

**GLYCAEMIA AND UPPER GASTROINTESTINAL
FUNCTION IN HEALTH AND CRITICAL ILLNESS**

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

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by

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NOTE: Statements of authorship appear in the print copy of the thesis held in the University of Adelaide Library.

Abstract

This thesis comprises four distinct but complementary chapters with a broad focus of glycaemia and upper gastrointestinal function in health and critical illness, encompassing four literature reviews, two epidemiological-observational and two interventional studies in the critically ill and three proof-of-principle studies in healthy volunteers.

Hyperglycaemia occurs frequently in the critically ill, both in patients with diabetes, and in those with previously normal glucose tolerance. The literature is reviewed on the impact of dysglycaemia in the patient with sepsis, emphasising the interaction between acute dysglycaemia, chronic hyperglycaemia and outcomes (*chapter 1.2*). Observational studies were performed to estimate the prevalence of stress induced hyperglycaemia in the critically ill and to evaluate the subsequent risk of diabetes. I established that: (i) stress induced hyperglycaemia occurs in ~50% of patients, and (ii) peak blood glucose concentrations are associated with greater mortality in patients with adequate pre-morbid glycaemic control but not in those with chronic hyperglycaemia (*chapter 1.3*). In a large, state-wide retrospective observational study I established that stress induced hyperglycaemia doubles the risk for subsequent type 2 diabetes (*chapter 1.4*).

Dysregulated enterohormone secretion is thought to mediate critical illness induced abnormalities of glycaemia and upper gastrointestinal function. A review of the literature is presented on the clinically relevant enterohormone disturbances (*chapter 2.2*). In a prospective comparative study of critically ill patients and healthy volunteers, I quantified gallbladder dysmotility in critical illness, a phenomenon that was independent of plasma concentrations of the enterohormone cholecystokinin (*chapter 2.3*).

The therapeutic potential for the incretin enterohormones in the management of stress induced hyperglycaemia is reviewed in detail with a focus on glucagon-like peptide 1 (GLP-1) (*chapter 3.2*). Upper gastrointestinal function and glycaemia are inextricably linked and in healthy volunteer studies I examined the effect of GLP-1 on gastric emptying during extremes of glycaemia. I demonstrated that GLP-1 attenuated the acceleration of gastric emptying engendered by hypoglycaemia (*chapter 3.3*) and that

the slowing of gastric emptying induced by hyperglycaemia is potentiated by GLP-1 (*chapter 3.4*). In a study investigating the islet cell effects of GLP-1 I demonstrated that intravenous pulsatile delivery of GLP-1 has an equivalent insulinotropic effect to continuous delivery in healthy volunteers (*chapter 3.5*).

Prophylactic administration of proton pump inhibitors for the prevention of gastric stress related mucosal injury is widely prescribed yet has the potential to cause harm and has been inadequately evaluated in the critically ill. A review of the literature highlighting this paradox is presented in *chapter 4.2*. In a prospective, double-blind randomised, placebo-controlled trial I demonstrated that prophylactic administration of a proton pump inhibitor was neither superior nor inferior to placebo in preventing clinically significant upper gastrointestinal bleeding during critical illness (*chapter 4.3*).

In summary, these studies have yielded a number of important insights including; the incidence of stress induced hyperglycaemia and the subsequent risk of diabetes, quantification of gallbladder motility in critical illness, the gastrokinetic effects of GLP-1 during extremes of glycaemia, the insulinotropic effects of pulsatile GLP-1 delivery, and an evaluation of stress ulcer prophylaxis in the critically ill.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Mark Philip Plummer

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Format of Thesis

This thesis is by publication, supplemented by narrative, as per University of Adelaide Guidelines. The thesis comprises four distinct chapters each with a brief narrative introduction followed by a published literature review and clinical trials. In total the thesis comprises eleven manuscripts; 4 reviews of the literature and 7 clinical trials. At the time of submission of this body of work, ten of the manuscripts have been published or accepted for publication. The manuscript that comprises chapter 1.4 is currently under review with *Intensive Care Medicine*. None of the manuscripts were solicited. Each of the four chapters are followed by a narrative conclusion of the major findings and future directions.

The eleven manuscripts are presented in the style of the publication to which they were submitted, accounting for the heterogeneity in American and UK English, and the variance in referencing style and manuscript structure. The references for the 11 publications are included in each respective manuscript and, for consistency, references for the introductions, conclusions and future directions of each chapter follow each section.

The publications are as follows.

Plummer MP, Deane AM. Dysglycemia and glucose control during sepsis. *Clinics in Chest Medicine* [accepted for publication] (IF 2.1). Relevant section in this thesis; Chapter 1.2.

Plummer MP, Bellomo R, Cousins CE, Annink CE, Sundararajan K, Reddi BA, Raj JP, Chapman MJ, Horowitz M, Deane AM. Dysglycaemia in the critically ill and the interaction of chronic and acute glycaemia with mortality. *Intensive Care Medicine* 2014, 40(7):973-980. (IF 5.3). Relevant section in this thesis; Chapter 1.3.

Plummer MP, Finnis ME, Phillips LK, Kar P, Bihari S, Biradar V, Moodie S, Horowitz M, Shaw JE, Deane AM: Stress induced hyperglycaemia and the subsequent risk of type 2 diabetes in survivors of critical illness. [under review with *Intensive Care Medicine*]. Relevant section in this thesis; Chapter 1.4.

Plummer MP, Reintam Blaser A, Deane AM: Enterohormones and the response to critical illness. Book Chapter: *The Stress Response of Critical Illness: Metabolic and Hormonal Aspects*. Springer. [accepted for publication]. Relevant section in this thesis; Chapter 2.2.

Plummer MP, Kar P, Cousins CE, Hausken T, Lange K, Chapman MJ, Jones KL, Horowitz M, Deane AM: Critical illness is associated with impaired gallbladder emptying as assessed by 3D ultrasound. *Critical Care Medicine* [accepted for publication] (IF 6.3). Relevant section in this thesis; Chapter 2.3.

Plummer MP, Horowitz M, Deane AM: Incretins and the intensivist: what are they and what does an intensivist need to know about them? *Critical Care* 2014, 18(1):205.(IF 4.7). Relevant section in this thesis; Chapter 3.2.

Plummer MP, Jones KL, Annink CE, Cousins CE, Meier JJ, Chapman MJ, Horowitz M, Deane AM: Glucagon-like peptide 1 attenuates the acceleration of gastric emptying induced by hypoglycemia in healthy subjects. *Diabetes Care* 2014, 37(6):1509-1515.(IF 8.1). Relevant section in this thesis; Chapter 3.3.

Plummer MP, Jones KL, Cousins CE, Trahair LG, Meier JJ, Chapman MJ, Horowitz M, Deane AM: Hyperglycaemia potentiates the slowing of gastric emptying induced by exogenous GLP-1. *Diabetes Care* 2015, 38(6):1123-9. (IF 8.1). Relevant section in this thesis; Chapter 3.4.

Plummer MP, Kar P, Cousins CE, Lange K, Chapman MJ, Nauck MA, Horowitz M, Meier JJ, Deane AM: The insulinotropic effect of pulsatile compared with continuous intravenous delivery of GLP-1. *Diabetologia* [accepted for publication] (IF 6.7). Relevant section in this thesis; Chapter 3.5.

Plummer MP, Reintam Blaser A, Deane AM: Stress Ulceration: prevalence, pathology and association with adverse outcomes. *Critical Care* 2014 18;18(2):213. (IF 4.7). Relevant section in this thesis; Chapter 4.2.

Selvanderan SP, Summers MJ, Finnis ME, **Plummer MP**, Ali Abdelhamid Y, Anderson MB, Chapman MJ, Rayner CK, Deane AM: Pantoprazole or Placebo for stress Ulcer Prophylaxis (POPUP): Randomized double blind exploratory study. *Critical Care Medicine* [accepted pending minor edits] (IF 6.3). Relevant section in this thesis; Chapter 4.3.

CHAPTER 1

DYSGLYCAEMIA IN THE CRITICALLY ILL

1.1 INTRODUCTION

Hyperglycaemia during critical illness occurs frequently even in patients with previously normal glucose tolerance; so-called ‘stress hyperglycaemia’ or ‘critical illness associated hyperglycaemia’ (terms which are used interchangeably throughout the publications in this body of work, as tailored to Editors’ preferences). While it is generally accepted that marked hyperglycaemia is associated with adverse pathological outcomes and should be avoided, the threshold at which hyperglycaemia becomes harmful remains contentious. Furthermore, recent retrospective observational data suggest that hyperglycaemia does not represent an equivalent insult to all critically ill patients and may, in fact, be modified by the premorbid glycaemic control in an individual patient.

The literature review presented in chapter 1.2 focuses on dysglycaemia in patients with critical illness due to sepsis. In this review the mechanisms underlying dysglycaemia in this group are outlined and the pathophysiological consequences of hyperglycaemia, hypoglycaemia and glycaemic variability are discussed. Finally, evidence is presented to support the evolving concept that the harm of acute hyperglycaemia is modulated by pre-existing chronic hyperglycaemia. Despite the increasing awareness of the importance of this interaction in the critically ill there is limited information about the respective prevalence of these conditions. It was, therefore, important to obtain epidemiological data and prospectively and accurately quantify recognised diabetes, undiagnosed diabetes and stress induced hyperglycaemia in the critically ill (chapter 1.3). Previous studies have, for the main part, regarded patients with diabetes as a homogenous cohort, which compromises assessment of the relationships between acute glycaemia, chronic blood glucose control and outcome. Accordingly, it was logical to quantify chronic blood glucose control using glycated haemoglobin in a prospective evaluation of the impact of acute glycaemia and mortality outcomes (chapter 1.3).

Finally, it is intuitively plausible that stress hyperglycaemia identifies patients at risk for the subsequent development of type 2 diabetes as a result of limited pancreatic

reserve, and/or increased insulin resistance. While the outcome of studies in hospitalised, but not critically ill, patients support the concept that hyperglycaemia during acute illness is predictive of future disordered glucose tolerance, data in the critically ill are lacking. For this reason a large epidemiological cohort study in non-diabetic critically ill patients was undertaken to determine whether stress hyperglycaemia signals a future risk for type 2 diabetes (chapter 1.4).

1.2 Literature review: Dysglycaemia and glucose control during sepsis

Full title

Dysglycemia and glucose control during sepsis

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Duality of interest: M.P.P and A.M.D have no duality of interest to declare.

Synopsis

Sepsis predisposes to disordered metabolism and dysglycemia; the latter is a broad term that includes hyperglycemia, hypoglycemia and glycaemic variability. Dysglycemia is a marker of illness severity and extremes of disordered glucose control are associated with adverse outcomes including increased mortality. Large randomized controlled trials have provided considerable insights into the optimal blood glucose targets for critically ill patient with sepsis. Broadly, targeting blood glucose concentrations < 10 mmol/L; 180 mg/dL throughout a patient's illness is accepted as best practice. However, it may be that the pathophysiological consequences of dysglycemia are dynamic throughout the course of a septic insult and also altered by pre-morbid glycaemia. Accordingly, a more sophisticated and nuanced approach to glycaemia may be necessary. This review highlights the relevance of hyperglycemia, hypoglycemia and glycaemic variability in the patient with sepsis with an emphasis on a rational approach to management.

Key words

Sepsis, hyperglycemia, hypoglycemia, glycaemic variability

Key points

The three domains of sepsis induced dysglycemia; hyperglycemia, hypoglycemia and glycaemic variability, occur frequently in the patient with sepsis and are associated with increased mortality.

Dysglycemia may not represent the same insult to all septic patients and may be altered by a patient's long term blood glucose control.

Future randomized controlled trials should consider all three domains of dysglycemia as important outcomes with variable associations with mortality based on pre-morbid glycaemic control.

Introduction

The physiological stress of sepsis results in marked disturbances in metabolism and glucose regulation. Disordered metabolism can be divided into three separate but interrelated categories, otherwise known as ‘three domains of critical illness dysglycemia’; which are hyperglycemia, hypoglycemia and glycemically variability [1-3]. These dysglycemic states occur frequently, with the prevalence of hypo and hyperglycemia increasing along the continuum from sepsis through severe sepsis and septic shock [4]. While hyperglycemia, hypoglycemia and glycemically variability are all associated with increased mortality [2, 3, 5] the management goals of the patient with sepsis and hyperglycemia remain contentious. This review focuses on the relevance of the three domains of dysglycemia in the septic patient, with particular emphasis on a rational approach to blood glucose management.

Definitions, prevalence and pathogenesis

Hyperglycemia occurs frequently in patients who are critically ill due to sepsis and is a marker of illness severity [6]. Many of these patients have previously been diagnosed with diabetes mellitus. A smaller proportion of patients may have diabetes that was unrecognized prior to the onset of sepsis. Furthermore, patients may have hyperglycemia in the absence of pre-existing glucose intolerance (whether diagnosed or not), so-called ‘stress hyperglycemia’. The distinction between these clinical entities is important as recent retrospective and prospective observational data indicate that the association between hyperglycemia and mortality may be modulated by a patient’s chronic glycemically state [7-10].

Stress hyperglycemia

In the critically ill the precise threshold blood glucose concentration that causes harm and therefore constitutes pathological hyperglycemia remains controversial. The American Diabetes Association (ADA) Diabetes in Hospitals Writing Committee Guidelines recommend thresholds of fasting glucose >6.9 mmol/L; 124 mg/dL or random glucose >11 mmol/L; 198 mg/dL [11] as identifying disordered glucose metabolism, but these are based on pathological thresholds in health [12]. While these values facilitate standardization in the critically ill, the blood glucose threshold/s that

cause harm is likely more complex in the patient with sepsis and may fluctuate throughout an individual patient's illness. Regardless of definitive values, it appears that hyperglycemia occurs frequently in critically ill patients with sepsis, even in those who did not previously have diabetes. We prospectively studied 1000 consecutively admitted patients and classified them as having recognized diabetes, unrecognized diabetes, stress hyperglycemia or normal glucose according to their past medical history, glycated hemoglobin (HbA_{1c}) obtained on admission, and peak blood glucose in the first 48 hours [10]. Patients were deemed to have stress hyperglycemia if their blood glucose exceeded the aforementioned ADA thresholds and HbA_{1c} was <6.5% (47.5 mmol/mol). Of the 1000 patients, 67 were admitted with a primary diagnosis of sepsis and in this sub-group pre-existing diabetes (recognized or not) occurred in ~45% and stress hyperglycemia in ~40% of patients (Fig 1.), consistent with the concept that disordered glucose metabolism occurs frequently during sepsis.

Mechanism of stress-induced hyperglycemia

Sepsis induced hyperglycemia is initiated by the overwhelming activation of pro-inflammatory mediators and the release of counter-regulatory hormones leading to excessive hepatic gluconeogenesis and peripheral insulin resistance [13]. Cortisol, catecholamines, interleukin-6, tumour necrosis factor- α and glucagon independently, and synergistically, stimulate hepatic glucose production with hyperglucagonemia appearing to be of pivotal importance [13-15]. Peripheral insulin resistance is directly proportional to the severity of the stress response [16] and results from defects in post-receptor insulin signalling, with subsequent down regulation of insulin-mediated GLUT-4 glucose transporters [17]. The exact mechanism/s whereby sepsis induces defective translocation of GLUT-4 transporters is unclear, however data from animal studies implicate cortisol [18], catecholamines [19], growth hormone [20] and tumour necrosis factor- α [21] as particularly important. The hyperglycemia attributed to these metabolic derangements is further exacerbated by therapeutic interventions such as administration of catecholamines, dextrose, corticosteroids and nutrition.

Harm secondary to hyperglycemia

Acute hyperglycemia has been recognized as a marker of the severity of illness [6, 22, 23]. Moreover, various investigators have repeatedly reported that the magnitude of hyperglycemia is associated with increased mortality, even after adjusting for illness

severity scores, suggesting that at some threshold hyperglycemia is harmful in patients with sepsis [6, 22, 23]. However, recent data from Kaukonen, et al suggests the relationship between hyperglycemia and harm may be more complex; and the variables used in previous studies to adjust for risk may have been imprecise [24]. In a retrospective observational study of patient's concurrent glucose and lactate samples (n=7925 critically ill patients) they utilized multivariable analysis and reported no association between hyperglycemia and mortality once lactate levels were incorporated into the model [24]. These data challenge the causal relationship of hyperglycemia with mortality within the spectrum of moderate-glucose control targets. It should be highlighted however that these patients had well controlled glycemia with the mean (SD) blood glucose during ICU stay reported as 7.6 (2.1) mmol/L; 137 (38) mg/dL in survivors and 8.1 (2.5) mmol/L; 146 (45) mg/dL in non-survivors [24]. It is plausible that extremes of hyperglycemia have increased toxicity and at higher levels may be independently associated with mortality.

While the degree of hyperglycemia required for harm and the association between hyperglycemia and mortality remain uncertain, *in vivo* and *in vitro* studies have highlighted a number of putative pathophysiological consequences of acute hyperglycemia. Glucose has been shown to be a powerful pro-inflammatory mediator stimulating cytokine production and exacerbating the oxidative stress response thereby setting up a cycle whereby hyperglycemia leads to further hyperglycemia [25]. In human clinical trials hyperglycemia has also been shown to exert pro-thrombotic effects [26], reduce endothelial vascular reactivity [27] and impair neutrophil chemotaxis and phagocytosis [28].

Diabetes and the impact of chronic hyperglycemia

The relative risk of sepsis in patients with diabetes is two to six-fold greater than in normal age matched persons without diabetes [29, 30]. It is therefore unsurprising that diabetes is a common co-morbid illness in the septic critically ill population, with a reported prevalence between 17 - 45% [1, 10, 22, 23]. However, somewhat counter-intuitively the diagnosis of diabetes does not identify critically ill patients at risk of dying in ICU. Indeed outcomes in patients presenting with sepsis appear comparable despite patients with diabetes being older, sicker and having higher blood glucose

concentrations than patients without diabetes [22, 23, 31]. In the largest epidemiological study to date into the effect of pre-morbid diabetes status on outcomes in sepsis, Esper and colleagues analyzed data from 12.5 million acute-care admissions, of which 17% (2,070,459) were identified as having diabetes mellitus [23]. Compared with patients with severe sepsis that were not known to have diabetes, patients with diabetes had a lesser case-fatality rate (18.5% versus 20.6% ($p < 0.05$)), shorter length of hospital stay, and were less likely to develop acute respiratory failure and Acute Respiratory Distress Syndrome (9% vs 14%) [23]. Furthermore, while there is a strong association between the magnitude of hyperglycemia and mortality in the non-diabetic septic population (as described above), the strength of this association is either markedly reduced, or absent, in studies that have adjusted for patients with recognized diabetes [22].

A limitation of these epidemiological studies is that patients with unrecognized diabetes have not been identified and, more importantly, patients with diabetes have been viewed as a homogenous cohort irrespective of their premorbid glycemic control. This relatively crude approach is likely to be flawed, as it is increasingly recognized that chronic blood glucose control is important when evaluating associations between acute hyperglycemia and outcomes in the general intensive care population [3, 8-10, 32]. In the study we recently undertook to evaluate the prevalence of unrecognized diabetes we concurrently evaluated the interaction between pre-existing chronic hyperglycemia, acute glycemia and mortality [10]. Using admission glycated hemoglobin (HbA_{1c}) as a measure of chronic glycemic control we observed that acute hyperglycemia (glucose > 11 mmol/L; 198 mg/dL) was associated with increased mortality in patients without diabetes and in those with adequately controlled diabetes ($HbA_{1c} < 7\%$; 53 mmol/mol). However there was no association between acute glycemia and mortality in patients with 'insufficiently controlled' diabetes ($HbA_{1c} \geq 7\%$) i.e. those with chronic hyperglycemia, such that blood glucose concentrations in excess of 15 mmol/L (270 mg/dL) were well tolerated (Fig. 2) [10]. These prospective data support previous retrospective studies that indicated the benefit in treating hyperglycemia during critical illness may be diminished in patients with known diabetes [3, 7, 8, 31, 33, 34]. Even more startling are observations from Egi and colleagues who conducted a retrospective observational study and stratified patients with diabetes according to premorbid blood glucose control [8]. Using this

approach, higher blood glucose concentrations were associated with a reduction in mortality in patients with insufficiently controlled diabetes, suggesting that treating hyperglycemia in this population may actually be harmful [8].

The mechanisms governing the interaction between acute hyperglycemia during sepsis and outcome in patients with poorly controlled diabetes and chronic hyperglycemia remain poorly understood. The effect of diabetes on the immune system has been hypothesized to play a role, possibly through impaired neutrophil function blunting the exaggerated inflammatory response [23, 35]. Conditioning to chronic hyperglycemia has also been proposed to cause cellular adaptation with preferential down-regulation of insulin independent GLUT-1 and GLUT-3 glucose transporters preventing intracellular glucotoxicity [32]. Prospective studies classifying diabetic patients according to pre-morbid glycemic control are required to further delineate the importance of, and mechanisms for, the protective effect of chronic hyperglycemia in patients presenting with sepsis who have pre-existing diabetes prior to their acute illness [36].

A protective effect of oral hypoglycemic agents has also been proposed as a putative contributory factor explaining the paradox of improved outcome in septic patients with diabetes. There are recent data that suggest that metformin use prior to ICU admission may have a protective effect via an attenuation of the inflammatory response [37]. Pre-clinical studies have reported that metformin, when compared to placebo, reduces mortality in murine models of endotoxemia and acute lung injury via a reduction in pro-inflammatory cytokines and neutrophil activation [38, 39]. In a parallel study of 40 non-diabetic mice utilizing a lipopolysaccharide model of mouse sepsis, Tsoyi and colleagues demonstrated a dramatic survival benefit in those mice pre-treated with metformin (n=20) compared to placebo (n = 20) (survival 75% vs. 17%; $P < 0.05$) [38]. There are however limited data in humans. In a retrospective observational study of 1284 patients with diabetes undergoing cardiac surgery, patients that were receiving metformin had fewer post-operative infections when compared to those patients not receiving metformin (0.7% in metformin users versus 3.2% in non-users; OR 0.2, 95% CI 0.1, 0.7) [40]. A retrospective, observational, multi-centre study of 7404 critically ill adult patients with type 2 diabetes reported that users of metformin, both as monotherapy and in combination with other anti-diabetic drugs, had less mortality at day 30 when compared to non-users after

adjustment for age, sex, diabetes duration, preadmission HbA1c, preadmission morbidity and use of concurrent cardiac medications [adjusted hazard ratio 0.84, 95% CI 0.75 – 0.94] [37]. Metformin use was identified by data-matching to a registry of filled prescriptions within 90 days prior to ICU admission and medication compliance is unknown, however any non-adherence would, if anything, bias estimates towards no association [37].

Treatment with metformin during critical illness is contentious due to the risk of lactic acidosis in patients with shock and severe renal, liver and cardiac failure [41]. Only a single study has examined the effect of metformin use during critical illness [42]. In this small (n=21) parallel randomized-controlled trial of critically ill patients with a diagnosis of systemic inflammatory response syndrome and hyperglycemia, Ansari and colleagues report a non-significant reduction in pro-inflammatory cytokines at day 7 and a reduction in insulin requirement when metformin was compared to placebo in combination with intensive insulin therapy [42]. However, interpretation of these results is limited considerably by a lack of clinical outcome data and further studies are required to assess the safety and efficacy of metformin use during critical illness.

Sulphonylurea drugs such as glibenclamide exert their glucose lowering effect via blockade of the K_{ATP} channel on pancreatic beta-cells, triggering insulin release [43]. The K_{ATP} channel is also present on vascular smooth muscle, the unchecked opening of which has been implicated in the pathogenesis of vasodilatory shock [44]. Several studies in animal model of septic shock have demonstrated improvement in vasopressor responsiveness post sulphonylurea administration via blockade of vascular smooth muscle K_{ATP} channels [45-47]. In a small (n=10) randomized double-blind cross-over pilot study of sulphonylurea administration in human sepsis, Warrillow and colleagues failed to demonstrate any difference in median norepinephrine requirement, hemodynamic parameters or lactate concentration with sulphonylurea use and observed a concerning lowering of plasma glucose, confirming absorption of the drug [48]. Thus, while animal data are encouraging, the K_{ATP} channel may be a less important target in human vasodilatory shock and the side effect of hypoglycemia makes sulphonylureas a less attractive therapy for further evaluation in human sepsis.

Unrecognized diabetes

Given the association between chronic hyperglycemia and sepsis [29, 30], it is conceivable that unrecognized diabetes occurs frequently in this population, but there are insufficient data to be certain of this statement. The lack of information occurs because distinguishing acute stress hyperglycemia from unrecognized diabetes is problematic in critically ill patients with sepsis and hyperglycemia. Validated tests for the diagnosis of diabetes in the ambulant population, including fasting plasma glucose and the oral glucose tolerance test, are inaccurate or impractical during critical illness [49]. In health, a glycated hemoglobin (HbA_{1c}) >6.5% (48mmol/mol) has been endorsed as a suitable diagnostic criterion for the diagnosis of diabetes mellitus in both ambulant and inpatient populations [49]. The HbA_{1c} may be less accurate in the critically ill than in outpatients, particularly if patients receive transfusion/s of erythrocytes, or in other conditions that interfere with erythrocyte survival [49]. Despite these limitations, the HbA_{1c} is the most robust test readily available for clinicians and a threshold of 6.5% (48 mmol/mol) appears reasonable. Using this cut-off we reported the prevalence of undiagnosed diabetes in a sample population admitted to a general ICU as 5.5%, and 7.4% in the sub-population of critically ill patients admitted with a primary diagnosis of sepsis (Fig. 1) [10].

Hypoglycemia

Severe hypoglycemia, when defined as a blood glucose <2.2 mmol/L (40 mg/dL) [50], occurs relatively frequently in patients presenting with sepsis [1, 51], with the prevalence strongly associated with illness severity [4]. Waeschle and colleagues analysed hypoglycemia in a cohort of 191 critically ill septic patients treated with intensive insulin therapy (targeting 4.4 – 7.8 mmol/l; 80 – 140 mg/dL) and stratified patients according to illness severity [4]. The percentage of patients with at least one episode of severe hypoglycemia was 2.1%, 6% and 11.5% for patients with sepsis, severe sepsis and septic shock respectively [4]. Intensive insulin therapy appears to be the most important risk factor for the development of hypoglycemia: the relationship between intensive insulin therapy and hypoglycemia has been consistently demonstrated in several large, prospective, randomized-controlled trials [1, 51].

In unselected critically ill patients there is a strong relationship between hypoglycemia and mortality [1, 50-53]. Because hypoglycemia occurs in patients with greater severity of illness and therefore greater risk of death, several investigators have incorporated severity of illness as a variable in regression analyses. When this is done, in general, hypoglycemia appears to be independently associated with death [3, 52-54]. Recent observational [3, 53, 54] and prospective [52] trial data suggest that even moderate hypoglycemia (blood glucose < 3.9 mmol/L; 70 mg/dl) is associated with increased mortality, with the lower the nadir in glucose concentration the stronger the association [52]. This is evident even after a single episode of mild hypoglycemia and the risk is apparent in both patients with and without pre-existing diabetes [3]. The severity and duration of hypoglycemia required to cause harm remains uncertain and the mechanisms by which hypoglycemia increases mortality in sepsis are yet to be fully elucidated. Neurons, as obligate users of glucose, have been purported to be at particular risk of damage in the setting of hypoglycemia; however long-term neurocognitive outcome studies are lacking [55].

It is important to recognize that pathologically low blood glucose also occur secondary to impaired endogenous glucose production even in the absence of exogenous insulin. Accordingly, episodes of hypoglycemia are also a marker of illness severity, e.g. a retrospective observational study of 102 critically ill patients with an episode of severe hypoglycemia reported that 27.5% had not received insulin within 12 hours of the hypoglycemic episode [2].

Glycemic variability

Glycemic variability is a broad term describing marked fluctuations in blood glucose. There is a paucity of prevalence data on glycemic variability in sepsis, which may reflect the lack of standardization on the definition of variability, the thresholds at which swings in blood glucose become pathological, and the metrics used to calculate it [4, 5, 56, 57]. Nevertheless, there is evidence to suggest that fluctuations of blood glucose concentrations are associated with increased mortality in septic patients independent of hypoglycemic episodes and mean blood glucose [4, 5]. Similar to hyperglycemia, patients with diabetes appear to be somewhat protected from the harmful effects of glycemic variability, and the association between glycemic

variability and mortality is reduced or absent in patients with diabetes [3, 58, 59]. However, none of these studies have assessed for interactions between chronic glycemia (HbA_{1c}), acute variability and outcomes. Mechanistic studies into the effects of glycemic variability in sepsis are lacking and the causal link between glucose fluctuations and adverse outcomes have been drawn from studies *in vitro* and in patients with type 2 diabetes in the community, where marked acute fluctuations in blood glucose have been linked to oxidative stress induced endothelial dysfunction and endothelial apoptosis [60, 61].

Glucose targets

Until quite recently stress hyperglycemia was viewed as a ‘normal’ physiological response to critical illness and was, therefore, often tolerated [62]. However, in 2001 the landmark study from Van den Berghe and colleagues caused a paradigm shift in the approach to blood glucose management in the critically ill. In this single-centre open-label study from a surgical ICU in Leuven, Belgium, patients were randomized to ‘intensive insulin therapy’ targeting 4.4 - 6.2 mmol/L; 80-110mg/dL or to conventional therapy targeting 180 - 200 mg/dL; 10 - 11.1 mmol/L [50]. The intervention caused a surprisingly large reduction in mortality (intensive care mortality: relative risk reduction of 43%; absolute risk reduction of 3.4%, and a number needed to treat with intensive insulin therapy to reduce one ICU death of 29 patients) [50]. While the number of patients with severe sepsis was not reported at baseline, intensive insulin therapy reduced episodes of nosocomial septicemia by 46% and reduced the proportion of patients requiring prolonged antibiotic therapy [50]. Given the sheer magnitude of observed effect it was essential to reproduce this study and determine external validity of the results [63, 64].

In 2006, the Leuven group performed a second randomized controlled trial of intensive insulin therapy using the same glucose targets and feeding regimen in medical ICU patients with an anticipated length of stay > 3 days [65]. In contrast to the initial study there was no overall difference in mortality between intensive and conventional glycemic control [65]. Furthermore, there was no difference in the proportion of patients dying from therapy-resistant septic shock [65]. Analysis of the predefined subgroup of interest (patients admitted for > 3 days) reported a more modest survival benefit from intensive insulin therapy, but this group experienced significantly more hypoglycemic episodes, with the prevalence of hypoglycemia over

6-fold higher than in the original study [50, 64]. The prevalence of hypoglycemia raised concerns for the use of intensive insulin therapy in patients with sepsis, particularly as post hoc analysis of pooled data from the two Leuven studies revealed an association between hypoglycemia and death that appeared to be independent [66]. The Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) trial was the first to specifically examine the effect of intensive insulin therapy in patients with severe sepsis. This study, conducted in 18 intensive care units in Germany, utilized a 2 x 2 factorial study design to compare intensive insulin therapy to conventional insulin therapy and resuscitation with intravenous hydroxyethyl starch to Ringer's lactate [1]. At the planned interim analysis (n=488 patients), the insulin comparative arm of the study was terminated by the data and safety monitoring board owing to a six-fold increased risk of hypoglycemia [3]. There was no evidence of any beneficial effect of intensive insulin therapy [27].

The Corticosteroid Treatment and Intensive Insulin Therapy for Septic Shock (COITSS) trial was conducted to determine whether intensive glucose control would be beneficial in patients treated with corticosteroid for septic shock with a secondary objective to compare hydrocortisone plus fludrocortisone therapy with hydrocortisone therapy alone [51]. This 2 x 2 factorial study randomly assigned 509 patients with septic shock to either 'Leuven' intensive insulin therapy or conventional blood glucose control. Patients treated with intensive glucose control had significantly more hypoglycemic episodes than the conventional arm however there was no difference in mortality, mechanical ventilation free days or ICU and hospital length of stay [51].

The NICE-SUGAR study provides data that are the most externally valid to inform blood glucose management during critical illness. Within this study 6104 critically ill patients were randomly assigned to 'intensive' therapy targeting 4.5 – 6.0 mmol/L; 81 – 108 mg/dL or conventional therapy targeting < 10 mmol/L; 180 mg/dL and maintaining blood glucose between 8 – 10 mmol/L; 144 – 180 mg/dL [67]. Intensive therapy was associated with a markedly greater risk of severe hypoglycemia (OR 14.7; CI 9 – 25.9) and, in contradistinction to the Leuven trials, the NICE-SUGAR study reported that patients assigned to the intensive therapy had an increased risk of death (27.5% vs. 24.9%, P = 0.02) [67]. Severe sepsis was a predefined subgroup of interest and outcomes were similar to the population as a whole [67].

Although the NICE-SUGAR study is the only study to report increased mortality with intensive glucose control, several other randomized-controlled trials in mixed

populations of medical and surgical ICU patients have reported that intensive insulin therapy does not convey a survival benefit, and is associated with a much higher incidence of severe hypoglycaemia (Table 1.) [68-70]. Accordingly, the use of intensive insulin therapy should be avoided. However, it is now apparent that because diabetic status was considered a binary phenomenon (rather than a continuous variable) in these trials, the impact of chronic blood glucose control on acute hyperglycemia and outcome is uncertain. Future trials are warranted to further delineate this relationship and explore the concept of individualized blood glucose targets (Fig. 3).

Interpretation

The use of short-acting insulin administered intravenously with the dose based on protocols is currently the gold-standard for the management of hyperglycemia during sepsis [71]. The results from the aforementioned large randomized-controlled trials are somewhat contradictory, which has created uncertainty on the ideal glycemic range to target. The early termination of the VISEP study due to safety concerns of the increased incidence of severe hypoglycemia and the increased mortality demonstrated in the NICE-SUGAR study for patients randomized to intensive insulin therapy suggest that the ideal upper limit may be around 10 mmol/L; 180 mg/dL, particularly for patients with normal glucose tolerance. The current Surviving Sepsis Guidelines do not distinguish stress hyperglycemia from hyperglycemia in patients with diabetes, but strongly recommend threshold blood glucose concentrations ≤ 10 mmol/L; 180 mg/dL [71].

While the Surviving Sepsis Guidelines are written by expert clinicians, it may be that the 'one-size fits all' strategy for the management of hyperglycemia in sepsis is inadequate, particularly given the dynamic course of disease/s and the increasing appreciation of the role of chronic hyperglycemia in modifying the relationship between acute hyperglycemia and outcome. To date, all studies to determine the optimal glycemic range in the critically ill have evaluated the effect of glucose targets that have been maintained for the duration of a patient's admission. However stress hyperglycemia is by definition transient and the pathogenesis driving high blood glucose will be labile in response to changes in the course of the disease. It is intuitively plausible that the optimal blood glucose range fluctuates throughout the course of an individual patient's illness. Higher blood glucose concentrations may be

tolerable, or even more desirable, for cellular function during the early phase of sepsis, particularly if concomitant hypoxia and hypotension are present, but less desirable as the insult resolves and the patient improves – with the risk of infection from higher blood glucose concentrations outweighing any potential protection against cellular hypoglycemia. We also speculate that the ideal glycemic range will vary between patients with diabetes depending on their pre-morbid glycemic control (Fig 3). A patient with well-controlled diabetes, e.g. HbA_{1c} < 7%; 53 mmol/mol, has a mean daily blood glucose of ~8 mmol/L; 144 mg/dL, whereas a patient with poorly controlled diabetes and an HbA_{1c} of 10%; 13.3 mmol/mol has a mean blood glucose of ~13 mmol/L; 234 mg/dL. We suggest that the metabolic milieu prior to the onset of sepsis will be different in such patients and will influence the optimal blood glucose range during critical illness. Accordingly, the patient with chronic hyperglycemia may benefit from more liberal glucose targets. However, prospective randomized controlled studies are required to definitely determine the importance of dynamic glucose targets for stress hyperglycemia and individualized targets according to chronic glycemic control. In the interim, an intravenous insulin infusion protocol that targets an upper limit of 10mmol/L; 180 mg/dL, avoids hypoglycemia and minimizes glycemic variability appears to be a reasonable strategy.

Conclusions

Hyperglycemia, hypoglycemia and glycemic variability occur frequently in patients with severe sepsis with and without pre-existing diabetes. All three domains of sepsis-induced dysglycemia are associated with adverse outcomes in patients without diabetes but current observational data indicate uncertainty as to whether hyperglycemia is harmful or protective in those with pre-existing chronic hyperglycemia (HbA_{1c} > 7%). The current Surviving Sepsis Campaign recommendations for all patients with severe sepsis are for intravenous insulin therapy to be commenced when the blood glucose exceeds 10 mmol/L; 180 mg/dL with the goal of maintaining blood glucose between ~ 8 – 10 mmol/L; 144 – 180 mg/dL. However, we remain cautious about the strength of the Surviving Sepsis Guideline recommendation regarding glucose targets for patients with pre-existing chronic hyperglycemia. We believe further information is required in this sub-group because sepsis-induced dysglycemia appears to be a heterogeneous entity with unique pathophysiological features that appear to be altered by long-term glycemia. It is also

increasingly evident that strategies are required to minimize all three domains of sepsis-induced dysglycemia. The design of future randomized controlled trials should include consideration of hyperglycemia, hypoglycemia and glycemic variability as important outcomes with variable associations with mortality based on pre-morbid glycemic control.

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Table 1: Important randomized controlled trials of insulin therapy targeted to specific blood glucose concentrations in critically ill patients with sepsis.

First author, year (Country)	Sites	N	N with sepsis (%)	Glucose goal (mmol/L)	Outcome of intensive therapy
Annane 2010 (France) [COITSS] [51]	8	509	509 (100%)	IIT 4.4 - 6.1 Control 10 - 11.1	No effect on hospital mortality ↑ hypoglycemia
Arabi 2008 (Saudi Arabia) [68]	1	523	122 (24%)	IIT 4.4 - 6.1 Control 10 - 11.1	No effect on ICU or hospital mortality overall or in subgroup of patients with sepsis ↑ hypoglycemia in all-comers
Brunkhorst 2008 (Germany) [VISEP] [1]	18	535	535 (100%)	IIT 4.4 - 6.1 Control 10 - 11.1	No effect on 90 day mortality ↑ hypoglycemia -> early termination
Ellger 2008 (Belgium)# [72]	1	950	950 (100%)	IIT 4.4 - 6.1 Control 10 - 11.1	No effect on ICU or hospital mortality ↑ hypoglycemia
Iapichino 2008 (Italy) [73]	3	72	72 (100%)	IIT 4.4 - 6.1 Control 10 - 11.1	No effect on ICU or 90 day mortality
NICE-SUGAR 2009 (Australia, New Zealand, Canada) [67]	42	6104	1299 (21%)	IIT 4.4 - 6.1 Control < 10	↑ 90 day mortality overall No effect on 90 day mortality in sepsis ↑ hypoglycaemia overall and in patients with sepsis
Savioli 2009 (Italy) [74]	3	90	90 (100%)	IIT 4.4 - 6.1 Control 10 - 11.1	No effect on 90 day mortality ↑ hypoglycemia

#Patients classified post-hoc to severe sepsis from two larger randomized controlled trials in surgical [50] and medical intensive care units [65]

IIT Intensive insulin therapy

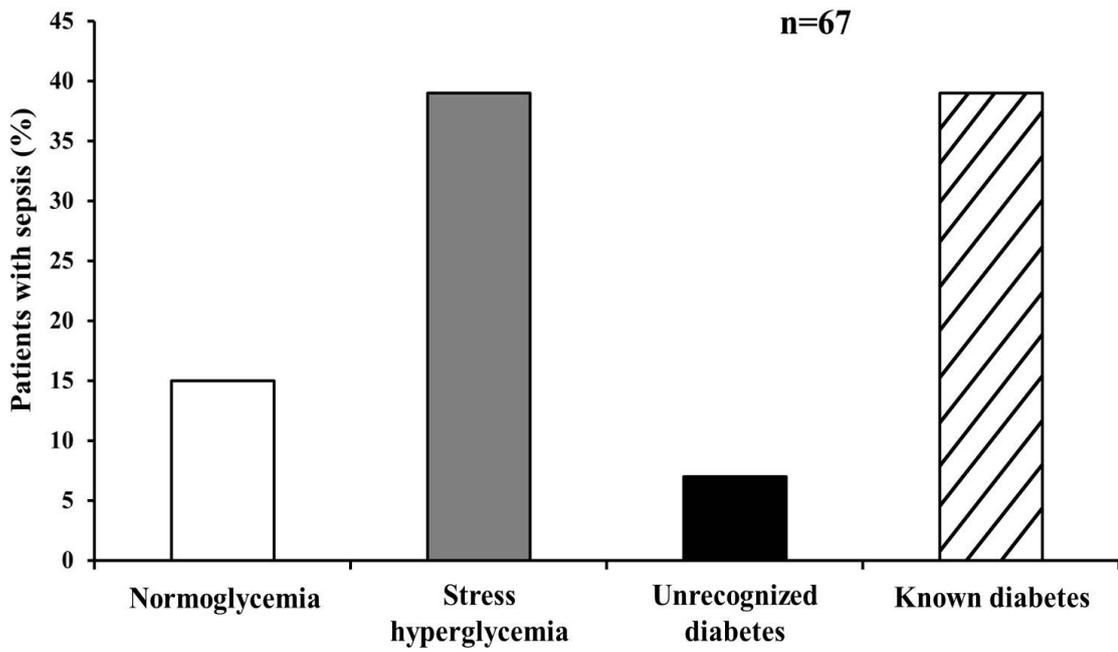


Fig 1. Glycemic category in critically ill patients with a primary diagnosis of sepsis

In a population of 1000 consecutively admitted patients there were 67 admitted with a primary diagnosis of sepsis. The majority of septic patients had pre-existing diabetes or 'stress hyperglycemia'.

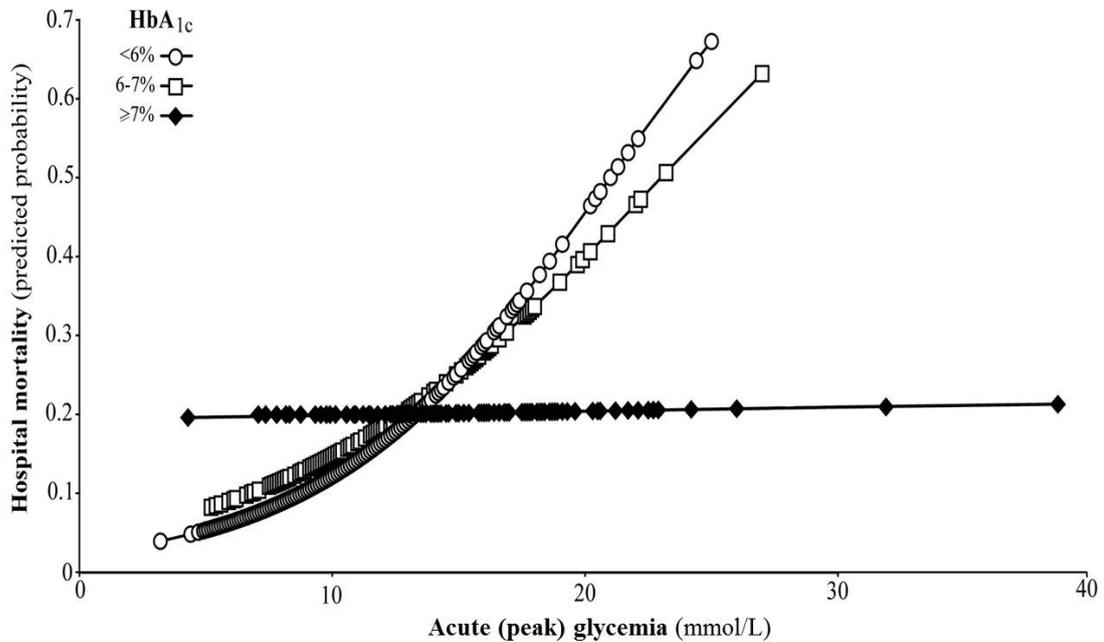


Fig 2. Relationship between hospital mortality and acute glycemia when categorised according to pre-morbid glycemia (HbA_{1c})

In patients without diabetes, and those with ‘stringently-controlled’ [open circles, HbA_{1c} < 6% (42 mmol/mol), n = 672, odds ratio=1.20 (95% CI 1.12, 1.28); P < 0.001] and ‘adequately-controlled’ diabetes [open squares, 6 ≤ HbA_{1c} < 7% (53 mmol/mol), n = 199, odds ratio=1.14 (95% 1.05, 1.25); P=0.003] increasing peak blood glucose concentrations were associated with increasing mortality. However there was no association apparent in patients with ‘insufficiently-controlled’ diabetes [filled diamonds, HbA_{1c} ≥ 7%, n = 129, odds ratio = 1.0 (95% CI=0.92, 1.1); P = 0.95]. The model was an adequate fit according to the Hosmer and Lemeshow goodness of fit test. Reproduced with permission; *Plummer et al, Intensive Care Medicine*, 2014 [10].

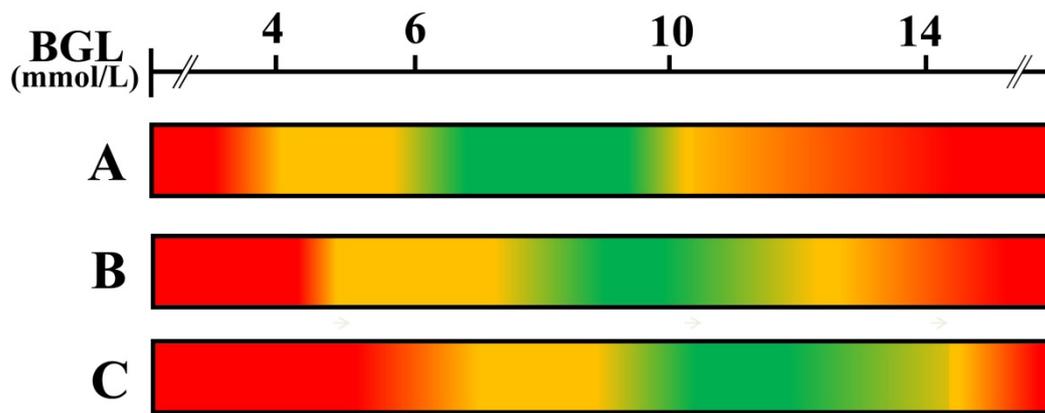


Fig 3. Schematic representation of the proposed model of personalized and dynamic optimal glucose targets (green zone)

A. Targeting a blood glucose <10 mmol/L while avoiding hypoglycemia may be appropriate for patients with normal glucose tolerance and those with well controlled diabetes in the recovery phase from a septic insult.

B. Patients with inadequately controlled diabetes (i.e. $HbA_{1c} > 7\%$) who were chronically hyperglycemic prior to their septic episode may benefit from shifting the optimal glucose threshold to the right to avoid ‘relative hyperglycemia’. Such a shift in targets could theoretically also benefit those patients without pre-existing diabetes who have severe septic shock and concurrent hypoxia.

C. A patient with poorly controlled diabetes who has developed cellular adaptation to chronic hyperglycemia may benefit from more liberal blood glucose targets and a further right-shift in the optimal glucose range when critically ill to reflect the mean blood glucose level prior to the onset of the acute illness.

1.3 Manuscript: Dysglycaemia in the critically ill and the interaction of chronic and acute glycaemia with mortality

Title

Dysglycaemia in the critically ill and the interaction of chronic and acute glycaemia with mortality

Running title

The impact of premorbid glycaemia on the association between acute hyperglycaemia and mortality

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Conflicts of Interest

M.H. has participated in advisory boards and/or symposia for Novo/Nordisk, Sanofi-Aventis, Novartis, Eli-Lilly, Boehringer Ingelheim, AstraZeneca, Satlogen and Meyer Nutraceuticals.

