Stress Hyperglycaemia and Arginine Metabolomics in Critical Illness

by

Tien Lee

Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy (PhD)

College of Medicine and Public Health

22 June 2021

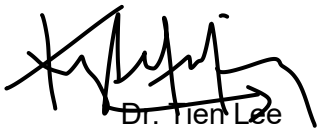
Table of Contents

Table of Figures

Table of Tables

Declaration

“I certify that this thesis: 1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and 2. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.”



Dr. Tien Lee

PhD Candidate

Thesis Summary

Hyperglycaemia in hospitalized patients, including those with an acute myocardial infarction (AMI) or critical illness, is associated with increased mortality. However, previous studies have reported that the acute rise in blood glucose concentration in hospital, termed stress or relative hyperglycaemia, is more strongly associated with mortality than the absolute glucose concentration (absolute glycaemia). The aim of this PhD project was to investigate whether relative hyperglycaemia is associated with mortality during AMI and critical illness and whether differences in arginine metabolites mediate the relationship between acute hyperglycaemia and mortality.

For AMI, I conducted a *post-hoc* analysis of data from The Hyperglycaemia: Intensive Insulin Infusion in Infarction (HI-5) study. For critical illness, 1,262 consecutively admitted patients to the Intensive Care Unit (ICU) at Flinders Medical Centre were studied prospectively. Relative hyperglycaemia was defined by the stress hyperglycaemia ratio (SHR), calculated by dividing a patient's admission glucose concentration by their estimated average glucose over the prior 3 months derived from glycosylated haemoglobin. Arginine and related metabolites have been associated with outcomes in critical care, and may provide a putative link for the effects of stress hyperglycaemia in this cohort. To study this, asymmetric dimethyl-L-arginine (ADMA) and L-homoarginine were measured by liquid chromatography mass spectrometry. An *in vitro* study was conducted to determine whether hyperglycaemia modulates the activity of dimethylarginine dimethylaminohydrolase-1 (DDAH1), the key enzyme that metabolises ADMA.

In the HI-5 study, SHR, but not absolute glucose, was positively associated with mortality, heart failure, arrhythmia, cardiogenic shock and a composite endpoint of a complicated AMI. These positive findings were not affected by diabetes status. These results show that relative hyperglycaemia is associated with mortality in patients with AMI across the glycaemic spectrum.

In critical illness, SHR was significantly associated with mortality after adjustment for the risk of death score, while the association with glucose was not statistically significant. The relationship between SHR and mortality was similar in patients with and without background hyperglycaemia. The results demonstrate that the relationship between relative hyperglycaemia and mortality is independent of known prognostic markers in ICU patients.

In this cohort of critically ill patients, ADMA was positively and L-homoarginine was negatively associated with mortality, independent of the risk of death score. The addition of ADMA and L-homoarginine significantly increased the area under the Receiver Operator Characteristic curve for mortality, compared to the risk of death score alone. These results suggest that measurement of ADMA and L-homoarginine may refine current models to predict mortality in ICU.

In critically ill patients there was not a significant correlation between ADMA and SHR. Consistent with this, *in vitro* DDAH1 activity was not affected by acute hyperglycaemia. L-homoarginine was significantly positively associated with mortality, but it is a negative predictor of mortality in this cohort. Therefore, changes in ADMA and L-homoarginine do not underlie the relationship between relative hyperglycaemia and mortality.

In summary, relative but not absolute hyperglycaemia is associated with mortality in AMI and critical illness. This association is not affected by background glycaemia and is independent of other prognostic factors. ADMA and L-homoarginine are also independently associated with mortality in critical illness. However, the relationships between relative hyperglycaemia and endothelial dysfunction and mortality are not mediated via these arginine metabolites.

Acknowledgment

The work presented in this thesis was performed at the Endocrine research unit at Flinders Medical Centre between 2015 and 2020, under the supervision of Associate Professor Morton Burt, Professor Arduino Mangoni and Associate Professor Leonie Heilbronn. The research was funded in part by grants from the FMC Foundation and Novo Nordisk Regional Diabetes Scheme. I was supported by scholarships from both the National Health Medical Research Council, Australia Postgraduate Research Scholarship and also by the Royal Australasian College of Physician's Kincaid-Smith Scholarship from 2015 to 2018.

The thesis would not have been possible without the support of my supervisors, collaborators and colleagues. First of all, I would like to thank my supervisor, Associate Professor Morton Burt for the tireless and patient review of manuscripts and presentations, help and guidance, and tutelage in aspects of research statistics. Credit should also go to him for the brilliant ideas that have formed the basis for many aspects of my research. He must be the most patient and understanding supervisor a PhD student can have. Secondly, I would like to thank my two co-supervisors. Professor Arduino Mangoni provided invaluable reviews and comments on manuscripts. His expertise in arginine metabolomics was crucial to the arginine studies in this thesis. Associate Professor Leonie Heilbronn provided guidance, encouragement, help with reviews of manuscripts and willingly shared her expertise. Thirdly, Prof Andrew Bersten, whose experience and support in the Intensive Care Unit at Flinders Medical Centre proved essential for the clinical research. Fourthly, Prof Wah Cheung and other investigators of the Hyperglycaemia: Intensive Insulin Infusion in Infarction (HI-5) study who graciously agreed to share the collected data for a *post-hoc* analysis, reviewed and provided important input for the research on acute myocardial infarction (AMI). Fifthly, I thank Dr. Sara Tommasi for help with the arginine samples storage, transport logistics and the *in vitro* arginine study. Sixthly, the arginine metabolite studies could not have been conducted in a timely manner without the

assistance of external collaborators from the University of Sassari, Italy, including Dr. Salvatore Sotgia, Assoc Prof Angelo Zinellu and Prof Ciriaco Carru. Finally, I acknowledge the support provided by Assoc Prof Stephen Stranks as the director of the Southern Adelaide Diabetes and Endocrine Services.

I would take the opportunity to acknowledge the help from the ICU administration staff and research nurses, many who kindly shared their computer terminals for the data collection, keeping medical records for data collection and providing the information necessary for the research. The support from the SA Pathology staff was instrumental to the success of sample retention, collection and processing for the prospective studies. Special thanks go to Mr Fotios Visvardis, Mr. Grant White and Mr. Darren Scott, who must have worked extra hours to support my research. I must also thank the research nurses and colleagues in the Southern Adelaide Diabetes and Endocrine Services for countless forms of help, including Dr. Jui Ho and Dr. Anjana Radhakutty for reviewing different chapters of this thesis.

On a personal note, I would like to thank my parents who always encouraged me to pursue knowledge, and together with my siblings, ensured I never gave up. Unfortunately, my father has departed to his eternal home before I was able to complete my doctoral studies, but I believe he would be proud to know that I completed it.

I also owe the debt of gratitude to my wife, Steph, for the initial moral support and reassurance to pursue the PhD, without which I would not have embarked on this long journey. I thank my two older children, Adam and Marissa, who had to persevere through occasionally testy times with an impatient father trying to provide them care, get his work done, and also complete his thesis. They have shown much brotherly and sisterly love, caring for the little baby Eevee, who came into this world in the year before the thesis was completed. Finally, to the many who have said a prayer for me to make it through the final hurdle of completing my thesis or have helped me, but I failed to mention here — thank you!

Publications Arising From This Thesis

The following are manuscripts arising directly from the work conducted during the candidature, which have been published or are in preparation for publication.

Manuscripts

Lee, Tien F., Morton G. Burt, Leonie K. Heilbronn, Arduino A. Mangoni, Vincent W. Wong, Mark McLean, and N. Wah Cheung. 2017. “Relative Hyperglycemia Is Associated with Complications Following an Acute Myocardial Infarction: A Post-Hoc Analysis of HI-5 Data.” Cardiovascular Diabetology 16 (1): 157.

Lee, Tien F., Sophie M. Drake, Gregory W. Roberts, Andrew Bersten, Stephen N. Stranks, Leonie K. Heilbronn, Arduino A. Mangoni, and Morton G. Burt. 2020. “Relative Hyperglycemia Is an Independent Determinant of In-Hospital Mortality in Patients With Critical Illness.” Critical Care Medicine 48 (2): e115–22.

Tien F Lee, Andrew Bersten, Leonie K Heilbronn, Angelo Zinellu, Ciriaco Carru, Salvatore Sotgia, Arduino A Mangoni, Morton G Burt. “ADMA and homoarginine independently predict mortality in critically ill patients: A prospective, observational cross-sectional study.” Manuscript submitted.

Tien F Lee, Sara Tommasi, Andrew Bersten, Leonie K Heilbronn, Salvatore Sotgia, Angelo Zinellu, Ciriaco Carru, Arduino A Mangoni, Morton G Burt. “Does hyperglycaemia affect arginine metabolites in critically ill patients?” Manuscript under preparation.

Abbreviations

Abbreviation	Phrase
ACE	Angiotensin converting enzyme
ADA	American Diabetes Association
ADMA	asymmetric dimethyl-L-arginine
AMI	acute myocardial infarction
ANZCTR	Australian New Zealand Clinical Trials Registry
ANZICS	Australian and New Zealand Intensive Care Society
ANZROD	Australian New Zealand Risk of Death
APACHE	Acute Physiology, Age and Chronic Health Evaluation
AUC	area under the curve
AUROC	area under the receiver operating characteristics
BGL	blood glucose level
BP	blood pressure
CABG	coronary artery bypass graft

Abbreviation	Phrase
CCB	calcium channel blocker
CCF	congestive heart failure
CI	confidence interval
CORE	Clinical Outcomes Research
CPK	creatinine phosphokinase
DDAH	dimethylarginine dimethylaminohydrolase
EAG	estimated average glucose
ECG	Electrocardiogram
EGFR	estimated glomerular filtration rate
EIC	extracted ion chromatogram
eNOS	endothelial nitric oxide synthase
FMC	Flinders Medical Centre
GCS	Glasgow Coma Score
GFR	glomerular filtration rate

Abbreviation	Phrase
HA	L-homoarginine
HbA1c	glycosylated haemoglobin-A1c
HI-5	Hyperglycaemia: Intensive Insulin Infusion in Infarction
HPLC	high performance liquid chromatography
HR	heart rate
ICU	intensive care unit
LC-MS/MS	liquid chromatography tandem mass spectrometry
LMWH	low molecular weight heparin
MAP	mean arterial pressure
MDM	Mortality Prediction Model
MDRD	Modification of Diet in Renal Disease Study
MMA	monomethyl-L-arginine
MODS	Multiple Organ Dysfunction Score
NHMRC	National Health and Medical Research Council

Abbreviation	Phrase
NO	nitric oxide
OR	odds ratio
PTCA	percutaneous transluminal coronary angioplasty
RACP	Royal Australasian College of Physician
ROC	receiver operating characteristics
ROD	risk of death
RR	respiratory rate
SAPS	Simplified Acute Physiology Score
SDMA	Symmetric dimethyl-L-arginine
SHR	stress hyperglycaemia ratio
SOFA	Sequential Organ Failure Assessment
STEMI	ST elevation myocardial infarction
TIC	total ion chromatogram
ToF	time of flight

Abbreviation	Phrase
WBC	white blood cell count

Chapter 1 Introduction

1.1 Introduction

The objective of this PhD project is to firstly investigate the relationship between the acute rise in glucose concentration during an acute illness such as a myocardial infarction or critical illness and mortality, and secondly, to assess whether differences in arginine and its metabolites mediate the relationship between acute hyperglycaemia and mortality.

By definition, hyperglycaemia is when the glucose concentration in the blood is above a generally accepted normal range. Hyperglycaemia can be classified as either: *chronic hyperglycaemia* — a long-term higher glucose concentration, such as in inadequately controlled diabetes mellitus; or *acute hyperglycaemia* — a short-term rise of blood glucose concentration that occurs during an intercurrent illness (Plummer et al. 2014; G. E. Umpierrez et al. 2002). The relationship between chronic hyperglycaemia and morbidity and mortality in patients with diabetes mellitus is well established, with treatment of chronic hyperglycaemia reducing morbidity and mortality. There is also evidence that acute hyperglycaemia in hospitalised patients may affect patient outcomes, but the evidence for this and the benefits of treatment of acute hyperglycaemia are not as clear.

In this introductory chapter, historical treatment of chronic hyperglycaemia will first be briefly reviewed. Next, major advances in the treatment of chronic hyperglycaemia will be summarized. The relationship between acute hyperglycaemia in hospital and patient outcomes will next be discussed, along with current approaches to identify acute hyperglycaemia and studies that have investigated the potential benefits of treating acute hyperglycaemia in the hospital. As one putative mechanism linking acute hyperglycaemia and adverse outcomes is endothelial and arterial dysfunction, the relationships between endothelial nitric oxide synthesis and arginine

metabolites with mortality and acute hyperglycaemia will be reviewed. A statement of the hypotheses and aims of this thesis will conclude this introduction.

1.2 Chronic Hyperglycaemia

1.2.1 History of Chronic Hyperglycaemia

The history of the management of chronic hyperglycaemia will be briefly summarised, as it potentially provides lessons for the treatment of acute hyperglycaemia. Chronic hyperglycaemia is the consequence of reduced insulin secretion and / or action in patients with diabetes mellitus and, if left untreated, is associated with increased mortality and morbidity (Garcia et al. 1974; Panzram 1987). The Egyptian physician, Hesy-Ra, has been credited with discovering diabetes more than 3,500 years ago, around 1500 B.C., after noting the adverse effects of polyuria and documenting it on papyrus (Zinman et al. 2017). The increased mortality and morbidity in patients with a hyperglycaemic state was first reported by Aretaeus of Cappadocia around AD 30-90, describing it as a “melting down of flesh into urine” and associated with “short survival.” (Reed 1954).

1.2.2 Treatment of Chronic Hyperglycaemia

1.2.2.1 Early Treatment Regimens

Given the significant impact of chronic hyperglycaemia, various treatment regimens have been proposed since the early times, often without much evidence and likely with detrimental effects. For example, Aretaeus tried a “non-irritating diet” of milk and carbohydrates and hiera, nardum, mastix, and theriak (Reed 1954). In the 1919 classic monograph, “Total Dietary Regulation in the Treatment of Diabetes”, Thomas Willis recommended “milk, rice, and starchy and gummy foods” in an attempt to “thicken the blood and supply salts,” limiting patients to a diet of milk and barley water boiled with bread (Frederick M. Allen, M.D., Edgar Stillman and Reginald Fitz

1919). He also proposed the use of opium, which was adopted by William Osler in his 1892 textbook as the only drug that "stands the test of experience as a remedy capable of limiting the progress of the disease." (Osler 1892). Another physician recommended "confinement" to "one room, with the utmost possible quiet and avoidance of exercise." (Frederick M. Allen, M.D., Edgar Stillman and Reginald Fitz 1919). Meanwhile, Frenchman, Apollinaire Bouchardat, considered by some to be the father of diabetology, proposed a low-carbohydrate diet, and "urged that patients eat as little as possible and masticate carefully." He was also a strong proponent on individualized treatment for patients.

These ancient and out-dated treatments are of historical interest, because they serve as lessons even today. These scholars correctly identified that: 1. Dietary restrictions and manipulations are important; 2. Addition of substances (drugs or medications) can help; 3. Individualised treatment for patients is the ultimate goal. One obvious lesson from this history is that expert-based opinions in treatment of diabetes, even by the most famous physicians of their times, often resulted in inappropriate recommendations, and a robust evidence-based approach is needed to manage hyperglycaemia.

1.2.2.2 Current Treatment Regimens

The discovery of insulin and its first clinical use in 1922 revolutionized the treatment of diabetes and led to Frederick Banting and John Macleod being awarded the Nobel Prize for Medicine and Physiology in 1923. Nearly 100 years later, insulin is still an important treatment for chronic hyperglycaemia. In addition, a number of other classes of oral and parenteral medications have been developed to treat type 2 diabetes, with a dramatic increase in new therapies over the last 10 years. This has facilitated individualised management of chronic hyperglycaemia in patients with type 2 diabetes (Zinman et al. 2017).

Advances in both measurements and therapeutics were highlighted in several large clinical trials reporting that treatment of chronic hyperglycaemia reduces mortality and morbidity (Table 1-1).

Reducing chronic hyperglycaemia has been associated with an improvement in outcomes (UKPDS 1998; Kosiborod, Inzucchi, Krumholz, et al. 2009). In all the landmark trials, microvascular complications such as retinopathy, nephropathy and peripheral neuropathies improve with better glycaemic control (Table 1-1). However, a J-shaped relationship between chronic glycaemia and mortality has been reported, with very tight glycaemic control in patients with type 2 diabetes associated with increased mortality (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008). There is also evidence that early good or intensive control of chronic hyperglycaemia may confer a "legacy effect" with reduced macrovascular disease long-term (Ritsinger et al. 2014; Nathan and DCCT/EDIC Research Group 2014; Holman et al. 2008). Sponsors of new therapies for type 2 diabetes are now required by the Food and Drug Administration (FDA) in the United States to undertake large cardiovascular outcomes trials. This has resulted in the identification of drugs that reduce mortality and / or cardiovascular events in addition to blood glucose (Table 1-2).

In summary, treatment of chronic hyperglycaemia has evolved over time. Current therapeutic regimens are associated with a reduction in microvascular disease, cardiovascular disease and all-cause mortality. However, overly aggressive treatment of chronic hyperglycaemia potentially increases mortality. The expanding range of therapeutic options permits individualised treatment regimens that optimise the health outcomes of patients with chronic hyperglycaemia. This thesis will explore whether similar concepts potentially apply to the management of acute hyperglycaemia.

	Trial	N	Study Cohort	Intervention	Cardiovascular Outcomes		Microvascular Events
					Events	Mortality	
1998	**UKPDS** (UKPDS 1998)	4,209	Newly diagnosed DM Type 2	Intensive sulphonylurea or insulin versus diet; Metformin versus diet.	⟷	⟷	↓
1998	**DCCT** (Diabetes Control and Complications Trial Research Group et al. 1993)	1,441	DM Type 1	Intensive insulin target HbA1c <6.5%	⟷	⟷	↓
2008	**ADVANCE** (ADVANCE Collaborative Group et al. 2008)	11,140	Long-standing DM Type 2	Intensive A1c target 6.5% (most on gliclazide MR and metformin)	⟷	⟷	↓
2008	**ACCORD** (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008)	10,251	Poorly controlled DM Type 2	Rapid, intensive A1c target 6.5% in 3 months (most on 3 oral agents and insulin)	⟷	↑	↓

Table 1-1 Summary of early landmark outcome trials of glycaemic control

See text for comments.

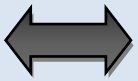
Trial	N	Intervention	CV Outcome	Other significant outcomes
SAVOR-TIMI 53, 2013 (Scirica et al. 2013)	16,492	Saxagliptin, 5 mg daily		**Negative**: increased heart failure
EXAMINE, 2013 (White et al. 2013)	5,380	Alogliptin, 25 mg daily		-
TECOS, 2015 (Green et al. 2015)	14,671	Sitagliptin 100 mg daily		-
ELIXA, 2015 (Pfeffer et al. 2015)	6,068	Lixisenatide 10-20 mcg daily		**Positive**: weight loss **Negative**: discontinuation due to side effects
EMPA-REG, 2015 (Zinman et al. 2015)	7,020	Empagliflozin, 10 or 25 mg daily		**Positive**: weight loss, decreased nephropathy, mild decrease in BP. **Negative**: genital infections
LEADER, 2016 (Marso, Daniels, et al. 2016)	9,340	Liraglutide 0.6-1.8 mg daily		**Positive**: weight loss, decreased nephropathy; decreased severe hypoglycaemia. **Negative**: cholecystitis/lithiasis; discontinuation due to side effects
SUSTAIN-6, 2016 (Marso, Bain, et al. 2016)	3,297	Semaglutide 0.5-1 mg SC/week		**Positive**: weight loss **Negative**: increased retinopathy
CANVAS, 2017 (Neal et al. 2017)	10,142	Canagliflozin 100 mg daily and 300 mg daily; OR 100 mg then 300 mg daily.		**Positive**: weight loss, decreased nephropathy, mild decrease in BP. **Negative**: genital infections, risk of lower limb amputations.
DECLARE-TIMI, 2019 (Wiviott et al. 2019)	17,160*	Dapagliflozin 10 mg		**Positive**: reduction in mortality and hospitalisation for heart failure, decreased nephropathy. **Negative**: genital infections.
VERTIS CV, 2020 (Cannon et al. 2020)	8,246	Ertugliflozin 5 mg and 15 mg		**Positive**: decreased nephropathy **Negative**: genital infections.

Table 1-2 Major randomised controlled cardiovascular outcomes trial for non-insulin agents in DM Type 2 with established atherosclerotic disease or at high risk

*10,186 (59.3%) of DECLARE-TIMI study population had no atherosclerotic disease.

1.3 Acute Hyperglycaemia: Hyperglycaemia in Hospital

By definition, acute hyperglycaemia is an elevation in blood glucose concentration that occurs over a short period of time (hours to days). During an intercurrent illness, such as a myocardial infarction, infection or surgery, counter-regulatory or stress hormones such as cortisol and adrenaline are secreted into the bloodstream. These hormones are critical to maintain vascular tone and survive a major illness, but can increase blood glucose. The increase in glucose above baseline concentrations during an acute illness has been termed “stress hyperglycaemia”, a concept first postulated by Claude Bernard in 1877 (Dungan, Braithwaite, and Preiser 2009). An alternative nomenclature is “relative hyperglycaemia”, which reflects the proportional glucose increase above baseline. For the purposes of this thesis, these terms will largely be used interchangeably. A subtle difference is the connotation that stress hyperglycaemia is an elevation in glucose that is caused by an acute illness (the "stress"), while relative hyperglycaemia does not imply any specific cause for the increase in glucose concentration.

Acute hyperglycaemia, quantified from the blood glucose concentration at admission to hospital, is associated with poorer outcomes including mortality during an acute illness (Kim et al. 2017; Fujino et al. 2014; G. E. Umpierrez et al. 2002). Paradoxically, this association has been noted to be stronger for patients who did not have diabetes, than those who had diabetes (Plummer et al. 2014; Kar et al. 2016; Luethi et al. 2018; Kotagal et al. 2014; James S Krinsley et al. 2013; G. Umpierrez et al. 2015; Capes et al. 2000; 2001). Figure 1-1 shows the results of one study demonstrating increased mortality with new hyperglycaemia in patients with critical illness (G. E. Umpierrez et al. 2002).

Image removed due to copyright restriction.

Figure 1-1 Increased ICU mortality in acutely unwell patients with new hyperglycaemia without known diabetes (G. E. Umpierrez et al. 2002)

Note that patients with diabetes did not have a significant increase in mortality.

It is not clear whether an increase in glucose is simply a marker of the severity of the underlying illness or contributes to mortality *per se*. However, a number of pathogenic mechanisms have been identified by which hyperglycaemia might contribute to adverse outcomes (Figure 1-2). These include indirect effects mediated through changes in the circulation and electrolytes causing volume depletion, hypoperfusion, electrolyte loss or acid-base imbalance, or direct tissue effects such as oxidative stress, enhanced inflammation, induction of apoptosis, activation of the coagulation cascade, and attenuation of endothelium-dependent vasodilation (Williams et al. 1998; Esposito et al. 2002; Stegenga et al. 2006; Jafar, Edriss, and Nugent 2016).

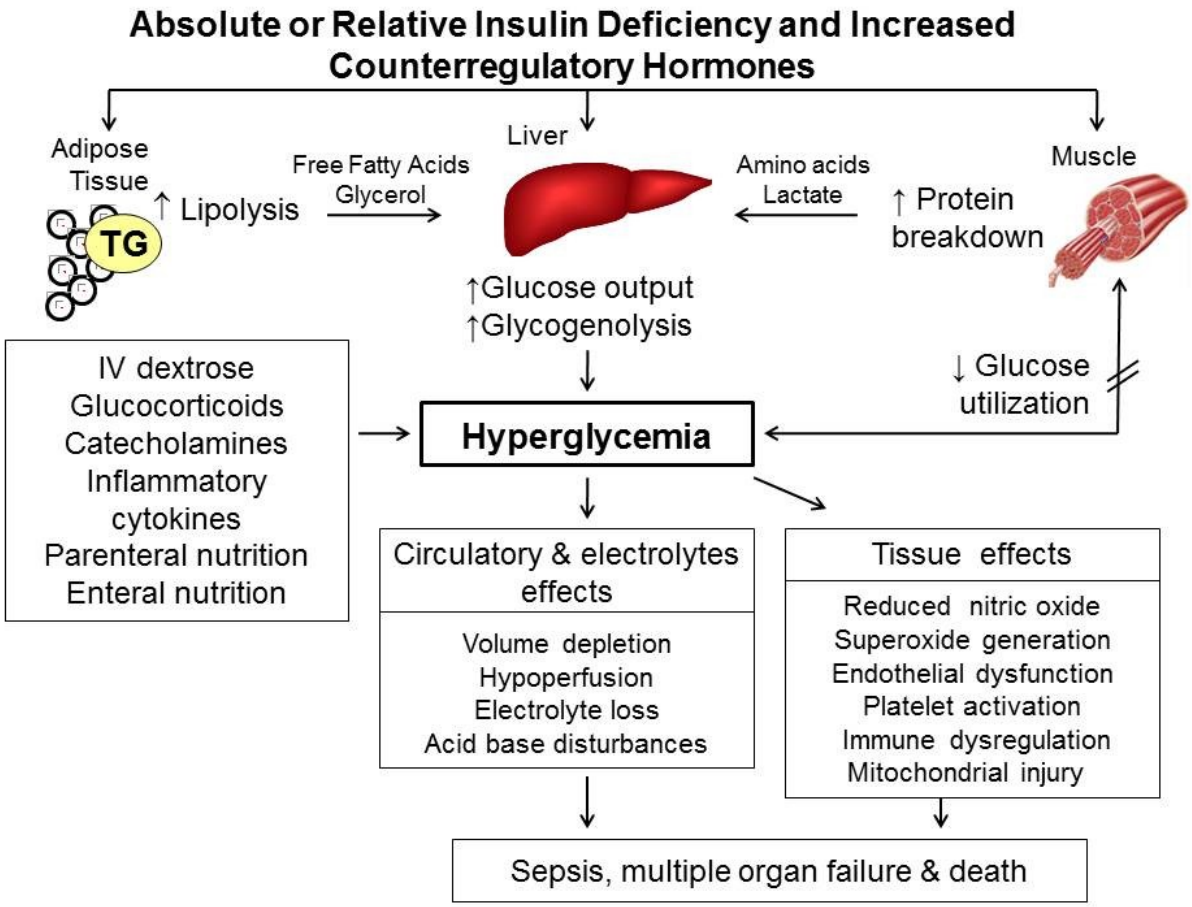


Figure 1-2 Postulated pathophysiology underlying and associated with acute hyperglycaemia

(Taken from EndoText)

1.3.1 Quantification of Stress Hyperglycaemia

The recognition that acute hyperglycaemia might be important has led to development of methods to quantify it. Initially a new classification for acute hyperglycaemia that was termed "hospital-related hyperglycaemia" was proposed by the American Diabetes Association (ADA), for patients with an elevated glucose in hospital but without known diabetes (Dungan, Braithwaite, and Preiser 2009). However, this definition includes patients presenting with an acute illness who have previously undiagnosed diabetes and these patients may have a glucose concentration that is similar to that present before the onset of the illness. Furthermore, this was

still a qualitative definition and varying thresholds have been used in different studies, making it difficult to compare across studies.

In recent years, several groups have described approaches to better quantify stress, or relative, hyperglycaemia. These methods all utilise measurements of glycosylated haemoglobin (HbA1c) to estimate background hyperglycaemia. HbA1c is a measure of glycated haemoglobin that is used to diagnose diabetes and guide therapeutic decisions in patients with diabetes. As the average life span of a red blood cell is typically 90-120 days, HbA1c provides an estimate of medium-term glycaemic control. In 2008 Nathan et al reported a large observational study of patients with type 1 and type 2 diabetes who underwent regular continuous glucose monitoring combined with measurement of HbA1c (Nathan et al. 2008). They used this data to derive a calculation for the average glucose concentration over the last 3 months, termed estimated average glucose (EAG). The details of this calculation are described in *Chapter 2 Methods*.

At the time of writing, there are 3 published methods to calculate relative hyperglycaemia. In 2013, Liao et al described the Glycaemic Gap in patients with pyogenic liver abscess (W.-I. Liao et al. 2013). The Glycaemic Gap is calculated by subtracting the EAG from the admission glucose, resulting in a measure expressed in the same glucose unit (typically mmol/L or mg/dL). The glycaemic gap has been studied *primarily in patients with diabetes*; patient groups include those admitted to hospital with pyogenic liver abscess, acute myocardial infarction, critically ill, community-acquired pneumonia and heart failure (W.-I. Liao et al. 2013; 2016; W. Liao et al. 2015; Donagaon and Dharmalingam 2018; Chen et al. 2019; W.-I. Liao et al. 2019; Jensen et al. 2019). In each of these patient cohorts, it was found that the glycaemic gap was positively associated with poorer outcomes and increased mortality.

In 2015, members of the Endocrinology Unit at Flinders Medical Centre described another method to measure relative hyperglycaemia, the Stress Hyperglycaemia Ratio (SHR) (Roberts et al. 2015). In this method, the acute admission glucose concentration was divided by the EAG,

resulting in a ratio without any units. In a *post hoc* analysis of adult patients admitted to the general wards of Flinders Medical Centre over a 3 month period, the odds ratio for critical illness, defined as death or admission to ICU, per 0.1 SHR increment was 1.20 ($p<0.001$). In the same analysis, the odds ratio per one mmol/L glucose increment was 1.03 ($p=0.31$). An advantage of SHR is that it is clinically intuitive; a SHR of 1.2 represents an increase of 20% over the patient's regular (or background) glycaemia. For the purposes of this thesis, SHR will be used as the measure of stress hyperglycaemia.

A third paper in 2017 introduced the Glucose-A1c Ratio (GAR), which is the ratio of the admission glucose to the HbA1c (Su et al. 2017). In the original paper, the unit of the admission glucose was in mg/dL and the HbA1c was in %, hence resulting with a ratio expressed as mg/dL/%. This is conceptually similar to SHR, but the Units are not standard in Australia and the implications of the GAR are not immediately clinically intuitive. For example, with SHR, one can easily define a predetermined range in a clinical context. A SHR between 0.8 to 1.2 would identify patients who are within 20% of their usual background glycaemia, and hence could be used clinically for targeted therapy, treatment threshold or research. The use of GAR would make it difficult to have such defined thresholds clinically.

A comparison of these methods of quantification of relative hyperglycaemia is beyond the scope of this thesis, but a *post hoc* analysis is planned using the data collected. In the only published comparison there was not a statistically significant difference in the relationship between the three measures and mortality in patients with stroke (C.-J. Yang et al. 2017). A limitation inherent to all 3 methods is that studies have usually employed a single glucose measurement to calculate indices of relative hyperglycaemia.

1.4 Stress Hyperglycaemia and Acute Myocardial Infarction

According to the Australian Institute of Health and Welfare analysis of the National Hospital Morbidity Database, there are more than 30,000 hospitalisations for acute myocardial infarction in Australia per annum, representing 252 hospitalisations per 100,000 people aged 35-84 years. An estimated 25% of patients presenting with acute myocardial infarction have diabetes (McQuillan and Thompson 2014). In patients with myocardial infarction, diabetes is associated with a 2.4 fold increased risk of dying (Mukamal et al. 2001). The proportion of patients with acute myocardial infarction and relative hyperglycaemia is not well established, nor is the relationship between relative hyperglycaemia and mortality.

A number of studies have been conducted to establish if a tighter control of blood glucose with insulin would provide immediate or subacute mortality benefits in patients with acute myocardial infarction (see Table 1-3). Other than DIGAMI 1, where a significant reduction in all-cause mortality after 12 months but not at 3 months was observed, none of these studies reported a statistically significant improvement in the primary end-point. There are potential explanations for these negative findings. Firstly, in these studies patients were treated with insulin to an absolute glucose target, regardless of diabetes status or background glycaemia. Secondly, in several of these studies the difference in blood glucose between intervention and control arms was small. Finally, these studies included exclusively or predominantly patients with diabetes and sub-group analyses based on diabetes status were not generally conducted. As hyperglycaemia is most strongly related to outcomes in patients without diabetes, the lack of non-diabetic subjects may have contributed to the inability to show any difference between the treatment groups (Wernly et al. 2016; Judith Jacobi 2016; James S Krinsley et al. 2013).

The Hyperglycaemia: Intensive Insulin Infusion in Infarction (HI-5) study was a prospective randomized-controlled trial conducted in Australia investigating the effect of intensive insulin therapy on mortality in patients with acute myocardial infarction (N Wah Cheung, Wong, and

McLean 2006). In the primary analysis, there was no significant difference in mortality in patients randomized to intensive insulin and conventional care. However, as finger prick BGLs were systematically recorded in both groups and the HbA1c was recorded for most patients, the study cohort provides an opportunity to investigate the association between relative hyperglycaemia during glucose-lowering treatment and adverse patient outcomes. These results are reported in Chapter 3 of this thesis.

In summary, there are varying results regarding the benefits of insulin therapy and tight glycaemic control in patients with acute myocardial infarction. While there are other potential explanations for the discordant results, it is possible that treating absolute rather than relative hyperglycaemia contributed to the negative results. In Chapter 3, I will investigate whether absolute or relative glycaemia is more strongly associated with outcomes in patients with acute myocardial infarction.

Trial	N	% with diabetes	Study Cohort	Intervention	Results
DIGAMI, 1995 (Klas Malmberg et al. 1995)	620	100%	Acute MI with or without ST elevation	24 hours IV glucose-insulin, then SC insulin for 3 months	All-cause mortality at 3 months: Not significant All-cause mortality at 12 months: Significant decrease with insulin infusion
ECLA-GIK pilot trial, 1998 (Díaz et al. 1998)	407	16%	Acute MI with or without ST elevation	High dose; low dose glucose-insulin-potassium, or no infusion	All-cause in-hospital mortality: Not significant
GIPS, 2003 (van der Horst et al. 2003)	940	11%	Acute ST elevation MI	8-12 hours of glucose-insulin-potassium infusion	All-cause mortality at 30 days: Not significant
DIGAMI-2, 2005 (K Malmberg et al. 2005)	1,253	100%	Acute MI with or without ST elevation	24 hours IV glucose-insulin, then SC insulin for 3 months; 24 hours IV glucose-insulin only; no insulin	All-cause mortality during follow up (mean of 2 years): Not significant
CREATE-ECLA, 2005 (The CREATE-ECLA Trial Group Investigators* 2005)	20,201	18%	Acute ST elevation MI	24 hours glucose-insulin-potassium infusion	All-cause mortality at 30 days: Not significant
GIPS-2, 2006 (Díaz et al. 2007)	889	10%	Acute ST elevation MI, Killip class I only	8-12 hours of glucose-insulin-potassium infusion	All-cause mortality at 30 days: Not significant
HI-5, 2006 (N Wah Cheung, Wong, and McLean 2006)	240	48%	Acute MI with or without ST elevation and admission glucose > 7.8 mmol/L	24 hours IV insulin to maintain glucose 4.0-9.9 mmol/L versus standard care	All-cause in-hospital death: Not significant
OASIS-6 GIK, 2007 (Yusuf et al. 2006)	2,748	14%	Acute ST elevation MI	24 hours glucose-insulin-potassium infusion	All-cause mortality at 30 days: Not significant

Table 1-3 Major trials of insulin infusion for patients with acute myocardial infarction

CREATE = Clinical Trial of Metabolic Modulation in Myocardial Infarction Treatment Evaluation; DIGAMI = Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infusion; ECLA = Estudios Cardiologicas Latin America; GIK = Glucose Insulin (K+) Potassium; GIPS = Glucose-Insulin-Potassium Study; HI-5 = Hyperglycaemia: Intensive Insulin Infusion in Infarction; OASIS = Outcome and Assessment Information Set

1.5 Stress Hyperglycaemia and ICU

1.5.1 Introduction

There are 125,000 ICU admissions in Australia every year (Carter and Hicks 2011). As such, an Australian resident has a 50% lifetime risk of an ICU admission (Warrillow and Raper 2019). There are two main categories of patients admitted to ICU: 1) admissions after elective major surgery requiring intensive monitoring, and 2) admissions for severe acute illness. The average mortality rate in Australian and New Zealand ICUs is around 15% (Moran et al. 2008). As such, any intervention that reduces mortality will have a significant public health benefit.

Hyperglycaemia is common in critically ill patients. For example, 75% of patients admitted to ICU had at least one glucose recording above or equal to 7 mmol/L fasting, or above or equal to 11.1 mmol/L during feeding (Plummer et al. 2014). About 50% of patients were thought to have stress hyperglycaemia, rather than hyperglycaemia because of known diabetes (Plummer et al. 2014).

1.5.2 Current Therapeutic Targets and Guidelines

Hyperglycaemia has been clearly associated with increased mortality and morbidity in the ICU (G. E. Umpierrez et al. 2012; Bochicchio et al. 2005; Koyfman et al. 2018; Egi et al. 2006; James Stephen Krinsley 2003). One of the early seminal studies on hyperglycaemia in this setting, a retrospective investigation of 1,826 patients admitted to a mixed ICU in Stamford, Connecticut serving medical, surgical, and coronary patients, reported a stepwise increase in mortality as mean blood glucose concentrations rose (James Stephen Krinsley 2003). In-hospital mortality was 9.6% among those with a mean blood glucose of 4.4-5.5 mmol/L, 29.4% among those with a mean blood glucose of 10-11.1 mmol/L, and 42.5% among those with a mean blood glucose greater than 16.6 mmol/L. These mortality rates were above and beyond

those expected based on the Acute Physiology, Age and Chronic Health Evaluation (APACHE) II prognostic scoring system. Observations such as these raised concern that acute hyperglycaemia was directly contributing to poor outcomes, potentially by rendering affected patients susceptible to at least some of the consequences observed among chronic diabetics, including high infection rates, poor wound healing, and polyneuropathy (Clain 2015).

