Section 3.1). A high level of non-dietary sodium administration has been similarly reported in cardiac patients (Tafreshi *et al.* 2011). Furthermore, although the selection of fluid type varied between participating sites, 0.9% saline was the most common i.v. fluid; contributing to 59.2% of all sodium administered. Whilst the reason(s) for choosing 0.9% saline cannot be ascertained from this point prevalence survey, it was noteworthy that recent studies have indicated that after a bolus of 0.9% saline, excretion of both water and sodium is slower (Ried *et al.* 2003) and may result in reductions in renal blood flow velocity and renal cortical tissue perfusion (Chowdhury *et al.* 2012).

Sodium retention might be more relevant in critically ill patients due to activation of the renin-angiotensin-aldosterone system . This is especially so in mechanically ventilated patients, where positive

pressure ventilation and positive end-expiratory pressure (PEEP) both raise intrathoracic pressure, which results in reduced venous return and a consequent complex neurohumoural response (Bersten 2006; Frazier 1999) leading to sodium and water retention. As seen in this current study, sodium administration on the study day had a weak correlation with the SOFA score and with a net positive fluid balance. Importantly, a positive fluid balance is associated with poor lung and kidney function (Wiedemann *et al.* 2006; Bouchard *et al.* 2009), delayed return of gastrointestinal function after surgery (Lobo *et al.* 2002) and an increased risk of mortality (Boyd *et al.* 2011). The adverse effects of positive fluid balance are probably due to extracellular fluid expansion. Therefore, both water and sodium may be important because water distributes to both intra- and extra- cellular spaces. In contrast, sodium distributes into the extracellular spaces leading to cellular dehydration and interstitial oedema in both the lungs and the systemic circulation. Current strategies using conservative fluid balance therapy without attention to concomitant sodium balance could potentially lead to intracellular dehydration and thereby may have been one of the mechanisms contributing to abnormal neurocognitive effects in patients with lung injury managed with conservative fluid balance (Mikkelesen *et al.* 2012).

Sodium administration is often coupled with chloride, usually as a 1:1, ratio except for fluids such as Hartmanns solution where the

ratio is 1.2:1. Although chloride administration was not directly measured in the present study it can be hypothesised that high sodium administration would have accompanying high chloride administration which may have adverse effects. Effects of chloride restriction on the acid base status of ICU patients has recently been investigated (Yunos *et al.* 2011) and implementation of a chloride-restrictive strategy in a tertiary ICU was associated with a significant decrease in the incidence of kidney injury and requirement for dialysis in ICU (Yonus *et al.* 2012).

Large amounts of administered sodium, together with the propensity for sodium retention and a conservative fluid balance, can lead to hypernatremia. Hypernatremia is not uncommon in the critically ill (Palevsky *et al.* 1996) and, in the present study, 19.3% of patients had a serum sodium ≥145 mmol/L and 5% had hypernatremia (≥ 150 mmol/L). This rate of hypernatremia is consistent with the literature (Lindner *et al.* 2009) and has previously been associated with poor outcomes (Hoorn *et al.* 2008; Lindner *et al.* 2009).

3.2.5 LIMITATIONS AND FUTURE DIRECTIONS

This single day point prevalence study conducted across multiple ICUs in Australia and New Zealand represents a snapshot of current practices for critically ill patients not receiving oral nutrition on a study day. Despite our best efforts to record all i.v. and enteral

fluids administered, it is possible that other sources of sodium were not included. Formal sodium balance and the indications for the prescription of high sodium containing fluids such as 0.9% saline were also not collected. Finally, inferences regarding sodium administration on day 1 are limited as data collection was less than 24 hours.

Inadvertent high levels of sodium administration are potentially modifiable as i.v sodium sources are primarily maintenance fluids, vehicles for infusions and drug boluses and flushes. Future studies should include both prospective, observational studies examining the relationship between sodium administration, sodium balance and important clinical outcomes (e.g. mortality) and interventional trials to assess whether it is desirable and/or safe to modify daily sodium administration in critically ill patients, and can be coupled with simultaneous measurement of chloride administration and balance.

3.2.6 SUMMARY

Currently there are high levels of sodium administration in multiple ICUs across Australia and New Zealand in a large cohort of patients. The majority of administered sodium is from inadvertent sources. However, there is wide variability in the use of infusions/vehicles for drug infusions and boluses.

3.3. Sodium administration in critically ill paediatric patients in Australia and New Zealand: a multi-centre point prevalence study

In the previous single-centre adult intensive care unit (ICU) study the amount of administered sodium given to critically ill adults receiving mechanical ventilation for greater than 5 days was more than twice the recommended daily intake (Section 3.1). The main sources of sodium were intravenous maintenance fluids, flushes and drugs (Section 3.1). These findings were later confirmed in a larger multi-centre, point prevalence study in adult ICU patients in which the main source of administered sodium was intravenous maintenance fluids, followed by fluid boluses and drug boluses (Section 3.2).

In critically ill paediatric patients sodium and water balance are no longer under the direct control of the individual's response to thirst or choice of dietary intake. Isotonic maintenance fluids are often administered in children, in order to reduce the risk of hyponatremia (Choong *et al.* 2011; Coulthard *et al.* 2012; Moritz *et al.* 2011; Balasubramanian *et al.* 2012) and this may increase the risk of sodium and fluid overload; especially in the critically ill where a complex interplay of several homeostatic mechanisms including activation of the renin-angiotensin-aldosterone system (Jungmann *et al.* 1987) and impaired activity of dopamine in the proximal tubule of the kidney (Seri *et al.* 1988) (dopamine normally inhibits sodium reabsorption (Seri *et al.* 1990) promote increased

sodium retention. Moreover, an increased level of arginine vasopressin released by the posterior pituitary in acute illness also promotes water reabsorption from the collecting ducts of the kidneys (Holmes *et al.* 2001; Iwasaki *et al.* 1995).

Data from the Prospective Paediatric Continuous Renal Replacement Therapy (ppCRRT) registry (Goldstein *et al.* 2004; Symons *et al.* 2007) and other observational studies (Goldstein *et al.* 2005; Michael *et al.* 2004; Foland *et al.* 2004) have shown that positive fluid balance is an independent predictor of mortality in children. The mechanism of the detrimental effects of fluid overload is probably multifactorial, but likely contributed to by the expansion of extracellular fluid space (Jacob *et al.* 2009) and increased distance for oxygen diffusion, as well as alterations in cell volume. Sodium is an extracellular ion and can affect the intra- and extracellular fluid distribution, with the potential to exacerbate interstitial edema in both the lung and other organs.

The aim of this study was to determine the total administered sodium and its sources and fluid balance over a single 24-hour period in infants and children in ICU in Australia and New Zealand.

3.3.1 METHODS

A multi-centre, single day, point prevalence study was undertaken in collaboration with the Paediatric Study Group (PSG) of the Australian and New Zealand Intensive Care Society (ANZICS)

Clinical Trials Group (CTG). Ten ICUs (8 dedicated paediatric and 2 mixed adult and paediatric) caring for the majority of critically ill or injured infants and children in Australia and New Zealand participated on the study day (Appendix 3). Neonatal ICUs, defined as units caring solely for newborn infants, were excluded from this study. Approval was obtained from individual participating site research ethics committees, with the requirement for individual subject consent waived at all sites. This ensured recruitment of all patients <16 years present in the ICU at 10 am on the study day.

Demographic and descriptive data including age, sex, weight (estimated and measured), admission paediatric index of mortality (PIM II) score (Baghurst *et al.* 2008), admission diagnosis and specific diagnoses on study day (acute lung injury [ALI], acute respiratory distress syndrome [ARDS], sepsis) were documented. Major treatment interventions (e.g. invasive mechanical ventilation, renal replacement therapy) on the study day were also collected. The respiratory component of the organ failure score as assessed by the sequential organ failure assessment (SOFA) respiratory score (Vincent *et al.* 1996) on the study day was also recorded. The highest and lowest serum sodium on the study day was recorded and patients categorised as having normal or hyper- or hyponatremia; serum sodium ≥ 150 mmol/l (Lindner *et al.* 2009) or < 130 mmol/l (Asadollahi *et al.* 2006) respectively.

Patients receiving normal oral diet who did not receive prescribed formula (enteral or parenteral) feed at any time during the study day were described as having “free oral intake” and were excluded from the study analysis. Data on all sources of administered sodium (excluding any non-prescribed oral diet) were recorded for the remaining patients. The absolute and relative contribution of the following potential sources of administered sodium were categorised as: (i) intravenous bolus, (ii) intravenous infusions -maintenance or replacement fluids, (iii) blood products i.e. red blood cells, platelets, fresh frozen plasma, (iv) intravenous drug boluses including fluid given as a diluent or a vehicle for administration, (v) intravenous drug infusions including fluid given as a diluent or a vehicle for administration (vi) intravenous flushes associated with haemodynamic monitoring of arterial or central venous catheter, (vii) prescribed enteral nutrition, (viii) prescribed parenteral nutrition.

Patients were categorised by the number of completed days in ICU at the onset of the study day (< 24 hours completed vs. ≥ 24 hours and <10 days, vs. ≥10 days). For patients in ICU < 24 hours at the time of the study census, data for individual patients was cohorted regardless of the exact number of hours spent in ICU. For all parenteral fluids and blood products, the type and volume administered over the previous 24-hour period was recorded and the amount of sodium administered was calculated based on their

sodium concentrations (Appendix 1). For drug infusions and boluses, sodium content was calculated from both the sodium content of the drug and the type and volume of carrier fluid or diluent. Antibiotics are reported as a subgroup of all drug boluses or infusions as it is known that they contribute a significant sodium load in adults in intensive care (Section 3.1 and 3.2). For prescribed enteral and parenteral nutrition, information on the type and volume of feed was recorded and the sodium content calculated accordingly. For custom parenteral nutrition, the sodium content was recorded. No data on sodium intake from oral intake was collected.

3.2.2 STATISTICAL ANALYSIS

Variables are reported as mean and standard deviation (SD) or median and interquartile range (IQR) and compared with either the Student t-test or Mann-Whitney U test, as appropriate. Sodium administration from each source is reported as percentage of total administered sodium with 95% CI.

Pearson's correlation was used to describe the association between administered sodium (log transformed for normal distribution) and the following variables: age, weight, day of ICU stay, SOFA respiratory score, serum sodium, fluid administered and 24 hour fluid balance on study day. Predictor variables for sodium administration were analysed using multiple linear regression

(stepwise method) (SPSS version 2.0, Chicago, IL, USA. Day 1 data was not included in the model as 24-hour data was incomplete (median [IQR] ICU length of stay 14[11-18] hours).

Data for infants and older children were also analysed separately as they may have different sources of fluid and sodium. Moreover infant kidneys may be undergoing developmental changes (Quigley 2012).

For all analyses, a P value of less than 0.05 was considered statistically significant.

3.3.3 RESULTS

3.3.3.1 Patient Characteristics

The study screened 65 subjects from 10 participating PICUs on a single study day. Seventeen subjects (26.2%) were excluded from the study because they had free oral feeds (n=15, 23.1%) or missing data (n=2, 3.1%)

Of the remaining 48 patients (24 males and 24 females), the contribution from each site was 4(1-9) children of which 21(44%) were infants [age (median, IQR) 4.0 (1.0-7.0) months; body weight 5.0 (3.5-6.1) kg], and 27 (56%) were > 1 year [age 3.0 (1.5-13.0) years; body weight 17.0 (9.5-47.5) kg]. The reason for PICU admission and other characteristics are shown in Table 3.4; the

profile was typical of PICU patients (Report of the Australian and New Zealand Paediatric Intensive Care Registry 2013).

Table 3.4: Patient characteristics in the paediatric ICUs on the point prevalence study day

Characteristic	**n=48**
Infants, n (%)	21 (43.7)
Age, months[$]	4.0 (1.0-7.0)
Weight, kg [$#]	5.0 (3.5-6.1)
Age > 1 year, n (%)	27 (56.3)
Age, years[$]	3.0(1.5-13.0)
Weight, kg [$#]	17.0 (9.5-47.5)
Male sex, n (%)	24 (50.0)
ICU admission source, n (%)	
Operating theatre	21 (43.7)
Elective surgery	20 (41.6)
Emergency surgery	1 (2.1)
Post cardiopulmonary bypass	9 (18.7)
Transfer from other ICU	10 (20.8)
Transfer from other hospital	7 (14.6)
Hospital ward	6 (12.5)
Emergency ward	4 (8.3)
ANZPICR diagnostic categories, n (%)	
Injury	5 (10.4)
Respiratory	13 (27.1)
Cardiovascular	7 (14.6)
Neurological	1 (2.0)
PIM ROD (Risk of Death) [$]	0.02 (0.01-0.06)
PIM HI-RISK	7 (14.6)
PIM LO-RISK	5 (10.4)
SOFA Respiratory score	2 (0-3)
Trauma	3 (6.3)
Burns	1 (2.1)
Sepsis on study day, n (%)	7 (14.6)

ALI/ARDS on study day, n (%)	6 (12.5)
Renal replacement therapy, n (%)	1 (2.1)
Mechanical ventilated in the first hour, n (%)	34 (70.8)
Discharged from ICU at 28-day (alive), n (%)	38 (79.2)
Discharged from hospital at 28-day (alive), n (%)	27 (56.3)
28-day ICU mortality, n (%)	2 (4.2)

 # Weight estimated or actual (38/48 [71.2%] measured)
$ Median and Interquartile range

3.3.3.2 Administered sodium and 24-hour fluid balance

Overall, the median (IQR) administered sodium in all patients (n = 48) was 4.9 (3.2 – 8.0) mmol/kg/24 hours on study day. Median administered fluid was 80.8 (49.8 - 111.4) ml/kg and median fluid balance was 9.0 (-1.4 to 41.0) ml/kg. Median urine output was 44 (24-78) ml/kg on the study day.

Twenty-four hour administered sodium, fluid and fluid balance according to day of stay in ICU are shown in Table 3.5. Total 24 hour administered sodium, administered fluid totals and fluid balance on the study day were not significantly different between patients in ICU for less than 10 days compared to patients in ICU for 10 days or more (p=0.18, p=0.39 and p=0.67 respectively).

Table 3.5: Daily administered sodium and fluid balance according to the day in PICU

ICU day	n	Daily administered sodium/kg mmol/kg	Daily administered fluid ml/kg	Daily fluid balance ml/kg
<1*	5	3.3 (1.2-5.0)	33.0 (25.3-48.2)	9 (5.3-17.7)
2-10	29	4.6 (3.2–7.9)	81.6 (53.9-106.2)	18.5 (1.1-41.3)
>10	14	6.1 (3.5–10.7)	106.5 (66.7-126.4)	0.5 (-10.4 - 19.5)
TOTAL	48	4.9 (3.2–8.0)	80.8 (49.8 - 111.4)	9 (-1.4 – 41.0)

**Median (IQR) hours 14 (11-18). Data expressed as median and IQR*

The twenty-four hour administered sodium, fluid and fluid balance in infants (n=21) and children >1year (n=27) was 6.0 (3.9–8.1) mmol/kg, 102.6 (80.0–127.7) ml/kg, 20.8 (3.5–47.2) ml/kg and 3.5 (3.1–7.8) mmol/kg, 58.1 (35.7–85.7) ml/kg, 5.3 (-2.7–17.7) ml/kg respectively. Although the total administered fluid volume recorded on the study day was greater in infants ($p<0.001$), administered sodium and 24-hour fluid balance were not statistically different between infants and children > 1 year of age ($p=0.53$, $p=0.08$ respectively).

Twenty-four hour administered sodium, fluid and fluid balance according to diagnostic category are shown in Table 3.6. Administered sodium in children with sepsis and children admitted with trauma were median (IQR) 6.8 (3.3- 16.1) and 8.9 (4.6-18.0) mmol/kg respectively. Median sodium administration across the

various sites was 5.3 (3.2 - 6.9) mmol/kg and it was not different between them.

Table 3.6: Sodium administration according to diagnostic category on study day in paediatric patients

Diagnostic category: n (%)	Administered sodium mmol /kg	Administered fluid ml /kg	Fluid balance ml/kg
Elective admission: 21 (43.8)	3.9 (2.9 -6.9)	80.0 (43.4 -121.2)	18.5 (-2.7 - 56.4)
Trauma: 3 (6.3%)	8.9 (4.6-18.0)	56.0 (63.7 -115.1)	19.5 (20.4 -84.1)
Post bypass surgery: 9 (18.8%)	3.4 (2.9 -6.2)	63.7 (43.4 - 94.9)	16.5 (-20.5-33.1)
Sepsis: 7 (14.6%)	6.8 (3.3- 16.1)	73.2 (46.4 -103.4)	5.3 (-10.4 - 24.3)
Burns : 1 (2.1%)	18.0	115.1	84.1
ALI/ARDS: 6 (10.6%)	5.4 (3.3 -12.4)	60.9 (42.2 -101.2)	-4.3 (-23.5 - 5.2)
Other*: 22 (45.8%)	5.0 (3.1-7.8)	90.1(58.9-125.0)	9.0 (0.3 - 48.3)
PIM HI-Risk: 7 (14.6%)	3.5 (3.2 - 7.9)	67.6 (50.0 -73.6)	17.7 (0.5 - 24.3)
PIM LO-Risk: 5 (10.4%)	3.9 (1.6 - 6.3)	63.4 (33.9 -91.8)	17.7 (8.9 - 31.7)
PIM ROD (Top 50%)	5.6 (3.5 -10.8)	87.7 (56.5 -117.3)	7.0 (0.5 - 24.3)
PIM ROD (Bottom 50%)	3.9 (2.7 -7.2)	75.1 (41.4-103.2)	13.4 (-5.9 - 43.5)

*Data expressed as median (IQR). Post–operative, trauma (operative and non-operative) and burns diagnostic categories were at ICU admission. Sepsis and ALI/ARDS diagnoses were on study day. Abbreviations: PIM, Paediatric Index of Mortality; ALI, acute lung injury; ARDS, acute respiratory distress syndrome. * Other included patients without trauma, post bypass surgery, sepsis, burns or ALI/ARDS.*

Sodium administration on the study day was correlated with day of stay on ICU ($p=0.003$, $r=0.47$), age ($p=0.005$, $r=-0.44$), 24-hour administered fluid/kg ($p<0.001$, $r=0.68$) and weight ($p=0.013$, $r=-0.38$). Using multiple linear regression modelling ($R^2=0.46$), administered sodium was only associated with 24-hour administered fluid/kg (unstandardized β coefficient (standard error) 0.08 (0.02), $p < 0.001$)

A number of patients (14/48; 29.2%) had only one serum sodium reading for the study day and hence we have reported the highest available serum sodium on the study day. The highest recorded serum sodium on the study day was (mean ±SD) 143.2 ± 6.6 mmol/L. Twelve patients (25%) had a serum sodium ≥145 mmol/L, 3 had hypernatremia and 1 had hyponatremia on the study day.

3.3.3.3 Sources of administered sodium

The contributions to the total sodium administered in all the patients (n = 48) is shown in Table 3.7.

Table 3.7: Contribution of sodium according to day of stay in PICU

Source of Administered Sodium (% total Na administered in 24 hours)	ICU stay on the study day <10 day* (n=29)	ICU stay on the study day >10 day (n=14)	Total (n=48)
Bolus	27.4 (25.5-29.3)	7.8 (6.4-9.2)	17.9 (16.7–19.1)
Maintenance/replacement infusions	25.4 (23.5-27.2)	17.6 (15.6-19.5)	23.9 (22.5-25.2)
Blood Products	2.2 (1.6-2.8)	5.2 (4.0-6.3)	3.1 (2.5-3.6)
Drug boluses	13.9 (12.5-15.4)	9.4 (7.9-10.9)	14.9 (13.7–16.0)
Antibiotics	10.1 (8.8-11.3)	10.9 (9.2-12.5)	10.5 (9.5–11.4)
Drug infusion	4.2 (3.3-5.0)	15.3 (13.4-17.1)	7.9 (7.1–8.7)
Flushes	5.9 (4.9-6.9)	3.1 (2.1-3.9)	4.4 (3.8–5.0)
Enteral feeds	10.6 (9.3-11.9)	27.6 (25.3-29.9)	16.2 (15.1–17.3)
Parenteral nutrition	0 (0.0-0.0)	3.1 (2.1-3.9)	1.1 (0.8–1.4)

Data represented as percentage and 95% CI
* Data excludes children whose stay in PICU was less than 24 hours

Overall, fluid infusions, boluses and catheter flushes were the major sources of administered sodium (46.2% total). Drugs, including antibiotics administered by intravenous bolus or infusion, also contribute substantially (33%) to administered sodium, with antibiotics accounting for the majority of sodium administered as drugs.

Table 3.7 shows subgroup analysis of patients by length of stay in ICU. For infants and children in ICU > 1 and < 10 days (n= 29), intravenous fluid administered as bolus or infusion contributed to the majority of administered sodium, whereas in infants and children who were in ICU > 10 days, the greatest proportion of administered sodium was administered via enteral feeds.

Intravenous maintenance or replacement fluid was administered to 34 patients (71%) in the study cohort on the study day. The type of fluid used for intravenous infusions (maintenance or replacement) is shown in Table 3.8. As well as being the most commonly used maintenance and replacement fluid, 0.9% sodium chloride was also the fluid used most commonly as a vehicle for intravenous drug boluses and infusions (65.8% and 68.2%, respectively). Heparinised 0.9% sodium chloride was the most common intravenous or intra-arterial flush fluid (97.1%).

Table 3.8: Types of maintenance / replacement fluid used on the study day in paediatric patients (n=34 patient, 71% of patients)

Type of maintenance or replacement fluid	n (%)
0.9% sodium chloride	8 (16.7%)
5% dextrose + 0.45% sodium chloride	6 (12.5%)
10% dextrose + 0.45% sodium chloride	5 (10.4%)
2.5% dextrose + 0.45% sodium chloride	5 (10.4%)
0.45% sodium chloride	4 (8.3%)
Hartman's solution	2 (4.2%)
4% dextrose + 0.18% sodium chloride	2 (4.2%)
5% dextrose	1 (2.1%)
3% sodium chloride	1 (2.1%)

Sources of sodium administration such as drug infusions, drug boluses, and intravascular flushes taken together inadvertently provide a high burden of daily administered sodium load (27.2%).

3.3.4 DISCUSSION

This study showed that children in PICU were administered much more sodium than expected. The median administered sodium was far greater than recommendations based on the Nutrient Reference Values for Australia and New Zealand guidelines (Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes 2013) with more than 75% of the study cohort receiving over 3 mmol/Kg of sodium on the study day. It is noteworthy that though the main source of sodium changed, with increased enteral sodium administration observed in patients in

PICU for more than 10 days, overall sodium intake remained high throughout the PICU admission.

The major source of sodium administration was intravenous fluid, mainly in the form of intravenous fluid infusions (for example maintenance fluids, replacement fluids), as well as intravenous drug infusions, boluses and flushes. This finding is similar to a single centre study in an adult ICU (Section 3.1) and to a report in adult cardiac patients (Tafreshi *et al.* 2011), but has not been previously reported in the paediatric intensive care population. It is noteworthy that 0.9% sodium chloride was the most common i.v. infusion fluid used as a vehicle for administration of drugs both as infusions and boluses and for flushing intra-vascular catheters, and contributed substantially to the high levels of administered sodium in children in this study. There is evidence to suggest that parenteral maintenance solutions with a high sodium concentration may be significantly safer than hypotonic solutions in protecting against acute postoperative hyponatremia in children. However, in adults recent studies have indicated that excretion of both water and sodium is slower after a bolus of 0.9% sodium chloride compared to balanced solutions (Reid *et al.* 2003), and may result in reductions in renal blood flow velocity and renal cortical tissue perfusion (Chowdhury *et al.* 2012).

The most commonly used intravenous fluid in our study was 0.9% sodium chloride. Chloride administration is a recognised cause of normal anion gap acidosis in paediatric intensive care (Taylor *et al.* 2006) and implementation of a chloride-restrictive strategy may have less deleterious effects, as manifested by kidney injury and requirement of dialysis (Yunos *et al.* 2012).

Although a portion of the administered sodium is inevitably required for the management of critically ill children, for example in bolus and maintenance fluids, some of the administered sodium, such as that present in the vehicle used for drug infusion, boluses and intravascular flushes, inadvertently provide more than one quarter of the administered sodium load, and are largely avoidable. Attention to minimizing these inadvertent sources of sodium is a possible target for a sodium restriction strategy.

Fluid balance was also positive in most patients, with 25% of patients in the cohort recorded to be more than 40ml/kg positive over the 24-hour study period. Compared to older children, infants less than 1 year of age received more sodium per kg body weight and had a more positive fluid balance, with 75% of the infants in the cohort recorded to have received more than 80 ml/kg fluid on the study day. Positive fluid balance in adults and children in ICU is associated with poor lung and kidney function (Wiedemann *et al.* 2006; Arikan *et al.* 2012; Bouchard *et al.* 2009), delayed return of

gastrointestinal function after surgery (Lobo *et al.* 2002) and an increased risk of mortality (Goldstein *et al.* 2004; Symons *et al.* 2007; Goldstein *et al.* 2005; Michael *et al.* 2004; Foland *et al.* 2004; Boyd *et al.* 2011). Valentine *et al.* have reported that a positive and increasing fluid balance on day 3 of ICU stay in children with acute lung injury in PICUs was independently associated with fewer ventilator-free days (Valentine *et al.* 2012). The adverse effects of positive fluid balance may be due to extracellular fluid expansion. Moreover, a positive fluid balance coupled with a high sodium administration, as seen in many of the patients in our study, can potentially lead to cellular dehydration and interstitial oedema in both the lungs and the systemic circulation, and it is postulated to be a potential mechanism contributing to abnormal neurocognitive effects in patients with lung injury managed with conservative fluid balance (Mikkelsen *et al.* 2012).

The serum sodium observed in this study is similar to studies performed in neonates (Balasubramanian *et al.* 2012). The prevalence of observed hypernatremia is consistent with the literature (Forman *et al.* 2012) and has been associated with poor outcomes (Holliday *et al.* 2003).

3.3.5 STUDY LIMITATIONS AND FUTURE DIRECTIONS

These data are from a single study day across multiple PICUs in Australia and New Zealand and thus represent a snapshot of current practice. Despite our best efforts (multiple pilot trials) to record all intravenous and enteral fluids administered, as with the adult study, errors in data collection cannot be excluded. Moreover, we excluded patients who were taking free oral intake as it was difficult to ascertain the sodium content of all oral feeds with the current study design. Despite this, some of the included patients were allowed oral feeds, hence the total administered sodium might be an underestimate. Indications for the prescription of high sodium containing fluids such as 0.9% sodium chloride were not recorded and they may have been used as a therapeutic modality. Our observations are based on calculated fluid balance in the study cohort; we did not weigh the patients before and after the study day as this was beyond the scope of our study. In addition, as sodium intake, calculated as volume times concentration for both i.v. and enteral fluids, was the main aim of the study, fluid balance has some advantages over weighing as it is internally consistent.

There were high levels of sodium administration in this cohort of PICU patients. This result should be confirmed in a longitudinal study in a larger group of patients. Future studies should include measurement of the estimated sodium balance from measured urinary sodium and examine this effect on oxygenation, serum

sodium and patient related outcomes (length of stay and mortality). Similarly, serum chloride and total chloride administration should also be measured and the effect on body pH should be examined. Furthermore specific subgroups such as children with renal failure and respiratory failure should be examined separately.

As the current level of administered sodium is high, future studies should examine strategies to decrease the amount of sodium administered via inadvertent sources, such vehicles for drug infusions and drug boluses and flush for intravascular catheters, and should examine the effect of such strategies on sodium balance and clinical outcomes.

3.3.6 SUMMARY

This study demonstrates high levels of administered sodium and positive fluid balance in the majority of patients across a cohort of paediatric patients in PICUs across Australia and New Zealand. The significant contributions of maintenance and bolus intravenous fluids, most commonly 0.9% sodium chloride, drug infusions including antibiotics, and enteral feeds, are noted. Future studies should evaluate the effect of this practice on overall sodium balance and patient outcomes.

3.4 Inadvertent sodium loading with renal replacement therapy in critically ill patients

Acute kidney injury (AKI) is a common finding among patients in the intensive care unit (ICU) (Hoste *et al.* 2006) and is an independent predictor of mortality (George *et al.* 2007; Uchino *et al.* 2005). Severe AKI requiring the use of renal-replacement therapy affects approximately 5% of patients admitted to the ICU and is associated with a mortality rate of 60% (Uchino *et al.* 2005).

Fluid overload is common in critically ill patients and is associated with poor lung and kidney function (Wiedemann *et al.* 2006; Bouchad *et al.* 2009), delayed return of gastrointestinal function after surgery (Lobo *et al.* 2002) and increased mortality (Boyd *et al.* 2011). In the previous section of this chapter it was shown that critically ill patients inadvertently receive high amounts of sodium during their stay in ICU (Section 3.1, 3.2) despite careful attention to negative fluid balance. Although renal replacement therapy (RRT) is often used to manage fluid overload, in addition to metabolic control in AKI, it has been recently reported that in patients requiring chronic haemodialysis, the dialysis procedure itself may become a de facto source of sodium loading rather than a means for sodium removal (Thijssen *et al.* 2011; Santos *et al.* 2010). This is mainly because low patient serum sodium results in a positive dialysate-to-serum sodium gradient (Keen *et al.* 2007). Such a phenomenon is also possible in critically ill patients during RRT; however, the effect on sodium balance is currently unknown.

We hypothesised that RRT contributes to patient sodium load independent of fluid balance, therefore we investigated the flux of sodium during RRT.

3.4.1 METHODS

Sixty patients (20 patients in each of 3 modes of RRT – see below) were recruited from September 2011 to December 2012 in a single centre tertiary level ICU. This study protocol was approved by the institutional human research ethics committee (#361/11) (Appendix 4). All ICU admissions underwent daily screening for eligible patients from 0800 to 1100 Monday to Friday during the study interval and patients who were initiated on dialysis during this time frame were consecutively included. Patients with a history of chronic haemodialysis, or peritoneal dialysis or those who were receiving citrate as a form of anticoagulation were excluded.

Either an intermittent technique - extended daily dialysis (EDD) (Fresenius 4008S; Bad Homburg, Germany) or a continuous technique - continuous renal replacement technique (CRRT) (Aquarius 6.01; Langenhagen, Germany) for RRT were studied. Blood flow from the patient was constant at 200 ml/minute.

3.4.1.1 EDD - Patients dialysed with intermittent RRT underwent extended haemodialysis (there were no patients with extended haemofiltration or haemodiafiltration). The affluent fluid going in to the dialysis was sampled; similarly the effluent fluid was sampled

for the amount of sodium present every hour throughout the duration of the dialysis. These samples were analysed for sodium with a Radiometer ABL 700 Series (Brønshøj, Denmark). The amount of sodium prescribed on the machine was noted.

3.4.1.2 CRRT - Patients undergoing continuous RRT were either undergoing continuous veno-venous haemofiltration (pre-filter fluid replacement) (CVVH) or continuous veno-venous haemodiafiltration (post filter fluid replacement) (CVVHDF). The amount of sodium in the dialysis bags (affluent) (5L Baxter Haemofiltration Replacement fluid or 5 L Gambro Hemosol BO fluids) was 140 mmol/litre. Twenty random samples were taken from these bags to check for the accuracy of these bags. The waste bags (effluent) during dialysis were weighed on a Conair weighing scale (model number-WW147A; Guangzhou, China) before discarding them (1 to 2 bags at a time) and a sample was taken from them for sodium analysis. These samples were also analysed for sodium with the Radiometer ABL 700 Series. The weighing scales were calibrated against a known set of weights ranging from 5 to 20 Kg (A&D Mercury Pty. Ltd.; NATA Accredited Laboratory no. 3811; Ref No A/m/037; File No: SA/M/027).

Sodium balance was calculated as the difference between the affluent and the effluent fluid sodium content corrected for volume.

The duration of study was either the duration of a single EDD session (6 to 10 hours) or 24 hours of CRRT. Each patient who may have received multiple sessions / days of RRT during their stay in ICU was enrolled only once for the study.

Data regarding patient demographics, dialysis duration, day of dialysis, total litres of dialysis/filtration, net fluid removal, interruption during dialysis were also recorded, in addition to their serum sodium (at initiation and end of study), serum urea, albumin, osmolality, serum glucose, requirement of invasive mechanical ventilation and vasopressors and total fluid balance on the study day was also recorded.

3.4.2 STATISTICAL ANALYSIS

Data are reported as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate for the distribution of each variable. Analysis was performed with the SPSS software (version 19.0, Chicago, IL, USA). Data from EDD, CVVH (pre-filter) and CVVHDF (post-filter) were analysed separately as it was hypothesised that they may have differential effect on sodium flux. Differences between the groups were analysed with Chi square test or Kruskall-Wallis test or one way ANOVA, as appropriate, and tukey's test was used for post hoc analysis. Change in serum sodium pre and post dialysis was tested with paired t test. Bland-Altman analysis was used to study the calibration and is reported as mean and SD of the difference. Pearson's correlation was used to

test for the association between the factors and the sodium flux during dialysis in ICU. In univariate analysis, variables were individually assessed as predictors of the sodium flux during dialysis in ICU. Variables significant at alpha ≤ 0.10 level were then assessed for possible inclusion in the multivariate model. A conventional alpha level of < 0.05 was used for all significance testing.

3.4.3 RESULTS

3.4.3.1 Demographics

Sixty suitable patients undergoing RRT were included in the study with 20 in each EDD, CVVH, and CVVHDF groups. Their demographics, serum sodium, albumin, urea, glucose and osmolality are shown in Table 1. There was no difference between the groups with respect to age, APACHE II score or serum sodium, albumin, urea, glucose and osmolality at the start of RRT. Use of invasive mechanical ventilation and vasopressors on the study day is also shown in Table 3.9. Fewer patients on EDD required vasopressors on the study day.

Table 3.9: Demographics, blood results of patients dialysed in the study

	EDD (n=20)	CRRT (n=20) CVVH (pre filter)	CRRT (n=20) CVVHDF (post-filter)
Gender (M/F)	12/8	10/10	13/7
Age (years)*	66 (28 – 81)	64 (28 – 90)	62(33-85)
APACHE II *	28 (16 – 40)	29(17 – 44)	28(15-42)
Mechanical ventilation on the study day n,(%) *	16 (80)	15 (75)	17 (85)
Vasopressor use on the study day n,(%)$	12 (60)	18(90)	17(85)
Dialysis episode / day*	2.0±2.3	3.0±2.2	2.1±2.1
Serum Sodium at start (mmol/l) *	137 (135-138)	135 (133 -138)	135 (134 -138)
Serum Sodium at end (mmol/l)*	138 (136-141)	137 (135-140)	137 (134-139)
Serum Albumin at start (g/L) *	28±6	28±5	27±4
Serum Urea at start (mmol/L) *	21±11	16±9	18±8
Serum Glucose at start (mmol/L) *	8±3	8±2	7.5±4
Serum Osmolality at start*	305±13	296±13	298±15

Data represented as median (IQR) or mean ± SD as appropriate.
** No statistically significant difference between groups was present for any of the listed parameters (Tested with ANOVA / Kruskall-Wallis test /Chi square as appropriate)*
$ There was a difference between the groups (p=0.048), tested with Chi square test

3.4.3.2 EDD:

Duration of dialysis was 8.2 ± 2.1 hours. Average sodium in the affluent dialysate was 139 ± 3 mmol/l. The difference (mean and SD) between the prescribed sodium in the Fresenius 4008S and measured sodium in the affluent fluid was 0.05 ± 0.51 mmol/l. Dialysis flow rate was 200 ± 31 ml/minute. Data regarding total dialysate used for EDD, total fluid removed during the dialysis, total flux of sodium in to the patient (total and per L of dialysate) and fluid balance on the study day are shown in Table 3.10.

Table 3.10: Total dialysate, sodium flux and fluid removed during dialysis in ICU

	EDD	CVVH	CVVHDF
Total dialysate used (L)*	106.3±40.3	53.3±22.8	55.3±26.2
Sodium flux (total mmol) *	186±155	305±196	355±180
Sodium flux (mmol per 1L exchange) *	2±2	5±3	6±2
Total fluid removed (L) *	0.9±0.9	1.3±1.5	1.4±1.1
Fluid balance on study day (ml) $	832 (121-1956)	746 (568-2544)	958 (429-2473)

** Data presented as mean ± standard deviation*
$ Data presented as median and IQR

Positive sodium flux in to the patient correlated with the gradient between RRT sodium and serum sodium and had a negative correlation with the total fluid removed. (Table 3.11).

Table 3.11: Correlations with sodium flux with dialysis

	EDD		CRRT			
			CVVH		CVVHDF	
	r	p value	r	p value	r	p value
Delta RRT sodium and initial serum sodium	0.56	0.009	0.64	<0.001	.66	<0.001
Total fluid removed	-0.53	0.01			-.34	.05
Total litre of exchange			0.73	<0.001	0.64	.001
Osmolality			-0.4	0.01		

Multivariate regression modelling identified factors [exp(b)(SE),Pvalue] which significantly affected sodium balance; these were gradient between RRT sodium and serum sodium [20.9(5.8),p<0.02] and total litres of exchange (dialysis) [1.5(0.68),p<0.04] (R^2=0.42).

There was no difference between serum sodium at the start (137 (135-138)mmol/l) and end (138 (136-141) mmol/l) of EDD (p=0.7).

3.4.3.3 CRRT:

The difference (mean and SD) between the sodium in replacement bags (affluent) (labelled as 140 mmol/l) and the sodium measured by the Radiometer ABL 700 Series was 0.00 ± 0.55mmol/l. The difference (mean and SD) between the weight of the replacement bags: two at a time; labelled as 5 kg each and then weighed on the weighing scale was 0.03 ± 0.18 kg. The difference (mean and SD) between the known weights measured on a standard scale ranging from 5 to 25 kg (total 30 measurements) and then measured on the weighing scale used for the study was 0.008 ± 0.05 kg.

CVVH (pre-filter fluid replacement): Duration of dialysis was 23.0 ± 1.0 hours. Total dialysate used for CVVH was 53.3 ± 22.8 litres. The replacement fluid flow rate was 2.6 ± 0.9 litre/hour. Data regarding total dialysate used, total fluid removed during the dialysis, total flux of sodium in to the patient (total and per L of dialysate) and fluid balance on the study day are shown in Table 3.10.

Positive sodium flux in to the patient correlated with gradient between the RRT sodium and serum sodium, total litres of exchange and had a negative correlation with serum osmolality. (Table 3.11). Multivariate regression modelling identified factors [exp(b)(SE),Pvalue] which significantly affected sodium balance. These were gradient between the RRT sodium and serum sodium [21.8(4.7),p<0.001] , dialysis day [-20.9(9.8), p<0.05] and total litres of exchange (dialysis and filtration) [5.2(0.96),p<0.001] (R^2=0.77). There was no difference between serum sodium at the start (135 (133 -138)mmol/l) and end (137 (135-140) mmol/l) of CVVH (p=0.4).

CVVHDF (post-filter fluid replacement): Duration of dialysis was 22.2 ± 1.1 hours. Total dialysate used for CVVHDF was 55.3 ± 26.2 litres. The replacement fluid flow rate was 2.8 ± 0.6 litre/hour. Data regarding total dialysate used, total fluid removed during the dialysis, total flux of sodium in to the patient (total and per L of dialysate) and fluid balance on the study day are shown in Table 3.10.

Positive sodium flux in to the patient correlated with gradient between the RRT sodium and serum sodium, total litres of exchange and had a negative correlation with total fluid removed. (Table 3.11) Multivariate regression modelling identified factors [exp(b)(SE),Pvalue] which significantly affected sodium balance were

gradient between the RRT sodium and serum sodium [23.8(3.7),p<0.001] and total fluid removal [-18.5(3.26),p<0.001], (R^2=0.73).There was no difference between serum sodium at the start (135 (134 -138)mmol/l) and end (137 (134-141)) of CVVHDF (p=0.5).

CVVH and CVVHDF resulted in similar sodium flux per litre exchange in to the patient (p=0.9) but both were greater than EDD (p<0.001 for both comparisons) despite greater fluid removal.

Though in each of the individual dialysis mode, there was no change in serum sodium at the start and end of dialysis, when all the serum sodium values were combined and examined as tertiles there was a significant change in serum sodium at the start and end of dialysis in the lowest (132.6 ± 3.0 to 136.4 ± 3.9 mmol/l, p<0.001) and the highest tertile (141.3 ± 4.1 to 138.5 ± 2.9 mmol/l, p=0.001) while there was no change in the middle one (136.0 ± 0.8 to 136.7 ± 2.4, p=0.21).

3.4.4 DISCUSSION

Critically ill patients undergoing RRT inadvertently receive a sodium load despite net fluid removal. The major reason for this appears to be the positive gradient between the dialysate/replacement fluid sodium and the patient's serum sodium at the start of the dialysis.

Why sodium is important in ICU

The association of positive fluid balance with adverse outcomes (Wiedemann *et al.* 2006; Bouchard *et al.* 2009; Lobo *et al.* 2002; Boyd *et al.* 2011) is believed to be due to extracellular compartment expansion, as water distributes to both intra and extracellular spaces. However, sodium primarily distributes into the extracellular space, which may have differential effects on extracellular volumes, cause cellular dehydration and exacerbate interstitial oedema in both the systemic and pulmonary circulations.

Furthermore, positive pressure ventilation, and positive end-expiratory pressure result in elevated intra-thoracic pressure, reduced venous return, and consequent complex neuro-humoral responses (Frazier 1999; Jungmann *et al.* 1987) that potentiate sodium retention in critically ill patients. Hence the additional contribution of RRT to positive sodium balance may be significant clinically.

Factors affecting the flux of sodium in to the patient

The gradient between the RRT sodium and patient's serum sodium appears to be the major factor driving the influx of sodium in to the patient. This makes intuitive sense and has been recognized as an important cause of sodium loading during chronic haemodialysis. The default sodium selected on the Fresenius 4008S is 140 mmol/l, (though there is an option to adjust the sodium from 127-

150mmol/L in EDD), whereas the replacement bags used for a typical CRRT device such as the Aquarius 6.01 are only available with a sodium concentration of 140 mmol/l as no other option is currently available. As most of the patients in this study had a serum sodium less than 140 mmol/l, this created a positive gradient between the two, resulting in net sodium flux in to the patient. Keen *et al.* have reported a positive dialysate-to-serum sodium gradient in approximately 98% of patients, and diffusive gain of 75 mmol in body sodium content at the end of each chronic haemodialysis session with a median dialysate-to-plasma sodium gradient of 6 mmol/l (Keen *et al.* 2007). Similar findings have been reported elsewhere (Peixoto *et al.* 2010). Lower predialysis serum sodium concentration has been associated with an increased risk of death in patients on chronic haemodialysis (Waikar *et al.* 2011) indicating a higher gradient will lead to a positive sodium flux in patients during a dialysis session. This may lead to extracellular fluid expansion and a rise in blood pressure, and has been linked to cardiovascular disease in patients with chronic kidney disease (Thijssen *et al.* 2011). Similarly, a high gradient between the RRT sodium and patient serum sodium can lead to extracellular fluid expansion in patients in ICU. Also, a higher dose of dialysis also adds an extra load of sodium to the patients, as seen in our study, as sodium flux is proportional to the exchange volume.

One of the other findings in this study was the negative correlation between fluid removal and sodium flux in to the patient. This was seen in patients with CVVHDF and indirectly in CVVH where dialysis day had a negative correlation with net sodium flux. The net fluid removal not only removes fluid but also sodium in patients on RRT. As ICU patients placed on RRT often are initially haemodynamically unstable they are treated with no net fluid removal in the initial days. The fluid removal seen in our study was less than that reported in the RENAL study (RENAL Replacement Therapy Study Investigators 2009) and patients had a positive fluid balance. Patients in the RENAL study were followed for an average of 6 days compared with 2 days in the current study, therefore this difference may have allowed time for recovery and greater fluid removal.

The lack of change in serum sodium in the study in each mode of dialysis was surprising as we expected it to rise with fluid removal and sodium loading during dialysis sessions (even though there was a rise in serum sodium in the combined lowest tertile of the data). A possible reason for this finding is that patients were early during their stay in ICU and were hemodynamically unstable; 78% of them were on vasopressors on the study, and they all had a positive fluid balance during the study day as they required additional fluids and drugs to resuscitate and treat them, thereby effecting the distribution of total body water and sodium. These results might

therefore be different if these patients were dialysed later during their stay when they would have been more stable and may have a different sodium profile.

Difference between the EDD and CRRT

We found that CRRT contributed a higher load of sodium to the patients when compared with EDD. A possible reason for this could be that in EDD there is an option of selecting the sodium prescription for the machine, hence, prescription of a better match to the patient's serum sodium can be achieved. However, as our average affluent fluid sodium in patients with EDD was 139mmol/l, which was very similar to patients on CRRT, this seems an unlikely explanation.

Overall, sodium balance seems to be more complex than previously considered. Current prescribing practices for dialysis for sodium flux rely primarily on convective losses (~78%) and less on diffusive losses (~22%) (Lambie *et al.* 2005). Thus sodium removal can be increased both by applying higher ultrafiltration volumes and by lowering dialysate sodium concentration. The principle of diffusive sodium removal is, however, more complicated. In both blood and dialysate, a significant, but differing, percentage of sodium is bound to anions and unavailable for diffusion. In plasma water, it is assumed that ±7 mmol/L of the total sodium concentration of ±150 mmol/L sodium is in a complexed form, versus ±4 mmol/L in

dialysate (Locatelli *et al.* 1989; Locatelli *et al.* 1999). No net diffusive sodium transport between blood and dialysate occurs when sodium in the plasma, as measured by flame photometry, is ± 2mmol/L lower than dialysate sodium concentration (Locatelli *et al.* 1984). This may explain the lower flux of sodium with EDD. Diffusive influx may occur with supraphysiologic dialysate sodium concentrations and in patients with low predialytic plasma sodium concentrations (Moret *et al.* 2002; Gelens *et al.* 2002). In ICU the serum albumin is often subnormal, making these predictions even more unreliable. Even less is known about sodium removal during haemofiltration. Sodium removal was found to be less during both predilution haemofiltration and postdilution haemodiafiltration compared with haemodialysis using the same sodium concentration in the dialysate and in the reinfusate (Locatelli *et al.* 2000), further supporting our findings.

Suggestions to reduce sodium influx during dialysis in ICU

Two achievable major interventions in ICU to potentially decrease the amount of sodium influx in patients are (i) use of better individualization of dialysate sodium concentration and (ii) earlier use of fluid removal. Several studies in which dialysate sodium concentration was individualized or randomly reduced have reported decreases in interdialytic weight gain and or blood pressure in patients on chronic haemodialysis (De Paula *et al.* 2004; Sayarlioglu *et al.* 2007; Thein *et al.* 2007). Individualisation of dialysate sodium

concentration is not possible currently with CRRT as, to the best of our knowledge, all available bags come with a fixed concentration of 140 mmol/l. Sodium profiling, decreasing the dialysate sodium during a session is controversial (Zhou *et al.* 2006; Iselin *et al.* 2001; Stiller *et al.* 2001) and probably can be counterproductive in ICU as seen in patients with chronic haemodialysis (Zhou *et al.* 2006; Stiller *et al.* 2001).

3.4.5 LIMITATIONS

These results are from a single centre and the sample size was relatively small. We studied only 2 types of dialysis machines and in total 3 modes of RRT. However, we believe this is the first study to examine the effect of dialysis on sodium in ICU, the 2 most common modes used in ICU – EDD and CRRT were studied. In addition, despite the small sample size the data was both predictable and had little variability.

Other short comings of the study are that most of the study data are from early on in the dialysis course and the sodium profile might be different later during a dialysis course and this should be a matter of future investigation. However, the effect on sodium will be most relevant early on in the stay of ICU patients when maximum stress will tend to retain sodium. We also acknowledge a lack of change of a body weight as study limitation for the present study, though it would have been useful and often reported with studies with chronic

dialysis, it was not practical to weigh high acuity ICU patients before and after the dialysis, instead we report the total fluid removed by the dialysis machines during the dialysis session and fluid balance on the study day. While other patient related data such as additional fluids administered, losses and sodium balance data are not available, this does not influence the conclusion of the study. However, in future studies these data would assist in defining the potential clinical consequences.

3.4.6 SUMMARY

In conclusion, these are the first data examining sodium balance during RRT in critically ill patients. Despite a net negative fluid balance, RRT contributes to sodium loading. We suggest further studies to examine the clinical relevance of this finding.

CHAPTER 4: SODIUM BALANCE IN ICU PATIENTS

4.1 Sodium balance is different from fluid balance in critically ill mechanically ventilated patients

The association of positive fluid balance with adverse outcomes is believed to be due to extracellular compartment expansion, as water distributes to both intra- and extracellular spaces. However, sodium only distributes into the extracellular space, which may have differential effects on extracellular volumes, cause cellular dehydration and exacerbate interstitial oedema in both the systemic and pulmonary circulations.

During current practice high amounts of sodium are administered; however, the clinical impact is unknown. In a single centre study critically ill patients were inadvertently administered more than twice (Chapter 3) the recommended intake of 100 mmol per day (Australian National Health and Medical Research Council, New Zealand Ministry of Health. Nutrient Reference Values for Australia and New Zealand including recommended dietary Intakes 2013) despite achieving fluid balance consistent with the conservative arm of the FACTT study (Wiedemann *et al.* 2006). This was confirmed in a point prevalence study across 40 ICUs in Australia and New Zealand (356 subjects), where total sodium administered on the study day (median, inter-quartile range) was 225 (145 – 368) mmol (Chapter 3). However, both studies only observed sodium

administration, neither estimated sodium balance, and no clinical correlates were made.

Both sodium and fluid need to be administered in balance and proportionate to the dynamic clinical state. For example, inadequate sodium and fluid administration may exacerbate haemodynamic instability, particularly during stress such as sepsis and mechanical ventilation, and an imbalance may result in hyper- or hypo-osmolality. Furthermore, positive pressure ventilation, and positive end-expiratory pressure result in elevated intra-thoracic pressure, reduced venous return, and consequent complex neuro-humoral responses (Bersten 2006; Frazier *et al.* 1999) that also lead (Jungmann *et al.* 1987) to sodium and water retention. Although conservative fluid administration may increase ventilator free days (Wiedemann *et al.* 2006), suggesting a direct effect on respiratory function, the contribution of sodium balance is unknown.

We hypothesised that the pattern of sodium balance in critically ill patients over the first few days following commencement of mechanical ventilation would differ from fluid balance, and that high sodium balance may be associated with greater respiratory dysfunction. Accordingly, this study aimed to prospectively estimate sodium balance, fluid balance and respiratory function in patients who were anticipated to require mechanical ventilation for ≥ 48 hours. We also aimed to qualify alterations in body composition.