M.P.P., R.B., C.E.C., C.E.A., K.S., B.J.R., J.P.R., M.J.C and A.M.D have no duality of interests to declare

Author Contribution

M.P.P. was responsible for acquisition of data, statistical analysis and drafting the manuscript.

R.B. and M.H. were responsible for the study conception and design, obtaining funding, interpretation of data and critical revision of the manuscript for important intellectual content.

C.E.C., C.E.A., K.S., B.J.R., J.P.R. and M.J.C contributed to the acquisition of data and critical revision of the manuscript for important intellectual content.

A.M.D was responsible for the study conception and design, obtaining funding, acquisition of data, interpretation of data and drafting the manuscript.

M.P.P. and A.M.D. are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of these data and the accuracy of the analysis.

List of abbreviations

ADA: American Diabetes Association

ANOVA: analysis of variance

APACHE: acute physiology and chronic healthy evaluation

BMI: body mass index

CIAH: critical illness-associated hyperglycaemia

EASD: European Association for the Study of Diabetes

HbA_{1c}: glycated haemoglobin

ICU: Intensive Care Unit

OGTT: oral glucose tolerance test

ABSTRACT

Purpose

Hyperglycaemia is common in the critically ill. The objectives of this study were to determine the prevalence of critical illness-associated hyperglycaemia (CIAH) and recognised and unrecognised diabetes in the critically ill as well as evaluate the impact of premorbid glycaemia on the association between acute hyperglycaemia and mortality.

Methods

In 1000 consecutively admitted patients we prospectively measured glycated haemoglobin (HbA_{1c}) on admission, and blood glucose concentrations during the 48 hours after admission, to the Intensive Care Unit. Patients with blood glucose ≥ 7.0 mmol/l when fasting or ≥ 11.1 mmol/l during feeding were deemed hyperglycaemic. Patients with acute hyperglycaemia and HbA_{1c} $< 6.5\%$ (48 mmol/mol) were categorised as 'CIAH', those with known diabetes as 'recognised diabetes', and those with HbA_{1c} $\geq 6.5\%$ but no previous diagnosis of diabetes as 'unrecognised diabetes'. The remainder were classified as 'normoglycaemic'. Hospital mortality, HbA_{1c} and acute peak glycaemia were assessed using a logistic regression model.

Findings

Of 1000 patients, 498 (49.8%) had CIAH, 220 (22%) had recognised diabetes, 55 (5.5%) had unrecognised diabetes and 227 (22.7%) were normoglycaemic. The risk of death increased by ~20% for each increase in acute glycaemia of 1mmol/L in patients with CIAH and those with diabetes and HbA_{1c} levels $< 7\%$ (53mmol/mol), but not in patients with diabetes and a HbA_{1c} $\geq 7\%$. This association was lost when adjusted for severity of illness.

Conclusions

CIAH is the most frequent cause of hyperglycaemia in the critically ill. Peak glucose concentrations during critical illness are associated with increased mortality in patients with adequate premorbid glycaemic control, but not in patients with premorbid hyperglycaemia. Optimal glucose thresholds in the critically ill may, therefore, be affected by premorbid glycaemia.

INTRODUCTION

Hyperglycaemia is common in the critically ill and may be secondary to either diabetes (recognised or not), or critical illness-associated hyperglycaemia (CIAH) [1; 2]. The latter condition refers to patients who have normal glucose tolerance following resolution of their acute illness. However, there is limited information about the respective prevalence of these conditions [2; 3; 4], with the majority of studies only assessing acute glycaemia and thus failing to discriminate between hyperglycaemia associated with unrecognised diabetes and true CIAH.

Such categorization may be important as retrospective data suggest that the benefit in treating hyperglycaemia during critical illness may be diminished in patients with known diabetes [5; 6; 7; 8; 9; 10]. Furthermore, in a retrospective observational study using glycated haemoglobin (HbA_{1c}) measured in the three months prior to ICU admission as a marker of premorbid glycaemia, it was reported that acute hyperglycaemia was associated with a reduction, rather than an increase, in mortality in patients with ‘insufficiently-controlled’ diabetes [7]. Given that in CIAH the magnitude of hyperglycaemia is associated with increased mortality [7; 11], it is plausible that the impact of acute hyperglycaemia on outcome is dependent on premorbid glycaemia. In this regard, a recent position statement from the European Association for the Study of Diabetes (EASD) and American Diabetes Association (ADA) emphasised that targets for chronic glucose control (HbA_{1c}) in ambulant patients with type 2 diabetes should be individualised [12]. However, the concept that during critical illness acute glycaemic targets should be individualised, based on premorbid glycaemia (HbA_{1c}), has apparently not been formally considered [13; 14].

Accordingly, in a cohort of critically ill patients, we aimed to determine the prevalence of CIAH and of recognised and unrecognised diabetes, and to evaluate prospectively the impact of premorbid glycaemia on the association between acute hyperglycaemia and mortality. The blood glucose thresholds for CIAH are based on The American Diabetes Association (ADA) Diabetes in Hospitals writing Committee Guidelines that state that thresholds used in health, that is fasting plasma glucose ≥ 7.0 mmol/l and/or random plasma glucose ≥ 11.0 mmol/l, are appropriate for use in hospitalized patients and allow standardization in this area [15].

PATIENTS AND METHODS

Patients

We performed a prospective single-centre observational study in a general Intensive Care Unit (ICU) and studied 1000 consecutively admitted patients aged ≥ 18 years who were admitted for ≥ 24 hours. The study was conducted between August 2012 and June 2013 at the Royal Adelaide Hospital ICU. The Royal Adelaide Hospital is a 680 bed quaternary university-affiliated hospital and is one of two centres for trauma and neurosurgical services in the state of South Australia. It is the only centre for burns and spinal injuries in the state. The ICU has 24 beds and admits all medical and surgical patients requiring organ support, with the exception of those admitted to the High Dependency and Cardiothoracic Surgical Units that admit patients not requiring organ support and post cardiac surgery respectively. Patients admitted to the latter two locations were not included in this analysis.

Protocol

This protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, with the need for informed consent waived. It was registered with the Australian New Zealand Clinical Trials Registry (www.anzctr.com.au; ACTRN12611000973910). We recorded age, Body Mass Index (BMI), administration of catecholamines and corticosteroids during the initial 48 hours of admission. We also obtained information on the Acute Physiology and Chronic Health Evaluation (APACHE) II score and hospital outcomes from the hospital electronic data repository. In all patients insulin was administered by continuous intravenous infusion according to an algorithm to target a blood glucose of 6.0 – 10.0 mmol/l, such that all patients with a blood glucose level >10 mmol/l were commenced on intravenous insulin (Supplementary Figure 2)[16]. On the first available blood sample after ICU admission, we measured glycated haemoglobin (HbA_{1c}) as a marker of premorbid glycaemia. Blood was collected into EDTA tubes and HbA_{1c} levels measured using high performance liquid chromatography (Biorad HPLC variant II turbo, California, USA [Qc: 2.44%]). We also collated all blood glucose concentrations during the initial 48 hours of admission with the peak blood glucose concentration recorded during the first 48 hours of admission used to define acute glycaemia. Blood gas analysers (Radiometer ABL800 Series Flex Q, Denmark) or bedside glucometers

(Optium Xceed; Abbott Laboratories, Bedford, MA) were used to measure blood glucose. Blood glucose was measured every hour unless it was between 6-10 mmol/l and insulin administration was unchanged from the previous measurement when the interval between measurements was extended to every two hours (Supplementary Figure 2).

Data analysis

We classified patients into the following groups according to their HbA_{1c} and peak blood glucose in the first 48 hours: CIAH, ‘recognised diabetes’, ‘unrecognised diabetes’ and ‘normoglycaemia’. CIAH was defined by a HbA_{1c} < 6.5% (48 mmol/mol) and any ‘fasting’ (i.e. patients not receiving enteral or intravenous nutrition) blood glucose \geq 7.0 mmol/l and/or random blood glucose \geq 11.1 mmol/l during feeding [15; 17].

We identified ‘recognised diabetes’ using the hospital case notes and history provided by family members. Any previous diagnosis of glucose intolerance (apart from gestational diabetes) was considered as recognised diabetes. We defined ‘unrecognised diabetes’ as an admission HbA_{1c} \geq 6.5% in the absence of a history of glucose intolerance. The latter was confirmed by contacting the patient’s family physician.

We defined ‘normoglycaemia’ as the combination of an admission HbA_{1c} < 6.5%, ‘fasting’ blood glucose < 7.0 mmol/l and random blood glucose < 11.1 mmol/l [15; 17].

Based on the current ADA/EASD position statement, we categorised patients with diabetes according to their pre-morbid glycaemia as ‘stringently-controlled’ [HbA_{1c} < 6% (42 mmol/mol)], ‘adequately-controlled’ [\leq 6% HbA_{1c} < 7% (53mmol/mol)], and ‘insufficiently-controlled’ [HbA_{1c} \geq 7 %] [12].

Statistical Analysis

Data are presented as mean (SD) or median (range) as appropriate and were evaluated using one-way analysis of variance (ANOVA), with Tukey posthoc tests for age, BMI, APACHE II, HbA_{1c} and peak blood glucose in the first 48 h. ICU and hospital

length of stay were analysed using non-parametric Kruskal-Wallis tests with posthoc Mann-Whitney tests and Bonferroni-Holm adjustment. Chi-squared tests were used to assess catecholamine use, steroid use and mortality, with post hoc Bonferroni-Holm adjustment for multiple comparisons. Patients still in ICU or hospital at the time of followup were censored at their observed length of stay. The outcome of hospital mortality was assessed in relation to HbA_{1c} and peak blood glucose using a logistic regression model. Predictors in both models were HbA_{1c} - assigned as categorical data in three bands: <6%; 6% ≤ HbA_{1c}<7%; and HbA_{1c} ≥ 7% - and peak blood glucose concentration as a continuous variable. The interaction term (peak glucose*HbA_{1c}) was used for the three categories. The Hosmer and Lemeshow Goodness of Fit test was used to evaluate the risk of mortality determined using the logistic regression model. Data were also analysed *post hoc* for potential confounding including age, APACHE-II score and type of admission (medical or surgical). A P value of 0.05 was used for significance. Analyses were run using SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA) by an independent biostatistician.

RESULTS

We studied 1000 patients (646 men), with a mean age of 55 (18) years, BMI 27.4 (7) kg/m² and an APACHE II score of 18 (7.6). The median hospital length of stay was 11.8 (1 – 258) days, with 145 (14.5%) patients dying in hospital and 16 patients still in hospital at the time of analysis. Patients were admitted with a primary disorder of: respiratory (22.8%); trauma (18.6%); neurological (17%); gastrointestinal (11%); cardiovascular (9.9%); sepsis (6.7%); metabolic (6.2%); haematologic (2.9%); renal/genitourinary (2.5%); and musculoskeletal/skin (2.3%). Overall median HbA_{1c} was 5.7 (3.8 – 16)% [39 (18 – 151) mmol/mol] and median peak blood glucose 9.4 (3.2 – 38.8) mmol/l.

Prevalence of CIAH, recognised and unrecognised diabetes, and normoglycaemia

In total, 498 (49.8%) patients had CIAH, 220 (22%) had ‘recognised diabetes’ (5% with known type 1), 55 (5.5%) had ‘unrecognised diabetes’ (all of whom were type 2), and only 227 (22.7%) were ‘normoglycaemic’.

Of the 275 patients with either recognised or unrecognised diabetes; 146 (53%) had ‘stringently-’ or ‘adequately-controlled’ diabetes and 129 (47%) had ‘insufficiently-controlled’ diabetes. Of the 220 patients with recognised diabetes, the treatment of hyperglycaemia prior to critical illness included oral medication in 70 (32%), diet alone in 56 (25%), insulin in 48 (22%), and both oral medications and insulin in 18 (8%) patients. We were unable to determine preexisting therapy in 28 (13%) patients.

Both recognised and unrecognised diabetes were associated with greater peak glucose concentrations than CIAH (Table 1). Patients with diabetes also had higher APACHE II scores, were older, and weighed more than CIAH or normoglycaemic patients (Table 1). Normoglycaemic patients required less exogenous catecholamine support, had shorter admissions, and had fewer deaths compared to patients with diabetes (recognised and unrecognised) or CIAH (Table 1). There were no significant differences in catecholamine use, length of stay, admission category (medical vs surgical) or mortality between patients with diabetes (recognised and unrecognised) and CIAH (Table 1).

Relationships between premorbid glycaemia, acute glycaemia and outcome

998 patients had data available for logistic regression analysis. There was a significant interaction between acute glycaemia, HbA_{1c} and mortality ($P = 0.04$), such that, in patients without diabetes and those with ‘stringently-controlled’ diabetes, the risk of death increased by 20% (95% CI: 1.12, 1.28) for each increase in acute glycaemia of 1 mmol/l ($P < 0.001$; Figure 1). There was also a significant relationship between mortality and acute glycaemia in patients with ‘adequately-controlled’ diabetes ($P=0.003$; Figure 1). In contrast, among patients with ‘insufficiently-controlled’ diabetes (i.e. chronic premorbid hyperglycaemia) there was no significant relationship between mortality and acute glycaemia ($P = 0.95$; Figure 1) such that the risk of death did not change, even when peak glucose concentrations increased above 15 mmol/l. The logistic regression model was an adequate fit ($P=0.29$). When data were analysed adjusting for potential confounders, the association between acute peak glucose and mortality was no longer significant. The adjusted model was interrogated for the significance of each individual term and APACHE-II was the only variable that indicated an independent association with mortality [OR 1.16, CI 1.13-1.20 ($P<0.001$)] (Supplementary Figure 1).

Patients with ‘insufficiently-controlled’ diabetes were comparable in age, BMI, admission category (medical vs surgical) and severity of illness to those with ‘adequately-controlled’ diabetes (Table 2). Despite significantly elevated peak blood glucose levels in the former group, ICU and hospital mortality as well of length of stay were not different when compared to the ‘adequately-controlled’ patients (Table 2).

DISCUSSION

In this prospective observational study critical illness-associated hyperglycaemia (CIAH) occurred in up to 50% of patients and while diabetes occurred in 27.5% of the cohort, this was apparently unrecognised in only 5.5%. While acute hyperglycaemia was associated with increased mortality in patients without diabetes and in those with ‘adequately-controlled’ diabetes, there was no association in patients with ‘insufficiently-controlled’ diabetes (i.e. chronic pre-morbid hyperglycaemia), even when glucose concentrations exceeded 15 mmol/l. After adjusting for age, BMI, APACHE-II and admission type, the predicted mortality curves were no longer significant for patients with HbA_{1c} < 6% and between 6-7%, probably reflecting the dominant association between APACHE II and mortality. Given observations from prospective multi-centre interventional studies of larger sample populations, marked hyperglycaemia should still be considered as harmful in patients without pre-existing diabetes and ‘stringently-’ or ‘adequately-controlled’ diabetes [16; 18; 19].

Two previous studies have provided data related to the prevalence of unrecognised diabetes in the critically ill. Cely and colleagues enrolled a sample of 75 patients admitted to a single medical ICU and, using a HbA_{1c} ≥ 6.5%, reported that 12% of patients had unrecognised diabetes [3]. Gornik and colleagues performed a 75 g oral glucose tolerance test (OGTT) at 4–6 weeks after discharge in 1105 critically ill patients and reported that unrecognised type 2 diabetes was evident in approximately 15% [20]. In ambulant populations, diabetes can be determined by using elevated fasting plasma glucose, 2-h glucose concentrations during an OGTT, or HbA_{1c} [21]. However use of fasting plasma glucose in the critically ill is impractical and the performance of the OGTT has clear limitations because gastric emptying, which is a major determinant of the glycaemic response [22], is often markedly impaired in this group [23].

Previous epidemiological studies in Australia suggest that 7% of the population aged > 25 years has diabetes with about half of these being unrecognised [24]. It is plausible that unrecognised diabetes predisposes to severe illness and, based on the studies by Cely and Gornik, we anticipated that the prevalence of unrecognised diabetes would be 10-15% [2]. However, we found that the prevalence of unrecognised diabetes (5.5%) was similar to that of the ambulant Australian population [24].

Arguably, the most accurate estimate of the prevalence of CIAH prior to this study was derived from the NICE-SUGAR study - in which approximately 60% of non-diabetic patients had at least one blood glucose measurement >10 mmol/l [16]. Limitations of using NICE-SUGAR data for this purpose are that HbA_{1c} was not measured and the WHO threshold for 'postprandial' hyperglycaemia is slightly greater at 11.1 mmol/l [17]. Accordingly, patients with unrecognised type 2 diabetes were not identified and CIAH may have been over-diagnosed [2]. Our observations are consistent with these perceptions.

While recently there has been a shift in focus to individualizing HbA_{1c} targets for ambulant patients with type 2 diabetes [12], the hypothesis that premorbid glycaemic control may modulate the response to hyperglycaemia during critical illness has not been tested [2; 13]. Previous studies have reported strong associations between hyperglycaemia and mortality in patients admitted with acute myocardial infarction [25] and heterogeneous groups of critically ill patients [5; 7; 10; 11; 26], with the strength of the relationship attenuated, or absent, in those patients with known diabetes [5; 9; 11; 25]. However, a limitation of all these studies is that patients with unrecognised diabetes were not identified and, therefore, were categorised as not having diabetes [1; 2]. Moreover, grouping patients with diabetes as a homogeneous cohort may well be flawed [2], particularly as retrospective data suggest that hyperglycaemia in patients with 'insufficiently-controlled' type 2 diabetes could be less harmful, and potentially may even be protective [27]. In their aggregate, these data provide a strong justification for future prospective, randomised studies to determine whether the optimal acute glucose range in critically ill patients should be individualised based on their premorbid glycaemic status (HbA_{1c}) [2].

Our study has several strengths. We prospectively measured HbA_{1c} levels in a large, heterogeneous cohort of consecutively admitted patients, limiting the likelihood of bias and type 2 error. Furthermore, by contacting the patient's family physician, we confirmed the categorisation of patients as 'unrecognised diabetes'. Based on the median APACHE II score our cohort was representative of a critically ill population and by excluding patients admitted to the cardiac care unit following acute myocardial infarction or cardiac surgery we minimised the bias from these subgroups [28; 29]. There are, however, inherent limitations related to a single centre design with the potential for recruitment bias, based on the prevalence of diabetes in the surrounding community [30]. We did not exclude patients with haemoglobinopathies, iron deficiency anaemia, or those who had received blood transfusions. Although these conditions are known to affect the measured HbA_{1c} [31; 32] and occur in the critically ill, we determined the HbA_{1c} on the first available blood sample to limit the likelihood of erroneous measurements.

A further limitation is that peak blood glucose level in the first 48 hours was the sole metric of glycaemic control. While glycaemic variability, mean blood glucose and hypoglycaemic events all have the capacity to influence outcome [25; 33] blood glucose concentrations within the first 24 hours are predictive of overall glycaemic control throughout ICU admission [34]. Furthermore, peak blood glucose is the trigger for intervention and is, therefore, clinically relevant. The influence of chronic hyperglycaemia on the other domains of glycaemic control during critical illness warrants further investigation.

In addition, we only measured deaths that occurred in hospital, and it is possible that the harm associated with acute glycaemia may become greater following discharge [16]. Based on previous recommendations[27] we categorised pre-morbid glucose control (HbA_{1c}) into three groups. However, the interaction between acute hyperglycaemia and pre-morbid glycaemia may be far more complex with outcomes differing between patients at the extremes within the 'insufficiently-controlled' range, i.e. outcomes in patients with HbA_{1c} of 7.1% and 10% may differ. Larger cohorts are required to further evaluate this relationship, as well as the interactions between diagnostic category, illness severity, catecholamine and steroid use. Finally, while it appears that pre-morbid hyperglycaemia may modulate the association of acute hyperglycaemia with increased mortality, we cannot prove causality and can only speculate on mechanisms. There is biological plausibility that the sudden correction

of chronic hyperglycaemia during acute illness may be harmful [27] and comparisons have been drawn with other fields of medicine in which there is a risk associated with the rapid correction of long-standing, abnormal physiological states (hypoxemia, hyponatremia, hypertension) [7]. A putative mechanism to account for these observations is that chronic hyperglycaemia is associated with adaptive changes at the cellular level resulting in relative neuroglycopenia [35].

In conclusion, acute hyperglycaemia secondary to CIAH or diabetes (both recognised and unrecognised) occurs frequently in the critically ill and appears to have a complex relationship with mortality. In patients with CIAH and ‘adequately-controlled’ diabetes, acute hyperglycaemia is associated with increased mortality, whereas in patients with ‘insufficiently-controlled’ diabetes it is not. These data provide a strong justification for controlled studies targeting the impact of different acute glucose levels according to the degree of premorbid glycaemia.

Take home message

While peak blood glucose concentrations during critical illness are associated with increased mortality in patients with adequate premorbid glycaemic control, this relationship is not evident in patients with premorbid hyperglycaemia, defined as a $HbA_{1c} > 7\%$. These data support undertaking prospective interventional studies in which glucose thresholds in the critically ill are titrated according to premorbid glycaemia.

Tweet

Peak BGL are associated with higher mortality in ICU patients with adequate glycaemic control but not in those with premorbid hyperglycaemia

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Table 1: Patient characteristics and outcomes according to category of glycaemia

	Recognised diabetes	Unrecognised diabetes	Critical illness-associated hyperglycaemia (CIAH)	Normoglycaemic	P value
Number of patients	220 (22%)	55 (5.5%)	498 (49.8%)	227 (22.7%)	-
Age (years) (mean (SE))	64.8 (0.9) §‡	60.9 (2.2) §‡	53.7 (0.8) #¶‡	48.7 (1.2) #¶§	<.001
BMI (kg/m ²) (mean (SE))	30.7 (0.7) §‡	29.4 (1.0) §‡	26.5 (0.2) #¶	26.0 (0.1) #¶	<.001
APACHE-II max during first 24hrs of ICU admission (mean (SE))	20.9 (0.5) §‡	19.7 (1.0) ‡	18.1 (0.3) #‡	14.5 (0.4) #¶	<.001
Medical admission (n(%))	183 (84%)	42(78%)	433(87%)	202 (89%)	0.110
HbA1c (%) (mean (SE))	7.2 (0.1) §‡	7.3 (0.2) §‡	5.5 (0.02) #¶	5.4 (0.03) #¶	<.001
Peak blood glucose (mmol/L) (mean (SE))	13.6 (0.3) §‡	12.3 (0.5) §‡	10.5 (0.1) #¶‡	7.3 (0.1) #¶§	<.001
Catecholamine use (n(%))	103 (47%) ‡	22 (40%) ‡	236 (47%) ‡	49 (22%) #¶§	<.001
Steroid use (n(%))	52 (24%) §‡	10 (18%)	79 (16%) #	22 (10%) #	.002
ICU mortality (n(%))	38 (17%) ‡	13 (24%) ‡	73 (15%) ‡	15 (7%) #¶§	<.001
Hospital mortality (n(%))	41 (19%) ‡	13 (24%) ‡	84 (17%) ‡	17 (7%) #¶§	0.004
ICU length of stay (days) (median (IQR))	3.1 (1.8-5.8) ‡	2.8 (1.6-5.2) ‡	3.0 (1.8-7.9) ‡	2.0 (1.3-4.4) #¶§	<.001
Hospital length of stay (days) (median (IQR))	12.5 (7.3-27.0) ‡	13.3 (6.3-21.2)	13.9 (7.2-31.0) ‡	11.3 (5.6-23.3) # §	0.019

BMI Body Mass Index, APACHE Acute Physiology and Chronic Health Evaluation, HbA1c glycated haemoglobin, ICU Intensive Care Unit
 Medical admission % = percentage of total (medical +surgical)

Significantly different to known diabetes in post hoc tests

¶ Significantly different to unrecognised diabetes in post hoc tests

§ Significantly different to CIAH in post hoc tests

‡ Significantly different to normoglycaemia in post hoc tests

Table 2: Patient characteristics and outcomes according to category of pre-morbid chronic glycaemia (HbA_{1c})

HbA _{1c}	<6%	≤6>7%	≥7%	P value
Number of patients	672	197	129	-
HbA _{1c} (%) (mean (SE))	5.4 (0.01)	6.3 (0.02)	8.6 (0.14)	-
Known Diabetes	55	57	108	-
Age (years) (mean (SE))	51.7 (0.7) ¶§	63.4 (1.0) #	62.6 (1.3) #	<0.001
BMI (kg/m ²) (mean (SE))	26.3 (0.2) ¶§	29.0 (0.5) #	30.9 (1.0) #	<0.001
APACHE-II max during first 24hrs of ICU admission (mean (SE))	17.0 (0.3) ¶§	20.1 (0.5) #	19.5 (0.7) #	<0.001
Medical admission (n(%))	583 (87%)	174 (89%)	103 (80%)	0.056
Peak blood glucose (mmol/L) (mean (SE))	9.4 (0.1) ¶§	11.5 (0.3) #§	15.1 (0.4) #¶	<0.001
Catecholamine use (n(%))	259 (39%)	94 (48%)	57 (44%)	0.053
Steroid use (n(%))	95 (14%)§	38 (19%)	29 (23%)#	0.027
ICU mortality (n(%))	77 (12%)¶	39 (20%)#	23 (18%)	0.005
Hospital mortality (n(%))	87 (13%)¶	42 (21%)#	26 (20%)	0.005
ICU length of stay (days) (median (IQR))	2.8 (1.6 – 6.2)	2.9 (1.8 – 6.1)	2.9 (1.7 – 6.9)	0.809
Hospital length of stay (days) (median (IQR))	15.0 (7.6 – 29.6)	13.3 (8.2 – 29.8)	13.5 (8.6 – 24.6)	0.748

BMI Body Mass Index, APACHE Acute Physiology and Chronic Health Evaluation, HbA_{1c} glycated haemoglobin, ICU Intensive Care Unit

Medical admission % = percentage of total (medical +surgical)

Significantly different to HbA_{1c}<6% in post hoc tests

¶ Significantly different to HbA_{1c} 6-7% in post hoc tests

§ Significantly different to HbA_{1c}>7% in post hoc tests

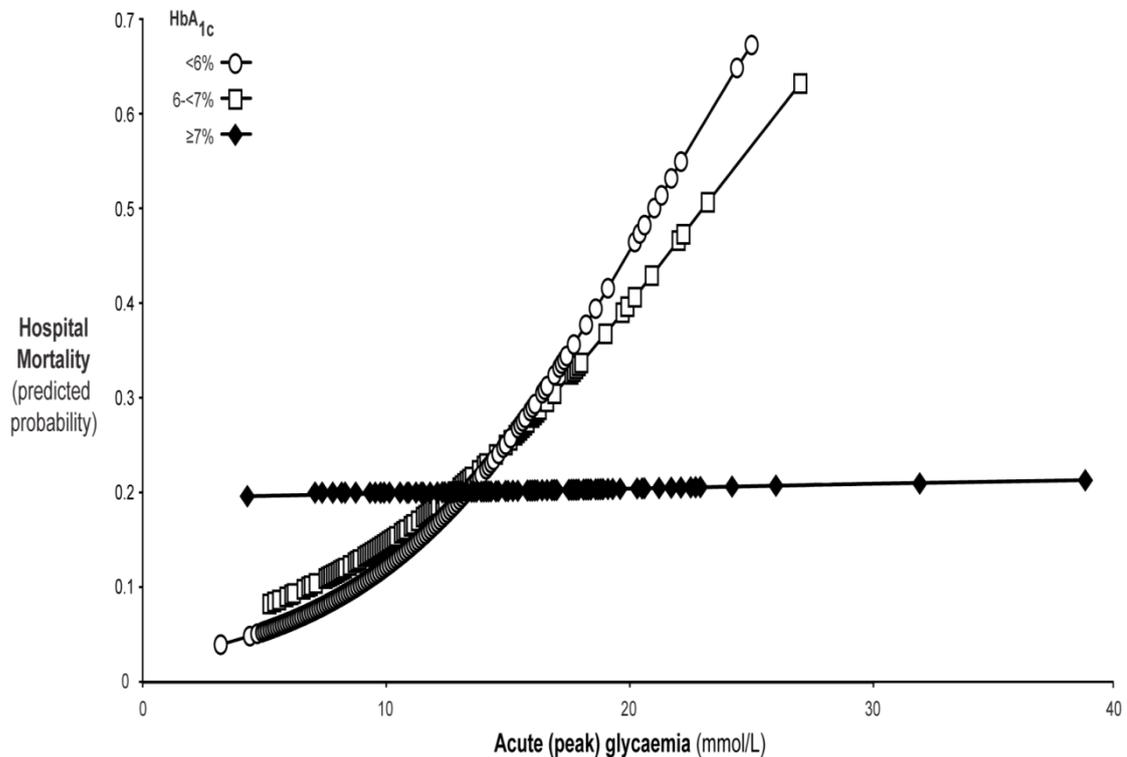
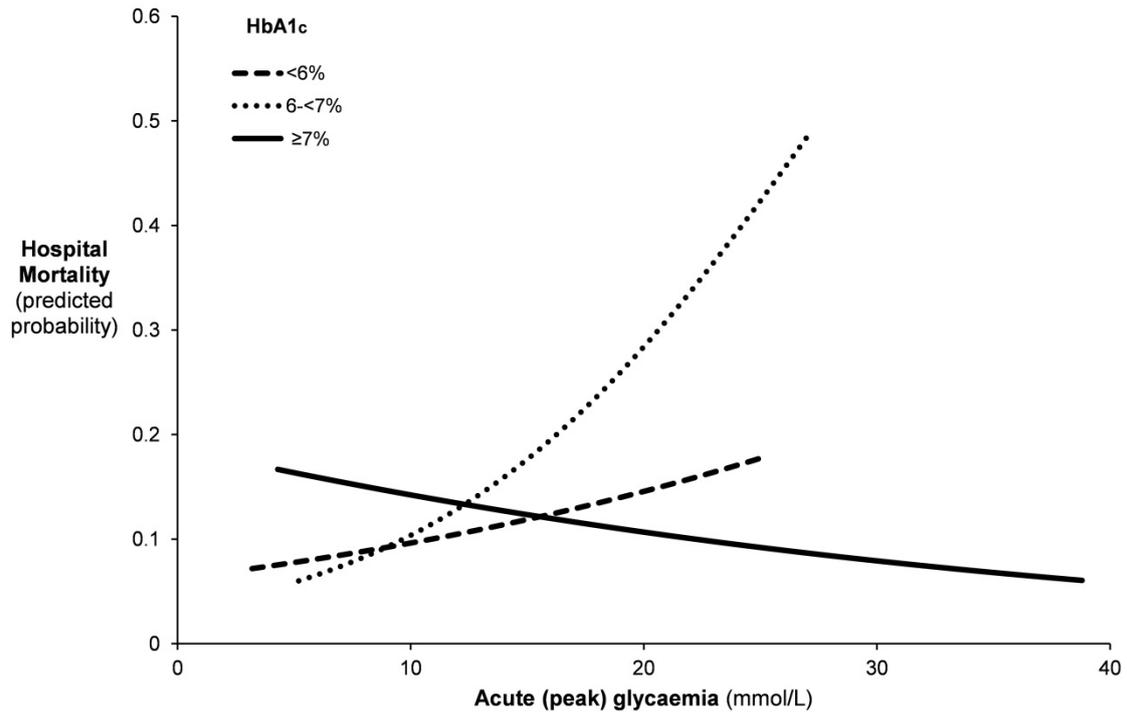


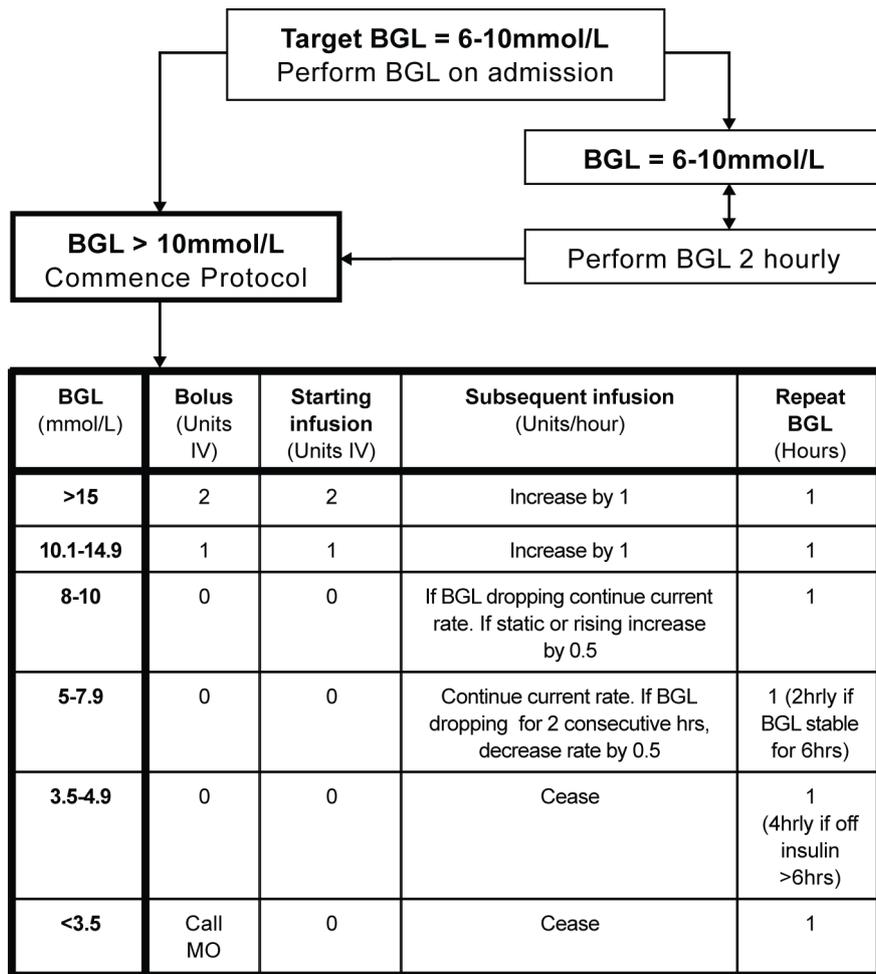
Fig 1. Hospital mortality versus acute glycaemia when categorised according to pre-morbid glycaemia (HbA_{1c})

In patients without diabetes, and those with ‘stringently-controlled’ [open circles, HbA_{1c} < 6% (42 mmol/mol), n = 672, odds ratio=1.20 (95% CI 1.12, 1.28); P < 0.001] and ‘adequately-controlled’ diabetes [open squares, 6 ≤ HbA_{1c} < 7% (53 mmol/mol), n = 199, odds ratio=1.14 (95%CI 1.05, 1.25); P=0.003] increasing peak blood glucose concentrations were associated with increasing mortality. However there was no association apparent in patients with ‘insufficiently-controlled’ diabetes [filled diamonds, HbA_{1c} ≥ 7%, n = 129, odds ratio = 1.0 (95% CI 0.92, 1.1); P = 0.95]. The model was an adequate fit (Hosmer and Lemeshow Goodness of Fit test).



Supplementary Fig 1. Hospital mortality versus acute glycaemia when categorised according to premorbid glycaemia (HbA_{1c}) adjusted for age, BMI, APACHE II and admission type (medical/surgical)

After adjustment the interaction term Peak BGL*HbA_{1c} interaction was no longer significant (P=0.13). The model was an adequate fit (Hosmer and Lemeshow Goodness of Fit test). Slope estimates for each category were HbA_{1c} < 6% (42 mmol/mol), odds ratio=1.05 (95% CI 0.97, 1.13); P=0.22, HbA_{1c} 6 ≤ 7% (53 mmol/mol), odds ratio=1.13 (95% CI 1.02, 1.25); P=0.016 and HbA_{1c} >7%, odds ratio=0.97 (95%CI 0.86, 1.09) P=0.575.



Supplementary Fig 2. Insulin and blood glucose protocol during study period

1.4 Manuscript: Stress induced hyperglycaemia and the subsequent risk of type 2 diabetes in survivors of critical illness

Title

Stress induced hyperglycaemia and the subsequent risk of type 2 diabetes in survivors of critical illness

Running title

Stress induced hyperglycaemia in ICU is associated with double the risk of diabetes

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Conflicts of interest

The authors report no potential conflicts of interest relevant to this paper.

Author Contribution

M.P.P. was responsible for study concept and design, securing funding, interpretation of the data, drafting the manuscript and approving the final version to be published.

M.E.F. was responsible for study concept and design, statistical analysis, interpretation of the data and drafting the manuscript and approving the final version to be published.

L.K.P. was responsible for securing funding, interpretation of data, revision of the manuscript for important intellectual content and approving the final version to be published.

P.K., S.B., V.B., and S.M. were responsible for acquisition of data, revision of the manuscript for important intellectual content and approving the final version to be published.

M.H., and J.E.S. were responsible for study concept and design, revision of the manuscript for important intellectual content and approving the final version to be published.

A.M.D, was responsible for study concept and design, securing funding, interpretation of the data, drafting the manuscript and approving the final version to be published.

M.P.P., M.E.F, and A.M.D. are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of these data and the accuracy of the analysis.

Key words

Stress induced hyperglycaemia, type 2 diabetes, critical illness

ABSTRACT

Word count: 218

Purpose

This retrospective cohort study evaluates associations between stress induced hyperglycaemia, the development of diabetes and mortality in survivors of critical illness.

Methods

All adult patients admitted to a tertiary intensive care unit (ICU) in South Australia between 2004 and 2011 were included. Stress induced hyperglycaemia was defined as blood glucose ≥ 11.1 mmol/l within the first 24 hours of ICU admission. Prevalent diabetes was identified through ICD-10 coding and/or prior registration with the Australian National Diabetes Service Scheme (NDSS). Incident diabetes was identified as NDSS registration > 30 days after hospital discharge until July 2015. The predicted risk of developing diabetes was described as sub-hazard ratios using competing risk regression. Survival was assessed using Cox proportional hazards regression.

Results

Stress induced hyperglycaemia was identified in 2,883 (17%) of 17,074 patients without diabetes who survived their hospital admission. The overall incidence of subsequent type 2 diabetes following critical illness was 4.8% (821 of 17,074). The risk of diabetes in patients with stress induced hyperglycaemia was approximately double that of those without (HR 1.91 (95% CI 1.62, 2.26), $p < 0.001$) and was sustained regardless of age group or severity of illness. Stress induced hyperglycaemia was not associated with increased mortality.

Conclusions

Stress induced hyperglycaemia within 24 hours of admission to the ICU identifies patients at greater risk of subsequent diabetes.

INTRODUCTION

Stress induced hyperglycaemia occurs in critically ill patients in whom glucose tolerance was previously normal, with hyperglycaemia resolving following resolution of the acute illness [1]. This acute derangement in glucose metabolism occurs frequently, with up to 50% of non-diabetic critically ill patients developing hyperglycaemia within 48 hours of admission to the intensive care unit (ICU) [2]. Stress induced hyperglycaemia is known to be a marker of illness severity, with the magnitude of hyperglycaemia strongly associated with short-term mortality, particularly in patients without a history of diabetes mellitus [2; 3].

The pathophysiology of stress induced hyperglycaemia is thought to reflect temporary insulin resistance coupled with a relative impairment in insulin secretion, in that plasma insulin concentrations are inadequate to compensate for hyperglycaemia [1]. Whether the episode of critical illness unmasks latent insulin resistance and/or impaired β -cell function has not been adequately explored. The identification of long-term metabolic derangements that are amenable to intervention is important, particularly because outcomes for those critically ill patients who survive hospital discharge remain poor, with up to 40% of patients dying within the subsequent five years [4].

The concept that transient hyperglycaemia during critical illness identifies patients at increased risk for developing type 2 diabetes is intuitively plausible. For example, there are similarities between critical illness and gestational diabetes where an acute period of glucose intolerance initially normalises following resolution of the physiological challenge [5; 6]. While gestational diabetes was once considered a temporary disorder, restricted to the period of pregnancy, it is now recognised that affected women are at high risk of developing type 2 diabetes [7; 8]. Moreover, screening programs are advocated to detect early impairment in glucose tolerance

because intervention strategies in young, high-risk, populations have been shown to reduce the progression to diabetes [8; 9].

The primary aim of this study was to evaluate the association between peak blood glucose in the first 24 hours following admission to ICU and the subsequent risk of developing type 2 diabetes in survivors of critical illness.

PATIENTS AND METHODS

The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital with the need for informed consent waived. Access to data for the purpose of performing this research was approved by the National Diabetes Service Scheme, maintained by Diabetes Australia, and by the South Australian Department of Health with third-party data matching approved by the Australian Institute of Health and Welfare (AIHW) EO215/1/146.

Patients

The study was a retrospective, multi-centre observational study across all public hospital ICUs in South Australia. Public intensive care services in South Australia (population 1.7 million) are exclusively provided by four tertiary hospitals (Flinders Medical Centre, Lyell McEwin Hospital, Queen Elizabeth Hospital and Royal Adelaide Hospital). Patient demographic, hospital episode and intensive care admission data were extracted from each contributing ICU from January 1 2004 to December 31 2011 inclusive. At each unit these data were collected prospectively prior to submission to the central de-identified Australia and New Zealand Intensive Care Society (ANZICS) Adult Patient Database [10]. The ANZICS Adult Patient Database captures clinical, physiologic, and laboratory data for the initial 24 hours of ICU admission along with outcome data for all patients admitted to contributing ICUs across Australia and New Zealand [11]. These data were then linked to population based datasets to match (i) International Classification of Diseases (ICD-10) coding of diabetes through the South Australian Department of Health Integrated South Australian Activity Collection dataset [12], (ii) mortality through the Australian National Death Index and (iii) registration with the Australian National Diabetes Service Scheme (NDSS) with a diagnosis of diabetes. The National Diabetes Service

Scheme is a database of Australian people diagnosed with type 1 and type 2 diabetes. As of September 2015, there were over one million people registered with type 2 diabetes [13].

The composite ICU data file was initially matched against the Integrated South Australian Activity Collection dataset to generate a “known diabetes” flag for each hospital separation. Socio-economic status was estimated from the separation postcode, using the Australian Bureau of Statistics Index of Relative Socio-Economic Advantage and Disadvantage score [14]. The patient identifier fields from this composite dataset were then forwarded to the Australian Institute of Health and Welfare who performed data linkage against the NDSS dataset and the National Death Index.

Patients over the age of 18 years who were discharged from hospital were assigned to one of three groups; (1) stress induced hyperglycaemia (SIH), where there was no known history of diabetes and at least one blood glucose level was ≥ 11.1 mmol/l [2], (2) normoglycaemia, where there was no known history of diabetes and all blood glucose levels were < 11.1 mmol/l, and (3) diabetes mellitus, where ICD-10 codes from the diabetes chapter were present in the current or any prior hospital separation, or the patient was registered with the NDSS as having diabetes prior to, or within 30 days of hospital separation. Patients in whom peak blood glucose was > 20 mmol/l were assumed to have undiagnosed diabetes even in the absence of registration [15] and were excluded from matching analysis. For patients with multiple hospital episodes during the study period only the index group admission was used.

Registration with the NDSS was used as a surrogate measure for the onset of type 2 diabetes, with time to registration from 30 days post hospital discharge, as per McAllister and colleagues [15], forming the primary study outcome. Secondary outcomes included the assessment of covariates potentially influencing the time to NDSS registration and description of the survival patterns between the normoglycaemic and stress induced hyperglycaemic cohorts.

Statistical analysis

Data are presented as frequencies and proportions for categorical variables and mean (standard deviation) or median [interquartile range] for continuous variables. For

NDSS registration, time to event analysis was described as sub-hazard ratios using competing risk regression, based upon the approach of Fine and Gray [16]. This was planned *a priori*, as death could not be considered a ‘non-informative’ censoring event in this clinical setting. A sensitivity analysis was performed using Cox proportional hazards regression, treating death as a censoring event. Patient survival was assessed using Cox proportional hazards regression. For competing risks and Cox proportional hazards models, between group effects are presented as sub-hazard ratio, SHR (95% CI) and hazard ratio, HR (95% CI) respectively. Between group comparisons were considered statistically significant at $p < 0.05$. Inclusion of covariates in the multivariate models was set at $p < 0.1$. All analyses were performed using StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp.

RESULTS

Baseline characteristics

A total of 31,007 patient separations were recorded across the four ICUs during the eight year capture period. Of these, 8,534 were excluded due to either missing data, non-index admission status or failure to meet the inclusion criteria, including 216 patients with a peak blood glucose > 20 mmol/l who, as indicated, were presumed to have undiagnosed diabetes, resulting in 22,473 index patient separations. There were 5,399 (24%) patients who had diabetes, 2,883 (13%) fulfilled the criteria for stress-induced hyperglycaemia and 14,191 (63%) were normoglycaemic (Figure 1); leaving 17,074 patients without recognised diabetes for subsequent analysis. Demographic data are presented in Table 1. Patients meeting the inclusion criteria had a mean age of 59 (19) years, a median APACHE III score of 56 [41- 74] and 59% were male. The four participating ICUs are all mixed medical/surgical units, with 61.3% of admissions categorised as medical and 38.7% surgical. Patients were followed-up for a maximum of 8 years post-discharge, with a median follow-up of 5.3 [3.6, 7.5] years.

NDSS capture rate of patients with known diabetes

Of the 5,399 patients with recognised diabetes according to the pre-stated criteria, 4,176 were diagnosed based on ICD-10 coding within the SA Health dataset.

Following matching with the NDSS, 3,363 of these patients were registered with the National Diabetes Service Scheme, reflecting a capture rate of 80.5%.

Risk of developing diabetes post ICU discharge

Within the follow-up period, 4.8% (821 of 17,074) of patients registered with the NDSS with a diagnosis of type 2 diabetes. The risk by category is presented in Table 2. Under a competing risks model, stress-induced hyperglycaemia increased the risk of developing diabetes with a relative sub-hazard ratio 1.88 (1.61, 2.20), $p < 0.001$ (Figure 2). In the multivariate model the covariates age, age-squared, severity of illness (APACHE III), hospital, socioeconomic status, acute renal failure, medical diagnosis and trauma were significant. After adjustment stress-induced hyperglycaemia remained an independent risk factor for developing diabetes, with an adjusted sub-hazard ratio of 1.91 (1.62, 2.26), $p < 0.001$.

Effect of age on risk of developing type 2 diabetes

The association of age with the risk of developing type 2 diabetes was non-linear, which required the inclusion of the age-squared term in the multivariate model. Subdividing age by approximate deciles, in each age-group the SIH cohort exhibited approximately two-fold the hazard rate of normoglycaemic patients (Figure 3). The risk of diabetes increased progressively with age to a peak in the 50-59 age group, with a SIH sub-hazard ratio 7.90 (5.38, 11.60), $p < 0.001$, and risk decreasing steadily thereafter (Figure 3).

Mortality

Within the follow-up period the mortality rate for survivors of critical illness who did not have diabetes on their index admission was 34% (5,843 of 17,074). Unadjusted proportional hazards regression suggested an increased risk of death associated with stress induced hyperglycaemia (Figure 4); however, this signal was no longer present after adjusting for age and severity of illness (Table 2).

DISCUSSION

The key finding of this study is that stress induced hyperglycaemia appears to approximately double the risk of developing type 2 diabetes in survivors of critical illness. This is the largest study which has assessed the long term risk of diabetes in survivors of critical illness. These results are biologically plausible and consistent with epidemiological studies reporting an association between hyperglycaemia during hospitalisation and subsequent diabetes [15; 17; 18; 19]. The most externally valid of these, a retrospective cohort study of over 86,000 admissions to Scottish emergency departments, reported that patients who are hyperglycaemic (blood glucose > 11 mmol/l) had a three-year risk of developing diabetes of 10%, compared to 2.3% in all patients admitted to the emergency department [15]. In this study, a subgroup analysis performed on 1,853 patients admitted to an ICU indicated that 37 patients (2.0%) developed diabetes after discharge and the authors suggested that the association between hyperglycaemia during hospital admission and subsequent diabetes was weaker in those admitted to ICU than in the general emergency department population, possibly reflecting the substantially greater physiological insult precipitating hyperglycaemia in the critically ill [15].