However, studies investigating *treatment* of hyperglycaemia have yielded conflicting results (see Table 1-4). In the first Leuven study of surgical ICU patients, there was a reduction in mortality benefit with intensive insulin therapy targeting a glucose concentration of 4.4-6.1 mmol/L compared to conventional glucose-lowering therapy (G van den Berghe et al. 2001). However, subsequent studies did not report a significant difference in mortality with intensive insulin therapy (see Table 1-4). Moreover, the large multi-centre NICE-SUGAR study, reported that intensive insulin therapy increased mortality in critically ill patients (Finfer et al. 2009).

Trial	N	Study Cohort	Intensive target	Control target	Mortality Findings	Favours Intensive Therapy
First Leuven Trial, 2001 (G van den Berghe et al. 2001)	1,548	Single-centre; surgical ICU	4.4-6.1 mmol/L	10.0-11.1 mmol/L	Favour intensive: reduced ICU and in-hospital mortality	Yes
Second Leuven Trial, 2006 (Greet Van den Berghe, Wilmer, Hermans, et al. 2006)	1,200	Single-centre; medical ICU	4.4-6.1 mmol/L	10.0-11.1 mmol/L	No mortality difference	Yes
Arabi et al, 2008	523	Single-centre; mixed ICU, including medical, surgical, and trauma patients	4.4-6.1 mmol/L	10.0-11.1 mmol/L	No mortality difference	No
Brunkhorst et al, 2008	537	Multicentre; mixed ICUs; all patients with severe sepsis or septic shock	4.4-6.1 mmol/L	10.0-11.1 mmol/L	No mortality difference	No
De La Rosa Gdel et al, 2008	504	Single centre; mixed ICU, including medical, surgical, and trauma patients	4.4-6.1 mmol/L	10.0-11.1 mmol/L	No mortality difference	No
Preiser et al, 2009	1,078	Multicentre; medical and surgical ICU	4.4-6.1 mmol/L	7.8-10.0 mmol/L	No mortality difference	No
NICE-SUGAR Trial, 2009 (Finfer et al. 2009)	6,104	Multicentre; medical and surgical ICU	4.5-6.0 mmol/L	7.9-10.0 mmol/L	Against intensive: Increased 90 day mortality	No
Annane et al, 2010	509	Multicentre; all patients with septic shock	4.4-6.1 mmol/L	10.0-11.1 mmol/L	No mortality difference	No
Macrae et al, 2014	1,369	Multicentre; medical and surgical paediatric patients	4.0-7.0 mmol/L	10.0-12.0 mmol/L	No mortality difference	No

Table 1-4 Major randomised controlled trials of intensive versus conventional glycaemic targets in critically ill patients with mortality and morbidity outcomes

Several reasons have been postulated to explain these discordant results. In NICE-SUGAR, a significant increase in hypoglycaemia was noted in the intensive therapy group (Table 1-4) and severe hypoglycaemia was thought to at least partially account for the negative findings (Investigators 2012). Another possible explanation is that these studies differed in the proportion of patients with and without diabetes, and the effect of insulin might differ in these patient groups. Finally, studies had different intensive and non-intensive glycaemic targets, which could also contribute to heterogeneity of results (Table 1-4). These findings, albeit conflicting, have led to various recommendations for glycaemic treatment targets in the ICU population (Table 1-5).

1.5.3 New Hyperglycaemia versus Known Diabetes

There is evidence that the duration of hyperglycaemia affects its relationship with mortality and the effect of treatment of hyperglycaemia in hospitalised patients. Observational studies have reported an increased mortality in patients with new hyperglycaemia in hospital, and especially in the ICU, compared to those who have known diabetes (G. E. Umpierrez et al. 2002) as shown in Figure 1-1. Most importantly, a retrospective study of critically ill patients reported that intensive insulin therapy was associated with reduced mortality in patients without diabetes but increased mortality in patients with diabetes (Lanspa et al. 2013), (Figure 1-3A and Figure 1-3B). In studies of critically ill patients that have stratified patients based on background glycaemia (Egi et al. 2011; Plummer et al. 2014), it was found that background hyperglycaemia increased the mortality and complication rates for patients receiving tight glucose control with insulin therapy. This has resulted in calls for different glycaemic targets for patients with different levels of background glycaemia (Egi et al. 2011; Marik and Bellomo 2013; Judith Jacobi 2016; James S. Krinsley et al. 2020) (also see Table 1-5). These recommendations, however, have minimal glycaemic target differences (e.g 1 mmol/L), dichotomize patients based upon a single absolute HbA1c value and have not been adopted by leading professional ICU societies.

Trial	Condition	Studies included	Recommendations
Furnary et al, 2004	Surgical ICU	1 prospective study: 4,864 patients across 17 years	If ICU >3 days, ventilator dependent, on dialysis or has cardiac history: <8.3 mmol/L
Wiener et al, 2008	Non-diabetic ICU	Systematic review: 29 studies, 8,432 patients	7.8-10 mmol/L
Griesdale et al, 2009	Non-diabetic ICU	Systematic review: 26 studies, 13,567 patients	7.8-10 mmol/L
NICE Guidelines, 2011	STEMI ICU	No high quality studies, consensus by NICE	Maintain < 11.1 mmol/L
Egi et al, 2011	Diabetic ICU	1 retrospective study with 415 patients	If HbA1c < 7%: 7.8-10 mmol/L If HbA1c ≥ 7%: maintain > 11.1 mmol/L
Marik et al, 2014	Non-diabetic Diabetic Cardiac Surgery, non-diabetic Cardiac Surgery, diabetic	No high quality studies, various small studies used to justify the recommendations.	Non-diabetic: 7.8-11.1 mmol/L Diabetic HbA1c < 7%: 7.8-11.1 mmol/L Diabetic HbA1c ≥ 7%: 8.8-12.2 mmol/L Cardiac surgery, non-diabetic: 7.8-10.0 mmol/L Cardiac surgery, diabetic HbA1c < 7%: 7.8-10.0 mmol/L Cardiac surgery, diabetic HbA1c ≥ 7%, 8.8-11.1 mmol/L
ADA Guidelines, 2019	ICU	No high quality studies	7.8-10 mmol/L; 6.1-7.8 mmol/L may be appropriate for certain patients providing no increased risk of hypoglycaemia

Table 1-5 Various publications suggesting different recommendations for ICU management of hyperglycaemia

ADA = American Diabetes Association; ICU = Intensive Care Unit; NICE = National Institute of Health and Care Excellence

Figure 1-3 Effect of intensive glycaemic control on mortality in patients without (A) and with (B) diabetes

(Lanspa et al. 2013)

1.5.4 Limitations of Observational Trials

As already highlighted, observational studies have often reported a significant association between hyperglycaemia and adverse outcomes while interventional studies have often failed to demonstrate a significant benefit arising from lowering blood glucose. While there are potentially other explanations for this discordance, a number of inherent limitations of observational studies, considered when planning my thesis, could contribute to these findings:

- Causal inferences cannot reliably be drawn
- Recall bias and missing data when trials are conducted retrospectively
- Selection bias when selecting cases for observational trials, especially retrospectively
- Confounders may exist that may explain an observed relationship.

While I cannot eliminate these factors completely, to minimise them I have considered:

- Prospectively registering my study in the Australian New Zealand Clinical Trials Registry (ANZCTR)
- Pre-specifying inclusion and exclusion criteria and the primary endpoint.
- Recruiting consecutive patients until an appropriate number of patients have been recruited based on *a priori* power analysis.
- Minimising confounding from other clinical variables by adjusting for them.
- Further studies to investigate plausible mechanisms that might explain my main findings.

1.5.5 Prognostic Scoring in ICU

As noted above, one limitation of observational studies is the potential confounding from other variables that are related to the variable being studied and influence the results. This limitation can be minimized by adjusting the results for other important variables that affect the outcome. This is possible in critically ill patients, as prognostic or severity scoring

systems in clinical use include the main clinical factors that affect mortality in ICU. These scoring systems are used to compare quality-of-care between different ICUs and stratification of variables in clinical trials (Rapsang and Shyam 2014).

Most scoring systems utilise data collected from the first day of ICU admission. These include the Acute Physiology and Chronic Health Evaluation (APACHE), Simplified Acute Physiology Score (SAPS) and the Mortality Prediction Model (MDM). Other scoring systems utilise daily data from the first three days of ICU stay such as the Sequential Organ Failure Assessment (SOFA) and Multiple Organs Dysfunction Score (MODS). There are also scoring systems developed and validated for a specific region, such as the Australian New Zealand Risk of Death (ANZROD) (Paul et al. 2016; Paul, Bailey, and Pilcher 2013). At the time of the research conducted in this thesis, the APACHE II, APACHE IIIj (a variant of APACHE III) and the simplified SAPS II scoring systems were used by the Australia and New Zealand Intensive Care Society Centre for Outcome and Resource Evaluation (ANZICS CORE) to benchmark ICUs in Australasia.

APACHE II, a validated ICU prognostic scoring system first published in 1985 (William A. Knaus et al. 1985), includes 12 acute physiology variables: rectal temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation status, arterial pH, serum sodium, serum potassium, serum creatinine, haematocrit, white blood count and Glasgow Coma Score (GCS). It assigns age points based on a number of age ranges, with a higher score for older patients. For patients with a history of severe organ system insufficiency or immunocompromise, a score is assigned depending on the reason for admission, higher points being given for non-elective admissions. The sum total of the scores from the acute physiology score, the age score and the chronic health points are totalled to calculate the APACHE II score. While this score has been used in research as a prognostication tool, the original intent was to compute a predicted death rate which further accounts for the diagnostic category that led to an ICU admission (William A. Knaus et al. 1985). This is often

referred to as the Risk of Death score, and the formula is available as part of the Appendix in the original paper.

The APACHE III scoring system was developed in 1991 in an attempt to improve upon the risk prediction based on APACHE II (W A Knaus et al. 1991). It included similar physiologic variables, but removed serum potassium and added urine output, blood urea nitrogen, serum albumin, serum bilirubin and importantly, admission glucose. Furthermore, the risk computation is no longer published, but has become proprietary and requires a licensing fee for use. As such, the APACHE II scoring system remains widely used in publications.

For this thesis, I used APACHE II in Chapter 4 to calculate the risk of death score as it does not include glucose and admission glucose was an independent variable in the analyses. In Chapter 5, APACHE III was used to calculate the risk of death score as admission glucose was not being studied.

1.6 Arginine Metabolomics and Hyperglycaemia

1.6.1 Introduction

As noted in Figure 1-2, hyperglycaemia has been postulated to exert direct tissue effects by modulating the nitric oxide pathway and causing endothelial dysfunction. The endothelium plays a key role in maintaining vascular homeostasis. A number of investigative techniques have been developed to quantify endothelial function in humans. Clinically, endothelial dysfunction can be evaluated using non-invasive techniques that measure post-ischaemic blood flow in large conduit arteries (flow-mediated dilation) or finger vasculature (reactive hyperaemia index). However, these techniques are impractical in critically ill patients, in whom consent is difficult and the requirement for multiple sites for venous access often preclude induction of ischaemia in an arm.

L-arginine and its metabolites are central to endothelial function (see Figure 1-4). The plasma concentration of L-arginine is affected by dietary intake, arginine synthesis in the

kidney and its metabolism by arginases. L-arginine then enters endothelial cells, predominantly through the y+ transporter system. In endothelial cells, L-arginine is the main substrate for the enzyme endothelial nitric oxide synthase (eNOS), which converts L-arginine to citrulline (Figure 1-4). This chemical reaction results in the synthesis of the potent endogenous vasodilator nitric oxide (NO) (L J Ignarro 2002; Louis J Ignarro, Napoli, and Loscalzo 2002). NO has anti-inflammatory, anti-thrombotic, anti-atherosclerotic effects, and also exerts effects on the vascular tone (Louis J Ignarro and Napoli 2005). Unsurprisingly, decreased eNOS activity, and hence decreased NO have been associated with increased cardiovascular morbidity and mortality (Lerman and Zeiher 2005; Versari et al. 2009).

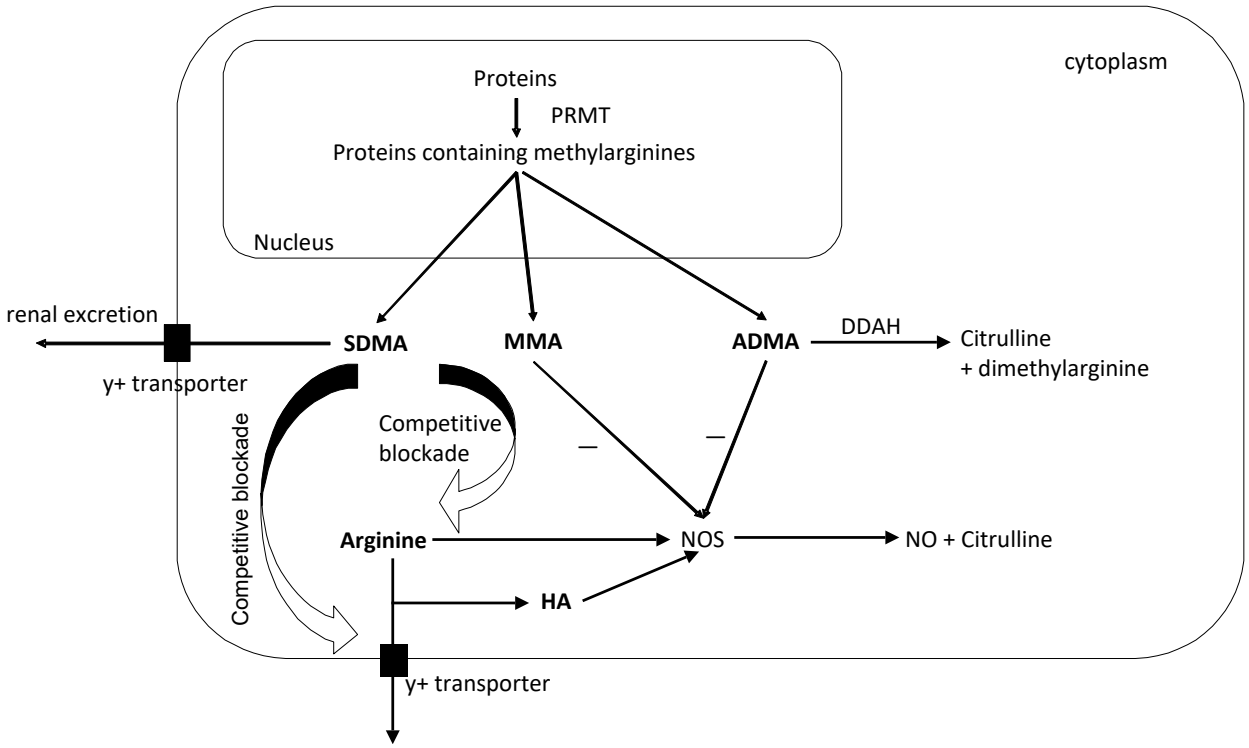


Figure 1-4 Interactions between arginine, asymmetric dimethyl-L-arginine (ADMA), symmetric dimethyl-L-arginine (SDMA), monomethyl-L-arginine (MMA) and L-homoarginine (HA) with nitric oxide synthase (NOS)

PRMT, protein arginine methyltransferases. Adapted from (Brinkmann et al. 2013), with additional information from (Davids, Ndika, et al. 2012; Jr 2016; Hecker et al. 1990).

1.6.2 Methylarginine Metabolites and the NOS Pathway

Metabolites of L-arginine play a key role in the nitric oxide pathway as shown in Figure 1-4. Proteins containing methylated arginine residues release, during proteolysis, asymmetric dimethyl-L-arginine (ADMA), symmetric dimethyl-L-arginine (SDMA) and monomethyl-L-arginine (MMA). ADMA and MMA are powerful competitive inhibitors of eNOS (Siroen et al. 2006). An increase in plasma ADMA reduces endothelial nitric oxide production, vascular tone and arterial blood flow (Mortensen et al. 2019). MMA has a similar action to ADMA, but circulates at a far lower concentration. In contrast, SDMA decreases nitric oxide production indirectly by reducing L-arginine availability and increased production of reactive oxygen species (Mangoni 2009). L-homoarginine is synthesized from lysine and arginine via arginine:glycine amidinotransferase (Kayacelebi et al. 2015). L-homoarginine is postulated to increase NOS activity by increase arginine availability by competitively inhibiting arginase (which metabolises arginine) and also acting as a substrate for eNOS in place of arginine, which despite its weaker affinity can be metabolised to NO and homocitrulline (Pilz et al. 2015). In studies of mice, L-homoarginine supplementation has been shown to contribute to more sustained NOS activation than arginine supplementation (Atzler, Schwedhelm, and Choe 2015).

1.6.3 Arginine Metabolites in Critical Illness

Several small studies have investigated the association of different arginine metabolites to mortality in the critically ill. Higher arginine concentration was weakly associated with better outcomes in critically ill patients (Brenner et al. 2012). In a recent meta-analysis, a higher ADMA level was associated with poorer outcomes (Mortensen et al. 2019). The ADMA/arginine ratio has been proposed as a good marker of mortality in critically ill patients, as it combines an inhibitor of nitric oxide synthase (ADMA) and its substrate (arginine) (Patel et al. 2016). However, in a meta-analysis the ADMA/arginine ratio was not significantly associated with mortality (Mortensen et al. 2019). Recent studies reported poorer outcomes in patients with higher SDMA and lower L-homoarginine concentrations in ICU (Ghashut et

al. 2017; Bode-Böger et al. 2006; Pilz et al. 2014; Zinellu et al. 2018). No studies to date have investigated MMA in the critical care or acute illness setting, although it has been previously associated with endothelial dysfunction (Karlsson, Sørensen, and Kruuse 2017; Jamwal and Sharma 2018). In this thesis I have measured L-arginine and its metabolites in the largest cohort of critically ill patients in the literature and assessed whether plasma contributions are associated with mortality (Chapter 5) and relative hyperglycaemia (Chapter 6). If so, changes in arginine metabolites could be one mechanism linking relative hyperglycaemia and mortality in ICU.

1.6.4 Dimethylarginine Dimethylaminohydrolase and Hyperglycaemia

As shown in Figure 1-4, dimethylarginine dimethylaminohydrolase (DDAH) is the primary enzyme that metabolises ADMA to citrulline and dimethylamine and thus its activity is the main determinant of circulating ADMA concentration. There are two DDAH isoforms in humans. DDAH1 is the key enzyme that metabolises ADMA and MMA and thus the main determinant of serum concentrations, while the contribution of DDAH2 is unknown (Leiper et al. 1999; Altmann et al. 2012). It was previously hypothesized that glucose reduces DDAH activity (Lin et al. 2002), thereby decreasing degradation of ADMA and MMA (Siervo et al. 2011). This could provide a putative link between hyperglycaemia, arginine metabolism and cardiovascular disease. In Chapter 6 of this thesis, I report an *in vitro* study investigating the effect of glucose concentration on DDAH1 activity to support the clinical data in critically ill patients.

1.7 Statement of Aims

In summary, management of acute hyperglycaemia has advanced through the years, but there remain questions to be answered. Preliminary, mainly retrospective, observational studies have reported that relative hyperglycaemia is more strongly associated with adverse health outcomes in hospitalised patients than absolute hyperglycaemia. However, it is not clear how best to diagnose relative hyperglycaemia, nor to what extent relative

hyperglycaemia is simply a marker of disease severity or whether it directly affects health outcomes. Clinical trials of intensive insulin therapy to lower blood glucose in critically ill and AMI patients have reported conflicting results. These studies have utilised absolute glucose targets and, if relative hyperglycaemia is a more important variable than absolute hyperglycaemia, it is possible that variability in treatment of relative hyperglycaemia between studies could have affected the results. Finally, there are data suggesting that lowering blood glucose in hospitalised patients has greater benefits in patients without diabetes than in patients with diabetes. These finding are consistent with the hypothesis that it is more important to treat relative, than absolute, hyperglycaemia.

In this PhD project I hypothesized that:

1. Relative rather than absolute hyperglycaemia is associated with mortality in acutely ill hospitalized patients.
2. The association between relative hyperglycaemia and mortality is similar in patients with and without diabetes.
3. Arginine metabolites independently affect mortality in critically ill patients.
4. The relationship between relative hyperglycaemia and mortality is mediated by changes in arginine metabolites.

Thus, the major aims of this thesis were to determine whether:

1. Relative hyperglycaemia is more strongly associated with mortality than absolute hyperglycaemia in patients with acute myocardial infarction and critical illness.
2. The association between relative hyperglycaemia and mortality is affected by background glycaemia.
3. Arginine metabolites are independently associated with mortality in critically ill patients.
4. Relative hyperglycaemia results in changes in arginine metabolites that may underlie the associated increase in mortality

Chapter 2 Methods

2.1 Introduction

This PhD project comprises two clinical studies and an *in vitro* study. Chapter 3 reports a retrospective analysis of the association between relative hyperglycaemia and mortality in the HI-5 study (N W Cheung et al. 2008). The data were collected under the supervision of the original study authors, Professor Wah Cheung, Associate Professor Vincent Wong and Professor Mark McLean. I performed the statistical analysis for this study and drafted the manuscript. Chapter 4, Chapter 5 and Chapter 6 report analyses of a prospective study investigating the relationship between relative hyperglycaemia and arginine and its metabolites with mortality in critically ill patients. For this study I assisted with study design, drafted the ethics application, collected the data, performed the statistical analysis and drafted the associated manuscripts. Arginine and its metabolites were measured in the laboratory of Professor Ciriaco Carru by Dr. Salvatore Sotgia with methodology developed with Assoc Prof Angelo Zinellu (University of Sassari, Italy). In Chapter 6 a small *in vitro* study is also included as it supports the clinical data. This was conducted by Dr Sara Tommasi, Department of Clinical Pharmacology, Flinders University.

2.2 Storage, Transportation and Analysis of Samples

For the prospective study reported in this thesis (Chapter 4-6), samples of blood collected for clinical purposes that are routinely stored at 4°C by SA Pathology for up to 10 days were utilised (Figure 2-1). If patients did not have HbA1c requested during the ICU admission this parameter was requested using blood in a stored EDTA tube. A further 0.2-0.5 mL of plasma was also taken from a sodium heparin tube for each patient and stored in a -70 or -80°C freezer for subsequent assessment of arginine and its metabolites. If patients did not have adequate suitable samples for these analyses they were excluded.

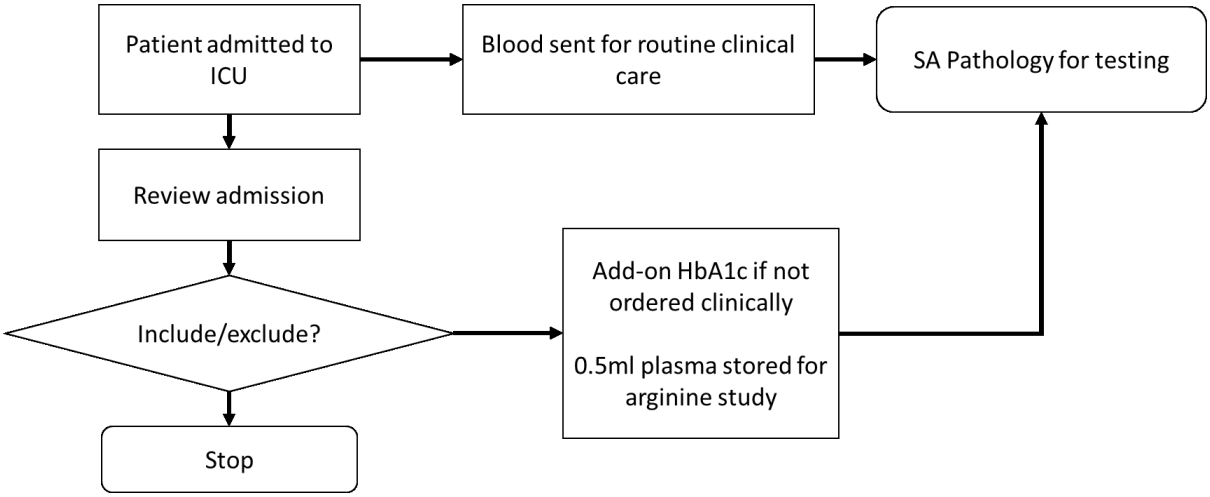


Figure 2-1 Recruitment and measurement of HbA1c

2.3 Assessment of Absolute and Relative Hyperglycaemia

2.3.1 Glucose

In Chapter 3, the original HI-5 study utilised capillary glucose measurements as their measure of absolute glycaemia (N Wah Cheung, Wong, and McLean 2006). As the HI-5 study was a randomized-controlled trial of treatment of hyperglycaemia during which glucose was systematically recorded I used mean glucose on treatment as the measure of absolute glycaemia.

Point-of-care glucose meters and capillary glucose measurements have been shown to be less reliable in critically ill patients (Pereira et al. 2015), and less reproducible in general (Colagiuri et al. 2003). Therefore, the studies conducted in the ICU and reported in Chapter 4-6 used venous plasma glucose measurements by the Flinders Medical Centre pathology service, SA Pathology, to measure absolute glycaemia. These were performed using a Roche P modular analyser (Hitachi High-technologies Corporation, Tokyo, Japan) using the hexokinase/glucose-6-phosphate dehydrogenase assay (between run CV 1.7% at glucose 4.9 mmol/L and 1.4% at glucose 15.7 mmol/L), As the prospective study reported in Chapter 4-6 was an observational study without systematic laboratory glucose measurements I pre-

specified admission glucose as the measure of absolute glycaemia. The closest glucose measurement to time of admission was used in analyses.

2.3.2 Glycosylated haemoglobin

In Chapter 3, HbA1c measurements were performed by the hospital pathology services in the six participating hospitals. The specific details for those measurements are not available. In Chapter 4-6, HbA1c measurements were measured by SA Pathology using High Performance Liquid Chromatography (HPLC) by PDQ, Primi's Diagnostics, Kansas City, Mo, USA and boronate affinity chromatography. The between-run CV was 2.2% at a HbA1c of 6.1% [43 mmol/mol] and 1.9% at HbA1c of 11.1% [98 mmol/mol]).

2.3.3 Calculation of relative hyperglycaemia

HbA1c levels were then translated into an estimated average glucose over the last 3 months using the equation derived by Nathan et al (Nathan et al. 2008):

$$\text{Estimated average glucose} = \left(1.59 \times HbA_{1c}\right) - 2.59$$

Relative hyperglycaemia was quantified using the Stress Hyperglycaemia Ratio using the formula (Roberts et al. 2015) :

$$SHR = \frac{\text{Current glucose}}{\text{Estimatedaverageglucose}}$$

2.4 Assessment of Risk of Death in Intensive Care

Risk of death in ICU can be predicted using various validated measures detailed in section *1.5.5 Prognostic Scoring in ICU*. The data for these calculations were collected as part of routine clinical care for submission to the Australian and New Zealand Intensive Care Society (ANZICS) Clinical Outcomes Research (CORE) audit by dedicated clinical research nurses and administration staff in the ICU. For retrieval of data, I worked closely with the clinical research nurses collecting ANZICS data, including the calculated risk of death scores

based on APACHE II and IIIj (the variant of APACHE III used by ANZICS). These data were available in an Excel spreadsheet which was then merged into my database for analysis.

In Chapter 4, risk of death scores were calculated from the APACHE II score, as it does not contain glucose and glucose was included in analyses as an independent variable (William A. Knaus et al. 1985). In Chapter 5, the risk of death score was derived from the APACHE IIIj score, as this is a more modern derivation of the APACHE scoring system and glucose was not a variable in the analysis. The risk of death score based on the APACHE II and APACHE IIIj scoring systems is computed for all patients by the ANZICS CORE database.

2.5 Assessment of Arginine and Metabolites

Arginine and related metabolites (monoethyl-L-arginine [MMA], L-homoarginine [HA], asymmetric dimethyl-L-arginine [ADMA], symmetric dimethyl-L-arginine [SDMA]), were measured in Italy at the University of Sassari under the supervision of Salvatore Sotgia (Sotgia et al. 2019).

The method was as follows:

1. 200 µL aliquots of plasma were spiked with 1 µL of internal standard solution containing L homoarginine-d4 dihydrochloride (d4-hArg), NG,NG-dimethyl-L-arginine-d6 dihydrochloride (d6-ADMA), and NG,NG'-dimethyl-L-arginine-d6 (d6-SDMA).
2. After vigorous vortex mixing, tubes were placed in a block heater for 5 min at 100 °C then cooled to room temperature.
3. After the addition of 400 µL of ultrapure water (Milli-Q grade), tubes were vortexed vigorously for 10 sec to displace the clot from the bottom of the vial.
4. Tubes with dislodged clots were then heat-treated again for 5 min at 100 °C, cooled down to room temperature, and centrifuged at 17,000×g for 5 min.
5. A 200-µL volume of clear supernatant was recovered and added with 20 µL of potassium phosphate monobasic buffer (100 mmol/L, pH 7.0) and 40 µL of diethylpyrocarbonate (33 mmol/L).

6. Tubes were mixed thoroughly and after leaving them at room temperature for 1 min, 20 µL of sample were analyzed by an Agilent liquid chromatography tandem mass spectrometry (LC-MS/MS) system (Agilent Italia, Milan, Italy) using a 100 mm × 4.6 mm Agilent Zorbax Eclipse Plus C18 3.5 µm column and a mixture of an aqueous solution of 0.4% v/v formic acid and acetonitrile (95:5) as a mobile phase, delivered isocratically at a flow-rate of 0.8 mL/min.
7. Mass detection was accomplished in positive ion mode by MRM of the precursor-product ion transitions m/z 247.14→142, 261.28→70, 261.28→84, 275.33→46, and 275.33→70 for arginine, MMA, L-homoarginine, ADMA, and SDMA, respectively, as well as m/z 265.28→88, 281.3→52, and 281.3→70 for d4-homoarginine, d6-ADMA, and d6-SDMA, respectively.

2.6 Assessment of Human DDAH1 Activity

In Chapter 6, the effect of glucose concentration on DDAH1 activity was assessed *in vitro* by Dr Sara Tommasi to support the clinical data I collected, using a method previously reported (S Tommasi et al. 2017). In this experiment, DDAH1 is incubated with its substrate, ADMA, and with varying concentration of glucose (0, 5, 7.5, 10, 15, 22.5mM). Citrulline is then a measure of DDAH1 activity, as ADMA is metabolised to citrulline in the absence of a DDAH1 inhibitor. As glucose has been postulated to modulate arginine metabolomics through inhibition of DDAH1, a decreasing amount of citrulline would be expected with higher concentrations of glucose (J. H. Lee et al. 2011).

The detailed process is as follows:

HEK293T cells expressing DDAH1 were lysed in 100 mM phosphate buffer (pH 7.4) by sonication at 16 × 1 sec bursts at 40% amplitude (Sonics Vibracell). Reactions for L-citrulline formation were undertaken in glass tubes at 37 °C in a 100 µL volume comprising 100 mmo/L phosphate buffer (pH 7.4), DDAH1 cell lysate (40 µg), glucose (0, 5, 7.5, 10, 15 and 22.5 mmol/L) and ADMA as substrate (45 µmol/L, equivalent to Km). The DDAH1

inhibitor ZST316 (compound 10a, (Sara Tommasi et al. 2015)) was used as a positive control for DDAH1 inhibition at a concentration of 1 µmol/L. Following a 60 min pre-incubation at 37 °C, reactions were initiated by the addition of ADMA, and were terminated after 30 min by the addition of 10 µL of an aqueous solution of the assay internal standard (L-citrulline-d6, 30 µmol/L) and 300 µl 0.1% formic acid in isopropanol. Each reaction mixture was vortex mixed, cooled on ice and centrifuged at 18,000 × g for 5 min. A 300 µL aliquot of supernatant was transferred into a glass tube and evaporated to dryness in a MiVac (50 °C, -OH programme, 25 min) (SP Scientific). Samples were reconstituted in 125 µL of 1:4 water/0.1% formic acid in isopropanol and a 3 µL aliquot assayed by UPLC-MS.

Citrulline analysis was performed using an Aquity Ultra Performance Liquid Chromatography, UPLC, (Waters, Sydney, Australia) coupled to a quadrupole time-of-flight (qToF) Premier high-resolution mass spectrometer (Waters, Sydney, Australia). Time-of-flight (ToF) data were collected in MS mode between 100 and 500 Da with an instrument scan time of 0.5 sec and inter-scan delay of 0.05 sec. Experimental parameters were as follows: capillary voltage 3.5 kV, source temperature 90°C, desolvation temperature 300°C, sampling and extraction cone voltages were 20 and 5 eV, respectively. The collision gas flow was 0.4 mL/min. Instrument control, data acquisition and data processing were performed using Waters MassLynx version 4.1 software.

Chromatographic separation was performed at a flow rate of 0.3 mL/min on a Waters ACQUITY UPLC® BEH HILIC column (1.7 µm, 2.1 mm x 100 mm) held at 35°C. Mobile phase composition was 0.1% v/v formic acid in acetonitrile (mobile phase A) and 10% v/v acetonitrile in water containing 0.1% v/v formic acid (mobile phase B). Initial conditions were 95% mobile phase A and 5% mobile phase B. The proportion of mobile phase B was increased linearly to 40% over 5 min and then returned to 5% for 2 min to re-establish equilibrium before injection of the following sample for analysis. Extracted ion chromatograms (EICs) were obtained with a mass window of 0.02 Da from total ion

chromatograms (TIC) employing the m/z corresponding to the 176.10→159.10 and 181.13→165.12 fragments of L-citrulline and L-citrulline-d6 respectively. For quantitation, L-citrulline was spiked into the incubation matrix at 6 concentrations (0, 1, 2, 3, 4 and 5 μM) then extracted and reconstituted in the same manner as incubation samples. A calibration curve was constructed by plotting the peak area ratio L-citrulline to internal standard versus the L-citrulline concentration.

2.7 Other Laboratory Tests

All other laboratory tests were performed under standard laboratory procedures at NSW Health Pathology (Chapter 3) and SA Pathology (Chapter 4-6). eGFR was calculated using the Modification of Diet in Renal Disease Study (MDRD) equation (Levey et al. 2006):

$$eGFR = 186 \times \left(\frac{\text{Creatinine}}{88.4} \right)^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female})$$

2.8 Determination of Mortality

The primary source of outcomes data was the data submitted to ANZICS. I also recorded all deaths manually for every ICU visit, to ensure data was robust, especially for in-hospital death after discharge from the ICU. For patients transferred to another hospital, patients were manually tracked for mortality up to 90 days post-transfer using the state-wide computing system (OACIS).

2.9 Data Storage and Linkage

The study reported in Chapters 4-6 of this thesis was prospectively registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) trial registry ACTRN12615001164583 on 2/11/2015. The registration information can be accessed at: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=369388.

For this study, I developed a password protected database using Filemaker 14.04 (Filemaker Inc, USA, an Apple subsidiary, 2015) and placed it within the hospital network. The database was developed to simplify collection of data, and to ensure appropriate inclusion and exclusion criteria were applied (Figure 2-2). The database also allowed immediate estimation of the relationship between Stress Hyperglycaemia Ratio and mortality rate (Figure 2-3). The use of a specialized database was important as the dataset was very large. It facilitated easier merging of the collected data with data from the intensive care registry, automated collection of HbA1c data from SA Pathology, merging of arginine metabolomic data from Italy and allowed relevant data to be exported to spreadsheets such as Excel or to statistical packages such as SPSS for further data analysis.

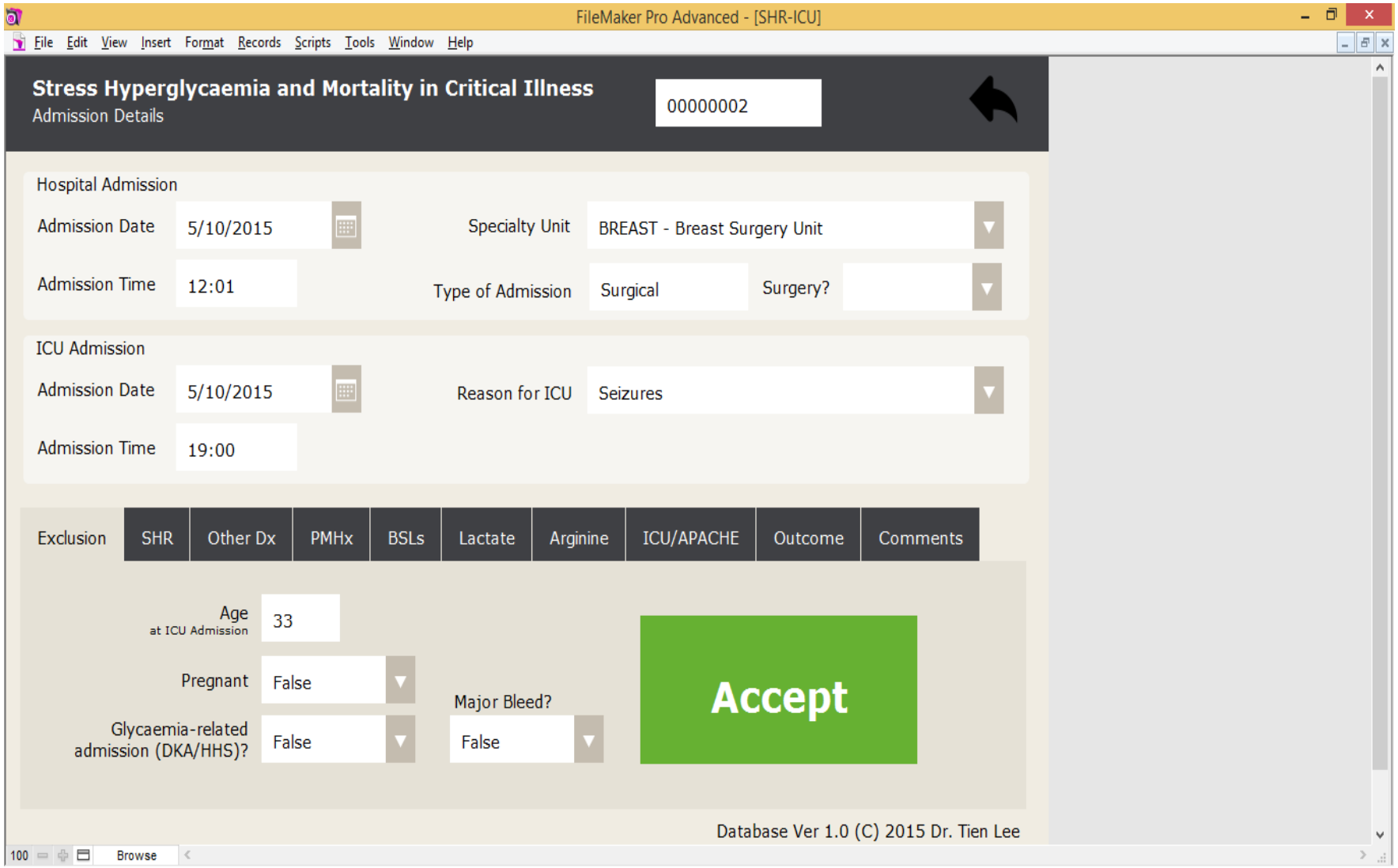


Figure 2-2 Database software to store data. It automatically checks for eligibility for recruitment to reduce recruitment error.