4.1.1 METHODS

This study protocol was approved by the institutional human research ethics committee (#036/11). Patients admitted to a single tertiary ICU were prospectively recruited. Patients who were anticipated to require invasive mechanical ventilation for at least 48 hours (based on the treating consultant's prediction), were eligible for participation at the time of intubation, providing they did not meet any of the following exclusion criteria: < 18 years of age; history of a chronic haemodialysis requirement; admission diagnosis of traumatic brain injury; diabetic ketoacidosis; hyperglycaemic hyperosmolar syndrome; surgical ICU admission; pregnancy or within 2 months post-partum; anticipated death within 24 hours or designated as not for active treatment. Surgical patients were excluded in an attempt to minimise other potential sources of fluid/sodium loss. Demographic details were collected with ICU admission diagnosis and measures of severity of illness APACHE versions II and III, simplified acute physiology score (SAPS II), and sequential organ failure assessment (SOFA) (Vincent *et al.* 1996). Outcome data included hours of ventilation, ICU mortality and both ICU and hospital lengths of stay. The presence of co-morbidity was assessed by the Charlson Co-morbidity Index (McGregor *et al.* 2005; Hall *et al.* 2004). The day that mechanical ventilation was commenced was designated as Day 0. The following variables were

then obtained daily for 5 days or until 24 hours after the day of extubation, whichever occurred first.

4.1.1.1 Estimated sodium and fluid balance

Sodium and fluid input were calculated as follows. For all solutions, the type and volume administered over each 24-hour study day was recorded. Furthermore, intravenous fluid/sodium sources were classified as *(i)* fluids administered by bolus or infusion for volume expansion/resuscitation, including crystalloids and colloids, *(ii)* transfusion of blood products including red blood cells, platelets, fresh frozen plasma, *(iii)* infusions given as maintenance or replacement fluids, *(iv)* antibiotics administered by a bolus together with its vehicle, as antibiotics significantly contribute to sodium load (Appendix 1), *(v)* other drugs administered by continuous infusion together with their vehicle, *(vi)* other drugs administered by a bolus together with their vehicle, and *(vii)* flushes of intra-vascular lines associated with haemodynamic monitoring, including arterial lines and central venous catheters. Fluid/sodium sources could also be classified as from, *(viii)* feeding via enteral nutrition or *(ix)* total parenteral nutrition (TPN). The amount of sodium administered was then calculated based on the sodium concentration (Appendix 1). Therefore, for drug infusions and boluses, sodium content was calculated from both sodium content of the drug and the type and volume of carrier fluid or diluent. For enteral nutrition, information on the type and volume of feed was recorded and the sodium

content calculated accordingly. No episodes of nasogastric intolerance were recorded in the study cohort. Similarly for custom TPN, the sodium content was recorded.

To estimate sodium output and therefore balance each day, urinary sodium concentration was measured from a 24 hour urine save, by the indirect ion selective electrode technique (Roche modular analyser, Hitachi High-Technologies Corporation, Tokyo, Japan), In addition, daily fluid balance, urine output, and serum sodium were collated. Hypernatremia was defined as a serum sodium ≥ 150 mmol/L.

4.1.1.2 Respiratory function

The chest radiograph was evaluated for pulmonary oedema by a blinded assessor and assigned a score from 0-390 using a scoring system validated by Halperin *et al.* (Halperin *et al.* 1985), with 0 representing no oedema and high scores representing severe oedema. More specifically, the chest x-ray score was calculated by the summation of sub-scores from 0 (normal) to 65 (alveolar oedema involving entire pulmonary region) assigned to each of 6 lung regions. The highest and lowest PaO_2/FiO_2 ratios were also calculated from routinely performed arterial blood gas analysis (ABL 700 Series, Radiometer Medical ApS, Bronshoj, Denmark)

4.1.1.3 Body composition

Body weight was measured to the nearest 0.1 kg at the same time each day using a Jordan frame attachment to a lifter with a weigh scale (IPL 150-em patient lifter and weighting device, Invacare, Cheltenham, VIC, AUS), which enabled patients to remain in a stable supine position. Care was taken to ensure that there was no additional weight of attachments or linen that had not been factored into the calculations and procedure for this measurement. Supine body length was also recorded to the nearest 0.1 cm using a tape measure (W606PM Executive Thinline, Lukfin, TX, USA) as a proxy measurement of height (Gray *et al.* 1985; Baldwin *et al.* 2012) on the initial test day.

Body composition analysis was then performed with bioelectrical impedance spectroscopy using a tetra-polar device (SFB7, ImpediMed Ltd, Pinkenba, QLD, AUS) and a previously described technique (Baldwin *et al.* 2012). Therefore, participants' rested supine, with the medial surfaces of the limbs abducted so as resting away from, and not touching the body. Single use gel electrodes (ImpediMed Limited, Pinkenba, Australia) were placed on the dorsal hand and foot of one side of the body, to form the tetra-polar electrode arrangement. Data were uploaded to the Bioimp Software (v. 5.3.1.1, Impedimed Limited, Pinkenba, Australia) and inbuilt prediction algorithms that used cole-cole modelling with details of weight, height, age and gender were used to approximate the volume

of the total body water (TBW), extracellular fluid (ECF) and intra cellular fluid (ICF) compartments. The relative volume of ECF was then expressed as a percentage of TBW.

4.1.1.4 Clinical parameters

Other clinical parameters that were recorded from routine monitoring/investigations included serum creatinine, urea, and albumin. The use of *a priori* selected therapies that were noted included the administration of diuretics, steroids and renal replacement therapy. Moreover, peripheral oedema was scored by a blinded and independent assessor, by the summation of sub-scores given to the pedal, lateral chest wall, hand and sacral regions (individually from score of 0 to 4) to form a total oedema score from 0-16 with high scores representing greater oedema (Urden *et al.* 2006). A SOFA score was also calculated daily (Vincent *et al.* 1996).

4.1.1.5 Statistical analysis

Data are reported as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate for the distribution of each variable. Analysis was performed with the SPSS software (version 19.0, Chicago, IL, USA). Differences between variables over time were analysed by a repeated measures analysis of variance (ANOVA) and correlations by Pearson's r. Cumulative fluid balance and sodium balance were correlated with next day's chest X-ray score

and the lowest PO_2/FiO_2 ratios. A conventional alpha level of < 0.05 was used for all significance testing.

4.1.2 RESULTS

Ten subjects aged 60 (12) years participated for 4.5 (3.2-5.0) days, with all subjects completing the study protocol. Other demographic details and characteristics at ICU admission are outlined in Table 4.1. With regards to clinical care, none of the patients required renal replacement therapy. All the patients were included in the study within 24 hours of initiation of mechanical ventilation.

Table 4.1: Subject demographics and details at ICU admission in the single centre sodium balance study

ID	Diagnosis	Age (years)	Gender	SBL (cm)	Weight kg)	APACHE II	APACHE III	SAPS II	SOFA	ICU LOS	Hospital LOS	MV hours	ICU mortality	CCI
1	Sepsis	68	F	149.8	68.2	19	79	46	10	7.4	57	56	Alive	10
2	Pneumonia	50	M	173.5	59.4	18	62	38	10	8.5	22	122	Alive	4
3	ARDS-aspiration	49	M	174.8	82.2	19	32	60	13	11.5	16	131	Alive	3
4	Sepsis	74	M	171.5	113.8	22	65	46	9	7.0	13	69	Alive	18
5	Cardiogenic shock	61	M	166.8	58.8	14	37	31	2	4.8	8	88	Alive	3
6	Sepsis	39	F	158.5	80.6	20	70	32	14	7.5	7	93	Alive	0
7	Acute liver failure	76	M	181.5	97.4	32	98	66	7	7.0	47	141	Alive	10
8	Pneumonia	63	M	179	98.8	29	113	72	9	26.8	52	138	Alive	4
9	Pneumonia	55	F	164.5	76.2	19	60	33	10	5.5	6	131	Dead	13
10	ARDS-aspiration	60	M	184.8	98.4	25	98	69	12	20.1	26	460	Alive	3
Mean (SD)	NA	60 (12)	NA	170.5 (10.8)	83.4 (18.4)	22 (6)	71 (26)	49 (16)	10 (3)	10.6 (7.2)	25.3 (19.6)	143 (115)	NA	7 (6)

Results for sodium and fluid parameters, are listed according to study day in Table 4.2 along with the respiratory function, body composition and clinical variables. More specifically, patients only ever achieved a relatively modest positive fluid balance, which was negative by study day 3. However, it took until day 5 for cumulative fluid balance to become negative -954 (3181) ml (Fig. 4.1A). Conversely, sodium administration was considered high throughout the study period, although it did progressively decrease each study day (Table 4.2). Furthermore, on study day 0, resuscitation fluids were the predominant source of sodium administration, with fluctuations in the contribution of other sources shown in Table 4.3. While 4 subjects met criteria for hypernatremia, the highest serum sodium did steadily increase from days 0-5 ($p = 0.046$). Furthermore, after 5 days of mechanical ventilation, patients had an estimated cumulative sodium balance of 253 (346) mmol (Fig. 4.1B). The increase in serum sodium was mirrored by an increase in serum urea but a decrease in serum creatinine and albumin between days 0-5 (Table 4.2).

Patients who received diuretics (total 15 diuretic days) during the study period did achieve a better urine output ($p = 0.04$) but did not achieve a higher or lower natriuresis when compared to subjects who did not receive diuretics ($p = 0.11$). There was no difference in the urine output or natriuresis with intravenous steroids (total 25 steroid days) ($p = 0.44$ and $p = 0.54$ respectively).

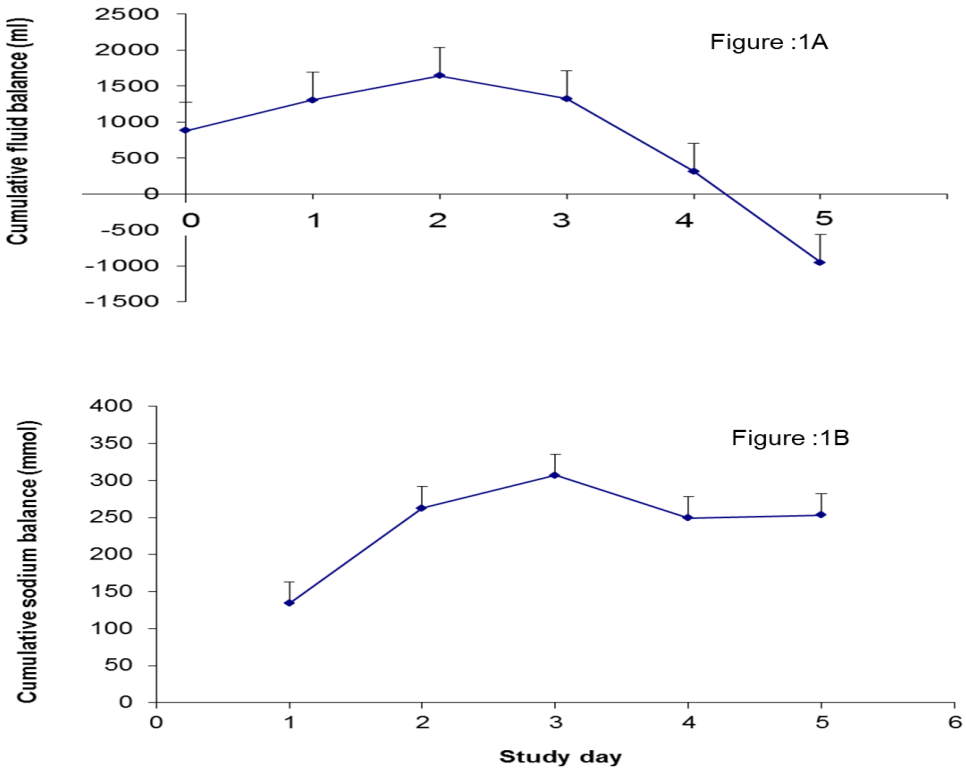
Table 4.2: Summary of fluid, sodium, respiratory, body composition and clinical data on each study day in the single centre sodium balance study

Parameter	Day 0[X]	Day 1[X]	Day 2[X]	Day 3[X]	Day 4[XX]	Day 5[XXX]
n	10	10	10	10	7	5
Sodium and fluid balance						
Total fluid administered (ml)	3563	2843	2563	2900	2510	2380
	(2109)	(1089)	(992)	(1126)	(793)	(953)
Urine output (ml)	883	1796	1589	2061	2018	2098
	(477)	(1074)	(604)	(603)	(1087)	(1137)
Fluid balance (ml)	1471	424	336	-321	-225	-451
	(542)	(1118)	(655)	(1666)	(1509)	(1761)
Total sodium administered (mmol)	488 (399)	198 (86)	149 (46)	135 (66)	112 (46)	94 (34)
Urine sodium (mmol/24 hours)	NA	64 (64)	56 (63)	56 (41)	128 (127)	95 (77)
Highest serum sodium (mmol/L)	140 (4)	141 (4)	142 (5)	145 (6)	146 (5)	147 (5)
Respiratory function						
Lowest PaO_2/FiO_2 ratio	96	194	237	231	258	205
	(24)	(109)	(64)	(47)	(95)	(100)
Chest x-ray score (*/390)	176	188	178	204	193	140
	(141)	(97)	(110)	(118)	(121)	(109)
Body composition						
Body weight (kg)	NA	83 (18)	84 (18)	79 (17)	83 (17)	90 (12)
TBW volume (L)	NA	43 (8)	42 (9)	39 (9)	40 (10)	44 (10)
TBW (% of body weight)	NA	52 (3)	50 (5)	49 (6)	48 (4)	49 (6)
ECF volume (L)	NA	21 (4)	21 (5)	20 (5)	20 (5)	22 (6)
ECF (% of TBW)	NA	50 (4)	50 (4)	50 (4)	50 (5)	51 (3)
Other clinical parameters						
Highest serum creatinine (umol/L)	89 (54)	95 (36)	87 (30)	84 (27)	62 (21)	69 (28)
Highest serum urea (mmol/L)	5 (4)	12 (6)	11 (5)	11 (5)	11 (5)	12 (7)

Highest serum albumin (g/L)	28 (6)	27 (6)	26 (6)	26 (4)	26 (4)	23 (4)
Total oedema score (*/16)	NA	2 (3)	2 (3)	5 (2)	4 (2)	5 (4)
SOFA	10 (5)	8 (4)	7 (4)	5 (3)	4 (2)	4 (2)

Data are mean (SD).
X: Data presented for 10 patients
XX: Data presented for 7 patients
XXX: Data presented for 5 patients

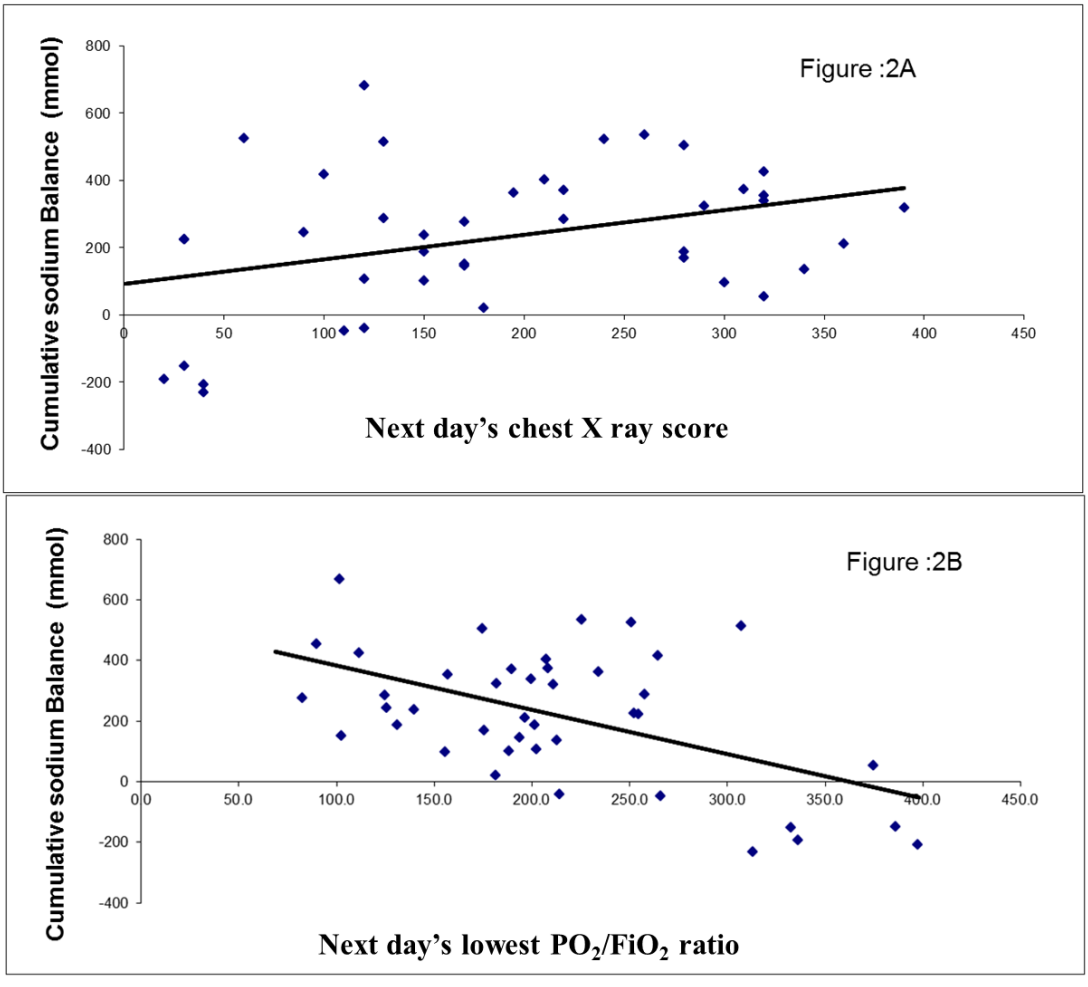
Figure 4.1: Cumulative fluid balance (A) and estimated cumulative sodium balance (B) during ICU stay in 10 mechanically ventilated patients



Data represented as mean (SEM)

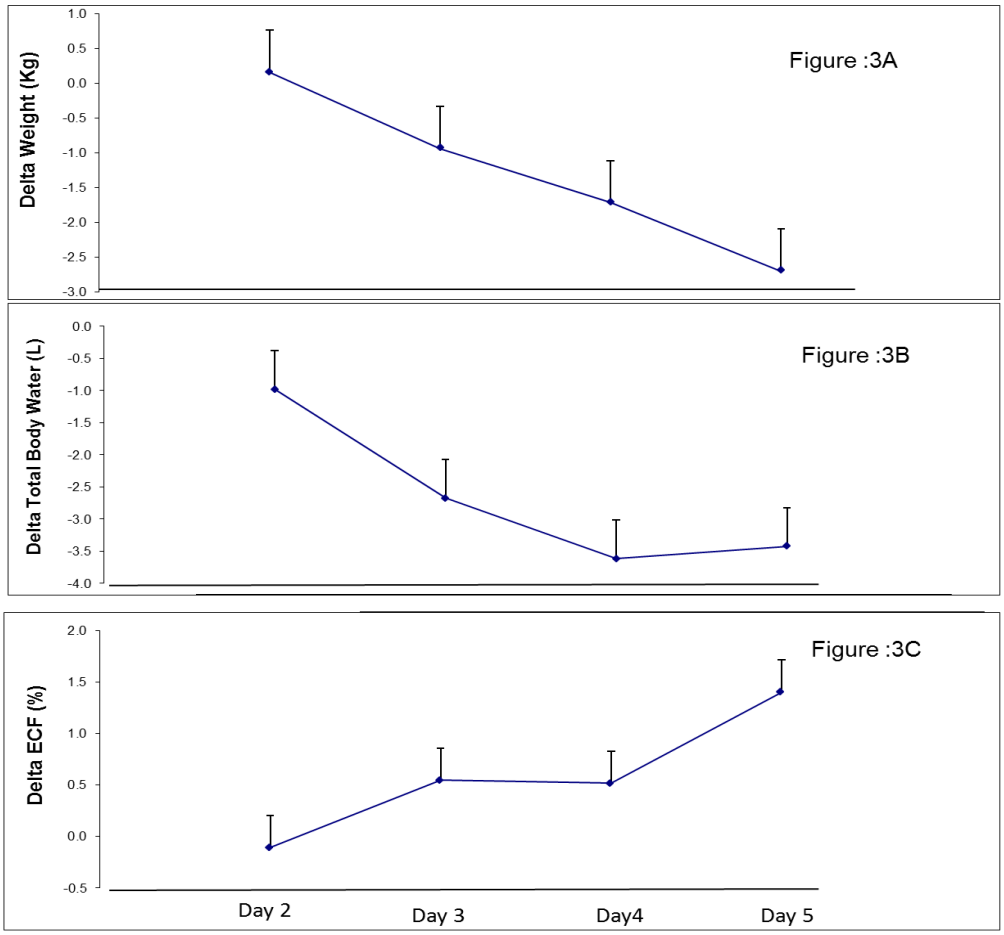
Using the data at each measurement for each subject, cumulative sodium balance was weakly correlated with: next day's worsening (higher) chest X-ray scores (r = 0.35, p = 0.004) (Fig. 4.2A); a next day's reduction in the lowest PaO_2/FiO_2 ratio (r = -0.52, p = 0.001) (Fig. 4.2B); and lower urinary sodium (r = -0.24, p = 0.02). However, there was not a significant association between cumulative fluid balance and either next day's chest X-ray scores (p = 0.24) or next day's PaO_2/FiO_2 ratios (p = 0.44). Neither was there an associating between cumulative sodium balance and either total oedema (p = 0.28) or SOFA scores (p = 0.11).

Figure 4.2: Correlation between cumulative sodium balance and next day's chest x-ray score (A) and next day's lowest PaO_2/FiO_2 ratio (B) in 10 mechanically ventilated patients



With regards to changes in participants body composition (Table 4.2), between days 1-5 (as compared with study day 1) body weight decreased (mean (SD), -2.7 (1.4) kg, p = 0.03) (Fig. 4.3A), and TBW volume decreased (-3.4 (1.3) L, p = 0.05) (Fig. 4.3B). However, there was a trend to an increase in the distribution of body water in the ECF compartment (1.4 (0.9) % of TBW, p = 0.08) (Fig. 4.3C). Delta ECF (as compared with study day 1) had a positive correlation with total sodium administered (r = 0.47, p = 0.008) and cumulative sodium balance (r = 0.42, p = 0.02), a negative correlation with highest serum albumin (r = -0.56, p = 0.001) and no correlation with either daily or cumulative fluid balance (p = 0.59 and p = 0.11 respectively).

Figure 4.3: Delta body weight (A), total body water (B) and extracellular fluid (% of total body water) (C), as compared with study day 1 in 10 mechanically ventilated patients



Data are mean (SEM).

Table 4.3: Sources of sodium administered during study days 0-5 in 10 mechanically ventilated patients

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Resuscitation mmol; %	305; 62.5	35;17.7	7; 4.8	9; 6.5	3;2.8	0; 0.0
[95%CI]	[58.7-65.1]	[15.9-19.1]	[4.4-5.2]	[5.9-7.1]	[2.3-3.2]	[0-0]
Transfusion mmol; %	40; 8.1	0; 0.0	3; 1.8	2; 1.4	3; 2.5	0; 0.0
[95%CI]	[7.8-9.4]	[0-0]	[1.6-2.1]	[1.1-1.8]	[2.2-3.0]	[0-0]
Infusion mmol; %	34; 7.0	18; 8.9	3; 1.9	4; 2.6	3; 2.5	0; 0.0
[95%CI]	[6.2-7.9]	[8.1-9.9	[1.6-2.2]	[2.2-2.9]	[2.2-3.0]	[0-0]
Drug Infusion mmol; %	17; 3.4	42; 21.1	15; 10.3	14; 10.5	1; 1.3	15; 15.5
[95%CI]	[3.1-3.8]	[19.2-22.3]	[9.2-11.0]	[9.1-12.1]	[0.9-1.5]	[14.1-17.1]
Drug Bolus mmol; %	0; 0.0	2; 1.0	8; 5.3	3; 2.0	23; 20.1	0; 0.0
[95%CI]	[0-0]	[0.7-1.5]	[4.9-5.8]	[1.5-2.6]	[18.8-22.3]	[0-0]
Antibiotics mmol; %	53;10.8	34; 17.0	32; 21.4	34; 24.9	23; 20.1	17; 17.8
[95%CI]	[9.4-11.9]	[15.9-18.1]	[20.0-23.2]	[22.9-26.4]	[18.9-22.3]	[15.9-19.1]
Feeds mmol; %	0; 0.0	26; 12.9	35; 23.7	28; 20.8	28; 25.4	22; 23.2
[95%CI]	[0-0]	[11.5-14.1]	[21.5-25.5]	[19.7-22.5]	[23.8-27.4]	[21.3-25.1]
TPN mmol; %	5; 1.1	5; 2.5	13; 8.4	2; 1.8	4; 3.2	7; 7.3
[95%CI]	[0.9-1.3]	[1.9-3.3]	[7.9-9.1]	[1.2-2.4]	[2.2-4.4]	[5.9-8.8]
Flush mmol; %	36; 7.3	37; 18.6	34; 22.5	40; 29.6	36; 31.8	34; 36.2
[95%CI]	[6.9-7.9]	[17.1-19.9]	[20.9-23.9]	[27.6-32.1]	[29.8-34.2]	[33.7-39.9]

Data are represented as mmol and % of the total administered sodium with 95% confidence interval [CI].

4.1.3 DISCUSSION

This study sampled a small group of mechanically ventilated subjects with a moderately high severity of illness at ICU admission and reports that despite achieving a negative fluid balance over the first 5 days of mechanical ventilation, estimated sodium balance remained positive and trended to increase. Furthermore, a more positive estimated sodium balance was weakly associated with both lower oxygenation and more severe pulmonary oedema, measured as the PaO_2/FiO_2 ratio and chest radiograph score, respectively. There was also a rise in serum sodium, an increase in the relative ECF volume, and losses in body weight and body water over the study period.

Sodium balance has not previously been separated from fluid balance in studies of critically ill patients who may be at high risk of retention. It is noteworthy that following shock pulmonary interstitial collagen may have an increased propensity for sodium absorption that is associated with interstitial oedema, as suggested by studies of primate lungs (Moss *et al.* 1972a; Moss *et al.* 1972b; Moss *et al.* 1973). This can potentially lead to intracellular dehydration; which may provide some explanation of recent reports of association between conservative fluid balance strategy's association and cognitive impairment in patients with acute lung

injury (Mikkelsen *et al.* 2012). Despite the pilot nature of this study, we also found novel associations between estimated sodium balance and clinically relevant parameters

Hypernatremia, (Palevsky *et al.* 1996) which has been associated with poor outcomes in critically ill patients (Hoorn *et al.* 2008; Lindner *et al.* 2009), may result from both sodium retention and inadvertent sodium loading during conservative fluid management. In the present study, with the exception of study day 0, sodium administration was at the lower end of the practice spectrum (Chapter 3) in our research locale, and the management of fluid balance in our study patients would be classified as conservative. Still, four patients met criteria for hypernatremia at some point during the study, and serum sodium did steadily increase. While the measured negative fluid balance only partly (approximately 3 mmol/L) accounts for the increase in serum sodium of 7 mmol/L, this is fully accounted for when faecal and skin losses are considered. We measured an average weight loss of around 3 kg with a corresponding loss in TBW, consistent with the cumulative negative fluid balance (-950 ml), plus an approximated (although unmeasured) fluid loss from faeces (-200 ml/per day), skin (-500 ml/per day) and net metabolic water production (+350 ml/per day) (Bersten 2006). This suggests that the positive sodium balance we estimated was either not or incompletely accounted for by standard measures of fluid balance and serum sodium.

The association of estimated sodium balance with both a lower PaO_2/FiO_2 ratio and a higher chest radiograph score may have been due to the distribution of sodium to extracellular spaces and the volume expansion of this compartment. At the whole body level, the result of our body composition analysis suggests that there was an increase in the relative volume of fluid distributed to the extracellular compartment. This was despite a reduction in total body water, which was reflected by changes in body weight. This observation is consistent with both our previous work (Baldwin *et al.* 2012), and other longitudinal observations of haemodynamically stable and mechanically ventilated critically ill patients early in the course of illness (Plank *et al.* 2000; Plank *et al.* 1998a; Finn *et al.* 1996). From these studies, fluctuations in body weight could mostly be attributed to changes in body water, amidst progressive cellular dehydration and fluctuations in extracellular overhydration at both the whole body (Plank *et al.* 2000; Plank *et al.* 1998; Finn *et al.* 1996) and tissue level (Garmin *et al.* 1996).The rise in the extracellular fluid compartment correlated with the cumulative sodium balance but not cumulative fluid balance. In relation to the lung, a small fractional rise in extracellular fluid compartment volume appears insufficient to explain the measured deterioration in PaO_2/FiO_2 ratio and chest radiograph score. Perhaps, both the propensity of interstitial collagen to retain sodium during mechanical ventilation and critical illness may lead to a ventilation

perfusion mismatch, resulting in worsening of the PaO_2/FiO_2 ratio and chest radiograph score, and act as a sink for sodium. While the lack of association between fluid balance and these variables in our study may be viewed as unexpected, it is consistent with the physiological understanding that the primary determinant of extracellular volume is sodium, in which case achieving negative fluid balance without consideration of sodium balance may be of little benefit.

The lack of effect of diuretics in producing natriuresis may seem surprising, but critically ill patients are at risk of sodium retention as a result of activation of the renin-angiotensin-aldosterone system, (Jungmann *et al.* 1987) and have impaired activity of dopamine in the proximal tubule of the kidney (Seri *et al.* 1988) where dopamine normally inhibits sodium reabsorption (Seri *et al.* 1990). Similar findings have been reported in chronic heart failure (Brater 1994).

With respect to the clinical implications of this study, we provide support for the suggestion of Trubuhovich (Trubuhovich 2012) that sodium balance should be considered daily as a part of routine management of critically ill patients, especially as it cannot be assumed that cumulative fluid balance reflects sodium balance. Furthermore, as positive sodium balance may be affected by the amount administered, it may be important for clinicians to

recognise the variety of sources by which inadvertent sodium loading can occur, and how this may change over the course of illness and recovery.

4.1.4 STUDY LIMITATIONS

The findings of the present study need to be considered in light of several limitations. Firstly, our sample was small and represents medical ICU patients. Although the small sample size limits our conclusions, we believe this is the first clinical study to correlate sodium balance and measures of respiratory function. This provides a basis to increase sample size and to examine sodium balance and body composition parameters with more objective and technically challenging measures that may include other measures of oxygenation and lung function, extravascular lung water and cognitive function. The recruitment of medical patients rather than surgical cases was done in an attempt to minimise other potential sources of fluid/sodium loss, particularly through the gastrointestinal system. While we were able to be accurate in our calculation of sodium administration, sodium balance was only estimated from urine loss, and did not include other gastro-intestinal or incidental losses. However, similar limitations apply to estimates of water balance. The difference between the calculated water balance and both body weight and TBW likely reflects unmeasured losses from the gastrointestinal tract and skin. Also, as subjects were only followed for up to 5 days, and there was some attrition by day 4 and 5 due to extubation, the data were

incomplete, and the pattern of sodium balance after 5 days remains unknown.

With respect to body composition variables, we did not use gold standard isotope dilution methods due to the technical expertise and equipment required - particularly for application to the critically ill. Alternatively, bioelectrical impedance spectroscopy may be used, which has been reported to have good agreement for monitoring the trend of changes over time in ICU patients despite under-estimating volumes by approximately 2-4 L, mostly in patients with sepsis (Plank *et al.* 2000, Plank *et al.* 1998b). Yet, only three of our patients had sepsis, and the average fluid balance of all patients at enrolment was (~1.5 L), being far less than in these previous studies (~12.5 L) (Plank *et al.* 2000; Plank *et al.* 1998a). The greatest inaccuracies of bioelectrical impedance analysis are evident in a severely fluid overloaded state and in patients with sepsis, supporting the reliability of assessment of changes over time, and that inaccuracies would have been minimised in our sample. Furthermore, the comparatively small magnitude of body water fluctuations we observed over the study period may have reflected a relatively modest initial fluid excess.

4.1.5 SUMMARY

This study related clinical squeal to estimated positive sodium balance in mechanically ventilated critically ill subjects who had a

conservative fluid balance. As there may be other clinically important and differential effects of sodium balance, further research is required to delineate the optimal balance of fluid and sodium in ICU subjects.

4.2 Sodium balance, not fluid balance, is associated with respiratory dysfunction in mechanically ventilated patients: a prospective, multi-centre study

Critically ill patients are at risk of a positive sodium balance due to both inadvertent excess administration, which may be over twice the recommended daily sodium intake for healthy individuals (Chapter 3) and decreased sodium clearance (Jungmann *et al.* 1987). Previously my small, single centre study suggested that the estimated positive sodium balance in ICU patients is high and that there may be dissociation between estimated sodium balance and fluid balance (Section 4.1). In mechanically ventilated patients, a cumulative positive fluid balance is associated with worsening oxygenation, prolonged mechanical ventilation and increased morbidity and mortality (Simmons *et al.* 1987; Mitchell *et al.* 1992; Sakr *et al.* 2005; Wiedemann *et al.* 2006; Boyd *et al.* 2011). The distribution of water between intracellular and extracellular compartments is strongly influenced by sodium concentration and its relative restriction to the extracellular fluid space. The adverse effects associated with a positive fluid balance, may, therefore, be related partly to a positive sodium balance.

Unfortunately, however, there is limited evidence about the factors contributing to sodium balance in critically ill patients and the clinical implications of a positive sodium balance (Saxena 2013).

The potential for excess sodium to exacerbate interstitial oedema in the systemic and pulmonary circulations, independent of fluid balance is supported by the recent single centre reports of adverse association between estimated positive sodium balance and PaO_2/FiO_2 ratio, radiological lung injury score and expanded extracellular fluid volumes in critically ill patients (Section 4.1). The aim of this study was to extend these initial single centre observations by examining sodium balance and its relationship with oxygenation (PaO_2/FiO_2 ratio) and length of mechanical ventilation in critically ill mechanically ventilated patients at multiple centres.

4.2.1 METHODS

A prospective, observational, multicentre study was conducted in 4 mixed medical/surgical Australian ICUs between April 2012 and September 2013. We included patients receiving invasive mechanical ventilation for less than 48 hours and who were anticipated to be ventilated for at least another 48 hours. Patients were also required to have an indwelling urine catheter in situ and a screening serum sodium concentration between 130 and 150 mmol/L. Exclusion criteria were age less than 18 years, traumatic brain injury, ICU admission diagnosis of diabetic ketoacidosis or hyperosmolar hyperglycaemic state, Child's C liver cirrhosis, pregnancy and anticipated survival less than 24 hours. Patients receiving renal replacement therapy or expected to require dialysis within the next 48 hours were also excluded due to the potential for

inadvertent excess sodium loading (Chapter 3). Ethics approval was obtained at all participating sites and the study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612000046808).

Data was collected on Days 1, 2 and 3 post-recruitment, with Day 1 being defined as the day of enrolment based on the ICU chart time. Data included: 1) demographics (age, sex, weight, height); 2) ICU admission diagnosis and severity of illness APACHE score II and III]; 3) daily highest and lowest PaO_2/FiO_2 ratio; 4) daily urine output and fluid balance; 5) daily serum creatinine, urea and albumin; 6) administration of diuretics, steroids, vasopressors and renal replacement therapy; 7) intravascular devices; 8) presence of shock (defined as the requirement for vasopressor infusion at any dose for more than 6 hours) and; 9) duration of invasive ventilation, ICU and hospital mortality, and ICU and hospital lengths of stay.

Sodium and fluid intake were calculated as follows: for all solutions, the type and volume administered over each 24 hour study day was recorded. Sodium and fluid sources were classified as (i) i.v. fluids administered by bolus or infusion for volume expansion/resuscitation, including crystalloids and colloids; (ii) transfusion of blood products such as red blood cells, platelets, and fresh frozen plasma; (iii) i.v. infusions given as maintenance or replacement fluids; (iv) i.v. antibiotics administered by a bolus

together with its vehicle; (v) other i.v. drugs administered by continuous infusion together with their vehicle (drug infusions); (vi) other i.v. drugs administered by a bolus together with their vehicle (drug boluses); (vii) intravascular line flushes associated with haemodynamic monitoring, including arterial lines and central venous catheters; (viii) TPN; (ix) enteral nutrition. The amount of sodium administered was then calculated based on sodium concentrations of each solution (Appendix 1). Therefore, for drug infusions and boluses, sodium content was calculated from both the sodium content of the drug and the type and volume of carrier fluid or diluent. For TPN and enteral nutrition, information on the type and volume of feed was recorded and the sodium content calculated accordingly. As the sodium and sodium content from sources such as drug boluses, drug infusions, antibiotics and flushes are often occult and may be considered unintentional, they were collated together as a single group and labelled 'inadvertent'.

Estimated sodium output was based on combined losses from urine, nasogastric drainage, other gastrointestinal losses and drains. For urinary sodium losses, sodium concentration was measured each day from a 24 hour urine collection. For all other losses (for pragmatic reasons), sodium concentration was estimated from published values (Bersten 2006).

4.2.2 STATISTICAL ANALYSIS

Data are reported as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate for the distribution of each variable. Shapiro-Wilk test and P-P plots were used to assess the distribution of data and independent sample t-test or Wilcoxon signed-rank test were used to compare the groups as appropriate. Repeated measures analysis of variance was used to analyse temporal data (day 1 to 3). Pearson's correlation was used to test for the association between continuous variables and chi-square test was used to compare proportions. Predictor variables predefined for length of mechanical ventilation (age, APACHE II, weight, PaO_2/FiO_2 ratio, cumulative sodium and fluid balance at day 3, the presence of shock and i.v. diuretic and steroid administration) were analysed using multiple linear regression analyses (stepwise), results are reported as beta coefficient, standard error and p value. Analysis was performed with the SPSS software (version 19.0, Chicago, IL, USA). A conventional two tailed alpha level of < 0.05 was used for all significance testing.

4.2.3 RESULTS

4.2.3.1 Demographics: Ninety-two patients were screened for eligibility and 50 patients were enrolled (33 males, 66%) (Figure 4.4). Data on fluid and sodium balance was acquired from all patients for 3 days and no patients were lost to follow-up (Figure 4.4). Details of the patients recruited are shown in Table 4.4. Sepsis was the most

common admission diagnosis (n=16, 32%), followed closely by patients with a respiratory diagnosis. More than half of the patients received vasopressors on any study day (Table 4.5). Daily physiological and biochemical data are shown in Table 4.5. The time between ICU admission and inclusion in the study; and intubation and inclusion in the study were 8 (0-17) and 4 (0-14) hours, respectively.

Figure 4.4: Flow diagram of subjects examined in the multicentre sodium balance study

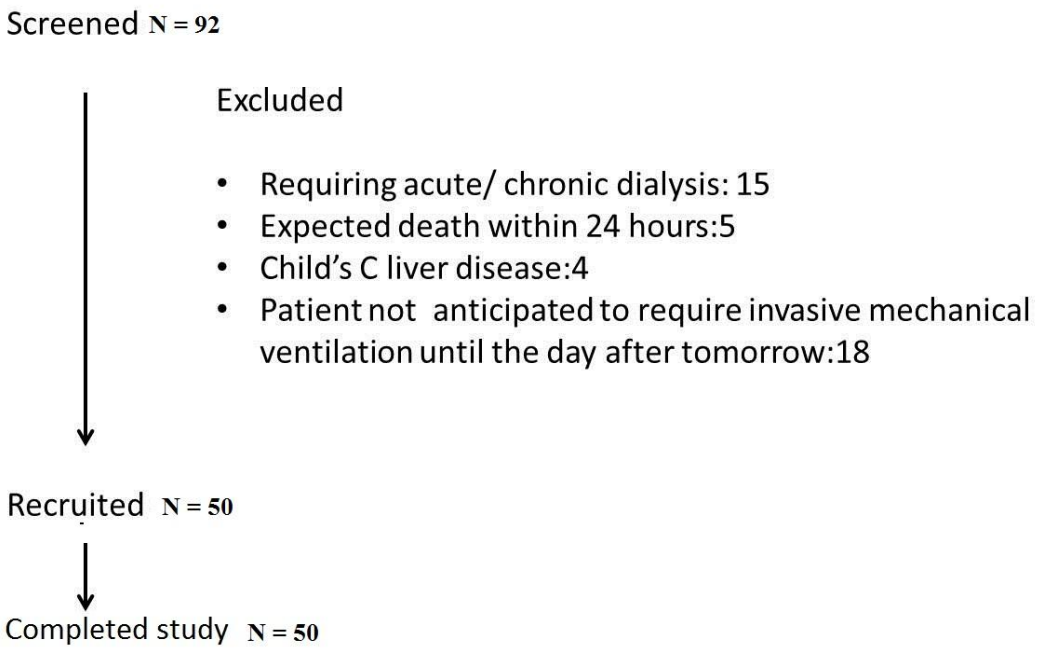


Table 4.4: Patient demographics and outcomes in the multicentre sodium balance study

Age (years)	62.8 (14.6)
Sex, male n (%)	33 (66)
Weight (kg)	79.4 (14.4)
Height (cm)	171.5 (8.9)
APACHE II[#]	25 (19-29)
APACHE III [#]	82 (61-99)
Length of mechanical ventilation (hours)[#]	120.0 (86.7-182.5)
Reason for ICU admission n (%) Sepsis Respiratory Cardiac Post-surgery Others	 16 (32) 15 (30) 5 (10) 5 (10) 9 (18)
ICU length of stay (days) [#]	7.5 (6.0 -12.7)
Hospital length of stay (days) [#]	17.9 (7.8-32.3)
ICU mortality, n (%)	4 (8%)
Hospital mortality, n (%)	7 (14%)

Data are presented as mean (SD) or as a proportion unless indicated
[#]median (IQR)

Table 4.5: Daily physiological and laboratory data in the multicentre sodium balance study

	Day 1	Day 2	Day 3
Serum sodium (mmol/l)	140.2 (5.3)	141.1 (5.2)	141.4 (5.5)
Serum albumin (g/L)	27.6 (5.1)	26.4 (5.1)	25.4 (4.6)
Serum creatinine (umol/L)	103.9 (51.9)	103.4 (59.7)	90.6 (50.1)
Serum urea (mmol/L)	10.5 (5.6)	11.4 (5.8)	10.9 (5.6)
Lowest PaO_2/FiO_2 ratio	171.3 (85.8)	192.8 (77.6)	204.4 (77.0)
Highest temperature (°C)	37.4 (1.1)	37.4 (0.7)	37.2 (0.7)
CVP (mm Hg)	14.5 (3.7)	14.6 (4)	13.3 (3.9)
Vasopressor requirement n (%)	32 (64)	31 (62)	26 (52)

All the reported laboratory and physiological values were the highest recorded on the study day, except PaO_2/FiO_2 which was the lowest recorded value.
Data are presented as mean(SD)

4.2.3.2 Fluid balance

Fluid balance was positive each day leading to a cumulative balance of +2668 (875-3507) ml by Day 3 (Table 4.6), but daily fluid balance was less marked on day 3 (P=0.01), due to lower fluid input (P=0.04) with an unchanged urine output (P=0.35). Examining patients on each study day (50 patients and 3 study days), those who were administered diuretics (n=39) had increased urine output and a lower daily fluid balance, while patients who were shocked (n=89) or were administered steroids (n=85) had no difference in their urine output and daily fluid balance (Table 4.7).

4.2.3.3 Sodium balance

Sodium balance was positive on all study days (Table 4.6), with a cumulative balance of +717 (422-958) mmol at the end of day 3. Daily sodium balance declined each day (P=0.03) due to both a lower sodium input (P=0.01) and increased urinary sodium losses (P=0.05), but remained positive on all 3 study days. Patients who were shocked or were administered steroids on the study day had reduced urinary sodium losses and a higher daily sodium balance; administration of diuretics made no difference either to their urinary sodium losses or daily sodium balance (Table 4.7).

Table 4.6: Daily and cumulative sodium and fluid balance in the multicentre sodium balance study

Daily balance			
	Day 1*	**Day 2**	**Day 3***
Fluid administered (ml)	2874 (1992-3788)	2995 (2144-3551)	2443 (1887-2845)
Urine output (ml)	1325 (918-2270)	1500 (1130-2285)	1493 (923-2364)
Fluid balance (ml)	+1054 (516-1650)	+1130 (-69–1788)	+619 (53-1388)
Sodium administered (mmol)	322 (213-504)	227(178-357)	199 (153-256)
Urine sodium losses (mmol)	16 (13-20)	43 (12-59)	54 (12-60)
Sodium balance (mmol)#	+299 (212-464)	+212 (116-319)	+158 (94-227)
Cumulative balance over days 1 to 3			
Sodium balance (mmol)*	+299 (212-464)	+565 (327-796)	+717 (422-958)
Fluid balance (ml)	+1054 (516-1650)	+2091 (413-2918)	+2668 (875-3507)

Data are presented as median and interquartile range
** Data collection on days 1 and 3 were over 23.0(16-24) and 23.3(3.9) hours respectively*
#Sodium balance is estimated from all sources of sodium administration minus the combined losses from urine, nasogastric drainage, other gastrointestinal losses and drains.

Table 4.7: The effect of diuretic, steroid and vasopressor administration on daily urine output, urinary sodium excretion, fluid balance and sodium balance in the multicentre sodium balance study

	Diuretics		P value
	No	Yes	
urine output (ml)	1370 (920-2160)	1765 (726-3191)	0.03
urinary sodium (mmol)	48 (11-47)	54 (19-72)	0.45
fluid balance (ml)	1035 (405 – 1604)	232 ((-421) - 1474)	0.005
sodium balance (mmol)	229 (143-352)	178 (113 – 285)	0.25
	Steroids		P value
	No	Yes	
urine output (ml)	1430 (1005-2352)	1487 (1050-2275)	0.38
urinary sodium (mmol)	43 (17-123)	19 (9-48)	0.004
fluid balance (ml)	1018 (25 – 1591)	831 (227 – 1600)	0.75
sodium balance (mmol)	208 (78 -310)	245 (158 – 352)	0.06
	Shock		P value
	No	Yes	
urine output (ml)	1585(1685-2375)	1275(903-2210)	0.2
urinary sodium (mmol)	48(25-109)	15(9-42)	0.01
fluid balance (ml)	574 (2-1448)	1108 (332 – 1814)	0.10
sodium balance (mmol)	135 (66 – 240)	259 (184 -390)	0.001

Data are presented as median(IQR)
**defined as vasopressor infusion at any dose administration for more than 6 hours on any particular study day*

4.2.3.4 Contributions to fluid and sodium

The sources of administered fluid and sodium each day are shown in Table 4.8. For both, the main source on day 1 was fluid boluses; 50.3% and 43.7% of total fluid and sodium intake, respectively. This was followed by inadvertent sources (fluid 22.2% and sodium 29.9%). The contribution of fluid boluses to total daily fluid and sodium intake declined significantly over the study period to 11.6% and 9.9%, respectively on day 3. (P<0.001). Inadvertent sources of fluid administration were unchanged over the three days (~23% for days 1-3) (P=0.84); the total daily sodium administered attributed to inadvertent sources increased significantly to approximately 50% of total sodium intake (P=0.003).

Table 4.8: Contribution of various sources to the administered daily fluid and sodium on different study days in the multicentre sodium balance study

	Fluid (%)			Sodium (%)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Bolus	50.3	36.5	11.6	43.7	19.8	9.9
Infusions	14.8	27.2	32.5	13.8	13.6	9.5
Enteral	2.6	11.2	26.7	4.1	13.1	22.3
Blood Products	9.5	2.1	1.1	7.7	4.6	3.0
TPN	0.6	0.9	3.2	0.8	1.2	2.2
Inadvertent	22.2	22.1	24.8	29.9	47.6	52.8

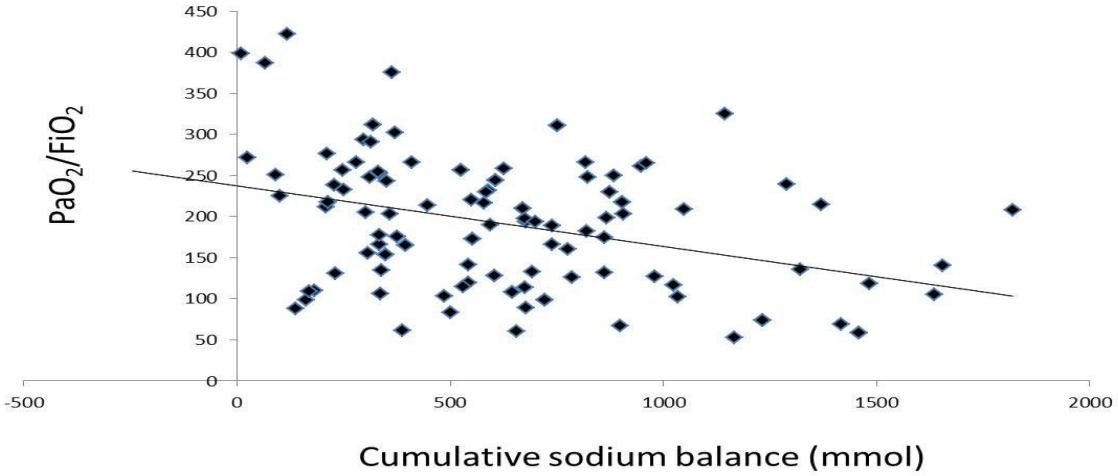
Inadvertent is defined as the total amount of fluid and sodium from sources such as drug boluses, antibiotics, drug infusions and flushes.

Contribution of infusions to total daily fluid administration doubled between day 1 (14.8%) and day 3 (32.5%); in contrast, infusions consistently contributed only ~10-13% to total daily sodium intake. There was a consistent increase in the contribution of enteral nutrition to total fluid and sodium intake over the three days (Table 4.8).

4.2.3.5 Oxygenation and length of mechanical ventilation

Cumulative estimated sodium balance had a negative correlation with the next day PaO_2/FiO_2 ratio (r= -0.36, P=0.001) (Figure 4.5). Factors (beta coefficient [standard error], p value) which related to the length of mechanical ventilation (r^2 = 0.56) were age (1.3 [2.3], P<0.01), cumulative estimated sodium balance at day 3(0.91[0.06], P <0.01), and steroid administration (0.44[3.0], P<0.001). Cumulative fluid balance neither correlated with oxygenation (r=0.10, P=0.23), nor was it a predictor for the length of mechanical ventilation in the linear regression analysis.

Figure 4.5: Correlation between cumulative sodium balance and next day PaO_2/FiO_2 ratio in the multicentre sodium balance study



4.2.3 DISCUSSION

The main findings of this multicentre, observational study are that sodium intake in the first 3 days of critical illness is ~200-300 mmol/ per day and inadvertent sources such as drug boluses and intravascular flushes are the predominant contributors. The positive cumulative estimated sodium balance (717 mmol) was associated with a worse PaO_2/FiO_2 ratio and increased length of invasive mechanical ventilation. These adverse respiratory outcomes were not related to the cumulative fluid balance.