There have only been two other studies which have evaluated the relationship between stress induced hyperglycaemia and the development of type 2 diabetes in survivors of critical illness [20; 21]. In a prospective observational study from a single-centre medical ICU, Gornik and colleagues performed annual oral glucose tolerance tests for a minimum of 5 years in 582 survivors of critical illness, stratifying patients into normoglycaemia (n = 389) and stress induced hyperglycaemia (n = 193) based on peak inpatient blood glucose [21]. Patients with stress induced hyperglycaemia (defined as a peak blood glucose > 7.8 mmol/l) had a five-fold increased risk of developing type 2 diabetes [21]. The external validity of these results is diminished by the lack of severity of illness data and the relatively high proportion of patients receiving total parenteral nutrition (32%) [21]. Moreover, given the lower glucose threshold used to define stress induced hyperglycaemia and the high proportion of patients who received total parenteral nutrition it is surprising that the incidence of 'hyperglycaemia' was only 40%, suggesting that perhaps the acuity of these patients was less than critically ill populations in other units. In a similar study design, in a single mixed medical-surgical ICU, Van Ackerbock and colleagues performed an oral glucose tolerance test 8 months post ICU discharge in 338 survivors of critical illness

[20]. Hyperglycaemia was again defined as a blood glucose > 7.8 mmol/l and, while at 8 months there was a greater proportion of hyperglycaemic patients that had developed type 2 diabetes (9 vs 4%), this did not achieve statistical significance, probably reflecting the short time period for diagnosis of incident diabetes and the relatively small sample size. Nevertheless, taken together these prospective studies support the concept that stress induced hyperglycaemia heralds the future onset of diabetes.

For the current study, the definition of stress induced hyperglycaemia was based on the American Diabetes Association (ADA) Diabetes in Hospitals Writing Committee Guidelines, i.e. random blood glucose ≥ 11.1 mmol/l, given that fasting status was unknown [22]. In the study by McAllister and colleagues, this threshold was observed to correlate with a five-fold increased risk of developing diabetes in a non-critically ill hospitalised population and it was suggested as the cut-off at which screening programs may be beneficial [15]. A particular strength of the current multi-centre study was the large sample size, the state-wide catchment area and the collection of multiple covariates, allowing assessment for potential confounders and hence, a less biased estimate of the risk effect. As such this allowed an evaluation as to whether the risk of developing diabetes varied according to age; the greatest risk being demonstrated in patients aged 50-59 with stress induced hyperglycaemia with over a seven-fold increased risk compared to patients aged 18-30 who did not develop stress induced hyperglycaemia. This is likely to be important as, for a diabetes screening program to be effective, early detection should yield benefits when compared to a delay in diagnosis and it is younger populations that have the greatest capacity to benefit [23]. Accordingly, these data provide a persuasive rationale for an evaluation of the efficacy of screening programs in this relatively young population of ICU survivors.

Adjusting for the major covariates of age and severity of illness there did not appear to be an increased risk of mortality with stress induced hyperglycaemia, suggesting that stress induced hyperglycaemia is simply a marker of illness severity in ICU survivors. This observation is in contradistinction to the association between hyperglycaemia and ICU mortality in non-diabetic critically ill patients [2; 3].

This study has several important limitations. Registration with the National Diabetes Service Scheme (NDSS) was used as a surrogate marker for incident diabetes. By including patients with 'known diabetes' from hospital ICD-10 coding in the NDSS matching we were able to provide direct validation and a capture rate of 80.5% for this cohort was obtained. While this suggests the true incidence of diabetes is almost certainly higher than reported in this study, we see no reason to expect different rates of NDSS registration for those patients developing diabetes within each group. Therefore, the estimated hazard ratios should be unbiased and this supports the use of NDSS registration as a valid surrogate for diabetes risk.

Peak blood glucose in the first 24 hours was utilised as the sole metric for classifying stress induced hyperglycaemia, missing those patients in whom hyperglycaemia was delayed. While the true hazard rate for subsequent diabetes may be greater than reported it is reassuring that blood glucose concentrations within the first 24 hours have been shown to be predictive of overall glycaemic control throughout ICU admission [24]. A further limitation of only having peak blood glucose concentrations within the first 24 hours is the inability to quantify the risk of sustained stress induced hyperglycaemia. Missing blood glucose or demographic data resulted in approximately 10% of patient separations being excluded from potential matching in this study. However, this exclusion rate is comparable to previous cohort studies using blood glucose data from the ANZICS Adult Patient Database and most frequently occurs with brief ICU admissions where no blood tests are recorded [25]. There was also no data available on body mass index (BMI) or other known risk factors for the development of diabetes such as smoking history, alcohol consumption or family history of impaired glucose tolerance. Whilst a previous study in a similar cohort demonstrated no significant difference in BMI between patients with and without stress induced hyperglycaemia [2], as this study was observational in nature unmeasured risk factors may have confounded the observations.

As glycated haemoglobin (HbA1c) is not routinely measured on ICU admission, the proportion of patients classified as having stress induced hyperglycaemia who actually had unrecognised type 2 diabetes is unknown. To limit this as a confounder, those patients with a blood glucose > 20 mmol/l or who registered as developing type 2 diabetes within 30 days of hospital discharge were considered to have had diabetes during their ICU admission and were excluded from analysis. In a recent study from

one of the four participating ICUs, glycated haemoglobin was collected on 1,000 consecutively admitted, mechanically ventilated patients with 5.5% being subsequently diagnosed with previously unrecognised diabetes (i.e. HbA1c \geq 6.5%) [2]. While these data suggest that the rate of undiagnosed type 2 diabetes appears to be relatively infrequent in South Australia, the potential that undiagnosed diabetes may have biased our hazard estimates cannot be excluded.

Clinical implications

This study indicates that stress induced hyperglycaemia substantially increases the risk of diabetes in survivors of critical illness. There are two major benefits in identifying individuals at risk of developing diabetes. Firstly, as type 2 diabetes may be asymptomatic for many years, a significant proportion of patients have established diabetic complications at the time of diagnosis [26] – and an early diagnosis of type 2 diabetes can facilitate earlier treatment and therefore reduce complication rates [27]. Secondly, identifying patients at risk of developing type 2 diabetes provides the opportunity for primary prevention through targeted programmes [28; 29]. For these reasons, current guidelines in many regions, including Europe, recommend screening high-risk individuals [30]. Studies are now warranted to determine whether hyperglycaemic survivors of critical illness would benefit from diabetes screening programs.

CONCLUSIONS

Acute hyperglycaemia during critical illness identifies patients at substantially greater risk of developing diabetes following hospital discharge. The risk of developing diabetes appears to be greatest in middle-aged patients, which may have implications for screening of this population.

Take home message

Stress induced hyperglycaemia is associated with almost double the risk of developing diabetes in survivors of critical illness. The risk of developing diabetes is highest in middle-aged patients and further studies are now warranted to assess the benefit of screening programs in this population.

Tweet

Stress induced hyperglycaemia doubles the risk of diabetes.

Acknowledgments

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Table 1. Demographic data by study group.

	Normoglycaemia	Stress-Induced Hyperglycaemia	Total
Separations, n (% total)	14,191 (63)	2,883 (13)	17,074
Male, n (% group)	8,522 (60)	1,635 (57)	10,157 (59)
Site, n (% group)			
A	5,009 (35)	961 (33)	5,970 (35)
B	5,180 (37)	996 (35)	6,176 (36)
C	2,245 (16)	576 (20)	2,821 (17)
D	1,757 (12)	350 (12)	2,107 (12)
ATSI, n (% group)	443 (3.1)	72 (2.5)	515 (3.0)
Age, mean (SD)	56.7 (20.0)	61.8 (17.6)	57.4 (19.7)
APACHE III, med (IQR)	52 (36, 69)	67 (50, 87)	54 (38, 72)
Length of Stay, med (IQR)			
ICU	1.9 (1.0, 3.9)	2.7 (1.4, 5.6)	2.0 (1.0, 4.1)
Hospital	11.1 (5.9, 21.1)	13.0 (7.2, 24.6)	11.4 (6.1, 21.7)
Acute Renal Failure, n (% grp)	308 (2.2)	111 (3.9)	419 (2.5)
Peak BG, med (IQR)	7.8 (6.6, 9.0)	12.7 (11.7, 14.4)	8.3 (6.9, 10)
Medical, n (% group)	8,463 (59.6)	1,910 (66.3)	10,373 (60.8)
Surgical, n (% group)	5,728 (40.4)	973 (33.8)	6,701 (39.3)
Trauma, n(% group)	1,552 (10.94)	165 (5.72)	1,717 (10.6)

ATSI Aboriginal and Torres Strait Islander peoples, APACHE Acute Physiology and Chronic Health Evaluation, BG Blood Glucose.

Table 2. Outcome Measures by group within 8 years of hospital discharge.

	Normoglycaemia
Separations, n (% total)	14,191 (83)
Subsequent NDSS Registration	
n (% Group)	603 (4.25)
Sub-Hazard Ratio (95%CI) ¹	1.0
Sub-Hazard Ratio (95%CI) ²	1.0
Death (National Death Index)	
n (% Group)	4,676 (33.0)
Hazard Ratio (95%CI) ³	1.0
Hazard Ratio (95%CI) ⁴	1.0

1. Univariate model, competing risks regression.

2. Multivariate model, competing risks regression, controlling for age, age-squared and APACHE III score.

3. Univariate model, Cox proportional hazards regression.

4. Multivariate model, Cox proportional hazards regression, controlling for age and APACHEIII score.

CI confidence interval, NDSS National Diabetes Service Scheme

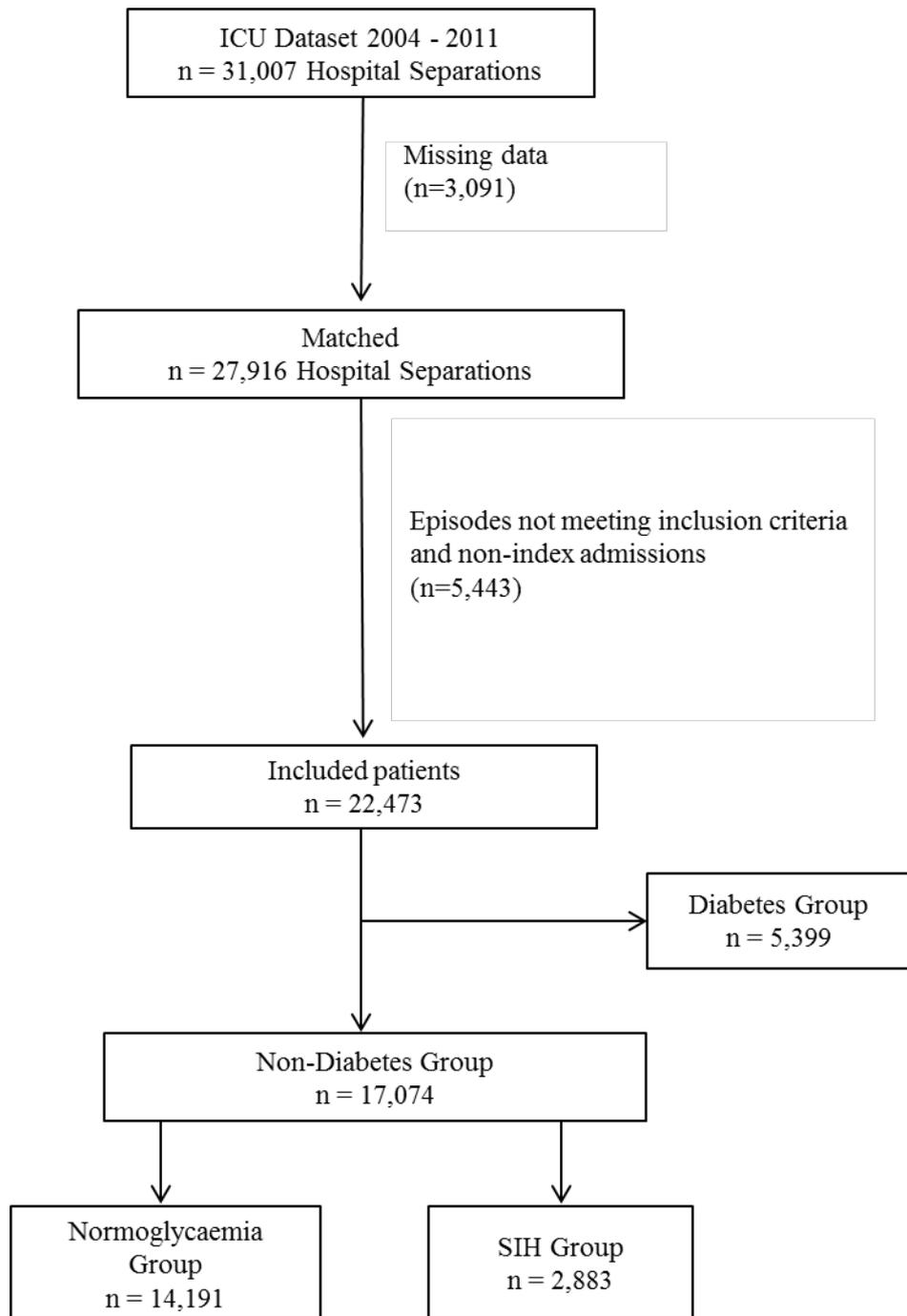
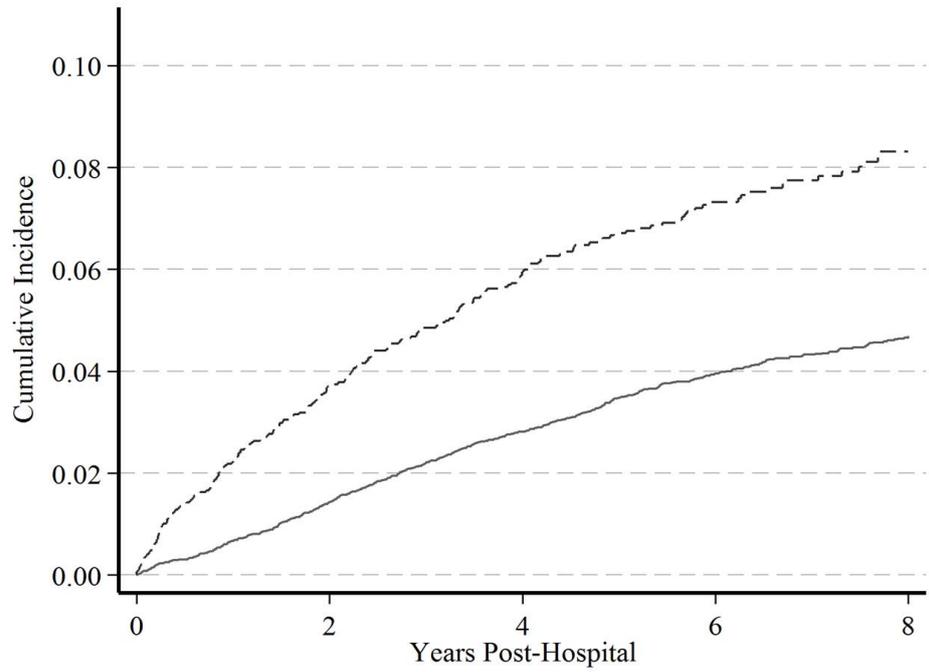


Fig 1. CONSORT style flowchart of patients included in analysis
 SIH, stress induced hyperglycaemia



Years Post-Hospital	Normal	Stress-Hyperglycaemia
0	14191	2883
2	11865	2249
4	10046	1740
6	6244	919
8	3028	433

Fig 2. Cumulative incidence for type 2 diabetes for normoglycaemia (solid line) versus stress induced hyperglycaemia (dashed line)

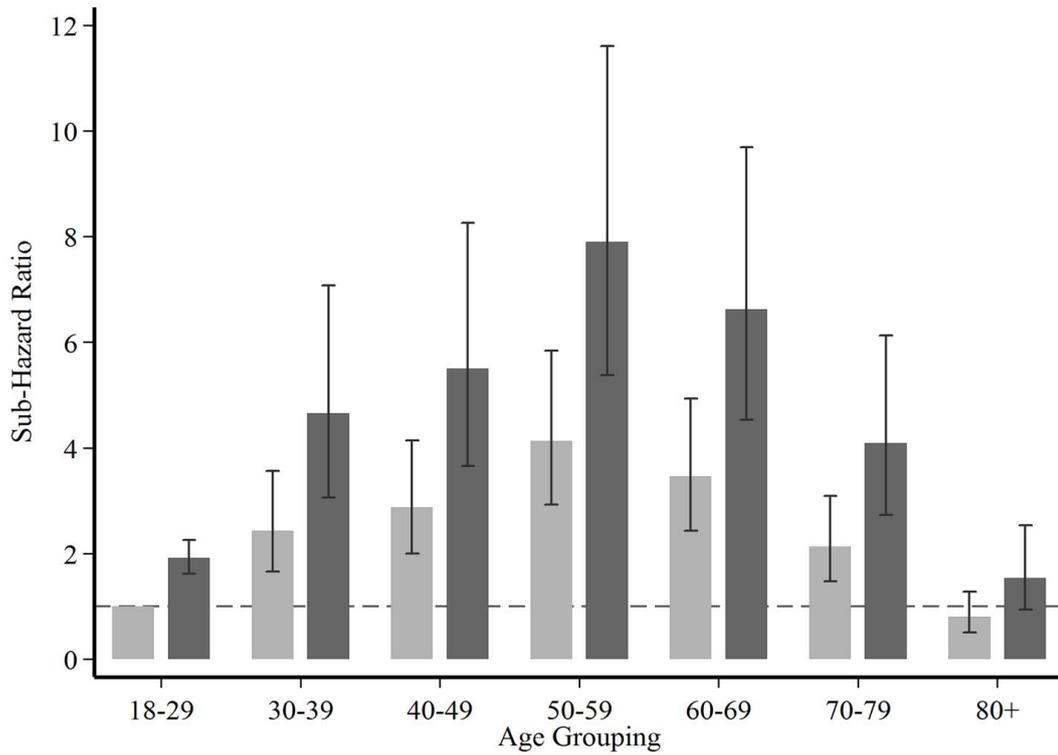


Fig 3. Sub-hazard ratios for the risk of type 2 diabetes by glycaemic category and age group

Normoglycaemia = light grey bars; stress induced hyperglycaemia = dark grey bars. Age is grouped approximately into deciles with normoglycaemia and age 18-29 years as the base reference; Data are sub-hazard ratios \pm 95% confidence intervals.

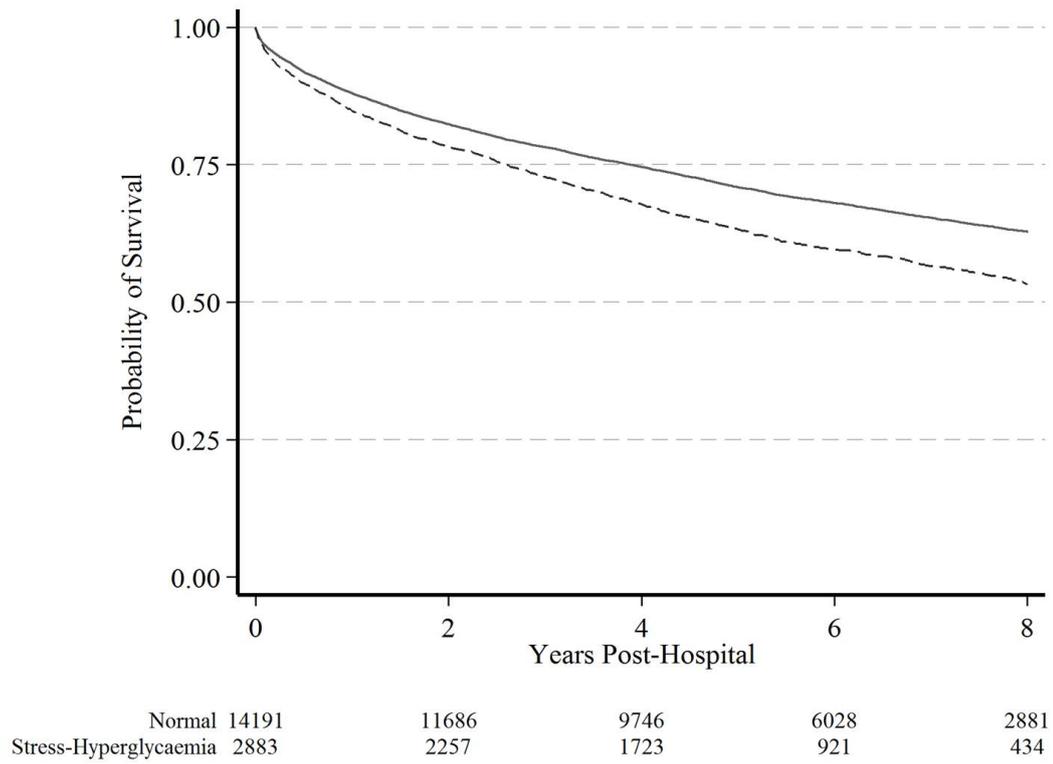


Fig 4. Kaplan-Meier survival curves for normoglycaemia (dashed line) and stress induced hyperglycaemia (solid line) from hospital discharge to 8 years

1.5 CONCLUSIONS

1.5.1 Introduction

The objectives of the studies that comprise this chapter were to (i) determine the prevalence of stress induced hyperglycaemia, recognised and unrecognised diabetes in the critically ill (ii) prospectively evaluate the impact of premorbid glycaemic control on the association between stress induced hyperglycaemia and mortality and (iii) evaluate the association between stress induced hyperglycaemia and subsequent type 2 diabetes in survivors of critical illness.

1.5.2 *Contribution of the work described in this thesis to the understanding of the prevalence of stress induced hyperglycaemia and unrecognised diabetes*

By measuring glycated haemoglobin (HbA1c) on admission and recording peak blood glucose levels in the first 48 hours of admission, the study reported in Chapter 1.3 is the first to quantify unrecognised diabetes and stress induced hyperglycaemia in a critically ill population accurately. This study established that in a metropolitan Australian Intensive Care Unit, stress induced hyperglycaemia occurred in approximately 50% of critically ill patients, 22% had recognised diabetes and 5.5% unrecognised diabetes. Subsequent to the publication of these data, these outcomes have been replicated in a larger population of critically ill patients admitted to nine intensive care units in Atlanta, USA [1]. In this study, HbA1c was measured in 15,735 patients on admission to the intensive care unit and using the same diagnostic criteria the incidence of recognised and unrecognised diabetes was reported as 26.5% and 9.3%, respectively. The higher incidence within the Atlanta population is not surprising given the increased prevalence of type 2 diabetes in the United States and differences in ethnicity between the cohorts. Caucasian patients, with a lower risk of type 2 diabetes, made up 88% of patients in the Australian study but only 47% of those in the United States study. Taken together these data establish that unrecognised diabetes occurs frequently in the critically ill and indicate that the prevalence is influenced by the rate of underlying diabetes within the catchment population.

1.5.3 *Contribution of the work described in this thesis to the understanding of the influence of chronic hyperglycaemia on the association between acute hyperglycaemia and mortality*

The study presented in chapter 1.3 was the first to stratify patients with type 2 diabetes according to pre-morbid glycaemic control and then prospectively interrogate the relationship between acute hyperglycaemia and mortality in the critically ill. While acute hyperglycaemia was associated with increased mortality in patients without diabetes and in those with adequately-controlled diabetes, there was no association in patients with insufficiently-controlled diabetes and chronic pre-morbid hyperglycaemia. Since the publication of these data, a prospective observational study from a medical ICU in Yale-New Haven, USA has yielded similar findings [2]. HbA1c was measured on 299 patients who became hyperglycaemic following admission to the ICU, with glycaemic control stratified according to an HbA1c \geq 6.5% or $<$ 6.5%. In this hyperglycaemic, critically ill population, HbA1c $<$ 6% was associated with increased hospital mortality compared to the chronically hyperglycaemic cohort (HbA1c \geq 6.5%), odds ratio 1.92, [95% CI, 1.3 – 2.85] P = 0.001. Accordingly, the outcomes of these two prospective observational studies are consistent with retrospective studies and indicate that chronic hyperglycaemia may confer a protective effect against acute hyperglycaemia during critical illness [3].

1.5.4 Contribution of the work described in this thesis to the understanding of the relationship between stress hyperglycaemia and subsequent type 2 diabetes in survivors of critical illness

The study presented in chapter 1.4 is the only large cross-sectional observational study that has precisely evaluated the association between stress induced hyperglycaemia and the subsequent development of type 2 diabetes. Utilising a multi-centre retrospective, observational, cohort study design, a two-fold increased risk of type 2 diabetes was observed in patients with stress hyperglycaemia. There was a non-linear association between the risk of diabetes and age such that middle-aged patients with stress induced hyperglycaemia were at over a seven-fold increased risk compared to adult patients less than age 30 who were not hyperglycaemic on admission to the ICU.

1.6 FUTURE DIRECTIONS

1.6.1 Variable blood glucose targets during critical illness based on pre-morbid glycaemic control

The findings reported in chapter 1.3 are consistent with the concept that acute hyperglycaemia during critical illness is well tolerated in patients with chronic premorbid hyperglycaemia and the outcomes from retrospective, observational studies suggest that higher blood glucose concentrations during critical illness may even be protective [2-5]. Taken together, there is clearly a pressing need to challenge the current practice of universal glycaemic targets in the critically ill which dictates the performance of a prospective interventional trial of variable blood glucose targets according to premorbid glycaemic control.

1.6.2 Mechanisms influencing the interaction between acute hyperglycaemia and outcome in critically ill patients with chronic premorbid hyperglycaemia

The mechanism(s) underlying the apparent protective effect of chronic hyperglycaemia on the interaction between acute hyperglycaemia during critical illness and mortality are poorly understood, however, it has been suggested that adaptive changes at the neuronal level are important [6]. In health the brain utilises glucose as an energy substrate, independent of insulin, such that even supraphysiological concentrations of insulin do not affect cerebral glucose metabolism [7,8]. There is evidence within the ambulant diabetic population that cerebral glucose metabolism is altered by pre-existing hyperglycaemia with studies demonstrating a reduction in basal cerebral glucose metabolism in type 1 diabetes [9] and insulin responsive cerebral glucose metabolism in patients with type 1 diabetes and impaired glucose tolerance [8,9].

Cerebral microdialysis is an established technique for analysing cerebral glucose delivery and utilisation in patients with severe brain injury [10]. With the advent of routine cerebral microdialysis in many neurointensive care centres, it would be logical to undertake observational studies in head injured patients with diabetes to determine the effect of glycaemic therapy targets, stratified according to premorbid glycaemia, on cerebral glucose metabolism. This research will form the basis of the candidates post-doctoral studies, which will be undertaken at the Neurosciences Critical Care Unit at Addenbrooke's Hospital, Cambridge, UK.

1.6.3 Role for type 2 diabetes screening programs for survivors of stress-induced hyperglycaemia

Type 2 diabetes is an insidious disease and a substantial proportion of patients have established microvascular complications at the time of diagnosis. In high-risk populations, such as women who have had gestational diabetes, screening programs in conjunction with primary prevention have been shown to both prevent disease progression and reduce the incidence of diabetes by 50% or more [11]. For these reasons the Australian National Health and Medical Research Council recommends screening programs in high risk individuals as an integral component of a diabetes prevention program [12]. The findings reported in Chapter 1.4 have highlighted that middle-aged survivors of critical illness who were hyperglycaemic in the first 24 hours of admission to the ICU represent a population at high-risk for developing diabetes. Determination of the efficacy and cost-effectiveness of screening programs in these patients represents a priority for future research in survivors of critical illness.

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CHAPTER 2

THE EFFECT OF CRITICAL ILLNESS ON NUTRIENT STIMULATED GALLBLADDER MOTILITY

2.1 INTRODUCTION

In the critically ill the presence of gastrointestinal dysfunction is associated with clinically important outcomes, including reduced survival. In large observational studies feed intolerance has consistently been associated with prolonged mechanical ventilation and ICU length of stay, as well as increased mortality [1, 2]. Feed intolerance encompasses both a functional delay in gastric emptying, slowing the delivery of nutrient to the small intestine [3], and impaired small intestinal absorption of macronutrients [4, 5]. The precise mechanisms underlying feed intolerance remain to be fully elucidated, but abnormalities of enterohormone secretion and function are likely important. ‘Enterohormone’ is a broad term describing hormones released by enteroendocrine cells of the gastrointestinal epithelium which together constitute the largest endocrine system in the body [6]. Chapter 2.2 contains a narrative review of enterohormones, with an emphasis on those that are most likely to be of relevance to the pathogenesis of feed intolerance, nutrient malabsorption and dysglycaemia, and a discussion relating to the therapeutic potential of manipulation of enterohormone axes. The enterohormones reviewed include ghrelin, motilin, cholecystokinin, glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 2 and polypeptide YY.

The enterohormone cholecystokinin is the dominant stimulant for gallbladder contraction and studies in the critically ill have reported elevated plasma cholecystokinin concentrations when compared to healthy subjects [7, 8]. Accordingly, there is physiological plausibility that gallbladder contraction should be vigorous in the critically ill but paradoxically, biliary stasis and gallbladder dysmotility are anecdotally cited to occur frequently in the critically ill, and believed to predispose to complications such as cholestasis, acute acalculous cholecystitis and acute pancreatitis [9-11]. Surprisingly, gallbladder motility has not been formally quantified in a critically ill population. The study presented in chapter 2.3 addresses the dissonance between documented cholecystokinin concentrations and gallbladder

dysmotility by accurately quantifying nutrient stimulated gallbladder motility in the critically ill.

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2.2 Literature review: Enterohormones and the response to critical illness

Full title

Enterohormones and the response to critical illness

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Introduction

The enteroendocrine cells constitute less than 1% of the total epithelial cell population of the gastrointestinal tract yet together form the largest endocrine system in the body [1]. These cells are responsible for the production of over 30 peptides which in health modulate gastrointestinal motility, secretory, absorptive and immune functions as well as mucosal growth and repair [2]. The physiological stress of critical illness and the treatments administered are associated with substantially disordered gastrointestinal and metabolic functions [3], many of which have been shown to be associated with adverse outcomes [4]. While it is not clinical practice to measure plasma enterohormone levels, which may contribute to the current paucity of data, it is increasingly evident that a number of enterohormones mediate, or have the potential to mediate, many of the functional gastrointestinal and metabolic abnormalities that occur during critical illness. This chapter will review the enterohormones most likely to be of clinical significance; ghrelin, motilin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 2 (GLP-2) and polypeptide YY (PYY). A summary for each hormone is provided with a focus on location of the secretory cell and receptor for hormone function, stimulus for secretion and, if there are sufficient data, the effect of critical illness on plasma concentrations and action is outlined (Table 1). In addition, studies relating to enterohormone receptor pharmacological agonism or antagonism and therapeutic potential in critical illness are presented where relevant.

Ghrelin

Ghrelin in health

Ghrelin is primarily secreted during fasting from parietal cells of the gastric fundus in the inactive (nonacylated) form [5]. Its secretion is suppressed in the postprandial phase as a result of the interaction of nutrient with the small intestine [5]. Ghrelin is a prohormone and requires posttranslational acylation for the majority of its biological activity [6]. Acylated ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) on the anterior pituitary, and therefore it is a natural secretagogue for growth hormone [7]. The GHS-R1a is expressed widely beyond the anterior pituitary including on pancreatic islets, B and T lymphocytes, neutrophils, myocardium, thyroid tissue and at multiple sites throughout the central nervous

system, which explains the diverse physiological actions of this hormone [8]. As well as regulating growth hormone secretion, ghrelin plays important roles in stimulating appetite and modulating glucose homeostasis; decreasing insulin secretion and increasing insulin sensitivity [8]. It also modulates stress, anxiety and sleep, protects against muscle atrophy, modulates taste sensation and has vasodilatory effects [8]. Studies using exogenous ghrelin at supra-physiological concentrations indicate that ghrelin accelerates gastric emptying in humans and in animal models of sepsis-induced gastroparesis [9, 10]. In ambulant patients with diabetic gastroparesis a ghrelin agonist has been shown to stimulate gastrokinesis [11].

Ghrelin in critical illness

In the largest study to date of endogenous ghrelin concentrations in critical illness, Koch et al. analysed plasma ghrelin in 170 critically ill patients and 60 healthy persons as a control group [12]. While they demonstrated that total ghrelin concentrations are increased during critical illness they did not differentiate between the active (acylated) and inactive form [12]. This is important as the majority of circulating ghrelin is in the inactive form and is renally cleared unlike the active form which undergoes organ independent enzyme metabolism with a short half-life of 10 minutes [13]. Inactive ghrelin accumulates in renal failure [11] and Koch et al. demonstrated an inverse association between renal function and ghrelin concentration in non-septic critically ill patients [12]. In the only study to date to measure both active and inactive ghrelin in critical illness, Crona and MacLaren demonstrated that compared to patients tolerating enteral nutrition, patients with feed intolerance had higher concentrations of total ghrelin but lower concentrations of active ghrelin [6]. These data suggest that while total ghrelin concentrations may be elevated during critical illness, active ghrelin levels may be decreased and contribute to slow gastric emptying. Studies into the effect of exogenous ghrelin, or its agonists, to manage feed intolerance in this population are warranted.

As well as influencing gut motility there is mechanistic plausibility that ghrelin may be protective in sepsis; in multiple animal models exogenous ghrelin has been found to down-regulate pro-inflammatory cytokines [14], protect against endotoxaemia induced acute kidney injury [15], ameliorate gut mucosal barrier function [16], attenuate sepsis-induced acute lung injury [17] and improve tissue perfusion [18].

Exogenous ghrelin has not been evaluated as a therapy in the critically ill but has been shown to reduce cachexia, increase appetite and improve exercise tolerance in patients with cancer, heart failure, end-stage renal disease and chronic obstructive pulmonary disease [19]. This is likely due to both anabolic growth hormone dependent and independent effects, for example improved appetite [4].

While growth hormone is suppressed in critical illness, trials with suprapharmacological doses of growth hormone have reported adverse outcomes [20]. Despite this adverse effect of growth hormone administration careful evaluation of ghrelin therapy in the critically ill appears warranted to establish the effects on gastric emptying, appetite and anabolism.

Motilin

Motilin in health

Motilin is synthesised by M cells in the proximal duodenum and regulates the fasting pattern of motility of the gut by binding to the motilin specific G-protein coupled receptor [21]. Motilin is predominantly secreted in the interdigestive state and the peak plasma motilin concentration coincides with the onset of the antegrade contractions during the fasting phase III migrating motor complex [22]. Pharmacological concentrations of exogenously administered motilin accelerate gastric emptying in health and in patients with gastroparesis [23]. The macrolide antibiotic erythromycin is a motilin receptor agonist and potently stimulates gastric emptying which has led to its additional use as a gastric prokinetic agent for the treatment of gastroparesis of multiple aetiologies [24].

Motilin in critical illness

Erythromycin potently stimulates gastric emptying in critically ill patients with feed intolerance and large gastric residual volumes [25-27]. Tachyphylaxis to stimulation of the motilin receptor develops quickly with erythromycin and the effects are diminished in 60% of critically ill patients within one week of regular administration [25]. While a more effective gastrokinetic drug than metoclopramide, observational data indicate that erythromycin is administered less frequently [28], perhaps because

of concerns regarding adverse effects; such as the potential to exacerbate bacterial resistance, interaction with other medications metabolised by the cytochrome P450 3A4 system and prolongation of the QT interval [29]. For these reasons there is increasing effort to identify a selective motilin receptor agonist without macrolide antibiotic properties for clinical use. There are preliminary data from a small phase 2 study that a non-macrolide selective motilin receptor agonist accelerates gastric emptying in the critically ill compared to placebo and larger randomised controlled trials are keenly awaited [30].

Cholecystokinin

Cholecystokinin in health

Cholecystokinin (CCK) is a peptide hormone secreted by I cells in the mucosa of the duodenum and jejunum in response to dietary fat, protein and to a lesser extent carbohydrates [31]. It binds to its specific G-protein coupled receptor on gastric, gallbladder and small intestinal mucosa, vagal afferents and centrally in the hypothalamus and hindbrain where it acts as a neuropeptide [32]. Endogenous CCK is the principal regulator of gallbladder contraction and has been shown to slow gastric emptying, relax the sphincter of Oddi and stimulate pancreatic enzyme secretion [33, 34]. Interaction of CCK with central satiation receptors in the hypothalamus reduces hunger and energy intake [35].

Cholecystokinin in critical illness

Our group has previously demonstrated elevated fasting and nutrient-stimulated plasma CCK levels in critical illness compared to healthy subjects [36]. Furthermore, fasting plasma CCK concentrations were higher in critically ill patients with delayed gastric emptying compared to those with normal emptying suggesting a role for CCK in the pathogenesis of delayed gastric emptying [37]. However, our experience from studies performed in healthy participants with normal rates of gastric emptying is that the magnitude of acceleration that occurs when antagonising endogenous hormones is much less than during administration of pharmacological concentrations [38, 39]. Given that gastric emptying is slow in many patients and can be due to many causes

our opinion is that CCK antagonists would have only a modest effect on gastric emptying and feed intolerance in the critically ill.

There are pre-clinical data to suggest that endogenous cholecystokinin mediates the beneficial immune and anti-inflammatory effects attributable to enteral nutrition in critical illness [40, 41]. In a rat model of haemorrhagic shock, CCK released in response to an enteral lipid load activates immunomodulatory receptors via vagal pathways, dampening the systemic inflammatory response; attenuating gastric epithelial permeability and bacterial translocation [40, 41]. Further studies are required to characterise the effect of critical illness on plasma CCK, the associations between plasma CCK and gastric emptying and the potential immunomodulatory role of CCK.

Glucagon-like peptide 1 (GLP-1)

GLP-1 in health and diabetes

GLP-1 is an incretin hormone stored in enteric L cells located predominantly in the distal small intestine and colon and is secreted in response to luminal fat, carbohydrate, protein and bile acids [42, 43]. Incretins are gut hormones that potentiate insulin secretion after a meal in a glucose-dependent manner [44]. Together with glucose-dependent insulinotropic polypeptide (GIP), GLP-1 accounts for the two to three-fold greater insulinotropic response to an oral glucose load compared to the equivalent intravenous glucose load (Fig 1.) [45]. The primary physiological role of endogenous GLP-1 is to lower blood glucose [46] via direct effects on pancreatic islet cell G-protein coupled receptors to propagate secondary messenger signals that stimulate insulin and suppress glucagon release; as well as indirect effects on the gut to slow gastric emptying and small intestinal motility [38, 46, 47]. The insulinotropic and glucagonostatic effect on the pancreatic α and β cells are strictly glucose dependent such that, below a blood glucose of ~ 6 mmol/L, even pharmacological doses of GLP-1 (and its agonists) have little or no impact on blood glucose [48]. In contrast, the ability of exogenously administered GLP-1 to slow gastric emptying persists during hypoglycaemia [39]. GLP-1 receptors are expressed widely beyond the pancreas and gut including in lung, kidney, skin, heart and brain [49]. A detailed

review of the extra-pancreatic effects of endogenous GLP-1 is beyond the scope of this chapter, however GLP-1 is thought to play a role in regulating appetite, learning and memory, preventing cardiac cell apoptosis, increasing bone formation and decreasing dermal cytokine expression [50].

The glucose-dependent insulinotropic effect of GLP-1 is preserved in patients with type 2 diabetes [51] making the GLP-1 receptor an attractive therapeutic target in this group [52]. Native GLP-1 is rapidly metabolised by dipeptidyl peptidase-4 (DPP-4) predominantly on capillary endothelia, imparting the enterohormone with a short half-life of 1-2 minutes [53, 54] which makes therapeutic delivery of native GLP-1 impractical. This has led to the development of subcutaneously administered GLP-1 receptor agonists that are resistant to DPP-4 degradation such as exenatide, and lixisenatide, as well as oral DPP-4 inhibitors such as sitagliptin, linagliptin and vildagliptin that have now been incorporated into standard algorithms for the management of type 2 diabetes [55].

GLP-1 in critical illness

In the critically ill endogenous GLP-1 concentrations are increased [56-58] when compared to nutrient stimulated physiological levels in health [59]. There appear to be associations between plasma concentration and biomarkers of inflammation, illness severity [57] and feed intolerance [58]. Murine studies have demonstrated inducible GLP-1 secretion by a range of inflammatory stimuli including endotoxin, IL-1 and IL-6 [57, 60]. Interestingly, when systemic inflammation is induced in healthy volunteers by a TNF- α infusion there is no demonstrable change in the incretin effect [61].

Therapeutic potential of GLP-1 based therapy in the critically ill

The rapid, organ-independent metabolism of a therapy that causes controlled, glucose-dependent glucagon suppression and insulin release makes GLP-1 a promising agent for the management of stress hyperglycaemia [43, 62]. To date, the use of GLP-1 in the critically ill is limited to small studies to establish proof of principle, albeit with promising results [63]. With pharmacological concentrations of intravenous GLP-1 marked glucose lowering has been observed in patients with type 2 diabetes post cardiac surgery [64, 65]. In a heterogeneous cohort of mechanically ventilated patients, exogenous GLP-1 has been observed to reduce the glycaemic response to

small intestinal nutrient delivery in patients with type 2 diabetes [66] and to intragastric and intestinal nutrient delivery in patients without pre-existing diabetes [67, 68]. In a small (n=18) randomised, double-blind, placebo controlled cross over study in critically ill surgical patients, GLP-1 in combination with intensive insulin therapy was also shown to reduce glycaemic variability when compared to intensive insulin therapy alone [69].

Administration of the commercially available GLP-1 agonist exenatide is also being explored. In an open-label, non-randomised, pilot study, Abuannadi and colleagues administered intravenous exenatide to 40 patients following major cardiac surgery [70]. Intravenous exenatide was associated with significantly reduced glycaemic variability compared to conventional intravenous insulin therapy and achieved equipotent blood glucose lowering with no episodes of severe hypoglycaemia [70]. Exenatide has also been administered subcutaneously in an open-label study in paediatric burn patients where it was shown to reduce exogenous insulin requirements [71].

While GLP-1 and its agonists have an inherently low risk of hypoglycaemia there is a dose-dependent relationship between GLP-1 and the slowing of gastric emptying and this has raised concerns that pharmacologically induced slower emptying may predispose to aspiration in mechanically ventilated critically ill patients [62]. Somewhat reassuringly, in a population of non-diabetic critically ill patients our group has demonstrated that acute infusion of GLP-1 at pharmacological concentration slows gastric emptying when gastromotor function is normal at baseline but has no effect when gastric emptying is already delayed [67].

There have been no human studies into the therapeutic potential of DPP-4 inhibitors in the critically ill which may be due to their oral route of administration and resultant variable pharmacokinetics.

Whether GLP-1, its agonists or the DPP-4 inhibitors could be used as stand-alone therapy or in combination with insulin for the management of stress hyperglycaemia warrants further investigation.

Glucose-dependent insulinotropic polypeptide (GIP)

GIP in health and type 2 diabetes

Glucose-dependent insulinotropic polypeptide, previously known as gastric inhibitory polypeptide, is secreted from duodenal and jejunal K cells in response to luminal fat and carbohydrate [44]. GIP exerts its incretin effect through distinct G-protein coupled receptors that are highly expressed in islet β cells and, like GLP-1, the insulinotropic action of GIP is strictly glucose-dependent [44]. GIP also has glucose-dependent effects on the α cell, dose-dependently stimulating glucagon secretion during hypo- and euglycaemia with no effect during hyperglycaemia [72]. GIP has no direct enterogastrone effect on either gastric acid secretion or gastric emptying but may slightly accelerate gastric emptying via indirect mechanisms through lowering systemic glycaemia [32]. GIP receptors are expressed widely and have been identified in fat, bone, brain and cardiac tissue with *in vitro* and murine studies suggesting potential roles for GIP in triglyceride metabolism, bone formation and neuroprotection [44]. GIP is also metabolised by DPP-4 with a resultant short half-life of ~4 minutes [73].

Unlike GLP-1, the insulinotropic effect of GIP is profoundly reduced in patients with type 2 diabetes and long-standing chronic hyperglycaemia [74]. This is likely due, at least in part, to the direct toxic effects of chronic hyperglycaemia down-regulating GIP receptor expression on the β cell [75]; an effect which may be reversible with Højberg et al. reporting that the insulinotropic property of GIP increased several-fold following 4 weeks of near-normal glycaemia in patients with type 2 diabetes [76].

GIP in critical illness

It does not appear that critical illness alters fasting or nutrient-stimulated GIP levels [56, 77]. There is a persuasive rationale for a potential therapeutic role for exogenous GIP in the management of stress hyperglycaemia, specifically its inherent safety profile; it stimulates glucagon release during hypoglycaemia and insulin release during hyperglycaemia and does not slow gastric emptying [78, 79].

In the only studies to date in the critically ill, our group has investigated GIP both as a solo agent and in combination with GLP-1 for the management of stress hyperglycaemia [78, 80]. Consistent with the lack of effect in patients with type 2 diabetes, we have shown that GIP has a negligible effect on glycaemia, gastric emptying, glucose absorption, insulin or glucagon secretion during critical illness [80] and provides no additional glucose lowering or insulinotropic effect when

administered in conjunction with GLP-1 [78]. Together, these data suggest that future studies should focus on GLP-1 or its agonists rather than GIP for the management of stress hyperglycaemia.

Glucagon-like peptide 2 (GLP-2)

GLP-2 in health

GLP-2 is co-secreted with GLP-1 in response to luminal nutrient from L cells that are located primarily in the distal ileum and colon [81]. GLP-2 is a pleiotropic hormone influencing multiple facets of intestinal physiology, the foremost of which is stimulation of intestinal mucosal growth in the small, and to a lesser extent the large bowel [82]. GLP-2 acts through G-protein coupled receptors primarily located in the small intestine [82]. While the receptor has been demonstrated on gastrointestinal endocrine cells, enteric neurons and myofibroblasts, its absence on both crypt epithelial cells and enterocytes suggest an indirect mechanism of its primary intestinotrophic action [81]. Like GLP-1 and GIP, GLP-2 is rapidly inactivated by the ubiquitous enzyme DPP-4, conferring a short half-life of ~7 minutes [83].

The majority of the gastrointestinal effects of GLP-2 have been elucidated following exogenous administration of GLP-2 or degradation resistant GLP-2 analogues such as teduglutide. The intestinotrophic effects of GLP-2 are mediated via an increase in intestinal crypt cell proliferation, a reduction in villous cell apoptosis and improved mesenteric blood flow; collectively increasing mucosal mass and surface area with an accompanied increase in intestinal digestive and absorptive capacity [81, 83]. GLP-2 administration also decreases gastric acid secretion and is glucagonotropic but unlike GLP-1 has no effect on insulin secretion, gastric emptying or post-prandial glycaemia [4].

Therapeutic role of GLP-2 in gastrointestinal disease

Exogenously administered GLP-2 and GLP-2 analogues significantly improve morbidity and increase gastrointestinal absorptive capacity in a diverse range of pre-clinical intestinal injury models, including small bowel resection [84], enteritis [85], necrotizing pancreatitis [86] and ischaemic-reperfusion injury [87]. Furthermore,

GLP-2 enhances epithelial barrier capacity, decreasing transcellular and paracellular permeability and reducing bacterial translocation [81, 86, 88]. These promising pre-clinical results encouraged human trials of the GLP-2 analogue, teduglutide which has since gained FDA approval for the management of short bowel syndrome after demonstrating increased gastrointestinal absorptive capacity and a reduction in fecal weight, energy expenditure and total parenteral nutrition (TPN) requirement [89].

Therapeutic potential of GLP-2 in critical illness implicate

Critically ill patients fasted for > 4 days demonstrate significant duodenal mucosal atrophy and increased gut permeability [90] and bacterial translocation has been implicated to play a role in the development of sepsis and multi-organ failure [91]. The physiological concentrations and potential effects of pharmacological concentrations of GLP-2 are yet to be studied in the critically ill. It is plausible that during critical illness the administration of GLP-2 may attenuate mucosal atrophy, improve nutrient absorption and reduce secondary infections.