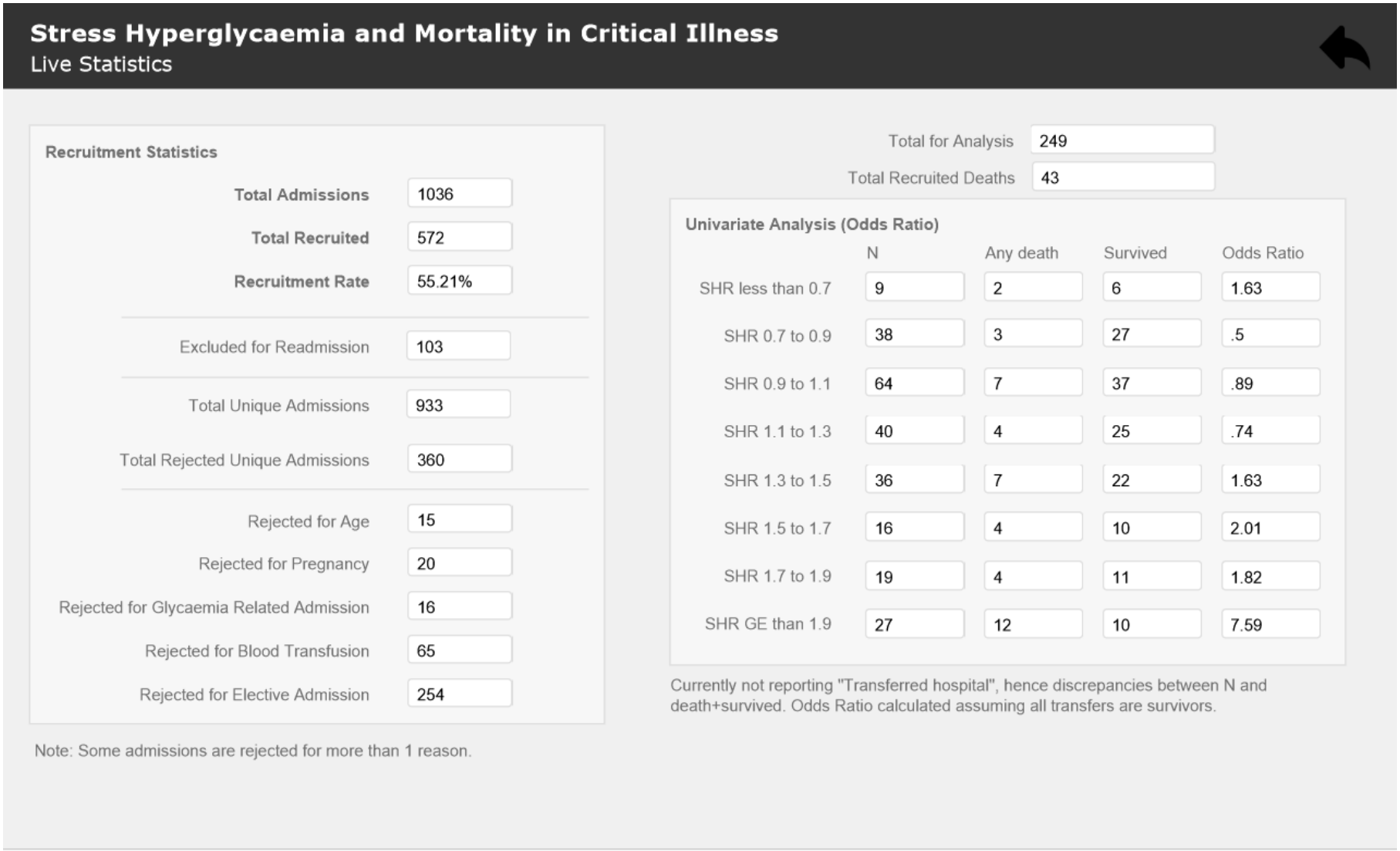


Figure 2-3 Live statistics from database software to check on progress during research.

2.10 Statistical analyses

Statistical analyses were performed using IBM SPSS version 23 for Windows (IBM, New York, USA) initially in Chapter 3 and Chapter 4 but subsequently on version 25 for Chapter 5 and Chapter 6. A p-value of <0.05 was considered statistically significant. Receiver Operating Characteristics (ROC) analyses were performed using MedCalc Software version 18.2.1 for Windows (MedCalc Software Ltd, USA). Subject characteristics were presented as mean +/- standard deviation if the data were normally distributed and median and interquartile range if they were not distributed normally. In Chapter 3 to 5, univariable binomial regression analyses were conducted and statistically significant variables were then included in multivariate analyses. In Chapter 6, simple linear regression analyses were used to compare the relationship between measures of glycaemic control and arginine metabolites. Variables were log-transformed when they were not normally distributed. Multiple linear regression was performed to ensure renal dysfunction did not affect results. Comparison of ROC analyses in Chapter 4 and Chapter 5 used a well-accepted methodology (DeLong, DeLong, and Clarke-Pearson 1988) for comparing the areas under two or more correlated ROC curves using a non-parametric approach. Odd ratios were computed and forest plots of the secondary outcomes after an acute myocardial infarction were constructed for Chapter 3.

Chapter 3 Relative hyperglycaemia is associated with complications following an acute myocardial infarction: a post-hoc analysis of HI-5 data.

Published without alteration to content as: Tien F. Lee, Morton G. Burt, Leonie K. Heilbronn, Arduino A. Mangoni, Vincent W. Wong, Mark McLean and N. Wah Cheung: Relative hyperglycaemia is associated with complications following an acute myocardial infarction: a post-hoc analysis of HI-5 data. Cardiovasc Diabetol 2017; 16:157

3.1 Author roles

TFL assisted with design of this analysis, performed the statistical analysis and drafted and revised the manuscript; MGB designed this analysis, assisted with the statistical analysis and drafted and revised the manuscript; LKH assisted with manuscript revision; AAM assisted with manuscript revision; VWW assisted with design of the HI-5 study, subject recruitment and manuscript revision; MM assisted with design of the HI-5 study, subject recruitment and manuscript revision; NWC designed the HI-5 study and assisted with subject recruitment and manuscript revision. All authors read and approved the final manuscript.

3.2 Abstract

Background: Hyperglycaemia is associated with increased morbidity and mortality in patients with an acute myocardial infarction (AMI). We evaluated whether complications after AMI are associated with absolute or relative glycaemia.

Methods: A total of 192 patients with AMI were randomized to intensive or conventional insulin therapy. Absolute glycaemia was defined as mean blood glucose level (BGL) during the first 24 hours following randomization. Relative glycaemia was defined by the stress hyperglycaemia ratio (SHR), calculated as mean BGL divided by average glucose concentration over the prior 3 months estimated from glycosylated haemoglobin. The primary endpoint was a "complicated AMI", defined as an AMI complicated by death, congestive cardiac failure, arrhythmia, cardiac arrest, reinfarction, cardiogenic shock, inotrope use or emergency revascularization.

Results: There was not a significant association between mean BGL and complicated AMI (odds ratio (OR) 1.05 per mmol/L glucose increment, 95% confidence intervals (CI) 0.93-1.19). In contrast, SHR was positively associated with a complicated myocardial infarction (OR 1.22 per 0.1 SHR increment, 95% CI 1.06-1.42), and individual complications of death (OR 1.55, 95% CI 1.14-2.11), congestive cardiac failure (OR 1.27, 95% CI 1.05-1.54), arrhythmia (OR 1.31, 95% CI 1.12-1.54) and cardiogenic shock (OR 1.42, 95% CI 1.03-1.97). The relationship between SHR and a complicated AMI was independent of diabetic status, intensive insulin therapy, sex and hypoglycaemia.

Conclusions: Relative, but not absolute, glycaemia during insulin treatment is independently associated with complications after an AMI. Future studies should investigate whether basing therapeutic glycaemic targets on relative glycaemia improves patient outcomes.

3.3 Background

Hyperglycaemia is associated with increased morbidity and mortality in hospitalized patients with a variety of medical conditions (Baker et al. 2006; Capes et al. 2001; 2000). However, while the association between glucose concentration in hospital and mortality is strong in patients without known diabetes, paradoxically glucose concentration is not as strongly associated with mortality in patients with diabetes (Capes et al. 2001; 2000; G. E. Umpierrez et al. 2002; Lanspa et al. 2013; Kim et al. 2017). This suggests that background glycaemia influences the relationship between glucose and mortality in patients admitted to hospital.

An elevated blood glucose in a hospitalized patient can occur because a patient has poor chronic glycaemic control or if there is an acute increase in glucose, often termed stress hyperglycaemia. (Dungan, Braithwaite, and Preiser 2009) Stress hyperglycaemia is the relative increase in glucose in response to an intercurrent illness. Our group has recently proposed a novel metric for relative glycaemia termed the *stress hyperglycaemia ratio* (SHR), whereby admission glucose concentration is corrected for background glycaemia estimated from glycosylated haemoglobin (HbA_{1c}) (Roberts et al. 2015). We reported that SHR was associated with critical illness in hospitalized patients, independent of absolute glycaemia. Moreover, the association between relative glycaemia and critical illness was present in patients with and without background hyperglycaemia. Since this publication, several other studies have reported that relative glycaemia at hospital admission predicts outcomes for patients admitted to hospital with a stroke (C.-J. Yang et al. 2017), acute illness (Su et al. 2017), acutely ill requiring intensive care unit (ICU) admission (W. Liao et al. 2015) and after percutaneous coronary intervention (Y. Yang et al. 2017). These studies demonstrate that quantifying relative hyperglycaemia at admission to hospital provides important prognostic information in patients with and without diabetes.

Calculation of relative hyperglycaemia could also potentially provide a basis for individualized glycaemic targets in patients treated with insulin in hospital. If relative hyperglycaemia during glucose-lowering treatment was associated with adverse patient

outcomes, it would support this hypothesis. However, in our previous study blood glucose levels (BGLs) were not systematically recorded in all patients throughout the hospital admission and this analysis could not be undertaken (Roberts et al. 2015).

The Hyperglycaemia: Intensive Insulin Infusion in Infarction (HI-5) study was a prospective randomized-controlled trial investigating the effect of intensive insulin therapy on mortality in patients with an acute myocardial infarction (N Wah Cheung, Wong, and McLean 2006). In the primary analysis, there was no significant difference in mortality in patients randomized to intensive insulin and conventional care. However, as finger prick BGLs were systematically recorded in both groups, the study cohort provides an opportunity to investigate the association between relative hyperglycaemia during glucose-lowering treatment and adverse patient outcomes.

We hypothesized that, in the HI-5 study cohort, relative hyperglycaemia during the first 24 hours following AMI would be more strongly associated with an adverse outcomes for patients than absolute glycaemia. If true, this would provide supportive evidence for a change in treatment paradigm whereby glucose-lowering treatment in patients following an AMI is targeted at relative, rather than absolute, hyperglycaemia. Therefore, the aim of this study was to assess the relationship between relative glycaemia and complications following a myocardial infarction and whether this relationship was affected by other potential confounding factors.

3.4 Methods

3.4.1 Study design

This is a secondary analysis of a prospective randomized-controlled trial that has previously been reported (N Wah Cheung, Wong, and McLean 2006). In brief, consecutive consenting patients presenting with an AMI at six hospitals in New South Wales, Australia with either known diabetes or without diabetes and BGL >7.8 mmol/L were randomized to receive insulin/dextrose infusion therapy for at least 24 hours to maintain finger prick BGL between 4

and 10 mmol/L or conventional therapy comprising their usual glucose-lowering therapy except metformin and supplemental subcutaneous short-acting insulin if finger prick BGL was ≥16 mmol/L. BGL were recorded at 8 pre-defined timepoints in all patients (0700, 0900,1200, 1400, 1700, 1900, 2200 and 0300 in all patients. Additional BGLs were performed on an hourly basis in patients on intensive insulin therapy. The study was approved by local ethics committees of Westmead Hospital, Nepean Hospital, Blacktown Hospital, Mt. Druitt Hospital and John Hunter Hospital. All subjects participating in the study provided written informed consent.

3.4.2 Subjects

This analysis included all participants in the HI-5 study in whom HbA_{1c} was measured at admission to hospital. Patients were treated with angioplasty, thrombolysis or anti-coagulation at the discretion of the admitting doctors. All patients received beta blocker therapy unless specifically contraindicated (N Wah Cheung, Wong, and McLean 2006). Demographic data, laboratory data, and in-hospital mortality and complications that were recorded at the time of the original study were used in the analysis. A Charlson Comorbidity Index was calculated from the co-morbidities recorded during the original data collection (Charlson et al. 1987).

3.4.3 Calculation of Stress Hyperglycaemia Ratio

Finger prick point of care capillary BGLs were measured at predefined time points during the first 24 hours as previously described (N Wah Cheung, Wong, and McLean 2006), and the readings were averaged to calculate mean BGL during glucose-lowering treatment for each subject. Estimated average glucose over the prior 3 months was calculated using the equation “estimated average glucose = (1.59 x HbA_{1c}) - 2.59” derived by Nathan et al. (Nathan et al. 2008) Relative hyperglycaemia during glucose-lowering treatment (SHR) was then calculated using the formula mean BGL divided by estimated average glucose.

3.4.4 Statistical methods

Before undertaking this secondary analysis we pre-specified a composite primary endpoint of a "complicated AMI," defined as AMI complicated by death during the hospital admission, congestive cardiac failure, arrhythmia, cardiac arrest, reinfarction, cardiogenic shock, inotrope use or the need for rescue percutaneous transluminal coronary angioplasty (PTCA) or emergency coronary arterial bypass graft (CABG). These events were defined as per the original HI-5 study (N Wah Cheung, Wong, and McLean 2006). Reinfarction was defined as a new AMI that occurred at least 72 hours following the index AMI. A patient was considered in congestive cardiac failure if there was documented dyspnea in the notes and the chest X-ray report confirmed pulmonary edema or interstitial edema. Cardiogenic shock referred to a state where the patient was in cardiac failure with a concomitant systolic blood pressure of less than 80 mmHg. The primary variables of interest were absolute hyperglycaemia, defined as mean BGL during glucose-lowering treatment and relative hyperglycaemia, defined as SHR during glucose-lowering treatment.

Characteristics of patients with and without a complicated AMI are reported as mean ± standard deviation if normally distributed and median (interquartile range) if the distribution was not normal. These variables were compared using unpaired t-tests, Mann-Whitney U-tests or Chi-squared tests as appropriate. Univariable binomial logistic regression analyses were undertaken to calculate the odds ratio of a complicated AMI for each BGL and SHR increment. Odds ratios for the individual components of the composite primary endpoint for each BGL and SHR increment were also calculated. We then included treatment group and known diabetes and interaction terms for these variables as co-variates in analyses to assess whether they moderated the relationship between SHR and a complicated AMI. Finally, univariable binomial logistic regression analyses were undertaken to examine whether other variables of interest such as sex, age, Charlson Comorbidity Index, hypoglycaemia, treatment type and peak creatinine phosphokinase were associated with a complicated AMI. If a variable was not normally distributed, it was log-transformed to achieve

a normal distribution before inclusion in regression analyses. Variables that were significantly associated with a complicated AMI in a univariable analysis were then included in a multiple binomial logistic regression analysis.

Statistical analysis was undertaken using SPSS version 23 for Windows (IBM, New York, USA). A two-tailed P-value of <0.05 was considered statistically significant.

3.5 Results

3.5.1 Patient characteristics

A total of 192 subjects were included in the final analysis; 48 patients out of the 240 patients included in the original study cohort were excluded as there were no recorded HbA_{1c}. In this cohort, 82 patients were defined as having a complicated AMI: 6 patients died, 22 developed congestive cardiac failure, 48 had an arrhythmia, 21 had a cardiac arrest, 3 had a re-infarction, 5 developed cardiogenic shock, 10 were treated with inotropic support, 26 required a rescue PTCA and 7 an emergency CABG. A number of subjects had more than one complication. Although subjects with a complicated AMI were more likely to be female, there were no significant differences in age, Charlson Comorbidity Index, intensive insulin therapy, known diabetes, other cardiovascular risk factors, cardiovascular medications, serum cholesterol, peak creatinine phosphokinase and use of anticoagulant, thrombolytic and angioplasty treatment and ST elevation on ECG between patients with a complicated and uncomplicated AMI (Table 3-1).

	Complicated AMI	Uncomplicated AMI	P value
Number	82	110	
Age (years)	63 (10)	61 (12)	0.108
Female (N, (%))	24 (29)	16 (15)	0.017
Charlson Comorbidity Index [a]	0.5 (0.0-1.0)	0.0 (0.0-1.0)	0.843
Intensive Insulin (N, (%))	44 (54)	58 (53)	0.898
Diabetes (N, (%))	38 (46)	53 (48)	0.801
Diagnoses prior to admission			
• **Hypertension (N, (%))**	50 (61)	57 (52)	0.287
• **Hyperlipidemia (N, (%))**	44 (54)	67 (61)	0.388
• **Smoker (N, (%))**	26 (32)	35 (32)	0.372
• **Previous AMI (N, (%))**	20 (24)	21 (19)	0.445
Medications at admission			
• **ACE Inhibitor (N, (%))**	16 (20)	23 (21)	0.845
• **Aspirin (N, (%))**	19 (23)	28 (25)	0.752
• **Beta-blocker (N, (%))**	17 (21)	13 (12)	0.098
• **CCB (N, (%))**	9 (11)	13 (12)	0.880
• **Fibrate (N, (%))**	1 (<1)	1 (<1)	0.826
• **Nitrate (N, (%))**	11 (13)	16 (15)	0.850
• **Statin (N, (%))**	22 (27)	31 (28)	0.918
Initial treatment			0.583
• **LMWH / Heparin (N, (%))**	22 (27)	39 (35)	
• **Thrombolysis (N, (%))**	33 (40)	20 (18)	
• **Acute PTCA (N, (%))**	24 (29)	49 (45)	
• **Not documented**	3 (4)	2 (2)	
Total Cholesterol (mmol/L)	4.67 (1.31)	4.92 (1.23)	0.197
Peak CPK [a]	923 (292-3130)	1115 (517-2844)	0.305
STEMI (N, (%)) [b]	62 (76%)	84 (76%)	0.780

Table 3-1 Characteristics of patients with and without a complicated myocardial infarction

Values represent mean (standard deviation) unless otherwise stated; [a] Median (interquartile range); [b] There were 6 patients missing STEMI status, 2 with uncomplicated AMI and 4 with complicated AMI.

ACE = Angiotensin Converting Enzyme; AMI = acute myocardial infarction; N = number of patients with endpoint; CCB = Calcium Channel Blocker; LMWH = low molecular weight heparin; PTCA =

percutaneous transluminal coronary angioplasty; Peak CPK = peak creatinine phosphokinase; STEMI = ST elevation on ECG or presence of Q-waves

3.5.2 Associations between absolute and relative glycaemia during glucose-lowering treatment and a complicated acute myocardial infarction

There was not a significant association between mean BGL and the incidence of complicated AMI (odds ratio (OR) 1.05 per mmol/L glucose increment, 95% confidence intervals (CI) 0.93-1.19, p=0.437) (Figure 3-1A). Furthermore, no individual component of the composite "complicated AMI" endpoint was significantly associated with mean BGL (Figure 3-1A). In contrast, SHR was associated with a complicated AMI (OR 1.22 per 0.1 SHR increment, 95% CI 1.06-1.42, p=0.006) (Figure 3-1B). Moreover, SHR was positively associated with death (OR 1.55, 95% CI 1.14-2.11, p=0.005), congestive cardiac failure (OR 1.27, 95% CI 1.05-1.54, p = 0.014), arrhythmia (OR 1.31, 95% CI 1.12-1.54, p = 0.001) and cardiogenic shock (OR 1.42, 95% CI 1.03-1.97, p = 0.033). SHR was not significantly associated with cardiac arrest, reinfarction, inotrope use, rescue angioplasty or emergency CABG (Figure 3-1B).

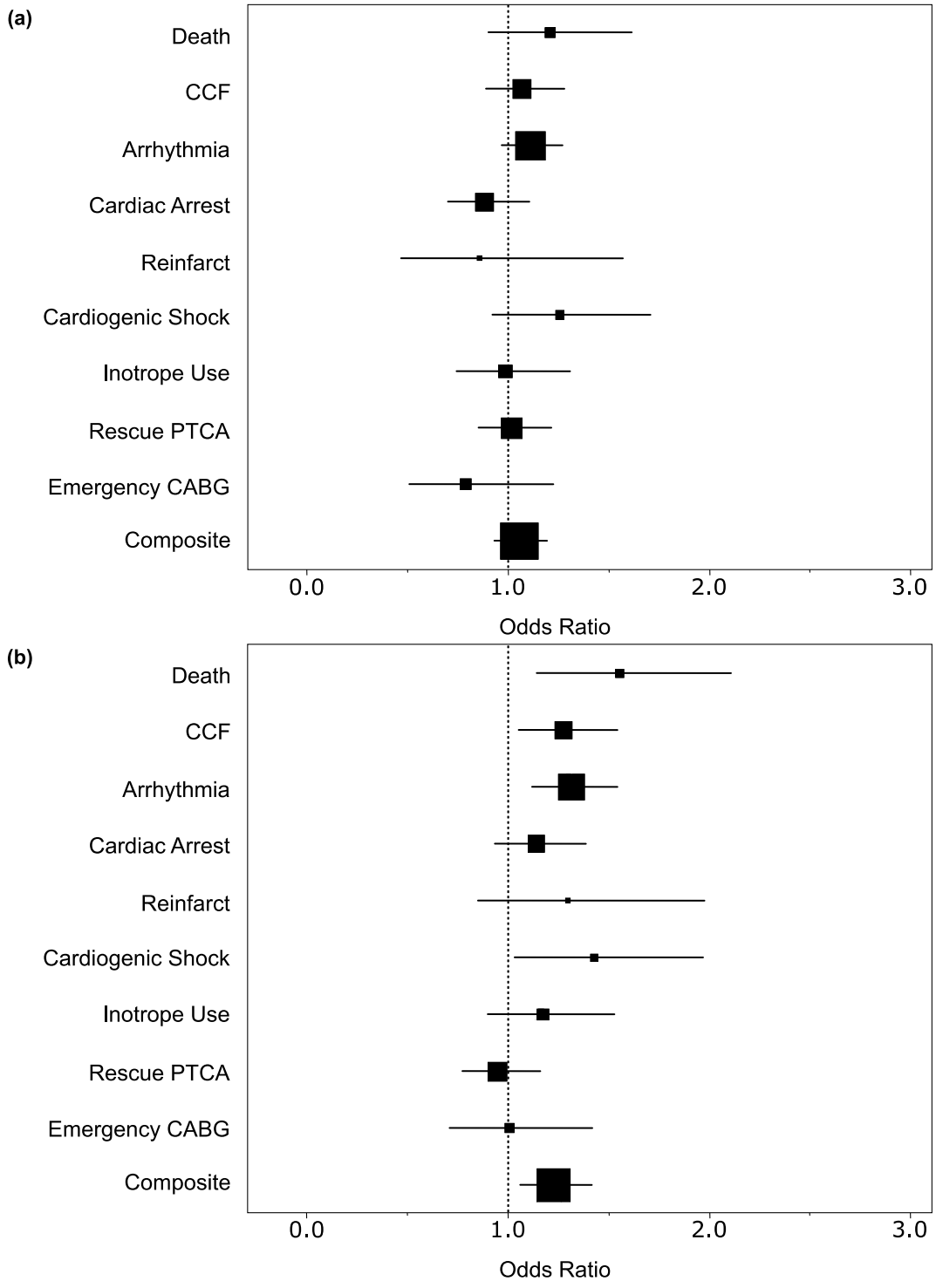


Figure 3-1 Odds ratios for the risk of complications of an acute myocardial infarction in 192 patients

(a) Complications per 1 mmol/L glucose increment and (b) 0.1 stress hyperglycaemia ratio increment. The size of the squares represents the observed frequency of each complication post myocardial infarction while the whiskers represent the 95% confidence intervals. CCF = congestive cardiac failure; PTCA = percutaneous transluminal coronary angioplasty; CABG = coronary arterial bypass graft.

3.5.3 Effect of diabetes and intensive insulin therapy on association between relative glycaemia and a complicated acute myocardial infarction

Figure 3-2A shows that the relationship between SHR and a complicated AMI was similar in patients with and without diabetes. The association between SHR and a complicated AMI was independent of diabetes status (OR 1.38 per 0.1 SHR increment, 95% CI 1.08-1.75 p=0.009), which was not independently associated with a complicated AMI (p=0.204). Furthermore there was not a significant interaction between SHR and diabetes status (p=0.216), demonstrating that diabetes status did not significantly modulate the relationship between SHR and a complicated AMI. Figure 3-2B shows that the relationship between SHR during insulin treatment and a complicated AMI was not different in patients randomized to intensive insulin and conventional therapy. The association between SHR and a complicated AMI was independent of intensive insulin therapy (odds ratio = 1.37 per 0.1 SHR increment, 95% CI 1.09-1.72, p=0.006), which was not associated with a complicated AMI (p=0.173). Moreover there was not a significant interaction between SHR and intensive insulin treatment (p=0.206), demonstrating that treatment group not significantly modulate the relationship between SHR and a complicated AMI.

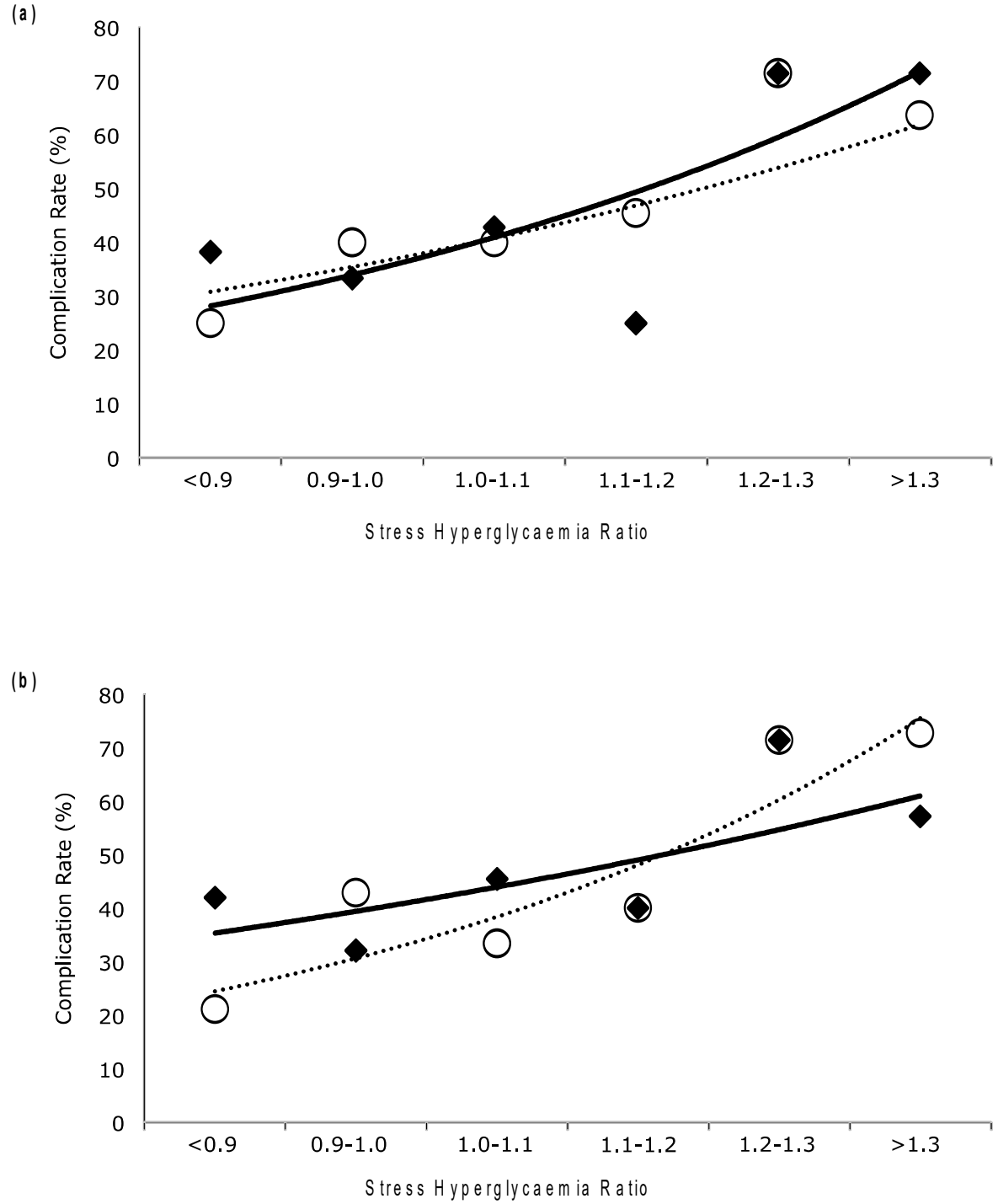


Figure 3-2 Rates of a complicated acute myocardial infarction per stress hyperglycaemia ratio range in 192 patients

(a) Patients with (◆, solid line) and without (○, dotted line) Diabetes Mellitus; and (b) patients receiving intensive (◆, solid line) or conventional (○, dotted line) glucose-lowering therapy.

3.5.4 Effect of other variables on a complicated acute myocardial infarction

In univariate analyses, female sex (p=0.015) and hypoglycaemia (p=0.015) were significantly associated with a greater risk of a complicated AMI (Table 3-2). Age, Charlson Comorbidity Index, treatment of the AMI, peak creatinine phosphokinase and ST elevation on ECG were not significantly associated with a complicated AMI. In a multiple regression analysis the relationship between SHR and a complicated AMI was independent of sex and hypoglycaemia (Table 3-2). Female sex and hypoglycaemia were also independently associated with a complicated myocardial infarction.

3.6 Discussion

Our secondary analysis of the HI-5 study observed no significant association between absolute glycaemia during glucose-lowering treatment, defined as the mean finger prick BGL during the first 24 hours post-AMI, and a complicated AMI. In contrast, when mean BGL was corrected for background glycaemia to estimate relative glycaemia, it was positively associated with a complicated AMI. Moreover, the association with relative glycaemia was independent of diabetes status, treatment group and a number of other variables that potentially affect the prognosis after an AMI. As relative glycaemia during glucose-lowering treatment is more strongly associated with patient outcomes than absolute glycaemia, we hypothesize that defining individualized therapeutic glucose targets based on relative, and not absolute, glycaemia could potentially reduce morbidity and mortality after an AMI.

Variable	Univariate		Multivariate	
	Beta	p-value	Beta	p-value
Sex	-0.888	0.015	-0.917	0.017
Age	0.021	0.108	NA	
SHR	0.202	0.006	0.245	0.002
Charlson Score [a]	1.105	0.740	NA	
Hypoglycaemia	1.109	0.049	1.218	0.038
Treatment [b]	-0.094	0.590	NA	
Peak CPK [a]	-0.382	0.169	NA	
STEMI [c]	0.102	0.779		

Table 3-2 Univariate and multivariate analyses of associations between selected variables and a complicated acute myocardial infarction

[a] Log transformed for statistical analysis; [b] Treatment groups were heparin or low-molecular weight heparin, thrombolysis or percutaneous transluminal coronary angioplasty. [c] There were 6 missing STEMI status which were excluded in this analysis.

NA = not assessed; SHR = stress hyperglycaemia ratio; Peak CPK = peak creatinine phosphokinase; STEMI = ST elevation on ECG or presence of Q-waves.

3.6.1 Absolute hyperglycaemia and mortality

A number of studies have reported that glucose concentration on admission to hospital is positively associated with mortality in patients with an AMI (Capes et al. 2000; Kosiborod et al. 2008; Wernly et al. 2016; Baker et al. 2006; Kosiborod et al. 2005; Goyal et al. 2009). Moreover, the association between mortality and mean glucose during hospitalization is even stronger than with admission glucose (Kosiborod et al. 2008; Deedwania et al. 2008; Suleiman et al. 2005; Goyal et al. 2006). In contrast, in the HI-5 patient cohort mean glucose concentration during glucose-lowering treatment was not significantly associated with a complicated AMI or any individual component of this composite endpoint. There are a number of potential explanations for this negative result. Firstly, approximately half the patients in the HI-5 study had diabetes and the association between glucose concentration and mortality is attenuated in this group (Capes et al. 2000; Kosiborod et al. 2008; G.

Umpierrez et al. 2015). Secondly, the primary endpoint of this analysis is a composite and there may be individual components of the endpoint that are not related to glucose concentration. Most importantly, the sample size in this analysis is relatively small compared to previous studies.

3.6.2 Relative hyperglycaemia and mortality

Measurement of HbA_{1c} in addition to glucose has revolutionized the management of chronic hyperglycaemia, where HbA_{1c} is both a diagnostic test for diabetes and the primary measurement used to guide the need for and efficacy of glucose-lowering therapy (Chamberlain et al. 2016). In addition to a clear relationship with microvascular disease, a lower HbA1c during long-term follow-up has been associated with reduced macrovascular disease (Wang et al. 2015; Bots et al. 2016). It has also been hypothesized that HbA_{1c} could be used to quantify stress or relative hyperglycaemia and assist management of acute hyperglycaemia (Braithwaite 2010; Marik and Egi 2014). However, other studies have utilized varying definitions of stress hyperglycaemia based on absolute glucose concentrations, sometimes taking a patients' diabetic status into account (Deedwania et al. 2008; Koracevic et al. 2008; Fujino et al. 2014). While it has been proposed that therapeutic targets should differ in patients with and without diabetes, the optimum method to quantify relative glycaemia remains to be determined (Koracevic 2016; Marik and Egi 2014). SHR is a novel metric to quantify relative glycaemia in a single numerical value that is cheap to measure and simple to calculate. It directly relates a patient's current glucose control during an acute presentation to their background glycaemia, providing an individualized quantification of stress or relative hyperglycaemia.

In contrast to absolute glycaemia, relative glycaemia during glucose-lowering treatment was positively and significantly associated with mortality, heart failure, arrhythmia, cardiogenic shock and a composite endpoint of a complicated AMI in the HI-5 study. These associations between relative glycaemia and a number of adverse patient outcomes are striking, given the fairly small sample size in this analysis. Our study extends previous observations by

demonstrating that relative hyperglycaemia during glucose-lowering treatment is also associated with adverse outcomes in patients with and without diabetes. This suggests that a management strategy that selectively targets glucose-lowering therapy for patients with an elevated SHR irrespective of prior diabetes status may improve outcomes after an AMI.

Others have reported that relative hyperglycaemia at admission to hospital has been associated with poor outcomes in patients with acute illness (Su et al. 2017), stroke (C.-J. Yang et al. 2017), acute myocardial infarction (W.-I. Liao et al. 2016; Fujino et al. 2014) and in cardiogenic shock (Kataja et al. 2017). In addition to SHR two other measures of relative glycaemia have been proposed. The “glycaemic gap” is the difference between the admission glucose and the estimated average glucose i.e it is the absolute rather than the relative difference in glucose concentration (W.-I. Liao et al. 2016; W. Liao et al. 2015; W.-I. Liao et al. 2013). Another proposal was for a “glucose concentration-to-HbA1c ratio”, in which the admission glucose is divided by the HbA1c (C.-J. Yang et al. 2017). This is theoretically similar to SHR. Whether one measure has advantages over the others has yet to be fully determined, although they are likely perform similarly (C.-J. Yang et al. 2017).

3.6.3 Effects of diabetes status and insulin use

During long-term follow-up of patients with AMI diabetes is associated with increased mortality (Kuhl et al. 2015; Pararajasingam et al. 2016). However, during a hospital admission the association between absolute glycaemia and mortality is stronger in patients without, as opposed to with, diabetes (Kosiborod et al. 2005; Capes et al. 2000; Kim et al. 2017). We previously reported that the relationship between relative glycaemia and critical illness was similar in patients with and without background hyperglycaemia. (Roberts et al. 2015) In this analysis, relative glycaemia was also associated with a complicated AMI independent of diabetes, which was not independently associated with a complicated AMI (Figure 2A). This suggests that relative hyperglycaemia is clinically important regardless of diabetes status.