The recommended sodium intake for healthy individuals is 100 mmol/day (Australian Government National Health and Medical Research Council, New Zealand Ministry of Health. Nutrient reference values for Australia and New Zealand including recommended dietary intakes 2014). Our mechanically ventilated

patients received 2 to 3 times this recommended amount. These results are consistent with those reported in our large, multi-centre point prevalence study of ICU patients (Chapter 3), in which the observed daily sodium intake was 224.5 (144.9–367.6) mmol.

This is the first multicentre study to report estimated sodium balance in critically ill mechanically ventilated patients. Although the daily estimated balance decreased from +309 mmol on day 1 to +158 mmol on day 3, there was a large cumulative estimated positive sodium balance (717 mmol). This amount is more than double the amount we previously reported in our single centre study of cumulative sodium and water balance in 10 mechanically ventilated patients (~300 mmol over 3 days) (Section 4.1). However, in the current study more patients were shocked (59%) or received either intravenous steroids (57%); both of which were associated with sodium retention.

The average daily fluid administered in our study population was nearly 3L, resulting in a cumulative positive fluid balance of 2.7L. Positive fluid balance is adversely associated with outcomes in critically ill patients. Several studies have described the association between a positive fluid balance and increased mortality and morbidity (prolonged ventilation, poor gas exchange, renal failure, prolonged ICU stay); presumed due to increased interstitial oedema, reduced cellular oxygen delivery and delayed recovery of failed

organs (D'Orio *et al.* 1987; D'Orio *et al.* 1991). In our study we did not find a similar relationship between cumulative fluid balance and either PaO_2/FiO_2 ratio or the duration of mechanical ventilation, once estimated sodium balance was accounted for in the regression model. In contrast, cumulative estimated sodium balance was negatively correlated with the PaO_2/FiO_2 ratio and was an independent predictor of duration of mechanical ventilation (along with age and day 1 PaO_2/FiO_2 ratio). This finding confirms the findings of the single centre study observation that sodium balance is an important determinant of respiratory function (Section 4.1).

A possible explanation for our findings is that sodium is the major contributor to extracellular tonicity and a driving force for fluid shifts across the cellular membrane towards the interstitium. Accordingly, a high sodium intake may exacerbate interstitial oedema in the systemic and pulmonary circulations, independent of fluid balance. We have previously reported that, whilst total body water decreases over time in mechanically ventilated patients, there is an increase in the relative volume of fluid distributed to the extracellular compartment (Section 4.1). This rise in extracellular fluid volume is also correlated with estimated positive sodium, but not fluid balance. Similar results have been reported in longitudinal observations of hemodynamically stable and mechanically ventilated critically ill patients early in the course of their illness (Plank *et al.* 2000; Plank *et al.* 1998; Finn *et al.* 1996; Garmin *et al.* 1996);

suggesting that fluctuations in body weight may be due to changes in body water and extracellular over-hydration, amidst progressive cellular dehydration (Plank *et al.* 2000; Plank *et al.* 1998; Finn *et al.* 1996).

Despite a large cumulative estimated sodium balance in our study, serum sodium remained unchanged over the 3 days. Calculations using distribution of free water across various compartments and sodium concentration, reveal that ECF has potentially increased up to 4.5 L during the study period. This increase is not explained by cumulative fluid balance, suggesting transcellular shift of approximately 2L to account for static serum sodium concentration.

In critically ill patients, activation of the renin–angiotensin–aldosterone system predisposes to sodium retention (Jungmann *et al.* 1987). This is particularly so in mechanically ventilated patients where positive pressure ventilation and positive end-expiratory pressure both raise intrathoracic pressure and reduce venous return, leading to a complex neurohumoral responses (Bersten 2006; Frazier 1999) with sodium and water retention. Upadaya *et al.* have reported that although a positive cumulative fluid balance can predict weaning failure, achieving a negative fluid balance using diuretics is not independently associated with weaning success (Upadaya *et al.*. 2005). Our finding that administration of diuretics increases urine output but not urinary sodium losses may, in part,

explain their results. Moreover, half of our patients were shocked which not only leads to sodium retention but also increases the propensity of pulmonary interstitial collagen to adsorb sodium (Moss *et al.* 1972a; Moss *et al.* 1972b; Moss *et al.* 1973) in primate lungs. This may explain the adverse associations of positive sodium balance with length of mechanical ventilation.

The main sodium source on day 1 was fluid boluses. On subsequent days, inadvertent sources contributed more to the total administered sodium. Over the 3 day study period 740 mmol of sodium was administered, of which 43.4% was from inadvertent sources. These inadvertent sources are potential target for sodium restriction strategies, such as using 5% dextrose as vehicles for drug boluses and infusion, whenever possible. Previous studies have shown that 0.9% saline is the most commonly used vehicle for i.v. drug boluses (75.6%) and infusions (64.4%) and heparinised saline was the most commonly used i.v. flush fluid (98.1%) (Chapter 3). Furthermore, inadvertent sources and infusions (maintenance or replacement fluids) were responsible for more than 50% of fluid sources by day 3. All of these are potentially modifiable and should be investigated in future studies.

4.2.4 LIMITATIONS

The findings of our study need to be considered in the light of several limitations. Firstly, this was a convenience sample; it was

small and represents mostly medical ICU patients. However, it supports the findings of our pilot study describing the adverse association between estimated sodium balance and respiratory function. Secondly it should also be noted that sodium balance after 3 days is unknown. Finally, we did not study chloride administration. Sodium administration is often coupled with chloride and recent evidence suggests that chloride restriction may impact positively on clinical outcomes, particularly the incidence of acute kidney injury and need for dialysis (Yunos *et al.* 2011; Yonus *et al.* 2012). Renal dysfunction was not evaluated in our study and the potential effect of chloride on mechanical ventilation and respiratory failure was not assessed.

4.2.5 SUMMARY

Sodium intake in mechanically ventilated patients is high and is predominantly attributable to fluid boluses and inadvertent sources such as drug infusion and boluses. A cumulative positive sodium balance is associated with adverse effects on respiratory function. Further research into the optimal sodium balance is warranted. Sodium restriction strategies may represent a novel therapeutic approach for mechanically ventilated patients in the future.

CHAPTER 5: FLUID BOLUS

5.1 Efficacy and prevalence of fluid boluses in resuscitated septic patients

Septic shock is an important cause of death in critically ill patients worldwide (Angus *et al.* 2001). It is characterised by a vasodilated state, sometimes complicated by early myocardial depression. Fluid boluses (FB) are often administered with the aim of improving tissue perfusion (Hollenberg *et al.* 2001; Zanotti-Cavazzoni *et al.* 2009) and are a key component in the effective management of such patients. However, it is also becoming increasingly evident that excessive volume administration can worsen outcome (Durairaj *et al.* 2008; Vincent *et al.* 2006; Wiedemann *et al.* 2006).

During sepsis the activity of the inflammatory response is highest in the initial hours after the insult, which has given rise to the concept of early and late resuscitation as distinct therapeutic entities (Dorresteijn *et al.* 2005). The role of early resuscitation (the first 6 hours) (Rivers *et al.* 2001; Jeon *et al.* 2012) has been well established. Early titrated fluid administration modulates inflammation, improves micro vascular perfusion, induces a shift towards an anti-inflammatory cytokine pattern, and improves organ function and outcome (Dorresteijn *et al.* 2005).

Use of FB after the initial resuscitation are a common practice in medical and surgical ICUs (Axler *et al.* 1997). In the Saline versus Albumin Fluid Evaluation (SAFE) (Safe study investigators 2004)

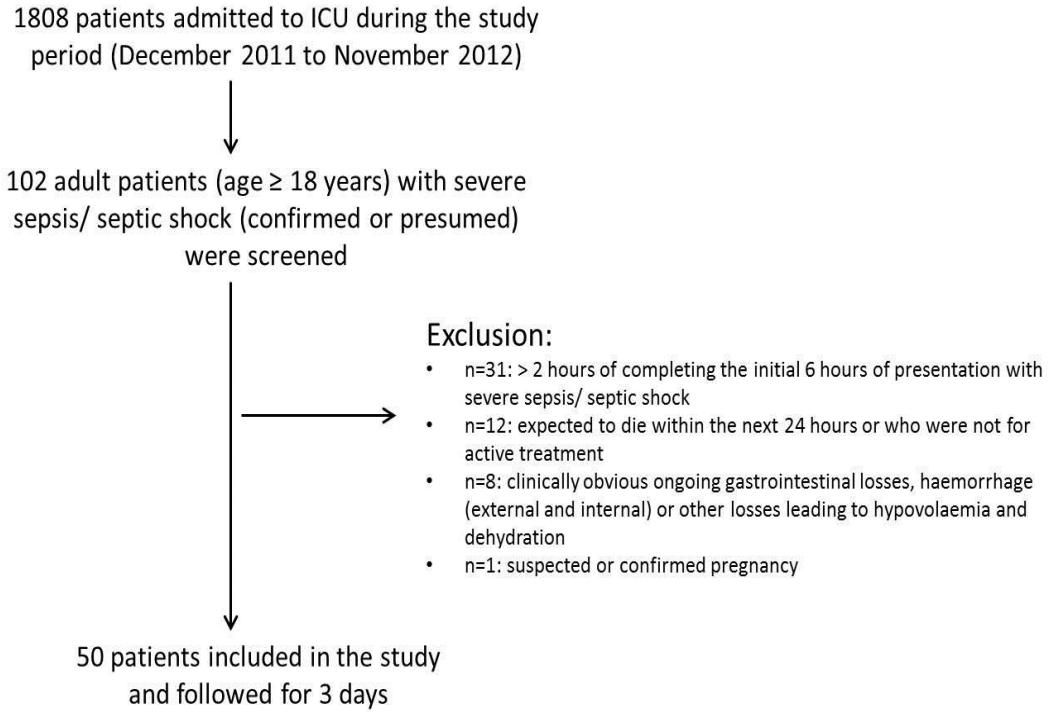
and CHEST (Myburgh *et al.* 2012) studies, 18.2% and 28.7% of patients with severe sepsis respectively, received FB at least for the first 4 days during their stay in ICU. In the recently conducted 6S (Perner *et al.* 2012) trial patients with severe sepsis had a median 1.5 litres of resuscitation fluid administered on day 1 and 2 and 1 litre on day 3 in addition to other fluids and blood products. Despite their frequent use in critically ill septic patients, little has been reported regarding the reasons why these fluid boluses are administered and whether they are efficacious.

Except for cases with obvious fluid loss, these FB can account for some of the positive fluid balance in ICU patients, this in turn has been associated with poor outcomes. Positive fluid balance has been associated with poorer lung, kidney and gastrointestinal function (Wiedemann *et al.* 2006; Bouchard *et al.* 2009; Lobo *et al.* 2002), and an increased mortality risk (Boyd *et al.* 2011). Given the growing evidence of disadvantages of positive fluid balance in ICU, it is important to understand the current practice, indications and effects (beneficial and adverse) of these FB in patients with severe sepsis / septic shock after their initial resuscitation. This study investigates the prevalence, efficacy and the possible harmful effects of these FB in a prospective cohort of septic patients after the initial fluid resuscitation phase. The hypothesis was that such FB are common, have limited efficacy, and are potentially harmful.

5.1.1 METHODS

In a single centre prospective observational study, we screened patients with severe sepsis or septic shock (ACCP/SCCM Consensus Conference Committee: American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference 1992) in a tertiary level ICU. We included adult (age ≥ 18 years) patients with severe sepsis/ septic shock (confirmed or presumed) within 2 hours of completing the initial 6 hours of resuscitation. 6 hours as the time frame for initial resuscitation was chosen as it is generally the most studied time point for initial resuscitation in previous and on-going studies (Rivers *et al.* 2001; Peake *et al.* 2009). Patients who were expected to die within the next 24 hours or who were not for active treatment, patients with clinically obvious ongoing gastrointestinal losses, haemorrhage (external and internal) or other losses leading to hypovolaemia and dehydration, and patients with suspected or confirmed pregnancy were excluded Figure 5.1).

Figure 5.1: Flow diagram showing the total number of patients screened and included in the fluid bolus study based on the inclusion and the exclusion criteria during the study period



The study was funded by the Australia and New Zealand Intensive Care Foundation, and was approved by the Southern Adelaide Clinical Human Research Ethics Committee (289.11). The requirement for consent was waived due to the observational nature of the study. All ICU admissions underwent thrice daily screening for eligible patients.

Beside the demographic data and requirement of organ supports at recruitment (ventilator, vasopressor, inotropes and dialysis), the number, type and volume of FB were also recorded. Daily data collection included fluid balance (daily and cumulative), SOFA score), Lung Injury Score (LIS), highest and lowest PaO_2/FiO_2 ratios,

renal function (serum creatinine and urea), and requirement of renal replacement therapy, diuretics and vasoactive medications. LIS was calculated from PaO_2/FiO_2 ratio, chest x-ray score, PEEP and static respiratory system compliance (if able to be measured). Delta SOFA scores (48-0 h and 72-0 h) were also calculated. Data was collected for 3 days or until ICU discharge or death which ever occurred earlier.

The decision to administer FB, the volumes and the resuscitation goals were made by the treating clinician. One fluid bolus was defined as the sum of a single type of fluid administered in less than half an hour. Each time a fluid bolus was administered, the prescribing clinician was asked to fill in a form (Figure 5.2) describing the indication for these FB and whether it was perceived to be successful. Simultaneously the bedside nurse was asked to fill in a form noting down the exact haemodynamic parameters immediately before and 1 hour after the administration of these FB (Figure 5.3). Data for serum lactate before and 1 hour after the FB were also recorded.

Figure 5.2: Data collection form which was filled in by the treating clinician every time a fluid bolus was administered describing the indication of the fluid boluses and whether it was perceived to be successful

	Indication for Fluid Bolus (Select & number the priority of all that apply)	Was the resuscitation target met? (Yes/No)
Low Blood Pressure		
Increase Vasopressor Dose		
Oliguria		
Low Filling Pressure		
Increase Heart Rate		
Increase Pulse Pressure Variation		
Clinical Signs		
Skin Turgor		
Sv02 / Scv02		
Low Cardiac Output		
Other (Please specifiy)		

Figure 5.3: Data collection form which was filled in by the bed side nurse noting the exact haemodynamic and other parameters immediately before and 1 hour after the administration of fluid boluses

	Before Fluid Bolus	1 hour after fluid bolus
Blood pressure (MAP)		
Temperature		
Vasopressors (Total last hr)	1.	
Name and Total Dose	2.	
Sedation (Name and Total Dose)	1.	
Name and Total Dose	2.	
Urine output (last hour - ml)		
CVP (mm Hg)		
PAOP (mm Hg)		
Heart rate (highest value)		
Pulse pressure variation (%)		
Sv02/Scv02 (%)		
PO_2/FiO_2		
Static lung compliance		
PEEP (cm H_2O)		
Cardiac output (L/min)		
Haemoglobin (gm/dl)		

5.1.2 STATISTICAL ANALYSIS

Data is presented as median (IQR). Analysis was performed with SPSS software (version 19.0, Chicago, IL, USA). Difference between variables over time was analysed with paired sample T test or Wilcoxon signed rank test, as appropriate, and correlations tested using either a Pearson's r or Spearman's rho. A conventional alpha level of < 0.05 was used for all significance testing. Predictor variables for increase in mean arterial pressure (MAP) before and 1 hour after the administration of FB were analysed using multiple linear regression analyses.

5.1.3 RESULTS

5.1.3.1 Demographics

Fifty patients were recruited out of 102 patients admitted with severe sepsis/septic shock during the study period (total ICU admissions during this period were 1808) based on the inclusion and the exclusion criteria (Figure 5.1). The demographic data, and use of ventilation, inotropes, vasopressors and dialysis and the hemodynamic profile at recruitment are shown in Table 5.1. Lung infection was the most common source of sepsis. There were no deaths during the study period of 3 days.

Table 5.1: Baseline characteristics of study patients in the fluid bolus study

Age*	72.5 (61.0-82.8)
Male #	33 (66%)
Weight (Kg)*	80.0 (71.8-85.0)
Illness severity score	
SOFA score at admission*	9 (6 -11)
APACHE III score*	80 (68 – 93)
Charlson co-morbidity index*	4 (2-6)
Ventilator #	13 (26.0%)
Inotropes #	16 (32.0%)
Vasopressors #	31 (62.0%)

Dialysis #	4 (8.0%)
Source of sepsis #	
Lung	16 (32%)
Abdomen	10 (20%)
Urosepsis	12 (24%)
Others	12 (24%)
Haemodynamic parameters*	
Mean Arterial pressure (mm Hg)	66 (62-75)
Heart rate	110 (92-130)
Central Venous Pressure (mm Hg)	11 (8-14)
PaO_2/FiO_2 ratio	273 (182-403)
Fluid Balance (ml) *	956 (655-2254)
Haemoglobin (g/L) *	126 (108-140)
Lactate (mmol/L) *	4.2 (3.1-6.2)

**Data presented as median (IQR)*
 # Data presented as number (%)

5.1.3.2 Fluid bolus prevalence

During the study period 47 out of 50 patients (94%) received FB, with a total of 184 FB [3(2-5) per patient], being administered over 72 hours after the initial resuscitation. The prevalence of these FB prescribed on each study day is shown in Table 5.2. The most common fluid used in the study was 4% albumin (80.9%) followed by red blood cells (7.6%), 20% albumin (3.2%), fresh frozen plasma (3.2%), Gelofusine® (3.2%), 0.9% saline (1%) and platelets (0.5%). Cumulative fluid balance, daily urine output, daily fluid balance and

the contribution of these FB to the daily fluid balance are also shown in Table 5.2.

Table 5.2: Fluid boluses (FB) prevalence and contribution to the daily fluid balance according to the days

Study Day	Patients receiving FB	No. of FB*	Volume of FB (ml) *	Urine output (ml) *	Daily Fluid balance (ml) *	Cumulative Fluid balance (ml) *	Proportion of daily fluid balance received as FB*
One	45 (90%)	2 (1-3)	750 (500-1720)	801 (288 – 1325)	1510 (561 –2727)	1510 (561–2727)	52.4% (22.1–124.2)
Two	26 (52%)	2 (1-2)	500 (250-769)	1189 (540 – 1789)	897 (161 –1694)	2727 (874–3842)	30.8% (15.0–90.7)
Three	8 (16%)	1 (1-3)	750 (250-1062)	2061 (1089 – 2612)	536 (-240–1191)	2820 (1337 –4689)	33.2% (-44.6 –72.5%)

** The data is per patient and presented as median (IQR)*

5.1.3.3 Fluid bolus indications and perceived efficacy

The initial indication and the efficacy as perceived by the prescribing clinician are shown in Table 5.3. Low blood pressure and increased vasopressor requirement were the two most common indications, while low filling pressure and clinical signs were perceived to be the most successful indications for FB.

Table 5.3: Indication and the success rate (as judged by the treating clinician) for a fluid bolus in patients with severe sepsis and septic shock after the initial 6 hours of resuscitation

Indication	Indication prevalence #	Success rate of the bolus as perceived by treating clinician #
Low blood pressure	76.0% (69.9-82.2)	37.1% (29.1-45.1)
Increasing vasopressor dose	60.3% (53.2-67.4)	29.7% (21.2-38.2)
Low filling pressure	30.9% (24.2-37.6)	70.9% (60.2-83.5)
Oliguria	26.1% (19.7-32.4)	31.2% (18.1-44.3)
Increased heart rate	20.1% (14.3-25.9)	54.1% (37.7-70.1)
Clinical signs	18.5% (12.8-24.1)	79.4% (65.8-93.0)

 # Data represented as percentage and 95% confidence interval

5.1.3.4 Fluid bolus efficacy 1 hour after its administration

One hour after FB, there was a small but significant increase in MAP ($p<0.01$) and CVP ($p<0.01$). However, there was also a significant increase in noradrenaline administered. There was also a significant decrease in PaO_2/FiO_2 ratio, haemoglobin and temperature, while the urine output remained unchanged. There was no difference in the central venous gas saturation and serum lactate 1 hour after the FB (Table 5.4). The drop in haemoglobin was more significant when FB with fresh red blood cells were removed from the analysis (104 (95-118) to 92 (82-109); $p<0.0001$).

Table 5.4: Effect of measured variables before and 1 hour after fluid boluses

Variables*	Before FB	1 hour after FB	P value
MAP (mm Hg)	71 (65-78)	73 (68-80)	0.001
Heart rate (per minute)	98 (88-110)	98 (86-110)	0.54
Temperature (ºC)	37.0 (36.3-37.9)	36.8 (36.1-37.7)	<0.001
CVP (mmHg)	9 (6-12)	11 (8-14)	<0.001
Noradrenaline (mcg/kg/min)	0.10 (0.06-0.18)	0.12 (0.05-0.19)	0.02
PaO_2/FiO_2 ratio	244 (163-290)	219 (120-287)	<0.001
Urine output (ml/hour)	40 (20-80)	40 (20-70)	0.2
Haemoglobin (g/L)	101 (93-114)	95 (85-111)	0.001
$ScvO_2$	70.8 (66.9-78.6)	71.2 (67.3-79.2)	0.45
Lactate (mmol/L)	2.4 (1.1-3.9)	2.2 (0.8-4.0)	0.15

**Data presented as median (IQR)*

The increase in MAP was significant only for the first 2 fluid boluses (90 /184 FB) and it became insignificant when analysed for FB administered after the first 2 FB. Out of 184 FB, 51 (27.7%) had an increase in MAP > 5 mmHg 1 hour after administration. When FB were analysed in patients who were not on vasopressors (71/184 FB) the increase in MAP was not significant (p=0.28). In patients who had 4% albumin as the resuscitation fluid (149/184) results were similar to the total FB cohort, in that there was a significant rise of MAP from 71 (65-78) to 72 (68-79) mmHg (p=0.01), but there

was also a significant increase in the noradrenaline administration from 0.10 (0.05-0.18) to 0.11 (0.05 – 0.20) mcg/kg/min (p=0.02)

Factors [Exp(b) (SE) p-value] which affected the increase in MAP using multiple linear regression analysis were the baseline MAP [-0.49 (.057) p<0.001] and the amount of fluid administered per bolus [-0.05 (.01) p=0.001] (Linear regression analysis, R^2=0.296).

Cumulative fluid balance had a weak positive correlation with delta SOFA score (r=0.32, p=0.001) and LIS (rho=0.13, p=0.02) and a weak negative correlation with PaO_2/FiO_2 ratio (r=-0.28, p=0.001) (Figure 5.4 and Figure 5.5).

Figure 5.4: Correlation between daily cumulative fluid balance and lowest PaO_2/FIO_2 ratio in the fluid bolus study

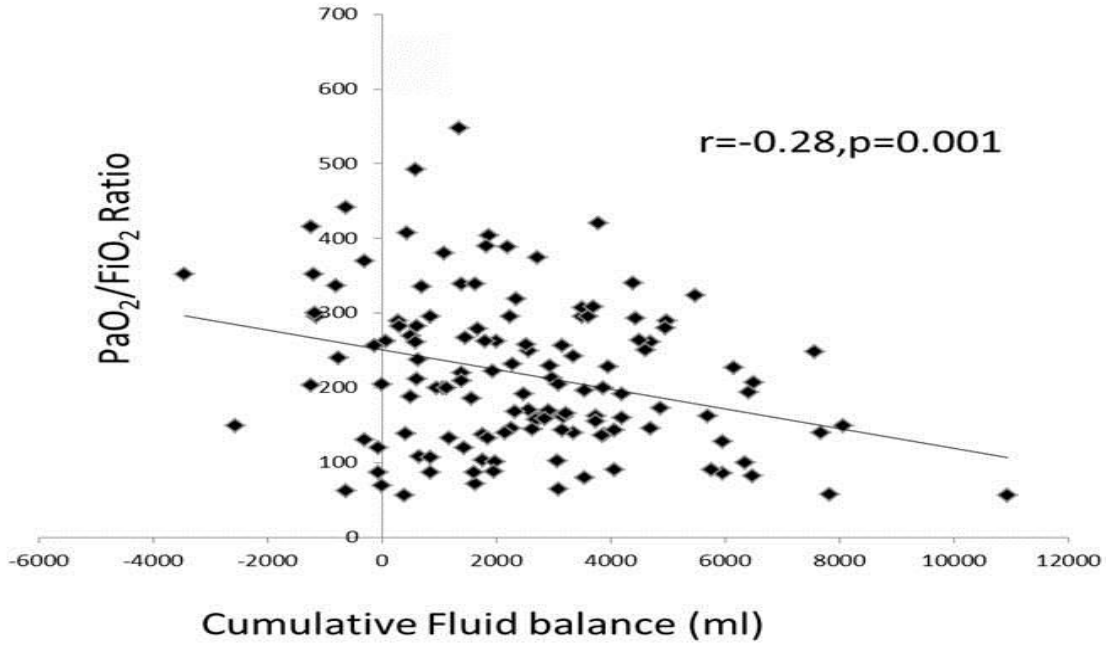
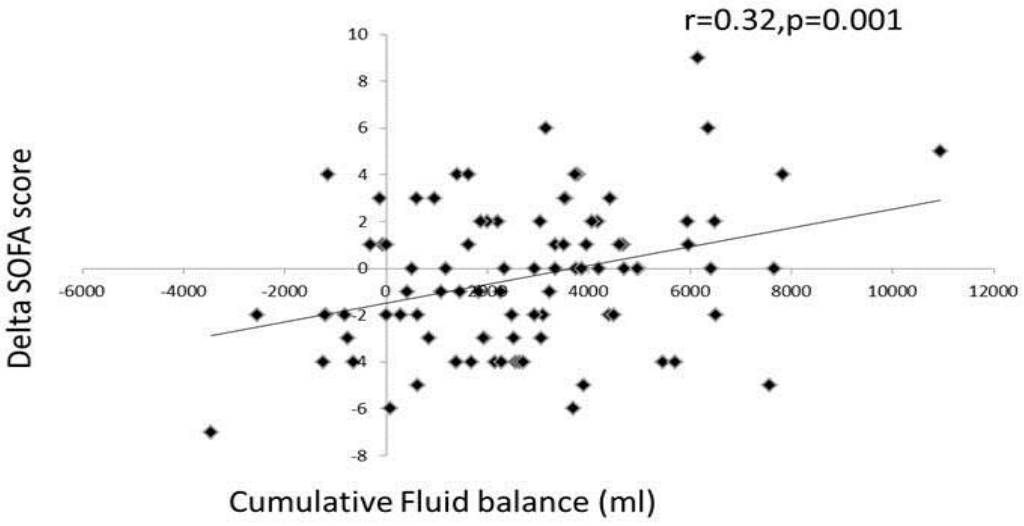


Figure 5.5: Correlation between daily cumulative fluid balance and delta (at 48 hours and 72 hours compared with baseline) SOFA (sequential organ failure assessment score) scores



5.1.4 DISCUSSION

In this study, 94% of patients with severe sepsis or septic shock received FB after the initial resuscitation period, these met with limited success and contributed to the daily fluid balance. The cumulative fluid balance negatively correlated with the oxygenation, LIS and the SOFA scores. This study highlights the common practice of fluid resuscitation after the initial 6 hours, suggests lack of any sustained benefit (1 hour period) following the FB resuscitation, and reports its possible adverse associations.

Patients with sepsis have higher volumes of extracellular water, lower intrathoracic blood volume index, higher pulmonary permeability ratios (extravascular lung water/pulmonary blood

volume) and higher systemic permeability ratios (interstitial/plasma volume) as compared to patients without sepsis (S'anchez *et al.* 2011). Hence any fluid administered, if not appropriately targeted, can lead to pulmonary and systemic oedema.

Axler et al (Axler *et al.* 1997) reported that rapid volume infusions are common therapeutic interventions in ICU (159 rapid volume infusions in 470 patient days) but the effect of a typical rapid volume infusion on haemodynamics was small. In 13 patients with sepsis, rapid volume infusion of an empiric 500 mL of saline did not significantly increase MAP but increased the pulmonary artery occlusion pressure immediately after the infusion. In our study FB led to a small rise in MAP, although there was a simultaneous rise in the dose of vasopressor administered, along with a rise in CVP. We chose 1 hour to judge the effectiveness of these FB in order to investigate the sustained effects (Ospina-Tascon *et al.* 2010) rather than the immediate ones. An increase in MAP > 5 mm Hg 1 hour later was present in only 27.7% of the FB and a difference in MAP before and after the FB were only statistically different for the first 2 FB and became insignificant thereafter. Also, in patients not on vasopressors (as a marker of patients in whom sepsis was under control) there was no significant effect of these FB on MAP. It is also noteworthy that even though we used larger amount of fluids, as compared with Axler and colleagues (Axler *et al.* 1997), and mostly

colloid in our study, the volume was comparable to the 6S study (Perner *et al.* 2012) and was administered for a clinical indication.

These FB administered in our study contributed significantly to the daily fluid balance and the cumulative balance, which had a negative correlation with the oxygenation, lung injury and the organ failure scores. Multiple studies have correlated positive fluid balance with reduced survival in ARDS (Humphrey *et al.* 1990; Simmons *et al.* 1987) and sepsis (Alsous *et al.* 2000). It is also known that sepsis impairs alveolar epithelial function by down regulating the epithelial Na-K-ATPase pump, and that alveolar fluid clearance is reduced when examined 24 and 48 hours after induction of sepsis (Berger *et al.* 2011). Our prospective study also provides support to the large retrospective study examining late fluid management of patients' septic shock complicated with acute lung injury (Murphy *et al.* 2009).

It is of some interest that, in the trial of early goal directed therapy (Rivers *et al.* 2001), those randomised to resuscitation guided by the $ScVO_2$ had improved survival and received significantly less fluid between 6 h and 72 h, whereas they received more fluid between 0 h and 6 h. Using a side stream dark field device, Ospina-Tascon et al (Ospina-Tascon *et al.* 2010) have evaluated the effects of fluids on the sublingual microcirculation in 60 patients with severe sepsis and have shown that fluid administration improved microvascular

perfusion in the early but not late phase of sepsis. Measures of commonly used resuscitation parameters (Kopterides *et al.* 2009) $ScvO_2$ and lactate did not change 1 hour after the FB in our study providing further support to Ospina-Tascon *et al* findings. They used a bolus of either 1,000 ml of a Ringer's lactate solution or 400 ml of a 4% albumin solution over 30 min with a response rate of 55%, defined as an increase in MAP by 5 mm Hg. Our results are consistent with this and with previous observations (Hauser *et al.* 1977; Calvin *et al.* 1981) and suggest that in many patients, large volumes of fluid must be infused rapidly in order to alter haemodynamics. After a 1-L infusion of lactated Ringer's solution over 1 hour in critically ill patients, Hauser and colleagues (Hauser *et al.* 1977) did not observe a significant increase in MAP or cardiac index. Similarly, after a 250-mL infusion of 5% albumin over 30 mins in critically ill patients, Calvin and colleagues (Calvin *et al.* 1981) observed only a small increase in cardiac index from 3.25 +/- 1.08 to 3.47 +/- 1.23 L/min.

The increase in CVP with FB was predictable, but did not predict a rise in MAP after these FB. CVP is probably the most used parameter for judging whether fluids should be administered, nevertheless, a large number of studies (Magder *et al.* 2007; Michard *et al.* 2000; Kumar *et al.* 2004; Reuse *et al.* 1990; Wagner *et al.* 1998) have shown that CVP fails to discriminate fluid responders from non-responders when defined as an increase in

cardiac output or MAP. When CVP is significantly elevated (>10 mm Hg), fluids are generally unlikely to increase perfusion, (Magder *et al.* 2007) but there are occasional exceptions. Overall, the predictive power of the CVP is poor. When CVP is greater than 12 mm Hg, the positive predictive value is only 47% (Osman *et al.* 2007). Even when CVP is much lower in patients receiving ventilation (about 5 mm Hg), the positive predictive value is still only 47%. FB contributes to positive fluid balance which along with elevated CVP (as seen in our study) is associated with increased mortality in patients with septic shock (Boyd *et al.* 2011).

In the SAFE study (SAFE study investigators 2004) and the recently conducted 6S (Perner *et al.* 2012) and CHEST (Myburgh *et al.* 2012) trials, patients administered colloids required more blood transfusion than their control groups, consistent with the drop in haemoglobin we found in our study. Depending on the cardiac output response, this may reduce oxygen delivery directly, and may precipitate transfusion (Yu *et al.* 2011).]

Clinicians use various clinical signs such as “cold clammy skin”, capillary refill time, skin turgor, dry tongue or mucosa for their clinical judgement about the fluid requirement. When we were designing the study we added this point in the data collection sheet to capture these indications. What we found was that the indication rate was low but a high percentage of them were perceived to be successful, providing support for the recent review by Sevransky

(Sevransky 2009) that, despite limitations, the availability, low risk, and ability to perform repetitive tests ensure that clinical examination of the haemodynamically unstable patient will continue to be a useful tool for the intensivist.

Urine output was one of the commonest and least successful indications for these FB. The change in urine output 1 hour after fluid administration was not statistically different. If these FB fail to increase urine output it further adds to the positive fluid balance in patients with potential renal failure. These FB contributed 30.8% - 52.4% of the daily fluid balance. The SOAP study (Payen *et al.* 2008), found that positive fluid balance was associated with a worse outcome in patients with acute renal failure, a finding which has been recently been supported by the PICARD (Bouchard *et al.* 2009) study.

A rise in MAP is a common resuscitation target in patients of sepsis (Morelli *et al.* 2007). When analysed with regression analysis FB had a negative correlation with MAP before fluid administration (i.e. lower the MAP the greater was the response) and with the amount of FB used (i.e. the greater the amount of fluid used for FB the lower the response), hence for a low MAP, using a small amount of fluid (mostly 4% albumin in our study) was the best model for a rise in MAP.

5.1.5 Study Limitation

These findings are from a single centre, predominantly using 4% albumin as the resuscitation fluid, in a prospective study, examining the effect of post resuscitation FB, in a group of patients with severe sepsis or septic shock. Moreover these patients were heterogeneous with regards to their source, usage of vasopressors, treatment with other medications (e.g. diuretics) and variable levels of hypotension and shock. To study the effects of such unavoidable heterogeneity a larger sample size would be required.

The predominant use of 4 % albumin also limits our results especially with regards to the drop in haemoglobin. However, use of 4% albumin in sepsis is supported by a recent meta-analysis (Delany *et al.* 2011) and the subgroup analysis of the SAFE study (SAFE study investigators 2004). Moreover, improved microvascular perfusion with fluids does not appear to be affected by the type of fluid used (Ospina-Tascon *et al.* 2010).

One of the other short comings is the lack of use of dynamic measures (Lanspa *et al.* 2013) to test for fluid responsiveness in our unit. Although measures such as pulse pressure variation were present in the data collection sheet, this technique was rarely used. A possible reason for this could be lack of patients who were not spontaneously breathing, a key requirement for valid dynamic testing. Passive leg raising (Monnet *et al.* 2006) (PLR) has been used

in several studies as a surrogate for a volume challenge. A downside of PLR is that it requires some measure of cardiac output (preferably continuous). Measures of cardiac output were present in only 4 out of the 50 patients we studied. Although it would be an invaluable addition to our study, cardiac output has been shown to be marginally increased (Calvin *et al.* 1981) or not affected 1 hour (Hauser *et al.* 1977) after the fluid bolus. Though we found correlations between fluid balance (a large contribution from the FB) and PaO_2/FiO_2 it would be invaluable to examine similar correlations with extravascular lung water, we suggest this be considered in future studies.

Contribution of non-resuscitation fluids to the daily fluid balance was highlighted in our study, which has been seen in other larger fluid trials (SAFE study investigators 2004; Myburgh *et al.* 2012; Perner *et al.* 2012). We believe future fluid trails should aim to control this as they form a high percentage of the daily fluid balance of these patients and may contribute to the adverse effects of positive fluid balance.

5.1.6 SUMMARY

In patients with severe sepsis or septic shock, after the initial resuscitation, caution must be used in administering FB, as they are usually ineffective, especially after the first 2 boluses, and may lead to positive fluid balance and concomitant adverse outcomes.

5.2 Effect of fluid boluses on serum electrolytes and acid-base status

In addition to differences in their effectiveness as volume expanders, different intravenous (i.v.) fluids have different and sometimes unexpected adverse effects. As the absence of calcium and high chloride levels in 4% albumin *(CSL Biotherapies; which contains the following - sodium 140 mmol/l, chloride 128 mmol/l , albumin 40 g/l and octanoate 6.4 mmol/l)* may lead to clinically significant effects, we further analysed data from our recently conducted study (Section 5.1), after 149 fluid boluses with 4% albumin administration. The amount of 4% albumin administered was 500 (250-500) ml (median (IQR)) and acid-base status and serum electrolytes were analysed with an ABL700 series *(Radiometer Medical ApS, Brønshøj, Denmark)* before and 1 hour after fluid administration. Fluid blouses with 4% albumin resulted in a decrease in plasma ionized calcium (corrected for pH) and an increase in serum chloride and an expected change in bicarbonate, $PaCO_2$ and ultimately pH (Table 5.5). There was also a decrease in the PaO_2/FiO_2 ratio and a decrease in haemoglobin (Section 5.1, Table 5.4) with no change in sodium, potassium or lactate levels.

The decrease in calcium is likely due to binding of free calcium with the administered albumin which does not contain calcium. A delayed decrease in calcium has been reported before in hypovolemic trauma patients who also had multiple blood transfusions with infusion of albumin (Kovalik *et al.* 1981), but the

immediate effect is not known. The rise in chloride levels seen in our study is likely a reflection of the high amounts of chloride present in 4% albumin. This then results in a metabolic acidosis and a compensatory decrease in $PaCO_2$ levels. The drop in PaO_2/FiO_2 ratio is consistent with lung oedema and a decrease in haemoglobin is suggestive of haemodilution.

Even though 4% albumin has been postulated to decrease the risk of death in patients with severe sepsis (SAFE study investigators 2011), we found a drop in PaO_2/FiO_2 ratio 1 hour after its administration in septic patients (section 5.1). The change in chloride and calcium levels may have a contradictory effect on the development of lung oedema. Chloride transport-driven alveolar fluid secretion is now considered a major contributor to lung oedema (Solymosi *et al.* 2013), and a transient rise in serum chloride after 4% albumin administration may expedite this process. This may be counterbalanced by a transient drop in ionized calcium levels. One of the major pathways of lung injury is entry of calcium via endothelial channels and transient drop in calcium may impede this process. Of note calcium free perfusate is often used in isolated perfused lung injury models as a negative control (Kuebler *et al.* 1999). The interaction between the above two variables may contribute to the development of lung oedema in patients with sepsis. Future studies should focus on acute changes in these

electrolyte levels after interventions such as the administration of 4% albumin.

While the levels of calcium and chloride are not known after the first 1 hour, earlier but transient electrolyte effects might play a more important physiologic role in the development of pulmonary oedema than their daily levels.

Table 5.5: Effect on acid-base status and serum electrolytes before and 1 hour after albumin boluses

Variables*	Before	After 1 hour	P value#
iCa^{++} (mmol/L) (corrected for pH)	1.06 (0.99-1.12)	1.01 (0.95-1.05)	<0.001
Na^{+} (mmol/L)	134 (133-135)	134 (133-135)	0.77
Cl^{-} (mmol/L)	105 (102-108)	107 (104-110)	<0.001
K^{+} (mmol/L)	3.7 (3.2-3.9)	3.8 (3.1-4.0)	0.46
HCO_3^{-} (mmol/L)	18 (15-21)	17 (14-19)	0.04
pH	7.37 (7.31-7.40)	7.34 (7.30-7.41)	<0.001
PaO_2/FiO_2 ratio	244 (163-293)	224 (117-292)	<0.001
$PaCO_2$ mm Hg	35 (30-38)	33 (29-36)	0.02
Haemoglobin (g/L)	105 (96-117)	97 (88-112)	0.001
Lactate	2.3 (1.1-3.8)	2.2 (0.9-4.0)	0.19

**Data presented as median (IQR)*
 # analysed with Wilcoxon signed rank test

5.3 Bolus administration of intravenous fluids leads to permeability lung oedema via activation of TRPV4 channels in a rat model

Bolus administration of intravenous fluids is common in resuscitation of critically ill patients as seen in the previous section (Section 5.1) and other studies (Rivers *et al.* 2001; Perner *et al.* 2012). However, adverse effects of fluid resuscitation are increasingly recognised. Generally, i.v. fluid is associated with positive fluid balance and has been associated with increased mortality in patients with both lung injury (Sakr *et al.* 2005) and sepsis (Simmons *et al.* 1987; Murphy *et al.* 2009). In the CHEST study which compared starch or saline for fluid resuscitation in ICU patients (Myburgh *et al.* 2012), 25.6% of patients developed new onset respiratory failure in both the groups following fluid administration. Moreover, as seen in the previous section, fluid boluses were associated with worsening oxygenation, before and 1 hour after fluid bolus (Section 5.1).

Furthermore, serious concerns have been raised with regards to the safety of the fluids boluses since the publication of the FEAST study (Maitland *et al.* 2011). FEAST was a randomized trial which compared 0.9% saline (crystalloid) or 5% albumin (colloid) resuscitation with no fluid resuscitation in African children with severe infection. Unexpectedly the group receiving no fluid boluses had the lowest mortality at both 48 hours and at four weeks when compared with groups receiving either 0.9% saline or 5% albumin.

While there may be have been specific issues related to the study population and associated management, this well conducted trial challenges current concepts regarding fluid resuscitation for patients with severe infection. However, possible mechanisms for the increase in mortality remain speculative.

Over the last 20 years concepts regarding pulmonary fluid regulation have undergone substantial refinement (Londino *et al.* 2013). It is now estimated that only 30% of alveolar oedema following elevated pulmonary capillary pressure is due to direct hydrostatic effects with the remaining 70% due to active changes in bi-directional fluid flux (Kaestle *et al.* 2007). Recently the transient receptor potential (TRP) ion channel superfamily have been recognised as critical components of this active cellular process through sensing and transmission of a broad variety of external or internal stimuli, including mechanical stress (Yin *et al.* 2010). TRP vanilloid (TRPV)4 has mechanotransductive properties and is abundantly expressed in pulmonary blood vessels (Yin *et al.* 2010). Activation of these channels by shear forces (Troidl *et al.* 2009), stretch (Mochizuki *et al.* 2009), over-inflation (Hamanaka *et al.* 2007; Jurek *et al.* 2014), hypothermia (Hamanaka *et al.* 2007), increased hydrostatic pressure (Yin *et al.* 2008) and hypotonicity (Liedtke *et al.* 2003) leads to the rapid influx of calcium ions (Figure 1.1). The resultant increase in intracellular Ca^{2+} leads to increased permeability of the alveolocapillary barrier, and reduction in alveolar fluid clearance via down regulation of fluid channels, aquaporin

(AQP)5 (Sidhaye *et al.* 2008), and an increase in the proteolytic disruption of cell-cell or cell-matrix adhesion by matrix metalloproteinases (MMPs) (Villalta *et al.* 2014) leading to lung endothelial permeability (Villalta *et al.* 2013) which manifests as lung injury (Alvarez *et al.* 2006). Activation of TRPV4 channels has been implicated in the causation of lung injury in heart failure, ventilator induced and chemically induced lung injury (Hamanaka *et al.* 2007; Jurek etal 2014; Balakrishna *et al.* 2014).

It was hypothesised that administration of bolus intravenous fluids will lead to a transient rise in the shear forces across the pulmonary endothelium leading to activation of TRPV4 channels, increased lung endothelial permeability and disruption of the alveolar septal barrier which will manifest as lung injury, "Fluid Induced Lung Injury" (FILI). Further, that this disruption of the alveolar septal barrier with fluid administration will occur despite maintenance of safe hydrostatic pressure. Therefore, the aim of the current study was to examine the effect on the lung of rapid administration of intravenous fluids in a rat model. Following establishment of FILI in this model we aimed to determine whether the observed increase in permeability could be ameliorated by prophylactic administration of a recognised TRPV4 antagonist, i.v. ruthenium red.

5.3.1 METHODS

5.3.1.1 Ethics approval

All experiments were approved by the Flinders University Animal Welfare Committee and performed according to the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation (application number 812.12).

5.3.1.2 Animals

Specific pathogen-free male Sprague-Dawley rats (250-280g) were used in all experiments.

5.3.1.3 Fluids utilised

Animals were randomly assigned to administration of the following fluids - 0.9% saline (n=6) and 4% albumin (n=6). All the fluids were administered through the right femoral vein by utilizing a 3 way tap.

5.3.1.4 Controls

Negative control animals (n=6) did not received any i.v. fluids or were administered intra-tracheal LPS. Positive control animals (n=6) had induced acute lung injury using a previously established method of intra-tracheal LPS endotoxin (Dixon *et al.* 2009).

5.3.1.5 Study protocol

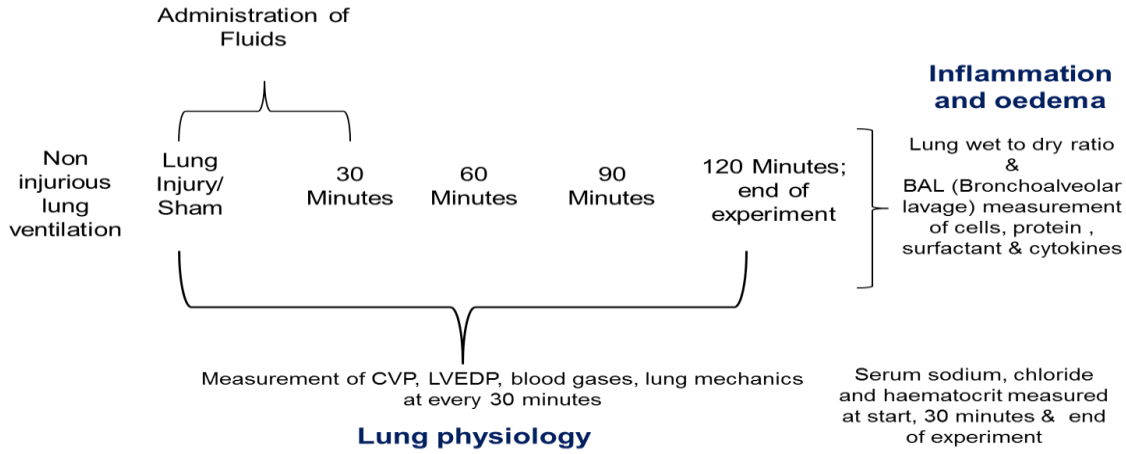
Rats were anaesthetised with intraperitoneal thiopentone sodium (60mg/kg; Abbott Australasia, Kurnell, Australia) and a right

femoral vein and artery catheterised for maintenance via continuous intravenous infusion of thiopental (50mg/kg/hr; Abbott Australasia) and for arterial blood sampling and pressure monitoring, respectively. Rats were paralysed with a bolus injection of pancuronium bromide (1mg/kg iv; Astra Zeneca, Bedfordshire, UK) maintained by continuous infusion (0.2mg/kg/h iv) and kept at 37°C with a temperature-controlled heat pad. A tracheotomy was performed and the lungs ventilated via a computer controlled small animal mechanical ventilator (flexiVent, SCIREQ Scientific Respiratory Equipment, Montreal, Canada) with 100% oxygen for 15 minutes to stabilise at a tidal volume (V_T) of 6ml/kg body weight, breathing frequency (f) of 120min^{-1}, and positive end expiratory pressure (PEEP) of 2 cmH_2O using a constant flow waveform where Ti/Ttot = 0.3.

LPS (*Escherichia coli* O55:B5, 15 mg/kg in saline, Sigma-Aldrich, St Louis, MO) (in positive control) or similar volume of saline was instilled through the tracheal catheter in 3 separate 0.1ml volumes, each volume followed by a 3ml air bolus and a respiratory recruitment manoeuvre of 2.5 × V_T and PEEP of 10cmH_2O for 15 seconds. The rat was left to stabilise on normal ventilation as above for 5 minutes after each instillation. Following LPS or saline instillation, rats were ventilated for 2 h, as above, while blood pressure was monitored continuously using a disposable pressure transducer (Sorenson Trans Pac; Abbott Critical Care Systems, Chicago, IL) connected to a MacLab system (AD Instruments,

Sydney, Australia). Study plan with description of various measurements at various time intervals is shown in figure 5.6.

Figure 5.6: Study protocol used in the animal study



Rt carotid artery: LVEDP; Rt femoral artery: BP and blood sampling; Rt femoral vein: for administration of drugs and fluids; Lt femoral vein: CVP; Tracheostomy- lungs ventilated via a computer controlled small animal mechanical ventilator (flexiVent, SCIREQ) aslo capable of measuring lung mechanics

5.3.1.6 Monitoring

Continuous monitoring of right heart pressure (CVP) was done by canulating the left femoral vein. Similarly continuous monitoring of the left heart pressure was done by cannulating the right carotid artery and advancing the cannula in to the left heart (left ventricular end diastolic pressure) (LVEDP). Both were monitored using a disposable pressure transducer (Sorenson Trans Pac; Abbott Critical Care Systems, Chicago, IL) connected to a MacLab system (AD Instruments, Sydney, Australia).

5.3.1.7 Dose finding experiment

In order to minimise development of hydrostatic oedema by rapid infusion of i.v. fluid, we conducted a dose response study of administration of 0.9% saline and 4% albumin administered as boluses of 20 ml/kg i.v. at 10 min intervals via the right femoral vein over 40 min. A clinically defined cut-off of an increase in LVEDP less than 18 mm Hg was utilised as hydrostatic pulmonary oedema has been described at higher levels (Forrester et al. 1976). Based on these findings 0.9 % saline and 4% albumin were administered in all subsequent experiments at 60 ml/kg over 30 min (Figure 5.6).

5.3.1.8 Measurement of respiratory mechanics

Respiratory mechanics (airway (Newtonian) resistance, Raw; tissue resistance, Gtis; and tissue elastance, Htis) were measured before instillation (baseline), 5 minutes after the last instillation of either LPS or saline (start) and every 30 min thereafter for the duration of the experiment (Figure 5.6), by measuring the lung's impedance (Z) using the computer-controlled ventilator, as described previously (Davidson *et al.* 2002). Briefly, 2 min after a recruitment manoeuvre ($2.5 \times V_T$), impedance of the respiratory system was measured following a forced oscillation. The data were fitted to a constant phase model (Bates *et al.* 1992) where $Z = Raw + jI + (Gtis - jHtis)/(2\pi f)^{\alpha}$, where I is inertance, j is the imaginary unit, f is

frequency and α = (2/π)arc tan (Htis/Gtis). Inertance was negligible and is therefore not reported.