Peptide YY

Peptide YY in health

Peptide YY (PYY) also known as peptide Tyrosine-Tyrosine is secreted by L cells located throughout the gastrointestinal tract but with the highest density in the colon [92]. PYY is released in response to enteral nutrient with fat being the most potent stimulus [93]. As PYY levels increase within 15 minutes of meal ingestion, an indirect mechanism mediated via CCK dependent pathways has been proposed to initiate the initial secretory response which is later maintained via direct enteral stimulation of the lower gastrointestinal tract [93]. PYY exerts predominantly inhibitory functions in health, slowing gastric and gallbladder emptying and inhibiting gastric acid and pancreatic exocrine secretion [32]. PYY receptors are also located centrally and exogenous PYY has been shown to be anorectic, inhibiting appetite and energy intake in overweight humans [94].

PYY in critical illness

Fasting and nutrient stimulated PYY concentrations are elevated two- to three-fold in critical illness which progressively normalise as critical illness resolves [95]. The PYY response is substantially greater in those critically ill patients with feed intolerance suggesting a role for PYY in critical illness-induced delayed gastric emptying [96]. This highlights a potential role for PYY receptor antagonists in the management of feed intolerance in the critically ill, however at present there are no PYY antagonists available for clinical use.

Conclusions

The secretion of a number of enterohormones is disordered in the critically ill which may mediate abnormalities in motility and glycaemia while also potentially serving a protective role, dampening inflammation and modulating the enteral immune response. There are over 30 recognised enterohormones and therapeutic manipulation of specific enterohormones or their receptors is a burgeoning area of critical care research with promising pre-clinical data and an increasing number of small clinical trials. Further characterisation of the effect of critical illness on the endocrine gut and how it can be manipulated to improve outcomes in critical illness warrants evaluation.

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Table 1: Function of enterohormones and impact of critical illness

Enterohormone	Site of secretion	Dominant effects	Effect of critical illness
Ghrelin	Pareital cells Gastric fundus	↑ growth hormone ↑ appetite ↑ gastric emptying Energy homeostasis	↑ total concentration ? ↓ active concentration
Motilin	M cells Proximal duodenum	↑ fasting intestinal motility ↑ gastric emptying (supra-physiological)	Unknown
CCK	I cells Duodenum and jejunum	↑ gallbladder contraction ↓ gastric emptying ↑ pancreatic enzyme secretion ↓ appetite	↑ concentration
GLP-1	L cells Distal ileum & colon	↑ insulin (glucose-dependent) ↓ glucagon (glucose-dependent) ↓ gastric emptying ↓ appetite	↑ concentration
GIP	K cells Duodenum and jejunum	↑ insulin (glucose-dependent) ↑ glucagon (glucose dependent)	No effect
GLP-2	L cells Distal ileum & colon	↑ intestinal mucosal growth ↑ intestinal absorptive capacity ↓ intestinal permeability ↑ intestinal mucosal blood flow	Unknown
Peptide YY	L cells Distal ileum, colon & rectum	↓ gastric emptying ↓ gallbladder contraction ↓ gastric acid secretion ↓ pancreatic exocrine secretion	↑ concentration

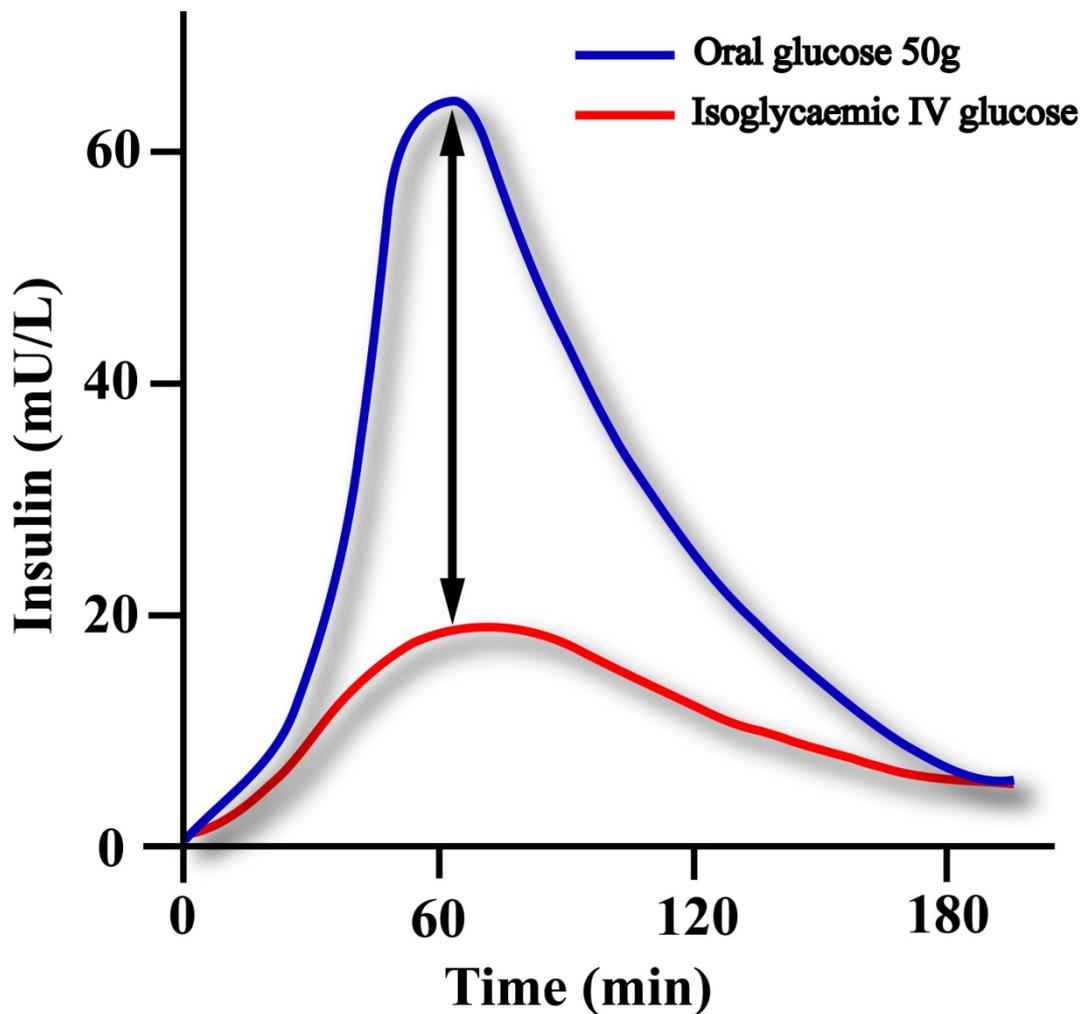


Fig 1. The incretin effect

Adapted from Nauck et al, *Diabetologia* 1986 [74]. There is a substantially greater release of insulin in response to oral glucose as compared to an isoglycaemic intravenous infusion of glucose. The difference between the plasma insulin, as demonstrated by the arrow, is the incretin effect and is mediated by the enterohormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).

2.3 Manuscript: Critical illness is associated with impaired gallbladder emptying as assessed by 3D ultrasound.

Title

Critical illness is associated with impaired gallbladder emptying as assessed by 3D ultrasound

Authors

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Key words

Gallbladder dyskinesia, gallbladder emptying, ultrasound imaging, critical illness, intensive care

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ABSTRACT

Objective:

To quantify gallbladder dysfunction during critical illness.

Design:

Prospective observational comparison study of nutrient stimulated gallbladder emptying in health and critical illness.

Setting:

Single-centre mixed medical/surgical ICU.

Patients:

Twenty-four mechanically ventilated critically ill patients suitable to receive enteral nutrition were compared to 12 healthy subjects.

Interventions:

Participants were studied after an 8 hour fast. Between 0-120 min high-fat nutrient (20% intralipid) was infused via a post-pyloric catheter into the duodenum at 2 kcal/min.

Measurements and Main Results:

3D images of the gallbladder were acquired at 30 minute intervals from -30–180 min. Ejection fraction (%) was calculated as changes between 0 – 120 min. Blood samples were obtained at 30 minute intervals for plasma cholecystokinin (CCK). Data are mean (SD) or median [IQR]. In the critically ill, fasting gallbladder volumes [critically ill 61[36-100] vs. health 22[15-25] ml; $P<0.001$] and wall thickness [0.45(0.15) vs. 0.26(0.08) mm; $P<0.001$] were substantially greater, and sludge was evident in the majority of patients (71% vs. 0%). Nutrient-stimulated emptying was incomplete in the critically ill after 120 min but was essentially complete in health [22[9–66] vs. 4[3-5] ml; $P<0.01$]. In five critically ill patients (21%) there was no change in gallbladder volume in response to nutrient and overall ejection fraction was reduced in the critically ill [50[8-83] vs. 77[72-84]%; $P=0.01$]. There were no differences in fasting or incremental CCK concentrations.

Conclusions:

Fasted critically ill patients have larger, thicker-walled gallbladders than healthy subjects and nutrient-stimulated gallbladder emptying is impaired with 'gallbladder paresis' occurring in ~20%.

INTRODUCTION

Biliary sludge, a precipitant of bile, is reported to occur frequently in the critically ill with a prevalence 50-fold greater than in health [1]. Gallbladder dysmotility and biliary stasis have been implicated in the pathogenesis of biliary sludge, as well as complications such as cholangitis, pancreatitis and acute acalculous cholecystitis [2, 3], with the importance of gallbladder dysmotility emphasised by reports that acute acalculous cholecystitis complicates up to 1% of all intensive care admissions [4] with an associated mortality of at least 30% [2]. It is, accordingly, surprising that gallbladder motility has never been formally evaluated in the critically ill.

In health, gastric emptying occurs promptly and the presence of nutrient, particularly lipid, in the proximal small intestine stimulates the secretion of cholecystokinin (CCK) [5], the major hormonal mediator of gallbladder contraction [6]. In the critically ill gastric emptying is frequently delayed [7] and to quantify postprandial gastrointestinal hormone secretion a predictable small intestinal stimulus is required [8].

Our group has previously demonstrated an increase in both fasting and small intestinal nutrient-stimulated plasma CCK concentrations in critical illness [9, 10], providing a mechanistic rationale that gallbladder motility will be vigorous and emptying complete. However gallbladder motility has never been correlated with CCK concentrations in critical illness.

The primary aim of this study was to quantify fasting gallbladder volumes and nutrient-stimulated gallbladder emptying in the critically ill. Secondary aims were to evaluate the relationship between plasma CCK concentrations and gallbladder emptying and to determine whether gallbladder volume calculation using 3D ultrasound correlates with the conventional 2D technique in the critically ill.

MATERIALS AND METHODS

Participants

Critically ill patients: Twenty-four, intubated, mechanically ventilated patients aged between 18 and 80 years who were suitable to receive enteral nutrition via a small intestinal feeding catheter were recruited from the Intensive Care Unit (ICU) at the Royal Adelaide Hospital. Written, informed consent was obtained from the patients' next of kin. Exclusion criteria were; parenteral nutrition administered within the previous 5 days, a history of biliary pathology, visible gallstones at the time of ultrasonography, pregnancy or a haemoglobin <80 g/L.

Healthy Controls: Twelve healthy participants aged between 18 and 80 years were studied as a 'control' group. Exclusion criteria were; a history of biliary disease, pregnancy, diabetes, impaired renal or liver function, anemia on screening bloods, currently smoking, consumption of >20 g/day of alcohol per day, or receiving medication known to affect gastrointestinal motility. All healthy participants provided written, informed consent.

The study was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and performed according to Australian National Health and Medical Research Centre guidelines for the conduct of research on unconscious patients.

Protocol

All critically ill patients were being fed via nasogastric tube and had nutrient ceased 8 hours prior to the study. A small intestinal feeding catheter was inserted using an electromagnetic guidance technique; correct duodenal placement of the catheter was confirmed by abdominal radiography [11]. Patient demographics, preceding nutritional delivery, liver function tests, plasma urea and creatinine concentration, glycated hemoglobin, requirement for narcotic analgesia and vasopressor agents, ventilation settings and antibiotic use were recorded. Death prior to day 90 was established using the hospital electronic database. All patients had an arterial catheter *in situ* which was used for blood sampling.

Healthy participants were instructed to drink 250 ml Ensure (Abbott laboratories, Holland), a 1 kcal/ml mixed-nutrient liquid, at 2200 hours before fasting overnight to replicate enteral feeding of the critically ill [8]. Healthy participants attended the hospital at 0830 hours and a small intestinal feeding catheter was inserted using the same electromagnetic guidance technique as for the critically ill patients. An intravenous cannula was placed in the right antecubital vein for blood sampling.

In both groups, a test meal consisting of 120ml of 20% Intralipid (Baxter Healthcare, USA; 20% soybean oil, 2 kcal/ml) was infused into the duodenum at a rate of 2 kcal/min (60 ml/h) between 0 and 120 minutes. 2D and 3D ultrasound measurements of gallbladder volume, blood glucose and plasma CCK concentrations were obtained every 30 minutes during the 'fasting' (t = -30 to 0), 'small intestinal nutrient-stimulated' (t = 0 to 120) and 'postprandial' (t = 120 to 180) periods.

Data analysis

Ultrasound Imaging

Ultrasonographic measurements of the gallbladder were performed by a single operator (M.P.P) using a Logic™ 9 ultrasonography system (GE Healthcare, USA) with Truscan Architecture, including built-in magnetically sensed 3D technology [12, 13]. Subjects were scanned supine at 30°. Gallbladder volumes were calculated during post-processing from 2D and 3D images obtained at 30 minute intervals from -30 to 180 min. The gallbladder ejection fraction (EF) was calculated from 3D volumes with the following equation: $EF = [(volume\ at\ t = 0 - volume\ at\ t = 120) / volume\ at\ t = 0] \times 100$. Where a 3D volume was not measurable the 2D volume was substituted for that time point.

Static images of the gallbladder at t = -30 were evaluated against diagnostic criteria for acute acalculous cholecystitis i.e. gallbladder wall thickening >3 mm, gallbladder wall striations, pericholecystic fluid, gallbladder distension (long axis >10 cm and/or transverse diameter >5 cm) and the presence of sludge [2, 3, 14-16].

Two-Dimensional Ultrasound Method

All 2D images were obtained with a 3.5C broad spectrum convex-array 2.5-4 MHz transducer and maximal length, width and height recorded [17] (Supplemental Fig. 1a and 1b). All measurements were obtained from inner wall to inner wall. The gallbladder volume was mathematically calculated using the equation for a prolate ellipsoid [17].

Three-Dimensional Ultrasound Method

For 3D positioning and orientation measurement a snap-on sensor was attached to the 3.5C broad spectrum convex transducer [12]. The gallbladder was scanned by a continuous translational movement along its long axis, starting at the cystic duct and moving through the neck, body and fundus to the gallbladder tip. Data processing and volume estimation were performed with the use of EchoPAC-3D software® (GE Vingmed Sound, Norway) [18]. Regions-of-interest were drawn around the gallbladder on transverse sections, which were subsequently interpolated to produce a 3D image of the total gallbladder (Supplemental Fig. 2a and b).

Blood glucose and Plasma CCK

Blood glucose concentrations were measured using a portable electrochemical coulometric glucose dehydrogenase glucose meter (Optium Xceed; Abbott Laboratories, USA) [19].

Blood samples (5 ml) were collected at 30 minute intervals from $t = -30$ to 180 for measurement of plasma CCK and blood glucose. Blood was collected from the intra-arterial catheter in the patients and intravenous catheter in the healthy volunteers. Samples for CCK were collected into chilled EDTA tubes, centrifuged at 4°C within 30 min of collection, and the plasma stored at -70°C for subsequent analysis [20]. Plasma CCK concentrations were measured by radioimmunoassay [21]. The intra-assay and inter-assay coefficients of variation were 5.4% and 13.4% respectively. The detection limit of the assay was 1 pmol/L.

Statistical Analysis

As there was no pre-existing data we were unable to perform a formal sample size calculation but estimated *a priori* that 24 critically ill patients and 12 healthy

participants would be sufficient to detect a clinically meaningful difference. Differences between groups were analyzed using Student's unpaired t-test or Mann-Whitney test as appropriate and data are presented as mean (SD) or median [IQR]. Gallbladder volumes of interest defined *a priori*, were compared at $t = 0$ to assess baseline differences in fasting gallbladder volume and at $t = 120$ to assess differences in the response to nutrient stimulation and allow the calculation of gallbladder ejection fraction. Unadjusted Pearson correlation analyses were performed for demographic, ultrasound, laboratory and CCK data. Limits of agreement between 2D and 3D measurements were calculated from a linear mixed effects model accounting for the repeated measurements from each subject as linked replicates [22]. The model included fixed effects for method and subject and random effects for the method-by-subject interaction, subject-by-replicate interaction and the measurement error within each method. Data were log transformed to account for non-constant variance in the model. All tests were two sided and the null hypothesis was rejected at the 0.05 significance level. Sensitivity analyses for group differences in gallbladder measurements were conducted using variance stabilising transformations. Statistical analyses were performed using SPSS (version 16.0; SPSS, USA). Method comparison plots and analyses were performed with R 3.1.2 [23].

RESULTS

Demographic data and characteristics of the critically ill patients and healthy participants are summarised in Table 1.

Fasting Gallbladder Characteristics

A diagnosis of acute acalculous cholecystitis had not been considered during the clinical management of any patient. However, 20 of 24 critically ill patients (83%) exhibited at least one criterion for an ultrasound diagnosis of acute acalculous cholecystitis (Table 2 and Supp. Video 1) [2, 3, 14-16]. In the critically ill, fasting gallbladder volumes ($t=0$) were greater (by about three fold) [ICU 61 [36-100] vs. health 22 [15-25] ml; $P < 0.001$] (Fig. 1), as was wall thickness (by more than 50%) [4.5 (1.5) vs. 2.6 (0.8) mm; $P < 0.001$] (Table 2).

Gallbladder Emptying

In the critically ill there was wide variation in motility in response to nutrient-stimulation; five patients (21%) had complete ‘gallbladder paresis’ with no demonstrable gallbladder emptying at either $t = 120$ or 180 (Fig. 2). Upon completion of the lipid infusion ($t = 120$) gallbladder emptying was essentially complete in all the healthy subjects and incomplete in the critically ill in whom volumes were ~four-fold greater [22 [9–69] vs. 4 [3-5] ml; $P < 0.01$] (Fig. 1). While there was no overall difference in the change in gallbladder volume [22[7-33] vs. 17[12-21] ml; $P = 0.18$] gallbladder ejection fraction was less in the critically ill [50 [8-83] vs. 77 [72-84]%; $P = 0.01$].

Plasma CCK Concentrations

Due to hemolysis of the samples, plasma CCK concentrations were not measurable in three critically ill patients. Fasting CCK concentrations [ICU 5.3 (2.5) vs. health 4.7 (1.2) pmol/L; $P = 0.5$] and Δ CCK concentration post nutrient infusion ($t = 0$ -120) [ICU 5.8 (3.8) vs. health 4.6 (3.7) pmol/L; $P = 0.37$] were comparable (Supplemental Fig. 3).

Relationships between gallbladder volume, motility, patient demographics and plasma CCK

There were no associations between fasting gallbladder volume or gallbladder ejection fraction with concentrations of ALT, AST, bilirubin, ALP, GGT, glycated hemoglobin, urea or creatinine, day of admission studied, fasting blood glucose or 90 day mortality. There was an association between gallbladder ejection fraction and both sequential organ failure assessment score on the day of study [$r = -0.50$; $P < 0.05$] and fasting gallbladder volume [$r = -0.50$; $P < 0.05$]. In the critically ill patients there were no associations between fasting CCK concentration and fasting gallbladder volume, or the incremental increase in plasma CCK during the fed period and gallbladder ejection fraction.

Two-Dimensional vs Three-Dimensional gallbladder volume calculation

Due to inadequate projections from the stored cine-clips, 3D volumes were not calculable from 20 of 288 clips. As a result, 268 2D vs. 3D comparisons were included in the mixed effects model. Volumes as determined by 2D ultrasound were 6% less than with the 3D technique (bias=0.94) with limits of agreement of (0.54, 1.54) and divergence at larger volumes (Fig. 3).

DISCUSSION

The major findings are that critically ill patients, when compared to healthy participants, appear to have larger, thicker walled gallbladders when fasting, that gallbladder emptying is sluggish in approximately 50% of patients and that impaired gallbladder emptying is associated with larger fasting volumes and greater severity of illness. There was no association between gallbladder emptying and fasting or nutrient-stimulated CCK.

This study is the first to formally and accurately quantify gallbladder motility in response to a standardized physiological stimulus in the critically ill. Our results are supported by Merrell and colleagues who reported diminished gallbladder emptying in 12 critically ill trauma patients randomized to receive a lipid load or water via the nasogastric route [24]. However, interpretation of the latter study is compromised by the route of feeding and the lack of a comparator group. Gastric emptying is markedly delayed in up to 50% of critically ill patients [7]. Because the rate of nutrient emptying into the small intestine is a major determinant of postprandial CCK release [25] and gallbladder contraction [26], nutrient must be infused directly into the small intestine to provide a standardised stimulus for gallbladder contraction.

This study has demonstrated marked heterogeneity in gallbladder contraction in response to small intestinal nutrient-stimulation during critical illness ranging from vigorous emptying to complete gallbladder paresis. Gallbladder ejection fraction was associated with fasting gallbladder volumes such that larger gallbladders had slower emptying. Sluggish gallbladder emptying has been proposed as a putative mechanism of acute acalculous cholecystitis leading to mucosal injury, hypoperfusion, gallbladder ischemia, and secondary infection [2, 27]. Slow or incomplete gallbladder contraction results in bile stasis which alters the chemical composition of bile by increasing the

concentration of irritants such as lysophosphatidylcholine predisposing to mucosal injury [2]. Bile stasis is also believed to increase intraluminal pressure thereby compromising gallbladder perfusion pressure which may be exacerbated in the critically ill by vasoactive drugs and hypotension [2, 27]. Bacterial invasion of ischemic and chemically injured mucosal tissue represents a secondary phenomenon in the subsequent development of acute acalculous cholecystitis [2]. Furthermore, gallbladder dysmotility has been suggested as a putative contributory factor in lipid malabsorption, impairing the mixing of lipid with bile salts [28, 29] and recent data suggest that small intestinal lipid absorption is impaired in critical illness [30].

There is a lack of consensus on suitable diagnostic criteria for acute acalculous cholecystitis with the identification of major and minor criteria considered to have reasonably acceptable sensitivity, but poor specificity [2, 3, 14-16]. In the current study both wall thickening >3mm (79%) and sludge (71%) occurred frequently, but no patients had been diagnosed clinically with acute acalculous cholecystitis. These data are consistent with previous ultrasound studies in the critically ill demonstrating that abnormalities of the gallbladder occur in 50-84% of patients even when acute acalculous cholecystitis is not considered as a diagnosis [3, 14, 31]. Together these data highlight that abnormalities occur frequently in a heterogeneous population of asymptomatic patients and suggest that the positive predictive value of ultrasound will be low without pre-test clinical suspicion of biliary pathology. Importantly, none of the aforementioned studies have assessed gallbladder motility as a criterion for diagnosing biliary pathology [3, 14, 31]. The current study demonstrates that gallbladder dysmotility occurs frequently and gross pathology can be reliably assessed by 2D ultrasound. Accordingly, further studies enrolling larger numbers of patients considered to be at increased risk of biliary pathology are warranted to assess the clinical importance of impaired gallbladder emptying.

While previous studies have demonstrated increased CCK concentration in the critically ill [10, 32], in the current study there was no difference in plasma CCK between the critically ill patients and healthy participants, and there was no association between CCK concentration and gallbladder ejection fraction. These data suggest the cause for gallbladder dysmotility is not due to insufficient hormonal stimulation. Purported risk factors for gallbladder dysmotility and bile stasis during critical illness include opioid medication [33], mechanical ventilation [34], total

parenteral nutrition [2], continuous enteral feeding [35], fasting and severe hypoalbuminemia [3]. Our study was not powered to evaluate the relationship of gallbladder ejection fraction with these predefined risk factors and using unadjusted correlation analyses, only the SOFA score was associated with diminished gallbladder emptying.

This is the first study to utilize 3D ultrasonography to determine gallbladder volume in the critically ill, a technique that has been shown to be the most accurate for diagnosing patients with gallbladder dyskinesia [12]. The 3D ultrasound system produces a spatially varying magnetic field which can be distorted by metal objects [36] and for practical reasons it was not possible for all metal objects around the patient to be removed. Magnetic distortion interfered with 3D volume calculation at 20 of 288 time points – a failure rate of 7%. With the correlations available, this study has demonstrated reproducibility between the 2D and 3D techniques in the critically ill; albeit with diminished correlations at higher gallbladder volumes. Furthermore, the 2D technique was technically more reliable and 2D volume was calculable at every time point. These data suggest that 2D ultrasound volume calculation is an accurate and reliable tool in the critically ill.

Limitations of this study should be recognised. In the critically ill cohort mean BMI was greater than in the healthy subjects and gallbladder volume has been reported to correlate positively with body size, albeit weakly [37]. We observed a major difference in fasting gallbladder volumes. Furthermore, there was no relationship between BMI and fasting gallbladder volume in the critically ill patients. Nutrient was only infused for 120 minutes and we cannot exclude the possibility that with ongoing stimulation complete emptying would have eventually occurred. However, in the subset of patients with no reduction in gallbladder volume after 180 minutes this appears unlikely. The sample size was relatively small and the majority of critically ill patients presented with a primary diagnosis of respiratory failure (15/24). Only three patients were admitted with a surgical diagnosis so any extrapolation of these results to the surgical intensive care population should be circumspect. However, given that anesthetic drugs, multiple blood transfusions [24], fasting [38] and ileus [2] have been implicated as risk factors for biliary stasis and occur frequently in a surgical cohort, gallbladder motility is likely to be impaired by at least a comparable magnitude. Finally, in our patient

population mortality was relative frequent (37.5%) and results may differ in a lower acuity critically ill population.

CONCLUSIONS

Fasted, critically ill patients have larger and thicker walled gallbladders than healthy subjects and about half of critically ill patients exhibit sluggish to parietic gallbladder emptying in response to a small-intestinal lipid meal which is associated with larger fasting gallbladder volumes and severity of illness. 2D ultrasound assessment of gallbladder volume correlates well with the 3D technique with the advantage of having increased reliability. It is intuitive that impaired gallbladder emptying will predispose to cholestasis, lipid malabsorption and acalculous cholecystitis and further research, including larger cohorts, is needed to define the strength of this relationship and its impact on patient outcomes.

Author Contribution

M.P.P. and A.M.D were responsible for study conception and design, obtaining funding, acquisition of data, statistical analysis and drafting the manuscript.

K.L. was responsible for statistical analysis and revision of the manuscript for important intellectual content.

P.K., C.E.C., M.J.C., K.L.J., T.H., and M.H contributed to the acquisition of data and critical revision of the manuscript for important intellectual content.

M.P.P. is guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of these data and the accuracy of the analysis.

List of abbreviations

ALP: alkaline phosphatase

ALT: alanine aminotransferase

APACHE: acute physiology and chronic health evaluation

AST: aspartate aminotransferase

BMI: body mass index

CCK: cholecystokinin
EDTA: ethylenediaminetetraacetic acid
EF: ejection fraction
GGT: gamma-glutamyl transpeptidase
HbA_{1c}: glycated hemoglobin
HIDA: hepatoiminodiacetic acid
ICU: intensive care unit
IQR: interquartile range
SD: standard deviation
SOFA: sequential organ failure assessment
2D: two-dimensional
3D: three-dimensional

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Table 1: Demographic data and characteristics of critically ill patients

	Critically ill patients	Healthy participants	P- value
Number	24	12	-
Age (years)	54 ± 16	55 ± 19	0.94
Male:Female	16:8	8:4	-
Body mass index (kg/m ²)	29 ± 6	24 ± 4	<0.01
Mean blood glucose (mg/dl)	126 ± 29 [#]	97 ± 9	<0.01
Admission diagnosis: <i>n</i> (%)	Respiratory: 15 (63%)	-	-
	Neurological: 3 (13%)	-	-
	Sepsis: 3 (13%)	-	-
	Post-surgery: 2 (8%)	-	-
	Trauma: 1 (4%)	-	-
APACHE II score	17 ± 5	-	-
SOFA score	7.5 ± 4	-	-
Day of ICU admission	5 ± 4	-	-
Vasopressor support <i>n</i> (%)	9 (38%)	-	-
↑ALP &GGT <i>n</i> (%)	8 (33%)	-	-
Acute renal failure* <i>n</i> (%)	7 (29%)	-	-
Antibiotic support <i>n</i> (%)	19 (79%)	-	-
Febrile with ↑ WCC [§] <i>n</i> (%)	4(16%)	-	-
Enteral nutrition: <i>n</i> (%)	24 (100%)	-	-
Hospital mortality <i>n</i> (%)	9 (38%)	-	-

ALP, alkaline phosphatase; APACHE, acute physiology and chronic health evaluation; GGT, Gamma-glutamyl transpeptidase; SOFA, sequential organ failure assessment.
*Acute renal failure was diagnosed as per RIFLE (Risk, Injury, Failure, Loss of kidney function, End-stage kidney disease) criteria.[39]

[§] Maximum temperature >38.4°C and a white cell count > 11.0 x 10⁹/L on the day of study
[#] Critically ill patients were managed with intravenous insulin to target a blood glucose <180 mg/dl.[40]

Data are mean ± SD unless specified.

Table 2: Fasting gallbladder characteristics (t = 0)

	Critically ill patients	Healthy participants	P-value
Volume (ml)	61 [36-100]	22 [15 - 25]	<0.001
Wall thickness (mm)	4.5 (1.5)	2.6 (0.8)	<0.001
Wall thickening > 3mm <i>n</i> (%)	19 (79%)	0	-
Sludge <i>n</i> (%)	17 (71%)	0	-
Gallbladder wall striations <i>n</i> (%)	4 (16%)	0	-
Gallbladder distension <i>n</i> (%)	2 (8%)	0	-
Pericholecystic fluid <i>n</i> (%)	1 (4%)	0	-
Mucosal sloughing <i>n</i> (%)	0	0	-
Intramural gas <i>n</i> (%)	0	0	-

Gallbladder distension was defined as a maximal length >10cm and/or width > 5cm [2, 3, 14-16].

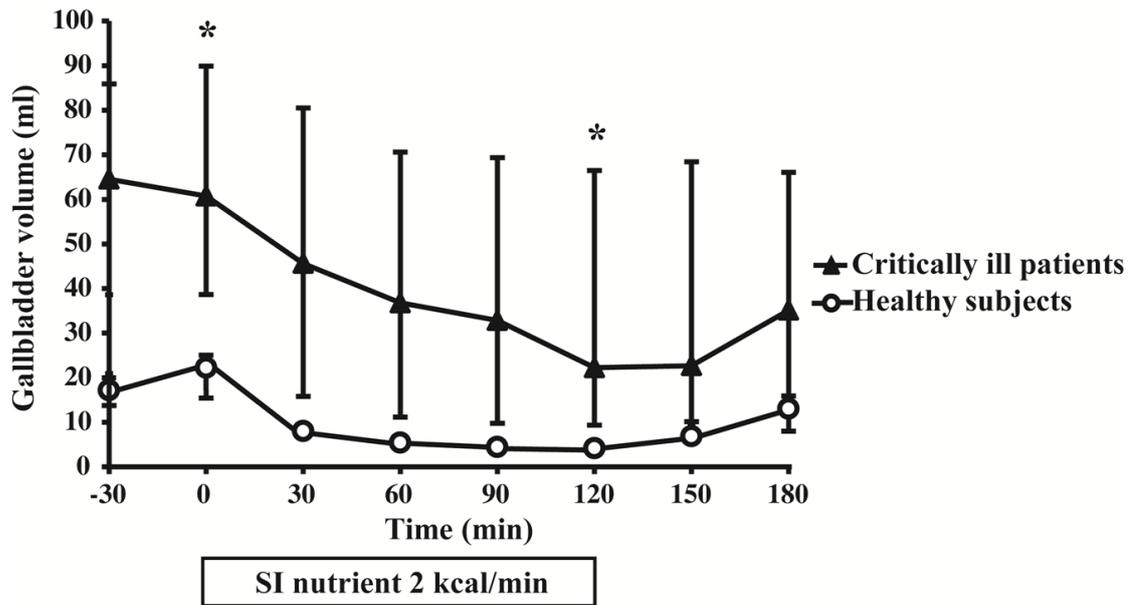


Fig 1. Gallbladder volumes

Gallbladder volumes during fasting (t=-30 to 0 min), small intestinal nutrient-stimulation (t=0 to 120 min) and post nutrient infusion (t=120 to 180 min) in critically ill patients [n=24] (filled triangles) and healthy participants [n=12] (open circles). Time points to compare defined *a priori*; 0 and 120 min. *P < 0.01. Data are median [IQR]. Gallbladder volumes were calculated using 3D ultrasonography.

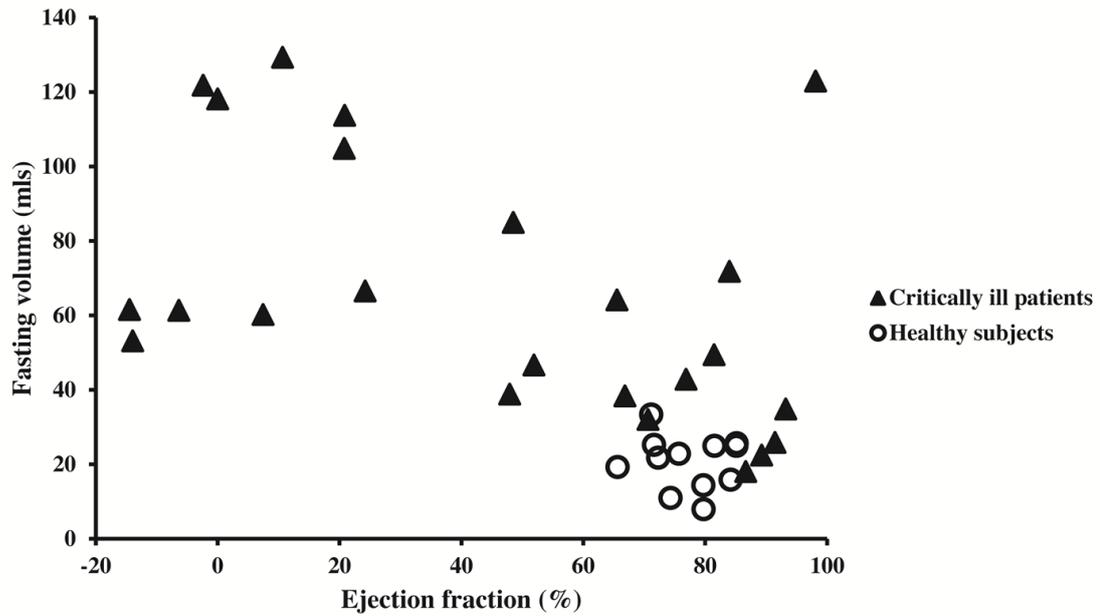


Fig 2. Fasting volumes vs. ejection fraction

Five critically ill patients had complete gallbladder paresis (ejection fraction $\leq 0\%$) and a further five patients had impaired emptying with an ejection fraction $< 25\%$. In critically ill patients (filled triangles) greater fasting gallbladder volumes were associated with lesser ejection fractions [$r = -0.50$; $P < 0.05$]. All healthy subjects (open circles) had ejection fractions $> 65\%$. Gallbladder volumes were calculated using 3D ultrasonography.

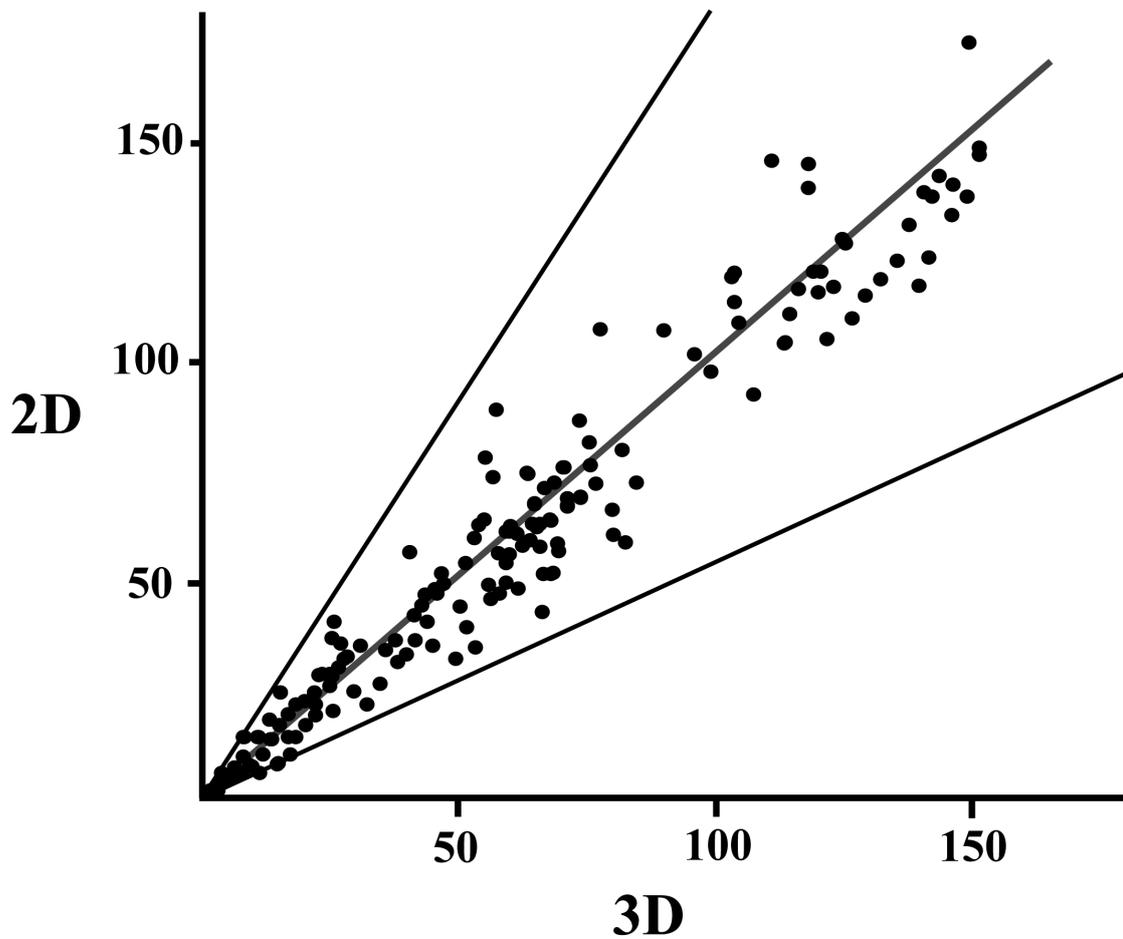
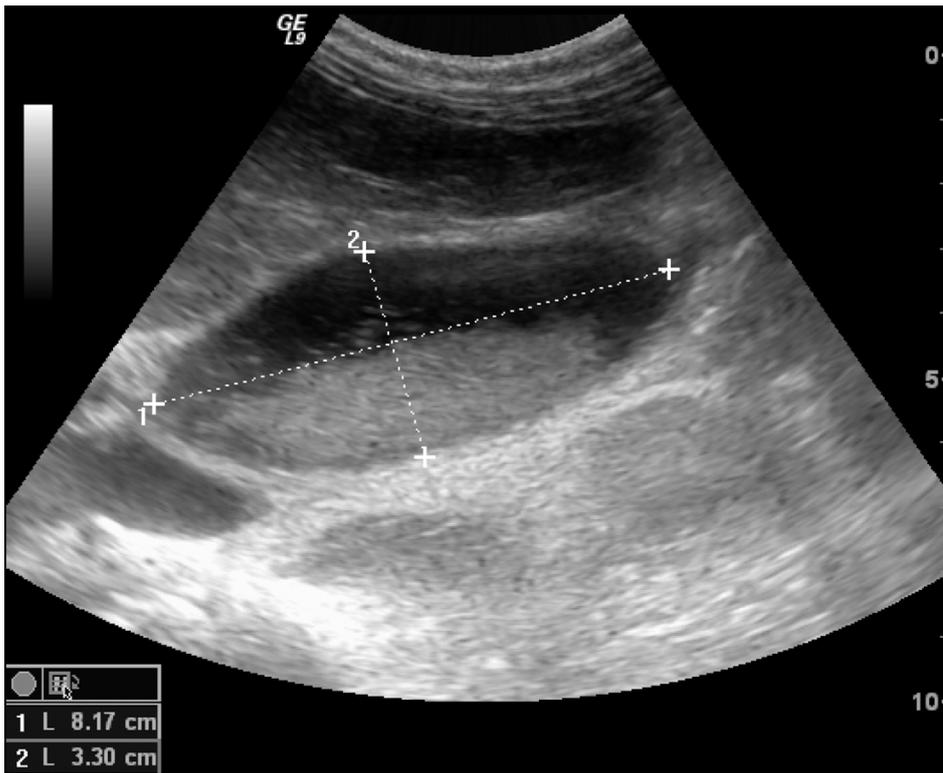
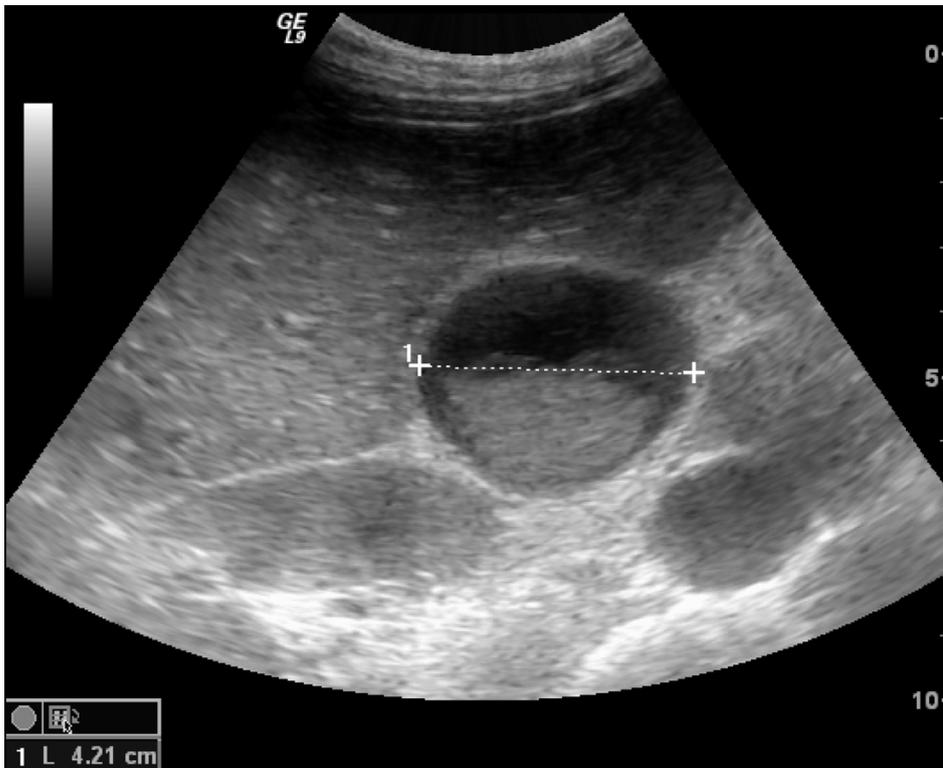


Fig 3. 2D vs 3D ultrasound

Limits of agreement between 2D and 3D measurements of gallbladder volume were calculated from a linear mixed effects model accounting for the repeated measurements from each subject as linked replicates. 2D volumes measured 6% less than with the 3D technique; bias=0.94 with limits of agreement of (0.54, 1.54).

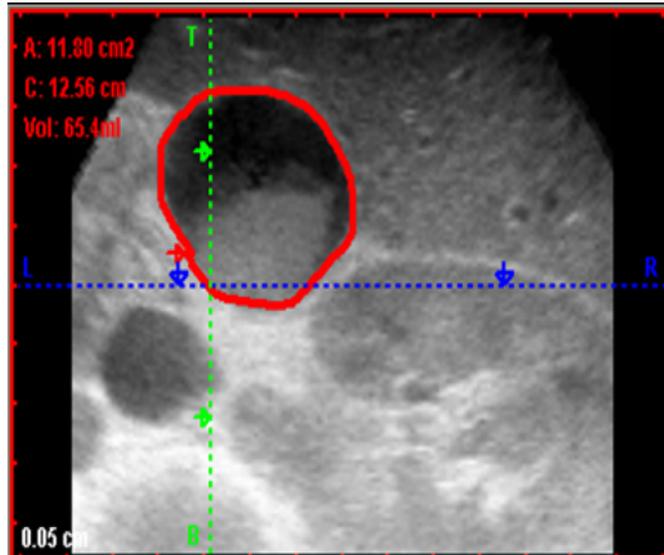


Supplemental Fig 1a. 2D ultrasound long axis view of the gallbladder



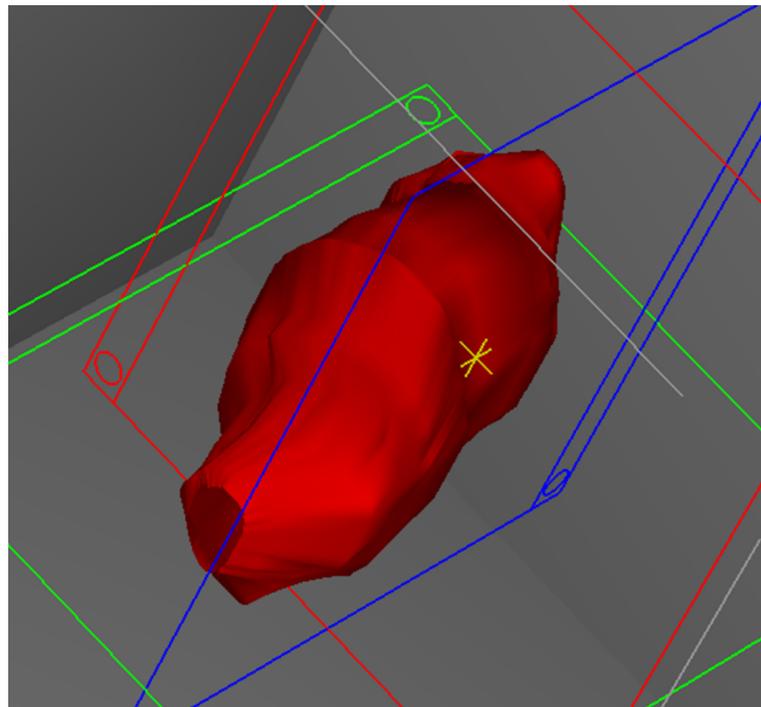
Supplemental Fig 1b. 2D ultrasound short axis view of the gallbladder

a. Long axis and **b.** short axis images in a fasted critically ill patient. Gallbladder wall thickening, pericholecystic fluid and sludge are observed with a volume of 59ml.