A potential confounder in this analysis is that half the cohort was randomized to intensive insulin therapy, which will lower absolute, and consequently relative, glycaemia. However, the 0.7 mmol/L difference in absolute glucose between the two groups was not statistically significant (N Wah Cheung, Wong, and McLean 2006). Furthermore, the positive association between relative glycaemia and a complicated AMI was independent of treatment group. Treatment group itself was not independently associated with a complicated AMI and there was not a significant interaction between treatment group and SHR in regression analysis. This suggests that insulin treatment did not confound the relationship between relative glycaemia and a complicated AMI.

3.6.4 Other variables affecting outcomes

The other variables that were independently associated with a complicated AMI were female sex and hypoglycaemia. The association between female sex and a poorer outcome after an AMI is consistent with previous studies (Maas and Appelman 2010; Hochman et al. 1999). An association between hypoglycaemia after an AMI and mortality is well described (Goyal et al. 2009; Kosiborod et al. 2008; Kosiborod, Inzucchi, Goyal, et al. 2009). However, previous studies have reported that the association is predominantly in patients with spontaneous hypoglycaemia while not on insulin therapy and at admission to hospital (Goyal et al. 2009; Kosiborod, Inzucchi, Goyal, et al. 2009). In our study, hypoglycaemia during glucose-lowering treatment was independently associated with a complicated AMI. This suggests that if a therapeutic approach targeting relative glycaemia were to be trialled, it will be important to avoid hypoglycaemia.

Some variables that are usually associated with poor outcomes after AMI were not statistically significant in our analyses. ST elevation was not a predictor of complicated AMI. Our analysis cannot determine the reason for this, but we postulate contributory factors include that only patients with STEMI were revascularized, the primary endpoint was composite and that the sample size was relatively small. The association between age and complicated AMI was positive but did not reach statistical significance (p=0.108). Peak CK

also did not show an association with poorer composite outcomes. Only peak CK was measured and recorded, as CKMB and troponin measurements were not routine at the time of the HI-5 study. Other variables can affect peak CK, such as procedures like PTCA.

3.6.5 Mechanisms linking stress hyperglycaemia and patient outcomes

An elevation in plasma glucose concentration is potentially an epiphenomenon and not directly contributing to cardiovascular events. For example, patients with an AMI have elevations of serum cortisol that could mediate both an increase in blood glucose and contribute to increased cardiovascular risk (Carmen Wong et al. 2010; Petersons et al. 2014; 2013). However, a number of mechanisms have been proposed by which stress hyperglycaemia could directly increase morbidity and mortality after an AMI. These include endothelial apoptosis, endothelial dysfunction and oxidative stress (Quagliaro et al. 2003; Monnier et al. 2006; Ceriello et al. 2008; Evans et al. 2002). Interventional studies demonstrating that lowering plasma glucose reduces cardiovascular events are needed to confirm that stress hyperglycaemia *per se* increases cardiovascular risk.

Studies that have lowered glucose concentration to a target absolute glucose range using insulin therapy have produced conflicting results (K Malmberg 1997; K Malmberg et al. 2005; N Wah Cheung, Wong, and McLean 2006). Consequently, the importance of this therapeutic approach has not been clearly defined, supported only by a low-level of evidence in clinical practice guidelines (Deedwania et al. 2008; Judith Jacobi 2016; G. E. Umpierrez et al. 2012). As relative glycaemia during glucose-lowering treatment is more strongly associated with adverse outcomes, we propose that SHR could be used to derive individualized glycaemic targets for patients with an AMI. If this improved patient outcomes it would represent a paradigm shift in the management of hyperglycaemia in hospitalized patients. Treatment of relative hyperglycaemia should be the subject of future interventional studies.

3.6.6 Strengths and Limitation

The strengths of this study include the use of a novel metric that quantifies stress hyperglycaemia as a continuous variable, a patient population that reflects usual clinical practice, patient data that was systematically and prospectively recorded, and defining a pre-specified primary endpoint before undertaking the analysis. However, we acknowledge that the study has limitations. This is a retrospective, post-hoc analysis, and is thus hypothesis generating. The study design cannot show causality, does not assess mechanisms by which relative hyperglycaemia could confer a poorer prognosis and does not distinguish whether spontaneous normalization of glucose or insulin treatment underlies a relationship between relative hypoglycaemia and a complicated AMI. In particular, it cannot distinguish whether relative hyperglycaemia is simply a marker of the severity or duration of an AMI, or directly contributes to poorer outcomes. Moreover, we did not assess the effect of other variables in this analysis that potentially affect cardiovascular outcomes, such as glycaemic variability and postprandial hyperglycaemia (Strojek et al. 2016; Okada et al. 2015). Another potential limitation is that glucose was assessed using point of care BGLs, which may differ from gold standard laboratory measurements. However, this approach reflects usual clinical practice. Management of AMI has also changed since the HI-5 study, notably the greater use of PTCA. This could potentially affect results, although treatment was not a predictor of outcome in this analysis. The mortality rate was fairly low in this study and the results may not be able to be extrapolated to patient groups with a higher mortality rate. Finally, the sample size was relatively small, which may have resulted in a type 2 error, especially in subgroup analysis of treatment groups as the numbers of patients in the insulin-treated group with a high SHR were low. Nevertheless, the study was of sufficient size to show a significant independent association between relative glycaemia and several cardiovascular endpoints.

3.7 Conclusion

Absolute glucose concentration during glucose-lowering treatment following an AMI was not significantly associated with a complicated AMI. In contrast, SHR was positively associated with mortality, heart failure, arrhythmia, cardiogenic shock and a composite endpoint of a complicated AMI. We conclude that relative glycaemia during glucose-lowering treatment is more strongly associated with adverse outcomes than absolute glycaemia in patients after an AMI. This research adds to other published research by demonstrating the prognostic utility of quantifying relative hyperglycaemia during glucose-lowering treatment after an AMI, and should provide a basis for prospective studies using relative, rather than absolute, glycaemic thresholds for intervention and therapeutic glycaemic targets.

3.8 Declaration

3.8.1 Ethics approval and consent to participate

The study was approved by local ethics committees of Westmead Hospital, Nepean Hospital, Blacktown Hospital, Mt. Druitt Hospital and John Hunter Hospital. All subjects participating in the study provided written informed consent.

3.8.2 Consent for publication

Not applicable.

3.8.3 Availability of data and materials

The data that support the findings of this study are available from the HI-5 group of researchers but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the original authors of the HI-5 study.

3.8.4 Competing interests

The authors declare that they have no competing interests.

3.8.5 Funding

The HI-5 study was supported by a project grant from the National Health and Medical Research Council, Australia. Novo Nordisk sponsored a dinner meeting for the HI-5 study. TL is supported by a scholarship from the National Health and Medical Research Council, Australia and the Royal Australasian College of Physicians Kincaid-Smith scholarship.

Chapter 4 Relative hyperglycaemia is an independent determinant of in-hospital mortality in patients with critical illness

Published without alteration to content as: Tien F Lee, Sophie M. Drake, Greg W. Roberts, Andrew Bersten, Stephen N. Stranks, Leonie K. Hielbronn, Arduino A. Mangoni, Morton G. Burt: Relative Hyperglycaemia Is an Independent Determinant of In-Hospital Mortality in Patients With Critical Illness. Crit Care Med 48:e115–e122.

4.1 Author Roles

TFL assisted with design of this study, recruited to patients, collected the data, performed the statistical analysis and drafted and revised the manuscript; SD assisted with collection of data; GWR assisted in manuscript revision; AB provided advice in study design and assisted with manuscript revision; SNS assisted with manuscript revision; LKH assisted with manuscript revision; AAM provided advice in analysis and assisted with manuscript revision; MGB designed this study, assisted with the statistical analysis and drafted and revised the manuscript. All authors read and approved the final manuscript.

4.2 Abstract

Objective: To determine whether relative hyperglycaemia was associated with in-hospital mortality in critically ill patients independent of other prognostic variables and whether this association is affected by background glycaemia.

Design: Prospective observational study.

Setting: Mixed medical-surgical Intensive Care Unit (ICU) in a metropolitan teaching hospital.

Patients: From 2,617 admissions to ICU between 27 January 2016 and 30 March 2017, 1,262 consecutive patients who met inclusion and exclusion criteria were studied.

Interventions: Glycosylated haemoglobin was used to estimate average glucose concentration over the prior 3 months. Glucose concentration on ICU admission was divided by estimated average glucose concentration to calculate the *stress hyperglycaemia ratio* (SHR), an index of relative glycaemia. Risk of death score was calculated using data submitted to the Australia and New Zealand Intensive Care Society.

Measurements and Main Results: In this study, there were 186 (14.7%) deaths. Admission glucose was significantly associated with mortality in univariate analysis (odds ratio = 1.08 per mmol/L glucose increment, $p<0.001$) but not after adjustment for risk of death score (odds ratio = 1.01, $p=0.338$). In contrast, SHR was significantly associated with mortality both in univariate analysis (odds ratio = 1.09 per 0.1 SHR increment, $p<0.001$) and after adjustment for risk of death score (odds ratio = 1.03, $p=0.014$). Unlike admission glucose concentration, SHR was significantly associated with mortality in patients with HbA1c <6.5% (odds ratio = 1.08 per 0.1 SHR increment, $p<0.001$) and HbA1c ≥6.5% (48 mmol/mol) (odds ratio = 1.08 per 0.1 SHR increment, $p=0.005$).

Conclusions: Unlike absolute hyperglycaemia, relative hyperglycaemia, as assessed by *SHR*, independently predicts in-hospital mortality in critically ill patients across the glycaemic spectrum. Future studies should investigate whether using measures of relative

hyperglycaemia to determine individualized glycaemic treatment targets improves outcomes in ICU.

4.3 Introduction

Hyperglycaemia in hospitalized patients is associated with increased mortality in a wide range of patient groups, including the critically ill (G. E. Umpierrez et al. 2002; James Stephen Krinsley 2003; Baker et al. 2006; Capes et al. 2001; 2000). However, the association between hyperglycaemia and mortality is stronger in patients without known diabetes than those with diabetes (Baker et al. 2006; G. E. Umpierrez et al. 2002). Furthermore, there have been discordant results from studies prescribing intensive insulin therapy in Intensive care Unit (ICU) patients, whereby patients without diabetes benefit from intensive insulin therapy, while patients with diabetes do not (Greet Van den Berghe, Wilmer, Milants, et al. 2006; Lanspa et al. 2013). Consequently, it may be important to identify and treat patients with new hyperglycaemia differently to those with chronic hyperglycaemia.

Hyperglycaemia in a hospitalized patient can either occur in patients with poor chronic diabetes control, and be “expected” for that patient, or represent a transient physiologic response to an intercurrent illness (stress hyperglycaemia). In this manuscript, absolute hyperglycaemia refers to the blood glucose concentration *per se*, while relative hyperglycaemia is the acute rise of blood glucose concentration because of stress hyperglycaemia above background levels. Several recent studies have consistently demonstrated that the relative increase in glucose concentration is more strongly associated with adverse patient outcomes than absolute hyperglycaemia (Roberts et al. 2015; W.-I. Liao et al. 2013; W. Liao et al. 2015; W.-I. Liao et al. 2016; C.-J. Yang et al. 2017; Su et al. 2017). Moreover, this association has been reported in patients with and without diabetes (T. F. Lee et al. 2017; Roberts et al. 2015).

A subject of ongoing investigation is whether relative hyperglycaemia is simply an indicator of the severity of underlying illness, or contributes *per se* to increased mortality. If relative

hyperglycaemia directly affects mortality, then the association should be independent of other variables affecting this end-point. One study reported that in critically ill patients with diabetes relative hyperglycaemia was associated with mortality independent of APACHE II scores (W. Liao et al. 2015). However, the relationship between APACHE II score and mortality varies for different disease states. For heterogeneous groups of patients, an algorithm can be applied to adjust APACHE II scores for the underlying disease state to calculate a patients risk of death score (William A. Knaus et al. 1985; Wong and Knaus 1991). The aim of this study was to evaluate whether relative glycaemia is more strongly associated with in hospital mortality in critically ill patients than absolute glycaemia and whether this association is independent of risk of death score.

4.4 Methods

4.4.1 Study population

We prospectively reviewed admissions to the Intensive Care Unit (ICU) at Flinders Medical Centre between 27 January 2016 and 30 March 2017. Flinders Medical Centre's ICU is a 32 bed general medical and surgical ICU; admissions include patients with an acute medical problem and surgical patients undergoing liver transplants, following trauma, cardiothoracic surgery and neurosurgery. All adult patients aged ≥18 years were potentially eligible for the study. Subjects were excluded if they were admitted more than once to Flinders Medical Centre ICU within the study period. Clinical exclusion criteria were pregnancy, if the primary reason for admission to ICU was glycaemic-related (diabetes ketoacidosis, hyperosmolar hyperglycaemia syndrome or hypoglycaemia), a blood transfusion within the hospital admission prior to ICU or within 24 hours of admission to ICU, and elective admissions for routine post-operative surgery (Figure 4-1). The reason for admission to ICU was recorded by the ICU team, and surgical and medical admissions were defined based on the unit they were admitted under. We included patients who were admitted to ICU for other reasons but had a secondary diagnosis of an acute glycaemic event, and patients undergoing elective

surgery but had an acute complication that resulted in ICU admission. Subjects were also excluded from the analysis if glucose concentration was not measured within 24 hours of admission to ICU, there was no sample available to measure HbA1c or no APACHE II score was available. All patients who were transferred while acutely unwell to another public hospital were tracked for mortality up to 3 months after transfer using the state-wide computing system. All patients discharged from the hospital or surviving for more than 3 months in-hospital are considered survivors.

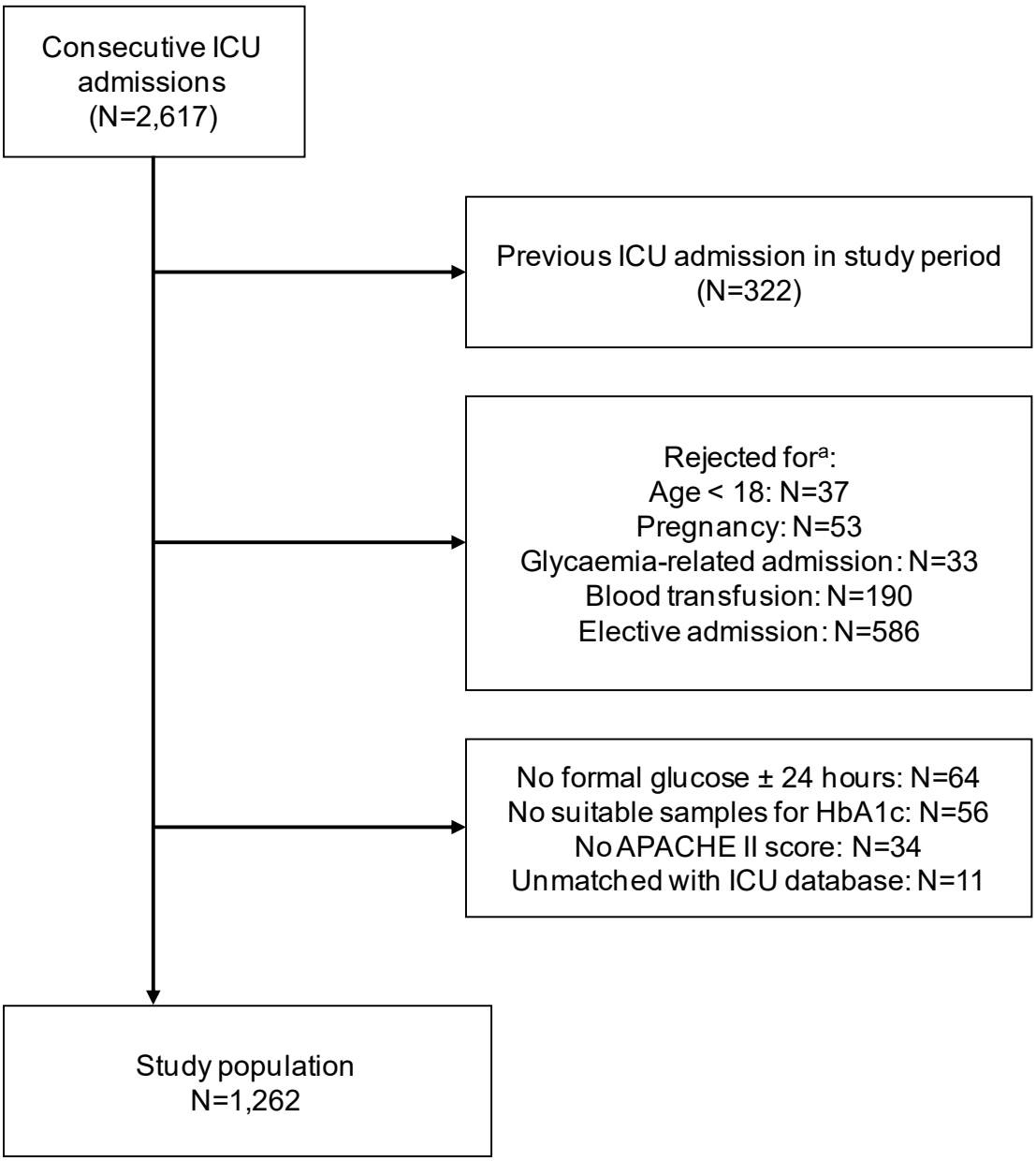


Figure 4-1 Patient recruitment summary

[a] 31 patients met more than one clinical exclusion criteria.

Ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee (reference number 268.15). Informed consent was not required as the study met all the requirements for a waiver under the Australian National Statement on Ethical Conduct in Human Research (updated 2015).

4.4.2 Assessment and Management of Hyperglycaemia

Glucose and HbA1c were measured by the hospital pathology laboratory, SA Pathology. HbA1c was measured by HPLC (PDQ, Primus Diagnostics, Kansas City, Mo, USA) using boronate affinity chromatography (between-run CV 2.2% at HbA1c 6.1% [43 mmol/mol] and 1.9% at HbA1c 1.1% [98 mmol/mol]), and venous plasma glucose was measured on a Roche P modular analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) using the hexokinase/glucose-6-phosphate dehydrogenase assay (between-run CV 1.7% at glucose 4.9 mmol/L and 1.4% at glucose 15.7 mmol/L).

4.4.3 Calculation of relative hyperglycaemia

HbA1c was measured from the first blood sample drawn after admission to ICU as part of routine clinical care. Estimated average glucose over the prior 3 months was calculated using the equation “estimated average glucose = (1.59 x HbA_{1c}) - 2.59” derived by Nathan et al (Nathan et al. 2008). We then calculated the *stress hyperglycaemia ratio* (SHR), defined as admission plasma glucose divided by estimated average glucose (Roberts et al. 2015). In this calculation, we used the closest formal plasma glucose concentration to admission to ICU in the 24 hours prior to ICU admission, which were available in 56% of patients. If glucose was not measured in the 24 hours before ICU admission, we used the first formal plasma glucose concentration within 24 hours after ICU admission. Hypoglycaemia was defined as any glucose below 4.0 mmol/L (72 mg/dl) in the first 24 hours of ICU admission. Subgroup analysis was performed in patients with HbA1c <6.5% and HbA1c ≥6.5% (48 mmol/mol).

4.4.4 ICU care and risk of death calculation

Patients received usual care from the ICU team, who had access to glucose concentrations, but not HbA1c, unless HbA1c was requested for routine clinical care. Data collected for submission to the Australia and New Zealand Intensive Care Society (ANZICS) were used to calculate the APACHE II score for each patient. This was then used to calculate a disease-specific risk of death score for each patient using the algorithm derived by Knaus et al. (William A. Knaus et al. 1985).

4.4.5 Treatment of Hyperglycaemia and Nutrition

The standard insulin protocol used in the ICU commences insulin infusion for any patient with a glucose concentration above 10 mmol/L (180 mg/dL). For patients with known diabetes, the insulin infusion is titrated to a target glucose of 8-10 mmol/L, and for patients without known diabetes, it is titrated to a target glucose of 6-8 mmol/L.

In the absence of contraindications, if adequate oral intake is not possible, enteral feeding is commenced within 12-24 hours of ICU admission and rapidly up-titrated to 30 kcal/kg during each 24 hour epoch.

4.4.6 Statistical Analysis

Odds ratios for in-hospital mortality per unit change in admission glucose concentrations and SHR were assessed in univariable logistic regression analysis. We then adjusted odds ratios for in-hospital mortality for glucose concentrations and SHR for risk of death scores in multiple binomial logistic regression analysis. Secondary endpoints included comparison of the association between SHR and in-hospital mortality in patients with and without background hyperglycaemia and evaluation of the potential for variables to predict mortality by calculating the area under receiver operating characteristics (ROC) curves.

Statistical analysis was undertaken using SPSS version 23 for Windows (IBM, New York, USA). A two-tailed P-value of <0.05 was considered statistically significant. The area under ROC curves was calculated using MedCalc Version 18.2.1.

4.4.7 Sample size calculation

The primary endpoint was in-hospital mortality, defined as death during the acute admission to Flinders Medical Centre or within 3 months of a hospital transfer for treatment of the acute illness. The primary variables of interest were glucose concentrations and SHR. Prior to study commencement, we conducted a simulation analysis using data from a previous study by our group (Roberts et al. 2015), assuming a mortality rate of 15%, to determine the required sample size. A sample size of 1,200 subjects has 80% power to detect an association between SHR and mortality at the two-tailed 0.05 significance level.

4.5 Results

4.5.1 Patient characteristics

There were 2,617 admissions to Flinders Medical Centre ICU during the study period (Figure 1). From this cohort, 322 patients were excluded as they had previously been admitted to Flinders Medical Centre ICU during the study period. A further 868 patients were excluded as they had one or more clinical exclusion criteria and 165 patients because glycaemic or APACHE II data were unavailable. Consequently, a total of 1,262 subjects were included in the final analysis.

In this cohort, 186 patients (14.7%) died while in hospital. Patients who died were older, less likely to be admitted under a surgical unit and had a higher admission glucose concentration and SHR than patients who survived (Table 4-1). There were no significant differences in sex, known diabetes, HbA1c and prevalence of hypoglycaemia between patients who survived and those who died (Table 4-1).

4.5.2 Association between glycaemia and mortality

In univariate analyses, admission glucose concentrations (odds ratio 1.08 per mmol/L glucose increment, p<0.001), SHR (odds ratio 1.09 per 0.1 SHR increment, p<0.001), insulin use (odds ratio = 1.57, p=0.008) and risk of death score (odds ratio 1.62 per 0.1 unit increment in risk of death score, p<0.001) were significantly associated with a greater risk of mortality (Table 4-2). By contrast, patient's sex, known diabetes status, and presence of hypoglycaemia within the first 24 hours of ICU admission, were not significantly associated with mortality. After adjustment for risk of death score, SHR was significantly associated with mortality (odds ratio 1.03 per 0.1 SHR increment, p=0.014) whereas admission glucose concentrations did not (odds ratio 1.01 per mmol/L glucose increment, p=0.338).

Patient Characteristic	Survivors	Deceased	p-value
Number	1,076	186	
Age (years)[a]	61 ± 18	70 ± 16	<0.001
Male sex (%)	55	57	0.617
Surgical Admission (%)	34	9	<0.001
Known diabetes (%) - Type 1 - Type 2	21.6 1.3 20.3	18.3 0.5 17.8	0.312 0.245 0.412
Glucose (mmol/L)[b]	7.4 (6.0-9.4)	8.9 (6.5-12.4)	<0.001
HbA1c (%)[b] (mmol/mol)	5.7 (5.4-6.5) 38.8 (35.5-47.5)	5.8 (5.3-6.3) 39.9 (34.4-45.6)	0.273
SHR[b]	1.10 (0.89-1.35)	1.34 (1.02-1.84)	<0.001
Hypoglycaemia (%)[c]	4.5	6.5	0.242
APACHE II	17.6 ± 6.65	25.13 ± 7.81	<0.001
ROD score	0.353 ± 0.234	0.649 ± 0.240	<0.001
Length of Stay (days)[d]	3 (2-5)	3 (1-7)	0.354
Insulin Use (%)[e]	24.2	33.3	0.008

Table 4-1 Clinical characteristics of patients who survived and died

[a] Values are mean ± standard deviation; b Values are median (interquartile range); c Hypoglycaemia defined as glucose <4.0 mmol/L (72 mg/dl) in first 24 hours. d Length of stay in ICU. e Insulin use is defined as having used any insulin infusion during the ICU admission. HbA1c = glycosylated haemoglobin, SHR = stress hyperglycaemia ratio, ROD = risk of death.

Measure	Univariate Analysis			Adjusted for Risk of Death		
	Odds ratio (mortality)	95% CI	P-value	Odds Ratio (mortality)	95% CI	P-value
Glucose[a]	1.08	1.04-1.11	<0.001	1.01	0.98-1.05	0.338
SHR[b]	1.09	1.06-1.11	<0.001	1.03	1.01-1.06	0.014
Insulin Use[c]	1.57	1.12-2.19	0.008	0.87	0.59-1.27	0.469
ROD score[d]	1.62	1.51-1.75	<0.001			
Sex	1.08	0.79-1.48	0.618			
Known diabetes	0.81	0.55-1.21	0.312			
Hypoglycaemia[e]	1.47	0.77-2.84	0.241			

Table 4-2 Univariate analyses of relationships between mortality and variables and after adjustment for Risk of Death Score in patients admitted to an Intensive Care Unit

[a] Glucose at admission to ICU, reported as per mmol/L change; b Stress Hyperglycaemia Ratio (SHR) is per 0.1 change, calculated as admission glucose divided by estimated average glucose based on HbA1c; c Insulin use is defined as having used any insulin infusion during the ICU admission. d Risk of death (ROD) score is calculated from APACHE II scores, and is reported as per 0.1 change; e Hypoglycaemia is defined as a blood glucose of <4.0 mmol/L (72 mg/dl) in the first 24 hours of ICU admission.

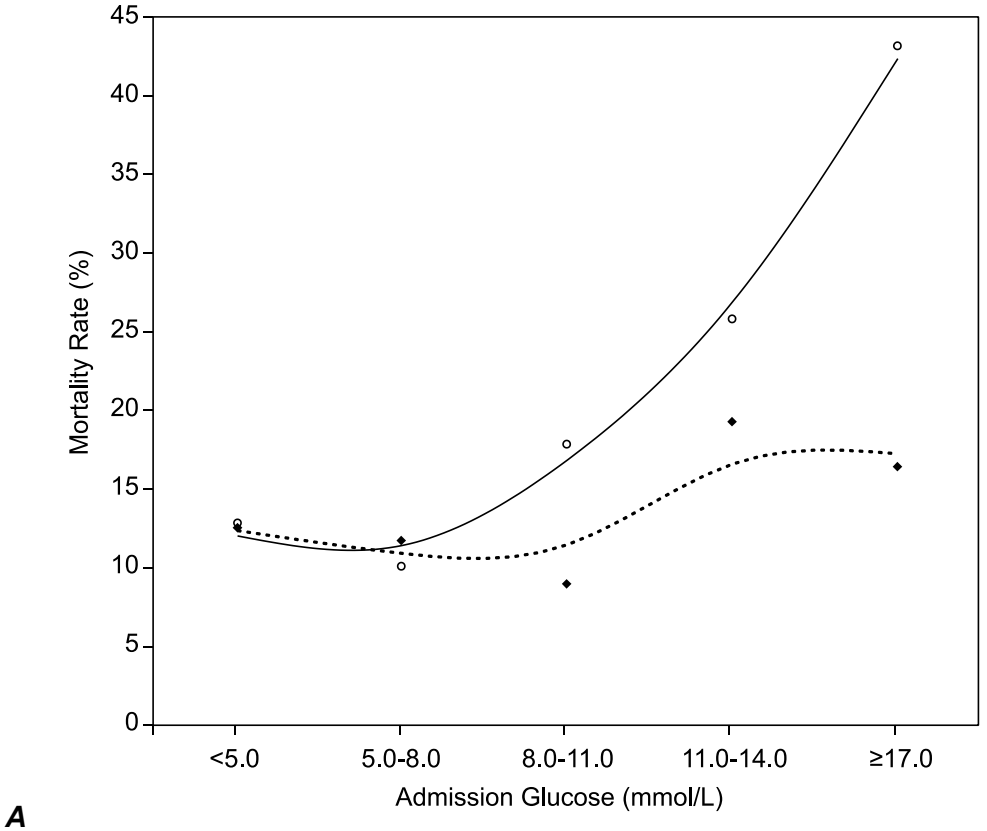
4.5.3 HbA1c subgroup analyses

Figure 4-2 shows that above glucose of 8 mmol/L (145 mg/dl) each increment in glucose concentrations was associated with a greater risk of mortality in patients with HbA1c <6.5% than in those with HbA1c ≥6.5% (48 mmol/mol). Admission glucose concentrations were associated with mortality in patients with HbA1c <6.5% (odds ratio = 1.14 per mmol/L

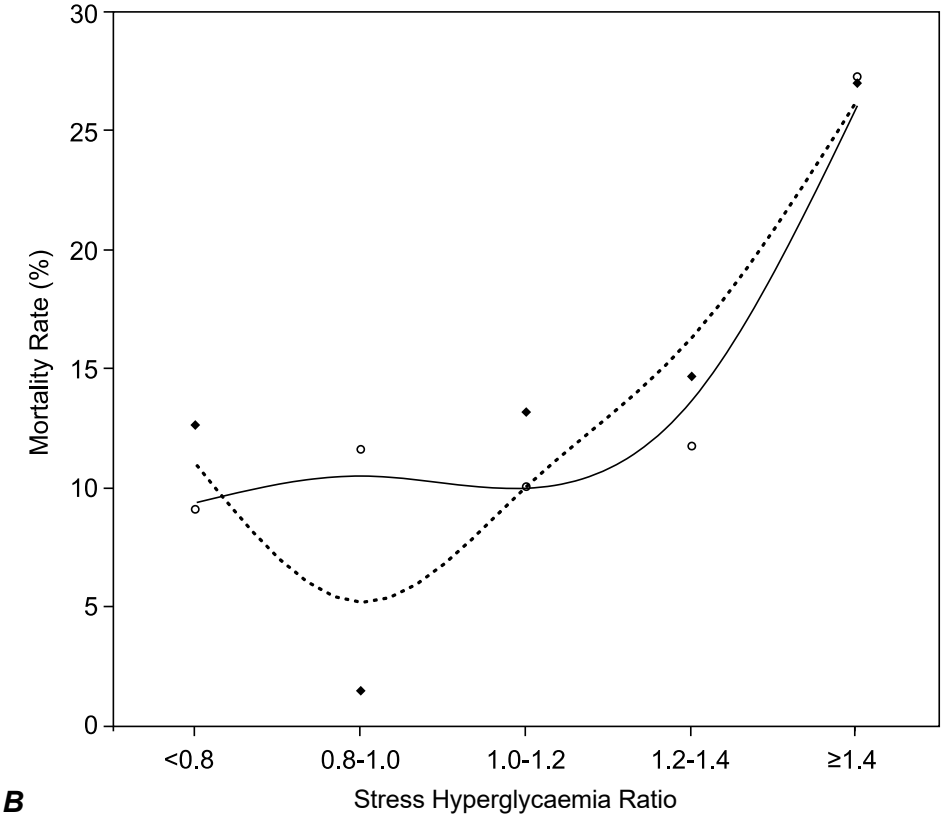
glucose increment, p<0.001) but not in those with HbA1c ≥6.5% (48 mmol/mol) (odds ratio = 1.04 per mmol/L glucose increment, p=0.124). Moreover, there was a statistically significant interaction between admission glucose concentrations and HbA1c status (p=0.002). In contrast, SHR was associated with mortality in patients with HbA1c <6.5% (odds ratio = 1.08 per 0.1 SHR increment, p<0.001) and in those with HbA1c ≥6.5% (48 mmol/mol) (odds ratio = 1.08 per 0.1 SHR increment, p=0.005). There was no interaction between SHR and HbA1c status (p=0.909). Above the threshold of 1.0, the relationship between SHR and mortality was similar for patients in either HbA1c groups. Below this threshold, SHR plateaued in patients with a HbA1c < 6.5%, whereas there appeared to be a J-shaped curve in patients with HbA1c >= 6.5% with an increase in mortality in patients with a low SHR (Figure 4-2).

4.5.4 Receiver Operating Characteristics (ROC) curves

The area under the ROC curve for risk of death score was significantly higher than that for APACHE II score (0.806, (95% CI: 0.783-0.828) vs 0.771, (0.747-0.794), p < 0.001), as shown in Figure 3. The area under the ROC curve for SHR was 0.642, (0.615-0.668). The area under the ROC curve for APACHE II score and SHR was slightly but significantly higher than that for APACHE II score alone (0.782, (0.758-0.804) vs 0.771, (0.747-0.794), p = 0.014). However, there was no significant difference between the area under the ROC curve for risk of death score and SHR and that for risk of death score alone (0.810, (0.787-0.831) vs 0.806, 95% CI: 0.783-0.828, p=0.163).

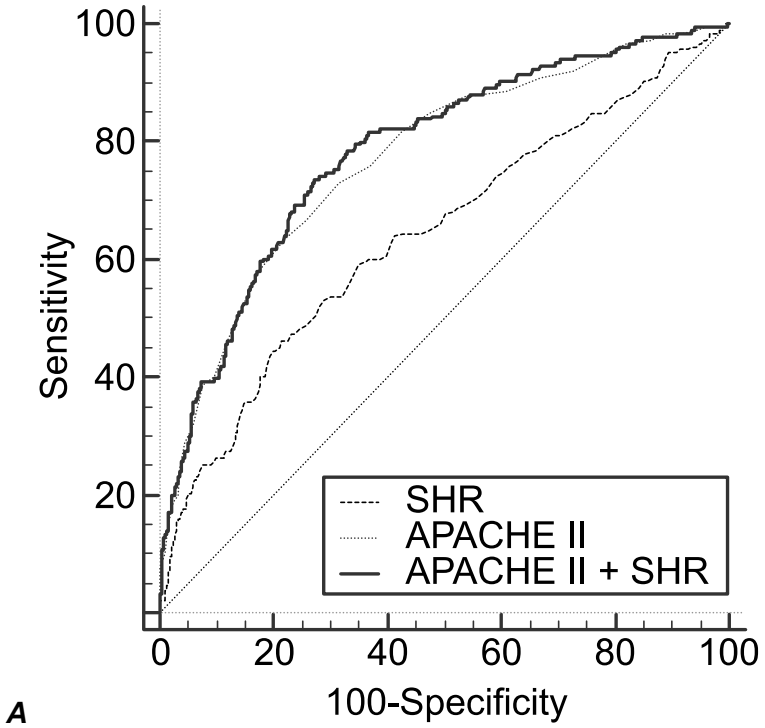


A

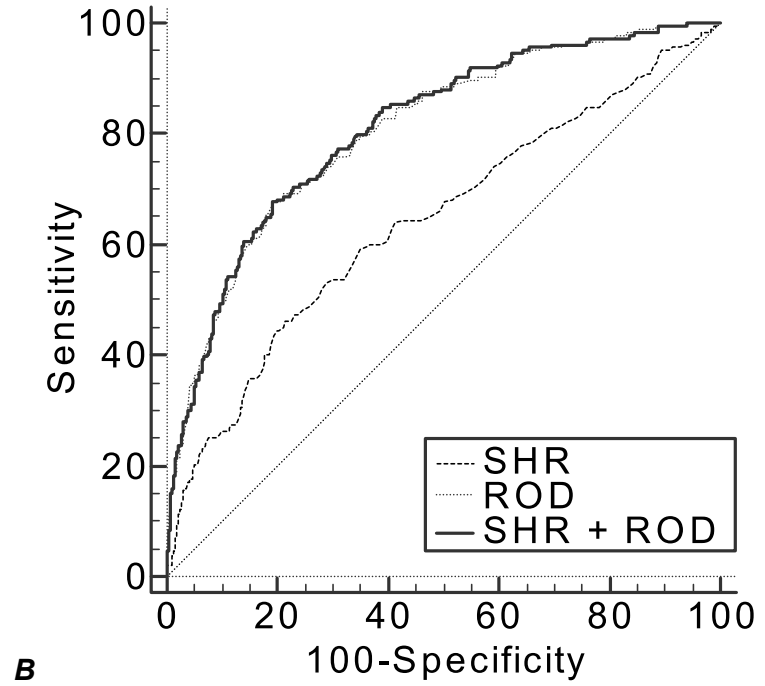


B

Figure 4-2 Relationship between glucose (A) and SHR (B) with mortality in patients admitted to an Intensive Care Unit with HbA1c <6.5% (48 mmol/mol) (º, continuous line) and ≥6.5% (⬥, dotted line).



A



B

Figure 4-3 Receiver operator characteristic curves for Stress Hyperglycaemia Ratio (SHR), APACHE II and combined SHR+APACHE II (A), and for SHR, risk of death (ROD) score, and combined SHR+ROD score (B)

4.6 Discussion

This study of 1,262 consecutive critically ill patients admitted to Intensive Care showed that relative hyperglycaemia independently was associated with in-hospital mortality after adjusting for risk of death score and the relationship between relative hyperglycaemia and mortality was not affected by background glycaemia. In contrast, absolute hyperglycaemia did not independently predict in-hospital mortality. Furthermore, its relationship with mortality differed in patients with and without diabetes. These results raise the hypothesis that treatment based upon relative, rather than absolute hyperglycaemia, might reduce mortality in patients across the glycaemic spectrum, which should be tested in an interventional study.