5.3.1.9 Assessment of lung injury

Blood samples were taken hourly throughout ventilation for measurement of arterial blood gas-pH (ABL 5, Radiometer, Copenhagen, Denmark) and bi-hourly for plasma cytokines. The lungs were removed and the right upper lobe resected for determination of wet-to-dry lung weight ratio, as described previously (Davidson *et al.* 2002). The remaining lung was degassed at 0.5 atm for 60 seconds and lavaged at 2°C with 3 separate 32ml/kg bodyweight volumes of 0.9% sodium chloride, each volume instilled and withdrawn three times. Percent recovery of lavage fluid was not different between the animals. The lung lavage fluid was centrifuged at 150 *g* for 5 minutes at 2°C. A sample was taken from the supernatant, aliquoted and stored at -80°C until analysis for cytokines. A further aliquot was taken for determination of total lung lavage protein with commercially available reagents (BioRad DC Protein Assay; BioRad Laboratories, Hercules, CA).

5.3.1.10 Surfactant analysis

The remaining supernatant was centrifuged at 1000 *g* for 25 minutes at 2°C to give a tubular myelin rich pellet (large aggregate) and a tubular myelin poor supernatant (small aggregate). Lipids were extracted from each fraction using the method of Bligh and

Dyer (Bligh *et al.* 1959) and total phospholipid content determined by measuring the amount of inorganic phosphorus with the method of Bartlett (Barlett *et al.* 1959). Measured surfactant was normalised to gram of dry lung weight. Results are expressed as a ratio of small and large aggregates.

5.3.1.11 Cytokine determination

Cytokine concentrations in lung lavage and plasma were analysed using commercially available enzyme linked immunosorbant assay (ELISA) kits for TNF-α, IL-8 (CINC-1) and PLA2 (R&D Systems), as per manufacturers instruction and described previously (Dixon *et al.* 2008).

5.3.1.12 Histological analysis

Following BAL, the right lung lobes were fixed at 20 cmH_2O with 10% buffered formalin. Paraffin-embedded sections (4 μm) were stained with hematoxylin and eosin for scoring of pulmonary inflammatory cell infiltration and alveolar wall thickening using a semi quantitative score (0-3) on blinded sections by two independent investigators (Dixon *et al.* 2009).

5.3.1.13 Electrolyte and haematocrit measurement

Serum sodium and chloride are measured using Ion Selective Electrodes (ISE). Haematocrit was measured with a haematocrit reader.

5.3.1.14 Administration of ruthenium red

The experiments were repeated with the control group (n=9), animals administered with i.v. 0.9% saline (n=8) or ruthenium red (1 micro mol/1 of blood) before the administration of 0.9% saline (n=7)). There were also control rats who were administered ruthenium red only (n=5). The protocol was similar to that used in experiment (Figure 5.6)

5.3.1.15 Statistical analysis

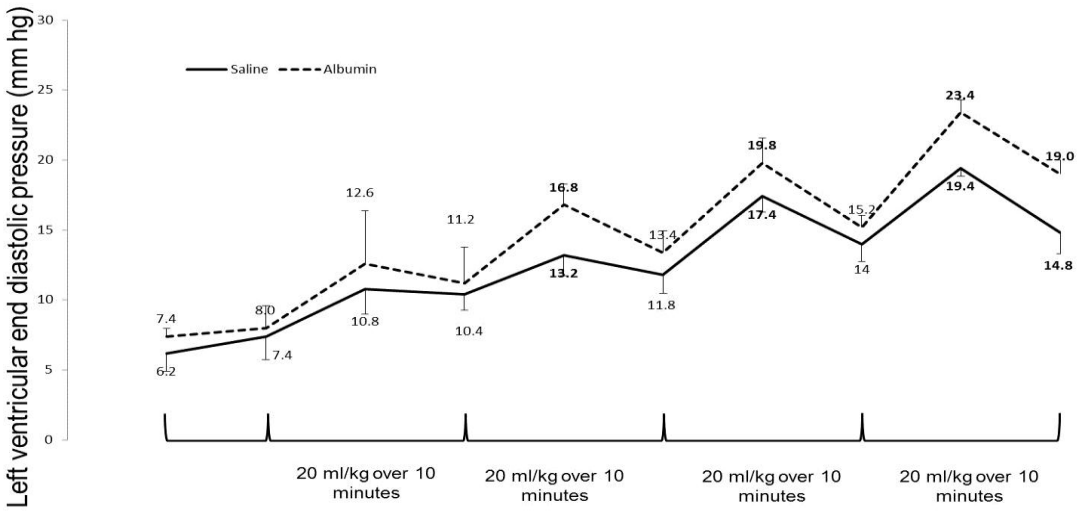
Statistical analyses were performed using PASW 22.0 software (SPSS Inc). All values are expressed as mean (SD). Two-way analysis of variance (ANOVA) and repeated measures analysis of variance with Tukey post-hoc tests were used to determine significant differences ($P \leq 0.05$).

5.3.2 RESULTS

5.3.2.1 Experiment : dose determination

The effect of administration of boluses of 0.9% saline and 4% albumin on the LVEDP is shown in Figure 5.7. LVEDP increased to more than 18 mm Hg after the fourth bolus of 20 ml/kg (total 80 ml/kg). Based on this we utilised 60 ml/kg administered over 30 minutes for both the fluids.

Figure 5.7: Effect of administration of 0.9% saline and 4% albumin on the left ventricular end diastolic pressure (LVEDP)

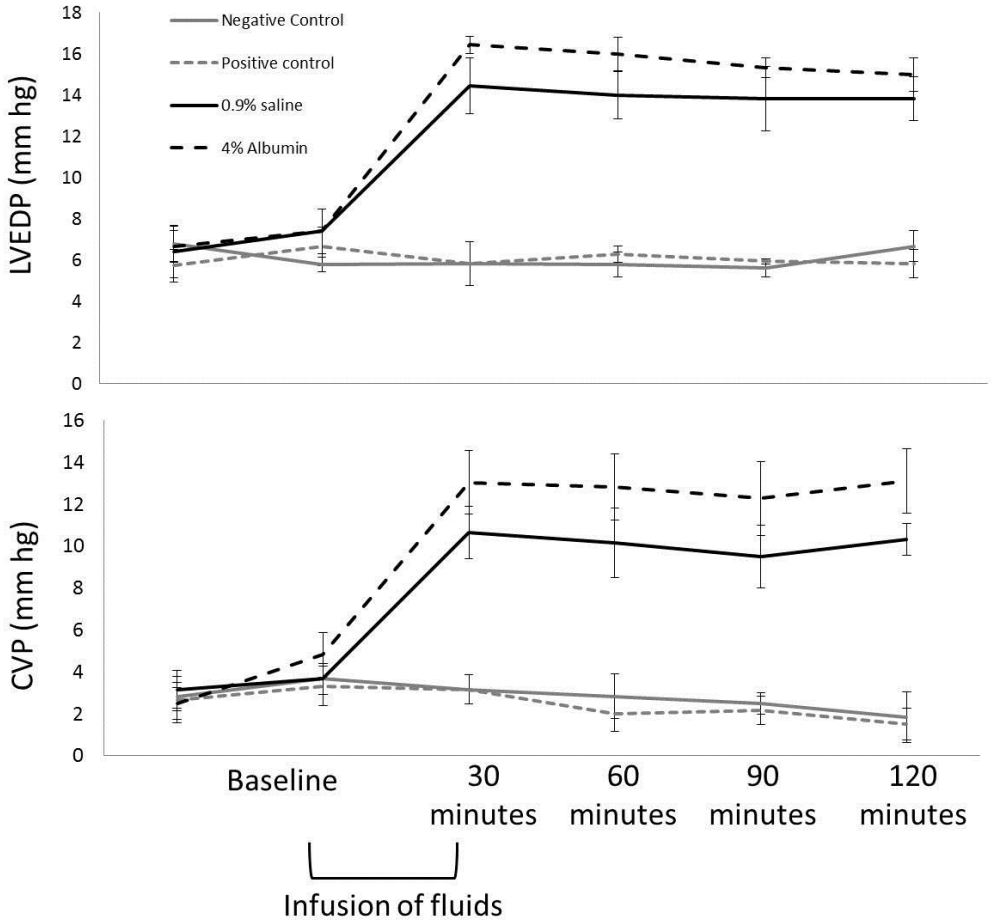


Each bolus was administered as 20 ml/kg each over 10 minutes. There was a difference between 0.9% saline and 4% albumin (p=0.008). LVEDP was less than 18 mm hg for both the fluids till the third bolus i.e. 60ml/kg (total volume).

5.3.2.2 Experiment: Effect of fluid boluses

There was an increase in LVEDP following administration of both 0.9% saline and 4% albumin (Figure 5.8). However, this remained under the clinically defined cut-off of 18 mm Hg in all animals. There was similarly an increase in CVP with administration of both intravenous fluids (Figure 5.8). Both the negative (no fluid) and positive (LPS-induced ALI) controls had no change in either LVEDP or CVP over the course of the experiment.

Figure 5.8: Effect of fluid administration on the left ventricular end diastolic pressure (LVEDP) and the central venous pressure (CVP) at the volume of 60 ml/kg administered over 30 minutes



Data presented as mean and SD.

Changes in serum sodium, chloride and haematocrit- Administration of 0.9 % saline increased serum sodium and chloride at 30 and 120 minutes, while 4% albumin lead to the largest decrease in the haematocrit levels. (Table 5.6).

Table 5.6: The effect of 0.9 % saline and 4% albumin infusion on serum sodium, chloride and the haematocrit

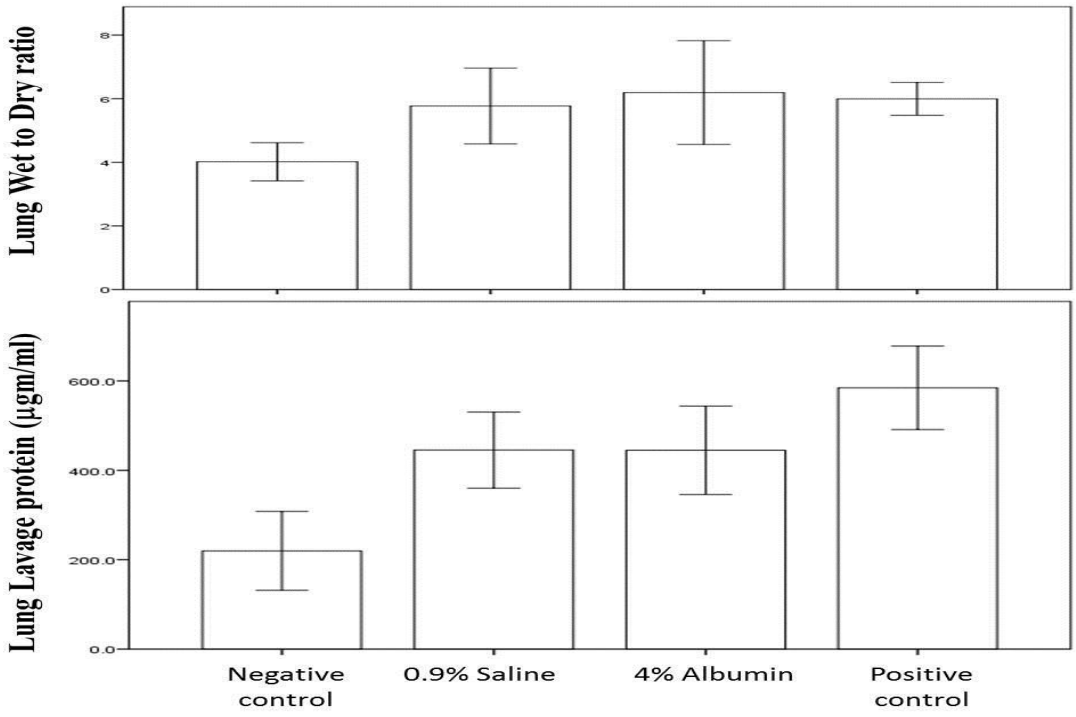
	Negative Control			0.9% Saline			4% Albumin			Positive Control			p
	Baseline	**30 min**	**120 min**	**Baseline**	**30 min**	**120 min**	**Baseline**	**30 min**	**120 min**	**Baseline**	**30 min**	**120 min**	
Haematocrit (%)	.45 (.02)	.47 (.05)	.48 (.03)	.46 (.01)	.41 (.03)	.44 (.04)	.45 (.04)	.33 (.02)	.36 (.03)	.44 (.05)	.44 (.02)	.46 (.02)	0.001
Serum Sodium (mmol/l)	136 (1)	137 (1)	138 (2)	137 (2)	140 (2)	142 (2)	137 (1)	133 (2)	135 (3)	135 (1)	137 (2)	138 (1)	0.001
Serum Chloride (mmol/l)	102 (1)	102 (1)	103 (1)	101 (1)	115 (2)	111 (2)	102 (1)	106 (2)	102 (2)	101 (1)	102 (2)	102 (1)	0.001

Data presented as mean and SD.

Fluid induced lung injury

Administration of both 0.9% saline and 4% albumin lead to an increase in the lung wet to dry weight ratio and lung lavage protein when compared with the negative control (Figure 5.9).

Figure 5.9: The effect of fluid boluses on lung oedema (wet to dry ratio) and lavage protein levels

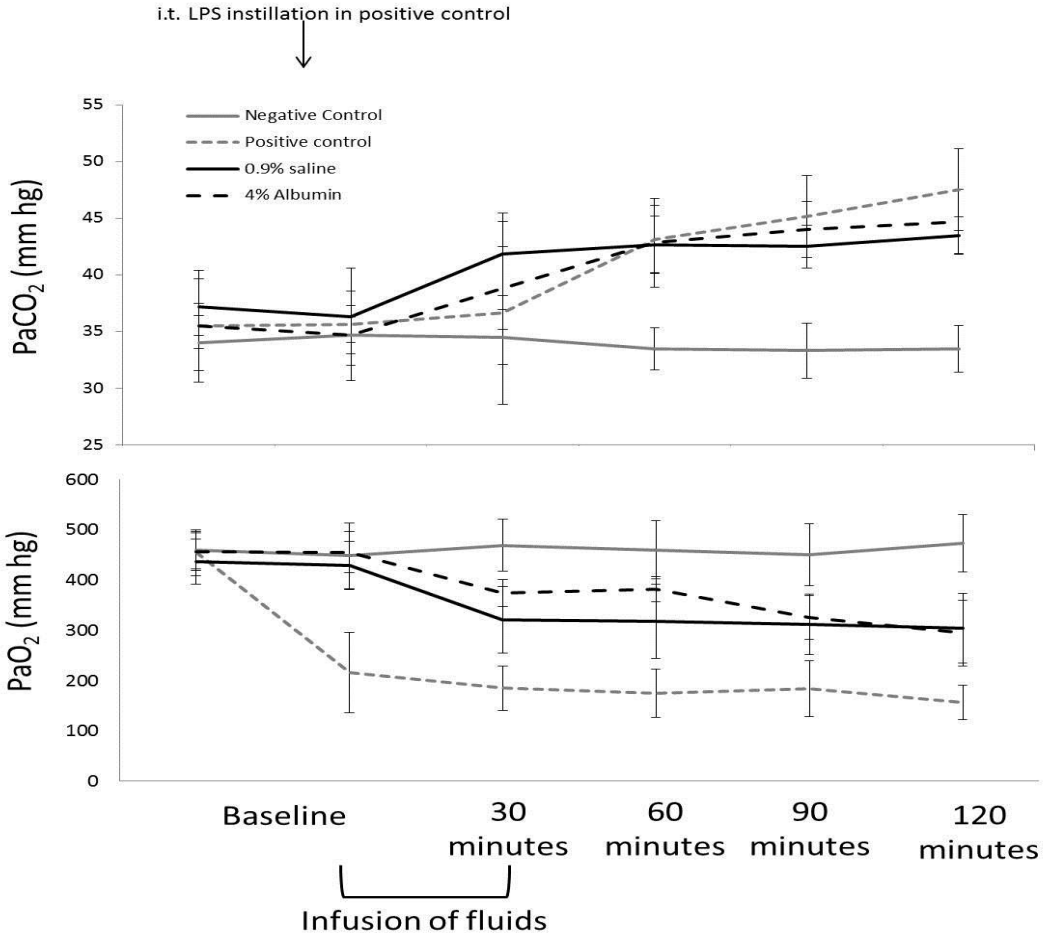


Data presented as mean and 2 SD.

Both fluids led to a decrease in the PaO_2 levels when compared with the negative controls but did not reach to levels seen in animals in the positive control group. Both fluids and animals in the positive control group, had an increase in $PaCO_2$ levels over time. (Figure 5.10)

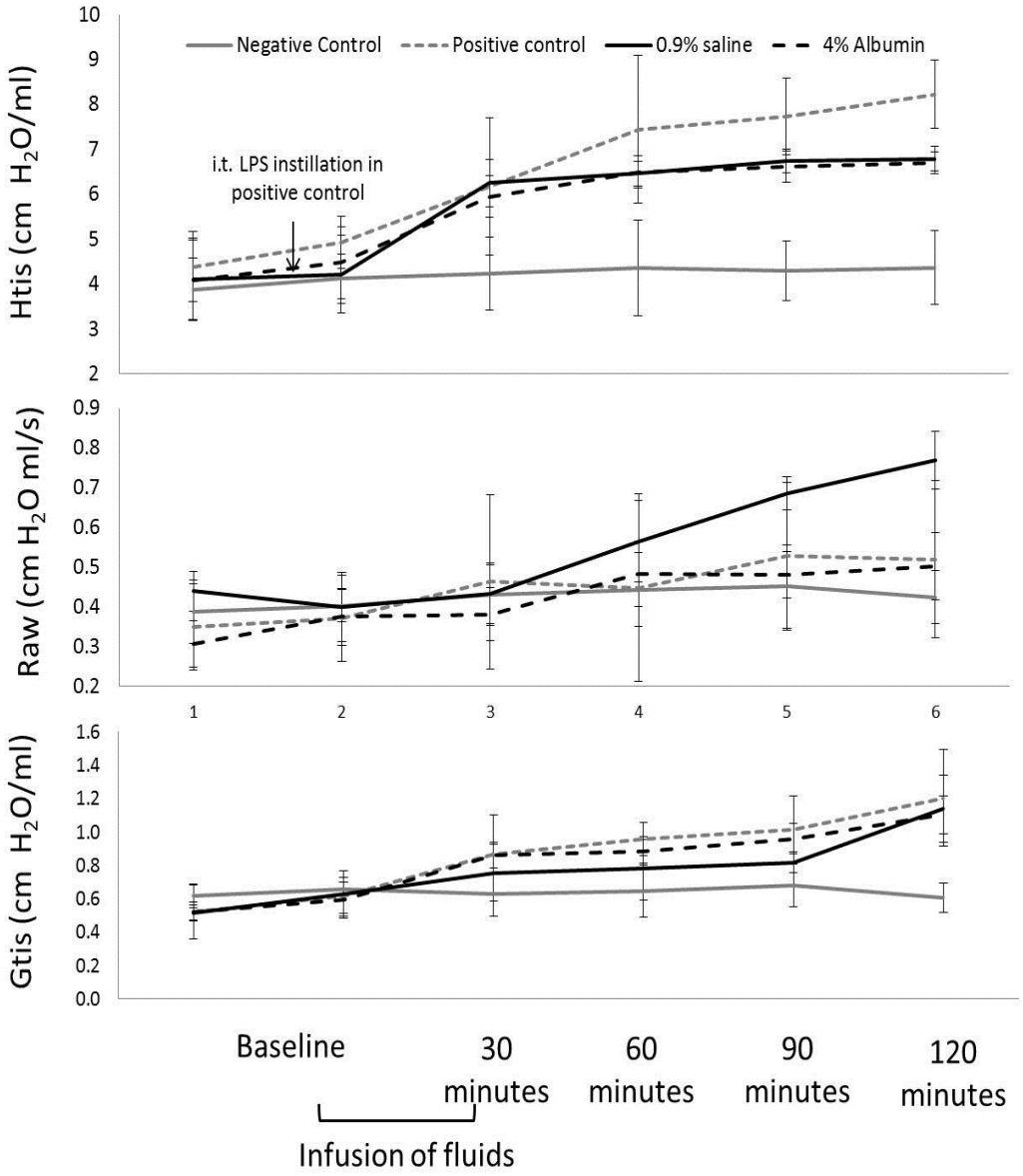
Both fluids, and animal in the positive control group had a rise in the Htis and Gtis over time. Only animals in the positive control had a rise in airway resistance over time (Figure 5.11).

Figure 5.10: The effect of fluid boluses on PaO_2 and $PaCO_2$ levels



Data presented as mean and SD.

Figure 5.11: The effect of fluid boluses on lung mechanics



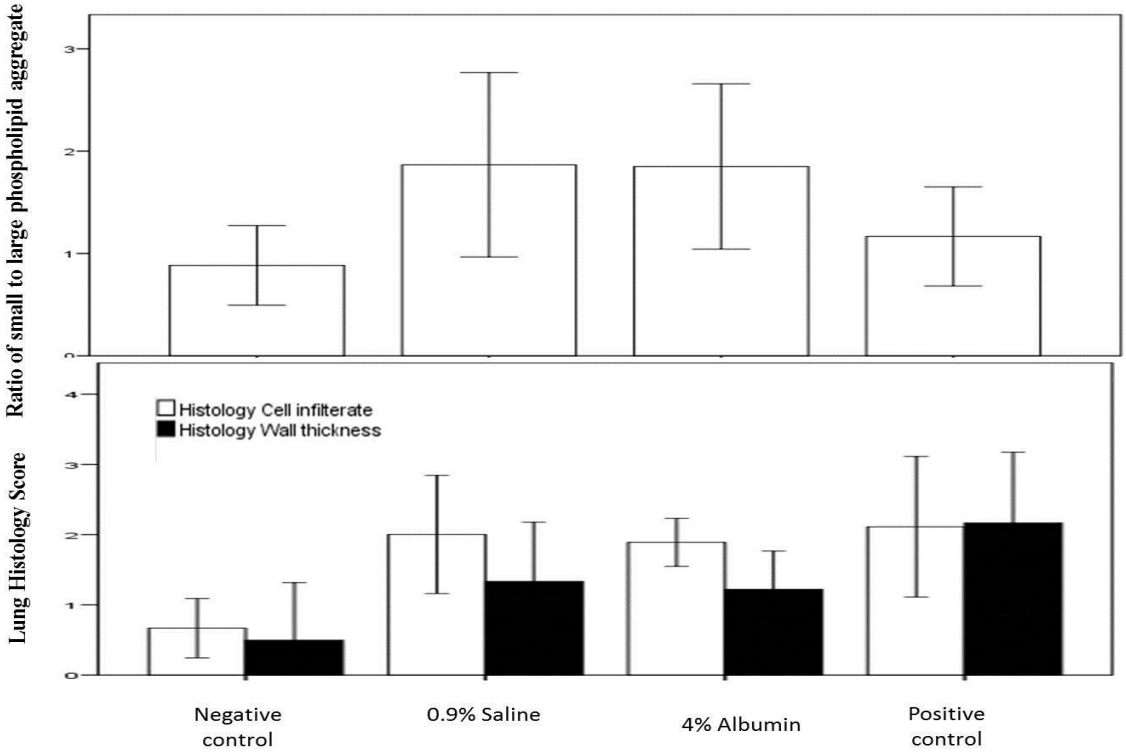
Data presented as mean and SD.

There was no change in the total amount of phospholipids but there was an increase in the ratio of small to large aggregates with both the fluids and the positive control animals (Figure 5.12)

There was an increase both in the cellular infiltrate and wall thickness oedema score with administration of fluids and animals in the positive control group (Figure 5.12).

Administration of fluids had no effect on the lung lavage cell count, neutrophil count, MPO activity assay, lung lavage and blood TNF-α and IL-8 levels. All of these parameters increased in the positive control animals.

Figure 5.12: The effect of fluid boluses on the ratio of small and large lung lavage phospholipid aggregate and lung histology (cell infiltrate and wall thickness score) score



Data presented as mean and 2 SD.

5.3.2.3 Experiment: Use of TRPV4 blocker- ruthenium red

Administration of ruthenium red had no effect on any measured variable when compared to the negative control animals.

Administration of ruthenium red before 0.9% saline led to lower lung oedema ($p<0.001$), lavage protein ($p=0.01$) and $PaCO_2$ and increased PaO_2 ($p>0.001$) and lung elastance at 2 hours ($p<0.001$) (Table 5.7). There was an increase in PLA2 levels in animals who were administered 0.9% saline, but the group of animals who received ruthenium red before the administration of fluids, had the same levels as the control group. (Table 5.7)

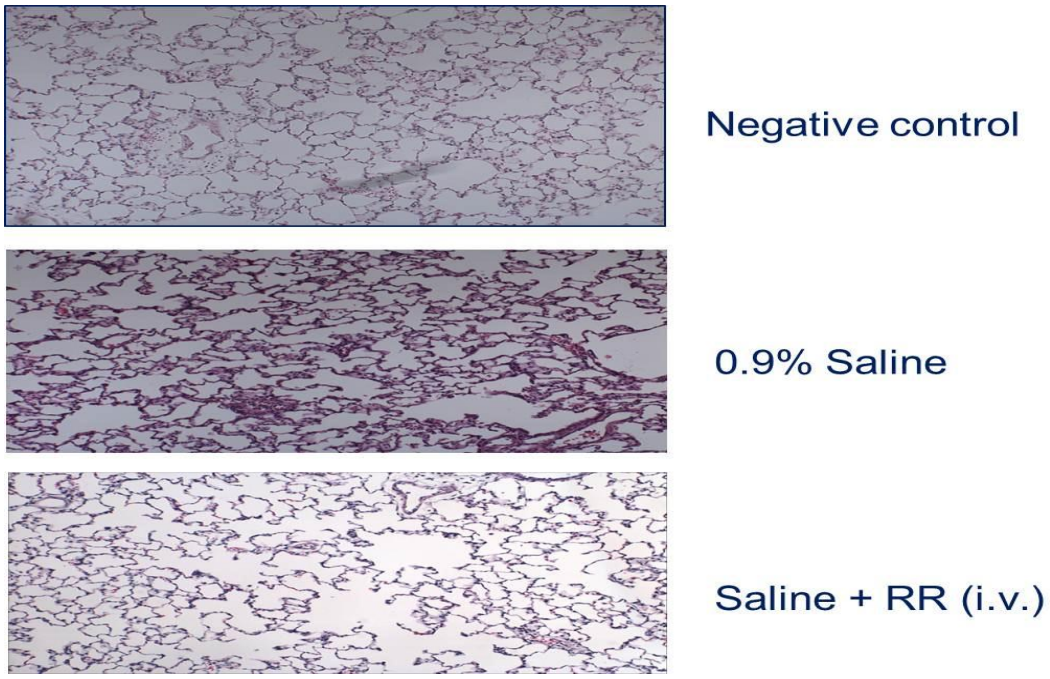
Lung histology score (cellular infiltrate and oedema score) were less in animals who were administered ruthenium red as compared with animals who were administered 0.9% saline (Figure 5.13).

Table 5.7: Parameters of lung injury 2 hours after initiation of mechanical ventilation and administration of i.v. fluids and RR.

	No fluid control (n=6)	**i.v. 0.9% saline (n=6)**	**i.v. 0.9% saline + RR (n=6)**	**p value**
Lung wet to dry ratio	4.1 (0.3)	5.9 (0.8)	4.6 (0.8)	0.01
Whole lung lavage protein (μgm/ml)	222.5 (15.2)	415.0 (76.9)	201.9 (52.1)	0.03
Whole lung lavage PLA2 (μm/min/ml)	0.16 (0.03)	1.63 (0.23)	0.89 (0.40)	0.04
Lung elastance cmH_2O/ml - 2 hours	4.4 (0.3)	6.5 (0.8)	4.8(0.7)	0.05
PaO_2 (mm Hg)- 2 hours	480 (17)	324 (77)	487 (18)	0.01
Lung histology score: cellular infiltrate	0.7 (0.3)	2.0 (0.4)	1.0 (0.6)	0.01
Lung histology score: wall thickness	0.6 (0.4)	1.5 (0.6)	1.1 (0.5)	0.01

Data presented as mean (SD). RR: Ruthenium red. Lung histology scores 0-3 of H&E tissue sections

Figure 5.13: Hematoxylin and eosin staining in the lung paraffin-embedded sections (negative controls, 0.9% saline and 0.9% saline with ruthenium red)



Administration of ruthenium red led to a decrease in worsening of lung histology with 0.9% saline administration.
RR; Ruthenium red

5.3.3 DISCUSSION

There are two main findings from this study. Firstly, it was shown that 0.9% saline or 4% albumin at 60 ml/kg administered over half an hour lead to permeability oedema in rats who are mechanically ventilated, despite a 'safe' LVEDP. Secondly, development of oedema with 0.9% saline is prevented by prophylactic administration of ruthenium red, suggesting activation of TRPV4 channels as a central event to the causation of the observed oedema.

5.3.3.1 Fluid induced permeability oedema – lung injury (FILI)

Multiple studies demonstrate lung oedema subsequent to a large increase in lung capillary pressure (West *et al.* 1991; Pietra *et al.*

1969; Nicolaysen *et al.* 1979; Rippe *et al.* 1984; Maron *et al.* 1989; Minnear *et al.* 1983). However, in the current study permeability oedema occurred at pressures traditionally considered as within clinically safe limits (Forrester *et al.* 1976). Previously, Wasserman *et al.* showed that small infusions of saline-dextran lead to increased lymph to protein concentration ratio for albumin and dextran. They hypothesised that capillary pore size is variable and can be increased by increasing the plasma volume (Wasserman *et al.* 1955). Similarly in 1968, horseradish peroxidase (HSP) injected in to mice was found by ultra structural cytochemistry to have passed through end cell junctions in to the basement membrane, however this has never been measured in the lung lavage protein (Schneeberger-Keeley *et al.* 1968). Even though anecdotally circulatory overload has been implicated as a cause for fluid induced pulmonary oedema, as often discussed in the context of transfusions (Andrzejewski *et al.* 2013), no direct relationship with fluid induced permeability oedema has been described. Fluid administration associated respiratory dysfunction is a common clinical conundrum, evidenced by more than 25% of patients in the CHEST study (in both the study arms) who developed new onset respiratory failure (Myburgh *et al.* 2012) after fluid administration.

5.3.3.2 Role of TRPV4 channels

In the last decade activation of TRPV4 channels leading to influx of calcium ions and increased endothelial permeability has been implicated in the causation of lung injury in heart failure, ventilator induced and chemical lung injury (Hamanaka *et al.* 2007; Jurek *et al* 2014; Balakrishna *et al.* 2014). In vitro activation of TRPV4 requires hydrolysis of membrane phospholipids via PLA2 and subsequent arachidonic acid metabolism by cytochrome P450 epoxygenases to form epoxyeicosatrienoic acids (EET) (Jian *et al.* 2008). In this study there was evidence of increased lung lavage PLA_2 with fluid administration. More importantly we found decreased permeability oedema evidenced by a decrease in the lung oedema (wet to dry ratio) and permeability (lung lavage protein) when the rats were prophylactically administered systemic ruthenium red. Ruthenium red (RR) is a well-studied, although non-specific, TRPV4 antagonist examined both in isolated perfused lungs and after nebulisation (Jurek *et al.* 2014; Yin *et al.* 2008; Jian *et al.* 2008). Systemic intravenous administration of ruthenium red per se did not lead to any pulmonary effects in our studied time frame. This suggests that administration of fluids though within "safe pressures", may lead to an increase in shear forces causing an activation of these vanilloid channels and intracellular influx of calcium. In vitro data suggests that TRPV4 can be activated within 5 minutes of stimulation by mechanical forces (Matthews *et al.* 2010), and in our study, fluid was administered over 30 minutes providing

enough time for its activation. In 1999 Kuebler and colleagues showed that an increase in pulmonary venular pressure lead to an increased expression of P-selectin via influx of intracellular calcium ions, while blockade of mechanogated Ca^{2+} channels with gadolinium, (physiological blocker of TRPV induced calcium influx (Kuebler *et al.* 1999), inhibited those responses. Gadolinium causes loss of TRPV4 mediated currents and is an inhibitor of endothelial stretch-activated channels (Lorenzo *et al.* 2008; Parker *et al.* 1985). It prevents pressure-induced permeability increases in isolated rat lungs (Parker *et al.* 1985), abolishes the pressure induced increase in calcium in lung venular capillaries (Kuebler *et al.* 1999) and blocks stretch-activated ion channels (Yang *et al.* 1989). This supports the hypothesis that the increase in calcium seen in the Kuebler study was due to activation of TRPV4 channels.

Weibel–Palade bodies (WPBs) are endothelial granules that store von Willebrand factor (VWF), P-selectin, angiopoietin 2 and other vascular modulators (Lowenstein *et al.* 2005). De-granulation of Weibel Palade bodies following increased intracellular calcium ions (via activation of TRPV4 channels) will release these pre-formed inflammatory mediators. Ang-2, sensitizes the endothelial cell to the inflammatory actions of TNF-α thus further perpetuating inflammation and circulatory collapse (Fiedler *et al.* 2006), while P-selectin facilitates cellular infiltration. In the FEAST trial (Maitland *et al.* 2011), i.v. fluids led to an increase in early mortality in children with severe infection, mostly due to cardiovascular

collapse. Using the TRPV agonist GSK1016790A Willette observed endothelial failure and circulatory collapse with systemic activation of the TRPV4 channel (Willette *et al.* 2008), thereby suggesting the hypothesis that activation of TRPV4 with administration of fluids may explain the FEAST result.

5.3.3.3 Dose of fluids

In the multi-centre (46 Intensive Care Units (ICU)) point prevalence study, (Section 3.2) fluid boluses were administered to 35% of ICU patients, and 42% of patients who were in the ICU for less than 3 days. In a more specific population, such as sepsis, 94% of patients continue to receive fluid boluses after the initial resuscitation period (Section 5.1). Moreover, the volume of these fluid boluses are often large, for example, in patients receiving early goal direct therapy 3499±2438 ml of fluid was administered with 4981±2984 ml administered to patients in the control arm in the first 6 hours (Rivers *et al.* 2001), and in the 6S study (Perner *et al.* 2012), patients on day 1 in the hydroxyethyl starch group received a median of 1500 ml in addition to open label fluid, other fluid and blood products (total 5825 ml (median) on day 1). As there are multiple reports and evidence of lung oedema with high pulmonary microvascular pressures (Forrester et al. 1976), we conducted initial dose finding experiment. It is noteworthy that 60 ml/kg is the same as the dose used in the FEAST study (60 ml per kilogram in stratum B) (Maitland *et al.* 2011) after a protocol amendment in June 2010.

5.3.3.4 Effect of fluid on surfactant and lung mechanics

Previously in healthy subjects, rapid infusion of 0.9% saline has been shown to reduce forced vital capacity, reduce forced expiratory volume in 1 second, increase airway resistance, produce premature airway closure and hypoxemia, reduce maximum oxygen consumption and impair diffusion of oxygen (Muir *et al.* 1975; Pellegrino *et al.* 2003; Farney *et al.* 1977). In the current study there was worsening of lung elastance with fluid administration which would be consistent with the findings from the healthy subjects. Several authors have proposed that the initial increase in airway resistance observed in experimentally induced pulmonary oedema is secondary to distension of the pulmonary veins within bronchovascular sheaths, with subsequent compression of the smaller peripheral airways (Hogg *et al.* 1972; Staub *et al.* 1967) which would explain the increase in the tissue resistance as seen in our study. Moreover we found an increase the ratio of small to large aggregates with fluid administration. One possible reason for this is the increase in permeability and alveolar protein which would increase the conversion from large to small aggregates (Davidson *et al.* 2000). As all the animals in this experiment were mechanically ventilated fluid-induced surfactant dysfunction could be a contributory factor to the currently understood ventilator-induced surfactant dysfunction, (Albert 2012; Greenfield *et al.* 1964) and should be an area of future studies

5.3.3.5 Limitations and future directions

1. Fluid dose- even though the dose was carefully selected the response of a different dose and a different rate of infusion was not investigated.
2. Animals were ventilated for only 2 hours in this study therefore longer term pulmonary effects of fluid boluses were not investigated.
3. Even though we did measure the LVEDP, pulmonary artery pressure and cardiac output were not directly measured.
4. We did not find any increase in lung lavage cells, even though there was an increase in the cellular infiltrate score on the lung histology. The ventilation period of 2 hours may not be enough for this change to occur.
5. As ruthenium red is a non-specific TRP inhibitor, it can also interact with many non-TRP proteins including Ca^{2+}-ATPase, mitochondrial Ca^{2+} uniporter, tubulin, myosin light-chain phosphatase and Ca^{2+} binding proteins such as calmodulin (Jurek *et al.* 2014), hence prevention of fluid induced lung injury with RR is consistent with, but not specific for a TRPV4 mechanism. We used ruthenium red as it was well studied and was readily available. Since, a more selective TRPV4 antagonist has become available and should be examined. Similarly, the effect of fluid bolus on TRPV4 knock out mice can also be examined in future studies to further define the role of TRPV4 channels for fluid induced lung injury.

Future directions

Administration of i.v. fluids and fluid boluses is ubiquitous in pre-hospital and hospital care and fluid resuscitation is frequently required to restore adequate perfusion to vital organs in critically ill patients. However, this may inadvertently contribute to morbidity and mortality. Future studies should examine the pulmonary and systemic effect of fluid resuscitation with the use if systemic TRPV4 blockers (under development with GSK) in both animal models and healthy volunteers. This should lead to safer fluid resuscitation in future.

5.3.4 SUMMARY

Bolus i.v. fluids can result in permeability pulmonary oedema despite a safe (non-hydrostatic) LVEDP. Administration of ruthenium red prevents such permeability oedema suggesting a TRPV4 mechanism.

5.4 Bolus administration of 0.9% saline leads to interstitial pulmonary oedema despite normal echocardiographic indices in healthy subjects when compared with 4% albumin and 5% glucose

After demonstrating fluid induced lung injury in an animal model, the effect of bolus fluid administration in healthy subjects was examined. Current clinical studies do not differentiate the effects of crystalloid and colloid resuscitation (Finfer *et al.* 2004; Perel *et al.* 2013; Choi *et al.* 1999), apart from toxicity attributable to specific synthetic colloids (Perner *et al.* 2012; Myburgh *et al.* 2012). However, the ratio of crystalloid and colloid required for resuscitation differs (Finfer *et al.* 2004; Brunkhorst *et al.* 2008; James *et al.* 2011) from that originally suggested (Twigley and Hillman 1985) and the central volume of distribution of crystalloid is substantially smaller than the anatomic extracellular volume (Hahn 2010). Moreover, recent evidence wherein both of these fluids led to unexpected increases in mortality in African children (Maitland *et al.* 2011) with severe infection and to the revision of the standard Starling equation (glycocalyx model) (Woodcock *et al.* 2012), has underscored some of the current lacunas in our understanding of the cardio- respiratory physiological effects of fluid boluses with crystalloid and colloids.

Previously, rapid infusion of 0.9% saline in healthy subjects has reduced forced vital capacity, forced expiratory volume in 1 second, and maximum oxygen consumption, and produced premature

airway closure and hypoxemia, and impaired diffusion of oxygen (Muir *et al.* 1975; Pellegrino *et al.* 2003; Farney *et al.* 1977). However, no current comparison with colloids has been performed in healthy subjects. In the current study, the effect of equivalent volumes (30 ml/kg) of a colloid (4% albumin- CSL Biotherapies), crystalloid (0.9% saline make - Baxter) and water (5% glucose- Baxter IV) on cardio respiratory parameters in healthy human volunteers was examined.

Based on the current convention, in which albumin will remain intravascular, 0.9% saline will expand the extracellular space, and 5% dextrose will expand both intra and extracellular spaces, it was hypothesised that 0.9% saline and 4% albumin would both lead to interstitial oedema;

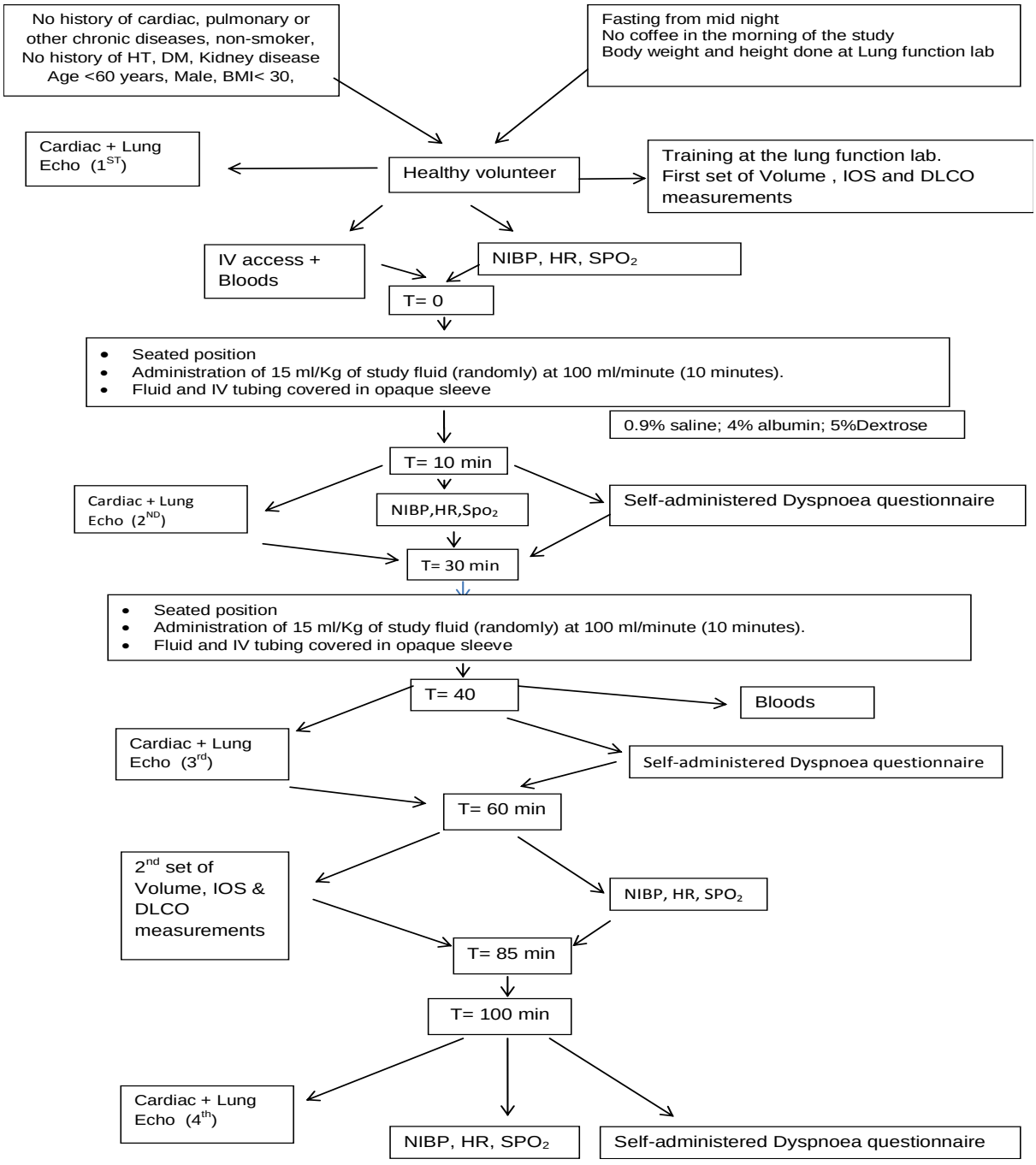
- 0.9% saline, due to its volume of distribution
- 4% Albumin due to cardiogenic (increase filtration) oedema.

5.4.1 METHODS

After approval from our ethics committee (Southern Adelaide Clinical Human Research Ethics Committee- approval number 263.12) four healthy male adult volunteers were selected for the study who had no history of cardiac, pulmonary or other chronic diseases (hypertension, diabetes mellitus or kidney disease). All of them were non-smokers, less than 40 years of age and body mass index less than 30. Informed consent was obtained from all of them. They were randomised in a double blind cross over study and

administered 30 ml/kg i.v. 0.9% saline, 4% albumin and 5% glucose at a rate of 100ml/minute on three separate days. The study protocol is shown in Figure 5.14.

Figure 5.14: Study protocol used in administrating fluid boluses in healthy subjects



T- Time, NIBP- Non-invasive blood pressure, DLCO- Diffusion capacity for carbon mono oxide, HR- Heart rate, Min- Minutes, Echo-Echocardiography

5.4.1.1 Respiratory measurement: Respiratory measurements were made in a specific sequence with respiratory resistance followed by slow and forced spirometry, single breath gas diffusing capacity and body plethysmography lung volumes before and after fluid administration. Respiratory resistance and reactance measurements were made by the impulse oscillometry method (CareFusion Jaeger Masterscreen IOS, CareFusion Germany 234 GmbH). Measurements were made during 30 seconds quiet tidal breathing, with cheeks supported, to obtain approximately 100 impulses. Tidal breathing was free of artefact, such as swallowing or glottic closure, and coherence was greater than 0.7 at 5 Hz and 0.9 at 20 Hz. Measurements were made in triplicate before and after intervention. Respiratory resistance and reactance measurements are the mean of the 3 measurements and represent the average from over 300 pulses during the tidal breathing cycle. Spirometry measurements were made on the same CareFusion Jaeger Mastersreen system. Slow spirometry measurements were made in duplicate with vital capacity (VC) repeatable within 150 mls. Forced spirometry measurements were made complying with ATS/ERS guidelines for test acceptability and repeatability. The best FEV1 and FVC measurements from each measurement session are reported. Diffusing capacity was measured using the single breath method (CareFusion Jaeger, Masterscreen PFT), corrected for haemoglobin, and adhering to the ATS/ERS Guidelines for test performance (Wanger *et al.* 2005; Macintyre *et al.* 2005; Miller *et al.*

2005). In all cases at least 2 measurements were made, repeatable to within 1.0 ml/min/mmHg. Total lung capacity (TLC) was measured by body plethysmography on the same system. Intra thoracic gas volume (ITGV) was estimated with supported cheeks at a breathing frequency of 15 to 20 breaths per minute. At least 3 measurements were obtained within 5% and TLC calculated from ITGV plus inspiratory capacity (IC). Residual volume (RV) was calculated as TLC – VC.

Oscillometry parameters dX/dV (Delta reactance/delta lung volume) was derived from the average change in reactance at 5 Hz divided by the average change in tidal volume during a 30 second period of tidal breathing. Similarly, dX/dflow (Delta reactance/delta flow rates) was derived from the average change in reactance at 5Hz divided by average expiratory tidal flow during the same period of tidal breathing.

All measurements of spirometry, diffusing capacity and total lung capacity were made according to the ATS/ERS guidelines for the individual measurements and were made by experience laboratory scientific staff with in excess of 10 years' experience and holding the Certified Respiratory Function Scientist Credential from the Australian and New Zealand Society of Respiratory Science. They were blinded to the type of the administered fluid on the study day. All the subjects underwent training in the respiratory function laboratory before the study day.

5.4.1.2 Cardiac and lung ultrasound: A Sonosite M-Turbo® ultrasound machine with phased array (1 to 5 MHz) transducer was used for echocardiographic assessments. Subjects were assessed in the supine position. Screening echocardiography was done before the study to rule out any major cardiac abnormality, also left ventricular out flow tract (LVOT) diameter was measured for calculating cardiac outputs on the study day. A single experienced person performed all the cardiac and lung ultrasound for the study and he was blinded to the type of administered fluid.

Lung ultrasound: Lung ultrasonography was performed before, half way, at the end of the infusion and 1 hour after the end of the infusion (Figure 5.14). It was performed along the posterior, middle and anterior axillary lines, and along the mid-clavicular and parasternal lines at the 2^{nd} to 5^{th} intercostal spaces bilaterally, specifically looking for B-lines. Performing echo scanning (right and left hemithorax, from the second to fourth intercostal spaces, from the parasternal to midaxillary lines), an individual patient comet score was obtained by summing the number of comets in each scanned space. A patient score was obtained by summing the number of B lines from each of the scanning spaces in the anterior right and left chest, from the second to fifth intercostal spaces.

Cardiac ultrasound measurement: Echocardiographic measurement of left ventricular outflow tract (LVOT) velocity time integral (VTI), inferior vena cava (IVC) size/variability, mitral inflow Doppler, and left ventricular annular tissue Doppler (both septal and lateral wall)

were performed prior to, half way and at the end of the fluid bolus administration and 1 hour after cessation. Each measurement was made four times and an average was used for the study. A ratio of mitral inflow Doppler E wave and A wave was calculated. Similarly the ratio between the mitral E wave and tissue Doppler E' wave was also calculated. ICV variability was calculated as (IVC maximum diameter – IVC minimum diameter) / IVC maximum diameter. Cardiac output was calculated from the calculation from LVOT VTI and LVOT diameter.

Non-invasive blood pressure (NIBP), heart rate and SpO_2 were measured prior to, half way and at the end of the fluid bolus administration and 1 hour after cessation (CASMED 740.vital sign monitor). Each measure was taken in triplicate and an average of the three was used in the study. A modified Borg's dyspnoea scale (Kendrick *et al.* 2000) was use to assess breathlessness in the volunteers. It was self-administered prior to, half way and at the end of the fluid bolus administration and 1 hour after cessation.

5.4.1.3 Blood samples were also taken before and after fluid administration. Serum electrolytes were measured with an indirect ion selective electrode technique. Serum albumin was measured with a colorimetric method (Bromocresol Purple). Haemoglobin was measured spectrophotometrically using the Cyan-Met Haemoglobin method and haematocrit was a calculated value from the red blood cell count and the mean cell volume. Angiopoietin 2 was measured using a commercially available kit, as per the manufactures

instructions (R&D Systems, MN). N-terminal pro B-type natriuretic peptide (NT pro BNP) was measured with electrochemiluminescence immunoassay (Cobas e immunoassay analysers). Blood volume at time 0 was estimated according to the method described by Nadler *et al.* (Nadler *et al.* 1962). Calculations for changes in blood volume and extravascular fluid volume were based on changes in haematocrit and body weight and were made using the formula described previously (Lobo *et al.* 2010; Chowdhury *et al.* 2012). Participants were permitted to pass urine as needed and, in all cases, at the end of the study. The time of each micturition was noted.

5.4.1.4 Statistical analysis: Data are reported as means with SD, or median with IQR, as appropriate for the distribution of each variable. Analysis was performed using SPSS version 22.0 (SPSS Inc.). Differences between variables over time were analysed by a repeated-measures analysis of variance (ANOVA) and the effect of the fluid was analysed as an interaction effect. Correlations was analysed using the Pearson correlation coefficient (r). The effect of individual fluid in the subjects wan analysed with paired t test. A conventional alpha level of < 0.05 was used for all significance testing.