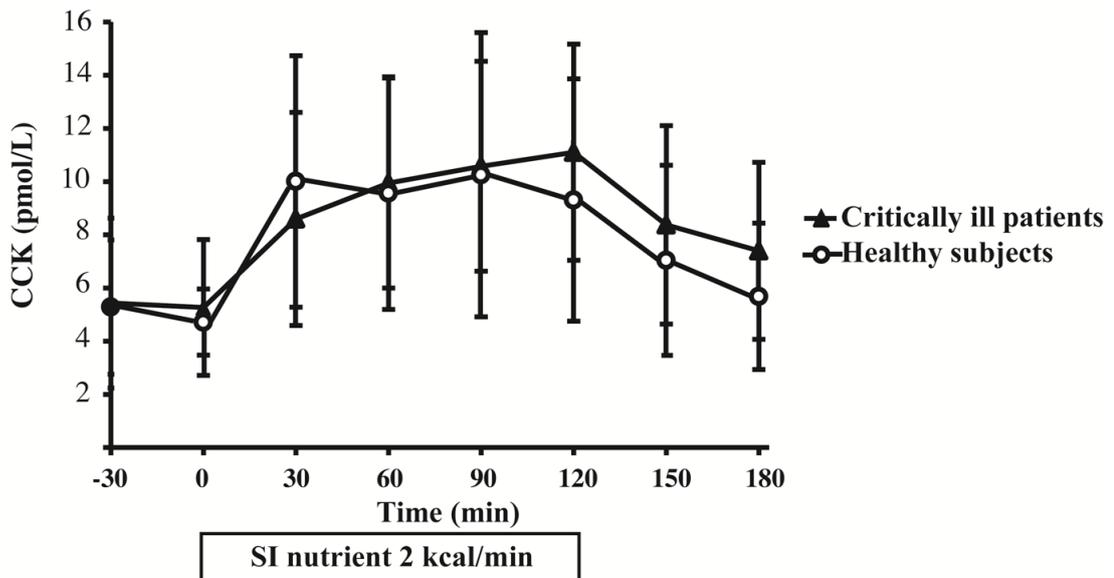
Supplemental Fig 2a. 3D ultrasound image acquisition

a. Screen image from the Echo-Pac 3D software® of the gallbladder as shown in Figure 1. The inner wall of the gallbladder perimeter is manually traced (red line) on at least six projections of the gallbladder as it is scanned from neck to tip. At completion of the last tracing, volume is automatically calculated (top left) = 65 ml.



Supplemental Fig 2b. 3D ultrasound image acquisition

b. 3D ultrasound image generated from surface-rendered perimeter tracings.



Supplemental Fig 3. Cholecystokinin

Plasma concentrations of cholecystokinin (CCK) during fasting ($t=-30$ to 0 min), nutrient-stimulation ($t=0$ to 120 min) and post nutrient infusion ($t=120$ to 180 min) in critically ill patients [$n=21$] (filled triangles) and healthy volunteers [$n=12$] (open circles). Data are mean (SD).

2.4 CONCLUSIONS

2.4.1 *Introduction*

The objective of the study that comprises this chapter was to quantify gallbladder motility in the critically ill (chapter 2.2). Cholecystokinin, a peptide hormone released by the gut in response to luminal nutrient is the primary hormonal stimulant of gallbladder contraction and previous studies have demonstrated elevated levels during critical illness. This would suggest that gallbladder emptying should be vigorous during critical illness which is in apparent contradistinction to the high prevalence of biliary sludge in this population – a phenomenon attributed to gallbladder dysmotility. Accordingly, it was important to quantify nutrient stimulated gallbladder motility in a critically ill population.

2.4.2 *Contribution of the work described in this thesis to the understanding of gallbladder motility in the critically ill*

The study reported in chapter 2.3 was the first to quantify gallbladder motility in the critically ill. A high fat meal was delivered directly and continuously into the small intestine to elicit maximal physiological stimulation for gallbladder contraction while avoiding confounding from variable gastric emptying rates. This study established that gallbladder dysmotility occurs frequently, with approximately half of critically ill patients observed to have sluggish to parietic nutrient-stimulated gallbladder emptying. Gallbladder emptying in this group appeared to be independent of plasma cholecystokinin concentrations.

2.4.3 *Contribution of the work described in this thesis to validating the novel technique of 3D ultrasound assessment of gallbladder volumes during critical illness*

The ultrasound technique reported in chapter 2.3 was the first to use 3D ultrasound to quantify gallbladder volume in the critically ill. It was established that when images were obtained with both 2D and 3D techniques there was close agreement between gallbladder volumes reported with minor divergence at higher volumes. However, due to magnetic distortion interfering with image acquisition, the 3D technique was

associated with a failure rate of 7% while images were obtained using the 2D technique at every time point. The clinical implication is that for future studies, 2D ultrasound is an accurate, cheap and reliable tool for determination of gallbladder volumes in a critically ill population.

2.5 FUTURE DIRECTIONS

2.5.1 The effect of gallbladder dysmotility on lipid absorption and outcome in critical illness

It is intuitive that gallbladder dysmotility and impaired delivery of bile salts into the small intestine will attenuate lipid emulsification and subsequent absorption. Lipid comprises 20-35% of most commercially available liquid nutrient formulae, but its absorption is markedly impaired during critical illness. Accordingly, studies to evaluate the effect of gallbladder dysmotility on lipid malabsorption are now warranted. While it was an essential initial step to evaluate the prevalence and severity of gallbladder dysmotility, the logical development of the study reported in chapter 2.3 is to determine whether this influences clinically important outcomes. As such, large prospective observational studies are indicated to determine the association between gallbladder dysmotility and serious complications, such as acute acalculous cholecystitis and acute pancreatitis, as well as mortality.

Finally, it is evident that there is a lack of consensus in relation to the ultrasound diagnostic criteria for acute acalculous cholecystitis. There was no clinical suspicion of acute acalculous cholecystitis in any of the patients recruited for the study presented in chapter 2.3, yet the majority fulfilled at least two of the ultrasound criteria for this pathological entity. This suggests that current criteria, at the very least, lack specificity and given the rarity of acute acalculous cholecystitis, ultrasound diagnosis will have a low positive predictive value. Modification of the diagnostic criteria for acute acalculous cholecystitis should be a priority.

CHAPTER 3

THE EFFECTS OF EXOGENOUS GLUCAGON-LIKE PEPTIDE-1 ON GASTRIC EMPTYING DURING EXTREMES OF GLYCAEMIA AND APPRAISAL OF A NOVEL INTRAVENOUS DELIVERY REGIMEN

3.1 INTRODUCTION

Relevant physiology of the incretin hormone glucagon-like peptide-1 (GLP-1) is briefly outlined in chapter 2.1, with greater detail provided in the literature review 3.2 ‘Incretins & the Intensivist: What are they and what does an intensivist need to know about them?’ This review also outlines the current commercial approach to utilise the glucose-dependent, glucose lowering properties of the incretin axis, with a particular emphasis on the potential role for these therapies in the critically ill.

GLP-1 receptor agonists are now routinely prescribed for the management of type 2 diabetes and it is well recognised that the dominant glucose lowering action of the short-acting agonists is via a slowing of gastric emptying rather than insulinotropic or glucagonostatic properties. A complex, bidirectional relationship exists between gastric emptying and glycaemia; gastric emptying is a major determinant of the variance in postprandial glycaemia yet the rate of gastric emptying is modulated by acute changes in systemic glycaemia. Given that patients with diabetes are taking GLP-1 receptor agonists in the setting of systemic hypo- and hyperglycaemia - and that GLP-1 treatment in the critically ill would also occur during extremes of systemic glycaemia - it was important to define the effects of GLP-1 on gastric emptying during extremes of glycaemia (chapters 3.3 and 3.4).

All previous studies of exogenous GLP-1 administration have utilised either continuous intravenous infusions, or depot subcutaneous injections that eventually reach relatively stable plasma concentrations. However, following nutrient-stimulation endogenous GLP-1 is released by small intestinal L cells into the blood stream in a pulsatile, rather than continuous, fashion. Insulin is similarly released in pulses and it is well established that administration of exogenous insulin in a pulsatile fashion results in greater glucose lowering potency. It was, therefore, logical to evaluate the insulinotropic effects of exogenous, pulsatile administration of pharmacological concentrations of GLP-1 (chapter 3.5).

3.2 Literature review: Incretins and the intensivist: what are they and what does an intensivist need to know about them?

Full title:

Incretins & the Intensivist: What are they and what does an intensivist need to know about them?

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Key words

Incretin hormones, glucagon-like peptide-1 (GLP-1), critical care, hyperglycaemia

ABSTRACT

Hyperglycaemia occurs frequently in the critically ill, even in those patients without a history of diabetes. The mechanisms underlying hyperglycaemia in this group are complex and incompletely defined. In health, the gastrointestinal tract is an important modulator of postprandial glycaemic excursions and both the rate of gastric emptying and the so-called ‘incretin’ hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) are pivotal determinants of postprandial glycaemia. Incretin-based therapies i.e. GLP-1 agonists and DPP-4 inhibitors, have recently been incorporated into standard algorithms for the management of hyperglycaemia in ambulant patients with type-2 diabetes and, inevitably, an increasing number of patients who were receiving these classes of drugs prior to their acute illness will present to intensive care units. This paper summarises current knowledge of the ‘incretin effect’, as well as the incretin-based therapies that are available for the management of type-2 diabetes and provide suggestions as to the potential relevance of these agents in the management of dysglycaemia in the critically ill, particularly to normalise elevated blood glucose levels.

Hyperglycaemia and critical illness: Prevalence and association with adverse outcomes

Both type 1 and type 2 diabetes mellitus (T2DM) increase the propensity for macrovascular and microvascular disease as well as infection, all of which are likely to predispose individuals to critical illness necessitating intensive care admission [1]. While the worldwide prevalence of formally diagnosed T2DM is about 3% [2], the prevalence in intensive care unit (ICU) patients is variably reported as 15-20% or even higher [3].

In the critically ill, hyperglycaemia also occurs frequently in patients without known diabetes; this group includes patients with undiagnosed type 2 diabetes and those with so-called 'stress hyperglycaemia'. We propose that the latter should be referred to as 'critical illness induced hyperglycaemia' (CIH), given that this describes the pathogenesis more appropriately. Several hormonal mechanisms appear to be important pathophysiological mediators of hyperglycaemia during critical illness. These include increases in counter-regulatory hormones, such as endogenous glucagon, catecholamines and glucocorticoids, inadequate insulin secretion for the degree of hyperglycaemia and insulin resistance [4, 5]. The exogenous administration of catecholamines, dextrose, corticosteroids and nutritional support has the potential to further exacerbate the elevation in blood glucose [4, 6].

CIH is therefore characterised by hyperglycaemia (fasting blood glucose ≥ 7 mmol/l or random blood glucose ≥ 11.1 mmol/l) in critically ill patients, who were glucose tolerant prior to their acute illness, and are shown in the longer-term not to have diabetes [1]. The true prevalence of CIH is unknown, at least in part because the vast majority of studies have failed to discriminate it from undiagnosed T2DM [3]. Nevertheless, it is clearly common - for example in the NICE-SUGAR study, >60% of patients without known diabetes in the 'control' arm had blood glucose concentrations >10 mmol/L requiring exogenous insulin [7].

Marked acute hyperglycaemia is associated with increased morbidity and mortality in the critically ill [8]. The magnitude of the elevation in blood glucose required to cause harm remains uncertain and is likely to differ between T2DM and CIH. In

observational studies, a robust association between increased mortality and hyperglycaemia has been reported consistently in patients with CIH, but not in those with pre-existing T2DM [9-11]. Moreover, preliminary, albeit retrospective and observational, data suggest that the adverse impact of hyperglycaemia is attenuated or abolished by pre-existing or chronic hyperglycaemia, and that hyperglycaemia during acute illness may, in fact, be protective [12]. Accordingly, glucose concentrations that are considered safe in patients with CIH may well be harmful in patients with type 1, (or more frequently) type 2 diabetes and chronic hyperglycaemia [12]. Furthermore, it is intuitively logical to tailor strategies for glycaemic control based on pre-existing glycaemia, rather than managing all critically ill patients as a homogenous group. Prospective studies are urgently required in this area to clarify these important issues.

The incretin effect – the historical context

The characterisation of the ‘incretin’ effect arguably began in 1902 when Bayliss and Starling, with the characterisation of secretin, speculated that the gastrointestinal tract could communicate with the pancreas via a messenger(s) in the blood [13]. Until that time, primarily as a result of Pavlov’s influence, it was thought that the functions of the body were regulated exclusively by nerves. However, it wasn’t until the early-1960s that it was demonstrated that the insulin response to enteral glucose was much greater than an intravenous (IV) glucose load, despite the latter resulting in substantially higher blood glucose concentrations [14, 15], suggesting that a hormone/s secreted from the gastrointestinal tract stimulates insulin secretion. When blood glucose concentrations resulting from oral and intravenous glucose load were matched, Perley and Kipnis observed that the plasma insulin response to intravenous glucose was approximately 40% of that resulting from the oral glucose load in health, quantifying the magnitude of the ‘incretin effect’ for the first time [16] (Figure. 1). The hormones responsible for the incretin effect were subsequently shown to be glucose-dependent insulintropic peptide (GIP) (in 1973) and glucagon-like peptide 1 (GLP-1) (in 1985).

GIP is secreted predominantly from duodenal K cells, in response to luminal fat and carbohydrate [17] while GLP-1 is secreted from intestinal L cells, located primarily in the distal ileum and colon, primarily in response to luminal fat, carbohydrate, protein

and bile acids [18, 19]. At least in health, both GIP and GLP-1 are insulinotropic and, in the case of GLP-1, glucagonostatic in a strictly glucose-dependent fashion, so that below a blood glucose threshold of ~8 mmol/L even pharmacological doses of these hormones have little or no effect on fasting blood glucose [20]. In addition to its insulinotropic and glucagonostatic properties, GLP-1 is a physiological modulator to slow gastric emptying [17, 21], while GIP probably has no effect on gastric emptying and is glucagonotropic during euglycaemia [22].

Attenuation of the incretin effect and pathogenesis of hyperglycaemia in type 2 diabetes

In 1986, Nauck et al reported that the magnitude of the incretin effect was markedly diminished in longstanding T2DM [23] and went on to show that the insulinotropic effect of GIP is also attenuated in this group, while that of exogenous GLP-1 is relatively preserved [24]. It is still uncertain whether the reduced incretin effect is a primary or epi-phenomenon of beta-cell failure [25] but it is apparent that the reduced insulinotropic effect of GIP is, in part, an effect of hyperglycaemia and, hence, reversible [25]. Nevertheless, these observations stimulated the development of GLP-1 based pharmacological therapy for the management of T2DM. It should be recognized that a limitation of these early studies is that gastric emptying was not quantified. This is important because the rate of nutrient entry into the small intestine is a major determinant of both GLP-1 and GIP secretion [26]. Studies employing direct, intraduodenal infusion of nutrients indicate that the secretion of GIP and GLP-1 is maintained in both T2DM [26, 27] and the critically ill [28].

Drug development of incretin therapies for the management of T2DM

Initial, proof of principle studies were performed using pharmacological doses of GLP-1 administered by intravenous or subcutaneous infusion. As well as stimulating insulin [29] and inhibiting glucagon secretion, both in a glucose-dependent manner [30], GLP-1 has been shown to have several important and unique pharmacodynamic properties that enhance its therapeutic potential. GLP-1 dose-dependently slows gastric emptying, thereby attenuating postprandial glycaemic excursions [31].

Exogenous GLP-1 also inhibits appetite resulting in reduced energy intake and sustained weight loss in obese subjects, features that are beneficial in patients with T2DM [32]. In-vitro and animal studies have shown that GLP-1 exerts trophic effects on the pancreatic β -cell, stimulating proliferation and differentiation [33], as well as reducing β -cell apoptosis [34]. Hence, it may have the potential to preserve β -cell mass in type 2 diabetics. Pharmacological administration of GLP-1 has significant extra-gastrointestinal effects with the potential cardioprotective properties of particular relevance to the critically ill. For example; in animal models GLP-1 attenuates ischaemia-induced myocardial damage [35], in patients with heart failure exogenous GLP-1 has been associated with improvements in left ventricular ejection fraction, myocardial oxygen uptake and 6-minute walking distance [36], and when administered post coronary artery bypass grafting GLP-1 is associated with less use of sympathomimetic drugs and fewer arrhythmias [37]. There is evidence, hitherto derived from animal studies, that GLP-1 may possess neurotropic effects [38] which have the potential to be beneficial in the management of neurodegenerative conditions such as Alzheimer's disease [39]. The implications in the neurocritically ill remain uncertain [40, 41].

Currently available incretin based agents for use in type II diabetes

The administration of GLP-1 to ambulant patients with diabetes proved clinically impractical. Endogenous GLP-1, and its synthetic peptide, has a plasma half-life of only 1-2 minutes [42], due to rapid metabolism by the ubiquitous, non-specific enzyme, dipeptidyl-peptidase 4 (DPP-4), which forms an inactive metabolite that is rapidly cleared by the kidneys [43], necessitating continuous infusion. The pharmaceutical industry has therefore focused on the development of stable DPP-4 resistant GLP-1 receptor agonists, as well as DPP-4 inhibitors that protect endogenously secreted GLP-1, GLP-2 and GIP from degradation. DPP-4 inhibitors act as incretin enhancers, increasing systemic and, perhaps of more pertinence to their effect, intestinal concentrations of endogenously secreted GLP-1, GLP-2 and GIP [44]. GLP-1 agonists are incretin mimetics, acting as agonists at the GLP-1 receptor and, in contrast to DPP-4 inhibitors, do not stimulate GIP or GLP-2 and may reduce

endogenous GLP-1 concentrations [45], possibly because of its effect on gastric emptying [21].

Incretin-based therapies are frequently prescribed and their use is expected to become even more common with recent international guidelines recommending that GLP-1 agonists and DPP-4 inhibitors be used as part of standard regimens for patients with T2DM [46].

GLP-1 receptor agonists

A GLP-1 agonist candidate was fortuitously identified in the peptide, exendin-4, from the salivary venom of the Gila Monster (*Heloderma suspectum*). The Gila Monster is a slow moving venomous lizard native to North America that ingests food 5-10 times per year and ingestion which results in a substantial increase in plasma exendin-4 concentrations that may have endocrine functions related to metabolic control in the lizard [47]. Exendin-4 is a peptide with ~50% amino acid sequence homology to GLP-1 and acts as a full agonist at the GLP-1 receptor. Exendin-4 is also resistant to metabolism by DPP-4, and is eliminated only by glomerular filtration, with a resultant plasma half-life of 30 minutes [48]. Exenatide was developed as the synthetic replica of exendin-4 and in 2005 was the first incretin based therapy approved by the US Food and Drug Administration. There are now at least 8 GLP-1 agonists undergoing clinical trials, however, twice daily exenatide, once daily lixisenatide, once daily liraglutide and once-weekly extended-release exenatide (exenatide-ER) are the only agents to have hitherto reached the market.

GLP-1 agonists can be categorised as short or long-acting compounds. All are given subcutaneously and stimulate fasting insulin secretion and reduce glucagon secretion in a glucose dependent manner [49]. Dose-dependent nausea, vomiting and diarrhoea occur frequently and while it is recognised as a far more prominent feature of the synthetic agonists when compared to the native peptide, it does decline with time and is attenuated by dose titration [50, 51]. Short and long-acting compounds do, however differ, in their effects on fasting and post-prandial glucose.

Short acting GLP-1 agonists

Exenatide and lixisenatide are considered ‘short-acting’ GLP-1 agonists and their short half-lives result in large fluctuations in circulating plasma concentrations [49]. The dominant effect of these drugs on glycaemia is mediated by the slowing of gastric emptying, thereby markedly blunting postprandial glycaemic excursions [52]. Therefore, patients with diabetes with significant postprandial hyperglycaemia and relatively well-controlled fasting glycaemia achieve the greatest benefit from these short-acting compounds. Additionally the combination of short-acting GLP-1 agonist and basal insulin confers complementary effects, with control of fasting hyperglycaemia by the basal insulin, control of postprandial glycaemic excursions with the GLP-1 agonists, a reduction in glycaemic variability and a low risk of hypoglycaemia [49].

Exenatide is approved in Europe as adjunctive therapy in patients with inadequately controlled T2DM who are taking oral hypoglycaemic agents, basal insulin or both. It has also been approved for use as monotherapy in the United States. In February 2013, lixisenatide was approved in Europe as adjunctive therapy in patients with inadequately controlled T2DM, who are taking oral hypoglycaemic agents, basal insulin or both. Their pharmacological and pharmacodynamics properties are summarised in tables 1 & 2 respectively.

Long-acting GLP-1 agonists

Exenatide-ER and liraglutide are ‘long-acting’ GLP-1 receptor agonists that have continuously elevated plasma concentrations between their recommended dosing intervals. They are approved in Europe and the United States to be used in the treatment, of T2DM as monotherapy or as second-line therapy in combination with oral hypoglycaemic agents. They achieve higher fasting insulin levels and greater reductions in fasting glucose concentrations [49]. The continuous activation of the GLP-1 receptor is thought to induce tachyphylaxis to the deceleration of gastric emptying, such that the slowing of gastric emptying diminishes markedly with long-term use, and consequently the lowering of postprandial glycaemia is only modest [53]. Long-acting compounds therefore seem to be advantageous when clinicians wish to target fasting hyperglycaemia in patients with T2DM.

DPP-4 Inhibitors

The DPP-4 inhibitors are orally administered agents and approved as monotherapy, or in combination with other oral hypoglycaemic agents for the management of T2DM. It is thought that the predominant mechanism of DPP-4 inhibitor action is via a gut effect involving local inhibition of intestinal DPP-4 activity, stimulation of gut incretin receptors and activation of gut-to-pancreas neural pathways, rather than a systemic increase in incretin plasma concentrations [44]. DPP-4 is expressed widely throughout the body, including on T-lymphocytes and macrophages, posing a theoretical risk of immune dysfunction with enzyme inhibition [54]. However, a meta-analysis reported a very minor increase in all-cause infection during sitagliptin treatment that was not evident in studies involving other DPP-4 inhibitors [55]. The reliability and significance of this association remains uncertain. The presentation, dose, contraindications and pharmacodynamics of the DPP-4 inhibitors are outlined in tables 1 and 2.

Extra-pancreatic effects of incretin-based therapies

The cardiovascular outcomes of the incretin based drugs are currently undergoing assessment in large, multicenter clinical trials [56]. Data from pre-clinical studies, short-term single center human studies and retrospective reviews of healthcare claims support a cardioprotective role for both GLP-1 receptor agonists and DPP-4 inhibitors [57]. The majority of clinical trials have reported reductions in body weight and blood pressure albeit with a mean increase in heart rate of 2-4 beats/min [57].

Finally, there are post-marketing reports of acute pancreatitis during use of GLP-1 agonists and DPP-4 inhibitors [58]. However, when compared to health, the risk of acute pancreatitis is more than doubled in T2DM, independent of treatment modality [59], and the incidence of pancreatitis appears to be no greater when starting these agents than in patients who are commenced on metformin or a sulfonylurea [60]. For these reasons we consider the risk of GLP-1 or DPP-4 inhibitor induced pancreatitis as negligible. There have also been concerns, principally from rodent studies, that the use of incretin-based therapies may be associated with chronic pancreatitis [61], particularly as this would intuitively also increase the risk of pancreatic cancer [62]. Hitherto these potential risks have not been substantiated [63] and while ongoing post-

marketing surveillance is important, any increase in risk is likely to be extremely small and unlikely to be relevant to short-term use in critically ill patients.

Rationale for the use of GLP-1 as a novel therapy in the critically ill

Administration of intravenous insulin guided by validated algorithms is the current standard of care for hyperglycemic critically ill patients [64]. While the optimal blood glucose for the critically ill remains contentious, concentrations > 10 mmol/L should be avoided, at least for those without pre-existing diabetes and chronic hyperglycaemia [7]. Several studies have demonstrated that insulin induced hypoglycaemia (blood glucose < 2.2 mmol/L) occurs more frequently with intensive insulin therapy and that it is an independent risk factor for mortality [7, 65, 66]. Moreover, in the NICE-SUGAR study ‘moderate’ (≥ 2.3 to ≤ 3.9 mmol/L) and ‘severe’ hypoglycaemia (< 2.3 mmol/L) were both associated with insulin use and increased mortality, with the lowest nadirs in glucose concentrations having the strongest association with death [67]. This may be of particular relevance in neurocritical care, particularly as there is evidence that systemic glucose concentrations even within the normal physiological range may be associated with cerebral hypoglycaemia and consequent adverse outcomes [68]. These data, accordingly, strongly suggest that treatment-induced hypoglycaemia should be avoided.

Regardless of target blood glucose, intravenous insulin therapy also carries the logistic limitations of frequent monitoring and training of nursing staff in complicated protocols. Additionally, increased glycaemic variability appears to be an independent risk factor for ICU mortality [69, 70] and although speculative, a therapy that reduces glycaemic variability may lead to improved outcomes.

By inhibiting glucagon secretion as well as increasing insulin secretion, GLP-1 or its agonists provide a plausible pharmacodynamic mechanism to counter the hyperglucagonemia and relative insulin resistance that typifies stress hyperglycemia. Furthermore, the cardioprotective properties hitherto demonstrated in incretin-based therapies are particularly attractive in the intensive care setting. In animal models, GLP-1 decreases ischaemia-induced myocardial damage [35], in patients with heart failure, exogenous GLP-1 has been associated with improvements in left ventricular

ejection fraction, myocardial oxygen uptake and 6-minute walking distance [36] and when administered post coronary artery bypass grafting is associated with less use of sympathomimetic drugs and fewer arrhythmias [37].

For the reasons outlined in table 3, a simplified therapy for the treatment of hyperglycaemia that avoided iatrogenic hypoglycaemia and limited glycaemic variability would be desirable.

Recommendations for incretin based therapies in critically ill patients.

Critical illness may markedly affect pharmacokinetics, particularly for the oral and subcutaneous routes [71]. Furthermore, multi-organ failure with evolving hepatic and renal dysfunction and the consequent derangements in metabolism and elimination may result in unpredictable half-lives and drug clearance [71]. For these reasons, and also because of limited experience with these incretin-based agents, it is appropriate to discontinue these drugs when critically ill patients are admitted to ICU.

In hyperglycaemic patients transitioning from intensive, to ward care, current recommended practice is to commence scheduled subcutaneous basal-bolus insulin regimens [72], although the ADA consensus statement concedes that noninsulin agents may be appropriate in selected, stable patients who are expected to consume meals at regular intervals [72]. There have been no randomised controlled trials to compare subcutaneous basal-bolus insulin with the incretin-based agents in this patient group. However, because of the limitations inherent in basal-bolus insulin administration, in patients who were on incretin-based therapies prior to their acute illness and who are improving clinically and transitioning from the ICU to the hospital ward, recommencement of the established incretin regimen would be reasonable and should be evaluated formally as a potential therapeutic option.

Experience using incretin-based therapy in the critically ill

Hitherto, the use of GLP-1 in the critically ill is limited to small studies to establish proof-of-principle [73]. A limitation of all of these studies has been the short duration of GLP-1 administration (<72 hours), however in patients with T2DM, glucose

lowering was maintained for up to 6 weeks during continuous subcutaneous GLP-1 infusion [74] suggesting that tachyphylaxis, at least to the glucose-lowering effect, does not occur. It should also be recognised that while synthetic GLP-1 is currently prohibitively expensive, there may be a substantial reduction in cost should a market become available.

With intravenous GLP-1 infusions at doses ranging from 1.2 to 3.6 pmol/kg/min, marked glucose-lowering has been observed in patients with T2DM post major surgery [75], coronary artery bypass grafting [37, 76] and acute myocardial infarction with primary angioplasty [77]. Our group has evaluated the effects of GLP-1 infusions in heterogeneous cohorts of mechanically ventilated patients, both with T2DM and CIIH [78-80]. GLP-1 infusions at 1.2 pmol/kg/min were shown to reduce the glycaemic response to small intestinal nutrient in mechanically ventilated patients with chronic hyperglycaemia [80] and to intra-gastric and small intestinal nutrient delivery in patients not known to have T2DM (figure 2) [78, 79]. At this dose, nutrient-induced hyperglycaemia was attenuated, but not suppressed completely, and the effect appears to be more prominent in patients without a history of T2DM. We also reported that GLP-1 slows gastric emptying when emptying is relatively normal, but has minimal effect when emptying is already delayed, consistent with studies in ambulant type 2 diabetics [79]. The infusions were well tolerated in all patient groups and there were no reported episodes of hypoglycaemia.

Administration of intravenous exenatide is also being explored, as currently these drugs are substantially cheaper than the synthetic peptide. As outlined (table 1), exenatide is only approved for subcutaneous administration in the ambulant type-2 patient, however, there is experience with its intravenous use in trial settings [81, 82]. Abuannadi and colleagues administered intravenous exenatide for 48 hours in an open-label, non-randomized, pilot study in 40 cardiac intensive care unit patients [83]. The effectiveness of exenatide was benchmarked to glucose concentrations previously recorded during intensive and moderate insulin therapies [83]. Mean blood glucose concentrations with intravenous exenatide were non-inferior to the NICE-SUGAR endorsed insulin protocol (i.e. moderate), and glycaemic variability was reduced [83].

No episodes of severe hypoglycaemia were reported in the exenatide group, however, nausea (40%) and vomiting (5%) occurred frequently [83]. It remains to be determined if these agents could be used as stand alone therapy or as ‘insulin-sparing’ agents with the benefits of reduced hypoglycaemia and diminished glycaemic variability. Further studies using the commercially marketed GLP-1 agonists are warranted [84].

While studies in the critically ill have focussed on GLP-1 and its agonists, the role of GIP and DPP-4 inhibitors also warrants consideration [84]. While it is known that the insulinotropic effect of GIP is markedly reduced in T2DM this may be primarily due to hyperglycaemia, rather than an inherent cellular defect [85]. In the critically ill, our group demonstrated that exogenously administered GIP with concurrent GLP-1 does not lower blood glucose concentrations, either in the fasted state or in response to small intestinal nutrient, more than GLP-1 alone [86]. However this was a small, single centre pilot study and further research to clarify whether GIP retains its insulinotropic effect in critically ill patients, in whom blood glucose concentrations have only been transiently elevated, merits further investigation.

The DPP-4 inhibitors have not yet been investigated as a therapeutic strategy for CIIH. Delayed gastric emptying occurs frequently in critical illness with absolute gastroparesis not infrequent [87], and also, at least in health, hyperglycaemia per se slows gastric emptying [88]. Accordingly, intragastric administration of an oral glucose-lowering drug may not be the most effective route. Nevertheless in patients with known normal gastric emptying or with post-pyloric administration in patients with delayed gastric emptying, the local intestinal mechanism of action supports the study of the renally ‘safe’ DPP-4 inhibitor linagliptin as a potential novel oral agent for glycaemic control in the critically ill.

Conclusions

The discovery of the incretin hormones has encouraged a burgeoning pharmaceutical industry that exploits the entero-insular axis. As the prevalence of T2DM and the use of incretin-based regimens continue to rise, the intensivist is likely to encounter more patients who were taking these drugs prior to their acute illness. Presently, there is little experience with continuing these agents in acute illness and more study is urgently required.

Nevertheless the pharmacologic strategy of administering incretin mimetics to manage acute hyperglycaemia in the critically ill, while minimising the risk of hypoglycaemia and mitigating glycaemic variability, is appealing. Future studies should focus on the identification of the patient groups most likely to benefit from the administration of incretin receptor agonists, which of the agents is most useful in the critically ill, and at what dose, the relevance of the route of feeding with concurrent incretin therapy and finally whether these agents should be used in combination with insulin or as single-agent therapy to provide a safer, more effective and simpler method of achieving glycaemic control.

List of abbreviations

CIIH: critical illness induced hyperglycaemia

DPP-4: dipeptidyl-peptidase-4

Exenatide-ER: extended release exenatide

GIP: glucose-dependent insulinotropic polypeptide

GLP-1: glucagon-like peptide-1

ICU: intensive care unit

T2DM: type 2 diabetes mellitus

Competing interests

Michael Horowitz has participated in advisory boards and/or symposia for Novo/Nordisk, Sanofi-aventis, Novartis, Eli-Lily, Boehringer Ingelheim, AstraZeneca, Satlogen and Meyer Nutraceuticals. All other authors have no competing interests to declare.

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Table 1: Marketed incretin based agents

Therapy	Approval	Dose	Route	Precautions
GLP-1 receptor agonists				
<i>Exantide</i> (Byetta®)	EMA, US FDA	5, 10 µg	SC, BD	Renal impairment†
<i>Lixisenatide</i> (Lyxumia®)	EMA	10, 20 µg	SC, OD	Renal impairment†
<i>Exenatide ER</i> (Bydureon®)	EMA, US FDA	2 mg	SC, QW	Renal impairment†
<i>Liraglutide</i> (Victoza®)	EMA, US FDA	0.6, 1.2, 1.8 mg	SC, OD	MTC of MEN2
DPP-4 Inhibitors				
<i>Sitagliptin</i> (Januvia®)#	EMA, US FDA	25, 50, 100 mg	Oral OD	Renal impairment‡
<i>Vildagliptin</i> (Galvus®)#	EMA	50 mg	Oral BD	Renal impairment† Hepatic impairment
<i>Alogliptin</i> (Nesina®)#	US FDA	25mg	Oral OD	Renal impairment‡
<i>Saxagliptin</i> (Onglyza®)	EMA, US FDA	2.5, 5mg	Oral OD	Renal impairment‡
<i>Linagliptin</i> (Trajenta®)	EMA, US FDA	5mg	Oral OD	-

Available in a fixed-dose combination with metformin

† Contraindicated in patients with severe renal impairment (CrCl <30mL/min) and end stage renal disease

‡ Dose adjustment required in moderate (CrCl ≥30 to <50 mL/min), severe and end stage renal disease

BD, twice daily; EMA, European Medicines Agency; ER, extended release; MEN, multiple endocrine neoplasia type 2; MTC, medullary thyroid carcinoma; OD, once daily; SC, subcutaneous; QW, once week; US FDA, U.S. Food and Drug Administration

Table 2: Pharmacodynamics: GLP-1 agonists vs DPP-4 inhibitors

	GLP-1 agonists	DPP-4 Inhibitors
Plasma GLP-1 concentrations	Pharmacological concentrations of the GLP-1 analogue (6-10 fold > than endogenous GLP-1)	2-3 fold increase
Glucose-dependent insulin secretion	Yes	Yes
Glucose-dependent inhibition of glucagon secretion	Yes	Yes
HbA _{1c} reduction	0.8-1.8%	0.5-1.1%
Inhibition of gastric emptying	Short acting only	No
Post-prandial glucose reduction	Yes: short-acting > long-acting	Yes (weaker)
Fasting plasma glucose reduction	Yes: long-acting > short acting	Yes (weaker)
Effects on weight	Significant weight loss	Weight neutral
Inhibition of food intake	Yes	No
Gastrointestinal adverse effects: nausea, vomiting and diarrhoea	Common	Uncommon

Table 3: Potential benefits of GLP-1 based therapies in the critically ill

Negligible risk of severe hypoglycaemia
Reduction in insulin requirement
Cardiovascular protection
Attenuated glycaemic variability
Amenable to continuous infusion without dose titration
Decreased blood glucose testing
Simplified protocol for medical and nursing staff

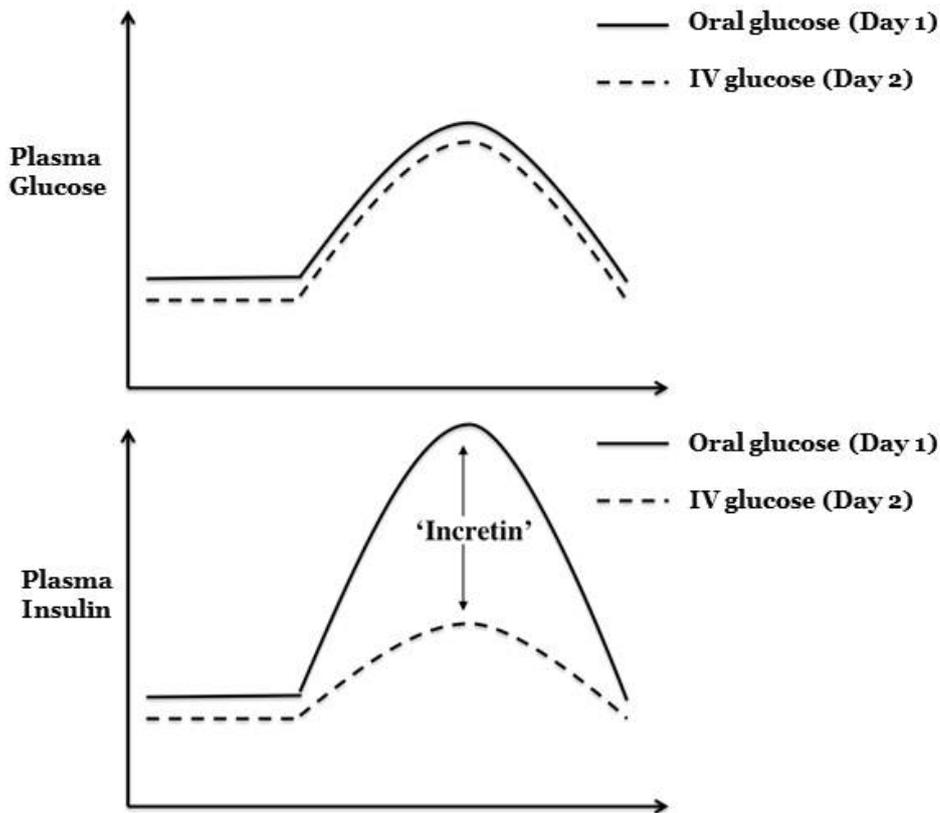


Fig 1. The Incretin Effect

Adapted from *Perley and Kipnis, J Clin Invest, 1967* [16]. There is a much greater release of insulin in response to oral glucose administration as compared to administering the same amount of glucose by intravenous infusion. Subjects were given oral glucose on day 1 with plasma insulin levels recorded. The same volunteers returned on a second day and an intravenous glucose infusion was titrated to match the plasma glucose excursion achieved with the oral load. The difference in the measured plasma insulin is the 'incretin effect', mediated by the hormones Glucagon-Like Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP).

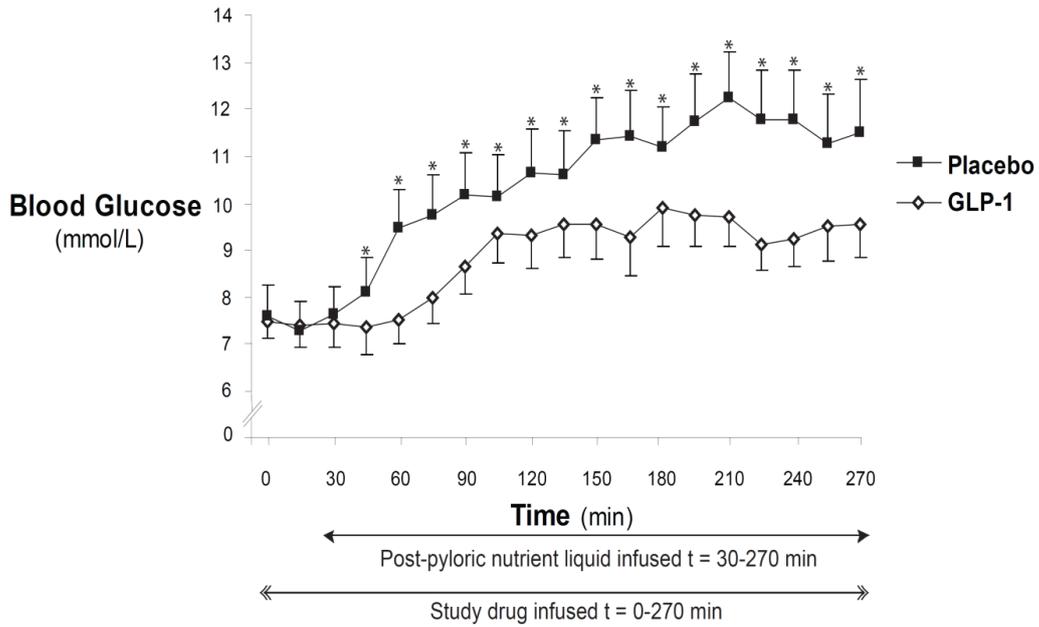


Fig 2. Glucose lowering effect of GLP-1 in critical illness

In critically ill patients without known diabetes the blood glucose excursion in response to small intestinal feeding is markedly attenuated by GLP-1 1.2pmol/kg/min (AUC_{240min} GLP-1: 2077 ± 145 mmol/l min vs. placebo 2568 ± 208 mmol/l min; $p < 0.01$). Data are mean \pm SEM (n=7). Reproduced with permission: *Deane et al, Crit Care, 2009* [78].

3.3 Manuscript: Glucagon-Like Peptide 1 attenuates the acceleration of gastric emptying induced by hypoglycaemia in healthy subjects

Full title

Glucagon-like peptide-1 attenuates the acceleration of gastric emptying induced by hypoglycemia in healthy subjects

Running Title

GLP-1 slows hypoglycemic gastric emptying

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ABSTRACT

Objective: Exogenous GLP-1 slows gastric emptying in health and diabetes leading to diminished glycemic excursions. Gastric emptying is markedly accelerated by hypoglycemia. The primary objective was to determine whether GLP-1 attenuates the acceleration of gastric emptying induced by hypoglycemia.

Design and methods: Ten healthy volunteers were studied on 4 separate days in a randomized double-blind fashion. Blood glucose was stabilised using a glucose/insulin clamp at hypoglycemia (2.6mmol/L; *hypo*; on 2 occasions), or euglycemia (6.0mmol/L; *eu*; on 2 occasions), between T=-15 to 45min, before clamping at 6.0mmol/L until 180min. During hypoglycemia and euglycemia subjects received intravenous GLP-1 (1.2pmol/kg/min) or placebo. At T=0min subjects ingested 100g of beef mince labeled with 20MBq ^{99m}Tc-technetium-sulfur-colloid and 3g of 3-O-methyl-glucose (3-OMG), a marker of glucose absorption. Gastric emptying was measured scintigraphically from T=0 to 180min and serum 3-OMG taken at 15min intervals. The areas under the curve (AUC) for gastric emptying and 3-OMG concentration were analysed using one-way RM-ANOVA with Bonferroni-Holm adjusted posthoc tests.

Results: Gastric emptying was accelerated during hypoglycemia (*hypo*/placebo vs *eu*/placebo; $P < 0.001$) as was glucose absorption ($P < 0.03$). GLP-1 slowed emptying during euglycemia (*eu*/placebo vs. *eu*/GLP-1; $P < 0.001$). However, hypoglycemia-induced acceleration of gastric emptying on placebo was markedly diminished by GLP-1 (*hypo*/placebo vs. *hypo*/GLP-1; $P < 0.008$) as was glucose absorption ($P < 0.01$).

Conclusions: Acute administration of exogenous GLP-1 attenuates, but does not abolish, the acceleration of gastric emptying by insulin-induced hypoglycemia in healthy subjects.

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) receptor agonists are now incorporated into standard treatment algorithms for the management of type 2 diabetes [1]. Several GLP-1 receptor agonists are available and are increasingly used as monotherapy, second-line therapy (particularly with metformin and/or sulphonylurea), and, more recently, in combination with basal insulin [1, 2].

The development of GLP-1 agonists was stimulated by characterization of the effects of exogenous GLP-1 to lower both fasting and postprandial glycemia [3]. During fasting the glucose-lowering effect of GLP-1 is predominantly mediated through effects on islet cell function to increase insulin and reduce glucagon secretion in a glucose-dependent manner [4, 5]. Accordingly, GLP-1 and its agonists are extremely unlikely to cause hypoglycemia when used as monotherapy.

In contrast to the fasted state, during the postprandial phase, glucose-lowering by acute administration of GLP-1 is mediated primarily through its effect to slow gastric emptying [6]. Indeed, postprandial insulin concentrations are reduced by administration of GLP-1 because of the delayed entry of nutrients into the small intestine [6]. This also appears to be the case with 'short-acting' GLP-1 agonists such as exenatide BD [7] and lixisenatide [8], which have a sustained effect to slow gastric emptying. Additionally, because the human stomach empties at an overall rate of 1-4 kcal/min in health [9], most humans are predominantly in the postprandial state with the duration of fasting limited to approximately 4 h before breakfast [10]. Hence, the effect of GLP-1, and its agonists, to slow gastric emptying is of fundamental significance and provides a persuasive rationale for their combination with basal insulin, given that this approach targets both fasting and postprandial glycemia, and doesn't lead to weight gain [10].

Given that GLP-1 agonists may be combined with a sulphonylurea or insulin there is, however, the potential for hypoglycemia. For this reason it is reassuring that in health pharmacological doses of GLP-1 have no effect on the counter-regulatory hormonal response to insulin-induced hypoglycemia [5]. Somewhat surprisingly however the effect of GLP-1 on gastric emptying during hypoglycemia has not been evaluated.

Acute glycemia *per se* is a major determinant of the gastric emptying rate, and hypoglycemia potently accelerates gastric emptying in both healthy subjects [11] and patients with type 1 diabetes [12]. Conversely, marked hyperglycemia slows gastric emptying [13, 14], and even glycemic perturbations that are within the normal physiological range influence gastric emptying in health and patients with type 1 diabetes [14]. Indeed systemic glycemia has been shown to alter the effect of gastrokinetic drugs, for example hyperglycemia potently attenuates the gastrokinetic effect of erythromycin [15]. However, the interaction of hypoglycemia with drugs that slow gastric emptying has not yet been investigated in humans.

The acceleration of gastric emptying during hypoglycemia is likely to be a physiological protective mechanism that increases delivery and absorption of carbohydrate. Indeed the current ADA guidelines for the treatment of hypoglycemia emphasise the importance of carbohydrate ingestion [16]. While the insulinotropic and glucagonostatic effects of GLP-1 and its agonists are established as glucose-dependent, the effect of hypoglycemia on the GLP-1 induced-slowness of gastric emptying is unknown. This is of particular importance considering the interest in combinations of GLP-1 agonists and basal insulin, which may increase the risk of hypoglycemia when compared to GLP-1 monotherapy. The primary aim of this study was to determine whether GLP-1 attenuates the acceleration of gastric emptying induced by acute hypoglycemia in health.

RESEARCH DESIGN AND METHODS

Subjects

Healthy volunteers aged 50-75 years were eligible, and those with diabetes (HbA1c>6.5%; 48 mmol/mol), BMI \geq 30 kg/m², impaired renal function or anemia, currently smoking, consuming >20g/day of alcohol, receiving medication known to affect gastrointestinal motility or glycemia, or with a history of gastric or small intestinal surgery, were excluded.

Protocol

All subjects were studied in random order on four separate occasions separated by a minimum of 4 days (*Fig. 1*). Each subject underwent concurrent measurements of

gastric emptying, blood glucose concentrations, and glucose absorption on each of the four occasions: twice with blood glucose concentrations maintained at euglycemia [blood glucose of 6 mmol/L (108 mg/dL); *eu*] and twice with a period of insulin-induced hypoglycemia [blood glucose of 2.6 mmol/L (47 mg/dL); *hypo*] (*Fig. 1 and 2*). Randomization was performed by the Department of Pharmacy at the Royal Adelaide Hospital who notified study investigators M.J.P., C.E.A. and C.E.C. of the glucose target on a particular study day. These investigators played no role in analysis of gastric emptying data. Randomization of study drug, either GLP-1 or placebo, was also performed by the Department of Pharmacy and allocation of the study drug was concealed to all investigators throughout the study. Either GLP-1 (1.2 pmol/kg/min) or placebo (0.9% saline) was administered intravenously during both euglycemia and hypoglycemia.

Each subject attended the Department of Nuclear Medicine, PET and Bone Densitometry at the Royal Adelaide Hospital at 0830 h after an overnight fast. Two intravenous cannulae were inserted into the right arm; one in the antecubital vein for an infusion of insulin and 25% dextrose, and another in the dorsal vein of the right hand for infusion of study drug. A third intravenous cannula was inserted into the left antecubital vein for blood sampling. Synthetic GLP-1 amide (Bachem, Weil am Rhein, Germany) was reconstituted by the Department of Pharmacy in 0.9% normal saline. After drawing baseline blood specimens, both GLP-1 (1.2 pmol/kg/min) and placebo (0.9% saline) infusions were commenced 60 min prior to meal ingestion, and infused at a rate of 1ml/min for the duration of the study (i.e. T=-60 to 180 min) [17]. The infusion rate of 1.2pmol/kg/min was based on previous studies and was known to raise plasma GLP-1 concentrations into the pharmacological range (approximately 3-4 fold higher concentrations in comparison to those measured after oral glucose) [5, 18, 19] and is representative of GLP-1 receptor stimulation that occurs during administration of commercial agonists [4, 20].