Previous studies have reported that glucose concentrations, either on or during admission to ICU, were positively associated with mortality in critically ill patients (Bochicchio et al. 2005; T. F. Lee et al. 2017; Kim et al. 2017; Roberts et al. 2015). However, while this association has been reported to be significant after correction for APACHE II score (James Stephen Krinsley 2003), in other studies glucose concentrations did not independently predict mortality (Kang et al. 2015; Terzioglu, Ekinci, and Berkman 2015; Koyfman et al. 2018; Ligtenberg et al. 2006). In our study, admission glucose concentrations were associated with mortality in univariate analysis, but not after correction for the risk of death score. Therefore, our data suggest that absolute glycaemia does not independently predict mortality in this setting.

Measurement of HbA1c has revolutionized the management of diabetes, as it can be used both as a diagnostic test and to evaluate the efficacy of glucose-lowering therapy (Chamberlain et al. 2016). Moreover, HbA1c is not acutely affected by critical illness (Luethi et al. 2016). In this study, we used HbA1c to estimate background glycaemia, allowing us to calculate relative or new hyperglycaemia. The main finding of this study is that, unlike absolute glycaemia, relative hyperglycaemia as defined by SHR, was significantly associated with mortality after correcting for risk of death score. This finding is concordant with our previous reports that SHR is an independent predictor of adverse patient outcomes

(T. F. Lee et al. 2017; Roberts et al. 2015), but extends them as the study was prospective and because we were better able to account for other variables that might impact this relationship. Risk of death score incorporates many factors that have been shown to impact prognosis, including 12 physiologic measurements, age and previous health status, along with a correction for the underlying diagnosis.

In this study each 0.1 increment in SHR was associated with a 3% increase in the odds ratio for mortality after adjustment for risk of death score. At first glance this increase appears small and its clinical significance modest. However, for a patient with HbA1c of 5%, an increase in glucose of just 0.55 mmol/L (9.9 mg/dL) above background levels is associated with a 3% increase in odds ratio for death. As the mortality in this cohort of patients is approximately 15%, a small change in odds ratio is associated with a major impact on survival. The SHR range in this cohort was 0.22 to 5.67, so the odds ratio for death varied by more than 150% across the cohort.

The relationship between SHR and mortality was similar in patients with HbA1c above and below 6.5% (48 mmol/mol), particular above a threshold of 1.0. However, the relationship between absolute glycaemia and mortality was much stronger in patients without diabetes. Furthermore, there was a significant interaction between glucose and diabetes status, demonstrating that the relationship between glucose and mortality differs in patients with and without diabetes. This is consistent with previous studies reporting that diabetes glucose is more strongly associated with mortality in patients without diabetes (T. F. Lee et al. 2017; Roberts et al. 2015; Lanspa et al. 2013; Plummer et al. 2014; N W Cheung et al. 2008; Egi et al. 2016; Kar et al. 2016; Kotagal et al. 2014). In contrast, measures of relative hyperglycaemia such as SHR are associated with adverse outcomes in patients across the glycaemic spectrum (Roberts et al. 2015; T. F. Lee et al. 2017). The apparent higher mortality for patients with HbA1c ≥6.5% (48 mmol/mol) and SHR below 1.0 (Figure 2), but not for those with HbA1c <6.5%, should be further investigated as it may signify that over-treating relative hypoglycaemia in patients with diabetes adversely affects outcomes.

We also assessed whether the inclusion of SHR increased the area under ROC curves for APACHE II and risk of death scores. Consistent with a previous study, the addition of SHR to APACHE II resulted in a small, but significant, increase in the area under the ROC curve (W. Liao et al. 2015). However, the addition of SHR did not significantly increase the area under the risk of death score ROC curve. Therefore, although the association between SHR and mortality is independent of risk of death score, it is unlikely to substantially increase the power of risk of death score to predict mortality. This is probably not surprising given the large number of variables already incorporated into the calculation of risk of death scores.

An optimum treatment target for glycaemia in critically ill patients remains uncertain. The results of randomized controlled trials from the early 2000s, showing a benefit of very tight glucose control, were not replicated in the largest prospective multi-centre trial, which reported an increase in mortality in patients prescribed intensive insulin therapy (G van den Berghe et al. 2001; Greet Van den Berghe, Wilmer, Hermans, et al. 2006; Finfer et al. 2009). This increase in mortality was postulated to be due, at least in part, to increased hypoglycaemia with intensive insulin therapy (Investigators 2012). In the future, implementation of technologies in ICU such as continuous glucose monitoring may alter the risk/benefit of intensive insulin therapy.

There is evidence that differences in background glycaemia could influence the outcomes of glycaemic therapy in ICU (Kar et al. 2016; Lanspa et al. 2013; Egi et al. 2016; Olariu et al. 2018; Luethi et al. 2018; Balintescu and Mårtensson 2019). A retrospective analysis of 3,529 critically ill patients reported that a glucose target of 5-7.8 mmol/L (90-140 mg/dl), was associated with higher mortality compared to a lower glucose target of 4.4-6.1 mmol/L (80-110 mg/dl) in patients without diabetes (Lanspa et al. 2013). However, in patients with diabetes the converse was found, with higher mortality in patients treated to a lower absolute glucose target. Although our research was not interventional, it is consistent with this. A patient without diabetes and HbA1c of 5% has an estimated average glucose of 5.4 mmol/L (98 mg/dl) over the preceding 3 months (Nathan et al. 2008), hence, any glucose elevation represents a large increase in relative glycaemia and treating to the tighter range (4.-6.1

mmol/L, 80-110 mg/dl) targets a SHR of about 1.0. In contrast, a patient with diabetes and HbA1c of 7% has an estimated average glucose of 8.6 mmol/L (155 mg/dl) over the last 3 months, hence, tight glucose control (4.4-6.1 mmol/L, 80-110 mg/dl) targets a glucose that is lower than their normal (equivalent to a SHR of approximately 0.6.).

As relative hyperglycaemia is independently associated with mortality, we propose that the benefits of treating relative rather than absolute hyperglycaemia in critically ill patients should be tested in a prospective randomized-controlled trial. Treating relative and not absolute hyperglycaemia will result in a lower glucose target for patients without diabetes than for patients with diabetes. While some authors already recommend different glycaemic targets for patients with and without diabetes using a HbA1c threshold (Marik and Egi 2014; James S Krinsley, Preiser, and Hirsch 2017; Luethi et al. 2018) and our ICU targeted slightly different glucose ranges for patients with and without diabetes, basing intervention and glycaemic targets on SHR has the potential to individualize treatment in critically ill patients and return them to their usual glycaemic status. This might be the answer to the call for an individualized glycaemic target (Judith Jacobi 2016; James S Krinsley, Preiser, and Hirsch 2017; James Stephen Krinsley 2018).

The strengths of this study include prospectively recruiting a large cohort of consecutive patients. Consequently, our results are likely to be generalizable to other cohorts of critically ill patients. The data collection was performed independent to clinical care and HbA1c requests for study purposes were unavailable to the treating clinicians. Patient data were systematically recorded from reliable sources. However, this study has limitations. It is an observational study, and has not proven causality nor does it provide a mechanism by which relative hyperglycaemia confers a poorer prognosis. Secondly, while we excluded pregnant patients and those receiving a blood transfusion in hospital prior to ICU admission and within 24 hours of admission, we do not have data on blood transfusions in the weeks prior to ICU admission and we did not exclude other conditions that might alter HbA1c including haemoglobinopathy, chronic renal impairment, hypothyroidism, use of iron infusion or

erythropoietin (Campbell, Pepper, and Shipman 2019; Radin 2014; Shepard et al. 2015). Thirdly, we did not study the severity or recurrence of hypoglycaemia. Fourthly, protocols for insulin therapy differed slightly in patients with and without diabetes. Finally, categorization of patients as medical or surgical is based on the team they were admitted under, and not whether they completed a surgical procedure.

In summary, relative hyperglycaemia, as assessed by SHR, is independently associated with in-hospital mortality in patients presenting to ICU with an acute critical illness, while absolute hyperglycaemia does not. The association between SHR and mortality is not affected by diabetes status. An interventional study should be performed to investigate the utility of using measures of relative hyperglycaemia to determine individualized glycaemic treatment targets in critically ill patients. If this showed a benefit, SHR is a clinically intuitive metric that quantifies relative glycaemia as a continuous variable and could rapidly be incorporated into clinical practice.

4.7 Declaration

4.7.1 Funding

Tien F Lee received post-graduate scholarships from the National Health and Medical Research Council and the Royal Australasian College of Physicians.

The study was supported by grants from the Flinders Medical Centre Foundation and the Novo Nordisk Regional Diabetes Scheme

4.7.2 Acknowledgment

The authors would like to acknowledge the help from Mr. Darren Scott, Mr. Grant White and Mr. Fotios Visvardis from SA Pathology for logistic support to locate blood samples and measure HbA1c. We would also like to thank the Clinical Research Nurses at the Intensive Care Unit at Flinders Medical Centre for logistic support and help with retrieving data from the ICU registry.

Chapter 5 ADMA and homoarginine independently predict mortality in critically ill patients: A prospective, observational cross-sectional study

Manuscript submitted

Tien F Lee [1,2], Andrew Bersten [1,3], Leonie K Heilbronn [4], Angelo Zinellu[5], Ciriaco Carru[5], Salvatore Sotgia[5], Arduino A Mangoni [6], Morton G Burt [1,2]

[1] College of Medicine and Public Health, Flinders University, Flinders Drive, Bedford Park, SA 5042, Australia

[2] Southern Adelaide Diabetes and Endocrine Services, Flinders Medical Centre, Flinders Drive, Bedford Park, SA 5042, Australia

[3] Department of Critical Care Medicine, Flinders University, Bedford Park, South Australia, Australia

[4] Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia

[5] Department of Biomedical Sciences, School of Medicine, University of Sassari, Sassari, Italy

[6] Discipline of Clinical Pharmacology, Flinders University and Flinders Medical Centre, Flinders Drive, Bedford Park, SA 5042, Australia

5.1 Author Roles

TFL assisted with design of this analysis, collected the clinical data, performed the statistical analysis and drafted and revised the manuscript; AB, LKH and AAM assisted with design of this study; SS measured arginine metabolites under the supervision of AZ and CC; LKH, AZ, CC, SS and AAM assisted with manuscript revision; MGB designed this analysis, assisted with the statistical analysis and drafted and revised the manuscript. All authors read and approved the final manuscript.

5.2 Abstract

Purpose: Arginine metabolites are associated with cardiovascular and all-cause mortality in several patient groups. The aim was to investigate whether arginine metabolites are associated with mortality in patients with critical illness and whether associations are independent of other factors affecting prognosis in an Intensive Care Unit (ICU).

Methods: 1,155 acutely unwell adult patients admitted to a mixed medical-surgical ICU were studied. Arginine, asymmetric dimethyl-L-arginine (ADMA), monomethyl-L-arginine (MMA), symmetric dimethyl-L-arginine (SDMA) and L-homoarginine were measured in a plasma sample collected at admission to ICU by liquid chromatography tandem mass spectrometry. Risk of death score was calculated using data submitted to the Australia and New Zealand Intensive Care Society.

Results: In this cohort, 163 patients (14.1%) died. ADMA (odds ratio = 1.159 (1.033-1.300) per 0.1 µmol/L increment, p = 0.012), L-homoarginine (odds ratio = 0.963 (0.934-0.992), p = 0.013) and risk of death score (odds ratio = 1.045 (1.037-1.053) per 1% increment, p<0.001) were independently associated with mortality in ICU patients. The area under the receiver operator characteristic curve for risk of death score, ADMA and L-homoarginine combined for mortality was greater than for risk of death score alone (0.815 (95% CI 0.790-0.837) vs 0.796 (95% CI 0.781-0.820), p = 0.019). Other arginine metabolites were not independently associated with mortality.

Conclusions: ADMA is positively and L-homoarginine negatively associated with mortality in ICU patients, independent of other clinical factors. Measuring ADMA and L-homoarginine may refine models to predict ICU mortality. Reducing ADMA and increasing L-homoarginine are potential therapeutic targets to reduce mortality in critically ill patients.

5.3 Introduction

The majority of patients admitted to an Intensive Care Unit (ICU) have an acute severe illness and consequently, the mortality rate is high. Mortality is dependent on many patient factors, especially the reason for admission, but averages more than 10% in ICUs across the United States (Zimmerman, Kramer, and Knaus 2013). Mortality in ICU has fallen dramatically in the last twenty years, demonstrating that advances in management of critically ill patients can substantially improve survival (Zimmerman, Kramer, and Knaus 2013).

There has been substantial interest in the contribution of microvascular dysfunction to mortality in ICU. The endothelium synthesizes nitric oxide which is a potent vasodilator and plays an important role in reducing vascular tone, the interaction of white blood cells and platelets with the endothelium and proliferation of vascular smooth muscle cells and thus maintenance of tissue perfusion in critically ill patients (Hollenberg and Cinel 2009; Yuyun, Ng, and Ng 2018). Endothelial dysfunction is present in many conditions that cause admission to ICU including trauma, hemorrhagic shock, ischaemia and sepsis (Ince, De Backer, and Mayeux 2020). However, currently there are no treatments that specifically target increasing endothelial function in critically ill patients.

Arginine, methylated arginines and L-homoarginine are important modulators of the nitric oxide pathway. Most interest has focused on asymmetric dimethyl-L-arginine (ADMA), which is a potent inhibitor of nitric oxide synthase, and its plasma concentration has been positively associated with mortality in many patient groups including those with acute coronary syndrome (Schulze et al. 2006), acute infection (Siroen et al. 2005; Nijveldt et al. 2003; Blackwell 2010; Davis et al. 2011) and end-stage renal failure (Zoccali et al., n.d.). Monomethyl-L-arginine (MMA) also inhibits nitric oxide synthase, and was previously shown to increase mortality when used therapeutically in acute myocardial infarction

(TRIUMPH Investigators et al. 2007), cardiogenic shock (TRIUMPH Investigators et al. 2007) and septic shock (López et al. 2004). Symmetric dimethyl-L-arginine (SDMA) does not directly inhibit nitric oxide synthase but inhibits cellular uptake of arginine, the substrate for nitric oxide synthase. SDMA is positively associated with mortality in the general population and patients with ischemic stroke (Schulze et al. 2010; Gore et al. 2013). In contrast L-homoarginine, which is produced by metabolism of arginine via arginine:glycine amidinotransferase, is negatively associated with cardiovascular and / or all-cause mortality in the general population (Gore et al. 2013) and patients with ischemic heart disease (Atzler, Baum, et al. 2016), congestive cardiac failure (Pilz et al. 2014) and stroke (Choe et al. 2013). It is not clear whether arginine, L-homoarginine or methylated arginines affect mortality directly or are an epiphenomenon (Karetnikova et al. 2019).

Several studies have explored the relationship between arginine and its metabolites and mortality in critically ill patients. ADMA and SDMA have been reported to be higher (Ghashut et al. 2017; Koch et al. 2013; Winkler et al. 2018; Gough et al. 2011) and L-homoarginine lower in patients who die in ICU (Ghashut et al. 2017; Zinellu et al. 2018). However, the sample sizes in the above studies were relatively small and independence from other variables was only partially assessed and only in some studies (Mortensen et al. 2019). The relationship between MMA and mortality in critically ill patients has not been previously studied.

This prospective study investigated whether arginine metabolites are independently associated with mortality in patients with critical illness. If true, arginine metabolites could enhance models predicting patient outcomes and be a potential therapeutic target in ICU patients.

5.4 Methods

5.4.1 Study design and ethical approval

This was a single centre, prospective, cross-sectional study. Ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee (reference number 268.15). Informed consent was not required as the study met all the requirements for a waiver under the Australian National Statement on Ethical Conduct in Human Research (updated 2015).

5.4.2 Patients

This patient cohort was recruited to assess the relationship between relative hyperglycaemia and mortality in critically ill patients, as previously described in detail (T. F. Lee et al. 2020). In brief, we prospectively included consecutive medical and surgical admissions to Intensive Care Unit (ICU) at Flinders Medical Centre between 27 January 2016 and 30 March 2017 that met inclusion and exclusion criteria. Subjects were excluded if they had previously been admitted to ICU within the study period or were admitted for routine post-operative monitoring following a surgical procedure, aged <18 years, pregnant or had a recent blood transfusion, were admitted primarily for treatment of hyper- or hypoglycaemia or had missing data preventing calculation of risk of death score. In the remaining subjects, we stored the plasma sample collected closest to admission to ICU at -70^{o} Celsius for subsequent measurement of arginine and metabolites. If no plasma sample was collected within 24 hours of admission to ICU, the subject was excluded from this analysis.

Patients received usual care from the ICU medical and nursing team. All patients who were transferred while acutely unwell to another public hospital were tracked for mortality up to 3 months after transfer using the state-wide computing system. Data collected for

submission to the Australia and New Zealand Intensive Care Society (ANZICS) were used to calculate the APACHE IIIj score for each patient. This was then used to calculate a disease-specific risk of death score for each patient (W A Knaus et al. 1991).

5.4.3 Laboratory measurements

Arginine and its chemically related metabolites and analogs MMA, L-homoarginine, ADMA and SDMA were measured according to the method developed by Sotgia et al (Sotgia et al. 2019). For this purpose, 200 µL aliquots of plasma were spiked with 1 µL of internal standard solution containing L-homoarginine-d4 dihydrochloride (d4-hArg), NG,NG-dimethyl-L-arginine-d6 dihydrochloride (d6-ADMA), and NG,NG'-dimethyl-L-arginine-d6 (d6-SDMA). After vigorous vortex mixing, tubes were placed in a block heater for 5 min at 100 °C then cooled to room temperature. After the addition of 400 µL of ultrapure water (Milli-Q grade), tubes were vortexed vigorously for 10 s to displace the clot from the bottom of the vial. Tubes with dislodged clots were then heat-treated again for 5 min at 100 °C, cooled down to room temperature, and centrifuged at 17,000×g for 5 min. A 200-µL volume of clear supernatant was recovered and added with 20 µL of potassium phosphate monobasic buffer (100 mmol/L, pH 7.0) and 40 µL of diethylpyrocarbonate (33 mmol/L). Tubes were mixed thoroughly and after leaving them at room temperature for 1 min, 20 µL of sample were analyzed by an Agilent liquid chromatography tandem mass spectrometry (LC-MS/MS) system (Agilent Italia, Milan, Italy) using a 100 mm × 4.6 mm Agilent Zorbax Eclipse Plus C18 3.5 µm column and a mixture of an aqueous solution of 0.4% v/v formic acid and acetonitrile (95:5) as a mobile phase, delivered isocratically at a flow-rate of 0.8 mL/min. Mass detection was accomplished in positive ion mode by MRM of the precursor-product ion transitions *m/z* 247.14→142, 261.28→70, 261.28→84, 275.33→46, and 275.33→70 for arginine, MMA, L-homoarginine, ADMA, and SDMA, respectively, as well

as *m/z* 265.28→88, 281.3→52, and 281.3→70 for d4-homoarginine, d6-ADMA, and d6-SDMA, respectively.

Other laboratory measurements were taken from the Intensive Care Unit's registry, and were measured by standard clinical laboratory methods as part of routine care. Estimated glomerular filtration (EGFR) was calculated using the Modification of Diet in Renal Disease Study (MDRD) equation (Levey et al. 2006).

5.4.4 Statistical Analysis

Data are presented as mean ± standard deviation if the distribution was normal or median (interquartile range) if they were not normally distributed. Comparisons between variables in patients who survived and died were assessed using unpaired t-tests or the Mann-Whitney U-test as appropriate. Odds ratios for in-hospital mortality per unit difference in arginine metabolites were first assessed in univariable binary logistic regression analyses. As many arginine metabolites are renally cleared, these odds ratios were then adjusted for GFR in a multiple binary logistic regression analysis to ensure that differences in renal function were not underlying results. The relationship between arginine metabolites and mortality were then adjusted for the risk of death score. As renal function is incorporated into the risk of death score it was not included as a separate variable in these analyses. The units in these analyses were chosen as they are 1-5% of the range for each variable, to allow some comparison of odds ratios for the different variables. We used the collinearity diagnostics in SPSS to confirm there was no collinearity in the multiple regression models. Finally, receiver operator characteristic (ROC) curves were generated for the variables that were independent predictors of mortality in regression analyses, to assess whether measurement of arginine metabolites improved the power of the risk of death score to predict mortality.

Statistical analysis was undertaken using SPSS version 23 for Windows (IBM, New York, USA) for Chapter 3 and subsequently version 25 for later chapters. A two-tailed P-value of <0.05 was considered statistically significant. The area under ROC curves was calculated using MedCalc Version 18.2.1 (MedCalc Software Ltd, Ostend, Belgium).

5.5 Results

5.5.1 Patient characteristics

There were 1,262 critically ill patients in our original study. In this cohort, 107 patients did not have an adequate plasma sample stored for arginine metabolomics studies. Therefore, 1,155 patients were included in this analysis (Table 5-1). In this cohort, 163 patients (14.1%) died while in hospital or within 3 months of a hospital transfer. Patients that died were older, admitted for non-surgical reasons, had a higher glucose, heart rate, respiratory rate and white blood count and a lower EGFR, systolic and diastolic blood pressure, albumin, arterial pH, and Glasgow Coma Scale (Table 5-1). Patients that died had a higher APACHE IIIj (89 (71-114) vs 61 (46-78), p<0.001) and risk of death score (0.53 (0.34-0.75) vs 0.22 (0.10-0.38), p<0.001) than those who survived.

	Survivors	Deceased	p-value
Number	992	163	
Age (years)[a]	61 ± 18	70 ± 15	<0.001
Male sex (%)	56	58	0.671
Surgical Admission (%)	34	16	<0.001
Glucose (mmol/L)[b]	7.4 (6.0-9.4)	8.9 (6.5-12.4)	<0.001
EGFR (ml/min/1.73m^2)	67 (38-96)	45 (24-72)	<0.001
Systolic BP (mmHg)	99 (89-160)	92 (80-150)	<0.001
Diastolic BP (mmHg)	60 (48-78)	54 (43-72)	0.005
Albumin (mmol/L)	29 (25-32)	27 (24-31)	0.003
APACHE II			
• Temperature	36.0 ± 1.4ºC	35.7 ± 1.8ºC	0.085
• MAP	85.5 ± 27.8	82.7 ± 31.3	0.29
• HR	91 ± 35	102 ± 39	0.001
• RR	22 ± 9	26 ± 9	<0.001
• Arterial pH	7.34 ± 0.10	7.28 ± 0.14	<0.001
• Serum Na+	139 ± 5	140 ± 6	0.365
• Serum K+	4.12 ± 0.81	4.23 ± 0.95	0.168
• Creatinine	89.0 (67.0-143.0)	122.5 (77-194.3)	0.049
• Haematocrit	0.34 ± 0.07	0.35 ± 0.08	0.334
• WBC	14.10 ± 7.52	15.62 ± 8.67	0.037
• GCS	15 (13-15)	14 (3-15)	<0.001

Table 5-1 Clinical characteristics of patients who survived and died

EGFR = estimated glomerular filtration rate; BP = blood pressure; MAP = Mean Arterial Pressure; HR = heart rate; RR = respiratory rate; WBC = white blood cell count; GCS = Glasgow Coma Score; ICU = intensive care unit. Values are mean ± standard deviation or median (interquartile range).

5.5.2 Mortality and arginine metabolites

Patients who died had higher plasma concentrations of ADMA, MMA and SDMA and lower concentrations of arginine and L-homoarginine than patients who survived (Table 5-2). The arginine / ADMA ratio was lower in patients who died (Table 5-2).

In univariate analyses, the odds ratios for death were significantly higher for ADMA, MMA and SDMA and lower for L-homoarginine and arginine / ADMA ratio (Table 3). The odds ratios for ADMA, MMA, L-homoarginine and arginine / ADMA ratio were independent of GFR. After correction for risk of death score, the odds ratios for mortality were significantly higher for ADMA and MMA and lower for L-homoarginine. The arginine / ADMA ratio was also independently associated with mortality, but the associated was not as strong as for ADMA *per se*.

Finally, in a multiple binary logistic regression model ADMA (odds ratio = 1.159 (1.033-1.300) per 0.1 µmol/L increment, p = 0.012), L-homoarginine (odds ratio = 0.963 (0.934-0.992), p = 0.013) and risk of death score (odds ratio = 1.045 (1.037-1.053) per 1% increment, p<0.001) were independent predictors of mortality, while MMA was not (odds ratio = 0.999 (0.947-1.054), p = 0.971).

	Survivors	Deceased	P value
ADMA (µmol/L)	0.54 (0.45-0.66)	0.60 (0.49-0.79)	<0.001
Arginine (µmol/L))	61.7 (42.9-83.4)	53.8 (32.8-81.7)	0.005
Homoarginine (µmol/L))	0.88 (0.55-1.36)	0.65 (0.35-1.16)	<0.001
MMA (nmol/L)	65.0 (50.8-82.6)	76.7 (57.1-106.2)	<0.001
SDMA (µmol/L))	0.69 (0.51-1.12)	0.85 (0.60-1.45)	<0.001
Arginine/ADMA	114.1 (81.1-156.3)	84.2 (53.5-130.6)	<0.001

Table 5-2 Arginine metabolites in patients who survived and died

Values are median (interquartile range).

ADMA = asymmetric dimethyl-L-arginine; MMA = monomethyl-L-arginine, SDMA = symmetric dimethyl-L-arginine

	Univariate		Adjusted EGFR		Adjusted Risk Of Death score	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
ADMA (per 0.1 µmol/L)	1.225 (1.143-1.314)	<0.001	1.203 (1.119-1.293)	<0.001	1.133 (1.045-1.228)	0.003
Arginine (per 10 µmol/L)	0.950 (0.901-1.001)	0.056	0.963 (0.914-1.014)	0.155	0.978 (0.928-1.031)	0.406
HA (per 0.1 µmol/L)	0.955 (0.928-0.983)	0.002	0.964 (0.936-0.992)	0.012	0.971 (0.943-0.1.000)	0.047
MMA (per 10 nmol/L)	1.088 (1.048-1.130)	<0.001	1.076 (1.036-1.118)	<0.001	1.040 (1.001-1.081)	0.047
SDMA (per 0.1 µmol/L)	1.020 (1.008-1.034)	0.002	1.011 (0.996-1.026)	0.155	1.009 (0.994-1.024)	0.232
Arginine/ADMA ratio (per 10)	0.929 (0.900-0.958)	<0.001	0.937 (0.907-0.968)	<0.001	0.965 (0.934-0.998)	0.036

Table 5-3 Odds ratios for in-hospital death for different arginine metabolites

EGFR = estimated glomerular filtration rate. ADMA = asymmetric dimethyl-L-arginine; HA = L-homoarginine; MMA = monomethyl-L-arginine, SDMA = symmetric dimethyl-L-arginine

5.5.3 Receiver Operator Characteristic Curves

The areas under the receiver operator characteristic curves (AUC) for risk of death score (AUC = 0.796, 95% confidence intervals (CI) 0.771-0.820, p<0.001), ADMA (AUC = 0.623, 95% CI 0.594-0.652, p<0.001) and L-homoarginine (AUC = 0.581, 95% CI 0.551-0.611, p<0.001) for mortality were significantly greater than 0.5 (Figure 5-1A). The area under the receiver operator characteristic curves for risk of death score, ADMA and L-homoarginine combined for mortality was significantly greater than for risk of death score alone (0.815 (95% CI 0.790-0.837) vs 0.796 (95% CI 0.781-0.820), p = 0.019, Figure 5-1B).

5.1 Discussion

This study assessed the relationship between 5 arginine metabolites and mortality in critically ill patients. We found that ADMA on admission to ICU is positively and L-homoarginine negatively associated with in-hospital mortality, independent of each other and other variables that predict mortality in ICU. Adding ADMA and L-homoarginine to the risk of death score increased its ability to predict mortality. These findings suggest that measurements of these arginine metabolites on admission to ICU might enable refinement of methods to predict mortality and potentially suggest new approaches to reduce mortality in critically ill patients.

In our study ADMA was positively associated with mortality. A recent meta-analysis of 15 smaller studies comprising a total of 1300 patients reported that ADMA was higher in non-survivors of critical illness (Mortensen et al. 2019). The authors of this meta-analysis noted that there was substantial heterogeneity in these studies and that only some studies accounted for other variables that affected prognosis (Mortensen et al. 2019). Our study provides the strongest evidence available that higher ADMA levels are associated with

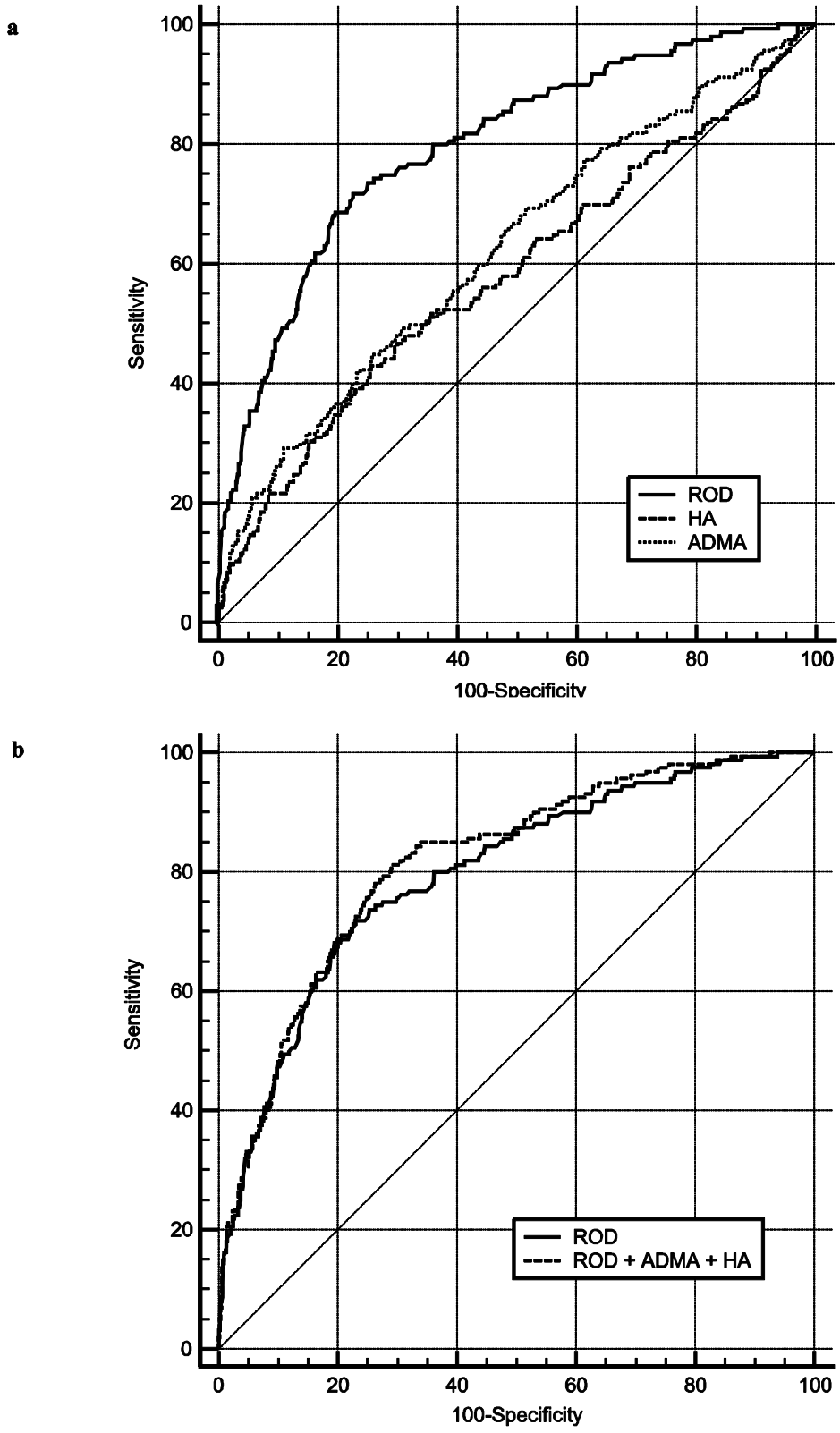


Figure 5-1 Receiver Operator Characteristic Curves assessing the sensitivity and specificity of Risk of Death score (ROD), L-homoarginine (HA) and asymmetric dimethyl-L-arginine (ADMA) individually (a) and combined (b) to predict mortality in critically ill patients (1A)

increased mortality in ICU. The sample size in our study is almost as large as the entire cohort in the recent meta-analysis. Most importantly, we show the positive association with mortality is independent of other arginine metabolites and risk of death score. Risk of death score applies an algorithm to adjust APACHE III scores for the underlying disease state in heterogeneous patient groups and is a better predictor of mortality in ICU than the raw APACHE scores (T. F. Lee et al. 2020; W A Knaus et al. 1991).

The arginine to ADMA ratio has been proposed as a good measure of arginine homeostasis as it incorporates a substrate and a suppressor of nitric oxide synthase (Patel et al. 2016). However, while a few smaller studies have reported that this ratio is inversely associated with poorer outcomes (Visser et al. 2012), most others have reported no significant relationship (Ghashut et al. 2017; Koch et al. 2013; Mortensen et al. 2016; Visser et al. 2012; Brenner et al. 2012). In our study the arginine to ADMA ratio was associated with mortality independent of the risk of death score, but this association was not as strong as for ADMA alone. Arginine itself was not significantly associated with mortality. As such, our data suggest that arginine does not provide additional information beyond measuring ADMA alone.

We found that L-homoarginine was negatively associated with mortality, independent of ADMA and risk of death score. A previous study reported similarly that L-homoarginine was lower in non-survivors of ICU (Ghashut et al. 2017). However, in that study, after adjustment for other prognostic factors the odds ratio for the association between L-homoarginine and mortality of 0.545 was not statistically significant. Our study cohort was more than 10 times the size of the study of Ghashut et al and their results could represent a type 2 error. Our data strongly suggest that L-homoarginine is negatively and independently associated with mortality in critically ill patients.

Other arginine metabolites potentially affect mortality in critically ill patients. Similar to previous studies (Gough et al. 2011; Koch et al. 2013), we found SDMA is higher in non-survivors of critical illness. One previous study reported that SDMA was associated with mortality after adjustment for several laboratory variables including serum creatinine (Koch et al. 2013). However, SDMA is not metabolized but eliminated by renal excretion (Bode-Böger et al. 2006), and in our study the association between SDMA and mortality was not significant after adjustment for renal function. No previous studies have investigated the relationship between MMA and mortality in critically ill patients. In our study the association between MMA and mortality was independent of risk of death score, but not of other arginine metabolites. MMA inhibits nitric oxide synthase with a similar potency to ADMA (Jarzebska et al. 2019). However, MMA circulated in plasma at a concentration <15% of ADMA and this may contribute to its lack of an independent association with mortality. Our data show that SDMA and MMA are not as important determinants of mortality as ADMA and L-homoarginine.

The APACHE / risk of death scoring system is an important tool used to benchmark outcomes (hospital mortality) from year to year within and between ICU sites and is also a simple measure of acuity for comparative research. In this study the addition of ADMA and L-homoarginine increased the area under the receiver operator characteristic curve for mortality, an integrated measure of the sensitivity and specificity of an investigation / clinical tool to identify an outcome. This suggests that measurement of ADMA and L-homoarginine on admission to ICU has the potential to further refine the ability of the APACHE / risk of death scoring system to predict mortality. As measurements of ADMA and L-homoarginine by LC-MS/MS are relatively cheap and simple, if future studies confirm their utility they could be readily incorporated into routine clinical practice.

Previous studies that have investigated the effect of treatment with arginine and MMA on mortality in critically ill patients have not shown a benefit (TRIUMPH Investigators et al.

2007; López et al. 2004; Patel et al. 2016). However, as ADMA is positively and independently associated with mortality in ICU patients, this raises the hypothesis that lowering ADMA could potentially reduce mortality in ICU. Early clinical studies demonstrated direct effects of ADMA that could increase mortality in ICU; infusion of ADMA causes endothelial dysfunction and reduces renal and cerebral blood flow (Vallance et al. 1992; Kielstein et al. 2004; 2006). ADMA is predominantly metabolized by dimethylarginine dimethylaminohydrolase 1 (DDAH1) and overexpression of DDAH1 in an animal model reduces plasma ADMA concentration (Johannes Jacobi et al. 2005). However, studies screening for modulators of DDAH, have not identified any activators of DDAH1 that could reduce ADMA *in vivo* (Jarzebska et al. 2019).

Homoarginine was negatively associated with mortality in this cohort of critically ill patients. This association was independent of other factors affecting prognosis and other arginine metabolites. This suggests that L-homoarginine may directly affect mortality and that increasing L-homoarginine concentration could potentially have a therapeutic benefit in ICU. In a mouse model of L-homoarginine deficiency cerebral infarction size was reduced by supplementation with L-homoarginine (Choe et al. 2013). Oral supplementation with L-homoarginine has been reported to be safe and well tolerated, but did not improve endothelial function in healthy volunteers (Atzler, Schönhoff, et al. 2016). Our data suggest it may be worth investigating the potential therapeutic benefits of L-homoarginine in critically ill patients if nasogastric or intravenous preparations were available.

The strengths of this study include that we prospectively recruited a large cohort of consecutive patients, pre-specified measurement of arginine metabolites as a key endpoint and systematically recorded patient data from reliable sources. However, we acknowledge this study has limitations. Firstly, we only measured arginine metabolites on admission to ICU. As a previous study reported that the increase in ADMA over the first

two days of ICU admission is associated with an increased risk of mortality (Siroen et al. 2005), measurements of ADMA and L-homoarginine at additional time points could further refine results. Secondly, while we adjusted for variables incorporated into the APACHE III scoring system, there may have been unknown variables that affected results. Thirdly, while it is likely that critically ill patients are fasting, we did not include any data on nutritional status which might influence the concentrations of arginine and its metabolites. Finally our study reports an association between ADMA and L-homoarginine and mortality but does not demonstrate a cause-and-effect relationship.