5.4.2 RESULTS

The median (IQR) age of the healthy volunteers (n=4) was 34 (25-37) years, weight was 80 (67-84) kg and height was 180.4 (173.0-188.7) cm, and all completed all arms of the study. None of the volunteers reported dyspnoea (modified Borg scale score of 0) at half way (15 ml/kg) with any of the fluids. One of the 4 volunteers reported slight breathlessness immediately after infusion of 0.9% saline (modified Borg scale score of 2) but it returned to baseline (modified Borg scale score of 0) after the recovery period.

5.4.2.1 Changes in the haemodynamic parameters

None of the administered fluids led to a change in the blood pressure or SpO_2. While the heart rate was unchanged with 4% albumin and 5% glucose, there was a decrease in the heart rate with 0.9% saline administration (Table 5.8).

Table 5.8: The effect of fluid boluses in hemodynamic parameters and lung ultrasound " B lines" before, halfway, at the end and 1 hour after completion of the fluid bolus.

	Before	Half	End	Recovery	
	0.9% Saline				P
Cardiac output (L/min)	5.03 ± 0.8	4.26(0.76)	4.7 ± 0.9	4.48(1.10)	0.12
MAP(mm Hg)	98(6)	101(6)	102(5)	97(7)	0.44
HR (beats /mt)	82(17)	65(8)	64(5)	65(7)	0.04
Lung USG "B" lines	0 ± 0	6.7(9)	12.5 ± 7	11(16)	0.01
	5% Glucose				P
Cardiac output (L/min)	4.71± 0.4	4.65(0.45)	4.55± 0.6	4.70(1.14)	0.52
MAP(mm Hg)	92(8)	95(8)	101(5)	99(1)	0.18
HR (beats/mt)	70(14)	66(8)	68(15)	74(7)	0.38
Lung USG "B" lines	0.07± 0.15	2(2)	4.7± 6.6	3(6)	0.28
	4% Albumin				P
Cardiac output (L/min)	4.75± 0.7	4.85(0.66)	5.31± 0.6	5.17(0.73)	0.39
MAP(mm Hg	98(9)	97(7)	95(9)	96(3)	0.79
HR (beats/mt)	78(19)	76(17)	76(19)	73(15)	0.44
Lung USG "B" lines	0 ± 0	0(0)	0.30 ± 0.5	0(0)	0.39

5.4.2.2 Changes in lung function

The administration of 4% albumin resulted in both a greater increase in estimated blood volume and diffusion capacity (corrected for alveolar volume), and a fall in FVC, FEV1 and FRC consistent with an increase in pulmonary blood volume, whereas 0.9% saline administration led to an increase in airway resistance at 5 Hz (p=0.03). There was also a decrease in dX/dflow with administration of 0.9% saline. There were no changes to lung function with administration of 5% glucose. (Table 5.9)

5.4.2.3 Changes in lung ultrasonography

Administration of 0.9% saline led to an increase in lung B-lines (p=0.02) which persisted through to the recovery lung ultrasonography. There was no statistically significant difference in B lines with administration of 4% albumin or 5% glucose. (Table 5.9)

5.4.2.4 Changes in cardiac ultrasound

None of the fluids led to a change in cardiac output (measured with LVOT VTI) or IVC parameters. Administration of 4% albumin led to an increase in mitral inflow E, and E/E' septal/lateral ratio suggesting an increase in pulmonary venous pressure. (Table 5.10)

5.4.2.5 Changes in blood parameters

Administration of 4% albumin led to a rise in NTproBNP, as well as the greatest observed decrease in haematocrit (greatest rise in estimated blood volume), with a simultaneous rise in serum albumin and a decrease in serum ionized calcium (corrected for pH). Administration of 0.9% saline led to a decrease in haematocrit and serum albumin, and an increase in serum chloride. Further, 0.9% saline also led to a rise in plasma angiopoietin 2 (corrected for blood volume) suggesting an inflammatory effect. Administration of 5% glucose led to a decrease in serum albumin, sodium and chloride, with no change in NTproBNP or Ang2. (Table 5.11)

The estimated change in blood volume was greatest with 4% albumin, followed by 0.9% saline then 5% glucose. There was a positive correlation between delta blood volume and cardiac output (r^2=0.43, p<0.01). However, cardiac output was only increased when the increase in delta volume exceeded 1 litre. (Figure 5.15)

Table 5.9: Effect of fluid boluses on the respiratory parameters.

	0.9% Saline (30ml/Kg)			5% Glucose (30ml/Kg)			4% Albumin (30ml/Kg)			P value #
	Before	After	P*	Before	After	P*	Before	After	P*	
Lung B-lines (ultrasound)	0 ± 0	12.5 ± 7	0.01	0.07± 0.15	4.7± 6.6	0.21	0 ± 0	0.30 ± 0.5	0.39	0.02
Airway resistance 5 Hz (kPa/(L/s))	0.3± 0.1	0.4 ± 0.1	0.05	0.3± 0.1	0.3± 0.1	0.7	0.3± 0.1	0.3± 0.2	0.09	0.03
DLCO (Alveolar volume) Ml/min/mmHg/L	4.8 ± 0.6	5.2 ± 0.4	0.06	4.9± 0.3	5.0± 0.6	0.38	4.8± 0.6	5.6± 0.6	0.01	0.04
FVC (L)	5.29± 1.5	5.17± 1.6	0.08	5.30± 1.6	5.28 ± 1.7	0.83	5.32± 1.6	5.01± 1.7	0.04	0.04
FRC (L)	3.9 ± 1.3	3.8 ± 1.4	0.38	3.8± 1.3	3.7± 1.2	0.07	3.7± 1.1	3.3± 0.9	0.05	0.03
FEV1(L)	4.06± 1.4	4.00± 1.4	0.43	4.05± 1.3	3.97 ± 1.3	0.11	4.13± 1.5	3.81± 1.6	0.04	0.03
PEFR (L/S)	10.05± 2.2	9.29± 2.5	0.02	10.2±2.4	10.2 ±2.3	0.99	10.4± 2.3	9.4±2.9	0.11	0.07
dX/dFlow 5 Hz kPa/(l/s)	0.04± 0.02	-0.04 ±0.03	0.01	-.03± 0.06	0.00 ± 0.03	0.39	0.01± 0.02	0.02± 0.03	0.82	0.01

0.9% saline lead to increase in lung ultrasound B lines and distal airway resistance and with a decrease in lung reactance (corrected for flow) while 4% albumin lead to decrease in FVC, FRC and FEV1 and increase in DLCO(corrected for alveolar volume).

*Data presented as mean ± sd * Paired t test , # ANOVA with fluid interaction*

Table 5.10: Effect of fluid boluses on the cardiac echocardiographic parameters.

	0.9% Saline (30ml/Kg)			5% Glucose (30ml/Kg)			4% Albumin (30ml/Kg)			P value #
	Before	After	P*	Before	After	P*	Before	After	P*	
Cardiac output (L/min)	5.03 ± 0.8	4.7 ± 0.9	0.29	4.71± 0.4	4.55± 0.6	0.71	4.75± 0.7	5.31± 0.6	0.30	0.24
LVOT VTI (cm)	16.1± 2.2	18.5± 2.9	0.09	17.9± 2.6	17.7± 1.1	0.81	16.2± 1.8	19.2± 2.1	0.12	0.17
IVC max diameter (cm)	1.5 ± 0.4	1.5 ± 0.5	0.88	1.6± 0.4	1.8± 0.4	0.28	1.7± 0.5	2.0± 0.6	0.11	0.65
Mitral E (cm/s)	62.3± 10.8	71.6± 6.7	0.09	62.4± 11.6	68.8± 9.4	0.08	56.7± 8.4	89.1± 7.9	0.01	0.003
E/E' (Lateral)	3.7 ± 1.3	4.3 ± 2.9	0.2	5.3± 0.9	5.6± 0.8	0.14	5.4± 0.7	8.2± 0.4	0.003	0.006
E/A	1.4± 0.1	1.7± 0.4	0.23	1.5± 0.1	2.0± 0.2	0.08	1.6± 0.2	2.4± 0.5	0.07	0.30

4% albumin leads to increase in mitral inflow Doppler E waves and E/E' ratio. None of the fluids lead to change in cardiac output, LVOT VTI or IVC parameters.

*Data presented as mean ± sd * Paired t test , # ANOVA with fluid interaction*

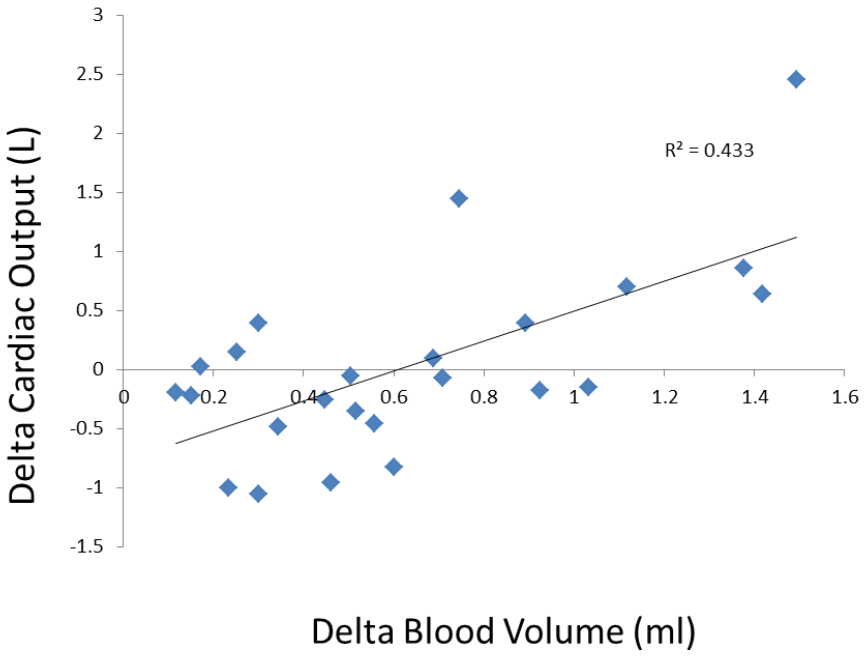
Table 5.11: Effect of fluid boluses on blood parameters.

	0.9% Saline (30ml/Kg)			5% Glucose (30ml/Kg)			4% Albumin (30ml/Kg)			P# value
	Before	After	p*	Before	After	p*	Before	After	p*	
Delta Blood volume (l)	0.86±0.2			0.44±0.3			1.35±0.2			0.001
Hct	0.47 ±0.01	0.40 ± 0.03	0.004	0.45± 0.04	0.41± 0.04	0.14	0.45± 0.02	0.36± 2.5	0.001	0.03
Serum Sodium (mmol/l)	142 ± 2.5	142± 3.6	0.49	140± 1.1	130± 1.8	0.01	142± 2.8	141± 0.0	0.37	0.01
Serum Albumin (g/l)	47.2 ± 1.1	37.2 ± 2.8	0.001	45.2± 2.0	37.0± 2.5	0.007	46.0± 2.2	52.2± 1.8	0.01	0.00
NT-proBNP (pg/ml)	16.4 ± 4.4	28.7 ± 8.6	0.06	14.7±7.9	15.1±5.5	0.9	13.7± 3.6	70.5± 18.5	0.01	0.00
Ang-2 ng/ml	3.06± 0.4	3.5± 0.5	0.02	3.36± 0.9	2.6± 1.1	0.08	3.87± 1.3	2.98± 0.9	0.13	0.02
iCa mmol/l	1.22 ± 0.04	1.21± 0.06	0.60	1.24 ± 0.05	1.22 ± 0.06	0.08	1.23 ± 0.04	1.15± 0.05	0.002	0.00
Serum chloride mmol/l	104 ± 2	110 ± 2	0.002	105 ± 1	97± 3	0.01	106 ± 1	108 ± 1	0.13	0.00
HCO3 mmol/l	24 ± 0.5	21 ± 0.6	0.001	24 ± 0.9	23 ± 2.2	0.25	24 ± 1.4	22 ±1.3	0.01	0.005
Time to micturition (Minutes)	108 ±17			37±15			58±17			0.001

4% albumin lead to a maximum rise in blood volume (calculated from change in haematocrit) and a simultaneous rise in NT pro BNP. 5% dextrose leads to a decrease in serum sodium. 0.9% saline lead to an increase in Angiopoietin 2 (marker for lung injury).

*Data presented as mean ± sd. * Paired t test , # ANOVA with fluid interaction*

Figure 5.15: Change in cardiac output with increase in blood volume in the healthy volunteers



5.4.3 DISCUSSION

The major finding of our study is that in healthy subjects' administration of 30ml/kg of 0.9% saline leads to interstitial lung oedema, evidenced via lung B lines, and an increase in airway resistance despite unchanged cardiac echocardiography. It also leads to a rise in plasma angiopoietin 2 suggesting an inflammatory effect of its administration. In comparison, administration of an equivalent volume of 4% albumin resulted in a greater increase in estimated blood volume, increase in diffusion capacity (corrected for alveolar volume) and a fall in FVC and FRC consistent with an increase in pulmonary blood volume. Lung ultrasound B-lines were unchanged despite changes in the echocardiographic parameters mitral inflow E, and E/E' ratio suggesting an increase in pulmonary venous pressure. Evidence of increased blood volume and increased

cardiac stretch is also evident from a rise in NTproBNP after administration. Such differences may have direct clinical relevance when these fluids are used as boluses in a patient population.

Dose of fluids used in the study:

Rapid intravenous infusion of 20 ml/kg saline does not impair resting pulmonary gas exchange in the healthy human lung as measured by the multiple inert gas elimination technique (Prisk *et al.* 2009), nor is there any change in lung density when analysed with magnetic resonance imaging (Henderson *et al.* 2012). We chose 30 ml/kg as previously 0.9% saline at this dose has altered lung volumes measurement (Muir *et al.* 1975; Robertson *et al.* 2004) and caused mild airflow obstruction and enhanced airway responsiveness (Pellegrino *et al.* 2003).

Changes with 0.9% saline

Rapid intravenous infusion of an iso-smotic solution, such as 0.9% saline, is expected to result in fluid filtration and leakage from both pulmonary and bronchial vessels (Wagner 1997; Wagner *et al.* 1996). We found that administration of 0.9% saline led to interstitial oedema as evidenced by an increase in lung ultrasound B lines, as well as a decrease in lung impedance (corrected for flow). Similar findings have been previously described on chest X-ray (Farney *et al.* 1977) but although our finding of no changes in the diffusion capacity has been reported before (Puri *et al.* 1999; Robertson *et al.*

2004), we did not find any dynamic changes in the measurement of lung volumes as previously described. As our protocol involved simultaneous echocardiographic measurement, there was a delay in performing the post infusion lung volumes which may have different if the test was performed earlier. We didhow ever find an increase in airway resistance at 5 Hz (distal airway) which supports the previous finding of an increase in airway wall oedema encroaching on the bronchial lumen (Pellegrino *et al.* 2003). Resistance values obtained by IOS at low frequency (R5rs) are reproducible and correlate well with spirometry and plethysmographic values (Olaguibel *et al.* 2005).

A 44% increase in the thickness of the airway wall, consistent with peribronchiolar oedema formation has been reported before in healthy subjects (King *et al.* 2002). Peripheral airways in the lung parenchyma lie within bronchovascular sheaths that also contain branches of the pulmonary vasculature and lymphatics. Several authors have proposed that the initial increase in airway resistance observed in experimentally induced pulmonary oedema is secondary to distension of the pulmonary veins within bronchovascular sheaths, with subsequent compression of the smaller peripheral airways (Millic-Emili *et al.* 1971; Hogg *et al.* 1972; Staub *et al.* 1967) but as the FEV1 and PEFR, are largely determined by the calibre of the larger airways they were not affected by rapid infusion of 0.9% saline in normal subjects. In addition, morphometric studies have failed to show any reduction in the calibre of airways in

experimental pulmonary oedema (Michel *et al.* 1987). Moreover we had evidence of lung B lines 1 hour after infusion of 0.9% saline suggesting a slow reabsorption of the interstitial oedema again confirming reports of slow clearance of 0.9% saline (Drummer *et al.* 1992). This is also corroborated by the fact that the time to micturition was maximal with 0.9% saline, a finding similar to that reported by Chowdhury *et al.* (Chowdhury *et al.* 2012).

Another important finding in our study is the rise of angiopoietin-2 with administration of 0.9% saline. This is not directly explained or described before. Angiopoietin-2 is an endothelial marker of lung injury and is lowered during conservative fluid therapy which in turn is associated with better outcomes in acute lung injury (Terpstra *et al.* 2013; Agrawal *et al.* 2013; Calfee 2012). Angiopoietin-2 is stored in the Weibel–Palade bodies (endothelial storage vesicles) (Romani *et al.* 2003) and its rise after administration of 0.9% saline suggests active signalling pathways leading to degranulation of Weibel–Palade bodies and a pro-inflammatory nature of 0.9% saline when administered as a bolus. It is noteworthy that angiopoietin-2 sensitizes the endothelial cell to the inflammatory actions of TNF-α thus further perpetuating inflammation (Fiedlet *et al.* 2006) and its regulation is flow dependent (Goettsch *et al.* 2008). Moreover, 0.9% saline also led to an increase in serum chloride levels which has been previously reported in healthy volunteers (Chowdhury *et al.* 2012; Ried *et al.* 2009) and critically ill patients (Yonus *et al.* 2011). Chloride

transport-driven alveolar fluid secretion is now considered a major contributor to lung oedema (Solymosi *et al.* 2013), and a transient rise in serum chloride levels after 0.9% saline administration may expedite this process.

Changes with 4% albumin

Administration of 4% albumin led to an anticipated increase in blood volume. There was also a simultaneous increase in the mitral inflow doppler and E/E' ratio. Such an increase in E velocity and E/E' has been correlated with an increase in pulmonary capillary wedge pressure. The levels reported by us have been correlated with a PCWP (mm Hg) of 20 mm Hg (Firstenberg *et al.* 2000) though the fluid used in that study was 0.9% saline instead of 4% albumin. As far as we are aware there is no similar data addressing the effect of administration of 4% albumin in healthy volunteers. Another indicator of increased blood volume and dilation of the cardiac chambers comes from the rise in NT proBNP with administration of 4% albumin. The decrease in lung volume with its administration can be explained by the rise in intra-thoracic blood volume leading to compression of the lungs and the resultant decrease in volumes. This is also corroborated by the rise in diffusion capacity of carbon monoxide (corrected for blood volume), which is well described in other conditions with increased intra thoracic blood volume (Addleman *et al.* 1985). Despite evidence of dilation of cardiac chamber and a rise in pulmonary artery wedge pressure, there was

no evidence of interstitial oedema with 4% albumin with absence of B lines.

There could be several counter-regulatory effects of administration of 4% albumin on the development pulmonary oedema. Though it did lead to an increase in pulmonary pressure and hence should have increased the net filtration of fluid into the interstitium, it also led to a simultaneous increase in serum albumin which in turn will increase in the colloidal osmotic pressure in part negating the effect of rise in pressure. The effect of the glycocalyx on fluid dynamics has led to a revision of the Starling equation, and albumin plays an important role in the modified Starling equation (Woodcock *et al.* 2012; Alphonsus *et al.* 2014). The rise of NT-proBNP with 4% albumin may lead to disruption of the endothelial glycolcalyx and enhanced vascular permeability (Bruegger *et al.* 2005; Jacob *et al.* 2013), even though this effect might be more systemic and not pulmonary (Zimmerman *et al.* 1990). In addition, albumin is protective to the glycocalyx structure, improves endothelial integrity (Jacob *et al.* 2009; Stevens *et al.* 2007), is a specific inhibitor of endothelial apoptosis (Zoellner *et al.* 1996) and also leads to release of endothelial derived relaxation factor (Kaufmann *et al.* 1995) leading to vasodilation and a decrease in shear force.

The adverse effects of an increase in pulmonary pressures is mediated via entry of intra-cellular calcium ions (Kuebler *et al.*

1999; Ichimura *et al.* 2003), but as seen in this study, bolus 4% albumin leads to a decrease in the ionized calcium levels. The decrease in calcium level is likely due to binding of free calcium with the administered albumin which does not contain any calcium. This decrease in calcium has been seen in patients with septic shock (Section 5.2) and in hypovolemic trauma patients who also had multiple blood transfusions (Kovalik *et al.* 1981). One of the major pathways of lung injury is entry of calcium via endothelial channels and a transient drop in calcium may impede this process. Of note, calcium free perfusate is often used in isolated perfused lung injury models as a negative control (Kuebler *et al.* 1999).

Effect of 5% glucose

Administration of 5% glucose did not cause any major cardio-respiratory changes, as anticipated, except for decrease in serum sodium and chloride levels, as it would be distributed in all the compartments of the body,

Change in cardiac echocardiographic variables

Previous studies have shown an increase in cardiac output with infusion of 0.9% saline (Muir *et al.* 1975), but unexpectedly we did not find any increase in cardiac output with any of the fluids. Our measures of cardiac output were based on the echocardiography measurement of the left ventricular outflow tract velocity time integral (averaged over 4 measurements) and were not continuous.

Even though we found a positive correlation between the delta blood volume and the cardiac output, the increase in cardiac output was only present when the increase in blood volume was more than 1 litre. Similarly we did not find any changes in the IVC maximum diameter or its variability. IVC variability has only been shown to have a weak correlation with simulated hypovolemia in healthy volunteers (Moore *et al.* 2010) and, despite its validation in ventilated patients (Barbier *et al.* 2004; Feissel *et al.* 2004), it has not been validated in healthy human volunteers with fluid administration. However, we did find an increase in mitral E wave, and E/E' ratio, and levels seen in our study have been shown to correlate with increased pulmonary artery wedge pressure (Firstenberg *et al.* 2000). Doyle *et al.* (Doyle *et al.* 1951) also have reported a similar finding of an increase in pulmonary arterial and pulmonary capillary pressure with infusion of saline in healthy subjects but with a variable response in the cardiac output to the intravenous infusions.

Validation of lung ultrasonography B lines for interstitial pulmonary oedema:

Lung ultrasound B-lines have been correlated with lung weight and density determined by computerised tomography (Baldi *et al.* 2013) and with wedge pressure and extravascular lung water (Agricola *et al.* 2005). They have satisfactory intraobserver and interobserver variability, around 5% and 7%, respectively (Jambrik *et al.* 2004). B-line resolution appears to occur in real-time as fluid is removed

from the body. These data support lung ultrasound as a useful method for evaluating real-time changes in extra-vascular lung water and in assessing a patient's physiologic response to fluid administration (Noble *et al.* 2009).

5.4.4 LIMITATIONS

This study should be viewed in light of several limitations

1. The number of subjects examined in our study was small (n=4). But as each subject was studied with 3 different fluids, they served as their own internal control providing validity to our data
2. Measurement error: Even though a strict protocol was utilised for the study and the measurements were made as per international standards and the person making those cardiac and lung measurement were blinded to the type of fluid utilised, we cannot rule out obscure sources of measurement errors
3. Long terms effects of these fluids on the cardiac respiratory systems are not known as the last measurements were made at the end of 1 hour after the fluid bolus. However, none of the subjects reported any episodes of dyspnoea for the next 24 hours after the fluid bolus with any of the fluids.
4. The effect of such fluid boluses on other organs such as the kidney are not known as it was not the focus of this study
5. This study did not examine the effect of a chloride balanced fluid and this should be area of future research.

5.4.5 SUMMARY

In healthy volunteers 0.9% saline and 4% albumin solutions have differential pulmonary effects not obviously attributable to passive fluid filtration. Compared with other fluids 0.9% saline leads to interstitial oedema whereas 4% albumin does not cause pulmonary oedema even though there was evidence of hypervolemia. This may reflect either differential effects of these fluids on active signalling in the pulmonary circulation, or a protective effect of albumin. This needs to be explored in future studies.

CHAPTER 6: EFFECT OF SERUM SODIUM AND OSMOLALITY ON LUNG INJURY

6.1 Admission high serum sodium is not associated with increased ICU mortality risk in respiratory patients

Hypernatremia is a serious electrolyte disturbance, which presents an independent risk factor for mortality in critically ill patients (Darmon *et al.* 2013; Palevsky *et al.* 1996; Lindner *et al.* 2007; Funk *et al.* 2010; Stelfox *et al.* 2008; Vandergheynst *et al.* 2013). High serum sodium leads to high serum osmolarity, which may alter the distribution of water between the extracellular and intracellular compartments, and cause intracellular dehydration potentially leading to adverse outcomes. However, there is experimental data to suggest that hyperosmolarity suppresses lung injury (Rabinovici *et al.* 1996; Pascual *et al.* 2003; Rizoli *et al.* 1998). The postulated mechanisms include expression of endothelial leukocyte adhesion molecules (Rizoli *et al.* 1998; Ochi *et al.* 2002), blockade of proinflammatory effects of lipopolysaccharide (Rabinovici *et al.* 1996; Ochi *et al.* 2002) and the effect of hyperosmolarity on remodelling of both the endothelial barrier and the actin cytoskeleton, to enhance barrier properties, and blockade of proinflammatory P-selectin expression (Safdar *et al.* 2003).

It was hypothesised that lung protective effects of hypernatremia would reduce its general adverse effects leading to amelioration of

the increase in mortality risk. As no clinical studies have examined this question we interrogated a large administrative data base.

6.1.1 Methods

6.1.1.1 Study design

A retrospective cohort design to evaluate the association between both the lowest and highest recorded serum sodium in the first 24 h of ICU admission and ICU and hospital mortality among all adult patients. Ethics approval was obtained from Southern Adelaide Human Research Ethics Committee South Australia (#118.12).

6.1.1.2 Patients

All patients with valid sodium data admitted to an adult ICU at one of 129 centres in Australia and New Zealand, and more than equal to 16 years of age, were eligible for inclusion in this study. We excluded patients with missing mortality data and data required to calculate an illness-severity-adjusted risk of death, or if their length of stay in ICU was less than 4 hours or if they were transferred to another ICU. Where patients were admitted to ICU more than once during a hospital admission, only the patient's first admission was included in the analysis.

6.1.1.3 Database

Data was extracted from the Australian and New Zealand Intensive Care Society Adult Patient Database (ANZICS–APD) (Stow *et al.* 2006). Data are collected under the Quality Assurance Legislation of

the Commonwealth of Australia (Part VC Health Insurance Act 1973, Commonwealth of Australia) which allows use of data for research purposes without individual patient consent or specific ethical approval. In New Zealand, use of anonymous quality data for research is classified as 'low risk audit activity' and is exempt from requirements for formal ethics approval.

6.1.1.4 Data extraction and categorisation

Data from January 2000 to December 2010 was reviewed. The following variables were extracted from the databases: age, sex, chronic co-morbidities, physiological measures in the first 24 hours including highest and lowest serum sodium, ICU admission diagnosis, admission source for hospital and ICU, hospital level, location and care type, year and month of admission, ICU and hospital mortality and illness severity determined using the APACHE III risk prediction model. Patients were further categorised into respiratory, medical, surgical, cardiac, neurological, liver, infection, ventilated (invasive) and non-ventilated categories using APACHE III codes.

6.1.1.5 Outcome

The study was designed to investigate the effect of high admission serum sodium on ICU mortality independent of illness severity in patients with or without a respiratory diagnosis admitted to ICU. To investigate severity of lung disease, respiratory patients were

stratified by a PaO_2/FiO_2 ratio less than or greater than 200. Primary outcome was ICU mortality, with analysis adjusted for illness severity (APACHE III risk of death with the serum sodium component removed) and year of admission. Similar analysis on hospital mortality was also done.

6.1.1.6 Statistical Analysis

All analyses were performed using SAS version 9.2 (SAS Institute Inc.,Cary,NC,USA). Continuously normally distributed variables were compared using student t-tests and presented as mean (standard deviation) while non-normally distributed variables are compared using Wilcoxon Rank Sum tests and presented as medians (interquartile range). Categorical variables were compared using chi-square tests for equal proportion and are reported as counts and percentages. The nature of the relationship between ICU mortality and serum sodium was determined using logistic regression with sodium treated as a categorical variable divided into increments of 5 mmol. Multivariate models were adjusted for centre, year of admission, patient severity and each patient's propensity to be admitted with a respiratory diagnosis. To account for a measure of illness severity that was independent of sodium, an APACHE III risk of death score was generated with the serum sodium component removed. To account for potential imbalances between patient's presenting with a respiratory diagnosis and those without, each patient was assigned a propensity score (probability of having

a respiratory diagnosis) by fitting a logistic regression model with respiratory diagnosis as the outcome. Model construction was made using a stepwise selection procedure (inclusion criteria $p<0.01$) with the following variables considered for inclusion: admission source for hospital and ICU, hospital level, location and care type, year and month of admission, age, gender and chronic comorbidities. Results are presented as odds ratios (OR) (95% confidence intervals; CI) referenced against an approximate normal range of 135 -144.99 mmol. To ascertain if the nature of the relationship between sodium and ICU mortality statistically differed in patients with a respiratory diagnosis compared to all others, an interaction term was fitted to the logistic regression model between sodium and respiratory diagnosis. Similar interaction variables were fitted among patients with admission PaO_2/FiO_2 ratio < 200 and those with the admission ratio ≥200 in (i) all patients; (ii) all respiratory patients; (iii) all mechanically ventilated patients. Furthermore, interaction models were studied among (i) respiratory patients who were mechanically ventilated with PaO_2/FiO_2 ratio < 200 within the first 24 hours of admission and other respiratory patients; (ii) all patients who were mechanically ventilated with PaO_2/FiO_2 ratio <200 within the first 24 hours of admission and other patients. To study the influence of different hospital levels (rural, metropolitan, tertiary and private) on study outcomes, additional sensitivity analysis was performed stratifying the data by hospital level. A two-sided p-value of ≤0.05 was considered to be statistically significant.

6.1.2 RESULTS

Admissions included in the ANZICS–APD database were drawn from 129 hospitals (26% rural, 22% metropolitan, 24% tertiary and 28% private). The majority of hospitals (61%) had less than 300 beds, with 24% of hospitals having between 300 and 500 beds and 15% having more than 500 beds.

Initial search of the database for the period 2000 to 2010 identified 510,362 subjects with recorded sodium measurements. Of these, 436,209 patients met screening criteria and were included in the study (Figure 6.1). Table 6.1 presents patient characteristics as ICU survivors and non-survivors.

Figure 6.1: Consort diagram of subjects examined in ANZICS–APD database study

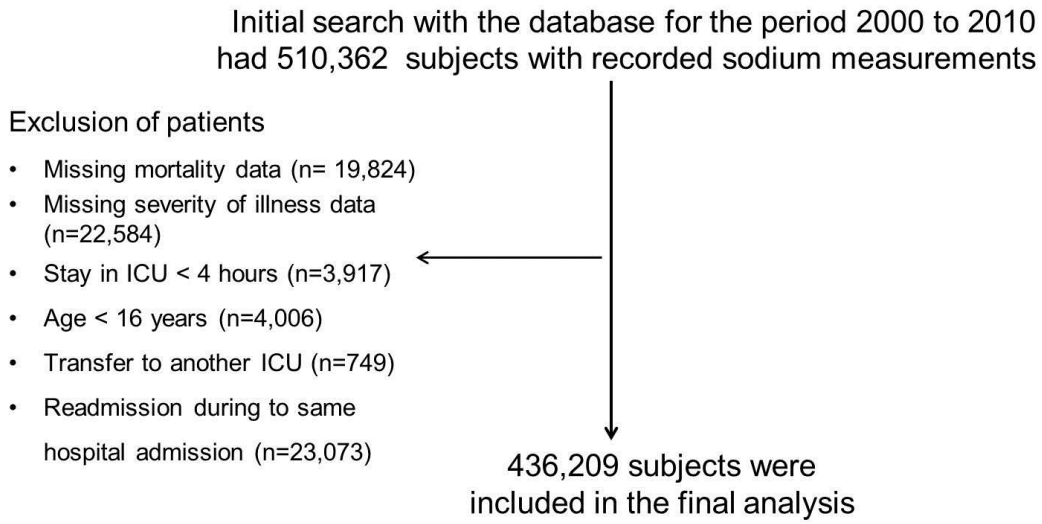


Table 6.1: Characteristics and outcomes of patients classified as ICU survivors and non-survivors (n=436,209)

	ICU* survivors	ICU* non-survivors	p-value
Number (%)	401938 (92)	34271 (8)	
Age, mean (SD†)	61.0 (18.3)	65.8 (16.6)	<0.001
Male gender, number (%)	234939 (54)	201270 (46)	0.02
APACHE‡ II score, mean(SD†)	15.3 (6.9)	27.7 (8.3)	<0.001
APACHE‡ III score, mean(SD†)	49.5 (23.9)	97.5 (32.5)	<0.001
SAPS‖ II, mean (SD†)	29.3 (14.2)	56.6 (18.0)	<0.001
APACHE‡ III risk of death, median (IQR§)	4.3 (1.4-13.8)	58.6 (30.4-80.5)	<0.001
ICU length of stay (hours,) median (IQR§)	44.7 (23.0-90.3)	62.3 (26.0–149.3)	<0.001

** Intensive care; † Standard deviation; ‡ Acute physiology and Chronic health evaluation; § Interquartile range; ‖ Simplified acute physiology score*

The number of patients in each serum sodium category and the raw mortality rate for ICU are shown in Table 6.2. The odds ratio (95% confidence interval) for both raw and adjusted ICU mortality (severity of illness) referenced against a range of 135 -144.9 mmol for highest and lowest serum sodium recorded in the first 24 hours of ICU admission are also shown in Table 6.2. Overall, mortality risk was U shaped at the extreme levels of dysnatremia. The highest adjusted OR's (95%CI) for ICU mortality for highest serum sodium (≥ 160 mmol/L) and lowest serum sodium (115 -120 mmol/L) over the 24 hours post ICU admission were 4.2 (3.6-4.9) and 1.6 (1.4-1.8) respectively (Figure 6.2).

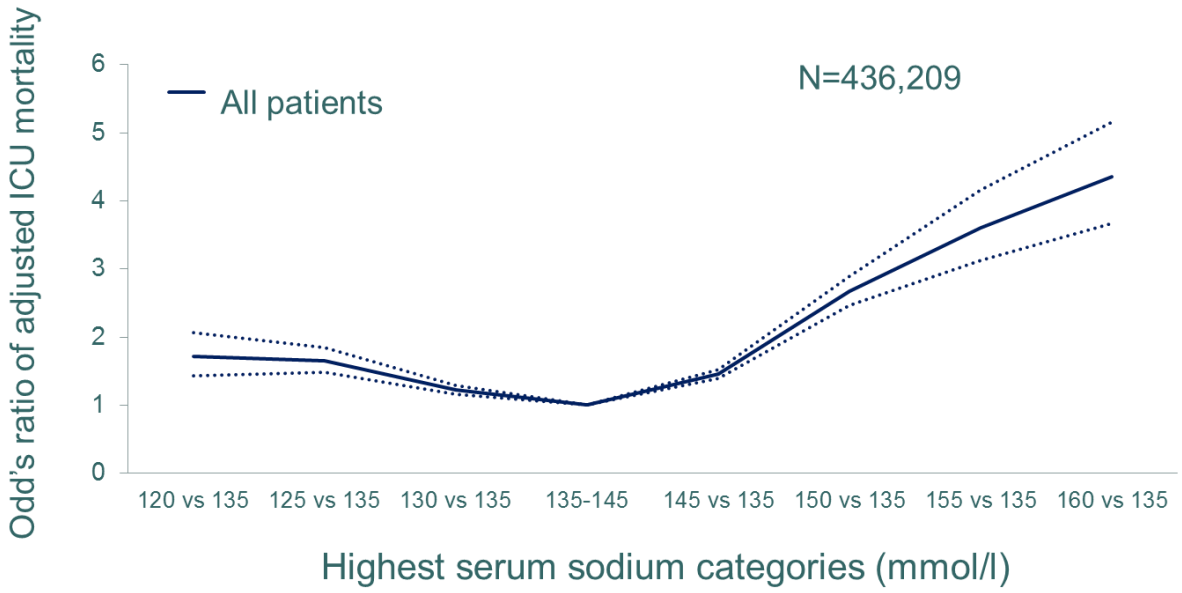
Table 6.2: ICU mortality and raw and adjusted OR (95% CI) for various serum sodium categories with reference to serum sodium of 135 -144.9 mmol for highest and lowest serum sodium in the first 24 hours of ICU admission

Admission serum sodium (mmol/l)	Number	ICU* mortality %	OR† (95% CI)	Adjusted OR‡ (95% CI)
Highest				
<125	2042	13.3	2.42 (2.13-2.76)	2.07 (1.77-2.43)
125 -129.9	5046	16.5	3.12 (2.89-3.37)	1.83 (1.66-2.01)
130 -134.9	30695	10.6	1.88 (1.80-1.96)	1.33 (1.27-1.40)
135-144.9	348121	6.3	1	1
145 -149.9	41041	13.2	2.39 (2.30-2.47)	1.51 (1.45-1.58)
150 -154.9	6488	26.3	5.64 (5.31-5.98)	2.58 (2.39-2.78)
155 -159.9	1620	32.8	7.71 (6.93-8.57)	3.40 (2.97-3.89)
≥160	1156	32.5	7.60 (6.71-8.61)	4.17 (3.55-4.89)
Lowest				
<120	2663	9.5	1.63 (1.43-1.86)	1.44 (1.23-1.68)
120 – 124.9	4126	16.8	3.14 (2.89-3.42)	1.58 (1.42-1.76)
125 – 129.9	15589	14.2	2.59 (2.47-2.72)	1.40 (1.32-1.49)
130 -134.9	82230	8.6	1.47 (1.43-1.52)	1.16 (1.12-1.20)
135-144.9	317733	6.7	1	1
145 -149.9	11859	17.4	3.28 (3.12-3.45)	1.75 (1.64-1.86)
≥150	2009	26.8	5.70 (5.16-6.31)	2.23 (1.97-2.53)

** Intensive care; † Odd's ratio; CI, Confidence intervals*

‡Adjusted for patient severity (APACHE-III with serum sodium component removed), attending hospital, year of admission and propensity to present with a respiratory diagnosis

Figure 6.2: Adjusted odds ratio (shaded lines are 95% confidence intervals) of ICU mortality relative to 135 -144.9 mmol/l for different categories of highest serum sodium in the first 24 hours in all patients



A similar trend was found in various apriori defined subgroups except for patients with respiratory diagnoses (n=52043) where the OR for ICU mortality was not significantly influenced by a high serum sodium [1.3 (0.7-1.2)] (Table 6.3). As there were apparent differences between respiratory and non-respiratory patients (Table 6.4), a propensity score was generated to account for these differences. The OR's for the risk of being admitted to ICU with a respiratory diagnosis are shown in Table 6.5.

Table 6.3: Serum sodium (highest in first 24 hours during an ICU admission) and adjusted ICU mortality for various diagnostic categories

Diagnostic category#	**Highest sodium (mmol/L)**	**OR* (95%CI†)**
Total (n=436,209)	≥160	4.2 (3.6-4. 9)
Medical (n=208,513)	≥160	2.7 (2.3-3.2)
Surgical (n= 227,696)	≥160	14.7 (10.5-20.5)
Ventilated (Invasive)$ (n=194,503)	≥160	5.8 (4.8-7.1)
Non-ventilated $ (n=241,699)	≥160	2.1 (1.8-2.5)
Selected sub groups		
Respiratory (n=52043)	**≥160**	**1.3 (0.7-2.2)**
Cardiac (n=25827)	≥160	2.3 (1.1-4.7)
Liver (n=10115)	≥160	2.3 (1.2-4.2)
Neurological (n=28663)	≥160	12. 8 (9.5-17.2)
Infection (n=50211)	≥160	1.7 (1.1-2.5)

** Odd's ratio; †CI, Confidence intervals*
$Total of ventilated and non-ventilated is 436,202 as there were small number of patients whose ventilation status was unknown
 # Based on APACHE III diagnostic codes

Table 6.4: Characteristics and outcomes of patients with non-respiratory or respiratory admission diagnosis

	Non-respiratory (n =384,166)	**Respiratory (n=52043)**	**p-value**
Age, mean (SD*)	61.4(18.2)	61.3 (17.8)	0.39
Male gender, number (%)	226,992 (59)	28193 (54)	<0.001
APACHE† II score, mean (SD)	15.97 (7.73)	18.79 (7.87)	<0.001
APACHE† III score, mean (SD)	55.48 (27.6)	61.41 (26.81)	<0.001
SAPS ‡ II, mean (SD)	31.03 (16.3)	34.76 (15.6)	<0.001
ICU§ LOS‖ (hrs), median (IQR¶)	43.5 (22.5-86)	75.3 (38.7-160)	<0.001
Physiological variables (first 24 hours)			
Highest MAP**, mean (SD)	97 (87-108)	99 (90-110)	<0.001
Highest heart rate, mean (SD)	100.1 (22.0)	113.4 (23.2)	<0.001
Highest temperature (ºC), mean (SD)	37.3 (0.8)	37.5 (0.9)	<0.001
Highest sodium (mmol/l), mean (SD)	139.9 (4.6)	139.8 (4.9)	<0.001
Lowest sodium (mmol/l,) mean (SD)	136.9 (4.6)	136. 9 (5.1)	0.47
ICU mortality, number (%)	27623 (8.0)	6648 (13.0)	<0.001
Chronic comorbidities at admission			
Respiratory, number (%)	21025 (5.5)	14372 (27.6)	<0.001
Cardiovascular disease, number (%)	47903 (12.5)	6032 (11.6)	<0.001
Renal disease, number (%)	13968 (3.6)	1921 (3.7)	0.52
Immune suppressive disease, number (%)	10802 (2.8)	3125 (6.0)	<0.001
AIDS***, number (%)	88 (0.0)	60 (0.1)	<0.001
Hepatic Failure, number (%)	1960 (0.5)	191 (0.4)	<0.001
Lymphoma, number (%)	2961 (0.8)	606 (1.2)	<0.001
Cirrhosis, number (%)	7312 (1.9)	768 (1.5)	<0.001

* Standard deviation; † Acute physiology and chronic health evaluation; ‡ Simplified acute physiology score; § Intensive care; ‖ Length of stay; ¶ Interquartile range; **Mean arterial pressure, ***Acquired immunodeficiency syndrome

Table 6.5: OR (95% CI) for the risk of being admitted to ICU with a respiratory diagnosis. The subsequent propensity score (predicted risk of having a respiratory diagnosis) was then included as a covariate in the prediction of ICU mortality

Effect	OR
ICU source (ref= other Hospital)	
Emergency	0.84(0.81-0.87)
OT	0.02(0.02-0.02)
Ward	1.31(1.26-1.37)
Hospital Admission source (ref=Home)	
Other Hospital	0.88(0.85-0.90)
Chronic Care	1.25(1.16-1.35)
Other ICU	1.10(1.01-1.20)
Unknown	0.69(0.65-0.73)
State or Country (ref=Victoria)	
Australia Capital Territory	1.32(1.24-1.41)
New South Wales	1.04(1.01-1.07)
Northern Territory	0.93(0.87-0.99)
New Zealand	0.95(0.90-1.00)
Queensland	0.90(0.87-0.93)
South Australia	1.26(1.21-1.32)
Tasmania	0.93(0.86-1.00)
Western Australia	1.29(1.20-1.39)
Gender (ref=male)	
Female	1.10(1.07-1.12)
Unknown	0.37(0.04-3.03)
Hospital Level (ref=Private)	
Rural Hospitals	1.40(1.34-1.46)
Metro Hospitals	1.38(1.32-1.44)
Tertiary Hospitals	1.06(1.02-1.11)
Care Type (ref=HDU)	
ICU	1.08(1.05-1.12)
Unknown	0.31(0.11-0.84)
Age	1.01(1.00-1.01)
Area under ROC	0.852

Effect	OR
Month (ref=February)	
January	1.04(0.99-1.10)
March	1.07(1.01-1.13)
April	1.12(1.06-1.18)
May	1.20(1.14-1.27)
June	1.35(1.28-1.43)
July	1.53(1.45-1.61)
August	1.51(1.44-1.59)
September	1.44(1.37-1.52)
October	1.21(1.15-1.28)
November	1.16(1.10-1.22)
December	1.10(1.04-1.16)
Year of admission (ref=2000)	
2001	0.90(0.83-0.98)
2002	0.92(0.85-1.00)
2003	0.91(0.84-0.98)
2004	0.89(0.82-0.96)
2005	0.91(0.84-0.97)
2006	0.83(0.78-0.89)
2007	0.89(0.83-0.95)
2008	0.87(0.81-0.93)
2009	0.92(0.86-0.99)
2010	0.89(0.84-0.95)
Chronic comorbidities (yes vs no)	
Respiratory	7.08(6.87-7.30)
Cardiovascular disease	0.55(0.53-0.57)
Renal disease	0.62(0.59-0.65)
Immune suppressive disease	1.62(1.54-1.70)
AIDS	3.61(2.51-5.19)
Hepatic Failure	0.57(0.49-0.67)
Lymphoma	0.88(0.80-0.97)
Cirrhosis	0.54(0.50-0.59)

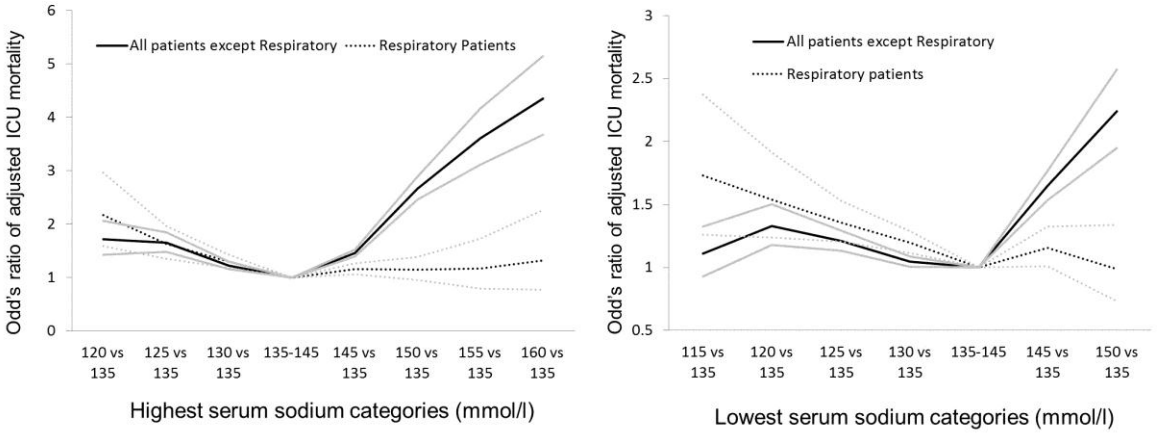
ICU, Intensive care Unit; HDU, High dependency unit; ref, Reference; OT, operation theatre; ROC, Receiver operating characteristic.

The relationship between sodium and ICU mortality was significantly different for those with a respiratory diagnosis compared to all other patients for both the highest serum sodium ($p<0.0001$) and lowest serum sodium ($p<0.0001$) in the first 24 hours of ICU admission (Figures 6.3). While examining the hospital mortality, a similar trend was found. The relationship between serum sodium and hospital mortality was significantly different for those with a respiratory diagnosis compared to all others, both for the highest serum sodium ($p<0.0001$) and the lowest serum sodium ($p<0.0001$), in the first 24 hours of ICU admission.

Patients with respiratory diagnoses were further analysed as those with admission PaO_2/FiO_2 ratio < 200 (n=26133) and ≥ 200 (n=17787). There was a significant interaction between PaO_2/FiO_2 ratio and highest serum sodium ($p=0.0005$), indicating that hypernatremia associated mortality was ameliorated further in more severe cases of respiratory failure ($PaO_2/FiO_2 < 200$). The interaction between lowest sodium and PaO_2/FiO_2 ratio was not statistically significant ($p=0.09$). Similarly, interaction was present with the highest serum sodium between respiratory patients who were mechanically ventilated with PaO_2/FiO_2 ratio < 200 (n=14474) in the first 24 hours of admission and the rest of the respiratory patients (n=29446) ($p=0.02$). The interaction for the lowest serum sodium was not statistically significant ($p=0.13$).

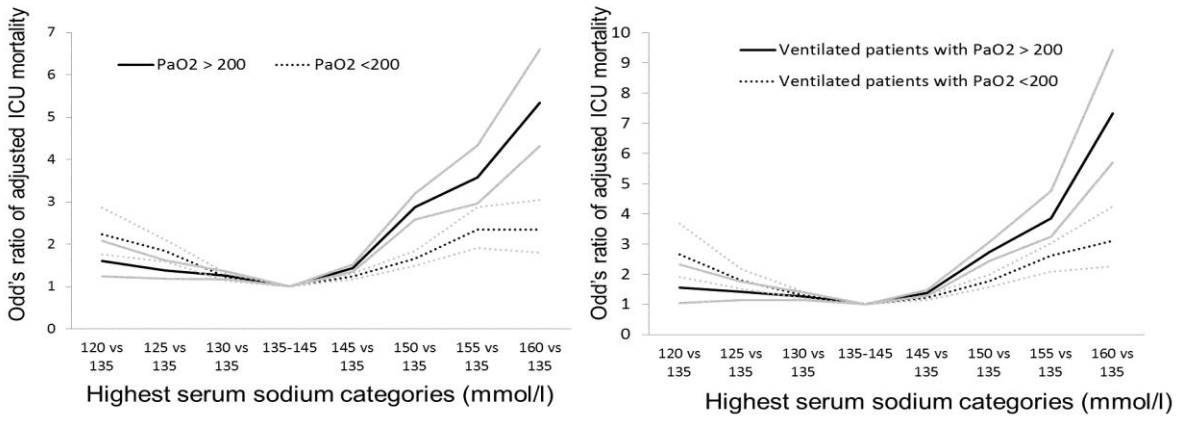
When all patients were stratified by PaO_2/FiO_2 ratio, 33% (n=114126) of patients fell below 200. There was significant statistical evidence to suggest that the nature of the relationship between serum sodium and ICU mortality differed according to PaO_2/FiO_2 ratio with both highest and lowest serum sodium in the first 24 hours of admission displaying significant interactions with PaO_2/FiO_2 ratio ($p<0.0001$ for both) (Figure 6.4). This significant interaction between serum sodium and PaO_2/FiO_2 ratio persisted when considering only mechanically ventilated patients. Of the 189004 ventilated patients, 38% (n=72421) had PaO_2/FiO_2 ratio < 200 and the interaction with PaO_2/FiO_2 ratio was highly significant for both highest and lowest serum sodium ($p<0.0001$ for both) (Figure 6.4). Similar results were seen while examining hospital mortality. When considering patients who were mechanically ventilated with PaO_2/FiO_2 ratio < 200 (n=72421) against all other patients (n=277778) there was again a significant interaction with both highest and lowest admission serum sodium. ($p<0.0001$ and $p=0.0009$ respectively) (Figure 6.5).