An insulin-glucose clamp was started 30 min later as described below. After blood glucose concentrations had been stabilized at the desired level for 15 min, subjects consumed the test meal within 5 min.

The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee and registered (www.anzctr.com.au; ACTRN12611000973910). Written informed consent was obtained from all subjects prior to their inclusion.

Stabilization of blood glucose concentrations

An insulin-glucose clamp was started at T=-30 min with a continuous infusion of human insulin (Actrapid; Novo Nordisk Pharmaceuticals, Auckland, New Zealand). 50 IU of insulin was drawn up into 50ml of normal saline. The protocol was modified from a hypoglycemic clamp algorithm administered to patients with type 1 diabetes [12]. The infusion for the current study was commenced at 125 mU/m²/min then titrated over 10 minutes to a maintenance rate of 40mU/m²/min. A 25% dextrose infusion was given simultaneously at a varying rate to maintain the blood glucose at the desired level [12]. On the euglycemic study days, blood glucose was maintained at 6.0 mmol/L (108 mg/dL) from T=-30 min to the completion of the study at T=180 min with 25% dextrose infused at rates of 50-200ml/h. During the hypoglycemic studies, the blood glucose was stabilised at 2.6 mmol/L (47 mg/dL) with infusion of 25% glucose at rates of 0-12 ml/h for 15 min prior to meal ingestion and then maintained at this blood glucose concentration for a further 45 min after completion of the meal [12]. The blood glucose was then titrated to 6.0 mmol/L over a 30 min period (i.e. to T=75 min) and maintained at 6.0 mmol/L for the remainder of the study (*Fig. 2*).

Starting immediately before the commencement of the insulin-glucose clamp, venous blood samples for measurement of glucose were taken every 5 min until T=90 min, and then every 15 min until study completion at T=180 min. Blood glucose concentrations were measured using a portable glucose meter (Optium Xceed; Abbott Laboratories, Bedford, Massachusetts, USA) [12].

Measurement of gastric emptying

The test meal comprised 100g lean minced beef, labeled with 20 MBq of ^{99m}Techetium-sulfur-colloid (Pharmalucence Inc; Bedford, Massachusetts, USA) [12], and 3g of 3-O-Methyl-D-glucopyranose (3-OMG; Sigma-Aldrich, Sydney, NSW, Australia) dissolved in 150ml of water. The solid meal was consumed over 5 min, followed by ingestion of the 3-OMG-labeled water. Scintigraphic data were

acquired with a gamma camera (Digirad, Poway, California, USA) placed over the abdomen of the participant to obtain a left anterior oblique (LAO) image. Subjects were lying in a supine position with the upper body at an angle of approximately 30 degrees. Data were acquired from meal completion (T=0 min) in 1 min frames for 180 min and corrected for radionuclide decay, gamma ray attenuation (using the LAO view), and subject movement [15]. Radioisotopic data were analysed by a nuclear medicine scientist (K.L.J.) who was not present during studies and remained blinded to both the treatment arm and the glycemic period assigned. A region-of interest was drawn around the total stomach and a gastric emptying curve, expressed as total retention over time, was derived from this region [15].

Measurement of glucose absorption

Serum 3-OMG was used as an index of intestinal glucose absorption [21, 22], and was measured using liquid chromatography/mass spectroscopy, with an assay sensitivity of 0.0103 mmol/L [22]. Following the test meal, blood was collected at T=15, 30, 45, 60, 90, 120, 150 and 180 min and, once clotted, centrifuged at 3,200 rpm for 15 min. Serum was then stored at -70 degree Celsius for subsequent measurement of 3-OMG concentrations, with the rate of glucose absorption indicated by the area under the 3-OMG concentration curve (AUC) [22].

Statistical analysis

Power calculations were performed using our data relating to the effect of exogenous GLP-1 on gastric emptying in health [23]. Overall effects for both gastric emptying and 3-OMG absorption were calculated as AUC_{0-180} . Given the strict hypoglycemic period occurred in the first 45 min (AUC_{0-45}), this period was also predefined as of interest. Data were evaluated using one-way repeated measures analysis of variance (ANOVA), with Bonferroni Holm adjusted post hoc tests for multiple comparisons. Data are shown as mean values \pm SEM, with the difference between groups (Δ) reported as mean (SD). Given that we have reported an association between glucose absorption and gastric emptying [21, 22], we tested for this relationship. This correlation was evaluated adjusted for repeated measures [24]. The null hypothesis was rejected at the 0.05 significance level. Statistical analyses were performed using SPSS (version 16.0; SPSS, Chicago, Illinois). All analyses were supervised by an independent professional biostatistician.

RESULTS

All of the subjects tolerated the study without adverse effects. During hypoglycemia all volunteers experienced a range of moderate symptoms including sweating, palpitations and visual disturbance but there were no major adverse events. One patient experienced mild nausea with GLP-1 during a euglycemic study day. Blood glucose concentrations were effectively clamped at hypoglycemic and euglycemic targets on both GLP-1 (2.7 ± 0.03 and 5.8 ± 0.05 mmol/L) and placebo (2.8 ± 0.04 and 5.9 ± 0.03 mmol/L) study days (*Fig.2*).

To maintain the glycemic clamps, substantially less (IV) dextrose was required during hypoglycemia when compared to euglycemia (hypo/placebo vs. eu/placebo; Δ 36 (11) g; $P = 0.018$). GLP-1 increased (IV) dextrose required during euglycemia (eu/GLP-1 vs. eu/placebo; Δ 25 (6) g; $P < 0.01$) and hypoglycemia (hypo/GLP-1 vs. hypo/placebo; Δ 23 (8) g; $P = 0.03$).

Solid gastric emptying

Gastric retention (%) over time is shown in *Figure 3*. The initial ANOVA for all four curves was significant at both 45 ($P = 0.003$) and 180 minutes ($P < 0.001$). Gastric retention was less during hypoglycemia at both 45 mins (AUC_{0-45} , hypo/placebo vs. eu/placebo; $P < 0.01$) and overall (AUC_{0-180} , $P < 0.001$), consistent with more rapid gastric emptying. Administration of exogenous GLP-1 increased gastric retention during euglycemia (AUC_{0-180} , eu/placebo vs. eu/GLP-1; $P < 0.001$) consistent with a profound slowing of gastric emptying. Despite GLP-1 administration, the induction of hypoglycemia accelerated gastric emptying at 45 and 180 minutes (hypo/GLP-1 vs. eu/GLP-1: AUC_{0-45} , $P = .03$ and AUC_{0-180} , $P < 0.01$). However, during hypoglycemia GLP-1 slowed gastric emptying at 180 minutes compared to placebo (AUC_{0-180} , hypo/placebo vs. hypo/GLP-1; $P < 0.008$), but not at 45 mins (AUC_{0-45} , $P = 0.10$).

Serum 3-O-Methylglucose concentrations

Serum 3-OMG concentrations are shown in *Figure 4*. The initial ANOVA for all four curves was significant at both 45 and 180 minutes ($P < 0.001$). During placebo and

hypoglycemic GLP-1 studies there was an initial linear rise in 3-OMG concentration tapering to a peak at 60 minutes followed by a gradual linear decline. In contrast, during euglycemic GLP-1 studies there was minimal 3-OMG absorption over the first 30 minutes with a mild rise to 60 minutes which then plateaued for the remainder of the studies. Overall, hypoglycemia increased 3-OMG concentrations, (AUC_{0-180} , hypo/placebo vs. eu/placebo; $P = 0.03$). However, GLP-1 markedly reduced 3-OMG concentrations during hypoglycemia (hypo/placebo vs. hypo/GLP-1; AUC_{0-45} , $P = 0.02$; and AUC_{0-180} , $P < 0.01$), as well as during euglycemia (AUC_{0-180} , eu/placebo vs. eu/GLP-1; $P < 0.001$).

Relationships between Glucose Absorption and Gastric Emptying

There was a strong association between 3-OMG absorption and gastric emptying (AUC_{0-180} % retention and AUC_{0-180} 3-OMG; $r = - 0.81$, $P < 0.001$).

DISCUSSION

The key finding of this study is that in health exogenous GLP-1 (1.2 pmol/kg/min) administered acutely attenuates the acceleration of gastric emptying secondary to insulin-induced hypoglycemia and, thereby, the rate of small intestinal carbohydrate absorption.

It is established that hypoglycemia markedly accelerates gastric emptying in healthy subjects [11] and patients with insulin-dependent diabetes [12]. The magnitude of the acceleration of emptying that we observed during hypoglycemia was consistent with these previous studies and while the exact mechanism(s) underlying this effect is incompletely understood, vagal stimulation appears to be important [25].

Furthermore, it is unequivocally established that acute administration of GLP-1 at pharmacological doses profoundly slows gastric emptying at 'normal' blood glucose concentrations in health and patients with diabetes [18, 23, 26]. While the underlying mechanisms also remain to be fully elucidated, vagal cholinergic pathways [3, 27] and direct centrally mediated effects [28] are probably important.

While the islet cell effects of GLP-1 are glucose-dependent, such that with increasing blood glucose concentrations the insulinotropic effects are more pronounced [29], during hypoglycemia the secretion of the counter-regulatory hormones are unaffected by GLP-1 [5]. Nauck and colleagues reported that during insulin-induced hypoglycemia (2.3 mmol/L) glucagon, catecholamines and cortisol concentrations were the same regardless of whether exogenous GLP-1 (1.2 pmol/kg/min) or placebo was infused [5]. Similar results have been reported using the commercial GLP-1 agonists exenatide and liraglutide at therapeutic concentrations in both health and type 1 diabetes [30, 31]. Furthermore, in health, GLP-1 and exenatide have no effect on C-peptide concentrations during insulin-induced hypoglycemic clamp conditions [5, 30]. Accordingly, during hypoglycemia even pharmacological doses of GLP-1 have no effect on islet cell secretion. This is the first study to evaluate the effect of a drug that slows gastric emptying during hypoglycemia in humans. A particular strength of our study is that we achieved glucose concentrations during hypoglycemia that are below the glycemic threshold for the glucagon and epinephrine counter-regulatory response in older adults, and comparable to a ‘severe’ hypoglycemic episode in the community [32]. At these glucose concentrations, and in contrast to the glucose-dependent β -cell response described above, we observed that during ‘severe’ hypoglycemia (2.6 mmol/L), GLP-1 continued to slow gastric emptying, albeit less potently than during euglycemia.

The mechanism(s) underlying the effect observed in our study can only be speculated upon. It is possible that GLP-1 induced slowing of gastric emptying, and retardation of delivery of carbohydrate to the small intestine, shares the same efferent vagal pathways as the ‘normal physiological’ response to hypoglycemia, which is to accelerate gastric emptying and increase delivery of carbohydrate to the small intestine [11]. Accordingly, the effects on gastric emptying we observed in health may not be reproducible in patients with autonomic dysfunction. However, it should also be recognized that the acceleration of gastric emptying by insulin-induced hypoglycemia is still evident in patients with type 1 diabetes who have autonomic dysfunction [12]. Studies in animals and/or studies in humans using specific cholinergic antagonists will be required to define the exact contribution of peripheral and central mechanisms [27, 33, 34].

Although the incretin-based therapies have an inherently low risk of hypoglycemia,

our findings may have important safety implications in patients where GLP-1 agonists are prescribed as ‘add-on’ therapy to a sulphonylurea or basal insulin. While these combinations with GLP-1 agonists are associated with a low risk of hypoglycemia [35], our data highlights that the subsequent management of hypoglycemia should it occur, is potentially problematic. Indeed our findings related to gastric emptying and glucose absorption suggest that the response to treatment of hypoglycemia with carbohydrate ingestion will be delayed in these patients. While only a trend towards attenuation of solid gastric emptying was observed during GLP-1 and the ‘strict’ hypoglycemic clamp period (0-45mins), the end-point of primary clinical relevance is small intestinal glucose absorption, which was substantially reduced when compared to placebo on the hypoglycemic days on GLP-1 at both 45 mins, and then for the duration of the study period. While we urge against over-interpreting these data, the observation that GLP-1 slows gastric emptying even during hypoglycemia may have implications for the prescription of other drugs which influence gastric emptying, such as opiates [36], when co-administered to patients who are receiving insulin. Studies of these other agents are now required.

There are, however, limitations to our study. We elected to evaluate the effects of GLP-1 on the response to hypoglycemia in subjects that were not diabetic, as patients with diabetes have the potential for abnormally slow gastric emptying at baseline [37], and it is known that the effect of GLP-1 to slow gastric emptying is dependent on the underlying rate of emptying [22]. The effect of hypoglycemia on gastric emptying in patients with type 2 diabetes is yet to be fully elucidated but the hormonal counter-regulatory response is similar, albeit occurring at higher blood glucose concentrations when compared to health [38], so the effect on gastric emptying is unlikely to be diminished.

A continuous infusion of synthetic GLP-1 was administered at a representative pharmacological concentration, rather than subcutaneous administration of a commercially available GLP-1 agonist. This ensured predictable GLP-1 concentrations, but the response to the commercially available drugs may not be so consistent. Furthermore, the study design was, of necessity, somewhat ‘artificial’ so that the effects in patients may potentially differ from those observed using a sustained period of hyperinsulinemic hypoglycemia and a predominantly protein ‘meal’. This, of course, does not reflect clinical practice where hypoglycemia is treated with a

carbohydrate load and the blood glucose allowed to normalize. It is reassuring that in large clinical trials of combined GLP-1 receptor agonist and basal insulin the occurrence of severe hypoglycemia (loss of consciousness, seizure or hypoglycemia requiring third party intervention) has been very low (0.0 - 2.5%) [2, 39]. The extent to which findings account for the low, but variable rate of minor hypoglycemia (4-53%) [2, 39], is unknown and warrants further investigation.

In conclusion, this study establishes that acute administration of exogenous GLP-1 attenuates, but does not abolish, the acceleration of gastric emptying induced by hypoglycemia in healthy subjects. This is in contrast to the well-documented glucose-dependent glucose-lowering effects of GLP-1 on the pancreatic islet cells. Our data highlight the potential safety implications for the combination of GLP-1 agonists with agents known to induce hypoglycemia, in particular sulphonylureas and insulin. Our observations support ongoing evaluation of the gastrokinetic properties of GLP-1 and its commercially available agonists during hypoglycemia in patients with type 2 diabetes.

List of abbreviations

ADA: American Diabetes Association

ANOVA: analysis of variance

AUC: area under curve

GLP-1: glucagon-like peptide-1

LAO: left anterior oblique

SD: standard deviation

SEM: standard error of the mean

3-OMG: 3-O-Methyl-D-glucopyranose

Author Contribution

M.P.P was responsible for study conception and design, acquisition of data, statistical analysis, interpretation and drafting the manuscript. He is guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

K.L.J. was responsible for analysis and interpretation of the scintigraphic data, contributed to the study design and critical revision of the manuscript for important intellectual content.

C.E.A. and C.E.C. contributed to the acquisition and interpretation of data.

J.J.M, M.J.C, and M.H. contributed to the study design and critical revision of the manuscript for important intellectual content.

A.M.D. was responsible for the study conception and design, obtaining funding, acquisition of data, interpretation, and critical revision of the manuscript for important intellectual content.

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Duality of Interest

J.J.M. has received consulting or lecture fees from the following companies: Astra Zeneca, Berlin-Chemie, BMS, Boehringer-Ingelheim, Eli Lilly, MSD, NovoNordisk, Novartis, Roche, Sanofi-Aventis

M.H. has participated in advisory boards and/or symposia for Novo/Nordisk, Sanofi-aventis, Novartis, Eli-Lily, Boehringer Ingelheim, AstraZeneca, Satlogen and Meyer Nutraceuticals.

M.P.P, K.L.J, C.E.A, C.E.C, M.J.C and A.M.D have no duality of interests to declare

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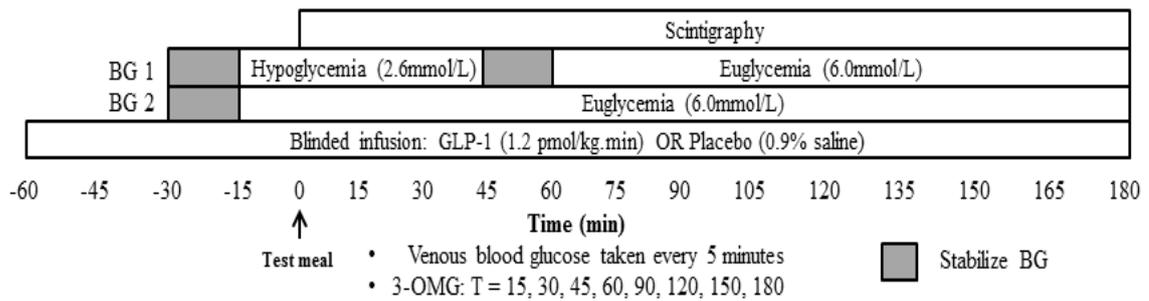


Fig 1. Schematic representation of the study protocol

Blood glucose concentrations (BG) were stabilised using a glucose/insulin clamp at hypoglycemia (2.6mmol/L; BG 1) or euglycemia (6.0mmol/L; BG2) between T=-15 to 45min before clamping at 6.0mmol/L until 180min. During hypoglycemia and euglycemia subjects received intravenous GLP-1 (1.2pmol/kg.min) or placebo between T=-60 to 180min. At T=0min subjects ingested a labeled meal. Gastric emptying was measured scintigraphically from T=0 - 180min and serum 3-OMG concentrations taken at 15 min intervals for the first hour, then half hourly until study completion.

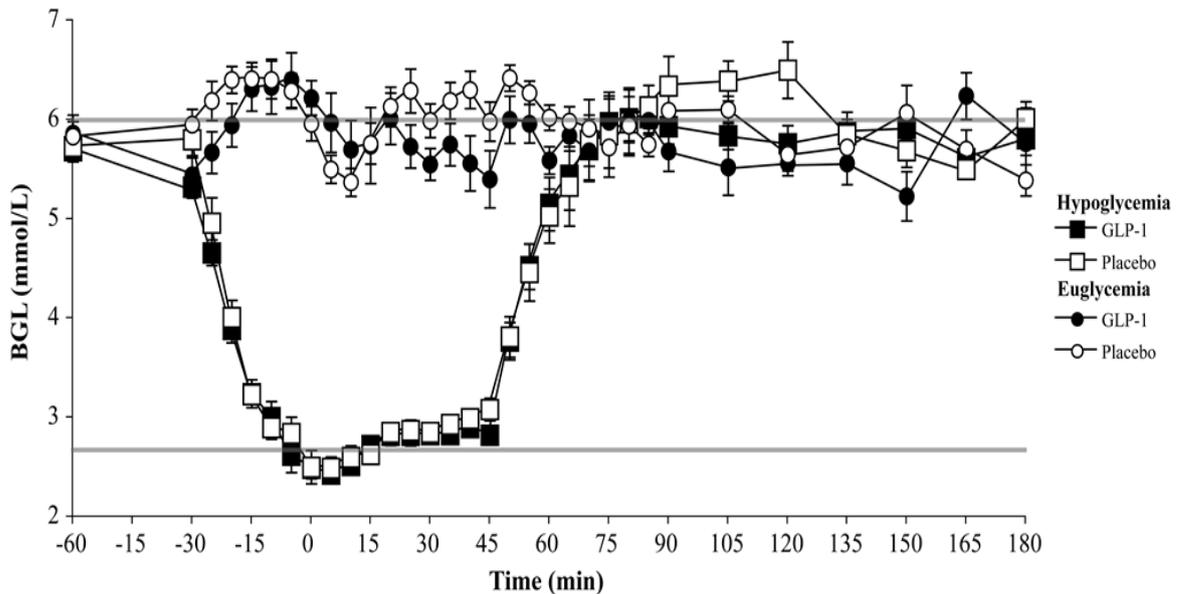


Fig 2. Blood glucose concentrations

Blood glucose (mmol/L) was stabilised using a glucose/insulin clamp at hypoglycemia (2.6mmol/L) or euglycemia (6.0mmol/L) between T=-15 to 45min before clamping at 6.0mmol/L until 180min. Data are presented as mean (SE)

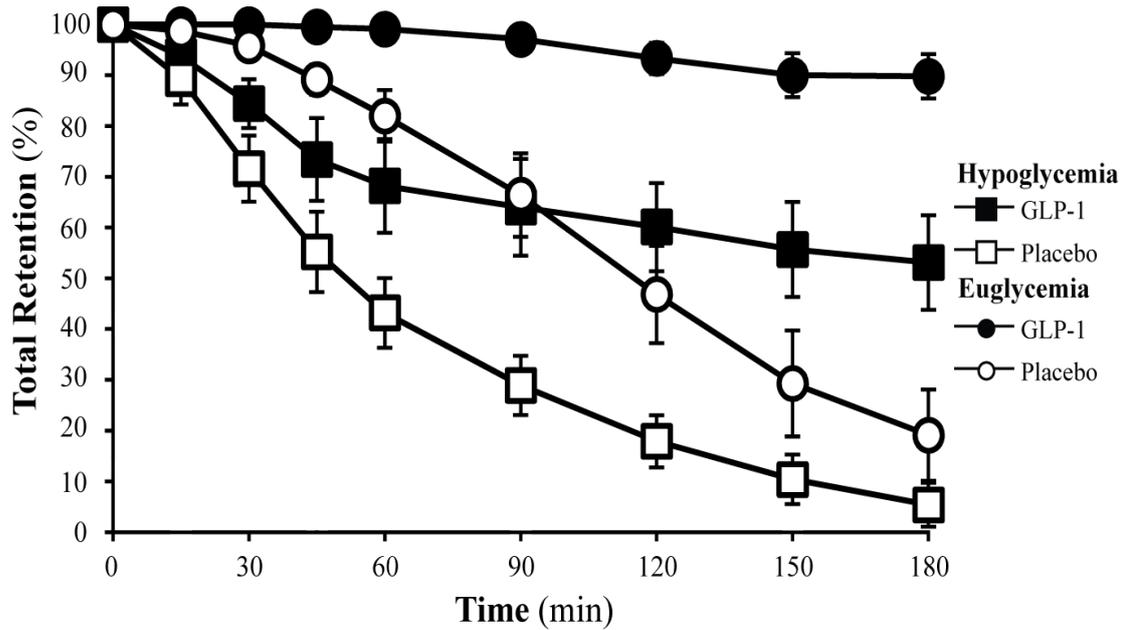


Fig 3. Gastric retention

Gastric retention (%) of test meal in 10 healthy volunteers with blood glucose clamped at either hypoglycemia (2.6mmol/L) or euglycemia (6.0mmol/L) with or without IV GLP-1 (1.2pmol/kg.min). Data are presented as mean (SE).

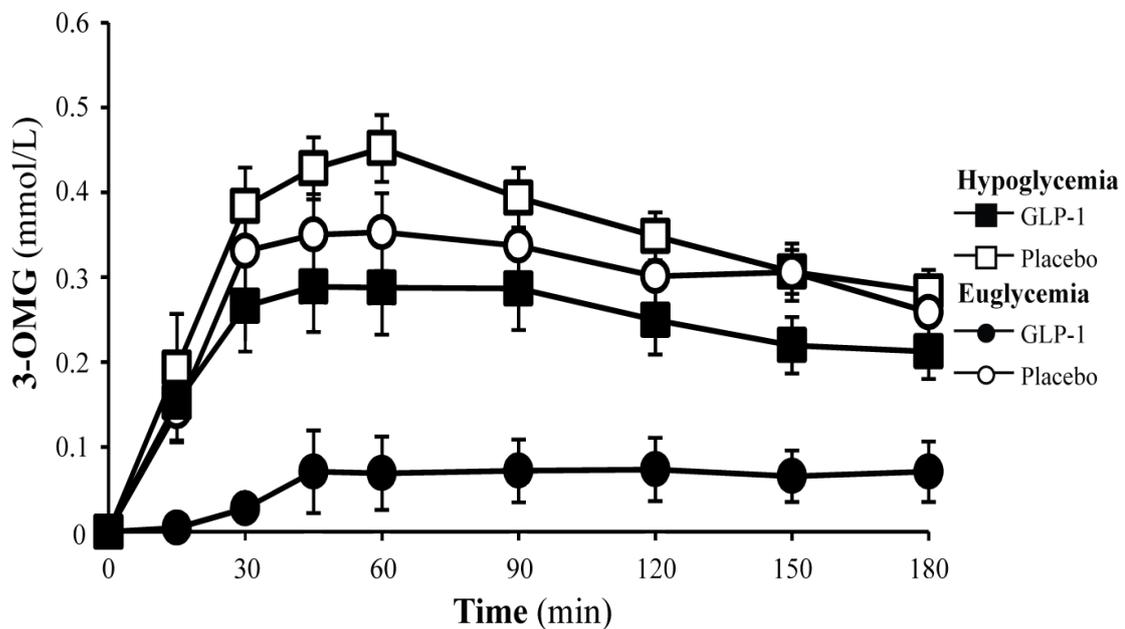


Fig 4. Glucose absorption (serum 3-OMG concentrations)

Serum 3-OMG (mmol/L) concentrations in 10 healthy volunteers with blood glucose clamped at either hypoglycemia (2.6 mmol/L) or euglycemia (6.0 mmol/L) with or without IV GLP-1 (1.2 pmol/kg.min). Data are presented as mean (SE).

3.4 Manuscript: Hyperglycemia potentiates the slowing of gastric emptying induced by exogenous GLP-1

Full title

Hyperglycemia potentiates the slowing of gastric emptying induced by exogenous
GLP-1

Running Title

Hyperglycemia potentiates GLP-1 induced slowing of gastric emptying

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ABSTRACT

Objective: Acute hyperglycemia markedly slows gastric emptying. Exogenous GLP-1 also slows gastric emptying leading to diminished glycaemic excursions. The primary objective was to determine whether hyperglycemia potentiates the slowing of gastric emptying induced by GLP-1 administration.

Design and methods: Ten healthy participants were studied on 4 separate days. Blood glucose was clamped at hyperglycemia using an intravenous infusion of 25% dextrose (~12mmol/L; *hyper*; on 2 days), or maintained at euglycemia (~6mmol/L; *eu*; on 2 days), between T=-15 to 240min. During hyperglycemic and euglycemic days participants received intravenous GLP-1 (1.2pmol/kg/min) and placebo in a randomized double-blind fashion. At T=0min subjects ingested 100g of beef mince labeled with 20MBq ^{99m}Tc-technetium-sulfur-colloid and 3g of 3-O-methyl-glucose (3-OMG), a marker of glucose absorption. Gastric emptying was measured scintigraphically from T=0 to 240min and serum 3-OMG taken at regular intervals from T=15 to 240min. The areas under the curve for gastric emptying and 3-OMG were analysed using one-way RM-ANOVA with Bonferroni-Holm adjusted posthoc tests.

Results: Hyperglycemia slowed gastric emptying (*eu*/placebo vs *hyper*/placebo; $P < 0.001$) as did GLP-1 (*eu*/placebo vs. *eu*/GLP-1; $P < 0.001$). There was an additive effect of GLP-1 and hyperglycemia, such that gastric emptying was markedly slower when compared to GLP-1 administration during euglycemia (*eu*/GLP-1 vs *hyper*/GLP-1; $P < 0.01$).

Conclusions: Acute administration of exogenous GLP-1 profoundly slows gastric emptying during hyperglycemia in excess of the slowing induced by GLP-1 during euglycemia. Studies are required to determine the effects of hyperglycemia on gastric emptying with the subcutaneously administered commercially available GLP-1 agonists in patients with type 2 diabetes.

INTRODUCTION

Gastric emptying is a major determinant of postprandial glycemia in health, as well as in type 1 and type 2 diabetes [1, 2], and accounts for approximately 35% of the variance in the initial glycemic response to oral carbohydrate [1]. Accordingly, dietary and pharmacological strategies that slow gastric emptying, including short-acting glucagon-like peptide-1 (GLP-1) agonists, are useful interventions to attenuate postprandial glycemic excursions and overall glycemia in type 2 diabetes [3].

GLP-1 receptor agonists are now incorporated into standard treatment algorithms for the management of type 2 diabetes [4]. During fasting, exogenous GLP-1 and GLP-1 agonists lower plasma glucose primarily via effects on the islet cell to increase insulin and reduce glucagon secretion in a glucose-dependent manner [5, 6], whereas during the postprandial phase glucose lowering is predominantly mediated through their effect to slow gastric emptying [7, 8]. Accordingly, in both health and in patients with type 2 diabetes, postprandial insulin concentrations are suppressed, rather than stimulated, during GLP-1 administration [7, 9]. The magnitude of the deceleration of gastric emptying induced by exogenous GLP-1 and its agonists is dependent on the baseline rate of gastric emptying, so that the emptying rate is markedly slowed in those with relatively rapid gastric emptying prior to GLP-1 administration, whereas the rate is largely unaffected when gastric emptying is already delayed at baseline [10, 11]. Furthermore, the reduction in postprandial glycemia induced by these agents is closely related to the magnitude of the slowing of gastric emptying [9-11]. Accordingly, when the effect of GLP-1 to slow gastric emptying is attenuated, such as with the concurrent administration of erythromycin [12], the glucose-lowering effect is observed to be similarly diminished.

As well as being a determinant of postprandial glycemia, gastric emptying is itself highly sensitive to acute changes in the blood glucose concentration [13]. Acute hyperglycemia (i.e., blood glucose level ~15mmol/L (270mg/dL)) substantially slows gastric emptying [14]. Indeed, even changes within the normal range of postprandial glycemia have marked effects on the rate of gastric emptying, with emptying being slower when blood glucose is clamped at 8mmol/L (144mg/dL) when compared to

emptying rates at 4mmol/L (72mg/dL) [15]. Conversely, insulin-induced hyperglycemia dramatically accelerates gastric emptying [16].

It also appears that the effect of drugs on gastrointestinal motor function is modified by systemic glucose concentrations. For example, acute hyperglycemia attenuates the gastrokinetic effect of erythromycin [12]. Moreover, we recently reported that exogenous GLP-1 attenuates the acceleration of gastric emptying by insulin-induced hypoglycemia (~ 2.6 mmol/l) [13].

Given that the ubiquitous feature in patients with diabetes is hyperglycemia and that GLP-1 agonists are frequently prescribed to this group, it was important to evaluate whether hyperglycemia potentiated or diminished the slowing of gastric emptying induced by GLP-1 administration.

RESEARCH DESIGN AND METHODS

Subjects

Participants aged 50-75 years who considered themselves healthy were eligible and attended a screening visit at the Royal Adelaide Hospital. Participants with known or undiagnosed diabetes (HbA1c>6.5%; 48 mmol/mol), impaired renal function (estimated creatinine clearance <107 ml/min) or anemia (haemoglobin < 11 g/dL in females and < 13g/dL in males), currently smoking, consuming >20 g/day of alcohol/day, receiving medication known to affect gastrointestinal motility or glycemia, or with a history of gastric or small intestinal surgery, were excluded.

Protocol

Participants were studied on four separate occasions separated by a minimum of four days. Each participant underwent concurrent measurements of gastric emptying, blood glucose concentrations and glucose absorption on each of the four occasions: twice with blood glucose concentrations within the euglycemic range [blood glucose of ~6 mmol/L (108 mg/dL); *eu*] and twice during acute hyperglycemia [blood glucose of ~12 mmol/L (216 mg/dL); *hyper*] (*Fig. 1*). Either GLP-1 (1.2 pmol/kg/min) or placebo (0.9% saline) was administered intravenously (IV) during both euglycemia

and hyperglycemia. The order of treatment (GLP-1, placebo, euglycemia and hyperglycemia) was determined by the Pharmacy Department at the Royal Adelaide Hospital using a computer generated randomization schedule. The investigators performing each study (M.P.P. and C.E.C.) were informed of the glucose target on a particular study day. These investigators had no role in the subsequent analysis of gastric emptying data. All investigators and participants were blinded to the study drug (GLP-1 or placebo) with blinding maintained throughout the entire study period, including analysis of data.

Each subject attended the Department of Nuclear Medicine, PET and Bone Densitometry at the Royal Adelaide Hospital at 0830 h after an overnight fast. Two intravenous cannulae were inserted into the right arm; one in the antecubital vein for an infusion of insulin and 25% dextrose, and another in the dorsal vein of the right hand for infusion of study drug. A third intravenous cannula was inserted into the left antecubital vein for blood sampling. Synthetic GLP-1 amide (Bachem, Weil am Rhein, Germany) was reconstituted by the Pharmacy Department in 0.9% normal saline. After drawing baseline blood specimens, both GLP-1 (1.2 pmol/kg/min) and placebo (0.9% saline) infusions were commenced 30 min prior to meal ingestion, and infused at a rate of 1ml/min for the duration of the study (i.e. T=-60 to 240 min) [13]. A 25% dextrose infusion was commenced concurrently to target a blood glucose concentration of 12 mmol/L (216 mg/dL). After the blood glucose concentration had been stabilized at the desired level for a minimum of 15 min, participants were instructed to eat the test meal as promptly as possible while remaining comfortable. The test meal comprised 100g lean minced beef, labeled with 20 MBq of ^{99m}Techneium-sulfur-colloid (Pharmalucence Inc; Bedford, Massachusetts, USA) [12], followed by 3g of 3-O-Methyl-D-glucopyranose (3-OMG; Sigma-Aldrich, Sydney, NSW, Australia) dissolved in 150ml of water [13]. Following study completion at T= 240 min, participants were monitored with half hourly blood glucose concentrations for a further 3 hours to detect delayed hypoglycemia, before being allowed to leave the laboratory.

The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee and prospectively registered (www.anzctr.com.au; ACTRN12611000973910). Written informed consent was obtained from all participants prior to their inclusion.

Stabilization of blood glucose concentrations

Hyperglycemia was achieved using a modified glucose clamp technique [12, 13]. Dextrose (25%) was infused IV with an initial rate over 2 min determined using body surface area (DuBois method) [17] and followed with a continuous infusion adjusted between 80 - 300 ml/hr to maintain the blood glucose concentration at 12 mmol/L (216 mg/dL) until T = 240 min. On the euglycemic study days, blood glucose was maintained at ~6 mmol/L (108 mg/dL) for the duration of the study i.e. T = -30 to 240 without external manipulation [13, 16]. The total amount of 25% dextrose required to maintain the blood glucose on each study day was recorded.

Commencing 30 min prior to the meal (i.e. T = -30 min) venous blood samples for measurement of glucose were taken at 5 min intervals until T= 90 min, and then every 15 min until study completion at T= 240 min. Blood glucose concentrations were measured using a portable electrochemical coulometric glucose dehydrogenase glucose meter with a co-efficient of variation of 3.8% in the targeted range (Optium Xceed; Abbott Laboratories, Bedford, Massachusetts, USA) [18].

Measurement of gastric emptying

On all study days the radiolabeled beef mince test meal was consumed within 5 min, followed by ingestion of the 3-OMG-labeled water. Scintigraphic data were acquired with a gamma camera (Digirad, Poway, California, USA) placed over the participant's abdomen to obtain a left anterior oblique (LAO) image. Subjects were in the supine position with the upper body at an angle of approximately 30 degrees. Data were acquired from meal completion (T=0 min) in 1 min frames for 240 min and corrected for radionuclide decay, gamma ray attenuation and subject movement [12]. Radioisotopic data were analysed by an experienced nuclear medicine scientist (K.L.J.) who was blinded to both the treatment arm and the glyceemic period assigned. A region-of-interest was drawn around the total stomach and a gastric emptying curve, expressed as intragastric retention over time, derived [12]. From these curves the following variables were considered; duration of the lag phase, determined visually as the time immediately before any of the solid meal had entered the small intestine, and total area under the curve T = 0 to 240 min (AUC₀₋₂₄₀).

Measurement of glucose absorption

Serum 3-OMG was used as an index of intestinal glucose absorption [10], and was measured using liquid chromatography/mass spectroscopy, with an assay sensitivity of 0.0103 mmol/L [10]. Blood samples were collected at T=15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min and, once clotted, centrifuged at 3,200 rpm for 15 min. Serum was then stored at -70 degrees Celsius for subsequent measurement of 3-OMG concentrations, with the rate of glucose absorption indicated by the area under the 3-OMG concentration curve (AUC) [10].

Statistical analysis

Sample size was determined using previous data relating to the effect of exogenous GLP-1 on gastric emptying in health and it was calculated that 10 participants completing all 4 study days would provide 80% power to detect a 25% difference in gastric emptying, the latter defined as the total area under the gastric emptying curve [9]. Overall effects for both gastric emptying and glucose (3-OMG) absorption were calculated as AUC_{0-240} . Data are shown as mean values \pm SEM, with the difference between groups (Δ) reported as median (IQR). Data were evaluated using one-way repeated measures analysis of variance (ANOVA), with Bonferroni Holm adjusted post hoc tests for multiple comparisons. Given that we have reported an association between glucose absorption and gastric emptying [13], we tested for this relationship. This correlation was evaluated adjusted for repeated measures. The null hypothesis was rejected at the 0.05 significance level. Statistical analyses were performed using SPSS (version 16.0; SPSS, Chicago, Illinois). All analyses were supervised by an independent professional biostatistician.

RESULTS

Ten healthy participants [5 men, age 71 (5) years, body mass index 26 (2.8) kg/m²] completed all four study days without significant adverse effects. However, five participants experienced leg cramping during the hyperglycemic study days. No participant experienced nausea or vomited. Blood glucose concentrations were effectively clamped at hyperglycemic and euglycemic targets on both GLP-1 (11.9 ± 0.2 and 5.1 ± 0.1 mmol/L) and placebo (12.0 ± 0.1 and 5.5 ± 0.1 mmol/L) study days

(Fig. 1). GLP-1 markedly increased (IV) glucose required to maintain hyperglycemia (hyper/GLP-1 vs hyper/placebo; Δ 91 (24) gm; $P = <0.001$) (Fig. 2).

Solid emptying

In all cases, gastric emptying approximated a linear pattern after an initial lag phase (Fig. 3). The lag phase and gastric retention (%) over time were markedly different across study days ($P < 0.001$). There were trends towards longer lag phases during clamped hyperglycemia with placebo (eu/placebo 14.1 ± 3.1 vs. hyper/placebo 24.3 ± 5.3 min; $P = 0.12$), during euglycemia with GLP-1 when compared to placebo (eu/placebo 14.1 ± 3.1 vs. eu/GLP-1 57 ± 17.9 min; $P = 0.09$), and with GLP-1 during hyperglycemia when compared to euglycemia (eu/GLP-1 57 ± 17.9 vs. hyper/GLP-1 98.4 ± 20.6 min; $P = 0.12$).

Total stomach: During placebo infusions the intragastric retention of the radioisotope was ~80% greater during hyperglycemia when compared to euglycemia (AUC_{240} , eu/placebo vs. hyper/placebo; $P < 0.001$) (Fig. 3). At euglycemia the administration of GLP-1 increased intragastric retention more than twofold (AUC_{240} , eu/placebo vs. eu/GLP-1; $P < 0.001$) and at T=240 more than 60% of contents were retained in the stomach compared to complete emptying during placebo. The rate of gastric emptying during GLP-1 administration at euglycemia was slower than the rate of emptying during placebo at hyperglycemia (AUC_{240} , hyper/placebo vs. eu/GLP-1; $P = 0.01$). During hyperglycemia GLP-1 slowed gastric emptying more than GLP-1 administered during euglycemia (AUC_{240} , hyper/GLP-1 vs eu/GLP-1; $P < 0.01$) so that there was only minimal emptying with GLP-1 administration during hyperglycemia (hyper/GLP at T=240 mean emptying of $15 \pm 3\%$).

Serum 3-O-Methylglucose concentrations

Serum 3-OMG concentrations are shown in Figure 4. The initial ANOVA for all four curves was significant ($P < 0.001$). During the placebo studies there was an initial steep linear rise in 3-OMG concentration which peaked at 30 minutes on the hyperglycemic day and 60 minutes on the euglycemic day followed by a gradual

linear decline. During euglycemic GLP-1 studies there was a linear rise in 3-OMG concentration to 45 minutes followed by a gradual increase to 120 minutes which then plateaued for the remainder of the study. In contrast, during hyperglycemic GLP-1 studies there was minimal 3-OMG absorption for the duration of the study with a gradual increase over 150 minutes which then plateaued for the remainder of the study. Overall hyperglycemia decreased 3-OMG concentrations, (AUC_{0-240} , hyper/placebo vs. eu/placebo; $P = 0.01$). GLP-1 markedly reduced 3-OMG concentrations during euglycemia, (AUC_{0-240} , eu/GLP-1 vs. eu/placebo; $P < 0.01$). During hyperglycemia, GLP-1 decreased 3-OMG absorption substantially more than GLP-1 administered during euglycemia, (AUC_{0-240} , hyper/GLP-1 vs. hyper/placebo; $P < 0.01$).

Relationships Between Glucose Absorption and Gastric Emptying

There was a strong association between 3-OMG absorption and gastric emptying (AUC_{0-240} % gastric retention and AUC_{0-240} 3-OMG; $r = -0.80$, $P < 0.001$).

DISCUSSION

The key finding of our study is that even during hyperglycemia the administration of GLP-1 (1.2 pmol/kg/min) retains its profound effect to slow gastric emptying and further limits the rate of small intestinal carbohydrate absorption. The implication of our observation in healthy participants is that GLP-1 agonists could well retain their potent effect to slow gastric emptying in patients with diabetes and preprandial hyperglycemia, as, at least in health, there appears to be a supplementary effect of GLP-1 during hyperglycemia to slow gastric emptying.

The novelty of our study is that, to the best of our knowledge, it is the first study to evaluate the effect of a drug that slows gastric emptying during hyperglycemia in humans. It is well established that acute hyperglycemia markedly slows the rate of gastric emptying. This phenomenon was initially observed in healthy participants [12] and then confirmed in patients with diabetes [19]. Gastric emptying of solids composes two phases; the lag phase corresponding to meal transportation from the

fundus to the antrum [20] and the post-lag phase corresponding to the propulsion of solid food particles through the pylorus [20]. Hyperglycemia slows gastric emptying of solids by prolonging the lag phase and decreasing the post-lag emptying rate [14], via reduced proximal gastric tone [21], suppression of antral pressure waves [22] and stimulation of pyloric contractions [23]. The magnitude of deceleration of emptying, the prolongation of the lag phase, and the slowing of the post-lag emptying rate that we observed during hyperglycemia are consistent with these previous studies [20, 22, 23]. Purported mechanisms governing this response include nitrergic pathways [24], direct stimulation of glucose dependent neurons within the myenteric plexus [25] and suppression of vagal cholinergic activity, which has been demonstrated via reduced pancreatic polypeptide levels during hyperglycemia [26].

Gastric emptying is the major rate-limiting step for glucose absorption from the gastrointestinal tract and therefore the primary determinant of postprandial glucose excursions [2, 27]. Acute GLP-1 administration at pharmacological doses has been shown to dose dependently relax the gastric fundus, increase gastric compliance, inhibit antral motility and increase pyloric tone [28, 29], thereby, slowing gastric emptying and attenuating postprandial glycaemic excursions [9]. Similar effects have been reported with the commercially available 'short-acting' GLP-1 agonists [11, 30]. The 'long-acting' GLP-1 agonists such as exenatide SR/LAR, when compared to the 'short-acting' GLP-1 agonists have diminished effect to slow emptying [31, 32], which is thought to reflect the development of rapid tachyphylaxis to sustained exposure to supraphysiological concentrations of GLP-1 [33]. The mechanisms underlying tachyphylaxis are uncertain - Nauck and colleagues have proposed a role for vagal pathways [34]. The relative importance of the plasma GLP-1 concentration, as opposed to the duration of its elevation is also uncertain. It should also be recognized that with prolonged GLP-1 receptor stimulation the slowing of gastric emptying is attenuated, but not abolished [31, 33, 34].

While the magnitude of the slowing of gastric emptying that we observed during euglycemia on GLP-1 is consistent with previous studies using acute administration of GLP-1 [13, 28, 29], this was dramatically potentiated during hyperglycemia such that mean gastric emptying was only ~15% at 4 hours. The mechanisms underlying the inhibitory effect of GLP-1 are incompletely understood however a number of studies indicate a putative role of vagal cholinergic pathways [28, 35] and nitric oxide has

been implicated as an important efferent neurotransmitter in GLP-1 induced gastric relaxation [36]. Accordingly, there appears to be substantial overlap between the mechanisms governing slower gastric emptying during both GLP-1 stimulation and hyperglycemia which we speculate may account for the summative interaction observed in our study.

Even minor variations in duodenal delivery of glucose (i.e. rate of gastric emptying) have major effects on postprandial glycemia [27] and postprandial glycemia is a major determinant of overall glycemic control [37]. The marked additional slowing of gastric emptying that we observed during hyperglycemia compared to euglycemia on GLP-1 study days highlights a role for ‘short-acting’ GLP-1 agonist in patients with type 2 diabetes who are hyperglycemic at the time of meal ingestion [38]. Furthermore, patients with type 2 diabetes frequently are prescribed one or more medications that are ingested, and the protracted period of gastric slowing (for up to 4 hours) induced by GLP-1 during hyperglycemia may have significant effects on the absorption of concomitant oral drugs, particularly modified release or enteric coated formulations.

There are, however, limitations to our study. We elected to evaluate the effects of GLP-1 on the response to hyperglycemia in healthy participants, rather than patients with diabetes. We chose a ‘healthy’ cohort for this proof of principal study as patients with diabetes have the potential for variable glycemic control, autonomic neuropathy and abnormally slow gastric emptying [39]. The latter variable may be particularly important as the capacity for GLP-1 to slow gastric emptying is dependent on the underlying rate of gastric emptying [10, 11]. However, patients with diabetes and autonomic neuropathy have impaired relaxation of the proximal stomach in response to exogenous GLP-1 [40], and there have been no studies to evaluate the effect of exogenous GLP-1 on gastric emptying in patients with gastroparesis. Additionally, our study design was somewhat artificial in that synthetic GLP-1 was administered acutely as a continuous intravenous infusion over 4 hours at a dose representative of pharmacological concentrations [8] rather than subcutaneous administration of a commercially available GLP-1 agonist. This was to ensure predictable plasma concentrations of GLP-1 across the four study days, however, the response may potentially be more variable with the subcutaneous route and the magnitude of slowing of gastric emptying possibly attenuated with prolonged administration [33].

Furthermore, increasing blood glucose acutely to a set level of 12 mmol/L (216 mg/dL) is not representative of the chronic hyperglycemia seen in the majority of patients with well to moderately controlled type 2 diabetes. Notably, exogenous GLP-1 was well tolerated with no episodes of nausea or vomiting during euglycemia or hyperglycemia, even with profound intragastric retention of the meal at four hours. This is consistent with previous reports of the low incidence of gastrointestinal side effects with intravenous GLP-1 [8] and the weak relationship between nausea and vomiting and delayed gastric emptying [11, 31]. However, it should be emphasized that gastrointestinal symptoms may be greater when using commercially available GLP-1 agonists. Studies are now warranted to determine if the potentiated effect of hyperglycemia to slow gastric emptying persists with prolonged infusions of intravenous GLP-1 and chronic administration of short- and long-acting GLP-1 agonists.

In conclusion, our study establishes that acute administration of exogenous GLP-1 in a healthy older population profoundly slows gastric emptying during hyperglycemia, in excess of the slowing induced by both GLP-1 during euglycemia and physiological hyperglycemia. This effect is associated with a substantial reduction in small intestinal carbohydrate absorption. The clinical relevance of our observations will be clarified further by studies with subcutaneous administration of commercially available short- and long-acting GLP-1 agonists in the target population of patients with type 2 diabetes stratified according to chronic glycemetic control.