In summary, ADMA concentration on admission to ICU is positively and L-homoarginine negatively associated with mortality in patients with an acute critical illness. These associations are independent of renal function, other clinical factors predicting mortality and other arginine metabolites. These findings suggest measurements of these two arginine metabolites at admission may allow refinement of models to predict ICU mortality. The data support exploring whether reducing ADMA and increasing L-homoarginine are therapeutic targets to reduce mortality in critically ill patients.

5.2 Declarations

5.2.1 Financial support

The study was supported by grants from the Flinders Medical Centre Foundation and the Novo Nordisk Regional Diabetes Scheme.

5.2.2 Conflicts of Interest/Competing Interests

Tien F Lee received post-graduate scholarships from the National Health and Medical Research Council and the Royal Australasian College of Physicians. Tien F Lee have received honorarium for presentations given on behalf of Novo Nordisk, Boehringer Ingelheim and Amgen.

5.2.3 Ethics Approval

Ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee (reference number 268.15).

5.2.4 Consent to Participate

Informed consent was not required as the study met all the requirements for a waiver under the Australian National Statement on Ethical Conduct in Human Research (updated 2015).

5.2.5 Consent for Publication

Informed consent was not required as the study met all the requirements for a waiver under the Australian National Statement on Ethical Conduct in Human Research (updated 2015).

Chapter 6 Does hyperglycaemia affect arginine metabolites in critically ill patients?

Manuscript Under Preparation

Tien F Lee [1,2], Sara Tommasi [3], Andrew Bersten [1,4], Leonie K Heilbronn [5], Salvatore Sotgia [6], Angelo Zinellu[6], Ciriaco Carru[6], Arduino A Mangoni [3], Morton G Burt [1,2]

[1] College of Medicine and Public Health, Flinders University, Flinders Drive, Bedford Park, SA 5042, Australia.

[2] Southern Adelaide Diabetes and Endocrine Services, Flinders Medical Centre, Flinders Drive, Bedford Park, SA 5042, Australia

[3] Discipline of Clinical Pharmacology, Flinders University and Flinders Medical Centre, Flinders Drive, Bedford Park, SA 5042, Australia

[4] Department of Critical Care Medicine, Flinders University, Bedford Park, South Australia, Australia

[5] Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia

[6] Department of Biomedical Sciences, School of Medicine, University of Sassari, Sassari, Italy

6.1 Author Roles

TFL assisted with design of this analysis, collected the clinical data, coordinated the plasma collection for arginine analysis, assisted with the statistical analysis and drafted and revised the manuscript; ST designed and performed the *in vitro* analysis; AB, LKH and AAM assisted with design of this study; SS measured arginine metabolites under the supervision of AZ and CC; ST, LKH, AZ, CC, SS and AAM assisted with manuscript revision; MGB designed this analysis, assisted with the statistical analysis and drafted and revised the manuscript.

6.2 Abstract

Purpose: Changes in the arginine metabolites asymmetric dimethyl-L-arginine (ADMA) and L-homoarginine and acute blood glucose concentration have been shown to cause endothelial dysfunction and have also been independently associated with mortality in critical illness. The aim of this study was to investigate whether hyperglycaemia potentially modulates these arginine metabolite concentrations to provide a mechanism that may link hyperglycaemia and mortality in Intensive Care Unit (ICU) patients.

Methods: Glucose, glycosylated haemoglobin-A1c and the stress hyperglycaemia ratio (SHR) (to quantify absolute, chronic and relative hyperglycaemia respectively) were measured in 1,155 acutely unwell adult patients admitted to a mixed medical-surgical ICU. ADMA, and L-homoarginine were measured in a plasma sample collected at admission to ICU by liquid chromatography tandem mass spectrometry. DDAH1 activity was assessed at varying glucose concentrations *in vitro* by quantifying conversion of ADMA to citrulline in HEK293 cells that overexpress DDAH1.

Results: Plasma ADMA was not significantly associated with any measure of hyperglycaemia. L-homoarginine was positively associated with glucose (β=0.067, p=0.018) and SHR (β=0.107, p<0.001) after correction for GFR. However, as L-homoarginine is a negative predictor of mortality, the direction of these associations are the opposite of those expected if hyperglycaemia was affecting mortality via changes in L-homoarginine. DDAH1 activity was not different at differing concentrations of glucose (p=0.506).

Conclusion: In critically ill patients the association between relative hyperglycaemia and mortality is not mediated by changes in arginine metabolites.

6.3 Introduction

Hyperglycaemia in critically ill patients is associated with increased mortality and morbidity (G. E. Umpierrez et al. 2012; Bochicchio et al. 2005; Koyfman et al. 2018; Egi et al. 2006; James Stephen Krinsley 2003). Furthermore, the relative increase in blood glucose is more strongly associated with mortality than absolute hyperglycaemia, and this relationship is independent of other variables that predict prognosis in ICU (see Chapter 4). However, these findings do not demonstrate that relative hyperglycaemia contributes to mortality directly or provide a mechanism linking hyperglycaemia and mortality.

A number of plausible pathogenic mechanisms have been postulated to link hyperglycaemia with adverse patient outcomes. Both direct and indirect effects have been implicated. Indirect effects include changes in circulation and electrolytes causing electrolyte imbalances, hypoperfusion, volume depletion or acid-base imbalance. Direct effects previously studied include oxidative stress, inflammation, induction of apoptosis, activation of the coagulation cascade, and down-regulation of the endothelium-dependent vasodilation in humans due to endothelial dysfunction. (Williams et al. 1998; Esposito et al. 2002; Stegenga et al. 2006; Jafar, Edriss, and Nugent 2016). Endothelial dysfunction is present in septic patients in ICU (Vassiliou et al. 2014; Szabo and Goldstein 2011), and is an independent predictor of mortality in critically ill patients (Duffy et al. 2011).

Endothelial dysfunction is often assessed by quantifying limb blood flow following localized ischaemia. However, this is impractical in large cohorts of critically ill patients. An alternative approach to assess endothelial function is measurement of L-arginine and related metabolites in plasma. As highlighted in Chapter 1, asymmetric dimethyl-L-arginine (ADMA), symmetric dimethyl-L-arginine (SDMA), monomethyl-L-arginine (MMA), and L-homoarginine (HA) directly or indirectly regulate the activity of the enzyme endothelial nitric oxide synthase (eNOS), which converts L-arginine to citrulline. This chemical reaction results in the synthesis of the potent endogenous vasodilator nitric oxide (NO), which also has anti-inflammatory, anti-thrombotic and anti-atherosclerotic effects (Louis J Ignarro, Napoli, and Loscalzo 2002).

Decreased NO production has been associated with increased cardiovascular morbidity and mortality in patients with cardiovascular disease and hypertension (Lerman and Zeiher 2005; Versari et al. 2009). Consistent with this, in Chapter 5 it was demonstrated that ADMA is positively and L-homoarginine negatively associated with increased mortality in patients with an acute critical illness. Acute hyperglycaemia is a well-recognized cause of endothelial dysfunction (Ceriello et al. 2008). Moreover, previous studies in a rodent model have reported that the activity of the main enzyme that regulates ADMA concentration in plasma, dimethylarginine-dimethylaminohydroxylase (DDAH), is down-regulated by hyperglycaemia (Lin et al. 2002). This would increase ADMA concentration, consequently reducing NO production, and potentially provide a mechanistic link between acute hyperglycaemia, endothelial dysfunction and mortality (Siervo et al. 2011).

We hypothesized that acute hyperglycaemia in critically ill patients reduces DDAH1 activity, thereby increasing plasma ADMA concentration and reducing endothelial-dependent vasodilatation. To investigate this we have undertaken: 1) a clinical study investigating whether measurements of glycaemia are associated with arginine metabolites in critically ill patients; and 2) an *in vitro* study assessing the effect of glucose concentration on DDAH1 activity.

6.4 Methods

6.4.1 Subjects

For the clinical study, we studied the patient cohort recruited to assess the relationship between relative hyperglycaemia and mortality in critically ill patients, as described in Chapter 4. In brief, we prospectively included consecutive medical and surgical admissions to Intensive Care Unit (ICU) at Flinders Medical Centre between 27 January 2016 and 30 March 2017 that met inclusion and exclusion criteria. Subjects were excluded if they had previously been admitted to ICU within the study period or were admitted for routine post-operative monitoring following a surgical procedure, aged <18 years, pregnant or had a recent blood transfusion, were admitted primarily for treatment of hyper- or hypoglycaemia or had missing data preventing calculation of risk of death score. In the remaining subjects, we stored the plasma sample collected closest to admission to ICU at -70°C

for subsequent measurement of arginine and metabolites. If no plasma sample was collected within 24 hours of admission to ICU, the subject was excluded from this analysis.

6.4.2 Arginine metabolites

Arginine and its chemically related metabolites and analogues MMA, L-homoarginine, ADMA and SDMA were measured according to the method developed by Sotgia et al (Sotgia et al. 2019) and described in detail in Chapter 2 and Chapter 5.

6.4.3 Absolute and relative hyperglycaemia

Glucose and HbA1c were measured by the hospital pathology laboratory, SA Pathology, using methodologies as previously described in Chapter 2 and Chapter 4. Relative hyperglycaemia was quantified using the Stress Hyperglycaemia Ratio, which was based on the admission glucose and the estimated average glucose derived from HbA1c (Roberts et al. 2015).

6.4.4 DDAH1 activity

In the *in vitro* study, cell lysate prepared from HEK293T cells overexpressing DDAH1, were used to investigate the direct effect of glucose on DDAH1 activity (Sara Tommasi et al. 2015). This enables a full kinetic characterisation of the inhibitory potency of glucose on DDAH1 activity. An established DDAH1 activity assay using isotope dilution UPLC-MS was used to measure the conversion of ADMA to citrulline (Sara Tommasi et al. 2015). When the substrate (ADMA) is mixed with DDAH1, ADMA will be metabolized to citrulline. Hence, DDAH1 activity in presence of glycaemia can be measured indirectly by measuring citrulline concentrations when ADMA and DDAH1 are combined with varying glucose concentrations.

All analyses were undertaken in triplicate. HEK293T cell lysate over expressing DDAH1 (0.4 mg/mL) in 0.1M phosphate buffer and glucose (0, 5, 7.5, 10, 15 and 22.5 mmol/L) were pre-incubated for one hour prior to the addition of ADMA (45 µmol/L, equivalent to Km) to initiate the reaction. After 30 min the reaction was terminated by the addition of acidified propanol, L-citrulline-d6 added as the assay internal standard and the sample centrifuged to remove the precipitated

proteins. Following evaporation and reconstitution of the supernatant fraction to achieve a more concentrated solution, the concentration of L-citrulline formed during the incubation was measured by UPLC-MS.

6.4.5 Statistical Analysis

Simple linear regression analyses were used to compare the relationship between measures of glycaemic control and arginine metabolites. As all variables were not normally distributed they were log-transformed to attain a normal distribution before the statistical analysis. As many arginine metabolites are renally cleared, these odds ratios were then assessed for independence from GFR in a multiple linear regression analysis to ensure that differences in renal function were not underlying results. Finally, the effect of glucose concentration on DDAH1 activity was assessed using one way analysis of variance.

Statistical analysis was undertaken using SPSS version 25 for Windows (IBM, New York, USA); A two-tailed P-value of <0.05 was considered statistically significant.

6.5 Results

6.5.1 Subject characteristics

There were 1,262 critically ill patients recruited for the study investigating the relationship between relative hyperglycaemia and mortality in critically ill patients (T. F. Lee et al. 2020). In this cohort, 107 patients did not have an adequate plasma sample for arginine metabolomics studies and excluded from further analysis. Therefore, 1,155 patients were included in this analysis. The subject characteristics of this cohort are reported in Chapter 5.

6.5.2 Arginine metabolites in Critical Illness

Homoarginine was weakly but significantly positively associated with HbA1c and SHR in univariate analyses and with glucose and SHR after correction for GFR (Table 1). SDMA was significantly associated with glucose, HbA1c and SHR in univariate analyses. After correcting for GFR, the correlations with glucose and SHR remained statistically significant (Table 1). ADMA, arginine, and

MMA were not significantly associated with any glucose parameter, either unadjusted or after correction for GFR (Table 1). GFR was associated with all arginine metabolites, an effect that was independent of all glucose parameters (data not shown)

6.5.3 DDAH1 activity

Citrulline production at different glucose concentrations *in vitro* is shown in Figure 1. There was no significant effect of glucose concentration on citrulline production (p = 0.506).

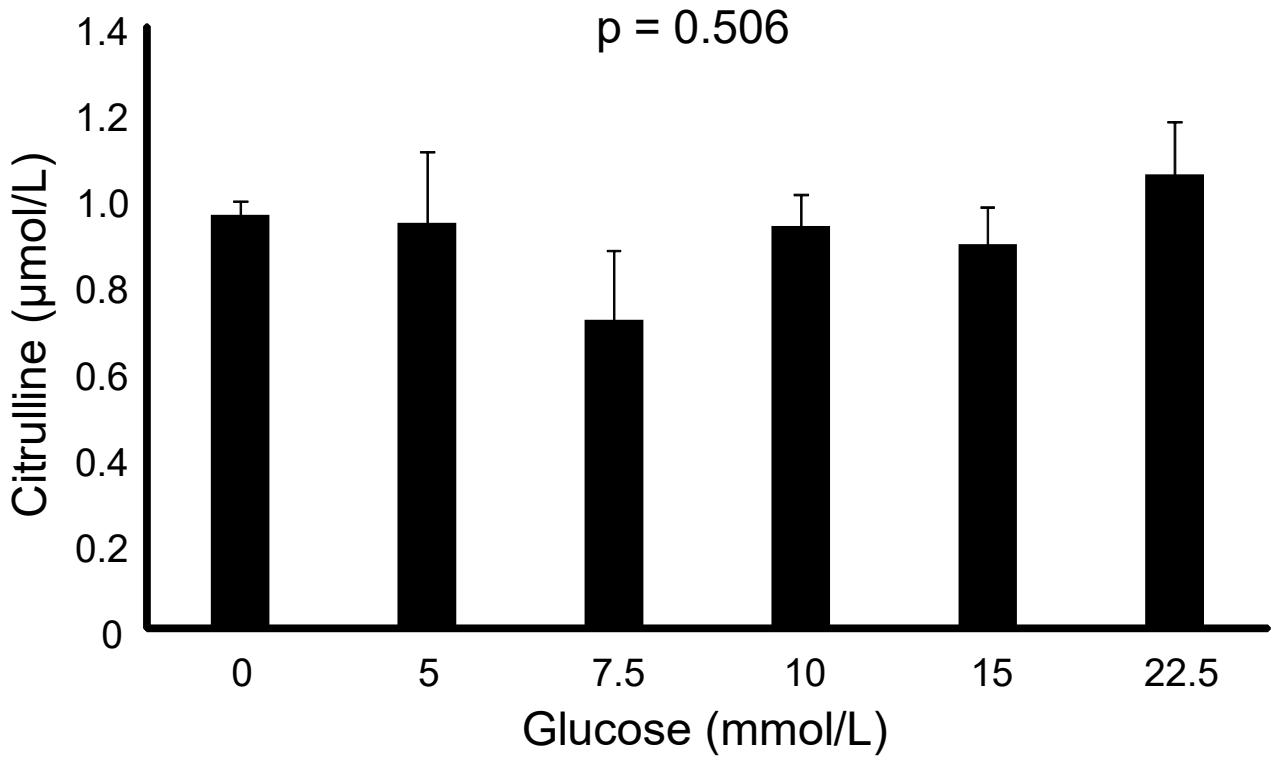


Figure 6-1 Citrulline production at different glucose concentrations

	Glucose		HbA1c		SHR		Glucose*		HbA1c*		SHR*	
	R	P value	r	P value	r	P value	β	P value	β	P value	B	P value
ADMA (nmol/L)	0.012	0.671	0.002	0.940	0.016	0.590	0.004	0.879	-0.046	0.109	0.038	0.190
Homoarginine (nmol/L)	0.057	0.054	0.086	0.004	0.123	<0.001	0.067	0.018	-0.048	0.097	0.107	<0.001
Arginine (µmol/L)	0.004	0.897	0.009	0.755	0.001	0.994	0.005	0.860	0.025	0.405	-0.009	0.762
MMA (nmol/L)	0.028	0.335	0.025	0.391	0.15	0.613	0.021	0.468	-0.017	0.559	0.036	0.206
SDMA (nmol/L)	0.132	<0.001	0.068	0.021	0.189	<0.001	-0.153	<0.001	-0.035	0.105	-0.139	<0.001

Table 6-1 Simple and multiple linear regression analyses between measures of glycaemic control and arginine metabolites

* Corrected for log GFR. Log GFR is independently associated with arginine metabolite concentrations in all analyses.

6.6 Discussion

We assessed the relationship between arginine metabolites and 3 different measurements of hyperglycaemia (absolute, relative and chronic hyperglycaemia) in a large cohort of critically ill patients. We found that there was no association between glucose parameters and plasma ADMA concentration. Concordant with these findings, differing glucose concentrations *in vitro* did not affect DDAH1 activity. There was a weak positive correlation between L-homoarginine and glucose parameters. However, as L-homoarginine is negatively associated with mortality (see Chapter 5), the direction of these associations are the opposite of those expected if hyperglycaemia was affecting mortality via changes in L-homoarginine. Taken together, the findings strongly imply that hyperglycaemia does not increase mortality by altering arginine metabolites.

In the clinical study there was not a significant association between ADMA and any of the 3 measures of hyperglycaemia. As we had reported that relative hyperglycaemia (Chapter 4) and ADMA (Chapter 5) were independently associated with mortality, we hypothesized that a glucose-mediated elevation in ADMA could be a mechanistic link between these two findings. Previous studies using non-invasive measures to assess endothelial function have reported that increasing ADMA (Çakar et al. 2014) and blood glucose acutely both cause endothelial dysfunction (Ceriello et al. 2008; Gordin et al. 2007). However, in this large cohort of critically ill patients there was no suggestion of a correlation between relative hyperglycaemia and ADMA.

Our *in vitro* study was consistent with a lack of effect of acute hyperglycaemia on ADMA concentration. Differences in glucose concentration across the range encountered in clinical practice did not affect DDAH1 activity, the main enzyme metabolizing ADMA and responsible for its plasma concentration. It was previously hypothesized that hyperglycaemia causes endothelial dysfunction by downregulating DDAH1 (Siervo et al. 2011) and this theory was supported by data from a rodent model of chronic hyperglycaemia (Lin et al. 2002). It is likely that differences in the biological model and duration of hyperglycaemia contribute to the differences between our study and that of Lin et al (Lin et al. 2002). Our combined clinical and *in vivo* data provide strong

evidence that changes in plasma ADMA do not mediate the relationship between acute hyperglycaemia and mortality in humans.

There was a weak but significant positive association between L-homoarginine and both glucose and SHR after correction for GFR. In Chapter 5, plasma L-homoarginine concentration was lower in patients who died than in survivors of critical illness and there was a negative association between L-homoarginine and mortality. This is consistent with reports of a negative association between L-homoarginine and mortality in other patient groups (Atzler et al. 2014; Drechsler et al. 2015; Zinellu et al. 2018). Therefore, if the relationship between relative hyperglycaemia and mortality was being mediated by changes in L-homoarginine, the association between L-homoarginine and SHR should be negative. As the association is weak and in the opposite direction, this suggests the relationship between acute hyperglycaemia and mortality is not via modulation of L-homoarginine.

After correction for renal function, there was a weak but significant association between SDMA and glucose and SHR. However, SDMA was not an independent determinant of mortality in this cohort (see Chapter 5). There was not a significant association between arginine and MMA and any of the 3 glucose parameters. Taken with the results of Chapter 5, it is unlikely that the relationship between relative hyperglycaemia and mortality is mediated by SDMA, MMA or arginine.

Acute hyperglycaemia has been clearly demonstrated to cause changes in vascular tone and endothelial dysfunction that are associated with increased mortality in critically ill patients (Siervo et al. 2011; Kawano et al. 1999; Williams et al. 1998). However, our study clearly demonstrates that endothelial dysfunction in the critically ill is not mediated by changes in arginine metabolites. Given the negative findings in this study, it is possible that relative hyperglycaemia: 1) is primarily linked to mortality with a different mechanism not related to endothelial dysfunction, and / or, 2) causes endothelial dysfunction through an arginine-independent pathway. We support further studies to elucidate mechanisms linking relative hyperglycaemia and mortality.

This study has several notable strengths. It comprised a large prospective population of consecutive critically ill patients. The combination of an *in vivo* and an *in vitro* study provides

confidence that the results are valid. However, there are a number of limitations of this study. The clinical study was observational, with inherent limitations, such as the inability to infer causality. Measurement of arginine metabolites was not the primary endpoint of the study. Another limitation is that only one mechanism by which arginine metabolites are modulated was assessed *in vitro*. However, as ADMA was the strongest independent predictor of mortality in Chapter 5, DDAH1 activity was considered the most important variable to study.

6.7 Conclusion

In summary, relative hyperglycaemia is not associated with plasma ADMA concentration in ICU patients and hyperglycaemia does not alter the activity of ADMAs main regulator DDAH1. Relative hyperglycaemia is weakly associated with L-homoarginine, but the direction of the association between L-homoarginine and SHR is the opposite to that expected if acute hyperglycaemia was increasing mortality by altering L-homoarginine concentration. Hence, we conclude that in critically ill patients, the association between relative hyperglycaemia and mortality is not mediated by changes in arginine metabolites.

Chapter 7 Summary and Conclusions

7.1 Introduction

Hyperglycaemia is common in hospitalized patients such as those with an acute myocardial infarction (AMI) or critical illness and is associated with increased mortality. Despite this, there is conflicting evidence in the literature as to whether treating hyperglycaemia in hospital reduces mortality. This has resulted in varied recommendations regarding how to treat hyperglycaemia in hospital from different authorities (ADA 2020; ADS 2012; G. E. Umpierrez and Pasquel 2017; Malcolm et al. 2018).

One possible explanation for the abovementioned discordant results is that hyperglycaemia has different implications in different patients. In a hospitalized patient with sub-optimally controlled type 2 diabetes, a high blood glucose concentration may be similar to their pre-admission glucose concentrations. In contrast, in a patient without diabetes the same blood glucose concentration represents a substantial increase above what is normal for them. Previous studies in this area have used an absolute glucose level (a single glucose concentration, usually at admission) to select which patients to treat, and have targeted the same absolute glucose range, regardless of whether they have acute or chronic hyperglycaemia.

The Stress Hyperglycaemia Ratio (SHR) is a method to quantify the acute rise of glucose concentrate in a hospitalized patient, thereby facilitating the differentiation between relative (stress) hyperglycaemia and chronic hyperglycaemia. SHR may provide a better understanding of the relative contributions of acute versus chronic to mortality in critical illness. SHR may help resolve the conflicting evidence in literature, and provide a means to tailor therapy in an individualised manner to hospitalized patients.

Arginine and its major metabolites, particularly ADMA and L-homoarginine, modulate the eNOS pathway, and have been: 1) considered a surrogate marker for endothelial function, and 2) associated with poorer outcomes in a range of patient groups. In small studies, endothelial

dysfunction is strongly associated with mortality in critically ill patients. By inference, the arginine metabolic pathway should be studied for its association with mortality in critical illness and also its potential as a risk marker for adverse outcomes.

Hyperglycaemia has both direct and indirect effects that potentially underlie an association with poorer outcomes in hospitalized patients. It has been postulated that hyperglycaemia modulates endothelial-dependent nitric oxide synthesis through an effect on the arginine metabolic pathway. This is a plausible mechanism by which acute hyperglycaemia exerts its negative effects and had not been studied in humans with critical illness.

The major aims of this thesis were to determine whether: 1) relative hyperglycaemia is more strongly associated with mortality than absolute hyperglycaemia in patients with AMI and critical illness, 2) the association between relative hyperglycaemia and mortality is affected by background glycaemia, 3) arginine metabolites are independently associated with mortality in critically ill patients, and 4) relative hyperglycaemia results in changes in arginine metabolites that may underlie the associated increase in mortality. For patients with AMI, the association between mortality and absolute and relative hyperglycaemia during insulin treatment were first studied in Chapter 3. Chapter 4 investigated whether relative hyperglycaemia is more strongly associated with mortality than absolute hyperglycaemia in patients with critical illness. The associations between arginine metabolites and mortality in patients with critical illness were studied in Chapter 5. Finally, in Chapter 6 the relationship between arginine metabolites and hyperglycaemia in critically ill patients were investigated.

7.2 Summary and Conclusions

The first aim was to assess whether relative hyperglycaemia is more strongly associated with mortality than absolute hyperglycaemia in patients with AMI and critical illness. For AMI, a *post-hoc* analysis was performed using data from 192 patients originally recruited prospectively for the HI-5 study (Chapter 3). The primary endpoint was a “complicated AMI”, which included death, congestive cardiac failure, arrhythmia, cardiac arrest, re-infarction during hospitalization, cardiogenic shock, inotropic support and emergency revascularization. SHR was associated with a

complicated AMI (odds ratio = 1.22 per 0.1 SHR increment, 95% CI 1.06-1.42, p=0.006), and the individual components of death, congestive cardiac failure, arrhythmia and cardiogenic shock, while absolute glucose concentration was not statistically associated with a complicated AMI. For critical illness, an observational prospective study is reported in Chapter 4, including 1,262 consecutively admitted ICU patients. After adjustment for other important prognostic markers in the risk of death score, SHR was significantly associated with mortality (odds ratio 1.03 per 0.1 SHR increment, p=0.014) whereas absolute glucose was not. The combined data from these two studies demonstrated that relative, but not absolute, hyperglycaemia is associated with mortality in hospitalized patients.

The second aim was to determine whether the association between relative hyperglycaemia and mortality is affected by background glycaemia. In patients with AMI the relationship between SHR and complicated AMI was independent of diabetes status and there was not a significant interaction between SHR and diabetes (Chapter 3). In patients with critical illness, SHR was associated with mortality in patients with HbA1c <6.5% (odds ratio = 1.08 per 0.1 SHR increment, p<0.001) and in those with HbA1c ≥6.5% (odds ratio = 1.08 per 0.1 SHR increment, p=0.005). There was no interaction between SHR and HbA1c in critically ill patients (p=0.909) (Chapter 4). These results show that the relationship between SHR and mortality is not significantly affected by background glycaemia, suggesting that SHR has prognostic utility in patients across the glycaemic spectrum.

The third aim was to investigate if arginine metabolites are independently associated with mortality in critically ill patients. In Chapter 5, L-arginine, ADMA, MMA, SDMA and L-homoarginine concentration were measured in plasma from 1,155 critically ill patients. In this cohort, ADMA was positively associated (odds ratio = 1.159 per 0.1 µmol/L increment, p=0.012) and L-Homoarginine negatively associated (odds ratio = 0.963, p=0.013) with mortality, independent of risk of death score and other arginine metabolites. Adding L-homoarginine and ADMA to the risk of death score significantly increased the area under the ROC curve for mortality. These results demonstrate that ADMA and L-homoarginine may refine models to predict ICU mortality. Reducing ADMA and

increasing L-homoarginine are potential therapeutic targets to reduce mortality in critically ill patients.

The final aim was to investigate whether relative hyperglycaemia results in changes in arginine metabolites that may underlie the associated increase in mortality. In a cohort of 1,155 critically ill patients ADMA was not significantly associated with any measure of glycaemia (Chapter 6). Moreover, *in vitro* DDAH1 activity, the main regulator of plasma ADMA concentration, was not different at differing concentrations of glucose ($p=0.506$). In the same cohort, L-homoarginine was positively associated with glucose ($\beta=0.067$, $p=0.018$) and SHR ($\beta=0.107$, $p<0.001$) after correction for GFR. However, as L-homoarginine is a negative predictor of mortality, the direction of these associations are the opposite of those expected if hyperglycaemia was affecting mortality via changes in L-homoarginine. Taken together, the results indicate that hyperglycaemia does not result in changes in arginine metabolites that underlie its association with mortality.

In conclusion, relative, but not absolute, hyperglycaemia is strongly associated with mortality in both patients with AMI and critical illness. Unlike absolute hyperglycaemia, the association between relative hyperglycaemia and mortality is not affected by background glycaemia. The arginine metabolites ADMA and L-homoarginine are independent predictors of mortality in critically ill patients. However, ADMA is not associated with relative hyperglycaemia and L-homoarginine is positively associated with relative hyperglycaemia. These results indicate that SHR, ADMA and L-homoarginine are markers of mortality in critical illness, independent of each other and of the risk of death score. However, changes in arginine metabolism are not the mechanism that links hyperglycaemia with endothelial dysfunction and increased mortality in critical illness.

7.3 Future Directions

This body of work represents the first assessment of relative hyperglycaemia in AMI, and also the first *prospective* and the largest study of relative hyperglycaemia in critical illness. This was also the first study that corrected the association between hyperglycaemia for the severity of the underlying illness using the risk of death score, a severity of illness marker that corrects for the reason for admission to ICU. Both studies reported that relative, but not absolute, hyperglycaemia

is strongly associated with mortality. These positive findings suggest a need for further research into the importance of relative hyperglycaemia in hospitalized patients.

An important area that requires clarification is whether the concept of relative hyperglycaemia could resolve reports in the literature that the benefits of tight glycaemic control in ICU differ in patients with and without diabetes. Several retrospective studies have reported that intensive insulin therapy was associated with lower mortality in patients without diabetes than more liberal glucose control. However, in patients with diabetes the converse was found, with higher mortality in patients prescribed intensive insulin (Luethi et al. 2018; Lanspa et al. 2013; Kar et al. 2016; Balintescu and Mårtensson 2019) (see Figure 1-3). These studies have utilised the same absolute glucose targets for all patients. This results in patients without diabetes who have relative hyperglycaemia not being treated, whereas a patient with diabetes who has absolute, but not relative, hyperglycaemia is treated with insulin (Table 7-1).

Patient	Diabetes	Glucose (mmol/L)	HbA1c (%)	SHR	Insulin
1	No	8	5	1.5	No
2	Yes	12	9	1.0	Yes

Table 7-1 Theoretical patients and current insulin treatment recommendations in ICU

There is a need is for further studies testing a new paradigm, whereby insulin therapy in hospital is predominantly targeted at treating relative hyperglycaemia to maintain a usual glucose concentration for an individual patient, rather than treating to an absolute glucose target. In this paradigm and contrary to current treatment, Patient 1 without diabetes in Table 7-1 would be prescribed an insulin infusion, while Patient 2 with diabetes would not. This could first be assessed retrospectively, with *post-hoc* analyses of previous studies, similar to my re-analysis of the HI5 study. However, ideally a randomized controlled trial should be performed, with patients randomized to either an individualised relative glucose target (e.g SHR 0.8-1.2) or the current gold-standard of care targeting an absolute glucose range (ADA 2020; Malcolm et al. 2018; Moghissi et

al. 2009; Qaseem 2011; Judith Jacobi et al. 2012). This concept has recently been supported by experts in the management of hyperglycaemia in ICU (James S. Krinsley et al. 2020). The results of Chapter 3 support a similar study in patients with an AMI.

In Chapter 5, ADMA and L-homoarginine increased the area under the ROC curve for death compared to risk of death alone (see Chapter 5). These results suggest that measurement of these arginine metabolites on admission to ICU may improve upon current models to predict mortality in critically ill patients. However, this would need to be further evaluated and validated with established methodologies after incorporating SHR into a prognostic model and testing in an independent cohort (Leisman et al. 2020). As ADMA and L-homoarginine are independently associated with mortality, future studies should also explore whether reducing and increasing levels of these arginine metabolites respectively has an impact on mortality in ICU.

The main negative finding in this thesis is that relative hyperglycaemia was not associated with arginine metabolites in critically ill patients (Chapter 6). As such, my thesis does not provide insights into mechanisms linking relative hyperglycaemia and mortality. Although this relationship was significant after correction for other known factors associated with mortality in ICU (Chapter 4), it remains possible that relative hyperglycaemia is only a biomarker of disease severity and not pathogenic. This conundrum is best addressed by a randomized controlled trial, but the rationale for treating relative hyperglycaemia would be further strengthened by studies investigating other potential mechanisms link that might link relative hyperglycaemia and mortality.

Finally, three measures of relative hyperglycaemia have been proposed in the literature (Glycaemic Gap, SHR, Glucose-A1c Ratio; see Section 1.3.1). I plan to undertake a *post hoc* comparison of these 3 measures of relative hyperglycaemia using the data from Chapter 4.

References

Action to Control Cardiovascular Risk in Diabetes Study Group, Hertzel C Gerstein, Michael E Miller, Robert P Byington, David C Goff, J Thomas Bigger, John B Buse, et al. 2008. “Effects of Intensive Glucose Lowering in Type 2 Diabetes.” *The New England Journal of Medicine* 358 (24): 2545–59. https://doi.org/10.1056/NEJMoa0802743.

ADA. 2020. “15. Diabetes Care in the Hospital: Standards of Medical Care in Diabetes—2020.” *Diabetes Care* 43 (Supplement 1): S193–202. https://doi.org/10.2337/dc20-S015.

ADS. 2012. *Australian Diabetes Society Guidelines for Routine Glucose Control in Hospital*. 2012th ed. Australian Diabetes Society.

ADVANCE Collaborative Group, Anushka Patel, Stephen MacMahon, John Chalmers, Bruce Neal, Laurent Billot, Mark Woodward, et al. 2008. “Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes.” *The New England Journal of Medicine* 358 (24): 2560–72. https://doi.org/10.1056/NEJMoa0802987.

Altmann, Karin S, Antje Havemeyer, Eric Beitz, and Bernd Clement. 2012. “Dimethylarginine-Dimethylaminohydrolase-2 (DDAH-2) Does Not Metabolize Methylarginines.” *Chembiochem : A European Journal of Chemical Biology* 13 (17): 2599–2604. https://doi.org/10.1002/cbic.201200499.

American Diabetes Association. 2019. “15. Diabetes Care in the Hospital: Standards of Medical Care in Diabetes—2019.” *Diabetes Care* 42 (Supplement 1): S173–81. https://doi.org/10.2337/dc19-S015.

Annane, Djillali, Alain Cariou, Virginie Maxime, Elie Azoulay, Gilles D’honneur, Jean François Timsit, Yves Cohen, et al. 2010. “Corticosteroid Treatment and Intensive Insulin Therapy for Septic Shock in Adults.” *JAMA* 303 (4): 341. https://doi.org/10.1001/jama.2010.2.

Arabi, Yaseen M., Ousama C. Dabbagh, Hani M. Tamim, Abdullah A. Al-Shimemeri, Ziad A. Memish, Samir H. Haddad, Sofia J. Syed, et al. 2008. “Intensive versus Conventional Insulin Therapy: A Randomized Controlled Trial in Medical and Surgical Critically Ill Patients*.” *Critical Care Medicine* 36 (12): 3190–97. https://doi.org/10.1097/CCM.0b013e31818f21aa.

Atzler, Dorothee, Christina Baum, Francisco Ojeda, Till Keller, Kathrin Cordts, Renate B. Schnabel, Chi Un Choe, et al. 2016. “Low Homoarginine Levels in the Prognosis of Patients with Acute Chest Pain.” *Journal of the American Heart Association* 5 (4). https://doi.org/10.1161/JAHA.115.002565.

Atzler, Dorothee, M. Odette Gore, Colby R. Ayers, Chi Un Choe, Rainer H. Böger, James A. De Lemos, Darren K. McGuire, and Edzard Schwedhelm. 2014. “Homoarginine and Cardiovascular Outcome in the Population-Based Dallas Heart Study.” *Arteriosclerosis, Thrombosis, and Vascular Biology* 34 (11): 2501–7. https://doi.org/10.1161/ATVBAHA.114.304398.

Atzler, Dorothee, Mirjam Schönhoff, Kathrin Cordts, Imke Ortland, Julia Hoppe, Friedhelm C. Hummel, Christian Gerloff, et al. 2016. “Oral Supplementation with L-Homoarginine in Young Volunteers.” *British Journal of Clinical Pharmacology* 82 (6): 1477–85. https://doi.org/10.1111/bcp.13068.

Atzler, Dorothee, Edzard Schwedhelm, and Chi-un Choe. 2015. “L-Homoarginine and Cardiovascular Disease.” *Current Opinion in Clinical Nutrition and Metabolic Care* 18 (1): 83–88. https://doi.org/10.1097/MCO.0000000000000123.

Baker, E H, C H Janaway, B J Philips, A L Brennan, D L Baines, D M Wood, and P W Jones. 2006. “Hyperglycaemia Is Associated with Poor Outcomes in Patients Admitted to Hospital with Acute Exacerbations of Chronic Obstructive Pulmonary Disease.” *Thorax* 61 (4): 284–89. https://doi.org/10.1136/thx.2005.051029.

Balintescu, Anca, and Johan Mårtensson. 2019. “Hemoglobin A1c and Permissive Hyperglycemia in Patients in the Intensive Care Unit with Diabetes.” *Critical Care Clinics* 35 (2): 289–300. https://doi.org/10.1016/j.ccc.2018.11.010.

Berghe, G van den, P Wouters, F Weekers, C Verwaest, F Bruyninckx, M Schetz, D Vlasselaers, P Ferdinande, P Lauwers, and R Bouillon. 2001. “Intensive Insulin Therapy in Critically Ill Patients.” *The New England Journal of Medicine* 345 (19): 1359–67. https://doi.org/10.1056/NEJMoa011300.

Berghe, Greet Van den, Alexander Wilmer, Greet Hermans, Wouter Meersseman, Pieter J Wouters, Ilse Milants, Eric Van Wijngaerden, Herman Bobbaers, and Roger Bouillon. 2006. “Intensive Insulin Therapy in the Medical ICU.” *The New England Journal of Medicine* 354 (5): 449–61. https://doi.org/10.1056/NEJMoa052521.