Figure 6.3: Adjusted odds ratio (shaded lines are 95% confidence intervals) of ICU mortality relative to 135 -144.9 mmol/l for different categories of highest serum sodium (left) and lowest serum sodium (right) in the first 24 hours in patients with respiratory (dotted) or without respiratory diseases (solid)



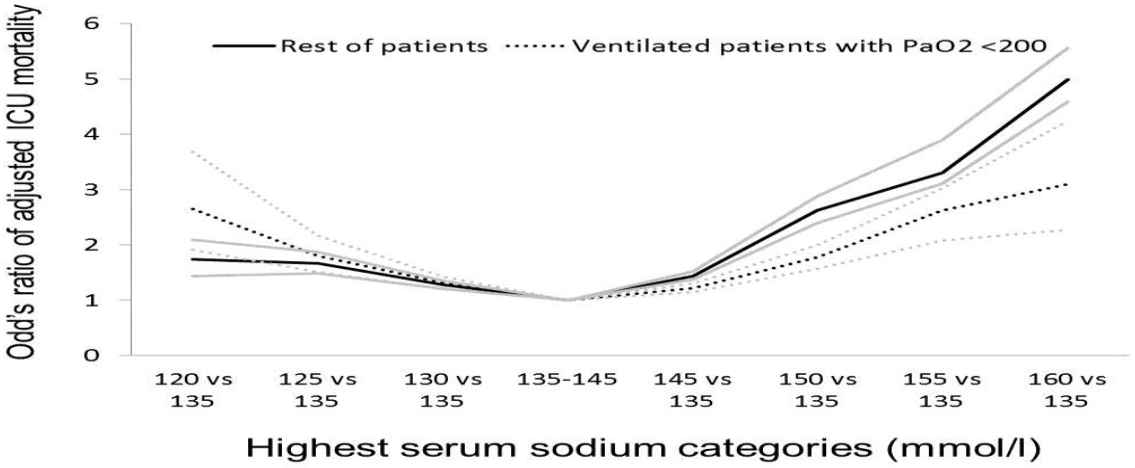
The relationship between sodium and ICU mortality was significantly different between respiratory and non-respiratory patients for the highest serum sodium (p<0.0001) and lowest serum sodium (p<0.0001) in the first 24 hours of ICU admission

Figure 6.4: Adjusted odds ratio (shaded lines are 95% confidence intervals) of ICU mortality relative to 135 -144.9 mmol/l for different categories of highest serum sodium in the first 24 hours in all patients (left) and mechanically ventilated patients (right) with PaO_2/FiO_2 ratio < 200 (dotted) or ≥ 200 (solid)



There was a significant interaction between PaO_2/FiO_2 ratio and highest serum sodium indicating the relationship between mortality and sodium is different in those with PaO_2/FiO_2 ratio < 200 compared to those with a PaO_2/FiO_2 ratio ≥ 200 (p<0.0001 for both)

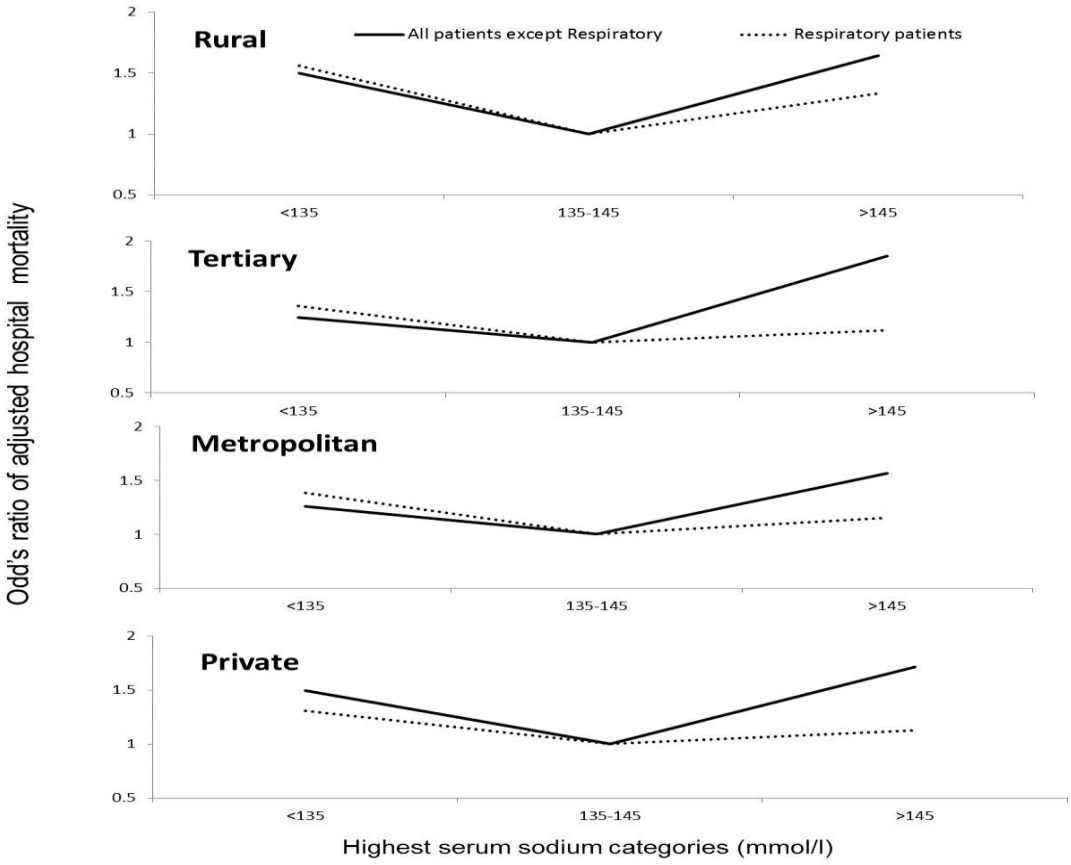
Figure 6.5: Adjusted odds ratio (shaded lines are 95% confidence intervals) of ICU mortality relative to 135 -144.9 mmol/l for different categories of highest serum sodium in the first 24 hours in all mechanically ventilated patients with PaO_2/FiO_2 ratio < 200 (dotted) vs all other patients (solid)



There was a significant interaction between this group and highest serum sodium indicating the relationship between mortality and sodium is different in those patients who were mechanically ventilated with PaO_2/FiO_2 ratio < 200 in the first 24 hours compared to all others (p<0.0001).

Whilst there were slight differences between hospital levels, the underlying difference between respiratory and non-respiratory patients for the relationship between sodium and mortality remained consistent throughout (Figure 6.6).

Figure 6.6: The relationship between sodium and mortality when the 129 hospitals in the study were categorised into hospital level (26% rural, 22% metropolitan, 24% tertiary and 28% private)



The difference between respiratory and non-respiratory patients remained consistent throughout.

6.1.3 DISCUSSION

ICU mortality (adjusted), overall, was U shaped with increased mortality at the extreme levels of dysnatremia. However, this pattern was not observed amongst patients admitted with respiratory diagnoses. While in patients without respiratory diagnoses, high serum sodium in the first 24 hours of ICU admission was associated with increased mortality. This difference was more evident when patients with severe impairment in oxygenation (admission PaO_2/FiO_2 ratio < 200) were analysed separately. To our knowledge

this study is the first to shown an association between hyperosmolarity and amelioration of its adverse mortality effects in patients with a respiratory diagnosis.

Possible reasons for differential effect

Hypernatremia is associated with increased mortality risk in critically ill patients, independent of age and severity of disease (Darmon *et al.* 2013; Palevsky *et al.* 1996; Lindner *et al.* 2007; Funk *et al.* 2010; Stelfox *et al.* 2008; Vandergheynst *et al.* 2013). In addition to causing intracellular dehydration, hypernatremia is thought to aggravate peripheral insulin resistance, leading to hyperglycaemia (Bratusch-Marrain *et al.* 1983). Hypernatremia also impairs hepatic gluconeogenesis and lactate clearance and is associated with neurological impairment that might lead to prolonged duration of mechanical ventilation and delayed weaning (Adrogue' *et al.* 2000). Hypernatremia can impair cardiac function, decrease left ventricular contractility (Kozeny *et al.* 1985), and cause rhabdomyolysis (Acquarone *et al.* 1989). There was an increase in mortality in all sub groups of patients, except respiratory. The rise in mortality was higher in patients in the surgical sub group (Table 6.3) as compared to medical sub-group. This is in contrast to that reported by Funk *et al.* (Funk *et al.* 2010) in a study sample, which was one third the size when compared to ours. Patients classified under "respiratory" in our study were also included under medical, thereby decreasing the mortality of the medical subgroups.

Moreover, our study presents significant methodological differences, which we believe are very important. Firstly, we have adjusted the mortality as outlined under the methods section; secondly, we used APACHE III risk of death with the serum sodium component removed; thirdly, we have adjusted analysis for year of admission and attending hospital to accommodate for significant changes in practice over 10 years and potential changes between hospitals. Finally, we have adjusted for each patient's propensity to be admitted with a respiratory diagnosis thus effectively controlling for the imbalance that exists between respiratory and non-respiratory patients. The odds ratio of mortality in the Funk (Funk *et al.* 2010) study surgical group with dysnatremia was calculated with their medical group as a reference. In our study, the odds were calculated by comparing hypernatremic patients within each diagnostic group to the patients with normal serum sodium in the same diagnostic group. There may be several reasons why mortality increases more rapidly in the surgical group as compared to the medical group with rising sodium. The incidence of emergency surgery is likely to be high in patients with dys-natremias, in contrast to patients with normal serum sodium (more likely to represent elective post-operative admissions). Patients undergoing emergency surgery are also known to have increased morbidity and mortality as compared to patients with elective surgeries (Ingraham *et al.* 2011).

However, hypernatremia may have some beneficial effects. High serum sodium leads to hyperosmolarity (effectively hypertonicity) which has been shown to rescue T-cells from suppression by trauma-induced anti-inflammatory mediators (Loomis *et al.* 2001), suppresses neutrophil activation (Angle *et al.* 1998; Junger *et al.* 2012; Deitch *et al.* 2003) and affects macrophage migration (Kim *et al.* 2013); all of which can mitigate lung injury (Angle *et al.* 1998).

Serum sodium is reflected in tonicity which can up- or down-regulate the TRPV4 ion channel which plays a critical role in lung vascular mechanotransduction (Chapter 5) (Yin *et al.* 2010). Specifically, hypotonicity can activate these channels (Chen *et al.* 2009a; Chen *et al.* 2009b; Becker *et al.* 2009; Garcia-Elias *et al.* 2008; Mizuno *et al.* 2003) leading to endothelial calcium influx, and a rise in pulmonary vascular permeability (Yin *et al.* 2010). We speculate that hypertonicity can suppress these channels leading to a decrease in pulmonary vascular permeability, which may be beneficial in patients with lung injury. In our study, patients with PaO_2/FiO_2 ratio < 200 had the maximum benefit from high serum sodium.

Hyperosmolarity increases type 1 alveolar epithelial cell repair (Wang *et al.* 2011), and augments actin filament formation and E-cadherin expression at the endothelial cell periphery (Safdar *et al.* 2003). Moreover it blocks TNF-α–induced P-selectin expression in an

actin-dependent manner which helps in remodelling of the endothelial barrier (Safdar *et al.* 2003). These results may explain the differential effects of high serum sodium in patients with and without respiratory diagnoses and furthermore the differential response of low admission PaO_2/FiO_2 ratio on ICU mortality in our study.

Use of hyperosmolar solutions

Indirect evidence to support our findings comes from animal studies which have studied the effect of hyperosmolar solutions. Hyperosmolar solutions have limited pulmonary injury after haemorrhagic shock in experimental models, improved splanchnic blood flow and reduced both adhesion and cytotoxicity of neutrophils compared with the use of isotonic solutions (Angle *et al.* 1998). Hyperosmolar sucrose strengthens the lung endothelial barrier, and enhances actin polymerization in the endothelium (Safdar *et al.* 2003; Quadri *et al.* 2003). Similarly, a brief period of vascular hyperosmolarity protects against acid-induced lung injury (Safdar *et al.* 2005). Though Bulger *et al.* (Bulger *et al.* 2008) using hypertonic resuscitation, did not demonstrate any difference in mortality, organ failure or in ARDS free survival, they did show improved ARDS-free survival among patients at risk of ARDS with massive transfusion (Zilberberg *et al.* 2007). In a randomised study that included 422 patients, evolution toward acute lung injury was less frequent when patients had received fluid loading with

hypertonic saline/dextran than with normal saline (Mattox *et al.* 1991). Similarly, previous meta-analysis has suggested survival advantage to hospital discharge in traumatic hypotensive patients when resuscitated with hypertonic saline dextran (Wade *et al.* 1997). Recently, hypertonic solution treatment decreased the need for ongoing fluid resuscitation in patients with septic shock (van Haren *et al.* 2012), and modulated gene expression implicated in leukocyte-endothelial interaction and capillary leakage (van Haren *et al.* 2011).

6.1.4 STRENGTHS AND LIMITATIONS

We have used a large multinational database and included a large cohort of patients (n=436,209). The data were independently collected by multiple trained data collectors for the purpose of audit and are unlikely to be subject to bias for the purpose of this study. ANZICS CORE database undergoes automated internal validation processes in addition to on-site audits by trained ANZICS auditors which indicate that serum sodium values are generally of high completeness, reliability and consistency. The outcome (ICU mortality) is objective and easily verifiable and unlikely to be affected by ascertainment error or bias. Collection of validated markers for severity of illness allowed the adjusted ICU mortality risk (OR) to be calculated by multivariate analysis. Finally, the differential association of serum sodium with outcomes is statistically strong and robust. While conventional multivariable regression methods will never be able to address bias resulting from

unmeasured confounders; by developing a score for each patient's propensity to present with a respiratory diagnosis, we were able to effectively control for all identifiable baseline imbalances that were found to exist between respiratory and non-respiratory diagnosis. This subsequently ensured that the observed results were independent of known baseline imbalances and effectively provided the equivalent of a matched analysis between respiratory and non-respiratory patients.

Although this was a large retrospective study, there are several limitations. Firstly, a number of patients with mortality and severity of illness data were excluded because of missing data, however they accounted for only 14% of the screened population. Secondly, whilst we controlled for severity of illness using APACHE III risk of death (with serum sodium component removed) year of admission, hospital and propensity (to have a respiratory diagnosis) in our adjusted model, there are multiple other factors contributing to ICU mortality. We also used APACHE III diagnostic codes to classify the patients and whilst this is a robust way to classify patients, we cannot rule out misclassification bias.

Being a retrospective study, it was not possible to control for any therapies administered to cause (for example- use of furosemide) or treat dysnatraemia prior to ICU admission. Also there was no information about the change in serum sodium during the ICU stay (and its potential effect on outcomes) and this should be a subject of

future studies. Similarly, the effect of fluid balance and its possible effect on serum sodium and hence outcome should also be the subject of a future trial.

Furthermore, I acknowledge that respiratory diagnoses include a wide variety of respiratory conditions, while hyperosmolarity may only benefit patients with lung injury in particular. In an attempt to examine the patients with ARDS (as these data are not routinely collected), we did examine all patients and patients admitted with a respiratory diagnosis with PaO_2/FiO_2 < 200 and those with PaO_2/FiO_2 < 200 receiving invasive mechanical ventilation in the first 24 hours using an interaction model, and found reduced risk of mortality with hypernatremia in these subsets of patients. Moreover, patients classified under a different diagnostic category may also have lung injury. Hence, we examined all patients with PaO_2/FiO_2 < 200 in addition to those who were mechanically ventilated, and found an interaction of serum sodium on ICU mortality between the 2 groups as well; high serum sodium also confirmed reduced risk of mortality in those patients who were mechanically ventilated with PaO_2/FiO_2 < 200 when compared with others. Examining the effect of hypernatremia in patients with ARDS should be a subject of future study.

6.1.5 SUMMARY

High levels of admission serum sodium did not lead to the expected increase in mortality observed in ICU patients in those who are

admitted with respiratory diagnoses. The difference was more pronounced in patients with more severely impaired oxygenation. These data could form the basis of future studies examining hyperosmolar therapies in patients with lung injury.

6.2 Admission high serum osmolarity is not associated with increased ICU mortality risk in respiratory patients

After examining the effect of serum sodium on mortality in respiratory patients the effect of serum osmolarity was directly investigated (serum osmolality is largely dependent on serum sodium). As increased serum osmolarity is lung protective we hypothesized that mortality associated with high admission serum osmolarity would be ameliorated in critically ill patients with an acute respiratory diagnosis.

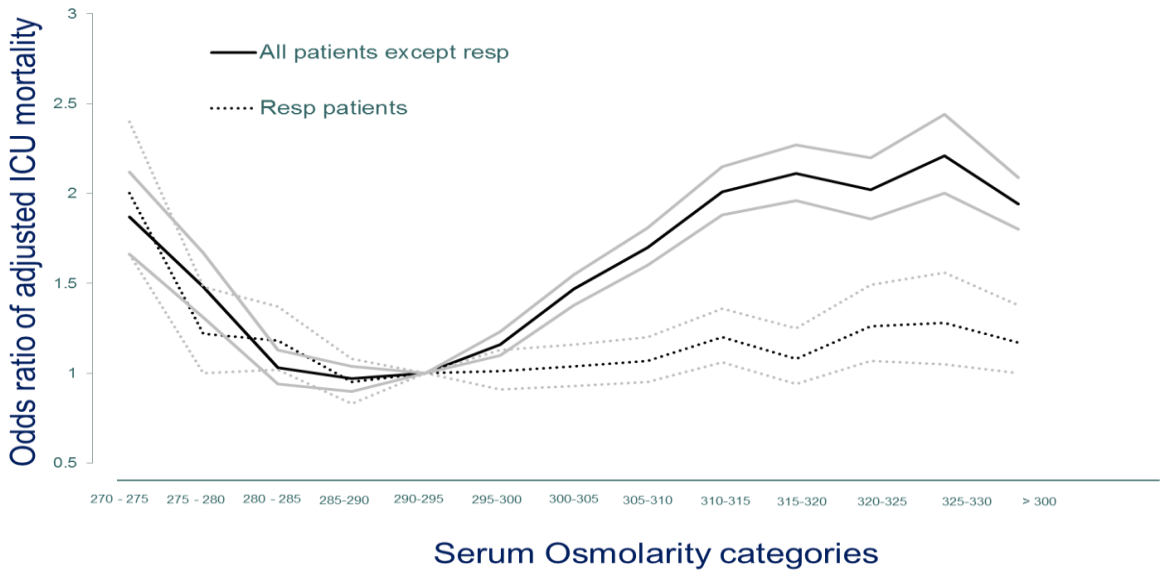
To examine the effect of the highest calculated admission serum osmolarity on ICU mortality in critically ill patients, data from January 2000 to December 2012 was accessed using the ANZICS CORE database. 509,180 patients were included. Serum osmolarity was calculated from the highest serum sodium, glucose and urea values in the first 24 hours of ICU admission. Predefined subgroups (based upon APACHE III diagnostic codes), including patients with acute respiratory diagnoses, were examined. The effect of serum osmolarity on ICU mortality was assessed with analysis adjusted for illness severity (serum sodium, glucose and urea component removed) and year of admission. Results are presented as odds ratio (OR) (95% CI) referenced against a serum osmolarity of 290 – 295 mmol/l.

Overall ICU mortality was increased at each extreme of calculated serum osmolarity (U- shaped relationship). A similar trend was found in various subgroups, with the exception of patients with respiratory diagnoses in whom ICU mortality was not influenced by high serum osmolarity and was different from other non-respiratory sub-groups ($P<0.01$) (Figure 6.7). Any adverse associations with high serum osmolarity in respiratory patients were confined to patients with a PaO_2/FiO_2 ratio ≥200. Using goodness of fit criteria,

area under the curve and the significance of the interaction term, serum osmolarity was a stronger predictor of ICU mortality than serum sodium for the separation of respiratory and non-respiratory patients.

High admission serum osmolarity was not associated with increased odds for ICU death in respiratory patients (unlike other subgroup of patients) and could be a potential area for a future interventional therapy.

Figure 6.7: Adjusted odds ratio (shaded lines are 95% confidence intervals) of ICU mortality relative to 135 -144.9 mmol/l for different categories of highest serum osmolarity in the first 24 hours in patients with respiratory (dotted) or without respiratory diseases (solid)



The relationship between sodium and ICU mortality was significantly different between respiratory and non-respiratory patients for the highest serum osmolarity (p<0.0001) in the first 24 hours of ICU admission.

6.3 Induced hypernatremia reduces acute lung injury, independent of fluid or sodium load

Based on the findings from the epidemiological studies (Section 6.1 and 6.2), administration of hypertonic saline (to induce hypernatremia) as a therapeutic option was tested in an animal model of lung injury. ARDS has a substantial impact on public health (Rubenfeld *et al.* 2005). While the incidence of ARDS varies considerably across the world (Rubenfeld *et al.* 2005; Luhr *et al.* 1999; Bersten *et al.* 2002), recent data suggest an incidence ranging from 15.3–58.7 cases per 100,000 person-years (Rubenfeld *et al.* 2005; Arroliga *et al.* 2002). Mortality also varies with reported ranges of 30–58% (Rubenfeld *et al.* 2005; The ANZIC influenza investigators 2009; Zilerberg *et al.* 1998). The problem of ARDS has been highlighted by the recent H1N1 influenza pandemic where ICUs treated an unprecedented number of cases of ARDS (The ANZIC influenza investigators 2009). Since the original description of ARDS (Ashbaugh *et al.* 1967), substantial progress has been made in understanding the natural history and pathogenesis of this lethal syndrome. However, lung protective ventilation (The Acute Respiratory Distress Syndrome Network 2000) remains the only current treatment strategy for ARDS. Similarly, besides some recent data suggesting role of neuro-muscular blockers (Papazian *et al.* 2010) no other therapeutic agent has been established as a treatment for lung injury.

Use of hyperosmolar solution to increase serum osmolarity could be utilised as a therapeutic option. As discussed in the section 6.1 hyperosmolarity increases type 1 alveolar epithelial cell repair (Wang *et al.* 2011) and augments actin filament formation and E-cadherin expression at the endothelial cell periphery (Safdar *et al.* 2003). Moreover, it blocks TNF-α–induced P-selectin expression in an actin-dependent manner which helps in remodelling of the endothelial barrier (Safdar *et al.* 2003). Hyperosmolar solutions have limited pulmonary injury after haemorrhagic shock in experimental models with improved splanchnic blood flow and reduced adhesion and cytotoxicity of neutrophils compared with the use of isotonic solutions (Angle et a 1998; Shi *et al.* 2002). A 15-min infusion of hyperosmolar sucrose, which increases vascular osmolarity by approximately 50 mOsm, strengthens the lung endothelial barrier, and enhances actin polymerization in the endothelial periphery (Safdar *et al.* 2003; Quadri *et al.* 2003). Similarly, a brief period of vascular hyperosmolarity (increase of 55 mOsm/kg) protects against acid-induced lung injury when the infusion of hyperosmolar sucrose is administered shortly before, or shortly after, acid instillation in the airway (Safdar *et al.* 2005).

As the safety of systemic administration of sucrose in human is not known and there are major concerns with use of synthetic colloids (Perner *et al.* 2012; Myburgh *et al.* 2012), it was hypothesised that induced hypernatremia (by the administration of hypertonic saline) would lead to an increase in serum osmolality and could be used to

ameliorate lung injury. As administration of intravenous resuscitation fluids is a common practise in patients with lung injury (Rivers *et al.* 2001; Perner *et al.* 2012; Maitland *et al.* 2005) we compared the hypertonic saline with other commonly utilised fluids in the ICU.

The present study was aimed to investigate whether hypertonic saline (HTS) can protect against LPS aspiration-induced lung micro-vascular injury in a rat model. Other fluid controls were also utilised. Lung injury was assessed during 2 h of mechanical ventilation by monitoring left and right heart filling pressures, lung compliance, and tissue oxygen tension, following which, lung oedema, MPO activity, and bronchoalveolar lavage (BAL) fluid protein concentration, leukocyte count, cytokines and surfactant activity were determined. Systemic parameters such as plasma TNF-α serum electrolytes and haematocrit were also evaluated.

6.3.1 METHODS

6.3.1.1 Ethics approval: All experiments were approved by the Flinders University Animal Welfare Committee and performed according to the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation (application number 812.12).

6.3.1.2 Animals: Specific pathogen-free male Sprague-Dawley rats (250-280g) were used in all experiments. The methods used is similar to that described in section 5.3.

6.3.1.3 Induction of Acute Lung Injury

Controls: Negative control animals (n=6) did not received any intravenous fluids nor were they administered intra-tracheal LPS. Positive control animals (n=6) had induced acute lung injury using a previously established method of intra-tracheal LPS endotoxin (Dixon *et al.* 2009).

Briefly, rats were anaesthetized with 1% inhaled isoflurane (Forthrane, Abbott Australasia) and the right femoral vein and artery catheterized for maintenance via continuous intravenous infusion of thiopental (60mg/kg/hr; Abbott Australasia) and for arterial blood sampling and pressure monitoring, respectively. Rats were paralysed with a bolus injection of pancuronium bromide (1mg/kg iv; Astra Zeneca, Bedfordshire, UK) maintained by continuous infusion (0.2mg/kg/h iv) and kept at 37ºC with a temperature-controlled heat pad. A tracheotomy was performed and the lungs ventilated via a computer controlled small animal mechanical ventilator (flexiVent, SCIREQ Scientific Respiratory Equipment, Montreal, Canada) with 100% oxygen for 15 minutes to stabilize at a tidal volume (VT) of 6ml/kg body weight, breathing frequency (f) of 120min-1, positive end expiratory pressure (PEEP) of 2 cmH2O using a constant flow waveform where Ti/Ttot = 0.3.

LPS (*Escherichia coli* O55:B5, 15 mg/kg in saline, Sigma-Aldrich, St Louis, MO) or saline was instilled through the tracheal catheter in 3 separate 0.1ml volumes, each volume followed by a 3ml air bolus

and a respiratory recruitment manoeuvre of 2.5 × V_T and PEEP of 10cmH_2O for 15 seconds. The rat was left to stabilize on normal ventilation as above for 5 minutes after each instillation. Following LPS or saline instillation, rats were ventilated for 2 h, as above, while blood pressure was monitored continuously using a disposable pressure transducer (Sorenson Trans Pac; Abbott Critical Care Systems, Chicago, IL) connected to a MacLab system (AD Instruments, Sydney, Australia). The study plan with description of various measurements at various time intervals is similar to the previous experiment (Section 5.3) and the protocol is shown in figure 5.6.

6.3.1.4 Monitoring

Continuous monitoring of the right heart pressure was achieved by canulation of the left femoral vein. Similarly continuous monitoring of the left heart pressure was done by cannulating the right carotid artery and advancing the cannula through to the left heart (left ventricular end diastolic pressure). Both were monitored using a disposable pressure transducers (Sorenson Trans Pac; Abbott Critical Care Systems, Chicago, IL) connected to a MacLab system (AD Instruments, Sydney, Australia).

6.3.1.5 Fluids utilised

Animals who were administered intra-tracheal LPS were randomly divided into various fluid groups. (No fluids-positive control, 20% saline, 0.9% saline, 4% albumin, 5% glucose, 20% albumin and 5% dextrose with 20% albumin). All the fluids were administered through the right femoral vein by utilising a 3 way tap. The dose of 20% saline was 2.6 ml/kg. The dose of the other fluids used in the study was 60 ml/kg for 0.9% saline, 4% albumin, and 5% glucose. At this dose the sodium load in 0.9% saline and 4% albumin were similar to 20% saline. 20% albumin had a similar amount of albumin present as 4% albumin and albumin with 5% dextrose had similar volume and albumin as 4% albumin but minimal amount of sodium. The comparative amount of sodium, albumin and volume of the different fluids used in the study is shown in Table 6.6. Negative control animals did not receive any intravenous fluids or were administered intra-tracheal LPS. All the fluids were administered over half an hour (Figure 5.6).

Table 6.6: The comparative amount to sodium, albumin and volume of the different fluids used in the animal study

	Lung Injury	*Sodium*	*Volume*	*Albumin*
Negative control	n	-		
Positive control	Y	-		
0.9% Saline	Y	Y	Y	-
4% Albumin	Y	Y	Y	Y
5% Dextrose	Y	-	Y	-
20% Saline	Y	Y	minimal	-
20% Albumin	Y	-	minimal	y
20% Albumin + 5% Dextrose	Y	-	y	y

20 % Saline had equal amount of sodium to 0.9% saline but less volume; Dose of 0.9% saline , 4% Albumin and 5% Glucose was 60 ml/kg

6.3.1.6 Measurement of respiratory mechanics

Following LPS or saline instillation, rats were ventilated for 2 h, as above (Figure 5.6), while blood pressure was monitored continuously using a disposable pressure transducer (Sorenson Trans Pac; Abbott Critical Care Systems, Chicago, IL) connected to a MacLab system (AD Instruments, Sydney, Australia).

Respiratory mechanics including (airway (Newtonian) resistance, Raw; tissue resistance, Gtis; and tissue elastance, Htis) were measured before instillation (baseline), 5 minutes after the last instillation of either LPS or saline (start) and every 30 min thereafter for the duration of the experiment (Figure 5.6), by measuring the lung's impedance (Z) using the computer-controlled ventilator, as described previously (Davidson *et al.* 2002). Briefly, 2 min after a recruitment manoeuvre (2.5 × V_T), impedance of the respiratory

system was measured following a forced oscillation. The data were fitted to a constant phase model (Bates *et al.* 1992) where Z = Raw + *j*I + (Gtis-*j*Htis)/(2πf)$^{\alpha}$, where I is inertance, *j* is the imaginary unit, f is frequency and α = (2/π)arc tan (Htis/Gtis). Inertance was negligible and is therefore not reported.

6.3.1.7 Assessment of lung injury

Blood samples were taken hourly throughout ventilation for measurement of arterial blood gas-pH analysis (ABL 5, Radiometer, Copenhagen, Denmark) and bi-hourly for plasma cytokines. The lungs were removed and the right upper lobe resected for determination of wet-to-dry lung weight ratio, as described previously (Davidson *et al.* 2002). The remaining lung was degassed at 0.5 atm for 60 seconds and lavaged at 2°C with 3 separate 32ml/kg bodyweight volumes of 0.9% sodium chloride, each volume instilled and withdrawn three times. Percent recovery of lavage fluid was not different between saline and LPS animals (80.6±7.8 v 79.6±7.5 % of total instilled lavage volume respectively; *P*=0.7). The lung lavage fluid was centrifuged at 150 *g* for 5 minutes at 2°C. A sample was taken from the supernatant, aliquoted and stored at -80°C until analysis for cytokines. A further aliquot was taken for determination of total lung lavage protein with commercially available reagents (BioRad DC Protein Assay; BioRad Laboratories, Hercules, CA).

Lung lobes were resected for determination of both wet-to-dry weight ratio and MPO activity (Elder *et al.* 2013). Bronchoalveolar lavage (BAL) was performed, following isolation of the top right two lobes, for determination of alveolar cytokine, total protein and cells (Elder *et al.* 2013).

6.3.1.8 Histological analysis

Following BAL, the right lung lobes were fixed at 20 cmH_2O with 10% buffered formalin. Paraffin-embedded sections (4 μm) were stained with hematoxylin and eosin for scoring of pulmonary inflammatory cell infiltration and alveolar wall thickening using a semi quantitative score (0-3) on blinded sections by two independent investigators (dos Santos *et al.* 2011).

6.3.1.9 Surfactant analysis

The remaining supernatant was centrifuged at 40000 *g* for 15 minutes at 2°C to separate the dense surface-active fraction, or large aggregates (LA) and less surface-active, small aggregates (SA). Lipids were extracted from each fraction using the method of Bligh and Dyer (Bligh *et al.* 1959) and total phospholipid content determined by measuring the amount of inorganic phosphorus with the method of Bartlett (Barlett *et al.* 1959). Measured surfactant was normalized to gram of dry lung weight

6.3.1.10 Cytokine determination

Cytokine concentrations in lung lavage and plasma were analysed using commercially available enzyme linked immunosorbant assay (ELISA) kits for TNF-α and IL-8 (CINC-1) (R&D Systems), as described previously (Dixon *et al.* 2008).

6.3.1.11 Electrolyte and haematocrit measurement

Serum sodium and chloride were measured using Ion Selective Electrodes (ISE) (Roche/Hitachi Modular Analyser- Japan). Haematocrit was measured with haematocrit reader.

6.3.1.12 Statistical analysis

All values are expressed as means (SD). Two-way analysis of variance (ANOVA) and repeated measures analysis of variance followed by a Tuckey post-test were used. Significant differences were determined where P was less than 0.05.

6.3.2 Results

Changes in serum sodium, chloride and haematocrit

Serum sodium, chloride and haematocrit over the course of the experiment is shown in Table 6.7. 20 % saline resulted in an increase in serum sodium and chloride at 30 and 120 minutes while glucose containing regimens led to a decrease in these electrolyte levels. Administration of all fluids led to decreased haematocrit; the greatest with albumin containing fluids. (Table 6.7)

Table 6.7: Effect of administered fluids on serum sodium, chloride and haematocrit.

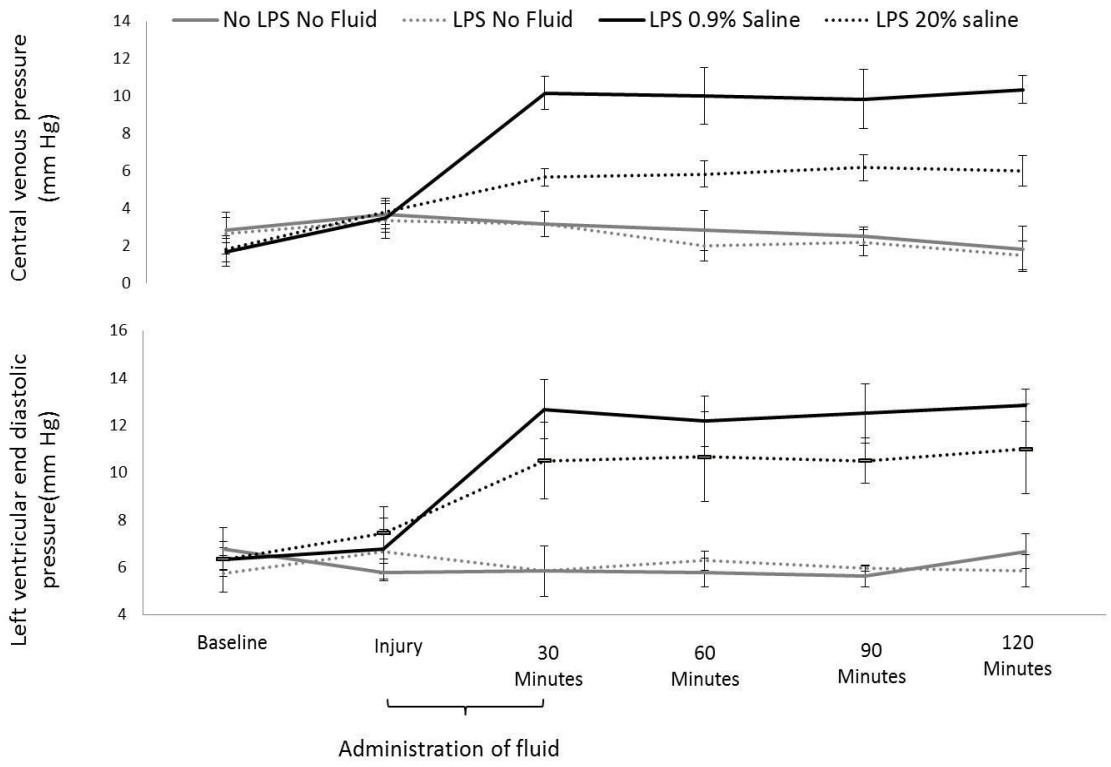
Groups	Serum Sodium (mmol/l)			Serum Chloride (mmol/l)			Haematocrit (delta)	
	Baseline	30 minutes	120 minutes	Baseline	30 minutes	120 minutes	30 minutes	120 minutes
Negative control (no LPS (i.t.) and no i.v. fluids)	136 (2)	137 (1)	138 (1)	102 (2)	102 (2)	103(1)	.03 (.03)	.05 (.03)
Positive control LPS (i.t.) with no i.v. fluids)	137 (2)	138 (2)	139 (1)	101 (3)	103 (2)	103 (4)	.02 (.01)	.04 (.01)
LPS (i.t.) with 20% saline	139 (2)	165 (3)	160 (1)	100 (2)	134 (3)	131 (2)	.02 (.02)	-.04 (.02)
LPS (i.t.) with 0.9% saline	138 (3)	140 (2)	142 (3)	101 (4)	115 (3)	111 (3)	-.07 (.02)	-.06 (.02)
LPS (i.t.) with i.v. 4% albumin	139 (3)	128 (4)	131 (3)	103 (3)	106 (3)	101 (4)	-.10 (.02)	-.08 (.02)
LPS (i.t.) with i.v. 5% glucose	139 (3)	128 (4)	131 (3)	103 (4)	100 (3)	99 (3)	-.04 (.02)	-.02 (.01)
LPS (i.t.) with i.v. 20% albumin	140 (3)	132 (3)	136 (4)	100 (2)	95 (3)	99 (3)	-.20 (.06)	-.08 (0.04)
LPS (i.t.) with i.v. 20% albumin + 5% glucose	138 (3)	118 (5)	124 (5)	101 (2)	91 (3)	93 (4)	-.16 (.06)	-.11 (.05)

Data presented as mean (sd). 20% saline led to an increase in serum sodium and chloride levels

LVEDP and CVP

There was an increase in LVEDP in all animals following administration of i.v. fluids however this was maintained at less than 18 mm Hg (Figure 6.8). Similarly there was increase in CVP following administration of all i.v. fluids (Figure 6.8). The rise in LVEDP and CVP with administration of 20 % saline was less when compared to all other fluids.

Figure 6.8: The effect of fluid administration on the left ventricular end diastolic pressure and the central venous pressure

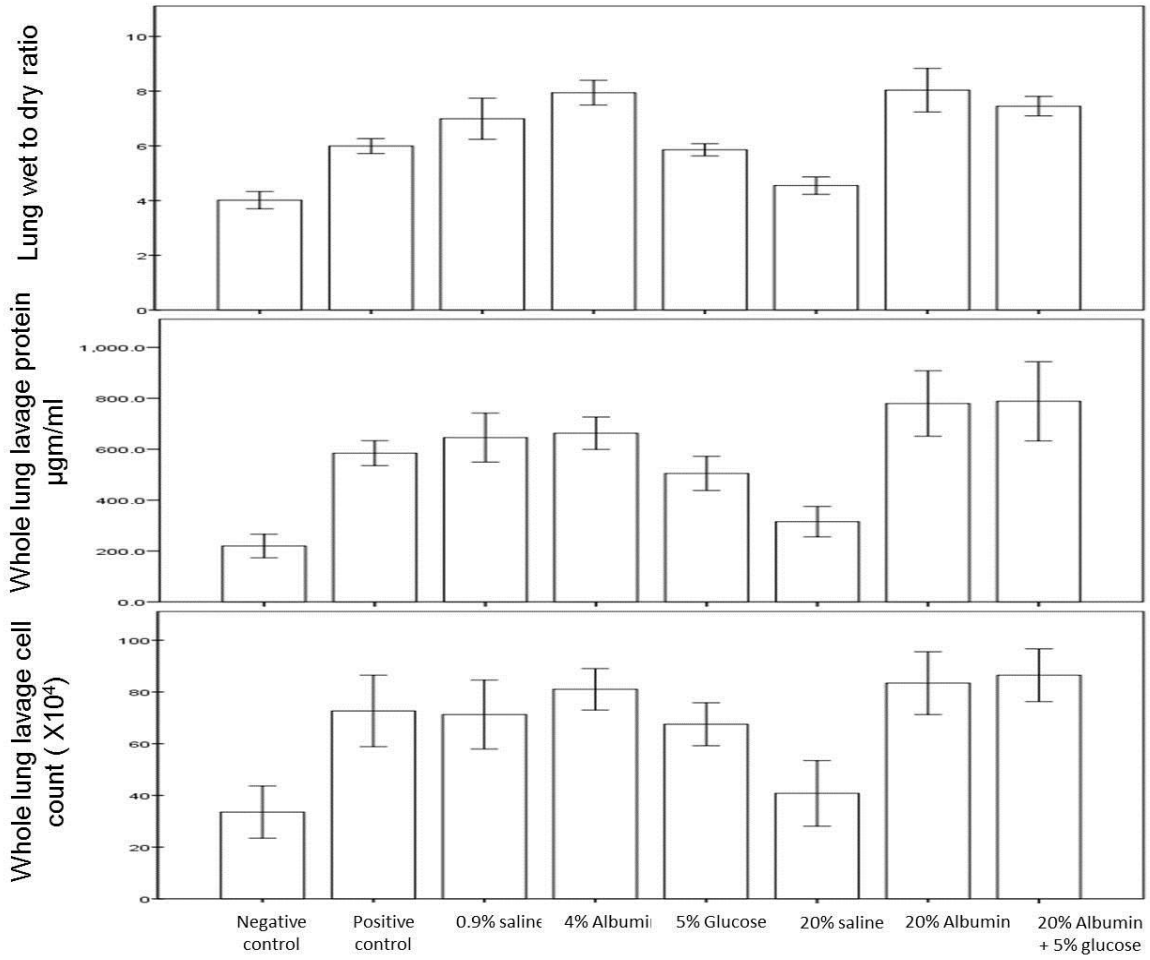


Administration of 20% saline lead to an increase in the left and right heart pressures but was less than the rest of the fluids (p<0.01). Result with 0.9% saline is shown here and it was not different from the rest of the administered fluid

Effect on Lung injury

Administration of 20% saline ameliorated the rise in lung wet to dry weight ratio apparent following induction of LPS-induced lung injury ($p<0.01$), as well as whole lung lavage protein ($p<0.01$), as compared with the positive control and other fluid controls (Figure 6.9). There were simultaneous decreases in total whole lung lavage cell($p<0.01$), neutrophil counts ($p<0.01$) and MPO levels ($p<0.01$) (Figures 6.9). Other fluids had no effect on these parameters.

Figure 6.9: Effect of study fluids on lung injury

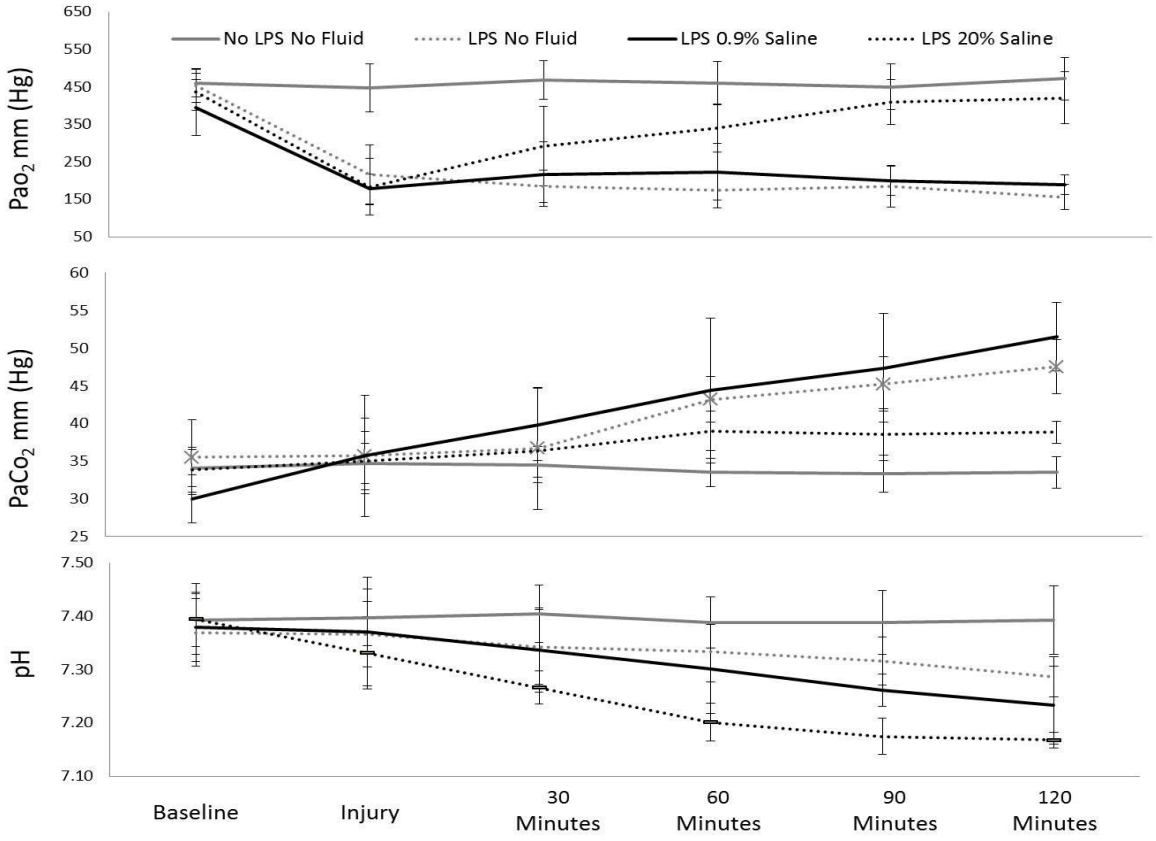


Data presented as mean and error bars as 95% CI

Blood gases

20% saline administration led to gradual improvement in PaO_2 levels while none of the other fluids had any effect on the oxygen levels. Similarly there was a lesser rise in $PaCO_2$ levels with 20% saline (Figure 6.10). Post-hoc analysis revealed the effect of 20% saline was different from the other fluids utilised in the study ($p<0.001$). There was greater drop in pH with administration of 20% saline as a result of hyperchloremia (Table 6.7)

Figure 6.10: Effect of the study fluids on blood gases

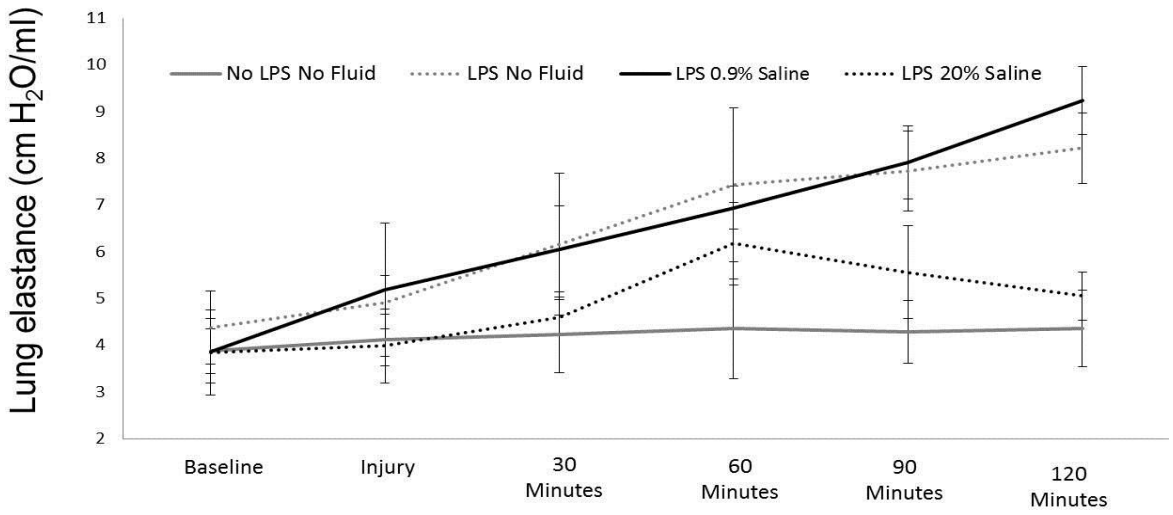


Data presented as mean and SD. Result with 0.9% saline is shown here and it was not different from the rest of the administered fluid

Lung mechanics

Administration of 20 % saline administration led to lower Htis and Gtis during the course of the experiments ($p<0.001$) (Figure 6.11). Post-hoc analysis revealed significant differences between the different kind of administered fluid and 20% saline ($p=0.01$).

Figure 6.11: Effect of the study fluids on lung elastance

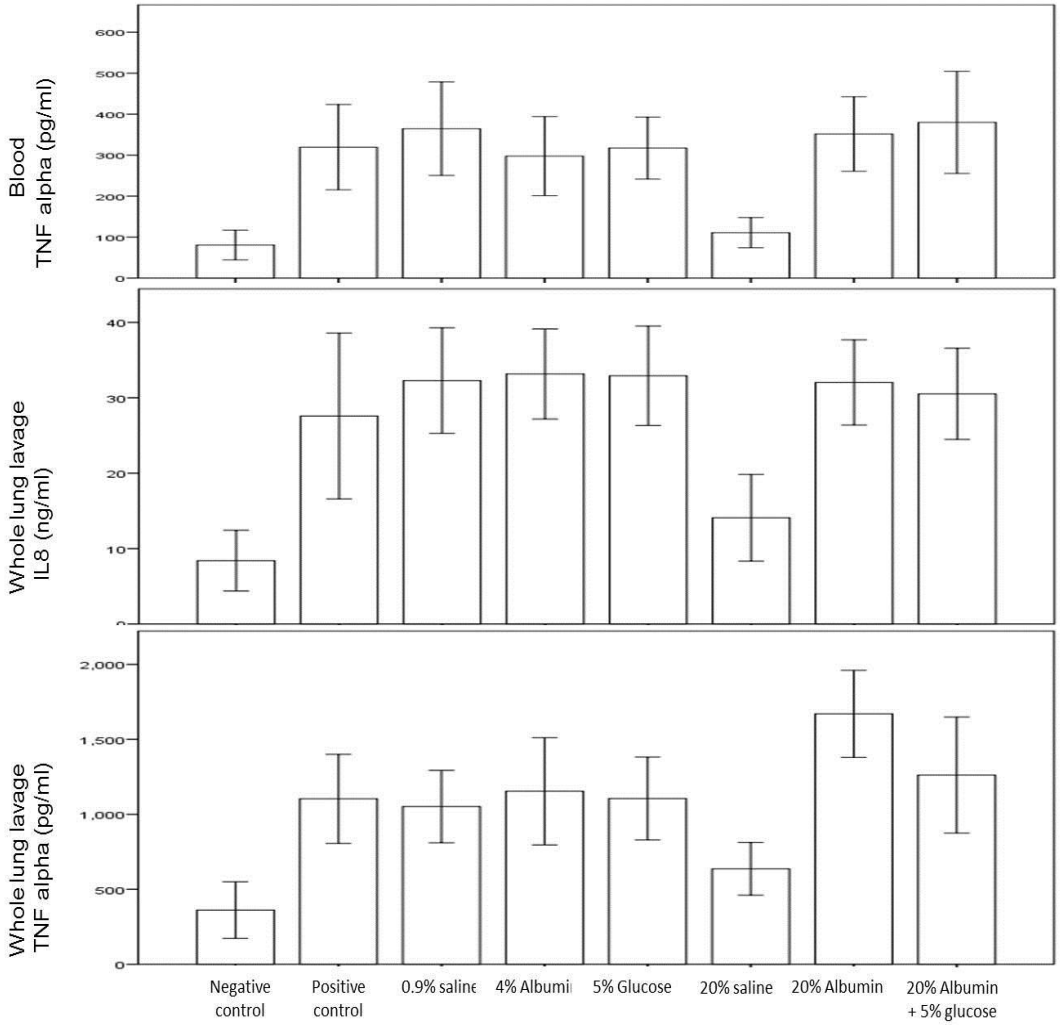


Data presented as mean and SD. Result with 0.9% saline is shown here and it was not different from the rest of the administered fluid

Lung lavage and serum TNF-α level and IL-8 levels

Administration of 20% saline resulted in lower serum and whole lung lavage TNF-α as compared with positive control and other fluids (p =0.03). Similarly the lung lavage IL-8 levels were also lower with 20% saline (p=0.01) whereas there was no difference in the serum IL-8. (Figure 6.12).