LIST OF ABBREVIATIONS

ANOVA: analysis of variance

AUC: area under curve

GLP-1: glucagon-like peptide-1

IQR: interquartile range

IV: intravenous

LAO: left anterior oblique

SEM: standard error of the mean

3-OMG: 3-O-Methyl-D-glucopyranose

Author Contribution

M.P.P. was responsible for study conception and design, acquisition of data, statistical analysis, interpretation and drafting the manuscript. He is guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

K.L.J. was responsible for analysis and interpretation of the scintigraphic data, contributed to the study design and critical revision of the manuscript for important intellectual content.

L.T. and C.E.C. contributed to the acquisition and interpretation of data.

J.J.M., M.J.C., and M.H. contributed to the study design and critical revision of the manuscript for important intellectual content.

A.M.D. was responsible for the study conception and design, obtaining funding, acquisition of data, interpretation, and critical revision of the manuscript for important intellectual content.

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Duality of Interest

J.J.M. has received consulting or lecture fees from the following companies: Astra Zeneca, Berlin-Chemie, BMS, Boehringer-Ingelheim, Eli Lilly, MSD, NovoNordisk, Novartis, Roche, Sanofi-Aventis

M.H. has participated in advisory boards and/or symposia for Novo/Nordisk, Sanofi-Aventis, Novartis, Eli-Lily, Boehringer Ingelheim, AstraZeneca, Satiogen and Meyer Nutriceuticals.

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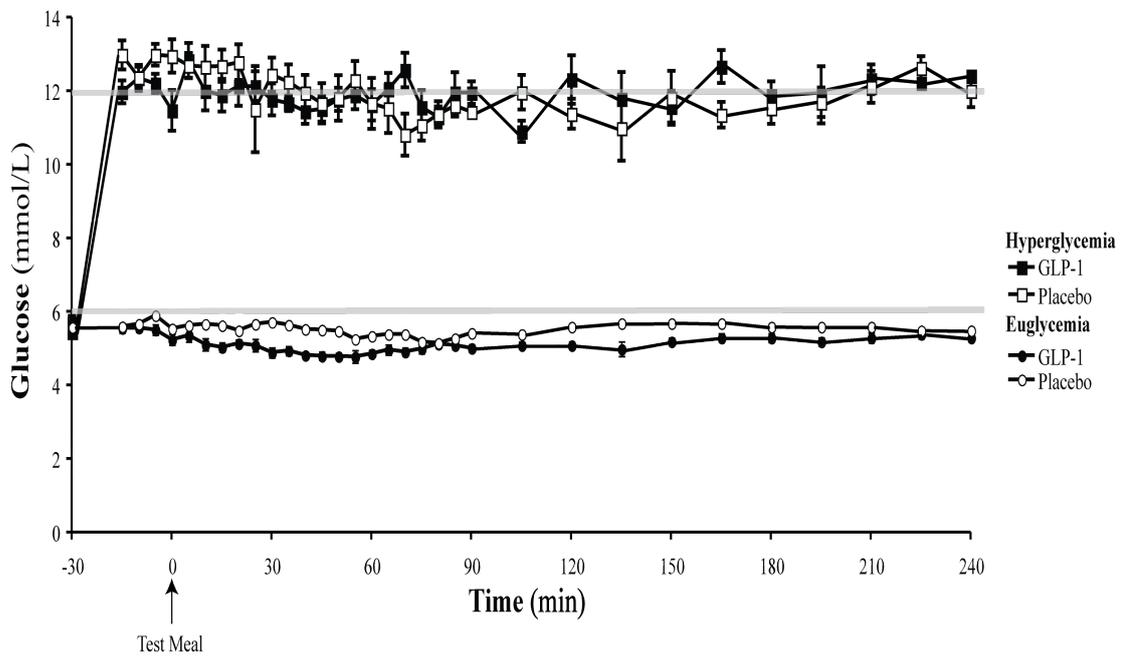


Fig 1. Blood glucose concentrations

Blood glucose (mmol/L) was stabilised using a dextrose infusion to target hyperglycemia (12mmol/L) or maintained without intervention at euglycemia (6.0mmol/L) between T=-15 to 240 min. GLP-1 (1.2 pmol/kg/min) or placebo (0.9% saline) was administered intravenously between T = -30 to 240 min. The test meal was ingested at T = -5 to 0 min. Data are mean (SE).

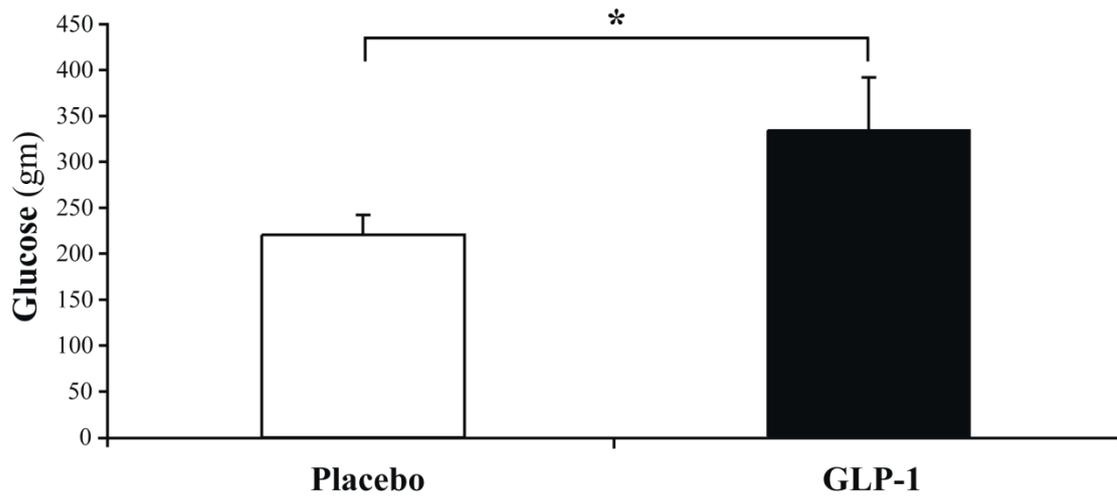


Fig 2. Exogenous glucose requirements during hyperglycemic clamps

GLP-1 markedly increased (IV) glucose required to maintain hyperglycemia [12mmol/L] (hyper/GLP-1 vs hyper/placebo; Δ 91 (24) gm; * $P = <0.001$). Data are mean (SE).

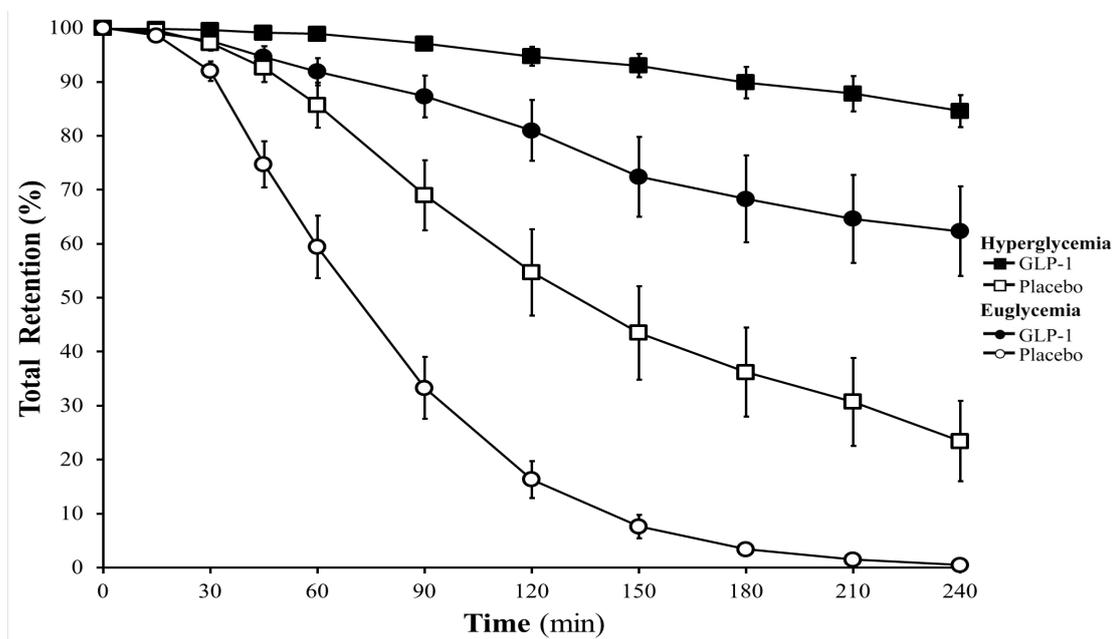


Fig 3. Gastric retention

Gastric retention (%) of test meal in participants with blood glucose clamped at either hyperglycemia (12mmol/L) or euglycemia (~6.0mmol/L) with or without IV GLP-1 (1.2pmol/kg.min). Data are mean (SE).

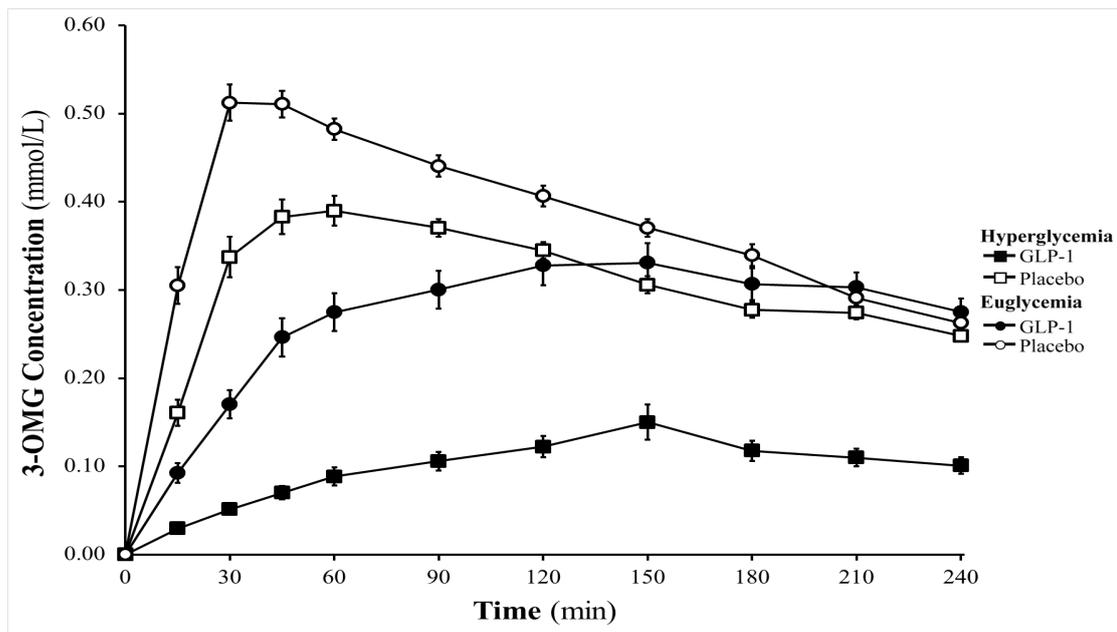


Fig 4. Glucose absorption (3-OMG concentrations)

Serum 3-OMG (mmol/L) concentrations in participants with blood glucose maintained at either hyperglycemia (12 mmol/L) or euglycemia (~6.0 mmol/L) with or without IV GLP-1 (1.2 pmol/kg.min). Data are presented as mean (SE).

Chapter 3.5 Manuscript: The insulinotropic effect of pulsatile compared with continuous intravenous delivery of GLP-1

Title

The insulinotropic effect of pulsatile compared with continuous intravenous delivery of GLP-1

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ABSTRACT

Aims:

In health, both insulin and glucagon-like peptide 1 (GLP-1) are secreted in a pulsatile fashion. Insulin has greater glucose-lowering properties when administered in pulses when compared to a constant intravenous infusion. The primary aim of this study was to compare the insulinotropic response to pulsatile and continuous intravenous infusions of equivalent doses of GLP-1.

Methods:

Twelve healthy participants were studied in a randomised fashion on three separate days; a continuous infusion day [GLP-1 at $0.6 \text{ pmol kg}^{-1} \text{ min}^{-1}$ (1ml/min) and a 1ml placebo bolus every 6 min], a pulsatile infusion day [placebo at 1ml/min and a 3.6 pmol/kg GLP-1 bolus every 6 min] and a placebo day [placebo at 1ml/min and a 1ml placebo bolus every 6 min]. Between 45 - 120 min a hyperglycaemic clamp was used to maintain blood glucose at 9 mmol/l. Venous blood glucose and plasma insulin concentrations were measured every 5 min from $t = 0 - 45$ min and every min from $t = 45 - 120$ min; plasma glucagon was measured every 15 min. Dextrose requirement and glucagon data were analysed using RM-ANOVA and insulin data with a linear mixed effects maximum likelihood model.

Results:

Continuous and pulsatile infusions of GLP-1 increased dextrose requirements by ~ three-fold [$p < 0.001$] and both increased insulin secretion by ~ nine-fold [$p < 0.001$] without any difference between them. While hyperglycaemia reduced plasma glucagon concentrations there was no difference between the study days.

Conclusion:

In health, pulsatile and continuous administration of intravenous GLP-1 appears to have comparable insulinotropic effects.

Trial registry number: ACTRN1261200104083

Key words: Glucagon-like peptide 1, insulin, pulsatile, glucagon,

Abbreviations

ANOVA: analysis of variance

AUC: area under curve

BMI: body mass index

GLP-1: glucagon-like peptide-1

HbA1c: glycated haemoglobin

IV: intravenous

INTRODUCTION

In health, a pulsatile pattern of hormone secretion is a fundamental property of a number of endocrine functions, including those of the parathyroid, pituitary, adrenal and islet-cell hormones [1]. It is well established that in addition to basal release, the majority of insulin is secreted in discrete high-frequency bursts [1]. This oscillatory pattern of insulin secretion is pivotal to optimal insulin action and in type 2 diabetes attenuation of the secretory burst mass, and a loss of pulsatile orderliness, are prominent pathophysiological features [2]. In health and in type 1 diabetes mellitus intravenous insulin has a more pronounced glucose-lowering effect when given in a pulsatile, rather than continuous fashion [3].

The incretin hormone glucagon-like peptide 1 (GLP-1) lowers blood glucose via glucose-dependent stimulation of insulin and inhibition of glucagon secretion, and slowing of gastric emptying [4]. Balks and colleagues reported that GLP-1 is secreted in a pulsatile manner during both basal and glucose-stimulated conditions with a pulse interval of ~7 minutes [5]. However, the effect of exogenous GLP-1 and GLP-1 agonists has hitherto only been determined with continuous intravenous or subcutaneous infusions and the effect of pulsatile intravenous administration has not been assessed.

The primary aim of this study was to determine whether intravenous pulsatile administration of GLP-1 increased insulin secretion during eu- and hyperglycaemia when compared to continuous administration.

METHODS

Subjects

Healthy participants aged 18-35 years were eligible and attended a screening visit at the Royal Adelaide Hospital. Written informed consent was obtained from all participants. The study protocol was approved by the hospital Research Ethics Committee and prospectively registered (www.anzctr.com.au; ACTRN1261200104083).

Protocol

Participants were studied on three occasions separated by a minimum of seven days. The study days comprised; a control 'continuous infusion day' [continuous infusion of GLP-1 at $0.6 \text{ pmol kg}^{-1} \text{ min}^{-1}$ (1ml/min) and a 1 ml bolus of 4% human albumin every 6 minutes], an intervention 'pulsatile day' [continuous infusion of 4% albumin and a 1ml bolus of GLP-1 at 3.6 pmol/kg every 6 minutes] and a 'placebo day' [continuous infusion of 4% human albumin and a 1ml bolus of 4% albumin every 6 minutes]. Each study lasted 2 hours with a total dose of 72 pmol/kg of GLP-1 delivered on both control and intervention days. On each study day a hyperglycaemic clamp [6] was commenced at $t = 45\text{min}$ to target a blood glucose level of 9 mmol/l that was maintained until study completion at $t = 120\text{min}$. The order of treatment was randomised by the Pharmacy Department.

Subjects attended the Royal Adelaide Hospital at 0830h after an overnight fast. Two intravenous cannulae were inserted into the left arm; one in the cubital fossa for an infusion of 25% dextrose, and the other in the hand for infusion of the study drug. A third intravenous cannula was inserted into the right cubital fossa for blood sampling. Synthetic GLP-1 amide (Bachem, Weil am Rhein, Germany) was reconstituted by the Pharmacy Department in 4% albumin and presented in a volume of 120ml for infusion at $0.6 \text{ pmol kg}^{-1} \text{ min}^{-1}$ or in 20 x 1ml syringes each with a dose of 3.6 pmol/kg such that placebo and GLP-1 looked identical in both syringes and bags. After drawing baseline blood samples the first bolus was given followed by a 2ml flush of 0.9% saline. The continuous infusion was started concurrently at 1 ml/min and maintained for the duration of the study. Boluses were repeated as described every 6 minutes until study completion at $t = 120\text{min}$. Commencing at $t = 0\text{min}$ venous blood samples for

measurement of glucose, insulin and glucagon were taken at 5min intervals until t = 45min, and then every minute until t = 120min. The total amount of 25% dextrose required to maintain blood glucose on each day was recorded.

Laboratory determinations

Glucose was measured using a portable electrochemical coulometric glucose dehydrogenase glucose meter with a CV of 3.8% in the targeted range (Optium Xceed; Abbott Laboratories, Illinois, USA).

Once clotted, blood samples were centrifuged at 3200 rpm for 15min. Serum was stored at -70° Celsius for subsequent measurement of insulin and glucagon. Insulin was analysed via ultrasensitive paramagnetic immunoassay (Access Immunoassay Systems, Beckman Coulter, California, USA) with analytical sensitivity to 0.21 pmol/l and inter-assay CV of 2.98%. Immunoreactive glucagon was measured by radioimmunoassay (GL-32K, Millipore, Germany) with analytical sensitivity to 20pg/ml, intra-assay CV of 3.8% and inter-assay CV of 8.2%.

Statistical analysis

Overall effects for insulin and glucagon were calculated as area under curve (AUC). Data are mean \pm standard deviation unless otherwise stated. Dextrose requirements and glucagon AUCs were evaluated using RM-ANOVA with Bonferroni correction and insulin AUCs were analysed using a linear mixed effects maximum likelihood model, with a fixed effect for treatment and an unstructured covariance matrix accounting for repeated visits per subject, and Bonferonni adjusted tests for pairwise posthoc comparisons. Statistical analyses were performed using SPSS (version 22.0; IBM, USA).

RESULTS

Twelve healthy participants [9 men, age 22 (2.6) years, body mass index 24 (2.4) kg/m²] completed all three study days without significant adverse events.

Blood glucose

During the euglycaemic period ($t_{0-45\text{min}}$) blood glucose decreased by ~ 1 mmol/l on both continuous and pulsatile GLP-1 days and remained stable on the placebo day. Blood glucose concentrations were effectively clamped at the hyperglycaemic target during placebo [9.0 (0.2) mmol/l], continuous [8.9 (0.5) mmol/l] and pulsatile [8.8 (0.2) mmol/l] days. Dextrose requirements to maintain the hyperglycaemic clamp were approximately three-fold greater on the continuous GLP-1 infusion and pulsatile GLP-1 days when compared to the placebo days [$p < 0.001$], without any significant difference between the continuous and pulsatile days [$p = 1.0$].

Serum insulin

In all participants insulin concentrations approximated a steady plateau from $t = 0 - 45\text{min}$. There was an abrupt rise after commencement of the hyperglycaemic clamp with a return to a higher plateau on the placebo day and a progressive linear increase on both the pulsatile and continuous infusion days (Fig. 1). Insulin concentrations were greater during the euglycaemic period ($t_{0-45\text{min}}$) and overall with both continuous and pulsatile infusions of GLP-1 compared to placebo; [$p < 0.001$] (Fig. 1.) without any difference between the continuous and pulsatile GLP-1 infusion days [$p = 1.0$].

Serum glucagon

In all cases glucagon concentration remained stable until commencement of the hyperglycaemic clamp, following which there was a progressive decline from $t = 45 - 120\text{min}$. There was no overall difference in glucagon suppression between the study days (Fig. 2).

DISCUSSION

The key observation of this study is that during both euglycaemia and hyperglycaemia there was no difference in the insulinotropic response to pulsatile intravenous delivery

of GLP-1 when compared to an equivalent dose ($0.6 \text{ pmol kg}^{-1} \text{ min}^{-1}$) administered continuously.

Our study is the first to investigate the insulinotropic efficacy of replicating physiological pulsatile GLP-1 secretion with administration of exogenous peptide at supra-physiological concentrations. Prior studies utilising intravenous or subcutaneous infusions of GLP-1 in health and diabetes have exclusively delivered GLP-1 as a continuous infusion and, of more clinical relevance, the subcutaneous route of delivery of the commercially available GLP-1 agonists produces similarly stable plasma concentrations [7]. Data from the present study suggest that pulsatile delivery of GLP-1 is unlikely to result in a greater insulinotropic response.

Both intravenous glucose infusions and exogenous GLP-1 suppress endogenous glucagon secretion, with the effect of GLP-1 being glucose dependent [4]. In the present study there was no difference in the magnitude of glucagon suppression during hyperglycaemia regardless of placebo or GLP-1 administration suggesting that, in health, glucagon suppression is maximal following an intravenous hyperglycaemic clamp .

Limitations of this study should be recognised. Based on physiological secretion only a single, six minutely, interval of pulses was tested and a different duration may potentially change the effect. The continuous GLP-1 dose was $0.6 \text{ pmol kg}^{-1} \text{ min}^{-1}$, as this is above the threshold known to have insulinotropic effect with the equivalent bolus dose of 3.6 pmol/kg below that which is likely to have adverse effects [8], but we cannot exclude that lesser or greater doses may have an effect, or if a ‘ceiling’ effect to intravenous GLP-1 boluses occurs, as is seen with other G protein-coupled linked pathways [9]. Hyperglycaemia markedly potentiates the insulinotropic response to GLP-1 in healthy subjects [10] and, for this reason a ‘moderate’ glycaemic clamp of 9 mmol/l was utilised to minimise the synergistic effects of exogenous glucose, but we did not test multiple glucose concentrations. We studied healthy volunteers and cannot exclude that the response may be different in the setting of diabetes. Finally, we did not measure GLP-1 levels and, accordingly, are unable to comment as to whether pulses administered peripherally translate to a pulse amplitude, or overall difference in GLP-1 concentration in either plasma or the portal bed compared to continuous delivery.

In conclusion this study indicates that pulsatile and continuous intravenous infusions of GLP-1 have comparable insulinotropic properties such that pulsatile delivery regimens of GLP-1 or its agonists are unlikely to yield greater glucose-lowering, however, the effects of different pulse intervals and doses of GLP-1 warrant investigation.

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Duality of Interest

J.J.M. has served on advisory boards for, received honoraria or consulting fees from, or received research funding from AstraZeneca, Berlin-Chemie, BMS, Boehringer-Ingelheim, Eli Lilly, MSD, NovoNordisk, Novartis, Roche, and Sanofi-Aventis.

M.A.N has served on advisory boards for, received honoraria or consulting fees from, or received research funding from Amylin, AstraZeneca, Berlin-Chemie, Boehringer Ingelheim, Bristol-Myers, Squibb, Diartis Pharmaceuticals, Eli Lilly, GlaxoSmithKline, F. Hoffman-La Roche, Intarcia Therapeutics, Janssen, MannKind, Merck, MetaCure, Novartis, Novo Nordisk, Roche Pharmaceuticals, Sanofi, Takeda, Versatis, and Wyeth research.

M.H. has participated in advisory boards and/or symposia for AstraZeneca, Boehringer Ingelheim, Eli-Lily, Meyer Nutriceuticals, Novartis, Novo/Nordisk, Sanofi-Aventis, and Satiogen.

M.P.P, P.K., C.E.C, K.L., M.J.C., and A.M.D have no duality of interests to declare.

Contribution Statement

M.P.P was responsible for study conception and design, acquisition of data, statistical analysis, interpretation and drafting the manuscript and approving the final version to be published. He is guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

K.L. was responsible for statistical analysis, revision of the manuscript for important intellectual content and approving the final version to be published.

C.E.C. contributed to the acquisition and interpretation of data, revision of the manuscript for important intellectual content and approving the final version to be published.

P.K., M.A.N., M.J.C, and M.H. contributed to the study design, critical revision of the manuscript for important intellectual content and approving the final version to be published.

J.J.M. and A.M.D. were responsible for the study conception and design, obtaining funding, acquisition of data, critical revision of the manuscript for important intellectual content and approving the final version to be published.

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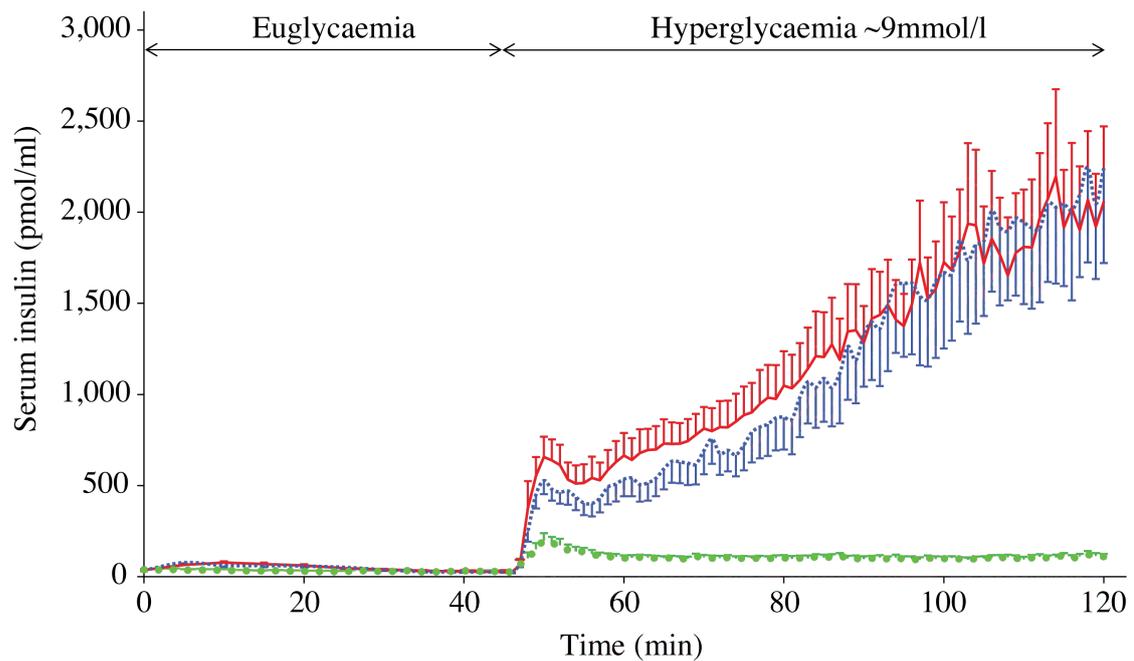


Fig 1. Serum Insulin concentrations

Continuous (red solid line) and pulsatile (blue dashed line) infusions of GLP-1 increased serum insulin by ~50% compared to placebo (green dashed line) during euglycaemia ($t_{0-45\text{min}}$) [$p < 0.001$]. Overall serum insulin was increased ~9-fold during continuous and pulsatile infusions of GLP-1 compared to placebo [$p < 0.001$]. There was no difference in serum insulin between continuous and pulsatile GLP-1 infusion regimens [$p = 1.0$]. Data are mean (SE).

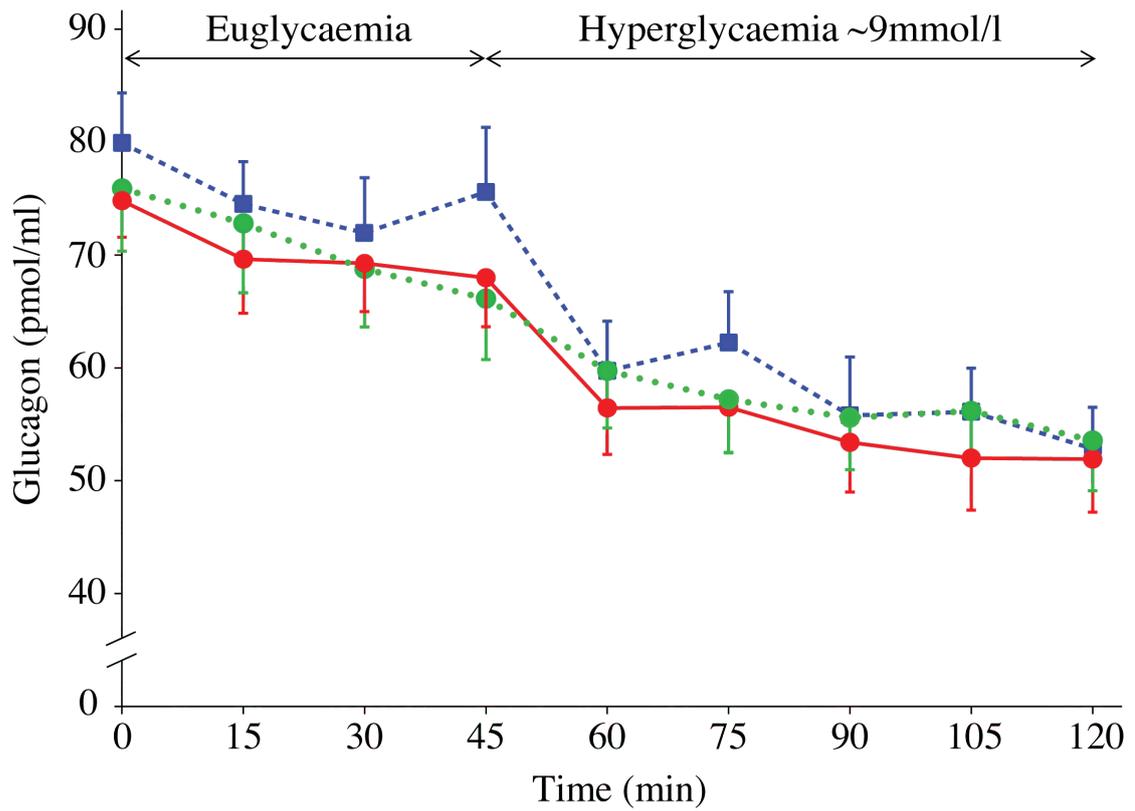


Fig 2. Serum glucagon concentrations

Continuous GLP-1 infusion is shown by red filled circles, pulsatile GLP-1 infusion by blue filled squares and placebo by green filled circles. There was no difference in plasma glucagon across all study days during both euglycaemia (t = 0-45min) and hyperglycaemia (t = 45 – 120min). Data are mean (SE)

3.6 CONCLUSIONS

3.6.1 Introduction

The studies included in this chapter characterised clinically important gastric and islet cell effects of exogenous glucagon-like peptide 1 (GLP-1). Because the hypotheses were novel and the heterogeneity inherent in ambulant patients with type-2 diabetes and/or critically ill patients considerable, it was appropriate to undertake these studies in healthy older participants using the synthetic peptide, rather than one of the commercially available GLP-1 agonists. That the study designs are somewhat artificial is implicit in this approach and extrapolation of the findings to the ‘real-world’ patient cohorts, i.e. patients with type 2 diabetes and/or critically illness, should accordingly be somewhat circumspect. Nevertheless, these studies have yielded important insights into the interaction between glycaemia, gastric emptying and GLP-1, as well as optimal delivery regimens of intravenous GLP-1.

3.6.2 Contribution of the work described in this thesis to the understanding of the effects of exogenous GLP-1 on gastric emptying.

Gastric emptying and glycaemia are inextricably linked, with gastric emptying mediating post-prandial glycaemia and systemic glycaemia being a major determinant of the gastric emptying rate. Short-acting GLP-1 agonists such as exenatide b.d, now incorporated into treatment algorithms for the management of diabetes, exert their glucose lowering effect primarily by slowing gastric emptying. Given that hypo- and hyperglycaemia (i) are prevalent in patients with type 2 diabetes and (ii) profoundly influence the gastric emptying rate, it is surprising that the potential impact of acute changes in glycaemic on the gastromotor effects of GLP-1 had not been quantified.

The study reported in chapter 3.3 was the first to evaluate the effects of pharmacological concentrations of GLP-1 on gastric emptying during insulin-induced hypoglycaemia. Gastric emptying was measured with the gold-standard technique, scintigraphy in healthy older subjects. Consistent with previous studies, a profound acceleration of gastric emptying was observed during marked hypoglycaemia (blood glucose ~2.6 mmol/l). Furthermore, consistent with its known pharmacological action, GLP-1 slowed gastric emptying during euglycaemia. The novel and important observation was that GLP-1 attenuated the acceleration of gastric emptying induced

by hypoglycaemia and, thereby, reduced the rate of carbohydrate absorption. This is in marked contrast to the well-documented 'islet cell' effects of GLP-1 whereby during hypoglycaemia GLP-1 has no effect on insulin secretion or counter-regulatory hormones. The clinical importance of this observation is highlighted by the recent recommendation for GLP-1 agonists to be prescribed as 'add-on' therapy to insulin, including the availability of 'fixed-dose' combinations of basal insulin and a GLP-1 agonist [1]. If patients were to become hypoglycaemic secondary to insulin therapy, these data suggest that glycaemic recovery from an oral carbohydrate load may be delayed secondary to a concomitant GLP-1 effect to slow gastric emptying. Accordingly, other emergency treatments for hypoglycaemia are likely to be more effective and preferable.

The study reported in chapter 3.4 was the first study to evaluate the effects of a medication known to slow gastric emptying during hyperglycaemia. In a similar design to the protocol utilised in chapter 3.3, scintigraphy was used to measure gastric emptying during euglycaemia and clamped hyperglycaemia in healthy older subjects. Consistent with the documented effect of acute hyperglycaemia, a marked slowing of gastric emptying was observed when blood glucose was clamped at 12mmol/L. A concurrent infusion of intravenous GLP-1 at 1.2pmol/kg/min during clamped hyperglycaemia resulted in a substantial further slowing of both gastric emptying and the rate of small intestinal carbohydrate absorption. These observations indicate that for patients with marked fasting hyperglycaemia who also have marked post-prandial glycaemic excursions short-acting GLP-1 agonists are likely to remain an effective glucose-lowering strategy targeting both fasting and post-prandial glycaemia.

3.6.3 Contribution of the work described in this thesis to the understanding of optimal delivery regimens for exogenous GLP-1

The study reported in chapter 3.5 was the first to evaluate the insulinotropic effects of intravenous pulsatile, compared to continuous, GLP-1 infusion. Contrary to the study hypothesis pulsatile GLP-1 regimen had equipotent insulinotropic and glucagonostatic effects to the equivalent dose administered as a continuous infusion. The implication is that pulsatile delivery regimens of GLP-1, or its agonists are unlikely to yield greater glucose-lowering and that continuous infusions are appropriate to achieve a maximal therapeutic response.

3.7 FUTURE DIRECTIONS

The outcomes from the studies reported in this chapter dictate the need for future research in several areas as a priority.

3.7.1 The effect of glycaemic extremes on the gastromotor effects of the commercially available GLP-1 agonists in patients with type 2 diabetes

As these studies were ‘proof-of-principle’ synthetic GLP-1 was administered as a continuous intravenous infusion to ensure predictable plasma concentrations, healthy volunteers were studied to standardise physiological gastric emptying and glycaemia was clamped with exogenous infusions of glucose and/or insulin. It is now appropriate to determine the gastromotor effects of the commercially available subcutaneous agonists in patients with type 2 diabetes, with and without delayed gastric emptying and autonomic dysfunction, at extremes of glycaemia.

3.7.2 Determine the utility of GLP-1 as a novel glucose lowering agent in the critically ill

As highlighted in Chapter 2.1 and 3.1, there is a persuasive rationale for the use of GLP-1 based therapies for the management of hyperglycaemia in critical illness. Potential benefits of GLP-1 include the negligible risk of hypoglycaemia and attenuated glycaemic variability with monotherapy. While there have been a small number of pilot studies in the critically ill, priorities for future research include evaluation of the dose response of GLP-1 on gastric emptying and glycaemia followed by a prospective, randomised controlled trial comparing GLP-1 and intravenous insulin. Furthermore, preliminary animal data suggesting cardiovascular and neuroprotective properties of GLP-1 therapy warrant definitive human trials and critically ill patients post cardiothoracic surgery or traumatic brain injury are logical populations to target.

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CHAPTER 4

STRESS ULCER PROPHYLAXIS IN THE CRITICALLY ILL

4.1 INTRODUCTION

Critical illness can cause stress ulceration of the gastric mucosa which sometimes results in clinically significant bleeding associated with increased morbidity and mortality. It is plausible that drugs that suppress acid secretion, such as the proton pump inhibitor pantoprazole, will reduce the incidence of bleeding from stress ulceration but this has not been demonstrated in a randomised controlled study. Furthermore, in interventional studies that have led to a reduction in the incidence of stress ulceration and bleeding have failed to observe any decrease in mortality or length of stay, suggesting that the association between the development of clinically significant bleeding and mortality may not be causal - rather clinically significant bleeding may just herald a poor outcome.

Despite the dearth of proven benefit, the routine use of stress ulcer prophylaxis has progressively crept into accepted dogma, highlighted by the recent publication of the 'Surviving Sepsis Campaign Guidelines' that strongly recommend the administration of stress ulcer prophylaxis to patients with risk factors, which included the need for invasive mechanical ventilation or coagulopathy [1]. What was most surprising about this recommendation was that in the accompanying discussion the authors acknowledged that there was an absence of data to support their recommendation. It is intuitive that widespread 'routine' administration of any drug to any large cohort may cause significant adverse effects. Gastric acid has an important role in controlling flora and preventing infection and it is mechanistically plausible that proton pump inhibitor prophylaxis may increase the risk of infective complications in a critically ill population. Indeed observational studies in critically ill and ambulant patients report that routine administration of acid suppressive medication increases complications such as pneumonia and *Clostridium difficile* and even mortality appears to be greater [2-6]. Based on the rationale that clinically significant bleeding occurs infrequently, that there is hitherto no evidence of survival benefit in an enterally fed critically ill population and the potential for infective related harm, the standard of care at the hospital at which these studies were undertaken, the Royal Adelaide Hospital Intensive Care Unit, was to not routinely prescribe stress ulcer prophylaxis.

Chapter 4.2 reviews the relevant literature relating to stress ulcer prophylaxis in the critically ill including prevalence, mechanisms, trials assessing prevention and potential adverse effects. The discordance between the published international guidelines and what, locally, is thought to be best practice represented the impetus to undertake the study outlined in chapter 4.3. The primary aim of this study was to evaluate whether prophylactic administration of the proton pump inhibitor pantoprazole was overtly beneficial or harmful in an enterally fed heterogeneous critically ill population. Secondary aims were to establish precise estimates of clinically significant gastrointestinal bleeding and infective complications.

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4.2 Literature review: Stress ulceration: prevalence, pathology and association with adverse outcomes

Full title

Stress ulceration: Prevalence, pathology and association with adverse outcomes

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Introduction

So-called 'stress related mucosal damage' (SRMD) is the broad term used to describe the spectrum of pathology attributed to the acute, erosive, inflammatory insult to the upper gastrointestinal tract associated with critical illness [1]. SRMD represents a continuum from asymptomatic superficial lesions found incidentally during endoscopy, occult gastrointestinal bleeding causing anemia, overt gastrointestinal bleeding and clinically significant gastrointestinal bleeding.

Prevalence

Stress ulceration was first described in 1969 when focal lesions in the mucosa of the gastric fundus were reported during post-mortem examinations in 7 (out of 150) critically ill patients [2]. Endoscopic studies have since identified that between 74 - 100% of critically ill patients have stress related mucosal erosions and subepithelial hemorrhage within 24 hours of admission (*figure 1a*) [3]. These lesions are generally superficial and asymptomatic, but can extend into the submucosa and muscularis propria and erode larger vessels causing overt and clinically significant bleeding (*figure 1b*). The prevalence of the latter two conditions depend on how they are defined, with the definitions by Cook and colleagues the most widely accepted [4]. These authors defined overt gastrointestinal bleeding as the presence of hematemesis, bloody gastrointestinal aspirate or melena, while clinically significant bleeding is the association of overt gastrointestinal bleeding and either hemodynamic compromise, or the requirement for blood transfusion, or surgery. It is important to emphasise that SRMD excludes variceal bleeding. However, bleeding *per se* is a clinical endpoint, and some studies may have incorrectly included bleeding attributable to varices, as well as that from the lower gastrointestinal tract, as part of the SRMD spectrum. This distinction is often not clear in the literature, particularly in observational studies of SRMD where clinically significant bleeding is a primary outcome, which may lead to investigators inappropriately including variceal, or non-SRMD bleeding. The importance of this distinction is highlighted in a prospective study by Cook and colleagues, which identified the cause of hemorrhage in 22 (of 33) patients with clinically significant gastrointestinal bleeding by the use of endoscopy or surgery [4]. In this study stress ulceration was identified as the sole source of bleeding in 14

patients, with evidence of ulceration noted in 4 (of the remaining 8) patients in whom another bleeding site was identified which included esophageal and gastric varices, vascular anomalies, and an anastomosis bleed [4]. Accordingly, variceal or non-SRMD pathologies, which will not be prevented by stress ulcer prophylactic therapies, are a frequent cause of overt and clinically significant bleeding. This distinction is often not identified in observational studies, whereas randomized controlled studies comparing different therapies for the prevention of SRMD have excluded patients with previous ulcer and variceal disease. For this reason prevalence data from the intervention studies may not be comparable to that from observational studies.

Nevertheless, data from earlier studies suggested that overt gastrointestinal bleeding occurred frequently, and in some studies up to 25% of critically ill patients developed overt gastrointestinal bleeding [5]. However, it is now accepted that the condition is far more infrequent, with the prevalence reported between 0.6 and 4% of patients [4, 6]. The variation in prevalence is due, at least in part, to the cohort of patients studied and their risk factors for developing SRMD and it has been estimated that episodes of clinically significant stress ulcer bleeding in patients without risk factors is negligible (~0.1%) [4]. The infrequency of the diagnosis in more recent epidemiological studies probably reflects an improvement in the overall management of the critically ill patient, including a focus on early aggressive resuscitation, attenuating mucosal hypoperfusion, and an awareness of the importance of early enteral nutrition [7].

Importance

Clinically significant gastrointestinal bleeding, as the name suggests, indicates that bleeding is substantive and important. It has been estimated that up to half of all patients with clinically significant upper gastrointestinal bleeding die in the Intensive Care Unit (ICU) and, in survivors, the length of ICU stay increases approximately 8 days [8]. It is therefore intuitive that preventing episodes of clinically significant gastrointestinal bleeding will lead to better patient outcomes. However, interventional studies that have reduced the incidence of stress ulceration have had no effect on either mortality or length of stay [6, 9]. Plausible explanations for this lack of effect following intervention are that: (i) a demonstrable proportion of clinically significant bleeding is not attributable to SRMD and will not respond to acid suppressive therapy;

(ii) previous studies were underpowered; (iii) the interventions studied have adverse effects that negate any benefit from a reduction in stress ulceration; and (iv) the association between development of clinically significant bleeding and mortality may not be causal, and that clinically significant bleeding may just be heralding a poor outcome.

Mechanisms

Putative mechanisms underlying SRMD include reduced gastric blood flow, mucosal ischemia and reperfusion injury, all of which have the capacity to occur frequently in the critically ill [9]. In a prospective observational study of 2200 critically ill patients, mechanical ventilation > 48 hours and coagulopathy were identified as substantial risk factors for clinically significant bleeding (odds ratio 15.6 and 4.3 respectively) [4]. Studies of smaller cohorts, which were performed over 30 years ago, also reported associations between clinically significant bleeding and hypotension, sepsis, hepatic failure, renal failure, burns and major trauma [10].

Prevention of stress ulceration

Although clinically significant bleeding occurs infrequently, the severity of the associated complications has encouraged preventative approaches. For example, the FAST HUG mnemonic reminds clinicians to consider the need for stress ulcer prophylaxis on a daily basis [11]. Moreover, the recent Surviving Sepsis Campaign guidelines recommended the use of stress ulcer prophylaxis in patients with severe sepsis who have a risk factor, one of which was the need for mechanical ventilation > 48 hours [12]. Somewhat surprisingly, the recommendation to prescribe a stress ulcer prophylaxis drug was listed as a 1B recommendation – translating to a ‘strong’ recommendation. This recommendation was endorsed despite the accompanying discussion acknowledging that there are no data to demonstrate mortality benefit when prescribing these drugs [12].

Several drugs/techniques have been described to reduce the incidence of SRMD including; sucralfate, histamine-2 receptor blockers (H2RBs) and proton pump

inhibitors (PPIs). Sucralfate acts by adhering to epithelial cells forming a physical cytoprotective barrier at the ulcer site, thereby protecting the gastric mucosa from the effects of acid and pepsin. Sucralfate is more effective than placebo in reducing overt bleeding, but has been shown to be inferior to H2RBs to reduce clinically significant bleeding [13]. Furthermore, sucralfate can impair the absorption of enteral feeds and co-administered oral medication [14], and there is a potential risk of bezoar formation (particularly in the setting of impaired gastric motility) when administering sucralfate to patients who are concurrently receiving enteral liquid nutrient [15]. Since intravenous H2RBs and PPIs are now widely available, sucralfate is rarely used as a first line therapy.

H2RBs competitively inhibit histamine binding to its G-protein coupled receptor on the basolateral membrane of gastric parietal cells, which results in a reduction of acid production and an overall decrease in gastric secretions. H2RBs were used in early studies as first line stress ulcer prophylaxis therapy, and were shown to significantly reduced the risk of clinically important bleeding when compared to placebo [13]. A limitation of H2RBs administration is that tachyphylaxis can occur rapidly. In health, the anti-secretory effect of continuously infused intravenous ranitidine is dramatically reduced within the first day of administration [16]. With intragastric pH monitoring, studies in health have demonstrated that 70% of patients have an intragastric pH >4 in the first 24 hours of ranitidine intravenous infusion which falls to 26% on the third day of continuous infusion [16]. While similar studies have not been performed in the critically ill these data raise concerns about the efficacy of H2RB's during longer term use in the critically ill [16].

Proton pump inhibitors inactivate the H^+/K^+ ATPase enzyme at the secretory surface of the parietal cell, inhibiting the secretion of H^+ ions thereby raising the pH of the gastric contents. In contrast to H2RBs the use of PPIs is not associated with the development of tolerance, with 100% of healthy subjects maintaining an intragastric pH >4 after 72 hours of continuous infusion of omeprazole [16]. In a recent meta-analysis, Alhazzani and colleagues reported PPIs to be more effective than H2RBs at reducing clinically important and overt upper gastrointestinal bleeding, without appearing to increase the risk of nosocomial pneumonia [6]. The Surviving Sepsis Campaign guidelines recommend the use of PPIs rather than H2RBs for stress ulcer prophylaxis citing level 2C evidence [12]. Previous studies of SRMD prophylaxis in

the critically ill with PPIs are summarised (*table 1*) [17-29]. While these studies have been subject to meta-analyses by various groups [6, 9], with somewhat divergent results, even when these analyses have shown a reduction in clinically significant bleeding with PPI use, there has been no corresponding reduction in mortality.

Potential adverse effects associated with stress ulcer prophylaxis therapy

Controversy surrounds the relationship between the use of stress ulcer prophylaxis and the development of infectious complications, particularly infection-related ventilator associated complications (IVAC) and *Clostridium difficile* infection. Gastric acid plays an important role in natural host defence, with an intragastric pH<4 being optimal for bactericidal action[30]. Accordingly, suppressing gastric acid production and raising the intragastric pH above this bactericidal threshold has the capacity to increase colonisation of the stomach with pathogenic organisms.