Berghe, Greet Van den, Alexander Wilmer, Ilse Milants, Pieter J Wouters, Bernard Bouckaert, Frans Bruyninckx, Roger Bouillon, and Miet Schetz. 2006. “Intensive Insulin Therapy in Mixed Medical/Surgical Intensive Care Units: Benefit versus Harm.” *Diabetes* 55 (11): 3151–59. https://doi.org/10.2337/db06-0855.

Blackwell, Scott. 2010. “The Biochemistry, Measurement and Current Clinical Significance of Asymmetric Dimethylarginine.” *Annals of Clinical Biochemistry* 47 (Pt 1): 17–28. https://doi.org/10.1258/acb.2009.009196.

Bochicchio, Grant V, Laura Salzano, Manjari Joshi, Kelly Bochicchio, and Thomas M Scalea. 2005. “Admission Preoperative Glucose Is Predictive of Morbidity and Mortality in Trauma Patients Who Require Immediate Operative Intervention.” *The American Surgeon* 71 (2): 171–74.

Bode-Böger, Stefanie M., Fortunato Scalera, Jan T. Kielstein, Jens Martens-Lobenhoffer, Günter Breithardt, Manfred Fobker, and Holger Reinecke. 2006. “Symmetrical Dimethylarginine: A New Combined Parameter for Renal Function and Extent of Coronary Artery Disease.” *Journal of the American Society of Nephrology* 17 (4): 1128–34. https://doi.org/10.1681/ASN.2005101119.

Bots, Sophie H, Yolanda van der Graaf, Hendrik M W Nathoe, Gert Jan de Borst, Jaap L Kappelle, Frank L. J. Visseren, and Jan Westerink. 2016. “The Influence of Baseline Risk on the Relation between HbA1c and Risk for New Cardiovascular Events and

Mortality in Patients with Type 2 Diabetes and Symptomatic Cardiovascular Disease." *Cardiovascular Diabetology* 15 (1): 101. https://doi.org/10.1186/s12933-016-0418-1.

Braithwaite, Susan S. 2010. "Through the Eyes of the A1C: A Call to Re-Examine Stress Hyperglycemia." *Critical Care Medicine* 38 (2): 717–19. https://doi.org/10.1097/CCM.0b013e3181cc8c6a.

Brenner, Thorsten, Thomas H. Fleming, Claudia Rosenhagen, Ute Krauser, Markus Mieth, Thomas Bruckner, Eike Martin, et al. 2012. "L-Arginine and Asymmetric Dimethylarginine Are Early Predictors for Survival in Septic Patients with Acute Liver Failure." *Mediators of Inflammation* 2012. https://doi.org/10.1155/2012/210454.

Brunkhorst, Frank M., Christoph Engel, Frank Bloos, Andreas Meier-Hellmann, Max Ragaller, Norbert Weiler, Onnen Moerer, et al. 2008. "Intensive Insulin Therapy and Pentastarch Resuscitation in Severe Sepsis." *New England Journal of Medicine* 358 (2): 125–39. https://doi.org/10.1056/NEJMoa070716.

Çakar, Mustafa, Fatih Bulucu, Murat Karaman, Seyit Ahmet Ay, Ömer Kurt, Şevket Balta, Sait Demirkol, et al. 2014. "Asymmetric Dimethylarginine and Augmentation Index in Newly Diagnosed Patients with Hypertension." *Angiology* 66 (1): 43–48. https://doi.org/10.1177/0003319713513145.

Campbell, Leon, Tessa Pepper, and Kate Shipman. 2019. "HbA1c: A Review of Non-Glycaemic Variables." *Journal of Clinical Pathology* 72 (1): 12–19. https://doi.org/10.1136/jclinpath-2017-204755.

Cannon, Christopher P., Richard Pratley, Samuel Dagogo-Jack, James Mancuso, Susan Huyck, Urszula Masiukiewicz, Bernard Charbonnel, et al. 2020. "Cardiovascular Outcomes with Ertugliflozin in Type 2 Diabetes." *New England Journal of Medicine* 383 (15): 1425–35. https://doi.org/10.1056/nejmoa2004967.

Capes, S E, D Hunt, K Malmberg, and H C Gerstein. 2000. "Stress Hyperglycaemia and Increased Risk of Death after Myocardial Infarction in Patients with and without Diabetes: A Systematic Overview." *Lancet* 355 (9206): 773–78. https://doi.org/10.1016/S0140-6736(99)08415-9.

Capes, S E, D Hunt, K Malmberg, P Pathak, and H C Gerstein. 2001. "Stress Hyperglycemia and Prognosis of Stroke in Nondiabetic and Diabetic Patients: A Systematic Overview." *Stroke* 32 (10): 2426–32.

Carmen Wong, K Y, Vincent Wong, Jui T Ho, David J Torpy, Mark McLean, and N Wah Cheung. 2010. "High Cortisol Levels in Hyperglycaemic Myocardial Infarct Patients Signify Stress Hyperglycaemia and Predict Subsequent Normalization of Glucose Tolerance." *Clinical Endocrinology* 72 (2): 189–95. https://doi.org/10.1111/j.1365-2265.2009.03654.x.

Carter, Rachel, and Peter Hicks. 2011. "Centre for Outcome and Resource Evaluation: Intensive Care Resources and Activity Report 2010 – 2011." *CORE Intensive Care Resources and Activity Report 2010-2011*.

Ceriello, Antonio, Katherine Esposito, Ludovica Piconi, Michael A Ihnat, Jessica E Thorpe, Roberto Testa, Massimo Boemi, and Dario Giugliano. 2008. "Oscillating Glucose Is

More Deleterious to Endothelial Function and Oxidative Stress than Mean Glucose in Normal and Type 2 Diabetic Patients." *Diabetes* 57 (5): 1349–54. https://doi.org/10.2337/db08-0063.

Chamberlain, James J, Andrew S Rhinehart, Charles F Shaefer, and Annie Neuman. 2016. "Diagnosis and Management of Diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes." *Annals of Internal Medicine* 164 (8): 542–52. https://doi.org/10.7326/M15-3016.

Charlson, M E, P Pompei, K L Ales, and C R MacKenzie. 1987. "A New Method of Classifying Prognostic Comorbidity in Longitudinal Studies: Development and Validation." *Journal of Chronic Diseases* 40 (5): 373–83.

Chen, Po-Chuan, Shih-Hung Tsai, Jen-Chun Wang, Yuan-Sheng Tzeng, Yung-Chih Wang, Chi-Ming Chu, Shi-Jye Chu, and Wen-I Liao. 2019. "An Elevated Glycemic Gap Predicts Adverse Outcomes in Diabetic Patients with Necrotizing Fasciitis." *PloS One* 14 (10): e0223126. https://doi.org/10.1371/journal.pone.0223126.

Cheung, N W, S Li, G Ma, and R Crampton. 2008. "The Relationship between Admission Blood Glucose Levels and Hospital Mortality." *Diabetologia* 51 (6): 952–55. https://doi.org/10.1007/s00125-008-1001-4.

Cheung, N Wah, Vincent W Wong, and Mark McLean. 2006. "The Hyperglycemia: Intensive Insulin Infusion in Infarction (HI-5) Study: A Randomized Controlled Trial of Insulin Infusion Therapy for Myocardial Infarction." *Diabetes Care* 29 (4): 765–70. https://doi.org/10.2337/diacare.29.04.06.dc05-1894.

Choe, Chi Un, Dorothee Atzler, Philipp S. Wild, Angela M. Carter, Rainer H. Böger, Francisco Ojeda, Olga Simova, et al. 2013. "Homoarginine Levels Are Regulated by L-Arginine: Glycine Amidinotransferase and Affect Stroke Outcome: Results from Human and Murine Studies." *Circulation* 128 (13): 1451–61. https://doi.org/10.1161/CIRCULATIONAHA.112.000580.

Clain, Jeremy. 2015. "Glucose Control in Critical Care." *World Journal of Diabetes* 6 (9): 1082. https://doi.org/10.4239/wjd.v6.i9.1082.

Colagiuri, S, A Sandbaek, B Carstensen, J Christensen, C Glumer, T Lauritzen, and K Borch-Johnsen. 2003. "Comparability of Venous and Capillary Glucose Measurements in Blood." *Diabetic Medicine : A Journal of the British Diabetic Association* 20 (11): 953–56. https://doi.org/10.1046/j.1464-5491.2003.01048.x.

Davis, Joshua S., Christabelle J. Darcy, Tsin W. Yeo, Catherine Jones, Yvette R. McNeil, Dianne P. Stephens, David S. Celermajer, and Nicholas M. Anstey. 2011. "Asymmetric Dimethylarginine, Endothelial Nitric Oxide Bioavailability and Mortality in Sepsis." *PLoS ONE* 6 (2): 1–6. https://doi.org/10.1371/journal.pone.0017260.

Deedwania, Prakash, Mikhail Kosiborod, Eugene Barrett, Antonio Ceriello, William Isley, Theodore Mazzone, Philip Raskin, and and Metabolism American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity. 2008. "Hyperglycemia and Acute Coronary Syndrome: A Scientific Statement from the American Heart Association Diabetes Committee of the Council on Nutrition, Physical

Activity, and Metabolism." *Anesthesiology* 117 (12): 1610–19. https://doi.org/10.1161/CIRCULATIONAHA.107.188629.

DeLong, E R, D M DeLong, and D L Clarke-Pearson. 1988. "Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach." *Biometrics* 44 (3): 837–45.

Diabetes Control and Complications Trial Research Group, D M Nathan, S Genuth, J Lachin, P Cleary, O Crofford, M Davis, L Rand, and C Siebert. 1993. "The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus." *The New England Journal of Medicine* 329 (14): 977–86. https://doi.org/10.1056/NEJM199309303291401.

Díaz, Rafael, Abhinav Goyal, Shamir R. Mehta, Rizwan Afzal, Denis Xavier, Prem Pais, Susan Chrolavicius, et al. 2007. "Glucose-Insulin-Potassium Therapy in Patients With ST-Segment Elevation Myocardial Infarction." *JAMA* 298 (20): 2399. https://doi.org/10.1001/jama.298.20.2399.

Díaz, Rafael, Ernesto A. Paolasso, Leopoldo S. Piegas, Carlos D. Tajer, Manuel Gil Moreno, Ramón Corvalán, Jesús E. Isea, Graciela Romero, and (Estudios Cardiológicos Latinoamé. 1998. "Metabolic Modulation of Acute Myocardial Infarction." *Circulation* 98 (21): 2227–34. https://doi.org/10.1161/01.CIR.98.21.2227.

Donagaon, Sandeep, and Mala Dharmalingam. n.d. "Association between Glycemic Gap and Adverse Outcomes in Critically Ill Patients with Diabetes." *Indian Journal of Endocrinology and Metabolism* 22 (2): 208–11. https://doi.org/10.4103/ijem.IJEM_580_17.

Drechsler, Christiane, Hege Pihlstrøm, Andreas Meinitzer, Stefan Pilz, Andreas Tomaschitz, Sadollah Abedini, Bengt Fellstrom, et al. 2015. "Homoarginine and Clinical Outcomes in Renal Transplant Recipients: Results from the Assessment of Lescol in Renal Transplantation Study." *Transplantation* 99 (7): 1470–76. https://doi.org/10.1097/TP.0000000000000568.

Duffy, Martin J, Brian A Mullan, Thelma R Craig, Murali Shyamsundar, Rob E MacSweeney, Gillian Thompson, Michael Stevenson, and Daniel F McAuley. 2011. "Impaired Endothelium-Dependent Vasodilatation Is a Novel Predictor of Mortality in Intensive Care." *Critical Care Medicine* 39 (4): 629–35. https://doi.org/10.1097/CCM.0b013e318206bc4a.

Dungan, Kathleen M., Susan S. Braithwaite, and Jean-Charles Preiser. 2009. "Stress Hyperglycaemia." *Lancet* 373 (9677): 1798–1807. https://doi.org/10.1016/S0140-6736(09)60553-5.

Egi, Moritoki, Rinaldo Bellomo, Eddie Stachowski, Craig J French, Graeme Hart, and Peter Stow. 2006. "Blood Glucose on Day of Intensive Care Unit Admission as a Surrogate of Subsequent Glucose Control in Intensive Care." *Journal of Critical Care* 21 (2): 197–202. https://doi.org/10.1016/j.jcrc.2006.03.003.

Egi, Moritoki, Rinaldo Bellomo, Edward Stachowski, Craig J French, Graeme K Hart, Gopal Taori, Colin Hegarty, and Michael Bailey. 2011. "The Interaction of Chronic and Acute

Glycemia with Mortality in Critically Ill Patients with Diabetes." *Critical Care Medicine* 39 (1): 105–11. https://doi.org/10.1097/CCM.0b013e3181feb5ea.

Egi, Moritoki, Simon Finfer, and Rinaldo Bellomo. 2011. "Glycemic Control in the ICU." *Chest* 140 (1): 212–20. https://doi.org/10.1378/chest.10-1478.

Egi, Moritoki, James S Krinsley, Paula Maurer, Devendra N Amin, Tomoyuki Kanazawa, Shruti Ghandi, Kiyoshi Morita, Michael Bailey, and Rinaldo Bellomo. 2016. "Pre-Morbid Glycemic Control Modifies the Interaction between Acute Hypoglycemia and Mortality." *Intensive Care Medicine* 42 (4): 562–71. https://doi.org/10.1007/s00134-016-4216-8.

Esposito, Katherine, Francesco Nappo, Raffaele Marfella, Giovanni Giugliano, Francesco Giugliano, Myriam Ciotola, Lisa Quagliaro, Antonio Ceriello, and Dario Giugliano. 2002. "Inflammatory Cytokine Concentrations Are Acutely Increased by Hyperglycemia in Humans: Role of Oxidative Stress." *Circulation* 106 (16): 2067–72. https://doi.org/10.1161/01.cir.0000034509.14906.ae.

Evans, Joseph L, Ira D Goldfine, Betty A Maddux, and Gerold M Grodsky. 2002. "Oxidative Stress and Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes." *Endocrine Reviews* 23 (5): 599–622. https://doi.org/10.1210/er.2001-0039.

Finfer, Simon, Dean R Chittock, Steve Yu-Shuo Su, Deborah Blair, Denise Foster, Vinay Dhingra, Rinaldo Bellomo, et al. 2009. "Intensive versus Conventional Glucose Control in Critically Ill Patients." *The New England Journal of Medicine* 360 (13): 1283–97. https://doi.org/10.1056/NEJMoa0810625.

Frederick M. Allen, M.D., Edgar Stillman, M.D., and M.D. Reginald Fitz. 1919. *Total Dietary Regulation in the Treatment of Diabetes*.

Fujino, Masashi, Masaharu Ishihara, Satoshi Honda, Shoji Kawakami, Takafumi Yamane, Toshiyuki Nagai, Kazuhiro Nakao, et al. 2014. "Impact of Acute and Chronic Hyperglycemia on In-Hospital Outcomes of Patients with Acute Myocardial Infarction." *The American Journal of Cardiology* 114 (12): 1789–93. https://doi.org/10.1016/j.amjcard.2014.09.015.

Furnary, Anthony P., YingXing Wu, and Stephen O. Bookin. 2004. "Effect of Hyperglycemia and Continuous Intravenous Insulin Infusions on Outcomes of Cardiac Surgical Procedures: The Portland Diabetic Project." *Endocrine Practice* 10 (Supplement 2): 21–33. https://doi.org/10.4158/EP.10.S2.21.

Garcia, M J, P M McNamara, T Gordon, and W B Kannel. 1974. "Morbidity and Mortality in Diabetics in the Framingham Population. Sixteen Year Follow-up Study." *Diabetes* 23 (2): 105–11. https://doi.org/10.2337/diab.23.2.105.

Ghashut, Rawia A., Scott Blackwell, Sylvia Ryan, Laura Willox, Donald C. McMillan, John Kinsella, and Dinesh Talwar. 2017. "Assessment of Asymmetrical Dimethylarginine Metabolism in Patients with Critical Illness." *European Journal of Clinical Investigation* 47 (4): 279–88. https://doi.org/10.1111/eci.12710.

Gordin, D, M Rönnback, C Forsblom, O Heikkilä, M Saraheimo, and P-H Groop. 2007. "Acute Hyperglycaemia Rapidly Increases Arterial Stiffness in Young Patients with Type 1 Diabetes." *Diabetologia* 50 (9): 1808–14. https://doi.org/10.1007/s00125-007-0730-0.

Gore, M. Odette, Nicole Lüneburg, Edzard Schwedhelm, Colby R. Ayers, Maike Anderssohn, Amit Khera, Dorothee Atzler, et al. 2013. “Symmetrical Dimethylarginine Predicts Mortality in the General Population: Observations from the Dallas Heart Study.” *Arteriosclerosis, Thrombosis, and Vascular Biology* 33 (11): 2682–88. https://doi.org/10.1161/ATVBAHA.113.301219.

Gough, Michael S, Mary Anne M Morgan, Cynthia M Mack, Denise C Darling, Lauren M Frasier, Kathleen P Doolin, Michael J Apostolakos, et al. 2011. “The Ratio of Arginine to Dimethylarginines Is Reduced and Predicts Outcomes in Patients with Severe Sepsis.” *Critical Care Medicine* 39 (6): 1351–58. https://doi.org/10.1097/CCM.0b013e318212097c.

Goyal, Abhinav, Kenneth W Mahaffey, Jyotsna Garg, Jose C Nicolau, Judith S Hochman, W Douglas Weaver, Pierre Theroux, et al. 2006. “Prognostic Significance of the Change in Glucose Level in the First 24 h after Acute Myocardial Infarction: Results from the CARDINAL Study.” *European Heart Journal* 27 (11): 1289–97. https://doi.org/10.1093/eurheartj/ehi884.

Goyal, Abhinav, Shamir R Mehta, Rafael Díaz, Hertzel C Gerstein, Rizwan Afzal, Denis Xavier, Lisheng Liu, Prem Pais, and Salim Yusuf. 2009. “Differential Clinical Outcomes Associated with Hypoglycemia and Hyperglycemia in Acute Myocardial Infarction.” *Circulation* 120 (24): 2429–37. https://doi.org/10.1161/CIRCULATIONAHA.108.837765.

Green, Jennifer B., M. Angelyn Bethel, Paul W. Armstrong, John B. Buse, Samuel S. Engel, Jyotsna Garg, Robert Josse, et al. 2015. “Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes.” *New England Journal of Medicine* 373 (3): 232–42. https://doi.org/10.1056/NEJMoa1501352.

Griesdale, D. E. G., R. J. de Souza, R. M. van Dam, D. K. Heyland, D. J. Cook, A. Malhotra, R. Dhaliwal, et al. 2009. “Intensive Insulin Therapy and Mortality among Critically Ill Patients: A Meta-Analysis Including NICE-SUGAR Study Data.” *Canadian Medical Association Journal* 180 (8): 821–27. https://doi.org/10.1503/cmaj.090206.

Hochman, J S, J E Tamis, T D Thompson, W D Weaver, H D White, F Van de Werf, P Aylward, E J Topol, and R M Califf. 1999. “Sex, Clinical Presentation, and Outcome in Patients with Acute Coronary Syndromes. Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes IIb Investigators.” *The New England Journal of Medicine* 341 (4): 226–32. https://doi.org/10.1056/NEJM199907223410402.

Hollenberg, Steven M., and Ismail Cinel. 2009. “Bench-to-Bedside Review: Nitric Oxide in Critical Illness--Update 2008.” *Critical Care (London, England)* 13 (4): 218. https://doi.org/10.1186/cc7706.

Holman, Rury R, Sanjoy K Paul, M Angelyn Bethel, David R Matthews, and H Andrew W Neil. 2008. “10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes.” *The New England Journal of Medicine* 359 (15): 1577–89. https://doi.org/10.1056/NEJMoa0806470.

Horst, Iwan C.C van der, Felix Zijlstra, Arnoud W.J van’t Hof, Carine J.M Doggen, Menko-Jan de Boer, Harry Suryapranata, Jan C.A Hoorntje, Jan-Henk E Dambrink, Rijk O.B

Gans, and Henk J.G Bilo. 2003. “Glucose-Insulin-Potassium Infusion Inpatients Treated with Primary Angioplasty for Acute Myocardial Infarction.” *Journal of the American College of Cardiology* 42 (5): 784–91. https://doi.org/10.1016/S0735-1097(03)00830-1.

Ignarro, L J. 2002. “Nitric Oxide as a Unique Signaling Molecule in the Vascular System: A Historical Overview.” *Journal of Physiology and Pharmacology : An Official Journal of the Polish Physiological Society* 53 (4 Pt 1): 503–14.

Ignarro, Louis J, and Claudio Napoli. 2005. “Novel Features of Nitric Oxide, Endothelial Nitric Oxide Synthase, and Atherosclerosis.” *Current Diabetes Reports* 5 (1): 17–23.

Ignarro, Louis J, Claudio Napoli, and Joseph Loscalzo. 2002. “Nitric Oxide Donors and Cardiovascular Agents Modulating the Bioactivity of Nitric Oxide: An Overview.” *Circulation Research* 90 (1): 21–28. https://doi.org/10.1161/hh0102.102330.

Ince, Can, Daniel De Backer, and Philip R. Mayeux. 2020. “Microvascular Dysfunction in the Critically Ill.” *Critical Care Clinics* 36 (2): 323–31. https://doi.org/10.1016/j.ccc.2019.11.003.

Investigators, The Nice-sugar Study. 2012. “Hypoglycemia and Risk of Death in Critically Ill Patients.” *New England Journal of Medicine* 367 (12): 1108–18. https://doi.org/10.1056/NEJMoa1204942.

Jacobi, Johannes, Karsten Sydow, Georges Von Degenfeld, Ying Zhang, Hayan Dayoub, Bingyin Wang, Andrew J. Patterson, Masumi Kimoto, Helen M. Blau, and John P. Cooke. 2005. “Overexpression of Dimethylarginine Dimethylaminohydrolase Reduces Tissue Asymmetric Dimethylarginine Levels and Enhances Angiogenesis.” *Circulation* 111 (11): 1431–38. https://doi.org/10.1161/01.CIR.0000158487.80483.09.

Jacobi, Judith. 2016. “Liberal Glucose Targets for Critically Ill Diabetic Patients: Is It Time for Large Clinical Trials with More Personalized Endpoints?” *Annals of Translational Medicine* 4 (18): 358. https://doi.org/10.21037/atm.2016.08.64.

Jacobi, Judith, Nicholas Bircher, James Krinsley, Michael Agus, Susan S. Braithwaite, Clifford Deutschman, Amado X. Freire, et al. 2012. “Guidelines for the Use of an Insulin Infusion for the Management of Hyperglycemia in Critically Ill Patients.” *Critical Care Medicine* 40 (12): 3251–76. https://doi.org/10.1097/CCM.0b013e3182653269.

Jafar, Nagham, Hawa Edriss, and Kenneth Nugent. 2016. “The Effect of Short-Term Hyperglycemia on the Innate Immune System.” *The American Journal of the Medical Sciences* 351 (2): 201–11. https://doi.org/10.1016/j.amjms.2015.11.011.

Jamwal, Shalini, and Saurabh Sharma. 2018. “Vascular Endothelium Dysfunction: A Conservative Target in Metabolic Disorders.” *Inflammation Research : Official Journal of the European Histamine Research Society ... [et Al.]* 67 (5): 391–405. https://doi.org/10.1007/s00011-018-1129-8.

Jarzebska, Natalia, Arduino A. Mangoni, Jens Martens-Lobenhoffer, Stefanie M. Bode-Böger, and Roman N. Rodionov. 2019. “The Second Life of Methylarginines as Cardiovascular Targets.” *International Journal of Molecular Sciences* 20 (18). https://doi.org/10.3390/ijms20184592.

Jensen, Andreas Vestergaard, Gertrud Baunbæk Egelund, Stine Bang Andersen, Pelle Trier Petersen, Thomas Benfield, Martin Witzenrath, Gernot Rohde, Pernille Ravn, Daniel Faurholt-Jepsen, and Members of the CAPNETZ study group except the authors: 2019. "The Glycemic Gap and 90-Day Mortality in Community-Acquired Pneumonia. A Prospective Cohort Study." *Annals of the American Thoracic Society* 16 (12): 1518–26. https://doi.org/10.1513/AnnalsATS.201901-007OC.

Kang, Li, Juan Han, Qun-Cao Yang, Hui-Lin Huang, and Nan Hao. 2015. "Effects of Different Blood Glucose Levels on Critically Ill Patients in an Intensive Care Unit." *Journal of Molecular Microbiology and Biotechnology* 25 (6): 388–93. https://doi.org/10.1159/000441655.

Kar, Palash, Mark P Plummer, Rinaldo Bellomo, Alicia J Jenkins, Andrzej S Januszewski, Marianne J Chapman, Karen L Jones, Michael Horowitz, and Adam M Deane. 2016. "Liberal Glycemic Control in Critically Ill Patients With Type 2 Diabetes: An Exploratory Study." *Critical Care Medicine* 44 (9): 1695–1703. https://doi.org/10.1097/CCM.0000000000001815.

Karetnikova, Ekaterina S., Natalia Jarzebska, Alexander G. Markov, Norbert Weiss, Steven R. Lentz, and Roman N. Rodionov. 2019. "Is Homoarginine a Protective Cardiovascular Risk Factor?" *Arteriosclerosis, Thrombosis, and Vascular Biology* 39 (5): 869–75. https://doi.org/10.1161/ATVBAHA.118.312218.

Karlsson, William K, Caspar G Sørensen, and Christina Kruuse. 2017. "L-Arginine and l-NMMA for Assessing Cerebral Endothelial Dysfunction in Ischaemic Cerebrovascular Disease: A Systematic Review." *Clinical and Experimental Pharmacology & Physiology* 44 (1): 13–20. https://doi.org/10.1111/1440-1681.12679.

Kataja, Anu, Tuukka Tarvasmäki, Johan Lassus, Jose Cardoso, Alexandre Mebazaa, Lars Køber, Alessandro Sionis, et al. 2017. "The Association of Admission Blood Glucose Level with the Clinical Picture and Prognosis in Cardiogenic Shock - Results from the CardShock Study." *International Journal of Cardiology* 226 (January): 48–52. https://doi.org/10.1016/j.ijcard.2016.10.033.

Kawano, H, T Motoyama, O Hirashima, N Hirai, Y Miyao, T Sakamoto, K Kugiyama, H Ogawa, and H Yasue. 1999. "Hyperglycemia Rapidly Suppresses Flow-Mediated Endothelium-Dependent Vasodilation of Brachial Artery." *Journal of the American College of Cardiology* 34 (1): 146–54.

Kayacelebi, Arslan Arinc, Jennifer Langen, Katharina Weigt-Usinger, Kristine Chobanyan-Jürgens, François Mariotti, Jessica Y Schneider, Sabine Rothmann, et al. 2015. "Biosynthesis of Homoarginine (HArg) and Asymmetric Dimethylarginine (ADMA) from Acutely and Chronically Administered Free L-Arginine in Humans." *Amino Acids* 47 (9): 1893–1908. https://doi.org/10.1007/s00726-015-2012-3.

Kielstein, Jan T., Frank Donnerstag, Sandra Gasper, Jan Menne, Anousheh Kielstein, Jens Martens-Lobenhoffer, Fortunato Scalera, John P. Cooke, Danilo Fliser, and Stefanie M. Bode-Böger. 2006. "ADMA Increases Arterial Stiffness and Decreases Cerebral Blood Flow in Humans." *Stroke* 37 (8): 2024–29. https://doi.org/10.1161/01.STR.0000231640.32543.11.

Kielstein, Jan T., Burcu Impraim, Solveig Simmel, Stefanie M. Bode-Böger, Dimitrios Tsikas, Jürgen C. Frölich, Marius M. Hoeper, Hermann Haller, and Danilo Fliser. 2004. "Cardiovascular Effects of Systemic Nitric Oxide Synthase Inhibition with Asymmetrical Dimethylarginine in Humans." *Circulation* 109 (2): 172–77. https://doi.org/10.1161/01.CIR.0000105764.22626.B1.

Kim, Eun Jung, Myung Ho Jeong, Ju Han Kim, Tae Hoon Ahn, Ki Bae Seung, Dong Joo Oh, Hyo-Soo Kim, et al. 2017. "Clinical Impact of Admission Hyperglycemia on In-Hospital Mortality in Acute Myocardial Infarction Patients." *International Journal of Cardiology* 236 (June): 9–15. https://doi.org/10.1016/j.ijcard.2017.01.095.

Knaus, W A, D P Wagner, E A Draper, J E Zimmerman, M Bergner, P G Bastos, C A Sirio, D J Murphy, Ted Lotring, and A Damiano. 1991. "The APACHE III Prognostic System. Risk Prediction of Hospital Mortality for Critically Ill Hospitalized Adults." *Chest* 100 (6): 1619–36. https://doi.org/10.1378/chest.100.6.1619.

Knaus, William A., Elizabeth A. Draper, Douglas P. Wagner, and Jack E. Zimmerma. 1985. "APACHE II- A Severity of Disease Classification System." *Critical Care Medicine*. https://doi.org/10.1097/00003465-198603000-00013.

Koch, Alexander, Ralf Weiskirchen, Julian Kunze, Hanna Dückers, Jan Bruensing, Lukas Buendgens, Michael Matthes, Tom Luedde, Christian Trautwein, and Frank Tacke. 2013. "Elevated Asymmetric Dimethylarginine Levels Predict Short- and Long-Term Mortality Risk in Critically Ill Patients." *Journal of Critical Care* 28 (6): 947–53. https://doi.org/10.1016/j.jcrc.2013.05.016.

Koracevic, Goran P. 2016. "Proposal of a New Approach to Study and Categorize Stress Hyperglycemia in Acute Myocardial Infarction." *The Journal of Emergency Medicine* 51 (1): 31–36. https://doi.org/10.1016/j.jemermed.2015.03.047.

Koracevic, Goran P, Sladjana Petrovic, Miodrag Damjanovic, and Teodora Stanojlovic. 2008. "Association of Stress Hyperglycemia and Atrial Fibrillation in Myocardial Infarction." *Wiener Klinische Wochenschrift* 120 (13–14): 409–13. https://doi.org/10.1007/s00508-008-0983-8.

Kosiborod, Mikhail, Silvio E Inzucchi, Abhinav Goyal, Harlan M Krumholz, Frederick A Masoudi, Lan Xiao, and John A Spertus. 2009. "Relationship between Spontaneous and Iatrogenic Hypoglycemia and Mortality in Patients Hospitalized with Acute Myocardial Infarction." *JAMA* 301 (15): 1556–64. https://doi.org/10.1001/jama.2009.496.

Kosiborod, Mikhail, Silvio E Inzucchi, Harlan M Krumholz, Frederick A Masoudi, Abhinav Goyal, Lan Xiao, Philip G Jones, Suzanne Fiske, and John A Spertus. 2009. "Glucose Normalization and Outcomes in Patients with Acute Myocardial Infarction." *Archives of Internal Medicine* 169 (5): 438–46. https://doi.org/10.1001/archinternmed.2008.593.

Kosiborod, Mikhail, Silvio E Inzucchi, Harlan M Krumholz, Lan Xiao, Philip G Jones, Suzanne Fiske, Frederick a Masoudi, Steven P Marso, and John a Spertus. 2008. "Glucometrics in Patients Hospitalized with Acute Myocardial Infarction: Defining the Optimal Outcomes-Based Measure of Risk." *Circulation* 117 (8): 1018–27.

https://doi.org/10.1161/CIRCULATIONAHA.107.740498.

Kosiborod, Mikhail, Saif S Rathore, Silvio E Inzucchi, Frederick A Masoudi, Yongfei Wang, Edward P Havranek, and Harlan M Krumholz. 2005. “Admission Glucose and Mortality in Elderly Patients Hospitalized with Acute Myocardial Infarction: Implications for Patients with and without Recognized Diabetes.” *Circulation* 111 (23): 3078–86. https://doi.org/10.1161/CIRCULATIONAHA.104.517839.

Kotagal, Meera, Rebecca G Symons, Irl B Hirsch, Guillermo E Umpierrez, E Patchen Dellinger, Ellen T Farrokhi, and David R Flum. 2014. “Perioperative Hyperglycemia and Risk of Adverse Events Among Patients With and Without Diabetes.” *Annals of Surgery* 00 (00): 1–7. https://doi.org/10.1097/SLA.0000000000000688.

Koyfman, Leonid, Evgeni Brotfain, Alexander Erblat, Inna Kovalenko, Yair-Yaish Reina, Yoav Bichovsky, Abraham Borer, Michael Friger, Amit Frenkel, and Moti Klein. 2018. “The Impact of the Blood Glucose Levels of Non-Diabetic Critically Ill Patients on Their Clinical Outcome.” *Anaesthesiology Intensive Therapy* 50 (1): 20–26. https://doi.org/10.5603/AIT.2018.0004.

Krinsley, James S., Peter Rule, Lovin Pappy, Afshan Ahmed, Camilla Huley-Rodrigues, Danielle Prevedello, and Jean-Charles Preiser. 2020. “The Interaction of Acute and Chronic Glycemia on the Relationship of Hyperglycemia, Hypoglycemia, and Glucose Variability to Mortality in the Critically Ill.” *Critical Care Medicine* Publish Ah (18): 1–8. https://doi.org/10.1097/ccm.0000000000004599.

Krinsley, James S, Moritoki Egi, Alex Kiss, Amin N Devendra, Philipp Schuetz, Paula M Maurer, Marcus J Schultz, et al. 2013. “Diabetic Status and the Relation of the Three Domains of Glycemic Control to Mortality in Critically Ill Patients: An International Multicenter Cohort Study.” *Critical Care (London, England)* 17 (2): R37+. https://doi.org/10.1186/cc12547.

Krinsley, James S, Jean-Charles Preiser, and Irl B Hirsch. 2017. “Safety and Efficacy of Personalised Glycemic Control in Critically Ill Patients: A 2-Year before and after Interventional Trial.” *Endocrine Practice : Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* 23 (3): 318–30. https://doi.org/10.4158/EP161532.OR.

Krinsley, James Stephen. 2003. “Association between Hyperglycemia and Increased Hospital Mortality in a Heterogeneous Population of Critically Ill Patients.” *Mayo Clinic Proceedings* 78 (12): 1471–78. https://doi.org/10.4065/78.12.1471.

———. 2018. “The Long and Winding Road Toward Personalized Glycemic Control in the Critically Ill.” *Journal of Diabetes Science and Technology* 12 (1): 26–32. https://doi.org/10.1177/1932296817728299.

Kuhl, Jeanette, Gun Jörneskog, Malin Wemminger, Mattias Bengtsson, Pia Lundman, and Majid Kalani. 2015. “Long-Term Clinical Outcome in Patients with Acute Coronary Syndrome and Dysglycaemia.” *Cardiovascular Diabetology* 14 (1): 120. https://doi.org/10.1186/s12933-015-0283-3.

La Rosa, Gisela D De, Jorge H Donado, Alvaro H Restrepo, Alvaro M Quintero, Luis G

Gonzalez, Nora E Saldarriaga, Marisol T Bedoya, et al. 2008. “Strict Glycemic Control in Patients Hospitalized in a Mixed Medical and Surgical Intensive Care Unit: A Randomized Clinical Trial.” *Critical Care* 12 (5): R120. https://doi.org/10.1186/cc7017.

Lanspa, Michael J., Eliotte L. Hirshberg, Gregory D. Phillips, John Holmen, Gregory Stoddard, and James Orme. 2013. “Moderate Glucose Control Is Associated with Increased Mortality Compared with Tight Glucose Control in Critically Ill Patients without Diabetes.” *Chest* 143 (5): 1226–34. https://doi.org/10.1378/chest.12-2072.

Lee, Jong Hoon, Gil Hong Park, Young Koo Lee, and Jun Hyung Park. 2011. “Changes in the Arginine Methylation of Organ Proteins during the Development of Diabetes Mellitus.” *Diabetes Research and Clinical Practice* 94 (1): 111–18. https://doi.org/10.1016/j.diabres.2011.07.005.

Lee, Tien F., Morton G. Burt, Leonie K. Heilbronn, Arduino A. Mangoni, Vincent W. Wong, Mark McLean, and N. Wah Cheung. 2017. “Relative Hyperglycemia Is Associated with Complications Following an Acute Myocardial Infarction: A Post-Hoc Analysis of HI-5 Data.” *Cardiovascular Diabetology* 16 (1): 157. https://doi.org/10.1186/s12933-017-0642-3.

Lee, Tien F., Sophie M. Drake, Gregory W. Roberts, Andrew Bersten, Stephen N. Stranks, Leonie K. Heilbronn, Arduino A. Mangoni, and Morton G. Burt. 2020. “Relative Hyperglycemia Is an Independent Determinant of In-Hospital Mortality in Patients With Critical Illness.” *Critical Care Medicine* 48 (2): e115–22. https://doi.org/10.1097/CCM.0000000000004133.

Leiper, J M, J Santa Maria, A Chubb, R J MacAllister, I G Charles, G S Whitley, and P Vallance. 1999. “Identification of Two Human Dimethylarginine Dimethylaminohydrolases with Distinct Tissue Distributions and Homology with Microbial Arginine Deiminases.” *The Biochemical Journal* 343 Pt 1 (October): 209–14.

Leisman, Daniel E., Michael O. Harhay, David J. Lederer, Michael Abramson, Alex A. Adjei, Jan Bakker, Zuhair K. Ballas, et al. 2020. “Development and Reporting of Prediction Models: Guidance for Authors from Editors of Respiratory, Sleep, and Critical Care Journals.” *Critical Care Medicine*, 623–33. https://doi.org/10.1097/CCM.0000000000004246.

Lerman, Amir, and Andreas M Zeiher. 2005. “Endothelial Function: Cardiac Events.” *Circulation* 111 (3): 363–68. https://doi.org/10.1161/01.CIR.0000153339.27064.14.