Figure 6.12: Effect of study fluids on lung lavage and serum TNF-α level and IL-8 levels

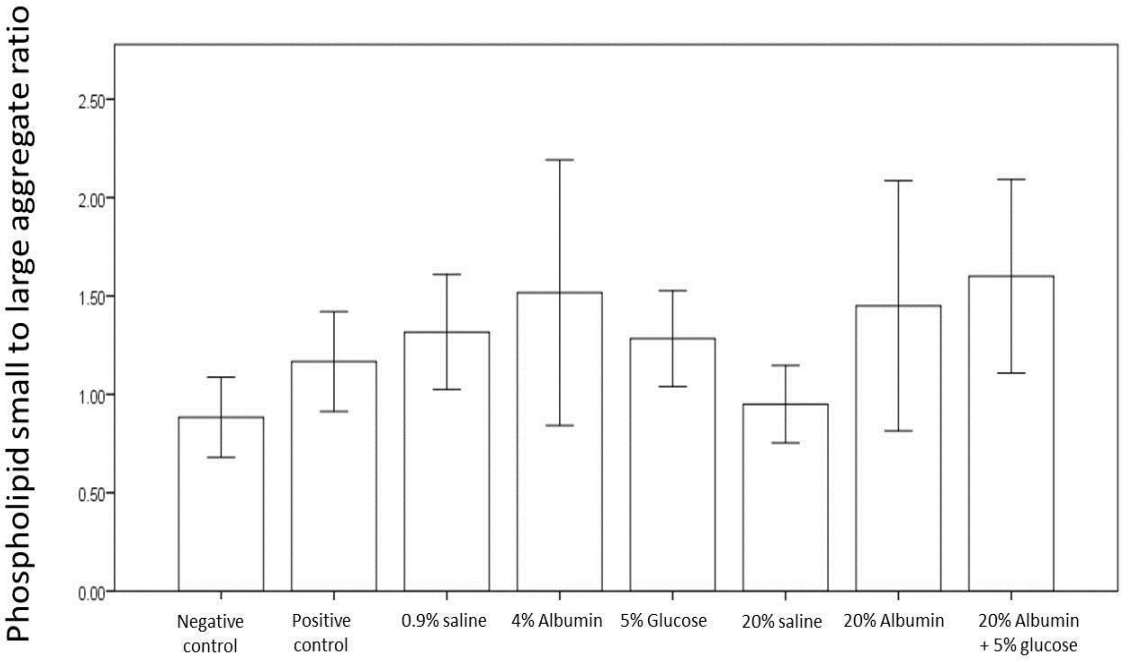


Data presented as mean and error bars as 95% CI

Surfactant

Although the total amount of phospholipids did not change with administration of 20% saline, the ratio of small to large aggregates did not increase with the administration of 20% saline, as compared to positive control or other fluids (p=0.02). (Figure 6.13)

Figure 6.13: Effect of study fluids on the ratio of small and large lung lavage phospholipid aggregate

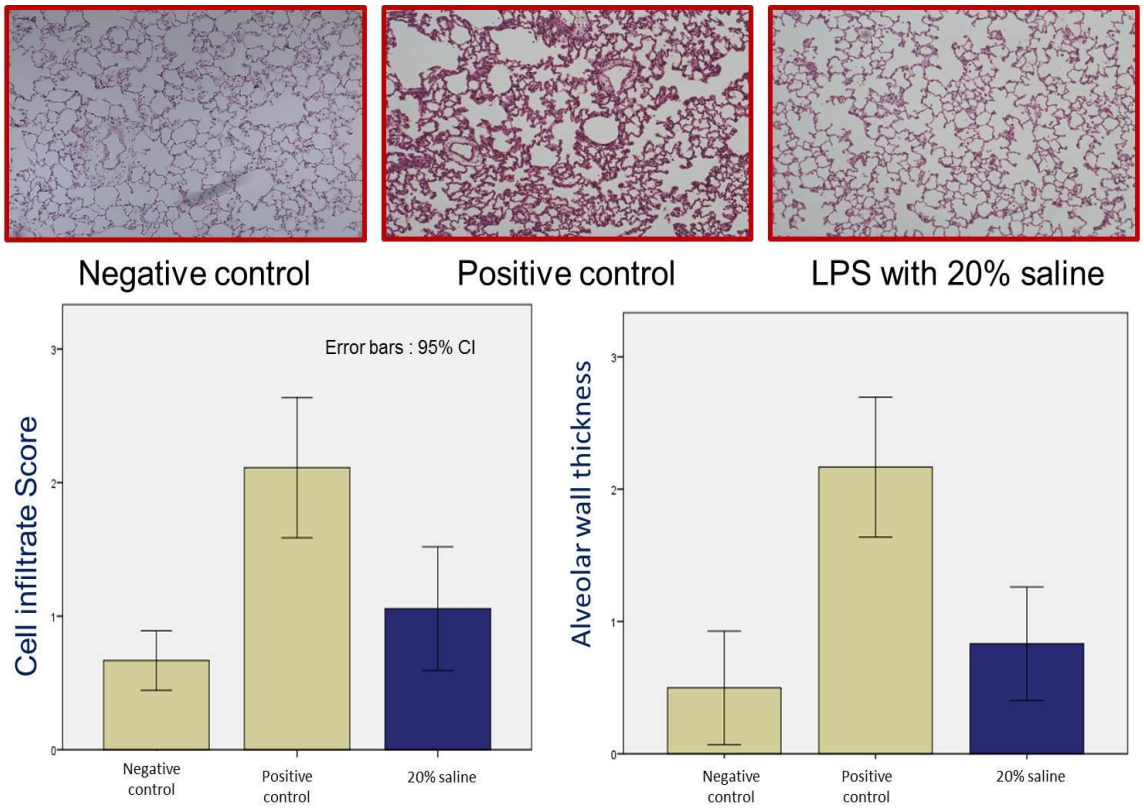


Data presented as mean and error bars as 95% CI

Lung histology

There was less cellular infiltrate (p=0.04) and wall thickness oedema (p=0.01) with the use of hypertonic saline (Figure 6.14)

Figure 6.14: Hematoxylin and eosin staining in the lung paraffin-embedded sections and lung histology scores (cell infiltrate and wall thickness score) in animals who were administered 20% saline compared with the positive and negative control



Data presented as mean and error bars as 95% CI

20% saline administration in animals with LPS induced lung injury led to less cellular infiltrate and wall thickness oedema

6.3.3 DISCUSSION

The main finding of this study was that in an epithelial model of lung injury, induced hypernatremia is lung protective. The beneficial effects of hypernatremia are likely due to the effect of increased serum osmolarity as other fluids (controlled for sodium and volume) had minimal/ worse effects on lung injury. This has clinical relevance as increasing serum sodium by administration of hypertonic sodium solution is an easy intervention with established clinical experience as it often used to manage patients with raised intracranial pressure.

Possible mechanisms for a lung protective effect of hypertonic saline

Administration of hypertonic saline leads to hyperosmolarity (effectively hypertonicity) which has been shown to rescue T-cells from suppression by trauma-induced anti-inflammatory mediators (Loomis *et al.* 2001), suppresses neutrophil activation (Angle *et al.* 1998; Junger *et al.* 2012; Deitch *et al.* 2003) and affects macrophage migration (Kim *et al.* 2013); all of which can mitigate lung injury (Angle *et al.* 1998). Pulmonary neutrophil sequestration, which is central to the mechanism of injury in ALI, is attenuated by hypertonic saline in diverse ALI models (Shields *et al.* 2003; Murao *et al.* 2003; Shields *et al.* 2000; Vialet et al 2003), as demonstrated here by a decrease in the lung lavage cells and cell infiltrate scores.

Hypertonic saline has been reported to exert protective hemodynamic effects in animal models of controlled post-traumatic hypotension (Rabinovici *et al.* 1991; Rabinovici *et al.* 1992) and in diverse clinical trials with trauma patients (Holcroft *et al.* 1987; Mattox *et al.* 1991). Also, HTS effectively resuscitated dogs (Horton *et al.* 1991), pigs (Kreimeier *et al.* 1991), and horses (Bertone *et al.* 1990) subjected to endotoxin shock. The mechanisms of action of hypertonic saline are increasingly well understood, and include inhibition of neutrophil adhesion molecule CD11b (Rizoli *et al.* 1999), reduced TNF-α and IL- 1 production (Shields *et al.* 2003a), and reduced activation of MAP kinase p38 and ERK-1 (Shields *et al.* 2003b). Similarly we found a decrease in lung lavage TNF-α and IL-8 level and serum TNF-α in our study. The therapeutic potential of HTS is demonstrated by its efficacy when used following initiation of the injury process, in both ischemia-reperfusion and pancreatitis induced ALI (Shields *et al.* 2003a; Shields *et al.* 2000). Effectiveness of HTS in other models of lung injury such as oleic acid injury and acid aspiration-induced lung injury (0.1 N HCl) have previously been shown through both reduction of inflammation and enhancing the resolution of lung injury (Kennedy *et al.* 2008; Rabinovici *et al.* 1996). Intra-tracheal instillation of LPS is a validated model of lung injury (Dixon *et al.* 2009). The effect of hypertonic saline in an LPS (intra-tracheal) model of lung injury has not been investigated before when controlled for the amount of sodium and fluid administered and compared with other commonly administered

fluids in ICU, with monitoring of left ventricular end diastolic pressure (in an attempt to control for hydrostatic oedema).

As discussed in section 6.1 serum sodium is reflected in tonicity which can up- or down-regulate the TRPV4 ion channel which plays a critical role in lung vascular mechanotransduction (Yin *et al.* 2010). Specifically, hypotonicity can activate these channels (Chen *et al.* 2009a; Chen *et al.* 2009b; Becker et al 2009; Garcia-Elias *et al.* 2008; Mizuno *et al.* 2003) leading to endothelial calcium influx, and a rise in pulmonary vascular permeability (Yin *et al.* 2010). We speculate that hypertonicity can suppress the TRPV4 channels leading to a decrease in pulmonary vascular permeability, which will ameliorate lung injury. We also found improved lung mechanics and blood gases with administration of HTS. This is reflected by a decrease in the ratio of small to large surfactant aggregates with fluid administration, possibly due to the decrease in the lung lavage protein concentration, as increased permeability and the presence of protein in the alveolus will increase the conversion from large to small aggregates (Davidson *et al.* 2000).

Clinical research

Hypernatremia is associated with increased mortality risk in critically ill patients, independent of age and severity of disease (Linder *et al.*, 2007; Hoorn *et al.* 2008). However, hypernatremia examined in these studies developed during the process of care for those patients, therefore induced hypernatremia in lung injury has

not been examined before. This in-vivo data supports our previous findings that high serum sodium was not associated with increased ICU mortality risk in respiratory patients (Section 6.1 and 6.2), and any adverse associations with hypernatremia in respiratory patients were confined to those with PaO_2/ Fio_2 ratios of greater than 200, indicating that lung protective effects of hypernatremia in patients with lung injury would reduce its general adverse effects leading to amelioration of the increase in mortality risk.

Administration of HTS in a patient population has been examined previously. Bulger et al (Bulger *et al.* 2008) using hypertonic resuscitation, did not demonstrate any difference in mortality, organ failure or difference in ARDS free survival. However, they did show improved ARDS-free survival among patients at risk of ARDS with massive transfusion (Zilberberg *et al.* 2007). In a randomised study that included 422 patients, evolution toward acute lung injury was less frequent when patients had received fluid loading with hypertonic saline/dextran than with normal saline (Mattox *et al.* 1991). Similarly, previous meta-analysis has suggested survival advantage in traumatic hypotensive patients when resuscitated with hypertonic saline dextran (Wade *et al.* 1997). Recently, hypertonic solution treatment was found to decrease the need for ongoing fluid resuscitation in patients with septic shock (van Haren *et al.* 2012), and modulated gene expression implicated in leukocyte-endothelial interaction and capillary leakage (van Haren *et al.* 2011).

Other fluids in lung injury

Besides 20% saline we also examined other commonly administered fluids in our epithelial model of lung injury. The dose administered was typically large but none of the administered fluids led to an increase in LVEDP more than 18 mmhg, the traditional cut off for hydrostatic pulmonary oedema. Moreover, recent studies have indicated that a large amount of fluid administration is a common practise in patients with lung injury. In a study investigating early goal direct therapy 39% of patients had pneumonia (the most common cause of ARDS) and they received 3499±2438 ml of fluid in the study arm and 4981±2984 ml in the control arm (Rivers *et al.* 2001). Similarly the recently conducted 6S study (Perner *et al.* 2012) where hydroxyethyl starch was compared with Ringer's acetate in patients with severe sepsis, 55% of patients with lung as the source of sepsis had a median 5235 ml of fluid (trial plus other fluid) administered on day 1 in the hydroxyethyl starch group. Similarly in the Feast study (Maitland *et al.* 2011) which showed adverse outcomes of either saline or albumin in children with severe infection, 83% of them were admitted with respiratory distress. Administration of such large volumes can contribute to a positive fluid balance which has been associated with poorer lung function (Wiedemann *et al.* 2006) though the effect of these fluid boluses per se on lung injury is not known. However, it is noteworthy that 25.6 % patients in the CHEST study (which compared starch and 0.9% saline) developed new onset respiratory failure during the study

(Myburgh *et al.* 2012). Hence the use of small volume resuscitation fluids such as hypertonic saline is an attractive option in patients with lung injury. Administration of 20% albumin also offers the advantage of small volume resuscitation but it not only led to a decrease in serum sodium it actually worsened, or had no effect on, the measured parameters of lung injury. This is in line with the recent findings by Caironi et al where in patients with severe sepsis, albumin replacement did not improve the rate of survival (Caironi *et al.* 2014). This is where HTS is different as it not only offers a smaller volume, it also causes a rise in serum sodium and hence serum osmolarity.

6.3.4 STUDY LIMITATION AND FUTURE DIRECTIONS

Our study has several limitations which should be addressed in future studies

1. Targeting a more physiological range of serum sodium: The current study was designed to simulate an increase in osmolality of 50 mosmol/l but future studies should target a more physiological range of serum sodium (145 to 150 mmol/l) as currently in practise for managing patients with raised intracranial pressure.
2. Preventing lung injury by prophylactic administration of HTS should be examined.
3. The time frame of lung injury was 2 hours in the current study. In future a longer time frame should be examined.

4. Rapid change in serum sodium can have adverse neurological effects and in the future a more physiological and a physiological increase in serum sodium levels should be examined.

6.3.5 SUMMARY

Induced hypernatremia leads to amelioration of lung injury and could be used a therapeutic option in patients with lung injury.

CHAPTER 7: SUMMARY AND RECOMMENDATIONS

7.1 Sodium administration and balance in ICU

- Sodium administration is high in both adult and paediatric patients which leads to a positive sodium balance
- Most of the administered source of sodium in ICU is from inadvertent sources. Sources such as vehicles for drugs, flushes for intravascular catheters and maintenance fluids inadvertently contribute a high proportion of the administered sodium
- Current dialysis practises in ICU remove water but lead to a positive sodium flux
- Frusemide leads to diuresis but not naturesis in ICU patients
- Positive sodium balance is associated with lung dysfunction

Future directions

As the majority of the administered sodium is from inadvertent and are from easily modifiable sources, a restrictive sodium strategy is possible and should be examined in future studies. Most drugs are soluble in 5 % glucose the use of which will lead to less sodium administration (Table 7.1 shows the list of handful of drugs which are incompatible with 5% glucose, the remainder can be administered with 5% glucose). Based on the data presented in section 3.2, sources of sodium can be divided into modifiable and

non-modifiable sources (Table 7.2). Modifiable sources should be a target of sodium restriction strategies. One such strategy could be

- 5% glucose as a vehicle for all drugs (infusion and bolus) unless contraindicated for reasons of drug solubility
- 5% glucose as maintenance fluid
- Heparinised 0.45% saline as flush for all intravascular catheters
- Use of low sodium enteral feeds

Table 7.1: Drugs which are incompatible with glucose

Alteplase	Enfuvirtide	Parecoxib sodium
Amifostine	Ertapenem sodium	Phentolamine
Ampicillin sodium	Erythromycin (IV)	Phenytoin
Apomorphine	Esomeprazole sodium	Rasburicase
Caspofungin acetate	Frusemide	EDTA
Clonidine	Hydralazine	Tenecteplase
Dantrolene	Infliximab	All the anti-neoplastic drug should be administered as per the manufacturers guidelines
Desmopressin	Interferon alfa	
Digoxin immune FAB	Iron polymaltose complex	
Dihydroergotamine	Iron sucrose	
Drotrecogin alfa (Xigris)	Oxytocin	
Efalizumab	Paraldehyde	

Table 7.2: Current contribution of sodium (mmol) from various sources

Source of sodium (intravenous and enteral nutrition)	**Current data (mmol) (Section 3.2)**
Modifiable	
Maintenance/replacement	69.3
Drug boluses	27.6
Enteral nutrition	26.5
Drug infusions	19.3
Flushes	16.6
Total Modifiable	**159.3**
Non-modifiable	
Fluid boluses	36.5
Blood products	13.5
Antimicrobial	11.2
Parenteral nutrition	4.3
Total non-modifiable	**65.5**
Grand total (mmol)	**224.8**

The feasibility of such an intervention should be investigated in future studies. Furthermore current dialysis strategies in the ICU have to be reviewed and modified to decrease sodium influx. Two

major interventions which can be easily done in ICU to potentially decrease the amount of sodium influx in patients are (i) use of better individualization of dialysate sodium concentration and (ii) earlier use of fluid removal as soon as feasible. Moreover drugs such as indapamide in combination with frusemide which have been shown to increase naturesis in outpatients (Tanaka *et al.* 2005) should be examined in ICU.

7.2 Fluid boluses

- Fluid boluses are common in septic patients, result in limited success, and may be harmful

- Fluids boluses can result in permeability pulmonary odema despite a safe (non-hydrostatic) LVEDP

- Administration of ruthenium red prevents such permeability oedema suggesting a TRPV4 mechanism

- In healthy volunteers 0.9% saline and 4% albumin solutions have differential pulmonary effects not obviously attributable to passive fluid filtration. Compared with other fluids 0.9% saline leads to interstitial oedema whereas 4% albumin does not cause pulmonary oedema even though there is evidence of hypervolemia.

Future directions

Indications and physiological effects of fluids boluses in other clinical areas such as the emergency department, post-surgery patients, and pre-hospital admission should be examined.

Fluid induced lung injury should be examined in other models, such as mice, sheep and healthy volunteers. The role of TRPV channels in such injury should be investigated by examining the effects of fluid boluses in TRPV4 knock out mice. Similarly, a more specific TRPV4 agonist (GSK1016790A) (Willette *et al.* 2008) and antagonist (GSK2193874) (Thorneloe *et al.* 2012) which are currently available should be used.

7.3 Hyperosmolality and lung injury

- Induced hypernatremia leading to an increase in serum osmolality might be a treatment option for lung injury

Future directions

As the administration of hypertonic saline leads to hyperosmolarity, hypernatremia and hyperchloremic acidosis, further studies should examine the differential effects of its administration in an animal model of lung injury.

Although I have reported a differential effect of high serum sodium in respiratory patients compared with others, patients with lung injury were not directly examined, future studies should examine the effect of high serum sodium in a database of patients with lung injury (such as DataMart Mayo Clinic, Rochester, Minnesota) (Herasevich *et al.* 2010).

Results of these studies could lead to a pilot study of hypertonic saline administration in patients with lung injury.

Appendix 1:

SODIUM CONTENT IN COMMONLY USED FLUIDS AND DRUGS.

Sodium Content (mmol)	
FLUID	
0.9% Saline	155 mmol/L
4% Dextrose & 0.18% Saline	31 mmol/L
20% Saline	3444 mmol/L
4% Albumin)	140 mmol/L
20% Albumin	74 mmol/L
Gelofusin	154 mmol/L
Hartmann (CSL)	131 mmol/L
TRANSFUSIONS	
PRC	31.3 mmol/U
FFP	44 mmol/U
Platelet	30.5 mmol/U
Nasogastric FEEDS	
Nutrison Concentrated	4.3 mmol/ 100 ml
Nutrison Energy	5.8 mmol/ 100 ml
Nutrison Energy multifibre	5.8 mmol/ 100 ml
Nutrison Low Sodium	1.1 mmol/ 100 ml
Nutrison Multifibre	4.3 mmol/100 ml
Abbotts Nepro	3.7 mmol/ 100 ml
Nutrison Standard	4.3 mmol/ 100 ml
FLUSH	
Heparin sodium	155.2 mmol/L (0.9% saline)
Daily 2 lines (8ml/hr)	29. 8 mmol/day (0.9% saline)

MEDICATIONS	
Acetazolamide *500 mg*	2.0 mmol/500 mg
Acetylcystein (NAC) *2g*	0 mmol/2g
Aciclovir *250 mg*	4.2 mmol/g
Adrenaline *1mg/1 ml*	0.2 mmol/ml
Adrenaline *1: 10000 (0.1 mg/ml)*	0.1 mmol/ml
Amiodarone *150mg/3 ml*	0 mmol/ml
Aminophylline *250 mg/10 ml*	0 mmol/ml
Amoxacillin *1g*	3.3 mmol/g
Ampicillin *1g*	2.7 mmol/g
Atropine *1mg/10ml*	1.5 mmol/mg
Azithromycin *500mg*	5.0 mmol/ 500 mg
Benztropine *2mg/2mL*	0.310 mmol/2 mg
Benzylpenicillin *1.2g/vial*	3.6 mmol/1.2 g
Betamethasone *5.7 mg/ml*	0.1 mmol/ml
Bupivacaine *50 mg/ 20ml vial*	0 mmol/vial
Cephazolin *1g*	2.0 mmol/g
Cefepime *1g*	0 mmol/vial
Cefotaxime *1g*	2.2 mmol/g
Ceftriaxone *1g/vial*	3.6 mmol/g
Chloramphenicol *1.2g*	2.3 mmol/g
Chlorpromazine *50mg/2 ml*	0.2 mmol/ 50 mg
Ciprofloxacin *200 mg/100 ml*	15.4 mmol/200 mg
Clonazepam *1 mg*	0 mmol
Clonidine *150 ug/1 ml*	0.2 mmol/ 150ug
Desmopressin (DDAVP)	0 mmol/vial
Dexamethasone *8mg/2 ml*	0 mmol/ 8 mg
Diazepam *10 mg/2 ml*	0.7 mmol/ 10 mg

Dicloxacillin *1g*	2.2 mmol/g
Digoxin *500 ug/2 ml*	0 mmol/ 500 ug
Dobutamine *250 mg*	0 mmol/ 250 mg
Dopamine *200 mg/ 5 ml*	0 mmol/ 200mg
Ephedine *30 mg/ml*	0.1 mmol/ 30 mg
Erythromycin *1g*	0 mmol
Esmolol *100 mg/10 ml*	0.3 mmol/100 mg
Flucloxacillin *1g*	2.2 mmol/g
Fluconazole *200mg/100ml (premix)*	15 mmol/200 mg
Flumazenil *0.5 mg/ 5ml*	0.8 mmol/0.5 mg
Frusemide *250mg/ 25 ml*	0.8 mmol/250mg
Frusemide *20mg/ 2ml*	0.3 mmol/ 20 mg
Gentamycin *80 mg/2ml*	0.1 mmol/80 mg
Glucagon *1 mg*	0 mmol
Glucose IV 50% *(25g/50 ml)*	0 mmol
Glyceryl *50 mg/10 ml*	0 mmol
Haloperidol *5 mg/ml*	0 mmol
Heparin Na	155.2 mmol/L (if 0.9% saline)
Hydralazine *20 mg/ampoule*	0 mmol
Hydrocortisone *100 mg/2ml*	0.3 mmol/100 mg
Imipenem-Cilastatin *500 mg*	1.6 mmol/500 mg
Ketamine *10 mg/ml*	0.2 mmol/10mg
Lignocaine *1% 5ml*	0 mmol
Lincomycin *600mg/2 ml*	0 mmol
Linezolid *2mg/ml*	5 mmol/300 ml
Meropenem *1g*	4.0 mmol/g
Metoclopramide *10 mg/2ml*	0.3 mmol/10 mg
Metoprolol *5mg/5ml*	0.8 mmol/5ml

Metronidazole *500 mg/100 ml*	13.5 mmol/500 mg
Methylprenisolone Na succinate *1g/16ml*	2 mmol/g
$MgSO_4$ *2.47g/5 ml*	0 mmol
Midazolam *5 mg/ ml*	0.1 mmol/5mg
Milrinone *10 mg/10 ml*	0 mmol
Morphine	0 mmol
Moxifloxacin *400 mg/250 ml*	34 mmol/400 mg
Naloxone *400 mg/ml*	0.2 mmol/ 400 mg
Nimodipine *100 mg*	0 mmol
Noradrenaline *2mg/ 2 ml*	0 mmol/ 2mg
Pantoprazole *40 mg*	0.1 mmol/ 40 mg
Phenobarbitone sodium *200 mg/ml*	< 1 mmol
Phenytoin *250 mg/ 5ml*	1.0 mmol/ 250 mg
Phytomenadine *10 mg*	0 mmol
Piperacillin *2g*	3.7 mmol/ 2g
Piperacillin *4g* **+ Tazobactam** *500 mg*	11.1 mmol/vial
Potassium	0 mmol
Propofol	0 mmol
Protamine *50mg/5 ml*	0.8 mmol/50 mg
Ranitidine *50 mg/2 ml*	0 mmol/ 50 mg
Rifampicin *600 mg*	0.1 mmol/vial
Rocuronium	< 1 mmol
Sodium bicarbonate *10ml*	10 mmol/10 ml
Sodium Nitroprusside *50 mg/ vial*	0.3 mmol/50 mg
Sotalol *40 mg/4ml*	< 1 mmol/100 mg
Suxamethonium *100 mg/2ml*	0 mmol
Thiamine *100mg/ml*	0 mmol/100 mg
Thiopentone *500 mg*	2.5 mmol/ 500 mg

Timentin *3.1g in 100 ml normal saline*	31 mmol mmol/3.1g
Theophyllin *200 mg/50 ml*	0 mmol
Trimethoprim *80 mg* **+ Sulfamethoxozole** *400 mg/ 5 ml*	0 mmol/5ml
Tropisetron *2 mg/ 2 ml*	< 1mmol
Vancomycin	0 mmol
Vasopressin *20 unit/ml*	0.2 mmol/ml
Vecuronium *10mg/vial*	0.1 mmol/10 ml
Verapamil *5 mg/2ml*	0.3 mmol/ 5mg
Voriconazole *200 mg*	10 mmol

PRC: Packed red blood cells; FFP: Fresh frozen plasma; U: Unit

Appendix 2

LIST OF PARTICIPATING SITES AND INVESTIGATORS IN THE ADULT POINT PREVALENCE STUDY

Albury Base Hospital, Albury, NSW, Australia: P Harrigan, C Mashonganyika

Alfred Hospital, Melbourne, VIC, Australia: A Davies, S Vallance, V Bennett

Auckland City Hospital, Auckland, New Zealand: S McGuiness, R Parke, V Cocharne

Auckland DCCM Hospital, Auckland, New Zealand: C McArthur, L Newby, C Simmonds

Austin Hospital, Melbourne, VIC, Australia: R Bellomo, G Eastwood

Box Hill Hospital, Melbourne, VIC, Australia

Calvary Mater Hospital, Newcastle, NSW Australia: Katrina Ellem

Canberra Hospital, Canberra, ACT, Australia: I Mitchell, E Crawford, R Ashley

Central Gippsland Health Service, Gippsland, VIC Australia:Jenny Dennett, Howard Connor

Christchurch Hospital, Christchurch, New Zealand: S Henderson,J Mehrtens

Concord Hospital, Sydney, NSW, Australia: D Milliss, H Wong

Dandenong Hospital, Melbourne, VIC, Australia: S Vij, B O'Bree, K Shepherd

Flinders Medical Centre, Adelaide, SA, Australia: S Verghese, E Ryan, A Waters

Geelong Hospital, Geelong, VIC, Australia: C Carrington, M Fraser,T Elderkin

Gold Coast Hospital, Southport,QLD, Australia: B Richards, M Tallot

Gosford Hospital, Central Coast, NSW, Australia: R Cameron, S Hatter

Hawke's Bay Hospital, Hastings, New Zealand: R Freebairn, L Chadwick

John Hunter Hospital, Newcastle, NSW Australia: Peter Harrigan

Liverpool Hospital, Liverpool, NSW, Australia: M Parr, S Micallef

Macquarie Hospital, Sydney, NSW, Australia: M Parr, D Bhonagiri

Middlemore Hospital, Auckland, New Zealand: T Williams, J Tai, A Tilsley

Maroondah Hospital, Ringwood East, VIC Australia: David Charlesworth

Nambour General Hospital, Nambour, QLD Australia: Peter Garrett

Nepean Hospital, Sydney, NSW, Australia: I Seppelt, L Weisbrodt

North Shore Private Hospital, Sydney, NSW, Australia: A Delaney, S Ash, DL Hogben

North Shore Hospital, Takapuna, New Zealand:Janet Liang

Princess Alexandra Hospital, Brisbane, QLD Australia: Chris Joyce

Royal Adelaide Hospital, Adelaide, SA, Australia: M Chapman, S O'Connor

Royal Darwin Hospital, Darwin, NT, Australia: D Stephens, J Thomas

Royal Hobart Hospital, Hobart, TAS, Australia: Andrew Turner & David Cooper

Royal Melbourne Hospital, Melbourne, VIC, Australia: C Macisaac, T Caf

Royal Perth Hospital, Perth, WA, Australia: S Webb, G McEntaggart, J Chamberlain

Royal Prince Alfred Hospital, Sydney, NSW, Australia: D Gattas, D Rajbhandari, H Buhr

Sir Charles Gairdner Hospital, Perth, WA, Australia: S Baker, B Roberts

St George Hospital, Sydney, NSW, Australia: J Myburgh, V Dhiacou

St Vincent's Hospital, Melbourne, VIC, Australia: J Santamaria, R Smith

St Vincent's Hospital, Sydney, NSW, Australia: P Nair, C Burns, C Reynolds

Tauranga Hospital, Tauranga, New Zealand: T Browne, J Goodson

The Queen Elizabeth Hospital, Adelaide, SA, Australia: S Peake, P Williams, C Kurenda

Toowoomba Hospital, Toowoomba, QLD Australia: Indranil Chatterjee

Townsville Hospital, Townsville, QLD, Australia: G Gordon, L Jones

Waikato Hospital, Hamilton, New Zealand: Mary La Pine

Wellington Regional Hospital, Wellington, New Zealand: D Dinsdale, D Mackle, L Andrews

Western Health, Melbourne, VIC, Australia: Craig French

Westmead Hospital, Sydney, NSW, Australia: A Bannerjee, C Skelly

Wollongong Hospital, Sydney, NSW, Australia: M Sterba, B Johnson

Appendix 3

LIST OF PARTICIPATING SITES AND INVESTIGATORS IN THE PAEDIATRIC POINT PREVALENCE STUDY

Children's Hospital Westmead, NSW, Australia: M Festa

John Hunter Children's Hospital, NSW, Australia: P Harrigan, M Hardie

Mater Children's Hospital, Brisbane, QLD, Australia:

Princess Margaret Hospital for Children, Perth, WA, Australia: S Erickson , J Abe

Royal Children's Hospital, Brisbane, QLD, Australia: A Slater, D Long, S Kendall

Royal Children's Hospital, Melbourne, VIC, Australia: W Butt, C Delzoppo

Royal Hobart Hospital, Hobart, TAS, Australia: A Turner. D Cooper, R McAllister

Starship Children's Hospital, Auckland, New Zealand: J Beca, L Segedin, C Sherring, M Rea

Sydney Children's Hospital, Sydney, NSW, Australia: ML Morritt, G Williams, J Young

Women's and Children's Hospital, Adelaide, SA, Australia: M Yung,G Letton

Appendix 4: LIST OF ETHICS APPROVALS

Ethics committee	**Study name**	**Relevant section**	**Approval number**
Southern Adelaide Human Research Ethics Committee South Australia	A Pilot Study of Retrospective Analysis of Sodium Administered in Patients with Prolonged Mechanical Ventilation	Section 3.1	216.09
Southern Adelaide Human Research Ethics Committee South Australia	Dialysis : Does it contribute or remove sodium in critically ill patients	Section 3.4	361.11
Southern Adelaide Human Research Ethics Committee South Australia	A prospective study evaluating the effects of daily sodium administration and balance on volume status, oxygenation and ventilation parameters.	Section 4.1	036.11
Southern Adelaide Human Research Ethics Committee South Australia	A prospective, multicentre observational study in patients admitted to intensive care to estimate daily sodium balance	Section 4.2	420.11
Southern Adelaide Human Research Ethics Committee South Australia	Post Resuscitation fluId boluses in severe sepsis or septic shoCk: prevalence and Efficacy (PRICE study)	Section 5.1	289.11
Southern Adelaide Human Research Ethics Committee South Australia	Early serum sodium and mortality in critically ill patients	Section 6.1	118.12
Southern Adelaide Human Research Ethics Committee South Australia	Is high serum osmolality be protective for critically ill patients admitted with respiratory diagnosis	Section 6.2	285.13
Southern	Do the commonly	Section 5.3	263.12

Adelaide Human Research Ethics Committee South Australia	administered fluids differ in their propensity to affect respiratory and cardiac variables in healthy human volunteers?		
Animal Ethics Review Committee Flinders University South Australia	Does the fluids and their components affect lung damage in an animal model of lung injury	Section 5.4 and 6.3	812.12

Appendix 5: LIST OF PUBLICATIONS AND PRESENTATIONS ARISING FROM CANDIDATURE

PEER REVIEWED ARTICLES

- Bihari S, Ou J, Holt AW, Bersten AD. Inadvertent sodium loading in critically ill patients. Crit Care Resusc. 2012;14:33-37. 7 citations
- Bihari S, Baldwin C and Bersten AD. Fluid balance does not predict estimated sodium balance in critically ill mechanically ventilated patients. Crit Care Resusc 2013; 15: 89-96. *Study had an editorial with it.*
- Bihari S, Prakash S, Bersten AD. Post Resuscitation Fluid Boluses In Severe Sepsis Or Septic Shock: Prevalence And Efficacy (PRICE Study). ajrccm-conference.2013.187.1_MeetingAbstracts.A3952.
- Bihari S, Prakash S, Bersten AD. Post resuscitation fluid boluses in patients with severe sepsis and septic shock (PRICE study). Shock 2013; 40: 28-34. 10 citations. *Study had an editorial with it.*
- Bihari S, Peake S, Bailey M, Pilcher D, Prakash S, Bersten AD High Admission Serum Sodium Does Not Affect ICU Mortality In Patients With Acute Respiratory Diseases. ajrccm conference. 2013.187.1_Meeting Abstracts.A1591.
- Bihari S, Peake SL, Seppelt IM, Williams P, Bersten AD. Sodium administration in critically ill patients in Australia and New Zealand: a multi-centre point prevalence study Crit Care Resusc 2013; 15: 294-300. *Study had an editorial with it.*
- Bihari S, Taylor S, and Bersten AD. Inadvertent sodium loading with renal replacement therapy in critically ill patients. J Nephrol. 2014 Feb 4. [Epub ahead of print]
- Bihari S, Festa M, Peake SL, Seppelt IM, Williams P, Wilkins B and Bersten AD. Sodium administration in critically ill paediatric patients in Australia and New Zealand: a multi-centre point prevalence study. Crit Care Resusc 2014; 16: 112-118.
- Bihari S, Peake S, Bailey M, Pilcher D, Prakash S, Bersten AD. Admission high serum sodium is not associated with increased ICU mortality risk in respiratory patients. J Crit Care. 2014 [Epub ahead of print].
- Bihari S, Dixon D and Bersten AD. Ruthenium Red Inhibits Intravenous Fluid Induced Permeability Lung Oedema Suggesting A TRPV4 Mechanism. ajrccm-conference. Meeting Abstracts. 2014 .ID A3275.
- Bihari S, Wiersema U, Schembri D, Depasquale C, Bowden J, Dixon D and Bersten AD. Interstitial Pulmonary Edema Despite Normal Echocardiographic Indices In Healthy Volunteers Following Bolus I.V. 0.9% Saline. ajrccm-conference Meeting Abstracts. 2014 .ID A3276.
- Bihari S, Dixon D and Bersten AD. Hypernatremia Reduces Acute Lung Injury, Independent Of Fluid Or Sodium Load. ajrccm-conference Meeting Abstracts. 2014 .ID A5001.
- Bihari S, Prakash S, Bersten AD. Low-dose vasopressin in addition to noradrenaline may lead to faster resolution of organ failure in patients with severe sepsis/septic shock accepted in Anaesth Intensive Care 2014; 42:
- Dixon D, Depasquale C, Bihari S and Bersten AD. Lung Mediators Of Leukocyte Infiltration And Activation In Chronic Heart Failure Patients, ajrccm-conference. Meeting Abstracts. 2014.ID A5853.

- Bihari S, Prakash S, Bersten AD. Early changes in serum electrolytes and acid-base status with administration of 4% albumin. Intensive Care Med. 2014; 40:1392-3.
- Bihari S, Peake S, Prakash S, Saxena M, Campbell V, Bersten AD. Sodium balance, not fluid balance, is associated with respiratory dysfunction in mechanically ventilated patients: a prospective, multi-centre study. manuscript accepted in Crit Care Resusc 2015.
- Bihari S, Dixon D, Lawrence M and Bersten AD. Induced hypernatremia reduces acute lung injury, independent of fluid or sodium load. Manuscript under review.
- Bihari S, Wiersema U, Schembri D, Depasquale C, Dixon D, Lawrence M, Bowden J, Dixon D and Bersten AD. Interstitial pulmonary edema despite normal echocardiographic indices in healthy volunteers following 0.9% Saline bolus when compared with 4% albumin and 5% glucose. Manuscript under review.
- Bihari S, Dixon D, Lawrence M and Bersten AD. Ruthenium red inhibits intravenous fluid induced permeability lung oedema suggesting a TRPV4 mechanism. Manuscript under review.

Book Chapter

- Bihari S and Bersten AD. Sodium loading in critical care - textbook "Diet and Nutrition in Critical Care" under "Specific nutrients" published by Springer 2013

Presentations (selected)

- 2014 National ANZICS Annual scientific meeting
- 2014 International American Thoracic society- San Diego USA
- 2014 National TSANZSRS Annual Scientific Meeting
- 2014 National ANZICS clinical trial group (CTG) annual meeting
- 2013 National Australian Society of medical research
- 2013 International American Thoracic society- Philadelphia USA
- 2012 International American Thoracic society – San Francisco USA
- 2012 National Australian Society of medical research
- 2012 National ANZICS Annual scientific meeting
- 2012 National ANZICS clinical trial group (CTG) annual meeting

References

Abotts, Nutritional Information Nepro, 2011, 'Nephro with Carb Steady-Therapeutic Nutrition for People on Dialysis' [Online] http,//nepro.com. (accessed October 2012).

ACCP/SCCM Consensus Conference Committee, American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference, 1992, 'Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis' Crit Care Med, 20, 864-874.

Acquarone N, Garibotto G, Pontremoli R, et al. 1989, 'Hypernatremia associated with severe rhabdomyolysis' Nephron, 51,441-442.

Addleman M, Logan AS, Grossman RF. 1985, 'Monitoring intrapulmonary hemorrhage in Goodpasture's syndrome' Chest, 87,119-20.

Adrogué HJ, Madias NE. 2000, 'Hypernatremia' N Engl J Med, 342,1493-1499.

Agrawal A, Matthay MA, Kangelaris KN, et al. 2013, 'Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients' Am J Respir Crit Care Med, 187,736-42.

Agricola E, Bove T, Oppizzi M, et al. 2005, '"Ultrasound comet-tail images", a marker of pulmonary edema, a comparative study with wedge pressure and extravascular lung water' Chest, 127,1690-5.

Albert RK. 2012, 'The role of ventilation-induced surfactant dysfunction and atelectasis in causing acute respiratory distress syndrome' Am J Respir Crit Care Med, 185,702-8.

Alphonsus CS, Rodseth RN. 2014, 'The endothelial glycocalyx, a review of the vascular barrier' Anaesthesia, 69, 777-84.

Alsous F, Khamiees M, DeGirolamo A, et al. 2000, 'Negative fluid balance predicts survival in patients with septic shock, a retrospective pilot study' Chest, 117, 1749-1754.

Alvarez DF, King A, Weber D, et al, 2006,'Transient receptor potential vanilloid 4-mediated disruption of alveolar septal barrier, novel mechanism of acute lung injury' Circ Res,99,988-95.

Andrivet P, Adnot S, Brun-Buisson C, et al. 1988, 'Involvement of ANF in the acute antidiuresis during PEEP ventilation' J Appl Physiol,65,1967–1974.

Andrzejewski C Jr, Casey MA, Popovsky MA. 2013, 'How we view and approach transfusion-associated circulatory overload, pathogenesis, diagnosis, management, mitigation, and prevention' Transfusion, 53,3037-47.

Angle N, Hoyt DB, Coimbra R, et al. 1998, 'Hypertonic saline resuscitation diminishes lung injury by suppressing neutrophil activation after hemorrhagic shock' Shock, 1998, 9,164-70.

Angus DC, Linde-Zwirble WT, Lidicker J, et al. 2001, 'Epidemiology of severe sepsis in the United States, analysis of incidence, outcome, and associated costs of care' Crit Care Med, 29,1303–1310.

Arikan AA, Zappitelli M, Goldstein SL, et al. 2012, 'Fluid overload is associated with impaired oxygenation and morbidity in critically ill children' Pediatr Crit Care Med, 2012,13,253-8.

Arroliga AC, Ghamra ZW, Perez Trepichio A, et al. 2002, 'Incidence of ARDS in an adult population of northeast Ohio' Chest,121,1972–1976.

Asadollahi K, Beeching N, Gill G. 2006, 'Hyponatraemia as a risk factor for hospital mortality' QJM, 99, 877–80.

Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. 1967, 'Acute respiratory distress in adults' Lancet, 1967,2,319–323.

Australian Government National Health and Medical Research Council, New Zealand Ministry of Health. Sodium. 2014, 'Nutrient reference values for Australia and New Zealand including recommended dietary intakes' http,//www.nrv.gov.au/nutrients/sodium.htm (accessed Apr 2014).

Awad S, Allison SP, Lobo DN 2008, 'The history of 0.9% saline' Clin Nutr,27,179–188.

Axler O, Tousignant C, Thompson CR, et al. 1997, 'Small hemodynamic effect of typical rapid volume infusions in critically ill patients' Crit Care Med, 25, 965-970.

Baghurst PA, Norton L, Slater A 2008, 'ANZICS Paediatric Study Group. The application of risk-adjusted control charts using the Paediatric Index of Mortality 2 for monitoring paediatric intensive care performance in Australia and New Zealand' Intensive Care Med,34,1281-8.

Balakrishna S, Song W, Achanta S, et al. 2014, 'TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury' Am J Physiol Lung Cell Mol Physiol, 15, 307, L158-72.

Balasubramanian K, Kumar P, Saini SS, et al. 2012, 'Isotonic versus hypotonic fluid supplementation in term neonates with severe hyperbilirubinemia – a double blind, randomized controlled trial' Acta Paediatr,101, 236–41.

Baldi G, Gargani L, Abramo A, et al. 2013, 'Lung water assessment by lung ultrasonography in intensive care, a pilot study' Intensive Care Med, 39, 74-84.

Baldwin CE, Paratz JD, Bersten AD. 2012, 'Body composition analysis in critically ill survivors, a comparison of bioelectrical impedance spectroscopy devices' J Parenter Enteral Nutr, 35, 306-15.

Barbier C, Loubières Y, Schmit C, et al. 2004, 'Respiratory changes in inferior vena cava diameter are helpful in predicting fluid responsiveness in ventilated septic patients' Intensive Care Med, 30, 1740-6.

Bartlett, G.R., 1959, 'Phosphorus assay in column chromatography' J. Biol. Chem, 234,466-468.

Bates JH, Daróczy B, Hantos Z. 1992, 'A comparison of interrupter and forced oscillation measurements of respiratory resistance in the dog' J Appl Physiol (1985), 72, 46-52.

Becker D, Bereiter-Hahn J, Jendrach M. 2009, 'Functional interaction of the cation channel transient receptor potential vanilloid 4 (TRPV4) and actin in volume regulation' European Journal of Cell Biology, 88, 141–152.

Bellomo R. 2014, 'Issue and challenges of fluid removal in the critically ill' Br J Anaesth, epub May 31.

Berger G, Guetta J, Klorin G, et al. 2011, 'Sepsis impairs alveolar epithelial function by downregulating Na-K-ATPase pump' Am J Physiol Lung Cell Mol Physiol, 301,L23-30.

Bersten AD, Edibam C, Hunt T, Moran J. 2002, 'Incidence and mortality of acute lung injury and the acute respiratory distress syndrome in three Australian states' Am J Respir Crit Care Med, 165, 443–448.

Bersten AD. 2006, 'Fluid management in the ventilated patient' Tobin MJ, editor, 'Principles and Practice of Mechanical Ventilation' McGraw-Hill Professional Publishing, 1323-1333.

Bertone JJ, Gossett KA, Shoemaker KE, et al. 1990, 'Effect of hypertonic vs isotonic saline solution on responses to sublethal Escherichia coli endotoxemia in horses' Am J Vet Med, 51, 999.

Bligh EG, Dyer WJ 1959, 'A rapid method of total lipid extraction and purification' Can J Biochem Physiol, 37,911-917.

Boemke W, Krebs M, Djalali K, et al. 1998, 'Renal nerves are not involved in sodium and water retention during mechanical ventilation in dogs' Anesthesiology, 89, 942–953.

Bouchard J, Soroko SB, Chertow GM, et al 2009, 'Fluid accumulation, survival and recovery of kidney function in critically ill patients with acute kidney injury' Kidney Int, 76,422–427.

Boyd JH, Forbes J, Nakada TA, et al. 2011, 'Fluid resuscitation in septic shock, a positive fluid balance and elevated central venous pressure are associated with increased mortality' Crit Care Med, 39, 259-65.

Brater DC. 1994, Pharmacokinetics of loop diuretics in congestive heart failure, Br Heart J, 72,S40-3.

Bratusch-Marrain PR, DeFronzo RA. 1983, 'Impairment of insulin-mediated glucose metabolism by hyperosmolality in man' Diabetes' 32,1028-1034.

Bruegger D, Jacob M, Rehm M, et al. 2005, 'Atrial natriuretic peptide induces shedding of endothelial glycocalyx in coronary vascular bed of guinea pig hearts' Am J Physiol Heart Circ Physiol, 289, H1993-9.

Brunkhorst FM, Engel C, Bloos F, et al. 2008, 'Intensive insulin therapy and pentastarch resuscitation in severe sepsis' N Engl J Med, 358, 125–39.

Bulger EM, Jurkovich GJ, Nathens AB, et al. 2008, 'Hypertonic resuscitation of hypovolemic shock after blunt trauma, a randomized controlled trial' Arch Surg, 143,139-48.

Burridge N. 2008, 'Australian Injectable Drugs Handbook' Collingwood, The Society of Hospital Pharmacists of Australia.

Caironi P, Tognoni G, Masson S, et al. 2014, 'Albumin replacement in patients with severe sepsis or septic shock' N Engl J Med, 370,1412-21.

Calfee CS, Gallagher D, Abbott J, et al. 2012, 'Plasma angiopoietin-2 in clinical acute lung injury, prognostic and pathogenetic significance' Crit Care Med, 40,1731-7.

Calvin JE, Driedger AA, Sibbald WJ. 1981, 'The hemodynamic effect of rapid fluid infusion in critically ill patients' Surgery, 90,61-75,.

Chen L, Liu C, & Liu L. 2009, 'Osmolality-induced tuning of action potentials in trigeminal ganglion neurons' Neuroscience Letters, 452, 79–83.

Chen L, Liu C, Liu L, Cao X. 2009, 'Changes in osmolality modulate voltage-gated sodium channels in trigeminal ganglion neurons' Neuroscience Research' 64, 199–207.

Choi PT, Yip G, Quinonez LG, Cook DJ. 1999, 'Crystalloids vs. colloids in fluid resuscitation, a systematic review' Crit Care Med, 27 ,200-10.

Choong K, Arora S, Cheng J et al. 2011, 'Hypotonic versus isotonic maintenance fluids after surgery for children, a randomized controlled trial' Pediatrics, 128, 857-66.

Chowdhury AH, Cox EF, Francis ST, Lobo DN. 2012, 'A Randomized, Controlled, Double-Blind Crossover Study on the Effects of 2-L Infusions of 0.9% Saline and Plasma-Lyte 148 on Renal Blood Flow Velocity and Renal Cortical Tissue Perfusion in Healthy Volunteers' Ann Surg, 256, 18-24.

Corcoran T, Rhodes JE, Clarke S, et al. 2012, 'Perioperative fluid management strategies in major surgery, a stratified meta-analysis' Anesth Analg, 114,640-51

Coulthard MG, Long DA, Ullman AJ, et al 2012, 'A randomised controlled trial of Hartmann's solution versus half normal saline in postoperative paediatric spinal instrumentation and craniotomy patients' Arch Dis Child, 97, 491-6.

D'Orio V, Mendes P, Carlier P, et al, 1991, 'Lung fluid dynamics and supply dependency of oxygen uptake during experimental endotoxic shock and volume resuscitation' Crit Care Med, 19,955–962.

D'Orio V, Wahlen C, Rodriguez LM, et al, 1987, 'Effects of intravascular volume expansion on lung fluid balance in a canine model of septic shock' Crit Care Med, 15, 863–868.

Darmon M, Diconne E, Souweine B, et al. 2013, 'Prognostic consequences of borderline dysnatremia, pay attention to minimal serum sodium change' Critical Care, 17, R12.

Davidson KG, Bersten AD, Barr HA, et al. 2002, 'Endotoxin induces respiratory failure and increases surfactant turnover and respiration independent of alveolocapillary injury in rats' Am J Respir Crit Care Med, 165, 1516-25.

Davidson KG, Bersten AD, Barr HA, et al. 2000, 'Lung function, permeability, and surfactant composition in oleic acid-induced acute lung injury in rats' Am J Physiol Lung Cell Mol Physiol, 279, L1091-102.

de Paula FM, Peixoto AJ, Pinto LV, et al. 2004, 'Clinical consequences of an individualized dialysate sodium prescription in hemodialysis patients' Kidney Int 2004, 66,1232–1238.

Deitch EA, Shi HP, Feketeova E, et al. 2003, 'Hypertonic saline resuscitation limits neutrophil activation after trauma-hemorrhagic shock' Shock, 19,328-33.

Delaney A, Peake SL, Bellomo R, et al. 2013, 'The Australasian Resuscitation in Sepsis Evaluation (ARISE) trial statistical analysis plan' Crit Care Resusc, 15,162-71.

Delaney AP, Dan A, McCaffrey J, Finfer S. 2011, 'The role of albumin as a resuscitation fluid for patients with sepsis, a systematic review and meta-analysis' Crit Care Med 39,386-91.

Dixon D-L, Barr H, Bersten AD, Doyle IR. 2008, 'Intracellular storage of surfactant and proinflammatory cytokines in co-cultured alveolar epithelium and macrophages in response to increasing CO2 and cyclic cell stretch' Exp Lung Res, 34, 37-47.

Dixon DL, De Pasquale CG, De Smet HR, et al. 2009, 'Reduced Surface Tension Normalizes Static Lung Mechanics in a Rodent Chronic Heart Failure Model' Am J Respir Crit Care Med, 180,181-7.

Dixon DL, De Smet HR, Bersten AD. 2009, 'Lung mechanics are both dose and tidal volume dependant in LPS-induced lung injury' Respir Physiol Neurobiol, 167, 333-40.

Dorresteijn MJ, van Eijk LT, Netea MG, et al. 2005, 'Iso-osmolar prehydration shifts the cytokine response towards a more anti-inflammatory balance in human endotoxemia' J Endotoxin Res, 11, 287–293.

dos Santos CC, Shan Y, Akram A, et al. 2011, 'Neuroimmune regulation of ventilator-induced lung injury' Am J Respir Crit Care Med, 183,471-82.

Doyle JT, Wilson JS, Estes EH, et al. 1951, 'The effect of intravenous infusions of physiologic saline solution on the pulmonary arterial and pulmonary capillary pressure in man'. J. Clin. Invest. 30, 345–352.

Druml W, Kleinberger G, Lenz K, et al. 1986, 'Fructose-induced hyperlactemia in hyperosmolar syndromes' KlinWochenschr, 64 ,615-618.

Drummer C1, Gerzer R, Heer M, et al. 1992, 'Effects of an acute saline infusion on fluid and electrolyte metabolism in humans' Am J Physiol, 262, F744-54.

Durairaj L, Schmidt GA. 2008, 'Fluid therapy in resuscitated sepsis, less is more' Chest 133, 252–263.

Elder ASF, Bersten AD, Saccone GTP, Dixon DL. 2013, 'Tripeptide feG prevents and ameliorates acute pancreatitis-associated acute lung injury in a rodent model' Chest, 143,371-8.

Farney RJ, Morris AH, Gardner RM, and Armstrong JD Jr. 1977, 'Rebreathing pulmonary capillary and tissue volume in normals after saline infusion' J Appl Physiol, 43, 246–253.

Feissel M, Michard F, Faller JP, Teboul JL 2004, 'The respiratory variation in inferior vena cava diameter as a guide to fluid therapy' Intensive Care Med, 30,1834-7.

Fiedler U, Reiss Y, Scharpfenecker M, et al. 2006, 'Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation' Nat Med, 12, 235-9.

Fiedler U, Scharpfenecker M, Koidl S, et al. 2004, 'The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies' Blood, 103,4150-6.

Finfer S, Bellomo R, Boyce N et al. 2004, 'A comparison of albumin and saline for fluid resuscitation in the intensive care unit' N Engl J Med, 350,2247-56.

Finn PJ, Plank LD, Clark MA, et al. 1996, 'Progressive cellular dehydration and proteolysis in critically ill patients' Lancet, 347, 654-6.

Firstenberg MS, Levine BD, Garcia MJ, et al. 2000, 'Relationship of echocardiographic indices to pulmonary capillary wedge pressures in healthy volunteers' J Am Coll Cardiol, 36,1664-9.

Foland JA, Fortenberry JD, Warshaw BL, et al. 2004, 'Fluid overload before continuous hemofiltration and survival in critically ill children, a retrospective analysis' Crit Care Med, 32,1771–1776.

Forman S, Crofton P, Huang H et al. 2012, 'The epidemiology of hypernatraemia in hospitalised children in Lothian, a 10-year study showing differences between dehydration, osmoregulatory dysfunction and salt poisoning' Arch Dis Child, 97, 502-7.

Forrester JS, Diamond G, Chatterjee K et al 1976, 'Medical therapy of acute myocardial infarction by application of hemodynamic subsets', N Engl J Med, 295,1356-62.

Frazier SK. 1999, 'Neurohormonal responses during positive pressure mechanical ventilation' heart & lung, 28, 149-165.

Funk GC, Lindner G, Druml W, et al. 2010, 'Incidence and prognosis of dysnatremias present on ICU admission' Intensive Care Med, 36, 304-11.

Gamrin L, Essen P, Forsberg AM, et al. 1996, 'A descriptive study of skeletal muscle metabolism in critically ill patients, free amino acids, energy-rich phosphates, protein, nucleic acids, fat, water, and electrolytes' Crit Care Med, 24, 575-83.

Gao J, Zhou L, Ge Y, et al. 2013, 'Effects of different resuscitation fluids on pulmonary expression of aquaporin1 and aquaporin5 in a rat model of uncontrolled hemorrhagic shock and infection' PLoS One, 8, e64390.

Garcia-Elias A, Lorenzo IM, Vicente R, et.al. 2008, 'IP3 receptor binds to and sensitizes TRPV4 channel to osmotic stimuli via a calmodulin-binding site' Journal of Biological Chemistry, 283, 31284–31288.

Gelens M, Luik AJ, Kleffens M, et al. 2002, 'A dialysate sodium concentration of 140 mmol/l may lead to net diffusive sodium gain' Blood Purif, 20,190.

George C, Bellomo R. 2007, 'Changes in the incidence and outcome for early acute kidney injury in a cohort of Australian intensive care units' Crit Care, 11,R68.

Goettsch W, Gryczka C, Korff T, et al. 2008, 'Flow-dependent regulation of angiopoietin-2' J Cell Physiol, 214, 491-503.

Goldsmith SR, 2006, 'The role of vasopressin in congestive heart failure' Cleve Clin J Med 2006, 3, S19–S23.

Goldstein SL, Somers MJ, Baum MA, et al. 2005, 'Pediatric patients with multi-organ dysfunction syndrome receiving continuous renal replacement therapy' Kidney Int, 67, 653–658.

Goldstein SL, Somers MJ, Brophy PD, et al. 2004, 'The Prospective Pediatric Continuous Renal Replacement Therapy (ppCRRT) Registry, design, development and data assessed' Int J Artif Organs, 27,9–14.

Gray DS, Crider JB, Kelley C, Dickinson LC. 1985, 'Accuracy of recumbent height measurement' J Parenter Enteral Nutr, 9, 712-5.

Greenfield LJ, Ebert PA, Benson DW. 1964, 'Effect of positive pressure ventilation on surface tension proterties of lung extracts' Anesthesiology, 25,312–316.

Hahn RG. 2010, 'Volume kinetics for infusion fluids' Anesthesiology, 113, 470-81.

Hall WH, Ramachandran R, Narayan S, et al. 2004, 'An electronic application for rapidly calculating Charlson comorbidity score' BMC Cancer, 4, 94.

Halperin BD, Feeley TW, Chiles C, et al. 1985, 'Evaluation of the portable chest roentgenogram for quantitating extravascular lung water in critically ill adults' Chest, 88, 649-52.

Hamanaka K, Jian MY, Weber DS, et al. 2007, 'TRPV4 initiates the acute calcium-dependent permeability increase during ventilator-induced lung injury in isolated mouse lungs' Am J Physiol Lung Cell Mol Physiol, 293, L923–L932.

Hauser CJ, Shoemaker WC, Turpin I, Goldberg SJ. 1977, 'Oxygen transport response to colloids and crystalloids in critically ill surgical patients' Surg Gynecol Obstet, 150, 811-816.

Henderson AC, Sá RC, Barash IA, et al. 2012, 'Rapid intravenous infusion of 20 mL/kg saline alters the distribution of perfusion in healthy supine humans' Respir Physiol Neurobiol, 180,331-41.

Herasevich V, Pickering BW, Dong Y, et al. 2010, 'Informatics infrastructure for syndrome surveillance, decision support, reporting, and modeling of critical illness' Mayo Clin Proc, 85, 247-54.

Hogg JC, Agarawal JB, Gardiner AJS, et al. 1972, 'Distribution of airways resistance with developing pulmonary oedema in dogs' J Appl Physiol, 32,20 –24.

Holcroft JW, Vassar MJ, Turner J E, et al. 1987, '3% NaCl and 7.5% NaCl/dextran 70 in the resuscitation of severely injured patients' Ann Surg, 206, 279-88.

Hollenberg SM, Dumasius A, Easington C, et al. 2001, 'Characterization of a hyperdynamic murine model of resuscitated sepsis using echocardiography' Am J Respir Crit Care Med, 164,891–895.

Holliday MA, Segar WE, Friedman A. 2003, 'Reducing errors in fluid therapy' Pediatrics, 111, 424–5.

Holmes CL, Patel BM, Russell JA, et al. 2001, 'Physiology of vasopressin relevant to management of septic shock' Chest, 120, 989–1002.

Hoorn EJ, Betjes MG, Weigel J, et al. 2008, 'Hypernatremia in critically ill patients, too little water and too much salt' Nephrol Dial Transplant, 2008, 23, 1562-8.

Horton JW, and Walker PB. 1991, 'Small-volume hypertonic saline dextran resuscitation from canine endotoxin shock' Ann. Surg, 214, 64.

Hoste EA, Clermont G, Kersten A, et al, 2006, 'RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients, a cohort analysis. Crit Care, 10, R73.

Humphrey H, Hall J, Sznajder I, et al. 1990, 'Improved survival in ARDS patients associated with a reduction in pulmonary capillary wedge pressure' Chest, 97,1176-1180.

Ichimura H, Parthasarathi K, Quadri S, et al. 2003, 'Mechano-oxidative coupling by mitochondria induces proinflammatory responses in lung venular capillaries' J Clin Invest, 111, 691-9.

Ingraham AM, Cohen ME, Raval MV, et al. 2011, 'Comparison of hospital performance in emergency versus elective general surgery operations at 198 hospitals' J Am Coll Surg, 212, 20-28.

Intersalt. 1988, 'An international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion' Intersalt Cooperative Research Group, BMJ, 297, 319–328.

Iselin H, Tsinalis D, Brunner FP, 2001, 'Sodium balance-neutral sodium profiling does not improve dialysis tolerance' Swiss Med Wkly, 131,635-9.

Iwasaki Y, Gaskill MB, Robertson GL. 1995, 'Adaptive resetting of the volume control of vasopressin secretion during sustained hypovolemia' Am J Physiol, 268, R349–R357.

Jacob M, Chappell D, Rehm M. 2009, 'The 'third space' – fact or fiction?' Best Pract Res Clin Anaesthesiol, 23, 145–157.

Jacob M, Paul O, Mehringer L, et al. 2009, 'Albumin augmentation improves condition of guinea pig hearts after 4 hr of cold ischemia' Transplantation, 87,956-65.

Jacob M, Saller T, Chappell D, et al. 2013, Physiological levels of A-, B- and C-type natriuretic peptide shed the endothelial glycocalyx and enhance vascular permeability, Basic Res Cardiol, 108, 347.

Jambrik Z, Monti S, Coppola V, et al. 2004, 'Usefulness of ultrasound lung comets as a nonradiologic sign of extravascular lung water' Am J Cardiol, 93, 1265-70.

James MF, Michell WL, Joubert IA et al. 2011, 'Resuscitation with hydroxyethyl starch improves renal function and lactate clearance in penetrating trauma in a randomized controlled study, the FIRST trial (Fluids in Resuscitation of Severe Trauma)' Br J Anaesth, 107, 693–702.

Jeon K, Shin TG, Sim MS, et al. 2012, 'Improvements in compliance with resuscitation bundles and achievement of end points after an educational program on the management of severe sepsis and septic shock' Shock, 37,463-7.

Jian Y, King A, Al-Mehdi AB, et al. 2008, 'High vascular pressure-induced lung injury requires P450 epoxygenase-dependent activation of TRPV4' Am J Resp Cell Mol Biol, 38, 386-92.

Junger WG, Rhind SG, Rizoli SB, et al. 2012, 'Resuscitation of Traumatic Hemorrhagic Shock Patients With Hypertonic Saline-Without Dextran-Inhibits Neutrophil and Endothelial Cell Activation, Shock, 38, 341-350.

Jungmann E, Schifferdecker E, Rümelin A, et al. 1987, 'Plasma renin activity and aldosterone behavior in critically ill patients' Klin Wochenschr, 65, 87-91.

Jurek SC, Hirano-Kobayashi M, Chiang H, et al. 2014, 'Prevention of Ventilator-Induced Lung Edema by Inhalation of Nanoparticles Releasing Ruthenium Red' Am J Resp Cell Mol Biol, (epub).

Kaczmarczyk G, Rossaint R, Altmann C, et al. 1992, 'ACE inhibition facilitates sodium and water excretion during PEEP in conscious volume expanded dogs' J Appl Physiol, 73, 962–967.

Kaestle SM, Reich CA, Yin N, et al 2007, 'Nitric oxide-dependent inhibition of alveolar fluid clearance in hydrostatic lung edema' Am J Physiol Lung Cell Mol Physiol, 293,L859-69.

Kaufmann MA1, Castelli I, Pargger H, Drop LJ. 1995, 'Nitric oxide dose-response study in the isolated perfused rat kidney after inhibition of endothelium-derived relaxing factor synthesis, the role of serum albumin' J Pharmacol Exp Ther, 273,855-62.

Keen ML, Gotch FA, 2007, 'The association of the sodium "setpoint" to interdialytic weight gain and blood pressure in hemodialysis patients' Int J Artif Organs, 30,971–979.

Kendrick KR1, Baxi SC, Smith RM. 2000, 'Usefulness of the modified 0-10 Borg scale in assessing the degree of dyspnea in patients with COPD and asthma' J Emerg Nurs, 26,216-22.

Kennedy MT, Higgins BD, Costello JF, et al. 2008, 'Hypertonic saline reduces inflammation and enhances the resolution of oleic acid induced acute lung injury' BMC Pulm Med, 8,9.

Kim JY, Choi SH, Yoon YH, et.al. 2013, 'Effects of hypertonic saline on macrophage migration inhibitory factor in traumatic conditions' Exp Ther Med, 5, 362-366.

King LS, Nielsen S, Agre P, Brown RH. 2002, 'Decreased pulmonary vascular permeability in aquaporin-1-null humans' Proc Natl Acad Sci U S A, 99,1059-63.

Kopterides P, Bonovas S, Mavrou I, et al. 2009, 'Venous oxygen saturation and lactate gradient from superior vena cava to pulmonary artery in patients with septic shock' Shock, 31,561-7.

Kotchen TA, Cowley AW Jr, Frohlich ED. 2013, 'Salt in health and disease--a delicate balance' N Engl J Med, 368, 1229-37.

Kovalik SG, Ledgerwood AM, Lucas CE, Higgins RF. 1981, 'The cardiac effect of altered calcium homeostasis after albumin resuscitation' J Trauma, 21, 275-9.

Kozeny GA, Murdock DK, Euler DE, et al. 1985, 'In vivo effects of acute changes in osmolality and sodium concentration on myocardial contractility' Am Heart J, 109, 290-296.

Kreimeier U, Frey L, Dentz J, et al. 1991, 'Hypertonic saline dextran resuscitation during the initial phase of acute endotoxemia, Effect on regional blood flow' Crit. Care Med, 19, 801-11.

Kuebler WM, Ying X, Singh B, et al. 1999, 'Pressure is proinflammatory in lung venular capillaries' J Clin Invest,104,495-502.

Kumar A, Anel R, Bunnell E, et al. 2004, 'Pulmonary artery occlusion pressure and central venous pressure fail to predict ventricular filling volume, cardiac performance, or the response to volume infusion in normal subjects' Crit Care Med, 32,691–699.

Lambie SH, Taal MW, Fluck RJ, et al. 2005, 'Online conductivity monitoring, validation and usefulness in a clinical trial of reduced dialysate conductivity' ASAIO J, 51,70–76.

Lanspa MJ, Grissom CK, Hirshberg EL, et al. 2013, 'Applying dynamic parameters to predict hemodynamic response to volume expansion in spontaneously breathing patients with septic shock' Shock, 39,155-60.

Lee JW. 2010, 'Fluid and electrolyte disturbances in critically ill patients' Electrolyte Blood Press, 8, 72-81.

Lenz K, Gossinger H, Laggner A, et al. 1986, 'Influence of hypernatremic-hyperosmolar state on hemodynamics of patients with normal and depressed myocardial function' Crit Care Med, 14, 913-914.

Levinson R, Epstein M, Sackner MA, et al 1977, 'Comparison of the effects ofwater immersion and saline infusion on central haemodynamics in man' Clin. Sci. Mol. Med. 52, 343–350.

Liedtke W, & Friedman JM. 2003, 'Abnormal osmoticregulation in trpv4-/- mice' Proceedings of the National Academy of Sciences of the United States of America, 100, 13698–13703.

Linde-Zwirble WT, Angus DC. 2004, 'Severe sepsis epidemiology, sampling, selection, and society' Crit Care 2004, 8, 222–226.

Lindner G, Funk GC, Schwarz C et al. 2007, 'Hypernatremia in the critically ill is an independent risk factor for mortality' Am J Kidney Dis, 50, 952-957.

Lindner G, Kneidinger N, Holzinger U et al. 2009, 'Tonicity balance in patients with hypernatremia acquired in the intensive care unit' Am J Kidney Dis, 54, 674-679.

Lobo DN, Bostock KA, Neal KR, et al. 2002, 'Effect of salt and water balance on recovery of gastrointestinal function after elective colonic resection' Lancet, 359, 1812-8.

Lobo DN, Stanga Z, Aloysius MM, et al. 2010, 'Effect of volume loading with 1 liter intravenous infusions of 0.9% saline, 4% succinylated gelatine (Gelofusine) and 6% hydroxyethyl starch (Voluven) on blood volume and endocrine responses, a randomized, three-way crossover study in healthy volunteers' Crit Care Med, 38, 464–470.

Locatelli F, Di Filippo S, Manzoni C, et al. 2000, 'Removal of small and middle molecules by convective techniques, Nephrol Dial Transplant, 15,37–44.

Locatelli F, di Filippo S, Manzoni C, 1999, 'Sodium kinetics during dialysis' Semin Dial, 12, S41–S44.

Locatelli F, Ponti R, Pedrini L, et al, 1989, 'Sodium and dialysis, a deeper insight' Int J Artif Organs, 12, 71–74.

Locatelli F, Ponti R, Pedrini L, et al. 1984, 'Sodium kinetics across dialysis membranes' Nephron, 38, 174–177.

Londino JD, Matalon S 2013, 'Chloride secretion across adult alveolar epithelial cells contributes to cardiogenic edema', Proc Natl Acad Sci USA,110,10055-6.

Loomis WH, Namiki S, Hoyt DB, et.al. 2001, 'Hypertonicity rescues T cells from suppression by trauma-induced anti-inflammatory mediators' Am J Physiol Cell Physiol, 281, C840-8.

Lorenzo IM, Liedtke W, Sanderson MJ, et al, 2008, 'TRPV4 channel participates in receptor-operated calcium entry and ciliary beat frequency regulation in mouse airway epithelial cells' Proc Natl Acad Sci USA, 105,12611-6.

Lowenstein CJ, Morrell CN, Yamakuchi M. 2005, 'Regulation of Weibel-Palade body exocytosis' Trends Cardiovasc Med, 15,302-8.

Luhr OR, Antonsen K, Karlsson M, et al. 1999, 'Incidence and mortality after acute respiratory failure and acute respiratory distress syndrome in Sweden, Denmark, and Iceland' Am J Respir Crit Care Med, 159,1849–1861.

Macintyre N, Crapo RO, Viegi G, et al. 2005, 'Standardisation of the single-breath determination of carbon monoxide uptake in the lung' Eur Respir J, 26,720-35.

Magder S, Bafaqeeh F. 2007, 'The clinical role of central venous pressure measurements' J Intensive Care Med, 22, 44–51.

Maitland K, Kiguli S, Opoka RO, et al. 2011, 'Mortality after fluid bolus in African children with severe infection' N Engl J Med, 364,2483-95.

Maitland K, Pamba A, English M, et al. 2005, 'Randomized trial of volume expansion with albumin or saline in children with severe malaria, preliminary evidence of albumin benefit' Clin Infect Dis, 40, 538-45.

Maron MB. 1989, 'Effect of elevated vascular pressure transients on protein permeability in the lung' J Appl Physiol, 67, 305-10.

Matthews BD, Thodeti CK, Tytell JD, et al. 2010, 'Ultrarapid activation of trpv4 ion channels by mechanical forces applied to cell surface beta1 integrins' Integr Biol (Camb), 2, 435-442.

Mattox KL, Maningas PA, Moore EE, et al. 1991, 'Prehospital hypertonic saline/dextran infusion for posttraumatic hypotension. The U.S.A. Multicenter Trial' Ann Surg, 213, 482-491.

McGregor JC, Kim PW, Perencevich EN, et al. 2005, 'Utility of the Chronic Disease Score and Charlson Comorbidity Index as comorbidity measures for use in epidemiologic studies of antibiotic-resistant organisms' Am J Epidemiol, 161, 483-93.

Michael M, Kuehnle I, Goldstein SL, 2004, 'Fluid overload and acute renal failure in pediatric stem cell transplant patients' Pediatr Nephrol, 19, 91–95.

Michard F, Boussat S, Chemla D, et al. 2000, 'Relation between respiratory changes in arterial pulse pressure and fluid responsiveness in septic patients with acute circulatory failure' Am J Respir Crit Care Med, 162, 134–138.

Michel RP, Zocchi L, Rossi A. 1987, 'Does interstitial lung edema compress airways and arteries? A morphometric study' J Appl Physiol, 62, 108–115.

Mikkelsen ME, Christie JD, Lanken PN, et al. 2012, 'The adult respiratory distress syndrome cognitive outcomes study long-term neuropsychological function in survivors of acute lung injury' Am J Respir Crit Care Med, 185, 1307–1315.

Miller MR, Hankinson J, Brusasco V, et al. 2005, 'Standardisation of spirometry' Eur Respir J, 26,319-38.

Millic-Emili J, Ruff F. 1971, 'Effects of pulmonary congestion and edema on the small airways' Bull Physiopathol Respir Nancy, 7, 1181–1196.

Minnear FL, Barie PS, Malik AB. 1983, 'Effects of transient pulmonary hypertension on pulmonary vascular permeability' J Appl Physiol Respir Environ Exerc Physiol, 55,983-9.

Mitchell JP, Schuller D, Calandrino FS, et al. 1992 'Improved outcome based on fluid management in critically ill patients requiring pulmonary artery catheterization, Am Rev Respir Dis, 145,990–998.

Mizuno A, Matsumoto N, Imai M, Suzuki M. 2003, 'Impaired osmotic sensation in mice lacking TRPV4' American Journal of Physiology Cell Physiology, 285, C96–C101.

MochizukiT, Sokabe T, Araki I, et al 2009, 'The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures' J Biol Chem,284,21257–64.

Monnet X, Rienzo M, Osman D, et al. 2006, 'Passive leg raising predicts fluid responsiveness in the critically ill' Crit Care Med, 34,1402–1407.

Moore CL, Tham ET, Samuels KJ, et al. 2010, 'Tissue Doppler of early mitral filling correlates with simulated volume loss in healthy subjects' Acad Emerg Med, 17,1162-8.

Morelli A, Lange M, Ertmer C, et al. 2007, 'Glibenclamide dose response in patients with septic shock, effects on norepinephrine requirements, cardiopulmonary performance, and global oxygen transport' Shock, 28, 530-5.

Moret K, Hassell D, Kooman JP, et al. 2002, 'Ionic mass balance and blood Volume preservation during a high, standard, and individualized dialysate sodium concentration' Nephrol Dial Transplant, 17,1463–1469.

Moritz ML, Ayus JC. 2011, 'Intravenous fluid management for the acutely ill child' Curr Opin Pediatr, 23, 186-93.

Moss GS, das Gupta TK, Newson B, Nyhus LM. 1972, 'Morphologic changes in the primate lung after hemorrhagic shock' Surg Gynecol Obstet, 134, 3-9.

Moss GS, das Gupta TK, Newson B, Nyhus LM. 1973, 'The effect of saline solution resuscitation on pulmonary sodium and water distribution' Surg Gynecol Obstet, 136, 934-40.

Moss GS. 1972, 'Pulmonary involvement in hypovolemic shock' Annu Rev Med, 23, 201-28.

Muir, AL, Flenley DC, Kirby BJ, et al. 1975, 'Cardiorespiratory effects of rapid saline infusion in normal man' J. Appl. Physiol, 38, 786–793.

Mulligan MS, Paulson JC, De Frees S, et al. 1993, 'Protective effects of oligosaccharides in P-selectin-dependent lung injury' Nature, 364, 149-51.

Murao Y, Loomis W, Wolf P, Hoyt DB, Junger WG. 2003, 'Effect of dose of hypertonic saline on its potential to prevent lung tissue damage in a mouse model of hemorrhagic shock' Shock, 20,29-34.

Murphy CV, Schramm GE, Doherty JA, et al. 2009, 'The importance of fluid management in acute lung injury secondary to septic shock' Chest, 136,102-109.

Myburgh JA, Finfer S, Bellomo R, et al 2012, 'Hydroxyethyl starch or saline for fluid resuscitation in intensive care' N Engl J Med, 367,1901-1911.

Nadler SB, Hidalgo JU, Bloch T. 1962, 'Prediction of blood volume in normal human adults' Surgery, 51,224–232.

Nicolaysen G, Waaler BA, Aarseth P. 1979, 'On the existence of stretchable pores in the exchange vessels of the isolated rabbit lung preparation' Lymphology, 12, 201-7.

Noble VE, Murray AF, Capp R, et al. 'Ultrasound assessment for extravascular lung water in patients undergoing hemodialysis. Time course for resolution' Chest, 135, 1433-9.

Nutricia. 2011, 'Product Information. Nutricia Advanced Medical Nutrition' [Online] http,//uk.nutricia.com. (accessed October 2012).

Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes.

http,//www.nrv.gov.au/resources/_files/n35-sodium.pdf (accessed October 2013).

Ochi H, Masuda J, Gimbrone MA. 2002, 'Hyperosmotic stimuli inhibit VCAM-1 expression in cultured endothelial cells via effects on interferon regulatory factor-1 expression and activity' Eur J Immunol, 32, 1821–1831.

O'Grady NP, Preas HL, Pugin J et al, 2001, 'Local inflammatory responses following bronchial endotoxin instillation in humans' Am J Respir Crit Care Med. 163,1591-8.

Olaguíbel JM, Alvarez-Puebla MJ, Anda M, 2005, 'Comparative analysis of the bronchodilator response measured by impulse oscillometry (IOS), spirometry and body plethysmography in asthmatic children' J Investig Allergol Clin Immunol, 15,102-6.

Opas LM, Adler R, Robinson R, et al. 1977, 'Rhabdomyolysis with severe hypernatremia' J Pediatr, 90, 713-716.

Osman D, Ridel C, Ray P, et al. 2007, 'Cardiac filling pressures are not appropriate to predict hemodynamic response to volume challenge' Crit Care Med, 35,64–68.

Ospina-Tascon G, Neves AP, Occhipinti G, et al. 2010, 'Effects of fluids on microvascular perfusion in patients with severe sepsis' Intensive Care Med, 36, 949-55.

Palevsky PM, Bhagrath R and Greenberg A. 1996, 'Hypernatremia in hospitalized patients' Ann Intern Med, 124, 197–203.

Papazian L, Forel JM, Gacouin A, et al. 2010, 'Neuromuscular blockers in early acute respiratory distress syndrome' N Engl J Med, ,363,1107-16.

Parker JC, Hashizumi M, Kelly SV, et al. 2013, 'TRPV4 calcium entry and surface expression attenuated by inhibition of myosin light chain kinase in rat pulmonary microvascular endothelial cells'. Physiol Rep.1,e00121.

Parker JC, Ivey CL, Tucker JA.1985, 'Gadolinium prevents high airway pressure-induced permeability increases in isolated rat lungs' J Appl Physiol, 84,1113-8.

Pascual JL, Khwaja KA, Ferri LE, et al. 2003, 'Hypertonic saline resuscitation attenuates neutrophil lung sequestration and transmigration by diminishing leukocyte-endothelial interactions in a two-hit model of hemorrhagic shock and infection' J Trauma, 54,121–130.

Payen D, de Pont AC, Sakr Y, et al. 2008, 'A positive fluid balance is associated with a worse outcome in patients with acute renal failure' Crit Care. 12, R74.

Peake SL, Bailey M, Bellomo R, et al. 2009, 'Australasian resuscitation of sepsis evaluation (ARISE), a multi-centre, prospective, inception cohort study' Resuscitation, 80, 811 - 818.

Peixoto AJ, Gowda N, Parikh CR, et al, 2010, 'Long-term stability of serum sodium in hemodialysis patients' Blood Purif, 29, 264–267.

Pellegrino R, Dellaca R, Macklem PT, et al. 2003, 'Effects of rapid saline infusion on lung mechanics and airway responsiveness in humans' J Appl Physiol, 95, 728–734.

Perel P, Roberts I, Ker K. 2013, 'Colloids versus crystalloids for fluid resuscitation in critically ill patients' Cochrane Database Syst Rev, 2, CD000567.

Perner A, Haase N, Guttormsen AB, et al 2012, 'Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis' N Engl J Med, 367,124-134.

Pietra GG, Szidon JP, Leventhal MM, Fishman AP. 1969, 'Hemoglobin as a tracer in hemodynamic pulmonary edema' Science, 166,1643-6.

Plank LD, Connolly AB, Hill GL. 1998, 'Sequential changes in the metabolic response in severely septic patients during the first 23 days after the onset of peritonitis' Ann Surg, 228, 146-58.

Plank LD, Hill GL. 2000, 'Similarity of changes in body composition in intensive care patients following severe sepsis or major blunt injury' Ann N Y Acad Sci, 904, 592-602.

Plank LD, Monk DN, Woollard GA, Hill GL. 1998, 'Evaluation of multifrequency bioimpedance spectroscopy for measurement of the extracellular water space in critically ill patients' Appl Radiat Isot, 49, 481-3.

Prisk GK, Olfert IM, Arai TJ, et al. 2010, 'Rapid intravenous infusion of 20 ml/kg saline does not impair resting pulmonary gas exchange in the healthy human lung' J Appl Physiol, 108, 53-9.

Puri S, Dutka DP, Baker BL, et al. 1999, 'Acute saline infusion reduces alveolar-capillary membrane conductance and increases airflow obstruction in patients with left ventricular dysfunction' Circulation, 99, 1190-6.

Quadri SK, Bhattacharjee M, Parthasarathi K, et al. 2003, 'Endothelial barrier strengthening by activation of focal adhesion kinase' J Biol Chem, 278, 13342–13349.

Quigley R. 2012, 'Developmental changes in renal function' Curr Opin Pediatr, 24, 184-90.

Rabinovici R, Vernick J, Hillegas L, Neville LF. 1996, ' Hypertonic saline treatment of acid aspiration-induced lung injury' J Surg Res, 60, 176-80.

Rabinovici R, Krausz MM, and Feuerstein G. 1991, 'Control of bleeding is essential for a successful treatment of hemorrhagic shock with 7.5% NaCl solution' Surg Gynecol Obstet, 173, 98-106.

Rabinovici R, Yue T-L, Krausz MM, et al. 1992, 'Hemodynamic, hematologic and eicosanoid-mediated mechanisms in 7.5% NaCl treatment of uncontrolled hemorrhagic shock' Surg Gynecol Obstet, 175, 341-54.

Reid F, Lobo DN, Williams RN, et al. 2003, '(Ab)normal saline and physiological Hartmann's solution, a randomized double-blind crossover study' Clin Sci (Lond), 104, 17-24.

RENAL Replacement Therapy Study Investigators, Bellomo R, Cass A, Cole L, et al. 2009, 'Intensity of continuous renal-replacement therapy in critically ill patients' N Engl J Med, 361,1627-38.

Report of the Australian and New Zealand Paediatric Intensive Care Registry. http,//www.anzics.com.au/downloads/doc_download/567-2010-anzpic-registry-annual-report. (accessed Sep 2013).

Reuse C, Vincent JL, Pinsky MR. 1990, 'Measurements of right ventricular volumes during fluid challenge' Chest 98,1450–1454.

Rippe B, Townsley M, Thigpen J, et al. 1984, 'Effects of vascular pressure on the pulmonary microvasculature in isolated dog lungs' J Appl Physiol Respir Environ Exerc Physiol, 57, 233-9.

Rivers E, Nguyen B, Havstad S, et al 2001, 'Early goal-directed therapy in the treatment of severe sepsis and septic shock' N Engl J Med, 345,1368-1377.

Rizoli SB, Kapus A, Fan J, et al. 1998, 'Immunomodulatory effects of hypertonic resuscitation on the development of lung inflammation following hemorrhagic shock' J Immunol, 161, 6288–6296.

Rizoli SB, Kapus A, Parodo J, Rotstein OD, 1999, 'Hypertonicity prevents lipopolysaccharide-stimulated CD11b/CD18 expression in human neutrophils in vitro, role for p38 inhibition' The Journal of trauma, 46, 794-8.

Robertson HT, Pellegrino R, Pini D, et al. 2004, 'Exercise response after rapid intravenous infusion of saline in healthy humans' J Appl Physiol, 97, 697-703.

Romani T, Rondaij MG, Hordijk PL, et al. 2003, 'Real-time imaging of the dynamics and secretory behavior of Weibel-Palade bodies' Arterioscler Thromb Vasc Biol. 23,755–761.

Rosner MH, Ronco C. 2010, 'Dysnatremias in the intensive care unit' Contrib Nephrol, 165, 292-8.

Rossaint R, Krebs M, Forther J, et al. 1993, 'Inferior vena caval pressure increase contributes to sodium and water retention during PEEP in awake dogs' J Appl Physiol, 75, 2484–2492.

Rubenfeld GD, Caldwell E, Peabody E, et al. 2005, 'Incidence and outcomes of acute lung injury' N Engl J Med, 353, 1685–1693.

Rudski LG, Lai WW, Afilalo J, et al. 2010, 'Guidelines for the echocardiographic assessment of the right heart in adults, a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography' J Am Soc Echocardiogr, 23,685-713.

Safdar Z, Wang P, Ichimura H, et al. 2003, 'Hyperosmolarity enhances the lung capillary barrier' J Clin Invest, 112,1541-9.

Safdar Z, Yiming M, Grunig G, Bhattacharya J. 2005, 'Inhibition of acid-induced lung injury by hyperosmolar sucrose in rats' Am J Respir Crit Care Med, 172, 1002-7.

SAFE Study Investigators, Finfer S, McEvoy S, Bellomo R, et al. 2011, 'Impact of albumin compared to saline on organ function and mortality of patients with severe sepsis' Intensive Care Med, 37, 86-96.

Sakamaki F, Ishizaka A, Handa M, et al. 1995, 'Soluble form of P-selectin in plasma is elevated in acute lung injury' Am J Respir Crit Care Med, 151,1821-6.

Sakr Y, Vincent J-L, Reinhardt K, et al. 2005, 'High tidal volume and positive fluid balance are associated with worse outcome in acute lung injury' Chest, 128,3098–3108.

Sakr Y, Vincent J-L, Reinhardt K, et al. 2005, 'High tidal volume and positive fluid balance are associated with worse outcome in acute lung injury' Chest, 128,3098–3108.

Sánchez M, Jiménez-Lendínez M, Cidoncha M, et al. 2011, 'Comparison of fluid compartments and fluid responsiveness in septic and non-septic patients' Anaesth Intensive Care, 39,1022-1029.

Santos SF, Peixoto AJ, 2010, 'Sodium balance in maintenance hemodialysis' Semin Dial, 23,549-55.

Saxena MK. 2013, 'Should sodium be the real target of fluid restriction?' Critical Care and Resuscitation, 15, 75-6.

Sayarlioglu H, Erkoc R, Tuncer M, et al. 2007' 'Effects of low sodium dialysate in chronic hemodialysis patients, an echocardiographic study' Ren Fail, 29, 143–146.

Schneeberger-Keeley EE, Karnovsky MJ. 1968, 'The ultrastructural basis of alveolar-capillary membrane permeability to peroxidase used as a tracer' J Cell Biol, 37,781-93.

Sedlacek M, Schoolwerth AC, Remillard BD. 2006, 'Electrolyte disturbances in the intensive care unit' Semin Dial, 19, 496-501.

Seri I, Kone BC, Gullans SR, et al. 1990, 'Influence of Na + intake on dopamine- induced inhibition of renal cortical Na(+)-K(+)-ATPase' Am J Physiol, 258, F52–F60.

Seri I, Kone BC, Gullans SR, et al. 1988, 'Locally formed dopamine inhibits Na + -K + -ATPase activity in rat renal cortical tubule cells' Am J Physiol, 255, F666–F673.

Sevransky J. 2009, 'Clinical assessment of hemodynamically unstable patients' Curr Opin Crit Care, 15, 234-8.

Shi HP, Deitch EA, Da Xu Z, et al. 2002, 'Hypertonic saline improves intestinal mucosa barrier function and lung injury after trauma hemorrhagic shock' Shock 17, 496-501.

Shields CJ, O'Sullivan AW, Wang JH, et al. 2003, 'Hypertonic saline enhances host response to bacterial challenge by augmenting receptor-independent neutrophil intracellular superoxide formation' Ann Surg, 238,249-257.

Shields CJ, Winter DC, Manning BJ, et al. 2003, 'Hypertonic saline infusion for pulmonary injury due to ischemia-reperfusion' Arch Surg, 138, 9-14.

Shields CJ, Winter DC, Sookhai S, et al. 2000, 'Hypertonic saline attenuates end-organ damage in an experimental model of acute pancreatitis' Br J Surg, 87,1336-1340.

Sidhaye VK, Schweitzer KS, Caterina MJ, et al, 2008, 'Shear stress regulates aquaporin-5 and airway epithelial barrier function' Proc Natl Acad Sci USA, 105,3345-50.

Simmons RS, Berdine GG, Seidenfeld JJ et al. 1987, 'Fluid balance and the adult respiratory distress syndrome' Am Rev Respir Dis, 135,924-929.

Solymosi EA, Kaestle-Gembardt SM, Vadász I, et al. 2013, 'Chloride transport-driven alveolar fluid secretion is a major contributor to cardiogenic lung edema' Proc Natl Acad Sci U S A, 110, E2308-16.

Staub NC, Nagano H, Pearce ML.1967, 'Pulmonary edema in dogs, especially the sequence of fluid accumulation in lungs' J Appl Physiol, 22, 227–240.

Stelfox HT, Ahmed SB, Khandwala F, et al. 2008, 'The epidemiology of intensive care unit-acquired hyponatraemia and hypernatraemia in medical-surgical intensive care units' Crit Care, 12,R162.

Stevens AP, Hlady V, Dull RO. 2007, 'Fluorescence correlation spectroscopy can probe albumin dynamics inside lung endothelial glycocalyx' Am J Physiol Lung Cell Mol Physiol, 293, L328-35.

Stiller S, Bonnie-Schorn E, Grassmann A, et al. 2001, 'A critical review of sodium profiling for hemodialysis' Semin Dial, 14, 337–347.

Stow P, Hart G, Higlett T, et al. 2006, 'Development and implementation of a high-quality clinical database, the Australian and New Zealand Intensive Care Society Adult Patient Database' J Crit Care, 21, 133-41.

Symons JM, Chua AN, Somers MJ, et al. 2007, 'Demographic characteristics of pediatric continuous renal replacement therapy, a report of the prospective pediatric continuous renal replacement therapy registry' Clin J Am Soc Nephrol, 2, 732–738.

Tafreshi J, Hoang TH, Grigorian T et al. 2011, 'Impact of Iatrogenic, Excessive, Nondietary Sodium Administration in Patients with Acute Heart Failure Exacerbation on Hospital Length of Stay' Pharmacotherapy, 31, 58–61.

Tanaka M, Oida E, Nomura K, et al. 2005, 'The Na+-excreting efficacy of indapamide in combination with furosemide in massive edema' Clin Exp Nephrol,9,122-6.

Taylor D, Durward A, Tibby SM, et al. 2006, 'The influence of hyperchloraemia on acid base interpretation in diabetic ketoacidosis' Intensive Care Med, 32, 295-301.

Terpstra ML, Aman J, et al. 2014, 'Plasma Biomarkers for Acute Respiratory Distress Syndrome, A Systematic Review and Meta-Analysis' Crit Care Med, 42, 691-700

The Acute Respiratory Distress Syndrome Network. 2000, 'Ventilation with Lower Tidal Volumes as Compared with Traditional Tidal Volumes for Acute Lung Injury and the Acute Respiratory Distress Syndrome' N Engl J Med, 342,1301-1308.

The ANZIC Influenza Investigators. 2009, 'Critical care services and 2009 H1N1 influenza in Australia and New Zealand' N Engl J Med, 361, 1925-34.

Thein H, Haloob I, Marshall MR. 2007, 'Associations of a facility level decrease in dialysate sodium concentration with blood pressure and interdialytic weight gain' Nephrol Dial Transplant, 22, 2630–2639.

Thijssen S, Raimann JG, Usvyat LA, et al. 2011, 'The evils of intradialytic sodium loading' Contrib Nephrol, 171, 84-91.

Thorneloe KS, Cheung M, Bao W et al 2012, 'An orally active TRPV4 channel blocker prevents and resolves pulmonary edema induced by heart failure' Sci Transl Med, 4,159ra148.

Trissel. 2001, Handbook on Injectable Drugs. Bethesda, American Society of Health System Pharmacists.

Troidl C, Troidl K, Schierling W, et al 2009, 'Trpv4 induces collateral vessel growth during regeneration of the arterial circulation' J Cell Mol Med, 13,2613-21.

Trubuhovich RV. 2012, 'Inadvertent sodium loading in critically ill patients — plus some pedantry' Crit Care Resusc, 14, 163-4.

Twigley AJ, Hillman KM. 1985, 'The end of the crystalloid era? A new approach to peri-operative fluid administration' Anaesthesia, 40, 860–71.

UBM Medica Australia. eMIMS. [Software] St Leonard , UBM Medica Australia Pty Ltd, 2010. (accessed October 2012)

Uchino S, Bellomo R, Goldsmith D, et al. 2006, 'An assessment of the RIFLE criteria for acute renal failure in hospitalized patients' Crit Care Med, 34,1913-7.

Uchino S, Kellum JA, Bellomo R, et al, 2005, 'Acute renal failure in critically ill patients, a multinational, multicenter study' JAMA, 294, 813-8.

Upadya A, Tilluckdharry L, Muralidharan V, et al. 2005, 'Fluid balance and weaning outcomes' Intensive Care Med, 31,1643–1647.

Urden LD, Lough ME, Stacy KM. 2006, 'Thelan's critical care nursing, diagnosis and management' St Louis, Mosby Elsevier.

Valentine SL, Sapru A, Higgerson RA, et al, 2012, 'Fluid balance in critically ill children with acute lung injury' Crit Care Med, 40, 2883-9.

van Haren FM, Sleigh J, Boerma EC, et al. 2012, 'Hypertonic fluid administration in patients with septic shock, a prospective randomized controlled pilot study' Shock, 37, 268-75.

van Haren FM, Sleigh J, Cursons R, et al. 2011, 'The effects of hypertonic fluid administration on the gene expression of inflammatory mediators in circulating leucocytes in patients with septic shock, a preliminary study' Ann Intensive Care, 1, 44.

Vandergheynst F, Sakr Y, Felleiter P, et al. 2013, 'Incidence and prognosis of dysnatraemia in critically ill patients, analysis of a large prevalence study' Eur J Clin Invest, 43, 933-48.

Vialet R, Albanese J, Thomachot L, et al. 2003, 'Isovolume hypertonic solutes (sodium chloride or mannitol) in the treatment of refractory posttraumatic intracranial hypertension, 2 mL/kg 7.5% saline is more effective than 2 mL/kg 20% mannitol' Crit Care Med, 31,1683-1687.

Villalta PC, Rocic P, Townsley MI. 2014, 'The role of MMP2 and MMP9 in TRPV4-induced lung injury' Am J Physiol Lung Cell Mol Physiol, epub.

Villalta PC, Townsley MI. 2013, 'Transient receptor potential channels and regulation of lung endothelial permeability' Pulm Circ, 3,802-15.

Vincent JL, Moreno R, Takala J, et al. 1996, 'The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure' Intensive Care Med, 22, 707-710.

Vincent JL, Sakr Y, Sprung CL, et al. 2006, 'Sepsis in European intensive care units, results of the SOAP study' Crit Care Med, 34, 344–3536.

Wade C, Grady J, Kramer G. 1997, 'Efficacy of hypertonic saline dextran (HSD) in patients with traumatic hypotension, meta-analysis of individual patient data' Acta Anaesthesiol Scand Suppl, 110, 77-79.

Wagner EM and Mitzner W. 1996, 'Effects of bronchial vascular engorgement of airway dimensions' J Appl Physiol, 81, 293–301.

Wagner EM. 1997, 'Effects of edema on small airway narrowing' J Appl Physiol 83, 784–791.

Wagner JG, Leatherman JW, 1998 'Right ventricular end-diastolic volume as a predictor of the hemodynamic response to a fluid challenge' Chest 113,1048–1054.

Waikar SS, Curhan GC and Brunelli SM. 2011 'Mortality Associated with Low Serum Sodium Concentration in Maintenance Hemodialysis' Am J Med, 124, 77–84

Wang S, Singh RD, Godin L, et al. 2011, 'Endocytic response of type I alveolar epithelial cells to hypertonic stress, Am J Physiol Lung Cell Mol Physiol, 300, L560-8.

Wanger J, Clausen JL, Coates A, et al. 2005, 'Standardisation of the measurement of lung volumes' Eur Respir J, 26, 511-22.

Wasserman K, Loeb L, Mayerson HS. 1955, 'Capillary permeability to macromolecules' Circ Res, 3, 594-603.

Wegiersk T, Lewandrowski U, Muller B, et al. 2009, 'Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli' Journal of Biological Chemistry, 284, 2923–2933.

West JB, Tsukimoto K, Mathieu-Costello O, Prediletto R. 1991, 'Stress failure in pulmonary capillaries' J Appl Physiol 4,1731-42.

Wetzel RC, Herold CJ, Zerhouni EA, and Robotham JL. 1993, 'Intravascular volume loading reversibly decreases airway cross sectional area' Chest 103, 865–87.

Wiedemann HP, Wheeler AP, Bernard GR, et al 2006, 'Comparison of two fluid management strategies in acute lung injury' N Engl J Med, 354,2564-2575.

Wilkins MA, Su X-L, Palayew MD, et al. 1995, 'The effects of posture change and continuous positive airway pressure on cardiac natriuretic peptides in congestive heart failure' Chest, 107, 909–915.

Willette RN, Bao W, Nerurkar S, et al. 2008, 'Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse, Part 2' J Pharmacol Exp Ther, 326, 443-52.

Woodcock TE, Woodcock TM. 2012, 'Revised Starling equation and the glycocalyx model of transvascular fluid exchange, an improved paradigm for prescribing intravenous fluid therapy' Br J Anaesth, 108,384-94.

Worthley LI, Guerin M, Pain RW. 1987, 'For calculating osmolality, the simplest formula is the best' Anaesth Intensive Care, 15, 199-202.

Yang XC, Sachs F. 1989, 'Block of stretch-activated ion channels in Xenopus oocytes by gadolinium and calcium ions' Science, 243,1068-71.

Yin J, Hoffmann J, Kaestle SM, et al. 2008, 'Negative-feedback loop attenuates hydrostatic lung edema via a cGMP-dependent regulation of TRPV4' Circ Res, 102, 966–974.

Yin J, Kuebler WM. 2010, 'Mechanotransduction by TRP channels, general concepts and specific role in the vasculature' Cell Biochem Biophys, 56,1-18.

Yu M, Pei K, Moran S, et al. 2011, 'A prospective randomized trial using blood volume analysis in addition to pulmonary artery catheter, compared with pulmonary artery catheter alone, to guide shock resuscitation in critically ill surgical patients' Shock, 35, 220-8.

Yunos NM, Bellomo R, Hegarty C, et al. 2012, 'Association between a chloride-liberal vs chloride-restrictive intravenous fluid administration strategy and kidney injury in critically ill adults' JAMA, 308, 1566-72.

Yunos NM, Kim IB, Bellomo R, et al. 2011, 'The biochemical effects of restricting chloride-rich fluids in intensive care' Crit Care Med, 39, 2419-24.

Zanotti-Cavazzoni SL, Guglielmi M, Parrillo JE, 2009, 'Fluid resuscitation influences cardiovascular performance and mortality in a murine model of sepsis' Intensive Care Med, 35,748–754.

Zhou YL, Liu HL, Duan XF et al, 2006, 'Impact of sodium and ultrafiltration profiling on haemodialysis-related hypotension' Nephrol Dial Transplant, 21, 3231–3237

Zilberberg MD, Carter C, Lefebvre P, et al. 2007, 'Red blood cell transfusions and the risk of acute respiratory distress syndrome among the critically ill, a cohort study' Crit Care, 2007, 11, R63

Zilberberg MD, Epstein SK. 1998, 'Acute lung injury in the medical ICU,comorbid conditions, age, etiology, and hospital outcome' Am J Respir Crit Care Med, 157, 1159–116.

Zimmerman RS, Trippodo NC, MacPhee AA, et al. 1990, 'High-dose atrial natriuretic factor enhances albumin escape from the systemic but not the pulmonary circulation' Circ Res, 67, 461-8.

Zoellner H, Höfler M, Beckmann R, et al. 1996, 'Serum albumin is a specific inhibitor of apoptosis in human endothelial cells' J Cell Sci, 109, 2571-80.