Levey, Andrew S, Josef Coresh, Tom Greene, Lesley A Stevens, Yaping Lucy Zhang, Stephen Hendriksen, John W Kusek, Frederick Van Lente, and Chronic Kidney Disease Epidemiology Collaboration. 2006. “Using Standardized Serum Creatinine Values in the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate.” *Annals of Internal Medicine* 145 (4): 247–54. https://doi.org/10.7326/0003-4819-145-4-200608150-00004.

Liao, Wen-I, Chin-Sheng Lin, Chien-Hsing Lee, Ya-Chieh Wu, Wei-Chou Chang, Chin-Wang Hsu, Jen-Chun Wang, and Shih-Hung Tsai. 2016. “An Elevated Glycemic Gap Is Associated with Adverse Outcomes in Diabetic Patients with Acute Myocardial

Infarction." *Scientific Reports* 6 (1): 27770. https://doi.org/10.1038/srep27770.

Liao, Wen-I, Wayne Huey-Herng Sheu, Wei-Chou Chang, Chin-Wang Hsu, Yu-Long Chen, and Shih-Hung Tsai. 2013. "An Elevated Gap between Admission and A1C-Derived Average Glucose Levels Is Associated with Adverse Outcomes in Diabetic Patients with Pyogenic Liver Abscess." *PloS One* 8 (5): e64476. https://doi.org/10.1371/journal.pone.0064476.

Liao, Wen-i, Jen-chun Wang, Wei-chou Chang, Chin-wang Hsu, Chi-Ming Chu, and Shih-Hung Tsai. 2015. "Usefulness of Glycemic Gap to Predict ICU Mortality in Critically Ill Patients With Diabetes." *Medicine* 94 (36): e1525. https://doi.org/10.1097/MD.0000000000001525.

Liao, Wen-I, Jen-Chun Wang, Chin-Sheng Lin, Chih-Jen Yang, Chia-Ching Hsu, Shi-Jye Chu, Chi-Ming Chu, and Shih-Hung Tsai. 2019. "Elevated Glycemic Gap Predicts Acute Respiratory Failure and In-Hospital Mortality in Acute Heart Failure Patients with Diabetes." *Scientific Reports* 9 (1): 6279. https://doi.org/10.1038/s41598-019-42666-0.

Ligtenberg, Jack J M, Sofie Meijering, Ymkje Stienstra, Iwan C C van der Horst, Mathijs Vogelzang, Maarten W N Nijsten, Jaap E Tulleken, and Jan G Zijlstra. 2006. "Mean Glucose Level Is Not an Independent Risk Factor for Mortality in Mixed ICU Patients." *Intensive Care Medicine* 32 (3): 435–38. https://doi.org/10.1007/s00134-005-0052-y.

Lin, Ken Y, Akira Ito, Tomoko Asagami, Philip S Tsao, Shanthi Adimoolam, Masumi Kimoto, Hideaki Tsuji, Gerald M Reaven, and John P Cooke. 2002. "Impaired Nitric Oxide Synthase Pathway in Diabetes Mellitus: Role of Asymmetric Dimethylarginine and Dimethylarginine Dimethylaminohydrolase." *Circulation* 106 (8): 987–92. https://doi.org/10.1161/01.cir.0000027109.14149.67.

López, Angel, Jose Angel Lorente, Jay Steingrub, Jan Bakker, Angela McLuckie, Sheila Willatts, Michael Brockway, et al. 2004. "Multiple-Center, Randomized, Placebo-Controlled, Double-Blind Study of the Nitric Oxide Synthase Inhibitor 546C88: Effect on Survival in Patients with Septic Shock." *Critical Care Medicine* 32 (1): 21–30. https://doi.org/10.1097/01.CCM.0000105581.01815.C6.

Luethi, Nora, Luca Cioccari, Peter Biesenbach, Luca Lucchetta, Hidetoshi Kagaya, Rhys Morgan, Francesca Di Muzio, et al. 2018. "Liberal Glucose Control in ICU Patients With Diabetes: A Before-and-After Study." *Critical Care Medicine* 46 (6): 935–42. https://doi.org/10.1097/CCM.0000000000003087.

Luethi, Nora, Luca Cioccari, Aiko Tanaka, Palash Kar, Emma Giersch, Adam M Deane, Johan Mårtensson, and Rinaldo Bellomo. 2016. "Glycated Hemoglobin A1c Levels Are Not Affected by Critical Illness." *Critical Care Medicine* 44 (9): 1692–94. https://doi.org/10.1097/CCM.0000000000001656.

Maas, A H E M, and Y E A Appelman. 2010. "Gender Differences in Coronary Heart Disease." *Netherlands Heart Journal : Monthly Journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation* 18 (12): 598–602.

Macrae, Duncan, Richard Grieve, Elizabeth Allen, Zia Sadique, Kevin Morris, John Pappachan, Roger Parslow, Robert C. Tasker, and Diana Elbourne. 2014. "A

Randomized Trial of Hyperglycemic Control in Pediatric Intensive Care." *New England Journal of Medicine* 370 (2): 107–18. https://doi.org/10.1056/NEJMoa1302564.

Malcolm, Janine, Ilana Halperin, David B. Miller, Sarah Moore, Kara A. Nerenberg, Vincent Woo, and Catherine H. Yu. 2018. "In-Hospital Management of Diabetes." *Canadian Journal of Diabetes* 42 (April): S115–23. https://doi.org/10.1016/j.jcjd.2017.10.014.

Malmberg, K. 1997. "Prospective Randomised Study of Intensive Insulin Treatment on Long Term Survival after Acute Myocardial Infarction in Patients with Diabetes Mellitus. DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study Group." *BMJ (Clinical Research Ed.)* 314 (7093): 1512–15.

Malmberg, K, L Rydén, H Wedel, K Birkeland, A Bootsma, K Dickstein, S Efendic, et al. 2005. "Intense Metabolic Control by Means of Insulin in Patients with Diabetes Mellitus and Acute Myocardial Infarction (DIGAMI 2): Effects on Mortality and Morbidity." *European Heart Journal* 26 (7): 650–61. https://doi.org/10.1093/eurheartj/ehi199.

Malmberg, Klas, Lars Rydén, Suad Efendic, Johan Herlitz, Peter Nicol, Anders Waldenstrom, Hans Wedel, and Lennart Welin. 1995. "Randomized Trial of Insulin-Glucose Infusion Followed by Subcutaneous Insulin Treatment in Diabetic Patients with Acute Myocardial Infarction (DIGAMI Study): Effects on Mortality at 1 Year." *Journal of the American College of Cardiology* 26 (1): 57–65. https://doi.org/10.1016/0735-1097(95)00126-K.

Mangoni, Arduino A. 2009. "Chapter 3 The Emerging Role of Symmetric Dimethylarginine in Vascular Disease." In *Advances in Clinical Chemistry*, 73–94. Elsevier Inc. https://doi.org/10.1016/S0065-2423(09)48003-X.

Marik, Paul E., and Rinaldo Bellomo. 2013. "Stress Hyperglycemia: An Essential Survival Response!" *Critical Care Medicine* 41 (6): e93–94. https://doi.org/10.1097/CCM.0b013e318283d124.

Marik, Paul E., and Moritoki Egi. 2014. "Treatment Thresholds for Hyperglycemia in Critically Ill Patients with and without Diabetes." *Intensive Care Medicine* 40 (7): 1049–51. https://doi.org/10.1007/s00134-014-3344-2.

Marso, Steven P., Stephen C. Bain, Agostino Consoli, Freddy G. Eliaschewitz, Esteban Jódar, Lawrence A. Leiter, Ildiko Lingvay, et al. 2016. "Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes." *New England Journal of Medicine* 375 (19): 1834–44. https://doi.org/10.1056/NEJMoa1607141.

Marso, Steven P., Gilbert H. Daniels, Kirstine Brown-Frandsen, Peter Kristensen, Johannes F.E. Mann, Michael A. Nauck, Steven E. Nissen, et al. 2016. "Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes." *New England Journal of Medicine* 375 (4): 311–22. https://doi.org/10.1056/NEJMoa1603827.

McQuillan, Brendan M, and Peter L Thompson. 2014. "Management of Acute Coronary Syndrome in Special Subgroups: Female, Older, Diabetic and Indigenous Patients." *The Medical Journal of Australia* 201 (10): S91-6. https://doi.org/10.5694/mja14.01248.

Moghissi, Etie S., Mary T. Korytkowski, Monica DiNardo, Daniel Einhorn, Richard Hellman, Irl B. Hirsch, Silvio E. Inzucchi, et al. 2009. "American Association of Clinical

Endocrinologists and American Diabetes Association Consensus Statement on Inpatient Glycemic Control." *Diabetes Care* 32 (6): 1119–31. https://doi.org/10.2337/dc09-9029.

Monnier, Louis, Emilie Mas, Christine Ginet, Françoise Michel, Laetitia Villon, Jean-Paul Cristol, and Claude Colette. 2006. "Activation of Oxidative Stress by Acute Glucose Fluctuations Compared with Sustained Chronic Hyperglycemia in Patients with Type 2 Diabetes." *JAMA* 295 (14): 1681–87. https://doi.org/10.1001/jama.295.14.1681.

Moran, John L., Peter Bristow, Patricia J. Solomon, Carol George, and Graeme K. Hart. 2008. "Mortality and Length-of-Stay Outcomes, 1993–2003, in the Binational Australian and New Zealand Intensive Care Adult Patient Database*." *Critical Care Medicine* 36 (1): 46–61. https://doi.org/10.1097/01.CCM.0000295313.08084.58.

Mortensen, Karoline Myglegård, Theis Skovsgaard Itenov, Nicolai Haase, Rasmus Beier Müller, Sisse Rye Ostrowski, Pär Ingemar Johansson, Niels Vidiendal Olsen, Anders Perner, Peter Søe-Jensen, and Morten Heiberg Bestle. 2016. "High Levels of Methylarginines Were Associated With Increased Mortality in Patients With Severe Sepsis." *Shock (Augusta, Ga.)* 46 (4): 365–72. https://doi.org/10.1097/SHK.0000000000000649.

Mortensen, Karoline Myglegård, Theis Skovsgaard Itenov, Marco Bo Hansen, Karen Hvid, Lars Hyldborg Lundstrøm, and Morten Heiberg Bestle. 2019. "Mortality in Critical Illness: The Impact of Asymmetric Dimethylarginine on Survival-A Systematic Review and Meta-Analysis." *Acta Anaesthesiologica Scandinavica* 63 (6): 708–19. https://doi.org/10.1111/aas.13339.

Mukamal, K J, R W Nesto, M C Cohen, J E Muller, M Maclure, J B Sherwood, and M A Mittleman. 2001. "Impact of Diabetes on Long-Term Survival after Acute Myocardial Infarction: Comparability of Risk with Prior Myocardial Infarction." *Diabetes Care* 24 (8): 1422–27. https://doi.org/10.2337/diacare.24.8.1422.

Nathan, David M., and DCCT/EDIC Research Group. 2014. "The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 Years: Overview." *Diabetes Care* 37 (1): 9–16. https://doi.org/10.2337/dc13-2112.

Nathan, David M., Judith Kuenen, Rikke Borg, Hui Zheng, David Schoenfeld, and Robert J. Heine. 2008. "Translating the A1C Assay into Estimated Average Glucose Values." *Diabetes Care* 31 (8): 1473–78. https://doi.org/10.2337/dc08-0545.

Neal, Bruce, Vlado Perkovic, Kenneth W. Mahaffey, Dick de Zeeuw, Greg Fulcher, Ngozi Erondu, Wayne Shaw, Gordon Law, Mehul Desai, and David R. Matthews. 2017. "Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes." *New England Journal of Medicine* 377 (7): 644–57. https://doi.org/10.1056/nejmoa1611925.

Nijveldt, R J, T Teerlink, B Van Der Hoven, M P C Siroen, D J Kuik, J a Rauwerda, and P a M van Leeuwen. 2003. "Asymmetrical Dimethylarginine (ADMA) in Critically Ill Patients: High Plasma ADMA Concentration Is an Independent Risk Factor of ICU Mortality." *Clinical Nutrition (Edinburgh, Scotland)* 22 (1): 23–30. https://doi.org/10.1054/clnu.2002.0613.

Okada, Kozo, Kiyoshi Hibi, Masaomi Gohbara, Shunsuke Kataoka, Keiko Takano, Eiichi Akiyama, Yasushi Matsuzawa, et al. 2015. “Association between Blood Glucose Variability and Coronary Plaque Instability in Patients with Acute Coronary Syndromes.” *Cardiovascular Diabetology* 14 (1): 111. https://doi.org/10.1186/s12933-015-0275-3.

Olariu, Elena, Nicholas Pooley, Aurélie Danel, Montserrat Miret, and Jean-Charles Preiser. 2018. “A Systematic Scoping Review on the Consequences of Stress-Related Hyperglycaemia.” *PloS One* 13 (4): e0194952. https://doi.org/10.1371/journal.pone.0194952.

Osler, William. 1892. *The Principles and Practice of Medicine*.

Panzram, G. 1987. “Mortality and Survival in Type 2 (Non-Insulin-Dependent) Diabetes Mellitus.” *Diabetologia* 30 (3): 123–31.

Pararajasingam, Gokulan, Dan Eik Høfsten, Brian Bridal Løgstrup, Michael Egstrup, Finn Lund Henriksen, Jørgen Hangaard, and Kenneth Egstrup. 2016. “Newly Detected Abnormal Glucose Regulation and Long-Term Prognosis after Acute Myocardial Infarction: Comparison of an Oral Glucose Tolerance Test and Glycosylated Haemoglobin A1c.” *International Journal of Cardiology* 214 (July): 310–15. https://doi.org/10.1016/j.ijcard.2016.03.199.

Patel, Jayshil J., Keith R. Miller, Cameron Rosenthal, and Martin D. Rosenthal. 2016. “When Is It Appropriate to Use Arginine in Critical Illness?” *Nutrition in Clinical Practice*. https://doi.org/10.1177/0884533616652576.

Paul, Eldho, Michael Bailey, Jessica Kasza, and David Pilcher. 2016. “The ANZROD Model: Better Benchmarking of ICU Outcomes and Detection of Outliers.” *Critical Care and Resuscitation : Journal of the Australasian Academy of Critical Care Medicine* 18 (1): 25–36.

Paul, Eldho, Michael Bailey, and David Pilcher. 2013. “Risk Prediction of Hospital Mortality for Adult Patients Admitted to Australian and New Zealand Intensive Care Units: Development and Validation of the Australian and New Zealand Risk of Death Model.” *Journal of Critical Care* 28 (6): 935–41. https://doi.org/10.1016/j.jcrc.2013.07.058.

Pereira, Adriano José, Thiago Domingos Corrêa, Francisca Pereira de Almeida, Rodrigo Octávio Deliberato, Michelle dos Santos Lobato, Nelson Akamine, Eliézer Silva, and Alexandre Biasi Cavalcanti. 2015. “Inaccuracy of Venous Point-of-Care Glucose Measurements in Critically Ill Patients: A Cross-Sectional Study.” *PloS One* 10 (6): e0129568. https://doi.org/10.1371/journal.pone.0129568.

Petersons, Carolyn J, Brenda L Mangelsdorf, Arthur B Jenkins, Anne Poljak, Malcolm D Smith, Jerry R Greenfield, Campbell H Thompson, and Morton G Burt. 2013. “Effects of Low-Dose Prednisolone on Hepatic and Peripheral Insulin Sensitivity, Insulin Secretion, and Abdominal Adiposity in Patients with Inflammatory Rheumatologic Disease.” *Diabetes Care* 36 (9): 2822–29. https://doi.org/10.2337/dc12-2617.

Petersons, Carolyn J, Brenda L Mangelsdorf, Campbell H Thompson, and Morton G Burt. 2014. “Acute Effect of Increasing Glucocorticoid Replacement Dose on Cardiovascular Risk and Insulin Sensitivity in Patients with Adrenocorticotrophin Deficiency.” *The*

Journal of Clinical Endocrinology and Metabolism 99 (6): 2269–76. https://doi.org/10.1210/jc.2013-4305.

Pfeffer, Marc A., Brian Claggett, Rafael Diaz, Kenneth Dickstein, Hertzel C. Gerstein, Lars V. Køber, Francesca C. Lawson, et al. 2015. “Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome.” *New England Journal of Medicine* 373 (23): 2247–57. https://doi.org/10.1056/NEJMoa1509225.

Pilz, Stefan, Frank Edelmann, Andreas Meinitzer, Götz Gelbrich, Ufuk Döner, Hans Dirk Düngen, Andreas Tomaschitz, et al. 2014. “Associations of Methylarginines and Homoarginine with Diastolic Dysfunction and Cardiovascular Risk Factors in Patients with Preserved Left Ventricular Ejection Fraction.” *Journal of Cardiac Failure* 20 (12): 923–30. https://doi.org/10.1016/j.cardfail.2014.09.004.

Pilz, Stefan, Andreas Meinitzer, Martin Gaksch, Martin Grübler, Nicolas Verheyen, Christiane Drechsler, Bríain Ó Hartaigh, et al. 2015. “Homoarginine in the Renal and Cardiovascular Systems.” *Amino Acids* 47 (9): 1703–13. https://doi.org/10.1007/s00726-015-1993-2.

Plummer, Mark P., Rinaldo Bellomo, Caroline E. Cousins, Christopher E. Annink, Krishnaswamy Sundararajan, Benjamin A.J. Reddi, John P. Raj, Marianne J. Chapman, Michael Horowitz, and Adam M. Deane. 2014. “Dysglycaemia in the Critically Ill and the Interaction of Chronic and Acute Glycaemia with Mortality.” *Intensive Care Medicine* 40 (7). https://doi.org/10.1007/s00134-014-3287-7.

Preiser, Jean-Charles, Philippe Devos, Sergio Ruiz-Santana, Christian Mélot, Djillali Annane, Johan Groeneveld, Gaetano Iapichino, et al. 2009. “A Prospective Randomised Multi-Centre Controlled Trial on Tight Glucose Control by Intensive Insulin Therapy in Adult Intensive Care Units: The Glucontrol Study.” *Intensive Care Medicine* 35 (10): 1738–48. https://doi.org/10.1007/s00134-009-1585-2.

Qaseem, Amir. 2011. “Use of Intensive Insulin Therapy for the Management of Glycemic Control in Hospitalized Patients: A Clinical Practice Guideline From the American College of Physicians.” *Annals of Internal Medicine* 154 (4): 260. https://doi.org/10.7326/0003-4819-154-4-201102150-00007.

Quagliaro, Lisa, Ludovica Piconi, Roberta Assaloni, Lucia Martinelli, Enrico Motz, and Antonio Ceriello. 2003. “Intermittent High Glucose Enhances Apoptosis Related to Oxidative Stress in Human Umbilical Vein Endothelial Cells: The Role of Protein Kinase C and NAD(P)H-Oxidase Activation.” *Diabetes* 52 (11): 2795–2804.

Radin, Michael S. 2014. “Pitfalls in Hemoglobin A1c Measurement: When Results May Be Misleading.” *Journal of General Internal Medicine* 29 (2): 388–94. https://doi.org/10.1007/s11606-013-2595-x.

Rapsang, Amy Grace, and Devajit C Shyam. 2014. “Scoring Systems in the Intensive Care Unit: A Compendium.” *Indian Journal of Critical Care Medicine : Peer-Reviewed, Official Publication of Indian Society of Critical Care Medicine* 18 (4): 220–28. https://doi.org/10.4103/0972-5229.130573.

Reed, J A. 1954. “Aretaeus, the Cappadocian: History Enlightens the Present.” *Diabetes* 3

(5): 419–21. https://doi.org/10.2337/diab.3.5.419.

Ritsinger, Viveca, Klas Malmberg, Anton Mårtensson, Lars Rydén, Hans Wedel, and Anna Norhammar. 2014. “Intensified Insulin-Based Glycaemic Control after Myocardial Infarction: Mortality during 20 Year Follow-up of the Randomised Diabetes Mellitus Insulin Glucose Infusion in Acute Myocardial Infarction (DIGAMI 1) Trial.” *The Lancet. Diabetes & Endocrinology* 2 (8): 627–33. https://doi.org/10.1016/S2213-8587(14)70088-9.

Roberts, Gregory W, Stephen J Quinn, Nyoli Valentine, Tariq Alhawassi, Hazel O’Dea, Stephen N Stranks, Morton G Burt, and Matthew P Doogue. 2015. “Relative Hyperglycemia, a Marker of Critical Illness: Introducing the Stress Hyperglycemia Ratio.” *The Journal of Clinical Endocrinology and Metabolism* 100 (12): 4490–97. https://doi.org/10.1210/jc.2015-2660.

Schulze, Friedrich, Angela M. Carter, Edzard Schwedhelm, Ramzi Ajjan, Renke Maas, Rouven Alexander von Holten, Dorothee Atzler, Peter J. Grant, and Rainer H. Böger. 2010. “Symmetric Dimethylarginine Predicts All-Cause Mortality Following Ischemic Stroke.” *Atherosclerosis* 208 (2): 518–23. https://doi.org/10.1016/j.atherosclerosis.2009.06.039.

Schulze, Friedrich, Henrike Lenzen, Christoph Hanefeld, Asja Bartling, Karl J. Osterziel, Lilia Goudeva, Caroline Schmidt-Lucke, et al. 2006. “Asymmetric Dimethylarginine Is an Independent Risk Factor for Coronary Heart Disease: Results from the Multicenter Coronary Artery Risk Determination Investigating the Influence of ADMA Concentration (CARDIAC) Study.” *American Heart Journal*. https://doi.org/10.1016/j.ahj.2006.06.005.

Scirica, Benjamin M., Deepak L. Bhatt, Eugene Braunwald, P. Gabriel Steg, Jaime Davidson, Boaz Hirshberg, Peter Ohman, et al. 2013. “Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus.” *New England Journal of Medicine* 369 (14): 1317–26. https://doi.org/10.1056/NEJMoa1307684.

Shepard, Jessica G, Anita Airee, Andrew W Dake, M Shawn McFarland, and Amit Vora. 2015. “Limitations of A1c Interpretation.” *Southern Medical Journal* 108 (12): 724–29. https://doi.org/10.14423/SMJ.0000000000000381.

Siervo, M., M. Corander, S. Stranges, and L. Bluck. 2011. “Post-Challenge Hyperglycaemia, Nitric Oxide Production and Endothelial Dysfunction: The Putative Role of Asymmetric Dimethylarginine (ADMA).” *Nutrition, Metabolism and Cardiovascular Diseases* 21 (1): 1–10. https://doi.org/10.1016/j.numecd.2010.10.003.

Siroen, Michiel P C, Paul A M van Leeuwen, Robert J Nijveldt, Tom Teerlink, Pieter J Wouters, and Greet Van den Berghe. 2005. “Modulation of Asymmetric Dimethylarginine in Critically Ill Patients Receiving Intensive Insulin Treatment: A Possible Explanation of Reduced Morbidity and Mortality?” *Critical Care Medicine* 33 (3): 504–10.

Siroen, Michiel P C, Tom Teerlink, Robert J Nijveldt, Hubert a Prins, Milan C Richir, and Paul a M van Leeuwen. 2006. “The Clinical Significance of Asymmetric Dimethylarginine.” *Annual Review of Nutrition* 26: 203–28.

https://doi.org/10.1146/annurev.nutr.26.061505.111320.

Sotgia, Salvatore, Angelo Zinellu, Panagiotis Paliogiannis, Gerard A Pinna, Arduino A Mangoni, Luciano Milanesi, and Ciriaco Carru. 2019. “A Diethylpyrocarbonate-Based Derivatization Method for the LC-MS/MS Measurement of Plasma Arginine and Its Chemically Related Metabolites and Analogs.” *Clinica Chimica Acta; International Journal of Clinical Chemistry* 492 (February): 29–36. https://doi.org/10.1016/j.cca.2019.02.004.

Stegenga, Michiel E, Saskia N van der Crabben, Marcel Levi, Alex F de Vos, Michael W Tanck, Hans P Sauerwein, and Tom van der Poll. 2006. “Hyperglycemia Stimulates Coagulation, Whereas Hyperinsulinemia Impairs Fibrinolysis in Healthy Humans.” *Diabetes* 55 (6): 1807–12. https://doi.org/10.2337/db05-1543.

Strojek, Krzysztof, Itamar Raz, György Jermendy, Anselm K Gitt, Rong Liu, Qianyi Zhang, Scott J Jacober, and Zvonko Milicevic. 2016. “Factors Associated With Cardiovascular Events in Patients With Type 2 Diabetes and Acute Myocardial Infarction.” *The Journal of Clinical Endocrinology & Metabolism* 101 (1): 243–53. https://doi.org/10.1210/jc.2015-1962.

Su, Y-W, C-Y Hsu, Y-W Guo, and H-S Chen. 2017. “Usefulness of the Plasma Glucose Concentration-to-HbA1c Ratio in Predicting Clinical Outcomes during Acute Illness with Extreme Hyperglycaemia.” *Diabetes & Metabolism* 43 (1): 40–47. https://doi.org/10.1016/j.diabet.2016.07.036.

Suleiman, Mahmoud, Haim Hammerman, Monther Boulos, Michael R Kapeliovich, Abeer Suleiman, Yoram Agmon, Walter Markiewicz, and Doron Aronson. 2005. “Fasting Glucose Is an Important Independent Risk Factor for 30-Day Mortality in Patients with Acute Myocardial Infarction: A Prospective Study.” *Circulation* 111 (6): 754–60. https://doi.org/10.1161/01.CIR.0000155235.48601.2A.

Szabo, Csaba, and Brahm Goldstein. 2011. “Endothelial Dysfunction as Predictor of Mortality in Sepsis.” *Critical Care Medicine* 39 (4): 878–79. https://doi.org/10.1097/CCM.0b013e31820a52ba.

Terzioglu, Berna, Osman Ekinci, and Zafer Berkman. 2015. “Hyperglycemia Is a Predictor of Prognosis in Traumatic Brain Injury: Tertiary Intensive Care Unit Study.” *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences* 20 (12): 1166–71. https://doi.org/10.4103/1735-1995.172984.

The CREATE-ECLA Trial Group Investigators*. 2005. “Effect of Glucose-Insulin-Potassium Infusion on Mortality in Patients With Acute ST-Segment Elevation Myocardial Infarction.” *JAMA* 293 (4): 437. https://doi.org/10.1001/jama.293.4.437.

Tommasi, S, D J Elliot, J A Hulin, B C Lewis, M McEvoy, and A A Mangoni. 2017. “Human Dimethylarginine Dimethylaminohydrolase 1 Inhibition by Proton Pump Inhibitors and the Cardiovascular Risk Marker Asymmetric Dimethylarginine: In Vitro and in Vivo Significance.” *Scientific Reports* 7 (1): 2871. https://doi.org/10.1038/s41598-017-03069-1.

Tommasi, Sara, Chiara Zanato, Benjamin C Lewis, Pramod C Nair, Sergio Dall’Angelo,

Matteo Zanda, and Arduino A Mangoni. 2015. “Arginine Analogues Incorporating Carboxylate Bioisosteric Functions Are Micromolar Inhibitors of Human Recombinant DDAH-1.” *Organic & Biomolecular Chemistry* 13 (46): 11315–30. https://doi.org/10.1039/c5ob01843a.

TRIUMPH Investigators, John H Alexander, Harmony R Reynolds, Amanda L Stebbins, Vladimir Dzavik, Robert A Harrington, Frans Van de Werf, and Judith S Hochman. 2007. “Effect of Tilarginine Acetate in Patients with Acute Myocardial Infarction and Cardiogenic Shock: The TRIUMPH Randomized Controlled Trial.” *JAMA* 297 (15): 1657–66. https://doi.org/10.1001/jama.297.15.joc70035.

UKPDS. 1998. “Intensive Blood-Glucose Control with Sulphonylureas or Insulin Compared with Conventional Treatment and Risk of Complications in Patients with Type 2 Diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group.” *Lancet (London, England)* 352 (9131): 837–53.

Umpierrez, Guillermo, Saumeth Cardona, Francisco Pasquel, Sol Jacobs, Limin Peng, Michael Unigwe, Christopher a. Newton, et al. 2015. “Randomized Controlled Trial of Intensive Versus Conservative Glucose Control in Patients Undergoing Coronary Artery Bypass Graft Surgery: GLUCO-CABG Trial.” *Diabetes Care* 38 (9): 1665–72. https://doi.org/10.2337/dc15-0303.

Umpierrez, Guillermo E., and Francisco J. Pasquel. 2017. “Management of Inpatient Hyperglycemia and Diabetes in Older Adults.” *Diabetes Care* 40 (4): 509–17. https://doi.org/10.2337/dc16-0989.

Umpierrez, Guillermo E, Richard Hellman, Mary T Korytkowski, Mikhail Kosiborod, Gregory A Maynard, Victor M Montori, Jane J Seley, Greet Van den Berghe, and Endocrine Society. 2012. “Management of Hyperglycemia in Hospitalized Patients in Non-Critical Care Setting: An Endocrine Society Clinical Practice Guideline.” *The Journal of Clinical Endocrinology and Metabolism* 97 (1): 16–38. https://doi.org/10.1210/jc.2011-2098.

Umpierrez, Guillermo E, Scott D Isaacs, Niloofar Bazargan, Xiangdong You, Leonard M Thaler, and Abbas E Kitabchi. 2002. “Hyperglycemia: An Independent Marker of In-Hospital Mortality in Patients with Undiagnosed Diabetes.” *Journal of Clinical Endocrinology & Metabolism* 87 (3): 978–82. https://doi.org/10.1210/jc.87.3.978.

Vallance, P, A Leone, A Calver, J Collier, and S Moncada. 1992. “Accumulation of an Endogenous Inhibitor of Nitric Oxide Synthesis in Chronic Renal Failure.” *Lancet (London, England)* 339 (8793): 572–75. https://doi.org/10.1016/0140-6736(92)90865-z.

Vassiliou, Alice G, Zafeiria Mastora, Stylianos E Orfanos, Edison Jahaj, Nikolaos A Maniatis, Antonia Koutsoukou, Apostolos Armaganidis, and Anastasia Kotanidou. 2014. “Elevated Biomarkers of Endothelial Dysfunction/Activation at ICU Admission Are Associated with Sepsis Development.” *Cytokine* 69 (2): 240–47. https://doi.org/10.1016/j.cyto.2014.06.010.

Versari, Daniele, Elena Daghini, Agostino Virdis, Lorenzo Ghiadoni, and Stefano Taddei. 2009. “Endothelium-Dependent Contractions and Endothelial Dysfunction in Human Hypertension.” *British Journal of Pharmacology* 157 (4): 527–36.

https://doi.org/10.1111/j.1476-5381.2009.00240.x.

Visser, Marlieke, Mechteld A R Vermeulen, Milan C Richir, Tom Teerlink, Alexander P J Houdijk, Piet J Kostense, Willem Wisselink, Bas A J M de Mol, Paul A M van Leeuwen, and Heleen M Oudemans-van Straaten. 2012. “Imbalance of Arginine and Asymmetric Dimethylarginine Is Associated with Markers of Circulatory Failure, Organ Failure and Mortality in Shock Patients.” *The British Journal of Nutrition* 107 (10): 1458–65. https://doi.org/10.1017/S0007114511004648.

Wang, Pin, Rong Huang, Sen Lu, Wenqing Xia, Haixia Sun, Jie Sun, Rongrong Cai, and Shaohua Wang. 2015. “HbA1c below 7 % as the Goal of Glucose Control Fails to Maximize the Cardiovascular Benefits: A Meta-Analysis.” *Cardiovascular Diabetology* 14 (1): 124. https://doi.org/10.1186/s12933-015-0285-1.

Warrillow, Stephen, and Raymond Raper. 2019. “The Evolving Role of Intensive Care in Health Care and Society.” *Medical Journal of Australia* 211 (7): 294. https://doi.org/10.5694/mja2.50340.

Wernly, Bernhard, Michael Lichtenauer, Marcus Franz, Bjoern Kabisch, Johanna Muessig, Maryna Masyuk, Malte Kelm, Uta C Hoppe, and Christian Jung. 2016. “Differential Impact of Hyperglycemia in Critically Ill Patients: Significance in Acute Myocardial Infarction but Not in Sepsis?” *International Journal of Molecular Sciences* 17 (9). https://doi.org/10.3390/ijms17091586.

White, William B., Christopher P. Cannon, Simon R. Heller, Steven E. Nissen, Richard M. Bergenstal, George L. Bakris, Alfonso T. Perez, et al. 2013. “Alogliptin after Acute Coronary Syndrome in Patients with Type 2 Diabetes.” *New England Journal of Medicine* 369 (14): 1327–35. https://doi.org/10.1056/NEJMoa1305889.

Wiener, Renda Soylemez, Daniel C. Wiener, and Robin J. Larson. 2008. “Benefits and Risks of Tight Glucose Control in Critically Ill Adults.” *JAMA* 300 (8): 933. https://doi.org/10.1001/jama.300.8.933.

Williams, S B, A B Goldfine, F K Timimi, H H Ting, M A Roddy, D C Simonson, and M A Creager. 1998. “Acute Hyperglycemia Attenuates Endothelium-Dependent Vasodilation in Humans in Vivo.” *Circulation* 97 (17): 1695–1701.

Winkler, Martin Sebastian, Axel Nierhaus, Gilbert Rösler, Susanne Lezius, Olaf Harlandt, Edzard Schwedhelm, Rainer H Böger, and Stefan Kluge. 2018. “Symmetrical (SDMA) and Asymmetrical Dimethylarginine (ADMA) in Sepsis: High Plasma Levels as Combined Risk Markers for Sepsis Survival.” *Critical Care (London, England)* 22 (1): 216. https://doi.org/10.1186/s13054-018-2090-1.

Wiviott, Stephen D., Itamar Raz, Marc P. Bonaca, Ofri Mosenzon, Eri T. Kato, Avivit Cahn, Michael G. Silverman, et al. 2019. “Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes.” *New England Journal of Medicine* 380 (4): 347–57. https://doi.org/10.1056/nejmoa1812389.

Wong, D T, and W A Knaus. 1991. “Predicting Outcome in Critical Care: The Current Status of the APACHE Prognostic Scoring System.” *Canadian Journal of Anaesthesia = Journal Canadien d’anesthesie* 38 (3): 374–83. https://doi.org/10.1007/BF03007629.

Yang, Chih-Jen, Wen-I Liao, Jen-Chun Wang, Chia-Lin Tsai, Jiunn-Tay Lee, Giia-Sheun Peng, Chien-Hsing Lee, Chin-Wang Hsu, and Shih-Hung Tsai. 2017. “Usefulness of Glycated Hemoglobin A1c-Based Adjusted Glycemic Variables in Diabetic Patients Presenting with Acute Ischemic Stroke.” *The American Journal of Emergency Medicine* 35 (9): 1240–46. https://doi.org/10.1016/j.ajem.2017.03.049.

Yang, Yeoree, Tae-Hoon Kim, Kun-Ho Yoon, Wook Sung Chung, Youngkeun Ahn, Myung-Ho Jeong, Ki-Bae Seung, Seung-Hwan Lee, and Kiyuk Chang. 2017. “The Stress Hyperglycemia Ratio, an Index of Relative Hyperglycemia, as a Predictor of Clinical Outcomes after Percutaneous Coronary Intervention.” *International Journal of Cardiology* 241 (August): 57–63. https://doi.org/10.1016/j.ijcard.2017.02.065.

Yusuf, Salim, Shamir R Mehta, Susan Chrolavicius, Rizwan Afzal, Janice Pogue, Christopher B Granger, Andrzej Budaj, et al. 2006. “Effects of Fondaparinux on Mortality and Reinfarction in Patients With Acute ST-Segment Elevation Myocardial Infarction: The OASIS-6 Randomized Trial.” *JAMA: The Journal of the American Medical Association* 295 (13): 1519–30. https://doi.org/10.1001/jama.295.13.joc60038.

Yuyun, Matthew F., Leong L. Ng, and G. André Ng. 2018. “Endothelial Dysfunction, Endothelial Nitric Oxide Bioavailability, Tetrahydrobiopterin, and 5-Methyltetrahydrofolate in Cardiovascular Disease. Where Are We with Therapy?” *Microvascular Research* 119 (December 2017): 7–12. https://doi.org/10.1016/j.mvr.2018.03.012.

Zimmerman, Jack E., Andrew A. Kramer, and William A. Knaus. 2013. “Changes in Hospital Mortality for United States Intensive Care Unit Admissions from 1988 to 2012.” *Critical Care* 17 (2): R81. https://doi.org/10.1186/cc12695.

Zinellu, Angelo, Panagiotis Paliogiannis, Ciriaco Carru, and Arduino A. Mangoni. 2018. “Homoarginine and All-Cause Mortality: A Systematic Review and Meta-Analysis.” *European Journal of Clinical Investigation*. https://doi.org/10.1111/eci.12960.

Zinman, Bernard, Jay S Skyler, Matthew C Riddle, and Ele Ferrannini. 2017. “Diabetes Research and Care Through the Ages.” *Diabetes Care* 40 (10): 1302–13. https://doi.org/10.2337/dci17-0042.

Zinman, Bernard, Christoph Wanner, John M. Lachin, David Fitchett, Erich Bluhmki, Stefan Hantel, Michaela Mattheus, et al. 2015. “Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes.” *New England Journal of Medicine* 373 (22): 2117–28. https://doi.org/10.1056/NEJMoa1504720.

Zoccali, C, S Bode-Böger, F Mallamaci, F Benedetto, G Tripepi, L Malatino, A Cataliotti, et al. n.d. “Plasma Concentration of Asymmetrical Dimethylarginine and Mortality in Patients with End-Stage Renal Disease: A Prospective Study.” *Lancet (London, England)* 358 (9299): 2113–17. https://doi.org/10.1016/s0140-6736(01)07217-8.