Stress ulcer prophylaxis and infection-related ventilator associated complications

For the purpose of this review the updated term infection-related ventilator associated complication (IVAC) has been used in preference to the previous term ventilator associated pneumonia (VAP). In 2013, the Centers for Disease Control and Prevention proposed new definitions for patients receiving mechanical ventilation, including IVAC to improve objectivity and facilitate comparability [31]. While prior studies investigating stress ulcer prophylaxis have exclusively used the term VAP to report data, with the inherent subjectivity associated with this diagnosis, we believe that using the recently proposed guidelines for IVAC in future studies will more accurately determine whether stress ulcer prophylaxis increases adverse events during mechanical ventilation. It should be recognised however that the previous studies all referred to VAP rather than IVAC.

A proposed mechanism contributing to IVAC is that reflux of gastric fluid contaminates the oropharyngeal area, with subsequent aspiration of the oropharyngeal bacteria to the lower airways [32]. Because numerous organisms are unable to live in an acidic environment the administration of drugs to increase gastric pH could

facilitate gastric colonisation with pathogenic organisms and predispose to respiratory infections [30]. In ambulant patients the use of PPIs has been associated with an increased risk of community acquired pneumonia [33]. Laheij et al., reported a 1.89 fold increase in the risk of community acquired pneumonia in those taking PPIs versus those who had stopped using PPIs [33], with a correlation between dose of PPI and risk of pneumonia [33].

In the critically ill however data relating intragastric pH and pulmonary infections are inconsistent. Supporting the importance of gastric acidity and the role of the entero-pulmonary route are studies that observed more IVAC in patients who received drugs to increase the gastric pH when compared to those who received sucralfate [34]. However, Heyland et al. reported that while the delivery of acidified enteral feeds (pH 3.5) preserved gastric acidity, and dramatically reduced gastric bacterial growth and lowered the rate of gram-negative bacterial growth in tracheal suction, there was no reduction in frequency of VAP [35]. In a meta-analysis of data comparing H2RBs and placebo that did not adjust for enteral nutrition Cook, *et al.* reported a trend towards increased rates of pneumonia with the routine use of H2RBs [13].

Despite PPI prophylaxis being a key recommendation of the Surviving Sepsis Guidelines there have been no large-scale prospective randomised trials that have compared PPIs and placebo to determine the efficacy and/or adverse events associated with their use [12]. Nevertheless, the rate of IVAC associated with PPI use is likely to be at least similar to that observed with H2RBs [6]. Furthermore, if tolerance to H2RBs occurs, and increasing pH increases the risk of IVAC, it is plausible that VAP rates will be even greater in patients receiving PPIs. Regardless of whether H2RBs or PPIs are more harmful in creating the ideal environment to alter bacterial colonisation of the stomach, this issue is likely to be particularly relevant for enterally fed patients, as enteral feeding *per se* may be a risk factor for IVAC [36].

Stress ulcer prophylaxis and *Clostridium difficile* infection

Symptomatic infection with *Clostridium difficile* occurs relatively frequently in mechanically ventilated critically ill patients. Using data from over 65,000 patients in the United States who required prolonged ventilation, *Clostridium difficile* associated

diseases were present in > 5% of patients [37]. Furthermore *Clostridium difficile* infections are important, as infection leads to a substantial increase in hospital length of stay (6.1 days; 95% confidence interval 4.9 - 7.4) [37].

There is a plausible biological mechanism that acid-suppression increases the risk of developing *Clostridium difficile* colonisation, as host immunity is compromised by a higher pH environment in the stomach [38]. Observational studies have reported an association between iatrogenic acid suppression and *Clostridium difficile* associated diseases [38]. In a prospective case-control study of 303 patients admitted to a general medical ward, Yearsley *et al.*, report a two fold increase in *Clostridium difficile* associated diseases in patients receiving PPIs [39]. However, to the best of our knowledge, there are no epidemiological data detailing *Clostridium difficile* associated diseases in critically ill patients receiving stress ulcer prophylaxis.

Complications associated with long-term use of drug therapies

While complications associated with the acute use of H2RBs and PPIs are of more relevance to critically ill patients it should be recognised that chronic use of PPIs has been associated with osteoporosis and fractures [40]. Adverse effects associated with chronic use may be important, as a recent observational study reported that around a third of patients given PPIs for stress ulcer prophylaxis went home on the drug despite there being no indication on discharge from hospital for their ongoing use [41].

Enteral feeds and the role of stress ulcer prophylaxis

The majority of the studies from which the current recommendations are based were performed over 20 years ago. Since that time there have been changes to the perceived importance of enteral nutrition, with intragastric feeds commenced sooner after admission [42]. Liquid nutrient buffers gastric acid, increases mucosal blood flow and induces the secretion of cytoprotective prostaglandins and mucus [43]. It is uncertain as to what influence the route of enteral feeding has on the effect of liquid nutrient. While it is intuitive that only liquid nutrient administered into the stomach could have these potentially beneficial effects, delivery directly into the small intestine may have

other advantages that lead to favorable outcomes [42]. Furthermore, because of duodenal-gastric reflux of liquid [32] and increase in mesenteric blood flow due to small intestinal delivery [44], postpyloric delivery may still prevent development of stress ulceration. Nevertheless the so-called ‘early’ administration of enteral nutrition into the stomach has been suggested to have contributed substantially to the diminishing frequency of stress ulcer related bleeding that has been observed over the last thirty years [7]. In the critically ill, continuous enteral nutrition has been shown to be more effective at increasing intragastric pH than H2RBs and PPIs [45] and, in rats, enteral nutrition provides better protection against stress ulceration than intravenous H2RBs [46]. Studies in humans to evaluate the effects of enteral nutrition on GI bleeding reduction have primarily been performed in patients post burn injury. Interpretation of these data are problematic because of inconsistencies around the definitions of SRMD, clinically significant upper gastrointestinal bleeding and enteral nutrition [47]. Marik *et al* performed a meta-analysis to evaluate the effects of H2RBs and placebo [9]. In the subgroup of patients who received enteral feeds, stress ulcer prophylaxis did not reduce the risk of bleeding but increased the VAP rates and mortality [9]. However, as acknowledged by the authors, subgroup analysis within a systematic review should be interpreted with caution. For this reason we consider the Marik review hypothesis generating, and prospective studies to determine the influence of enteral nutrition on SRMD and stress ulcer prophylaxis associated IVAC are urgently required.

Cost of routine prophylaxis

Models of cost effectiveness of stress ulcer prophylaxis advocate for prophylactic therapy being limited to patients with established risk factors for clinically significant bleeding [48]. In comparison to routine prophylaxis for all critically ill patients, this strategy has been shown to decrease H2RB drug costs by 80% without altering the frequency of gastrointestinal bleeding [49]. To our knowledge a cost analysis has not been performed with PPIs in the critically ill. Based on historical data, however, stress ulcer prophylaxis would need to be routinely administered to 900 hospitalized patients to prevent one episode of clinically significant bleeding [50]. Since clinically significant stress ulcer bleeding occurs infrequently in patients without risk factors,

routine stress ulcer prophylaxis is unlikely to be cost effective and should probably be avoided in this subgroup, particularly given the potential for harm with PPI and H2RB use. As described [41] almost a third of patients had PPIs continued on hospital discharge, which in itself will lead to increases in costs to individual patients and communities, independent of any long term health concerns.

Conclusions

Using current resuscitation and feeding practices, clinically significant gastrointestinal bleeding, as a consequence of SRMD, appears to occur infrequently. Nevertheless, should clinically significant bleeding occur it is associated with significant morbidity and at least a 4-fold increase in ICU mortality. Patients with respiratory failure requiring mechanical ventilation for >48 hours and those with coagulopathy are at the highest risk for clinically significant bleeding. Based on these observations there are current guidelines suggesting that this group is most likely to benefit from prophylactic therapy. The superior efficacy of PPIs has shaped recommendations that these agents be used as first line therapy. However, the routine use of stress ulcer prophylaxis in all critically patients may be harmful and is unlikely to be cost-effective. While controversy surrounds pharmacologically raising gastric pH, there is mechanistic plausibility that this may increase the rate of IVAC and *Clostridium difficile* infections – both of which are associated with substantial morbidity and increased costs – particularly in those ventilated for > 48 hours. In contrast to recent recommendations from the Surviving Sepsis Campaign we contend that the issue of stress ulcer prophylaxis is not settled and further prospective randomised trials are required to guide decision-making.

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Table 1: A summary of trials of proton pump inhibitors for stress ulcer prophylaxis

Author (year)	Population	Intervention	UGI bleeding	Pneumonia
Powell et al (1993) ¹⁷	Post CABG Age: 57; APACHE II: N/R	Omeprazole IV 80mg x1, then IV 40mg/day (n=10)	0 (0%)	N/R
		Omeprazole IV 80mg x1, then IV 40mg/8h (n=10)	0 (0%)	N/R
		Ranitidine IV 50mg/8h (n=11)	0(0%)	N/R
Risaliti et al (1993) ¹⁸	Surgical ICU. Age: 62; APACHE II: N/R	Omeprazole IV 40mg, then PO 20mg/day(n=14)	0 (0%)	N/R
		Ranitidine IV 150mg, then PO 300mg/day (n=14)	0 (0%)	N/R
Levy et al (1997) ¹⁹	Medical and surgical ICU. Age: 57; APACHE II: 19	Omeprazole NG 40mg/day(n=32)	1 (3%)*	5 (14%)
		Ranitidine IV 50mg bolus, then IV 50mg/day (n=35)	11 (35%)	6 (18%)
Lasky et al (1998) ¹⁰	Post trauma, ventilated. Age: N/A; APACHE II: N/R	Omeprazole NG 40mg x 2, then NG 20mg/day(n=60)	0(0%)	17(28%)
Phillips et al (1998) ²¹	General ICU. Age: N/A; APACHE II: N/R	Omeprazole NG 40mg x 2, then NG 20mg/day(n=33)	1 (3%)*	6(18%)
		Ranitidine IV 50mg x1, CIV 150-200mg/24h (n=25)	4(16%)	4(16%)
Azvedo et al (1999) ²²	General ICU. Age: 57; APACHE II: N/R	Omeprazole IV 40mg/12h (n=38)	0(0%)	5(13.1%)
		Ranitidine CIV 150mg/24h (n=38)	4(11%)	4(11%)
		Sucralfate NG 1gm/6h (n=32)	3(9%)	3(9%)
Kantorova et al (2004) ²³	Surgical ICU. Age: 47; APACHE II: 18	Omeprazole IV 40mg/day (n= 72)	1(1%)	8(11%)
		Famotidine IV 40mg/12h (n = 71)	2(3%)	7(10%)
		Sucralfate NG 1gm/6h (n=69)	3(4%)	6(9%)
		Placebo (n= 75)	1(1%)	5(7%)
Pan et al (2004) ²⁴	Severe acute pancreatitis. Age: 48; APACHE II: 12	Rabeprazole PO 20mg/day (n=20)	0(0%)	N/R
		Famotidine IV 40mg/12h (n = 10)	1(10%)	N/R
Conrad et al (2005) ²⁵	General ICU. Age: 55; APACHE II: 23	Omeprazole Ng 40mg x2, then NG 40mg/day (n=178)	7 (4%)	20 (11%)
		Cimetidine IV 300mg bolus, then CIV 1200mg/24 h (n = 181)	10 (6%)	17 (9%)
Hata et al (2005) ²⁶	Cardiac ICU. Age: 65; APACHE II: N/R	Rabeprazole PO 10mg/day (n=70)	0(0%)	N/R
		Ranitidine PO 300mg/day (n=70)	4 (6%)	N/R
		Teprenone NG 150mg/day(n=70)	4 (6%)	N/R
Kotlyanskaya et al (2008) ²⁷	Medical ICU. Age: 72; APACHE II: 28	Lanzoprazole PO (n=45) Dose not given	0(0%)	2(4%)
		Ranitidine (n=21) Dose and route not given	3(14%)	4(19%)
Somberg et al (2008) ²⁸	Mixed ICU. Age 42; APACHE II: 15	Pantoprazole IV 40mg/day (n=32)	0(0%)	3(9%)
		Pantoprazole IV 40mg/12h (n=38)	0(0%)	8(21%)
		Pantoprazole IV 80mg/day (n=23)	0(0%)	1(4.3%)
		Pantoprazole IV 80mg/12h (n=39)	0(0%)	2(5.1%)
		Pantoprazole IV 80mg/8h (n=35)	0(0%)	2(5.7%)
		Cimetidine IV 300mg bolus, then CIV 1200mg/24 h (n = 35)	0(0%)	3(9.1%)
Solouki et al (2009) ²⁹	General ICU. Age 50; APACHE II: N/R	Omeprazole NG 20mg/12h (n=61)	4 (7%)	8(13%)

*Study reported clinical significance,

Age and APACHE data are presented as mean. APACHE II = Acute Physiological and Chronic Health Evaluation II; CABG = coronary artery bypass graft; CIV = continuous intravenous infusion; IV = intravenous; NG = nasogastric; N/R = not recorded PO = per oral.



Fig 1a. Stress related mucosal disease: Gastric antral erosions



Fig 1b. Stress related mucosal disease: Pyloric ulcer with adherent clot

**4.3 Manuscript: Pantoprazole or Placebo for stress Ulcer Prophylaxis (POPUP):
Randomized double blind exploratory study**

Title

Pantoprazole Or Placebo for stress Ulcer Prophylaxis (POPUP): Randomized double blind exploratory study

Authors

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Author Contribution

SPS: Study concept and design, acquisition, analysis and interpretation of data and drafting of the manuscript.

MJS: Acquisition, analysis and interpretation of data and critical revision of the manuscript for important intellectual content.

MEF: Study concept and design, acquisition, analysis and interpretation of data, statistical analysis, and critical revision of the manuscript for important intellectual content.

MPP: Study concept and design, obtained funding, acquisition, analysis and interpretation of data, critical revision of the manuscript for important intellectual content.

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AMD: Study concept and design, obtained funding, acquisition, analysis and interpretation of data, drafting of the manuscript and study supervision.

All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest disclosures

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. No disclosures are reported.

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Role of the Funder

The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contribution

The authors are grateful for the assistance from the Department of Pharmacy, as well as the nursing and medical staff of the Royal Adelaide Hospital Intensive Care Unit as their support was crucial for performance of this study.

ABSTRACT

Objective

Pantoprazole is frequently administered to critically ill patients for prophylaxis against gastrointestinal bleeding. However, comparison to placebo has been inadequately evaluated and pantoprazole has the potential to cause harm. Our objective was to evaluate benefit or harm associated with pantoprazole administration.

Design

Prospective randomized double blind parallel group study.

Setting

University affiliated mixed medical-surgical intensive care unit.

Patients

Mechanically ventilated critically ill patients suitable for enteral nutrition.

Interventions

We randomly assigned patients to receive either daily IV placebo or pantoprazole.

Measurements

Major outcomes were clinically significant gastrointestinal bleeding, infective ventilator-associated complication or pneumonia and *Clostridium difficile* infection; minor outcomes included overt bleeding, hemoglobin concentration profiles and mortality.

Main results

None of the 214 patients randomized had an episode of clinically significant gastrointestinal bleeding, three patients met the criteria for either an infective ventilator-associated complication or pneumonia (placebo: 1 vs. pantoprazole: 2) and one patient was diagnosed with *Clostridium difficile* infection (0 vs. 1). Administration of pantoprazole was not associated with any difference in rates of overt bleeding (6 vs. 3; P=0.50) or daily hemoglobin concentrations when adjusted for transfusion rates of packed red cells (P=0.66). Mortality was similar between groups

(log-rank $P=0.33$: adjusted hazard ratio for pantoprazole: 1.68 (95% CI 0.97, 2.90); $P=0.06$).

Conclusions

We found no evidence of benefit or harm with the prophylactic administration of pantoprazole to mechanically ventilated critically ill patients anticipated to receive enteral nutrition. The practice of routine administration of acid suppressive drugs to critically ill patients for stress ulcer prophylaxis warrants further evaluation.

Trial Registration

Australian New Zealand Clinical Trials Registry (<http://www.anzctr.org.au>, trial ID: ACTRN12613000807752).

INTRODUCTION

Gastrointestinal (GI) bleeding that is severe enough to be termed ‘clinically significant’ is associated with increased mortality and longer intensive care unit (ICU) length of stay (1). However, recent data indicate the incidence of clinically significant stress-related GI bleeding has been decreasing over time (2-4), which may reflect changes in clinical practice such as the earlier initiation of enteral feeding that protects against stress related mucosal abnormalities via buffering gastric pH or increasing mesenteric blood flow (5, 6).

Trials comparing histamine receptor antagonists to placebo have reported that some form of ‘stress ulcer prophylaxis’ reduces the incidence of GI bleeding (7-9). Based on these historical data recently published guidelines, sponsored by prominent national and international organizations including the Society for Critical Care Medicine, strongly recommend prophylactic administration of acid-suppressive drugs for ventilated patients (10, 11). Consequently the use of acid-suppressive drugs has become widespread, with the majority of clinicians preferring to give proton pump inhibitors for this purpose (4, 12).

It is therefore somewhat surprising that only two studies have compared the use of proton pump inhibitors with placebo to prevent GI bleeding in critically ill patients (13, 14). While both studies were conducted in an open-label fashion and included only small numbers of patients (n=147 and 30 respectively), neither reported a reduction in GI bleeding events with prophylactic administration of proton pump inhibitors (relative risk of bleeding when prescribing a proton pump inhibitor: 1.04 [95% confidence intervals 0.07, 16.34]) (3, 13, 14). Accordingly, current recommendations and practice do not appear to be supported by robust evidence.

Recent observational studies have reported strong associations between the use of proton pump inhibitors and the prevalence of ventilator-associated pneumonia and *Clostridium difficile* infection (15, 16). Moreover, the use of proton pump inhibitors in the community is associated with an increased risk of similar infections and adverse cardiovascular events (17, 18). Prophylactic administration of proton pump inhibitors to critically ill patients may therefore be harmful.

Because proton pump inhibitors have been inadequately evaluated in the critically ill, yet are frequently prescribed and have the potential to cause harm, we designed the Pantoprazole Or Placebo for stress Ulcer Prophylaxis (POPUP) trial as a single-center exploratory study. Our primary objective was to evaluate whether prophylactic administration of a proton pump inhibitor is either overtly beneficial or harmful. Our secondary objectives were to: 1) establish estimates of event rates with and without prophylactic pantoprazole administration of clinically significant GI bleeding, infective ventilator-associated complication or pneumonia, *Clostridium difficile* infections and hemoglobin concentrations, and 2) ascertain whether the study drug could be administered promptly after commencing mechanical ventilation.

Methods

Patients

All patients admitted to the Royal Adelaide Hospital ICU between 28 January 2014 and 27 January 2015 were evaluated. The Royal Adelaide Hospital is the major quaternary referral center in the state of South Australia for trauma, neurological injuries and burn injuries. Patients who were anticipated to be invasively mechanically ventilated for greater than 24 hours and receive enteral nutrition within 48 hours of

admission were eligible for inclusion. Exclusion criteria included: 1) use of acid-suppressive therapy prior to admission, 2) admission with GI bleeding, 3) history of proven peptic ulcer disease, 4) administration of >100mg daily of prednisolone (or equivalent of other corticosteroid), 5) surgery on the upper GI tract or cardiac surgery during the current hospital admission, 6) pregnancy, 7) Jehovah's witnesses, 8) patients who could not receive their first dose of study medication within 36 hours of initiation of mechanical ventilation, 9) admission for the sole-purpose of providing palliative care and 10) patients readmitted to ICU.

The Research Ethics Committee of the Royal Adelaide Hospital approved the study protocol using delayed opt-out consent from the patient's surrogate decision maker. The rationale for this consent model was that at our institution both treatments are perceived as standard care. The delayed opt-out consent process allows surrogate decision makers or the patient to subsequently withdraw consent, with data for these patients then excluded from all analyses. The study was performed according to National Health and Medical Research Council of Australia guidelines for the conduct of research on unconscious patients and registered with the Australian New Zealand Clinical Trials Registry (<http://www.anzctr.org.au>, trial ID: ACTRN12613000807752).

Study design

We conducted a prospective randomized double blind parallel group study. Study participants were randomly assigned to receive pantoprazole (40mg in 10mL of 0.9% saline IV) or placebo (10mL of 0.9% saline IV). The intervention was administered as a once daily dose until the patient was no longer mechanically ventilated or for a maximum of 14 days. The intervention was also ceased at the discretion of the treating physician or if consent was withdrawn. All other treatment decisions, including interventions for any overt or clinically significant GI bleeding, were left to the discretion of the attending physician. The hospital Department of Pharmacy performed computer-generated 1:1 ratio randomization of the allocation and prepared the blinded study drug packs.

Outcome measures

Major outcomes of interest were clinically significant GI bleeding, infective ventilator-associated complication or pneumonia, and *Clostridium difficile* infection. We deemed the use of three major outcomes necessary and acceptable because the intervention had the potential for distinct and clinically important beneficial and harmful effects (19).

Unless specified we collected data regarding ventilator settings (for diagnosis of infective ventilator-associated complication or pneumonia) for the duration of each participant's ICU admission or for a maximum of 7 days after ceasing the study drug. All outcomes were assessed while the investigators remained blinded to treatment allocation. Clinically significant GI bleeding was defined as an episode of overt bleeding (hematemesis, bloody gastric aspirate, melena or hematochezia), accompanied by at least one of: 1) a reduction in mean arterial blood pressure of ≥ 20 mmHg within 24 hours in the absence of another cause, 2) a reduction in hemoglobin of ≥ 20 g/L within 24 hours, or 3) a need for endoscopy or surgery to achieve hemostasis (2). We utilized the Centers for Disease Control and Prevention (CDC) definitions for infective ventilator-associated complications and pneumonia (supplemental file) (20). Where a patient in ICU had ≥ 3 bowel movements in a 24 hour period, a single stool sample was sent for *Clostridium difficile* toxin B DNA polymerase chain reaction testing. Patients were followed after discharge from ICU until hospital discharge for stool testing, as ordered at the discretion of the attending physician.

We also collected data related to minor outcomes including: 1) time from initiation of mechanical ventilation to the first dose of study drug, 2) number of doses of study drug administered per patient, 3) overt bleeding, 4) daily hemoglobin concentrations and 5) units of packed red cells transfused, 6) clinician-adjudicated ventilator-associated pneumonia (according to criteria used previously by Davies et al. (21) [supplemental file]), 7) ventilator-free days at day 28 (22), 8) ICU and hospital length of stay and 9) 90 day all-cause mortality.

Because coagulopathy is an established risk factor for clinically significant gastrointestinal bleeding we categorized the presence of 'hemostatic dysfunction' on enrolment (defined as INR > 1.5 , APTT > 40 seconds or platelet count $< 100,000/\mu\text{L}$).

We also collected data related to enteral nutrition (feed intolerance was defined as gastric residual volume > 250 mL at least once on any day) (23) and medications.

All interventions other than the study drug were left to the discretion of the treating physician.

Statistical analysis

As our institution admits over 600 patients annually requiring mechanical ventilation for >24 hours we anticipated data from all eligible patients admitted and enrolled over a 12 month period would be sufficient for an exploratory study to identify overt benefit or harm and to provide event rate estimates for a phase III study.

Data are presented as frequencies and proportions for categorical variables and mean (standard deviation) or median [interquartile range] for continuous variables. Rate estimates for major outcomes are also presented as exact binomial 95% confidence intervals, except when there were no events i.e. the rate lies on a boundary, when we present the one-sided upper 97.5% interval. Patient characteristics, interventions and primary and secondary outcomes were compared between groups using either Fisher's exact or chi-squared test for categorical data, unpaired Student's t-tests for parametric continuous variables, and Mann-Whitney U tests for non-parametric continuous variables.

Sequential daily hemoglobin and transfusion data were analyzed using a linear mixed-effects model. Mortality is presented as Kaplan-Meier failure curves, with between group comparisons performed by log-rank test, and multivariate analysis performed using Cox proportional hazards, with results reported as hazard ratios (HRs) with 95% confidence intervals.

All analyses were conducted by intention to treat, with secondary sensitivity analysis performed per protocol. Notwithstanding the three major endpoints, we elected *a priori* not to adjust for multiple comparisons (19). A two-sided p-value < 0.05 was considered statistically significant. All analyses were conducted using Stata (version 14.0).

Results

Patients

During the study period 1632 patients were admitted to the Royal Adelaide Hospital ICU and 216 patients received study drug. Retention of data was subsequently refused for two patients so that 214 patients were included in the intention to treat analysis (Fig. 1). Five patients were withdrawn after commencing study drug and 209 patients were included in the per protocol analysis (Fig. 1).

The baseline characteristics of the treatment groups were similar (Table 1).

Processes of care

The study drug was administered promptly after initiation of invasive mechanical ventilation, with the mean time to the first dose of study drug similar between groups (Table 2). Likewise, the median number of doses administered per patient was similar between groups (Table 2). During the intervention period no patient other than those with overt bleeding (see minor outcomes) received an open-label acid-suppressive drug.

The majority of patients received enteral nutrition during the study period and there was no difference between the groups in terms of proportion fed, time-to-initiation, volume delivered, or feed-intolerance (Table 2).

Inotrope and corticosteroid administration was similar between the groups (Table 2). Approximately one-third of patients had 'hemostatic dysfunction' during the study but there was no difference between groups (Table 2).

Major outcomes

There were no episodes of clinically significant GI bleeding in either group (placebo: 0/108 (upper 97.5%CI 3.36%) vs. pantoprazole: 0/106 (upper 97.5%CI 3.42%); overall: 0/214 (upper 97.5%CI 1.71%)).

Infective ventilator-associated complication or pneumonia as per the CDC criteria occurred in three patients (placebo: 1/108 [0.9%, 95%CI (0.02, 5.1%)] vs. pantoprazole: 2/106 [1.9%, 95%CI (0.2, 5.1%)]).

Thirty patients assigned to pantoprazole and forty patients assigned to placebo were tested for *Clostridium difficile* infection with one patient who received pantoprazole

recording a positive sample (placebo: 0/108 [97.5%CI 3.4%] vs. pantoprazole: 1/106 [0.9%, 95%CI (0.02, 5.1%)]).

Minor outcomes

Nine patient had an episode of overt bleeding (placebo: 6/108 [5.6%, 95% CI (2.1, 11.7)%] vs. pantoprazole: 3/106 [2.8%, 95% CI (0.6, 8.0)%]; P=0.50). Upon recognition of overt bleeding, the study drug was replaced with open-label pantoprazole in eight of the patients, and in one patient study drug (placebo) was continued. No other interventions were required for management of the GI bleeding.

Daily hemoglobin concentrations, when adjusted for transfusion, revealed no significant between group difference (P=0.66) or group-by-time effect (P=0.16), with transfusion being a significant covariate (P<0.001) (Fig. 2). Given the apparent non-linearity of the mean hemoglobin profiles and visual separation between groups (Fig. 2), modelling was repeated incorporating either a linear spline at day 3 or including a polynomial term. Neither of these post hoc analysis models revealed a significant between group or group-by-time effect. In addition, modelling for the rate of transfusion revealed no significant difference between groups.

There were no differences in clinician-adjudicated ventilator-associated pneumonia (placebo: 8/108 [7.4%, 95%CI (3.3, 14.1)%] vs. pantoprazole 12/106 [11.3%, 95% CI (6.0, 18.9)%]; P=0.35) or ventilator free days (21 [4-25] vs. 21 [0-25] days; P=0.69). The length of stay in ICU (7 [4-14] vs. 6 [3-11] days; P=0.16) and hospital (18 [9-25] vs. 16 [8-31] days; P=0.88) were also similar.

There was no difference between groups in 90-day mortality (placebo: 25/108 [23.1%, 95% CI (15.6, 32.2)%] vs. pantoprazole 30/106 [28.3%, 95% CI (20.0, 37.9)%] (Fig. 3). Using a Cox proportional hazards model, APACHE III score, and age (grouped as tercile) were significant covariates (P<0.1) with an adjusted hazard ratio for the pantoprazole group of 1.68 (0.97-2.90); P =0.06.

Secondary analysis of all major and minor outcomes on a per protocol basis resulted in no change in inference.

DISCUSSION

We performed a prospective randomized double blind parallel group study of mechanically ventilated critically ill patients expected to receive enteral nutrition. We were able to initiate therapy promptly after commencing mechanical ventilation and analyzed data from nearly all patients who were eligible over a 12-month period and did not observe any benefit from the prophylactic administration of pantoprazole (40 mg IV once daily).

We did not observe a single episode of clinically significant GI bleeding in either group. While our upper confidence interval for overall incidence of bleeding at 1.7% is considerably less than recent epidemiological data (4) we believe these data are complementary and indicate that clinically significant GI bleeding in critically ill patients due to stress related mucosal damage now occurs infrequently (24). We also considered it desirable to avoid occult bleeding, particularly if such gradual bleeding increases transfusion of packed red cells. However, we were unable to demonstrate any protective effect of pantoprazole on either daily hemoglobin concentration or the requirement for red cell transfusion, providing supportive evidence that GI bleeding outcomes that are important to patients do not appear to be reduced by prophylactic pantoprazole administration.

Data from our study also suggest that administration of pantoprazole does not markedly increase risk of infective ventilator associated pneumonia or *Clostridium difficile* infection; a finding that contradicts previous observational studies (15, 16). The previous retrospective observational studies are at greater risk of bias and accordingly our data may represent a more precise estimate of the true effect. Nonetheless, given our relatively small sample and the infrequency of infective complications our study may have been underpowered to detect small but clinically important harmful effects of pantoprazole.

Significance of Study Findings

Whilst any relatively small exploratory study should not be seen as definitive we suggest that our data should, at the very least, encourage those clinicians who currently administer prophylactic pantoprazole to critically ill patients to re-evaluate their practice and recognize that there is uncertainty as to the benefits and harm associated with the use of these agents.

It should be noted that we enrolled only mechanically ventilated critically ill patients. This cohort was targeted because they are considered to be at greater risk of stress ulceration and GI bleeding (2). Moreover, illness severity scores, diagnostic categories, the use of inotropes and steroids and the prevalence of ‘coagulation dysfunction’ in these patients suggest that the cohort we studied was indeed at least at moderate risk of stress-related mucosal damage. This has implications for current clinical practice, as observational studies indicate that many critically ill patients considered to be at low risk of GI bleeding are administered acid-suppressive medications (4, 12, 26), whereas data from our study suggest that stress ulcer prophylaxis with acid-suppressive medication may not be warranted even in moderate to high risk patients and the benefit in any low risk cohort is therefore likely to be negligible. Moreover, once commenced in ICU, the administration of acid suppressing drugs appears to continue inappropriately after hospital discharge (27), and so the risks and costs associated with treatment may not be confined to the ICU period.

Strengths

Our study has robust internal validity with more rigorous methodology than that used for the two previously published trials comparing proton pump inhibitors to placebo for stress ulcer prophylaxis. To reduce selection bias we included consecutively admitted patients who were randomized to the intervention and to reduce performance bias we ensured all medical, nursing, research staff and patients were blinded to treatment allocation throughout the study period. We also believe that prompt administration of the first dose of study drug is a substantial strength, as recent observational data indicate that at least half of all clinically significant gastrointestinal bleeding episodes occur in the first two days of ventilation (4). Accordingly, delays in commencing the intervention risk a false negative result and should be minimized during any definitive trial of stress ulcer prophylaxis. Clinically important outcomes were prospectively evaluated so that clinically significant GI bleeding, infective ventilator-associated conditions and *Clostridium difficile* infections were quantified while blinded to reduce detection bias. Furthermore, outcomes that are at risk of subjectivity, such as ventilator-associated pneumonia, were diagnosed according to objective CDC definitions in addition to clinical criteria (28).

Limitations

There are however limitations to our study. Based on the findings of the meta-analysis by Marik and colleagues we only included patients that were anticipated to commence enteral feeding within 48 hours of admission (7). While this did not affect the internal validity of our study, only nine patients that were otherwise eligible were excluded, our findings may not be generalizable to units that favor longer periods of fasting. Treating doctors only had to expect that a patient would be mechanically ventilated for greater than 24 hours for them to be eligible, which may explain why duration of ventilation was relatively short for some patients. Our results may have varied had we only included patients that were artificially ventilate for longer periods of time but given ventilation per se, with the first two days of ventilation being the time of greatest risk (3), and coagulopathy are the two major risk factors for GI bleeding (2) we believe our cohort represented a cohort at considerable risk. It should also be recognized that a large number of patients were excluded (n=245) because they were receiving acid suppressive drugs prior to ICU admission. We excluded these patients on the basis that those allocated to placebo would have the potential for rebound acid hypersecretion (29, 30) but the unexpected prevalence of prior acid suppressive medication use in our cohort did reduce our sample size considerably. We also did not conduct a traditional power calculation to determine sample size; rather we studied a convenience sample and enrolled all eligible patients during a twelve month period. Our approach has been used by other investigators undertaking exploratory studies (31, 32). Moreover, this approach has the advantage of almost eliminating selection bias (31, 32); as in our study we were able to analyze data from > 98% of patients that met the eligibility criteria during the 12 month study period.

Future Directions

Whilst exploratory, our findings emphasize the pressing need to challenge current guidelines that recommend prophylactic pantoprazole be administered to mechanically ventilated critically ill patients. Given the relative infrequency with which we observed major outcomes we believe to detect meaningful differences any definitive trial will need to be powered to detect relatively modest effect sizes, include larger cohorts of patients at 'high risk', precisely quantify the risks (such as *Clostridium difficile*) and benefits (reduction in blood transfusions) associated with the use of pantoprazole, and/or evaluate whether placebo is non-inferior to pantoprazole to

prevent episodes of clinically significant GI bleeding (24, 33). These phase III studies will be commencing soon (34, 35).

CONCLUSIONS

In conclusion, we found no evidence that for mechanically ventilated critically ill patients who were expected to receive enteral nutrition that the prophylactic administration of pantoprazole is of benefit or is harmful. Our data highlight the need for larger multi-center studies to determine the safety of this approach.

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Table 1: - Patient data at enrolment.

Data Field	Placebo (n=108)	Pantoprazole (n=106)
▪ Male sex (%)	72 (67%)	68 (64%)
▪ Age, years	52 (17)	52 (18)
▪ APACHE III score	66 (28)	66 (26)
▪ Medications prescribed at enrolment		
▪ Non-steroidal anti-inflammatory drug, number (%)	16 (15%)	15 (14%)
▪ Oral/IV corticosteroids, number (%)	15 (14%)	9 (8%)
▪ Inotrope/vasoconstrictor infusion, number (%)	57 (53%)	52 (49%)
▪ Maximum inotrope dose,* ug/min, median (IQR)	10 (6-18)	10 (5-21)
▪ Receiving vasopressin, number (%)	1	0
▪ Other Previous Medical History		
▪ Kidney failure (RIFLE score)		
- Risk	6	9
- Injury	5	5
- Failure	2	1
- End Stage Renal Failure	0	1
▪ <i>Cl. difficile</i> infection	0	0
▪ Immunosuppressed	9	3
- Drug related	4	0
- Hematological malignancy	0	0
- Disseminated malignancy	3	3
- Human Immunodeficiency Virus	1	0
- Chronic Liver disease	2	0
- Other cause	1	0
▪ Primary ICU diagnostic group (non-operative/post-operative):		
▪ Trauma	20 / 12	18 / 13
▪ Neurological	18 / 8	27 / 8
▪ Respiratory	14 / 10	15 / 4
▪ Cardiovascular	13 / 1	7 / 2
▪ Sepsis	3 / -	3 / -
▪ Gastrointestinal	1 / 4	0 / 3
▪ Metabolic	1 / -	5 / -
▪ Renal	1 / -	0 / -
▪ Source of ICU admission:		
▪ Operating Theatre	53 (49%)	47 (45%)
▪ Emergency Department	36 (33%)	31 (30%)
▪ General ward	12 (11%)	17 (16%)
▪ Transfer from other Hosp.	7 (6%)	8 (8%)
▪ Transfer from other ED/ICU	0	2 (2%)

Unless specified data are mean (standard deviation)

*Maximum inotrope dose= maximum combined noradrenaline and adrenaline dose (ug/min) recorded.

Table 2 :- Processes of care whilst receiving study drug.

Data Field	Placebo (n=108)	Pantoprazole (n=106)	P-Value
▪ Study Drug			
▪ Time from commencement of invasive mechanical ventilation to first dose of study drug, hour ^a	17 [16-19]	16 [15-18]	0.44
▪ Median number of doses administered ^b	3 [2-7]	3 [1-7]	0.58
▪ Enteral nutrition (EN)	94 (87%)	88 (83%)	0.41
▪ Number who received liquid EN			
▪ Time from commencement of invasive mechanical ventilation to start EN, hours ^a	16 [10-25]	16 [8-22]	0.37
▪ Volume of EN delivery per day, mL ^a	798 (697-898)	844 (729-958)	0.55
▪ Patient intolerant of EN ^f	42 (39%)	34 (32%)	0.30
▪ Inotrope/Vasoconstrictor administered			
▪ Number of patients receiving at any stage ^c	74 (69%)	67 (63%)	0.41
▪ Duration of inotrope, days ^b	3 [2-4]	3 [2-4]	0.40
▪ Maximum dose, ug/min ^b	12 [8-20]	13 [8-28]	0.88
▪ Corticosteroids ²			
▪ Patients receiving at any stage ^c	24 (22%)	17 (16%)	0.25
▪ Maximum dose ^b	75 [44-100]	75 [50-100]	0.69
▪ Hemostatic dysfunction ³	35 (32%)	39 (37%)	0.50
▪ INR > 1.5	20 (19%)	24 (23%)	0.46
▪ APPT > 40	27 (25%)	21 (20%)	0.36
▪ Platelet count < 100,000	18 (17%)	20 (19%)	0.78
▪ Antibiotics = number receiving:			
▪ At any stage	93 (86%)	85 (80%)	0.25
▪ Multiple antibiotics	74 (69%)	62 (59%)	0.13
▪ Course > 1 week	16 (15%)	14 (13%)	0.74

Data are presented and analyzed as ^a mean (95% confidence intervals) – Student's t-test, ^b median [25th percentile, 75th percentile] – Wilcoxon rank-sum, and ^c number (%) – Chi-squared.

¹ Intolerance defined as a single gastric residual volume > 250ml on any given day.

² Corticosteroid dose in converted to mg prednisolone equivalent

³ At any stage during admission and patients may be assigned to multiple categories

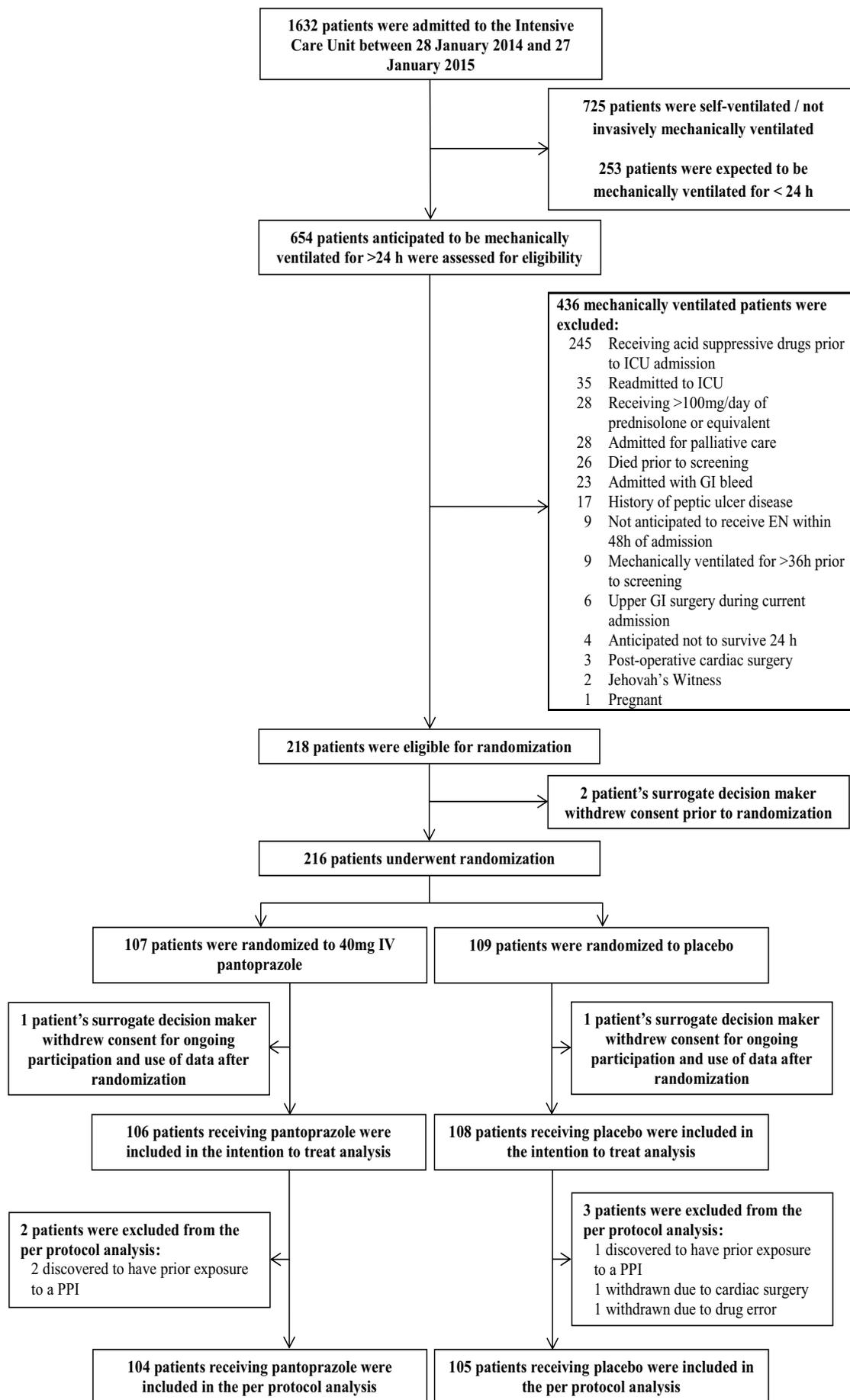
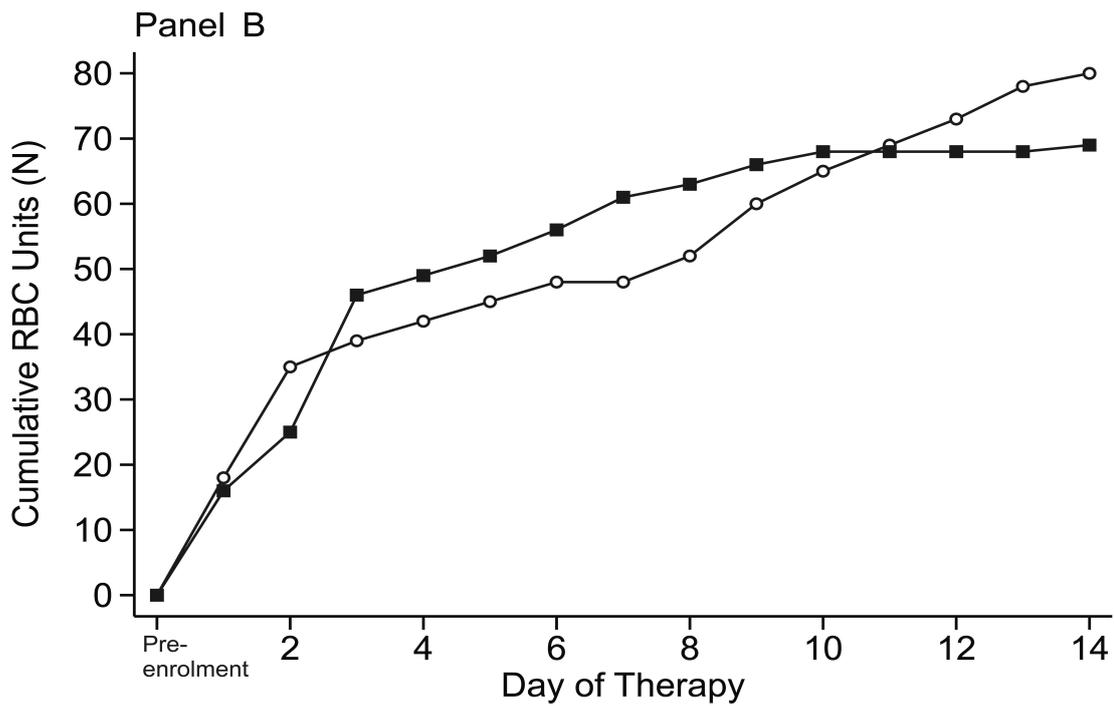
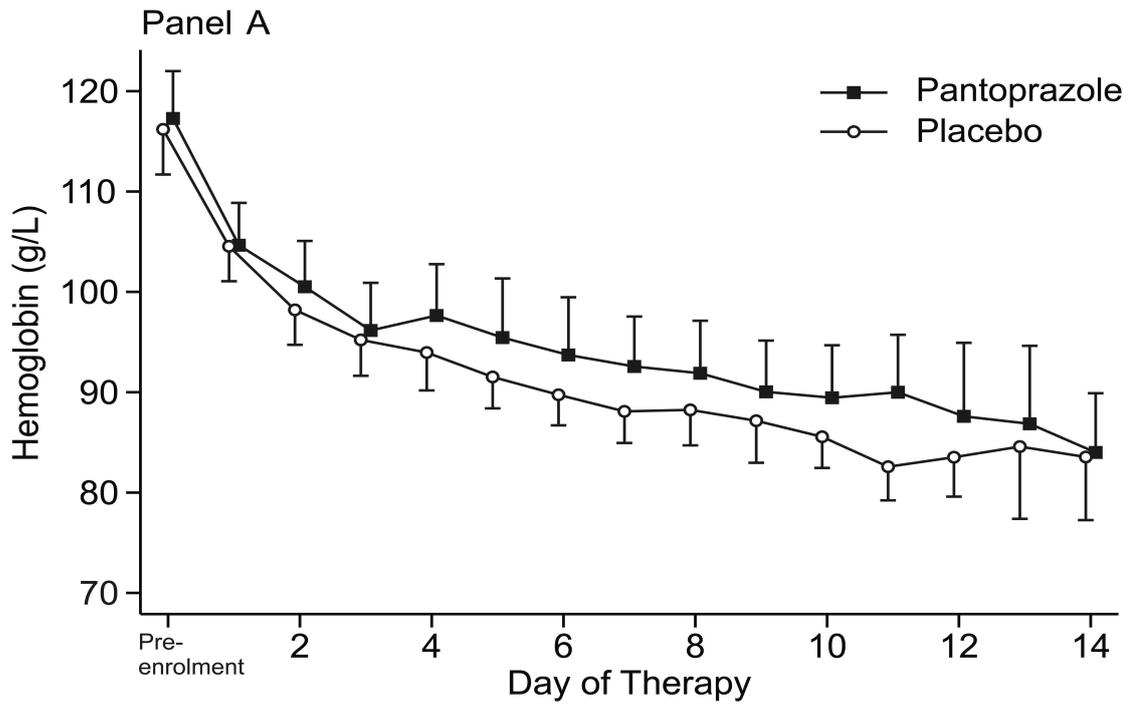


Fig 1. Assessment, randomization and follow-up of study patients

During the study period 218 patients were eligible for participation. Prior to randomization consent was refused from the surrogate decision maker for two patients and 216 patients received study drug. Consent for ongoing participation and retention of data was subsequently refused for two patients so that 214 patients were included in the intention to treat analysis. Five patients ceased study drug after randomization and were withdrawn: three patients after further history was made available indicating the use of regular PPI prior to admission, one patient had cardiac surgery during admission after randomization and one patient erroneously received open-label pantoprazole. Accordingly, 209 patients were included in the per protocol analysis.



	Number Remaining (n)														
Pantoprazole	106	106	78	58	48	38	35	30	28	25	20	19	15	14	11
Placebo	108	108	87	64	50	46	39	32	29	25	23	19	19	12	11

Fig 2. Daily hemoglobin and red blood cell transfusion according to treatment group

Panel A: Daily hemoglobin concentration (g/L). Day of therapy indicates first recorded hemoglobin concentration for that day.

Panel B: Number of units of packed red blood cells administered cumulatively.

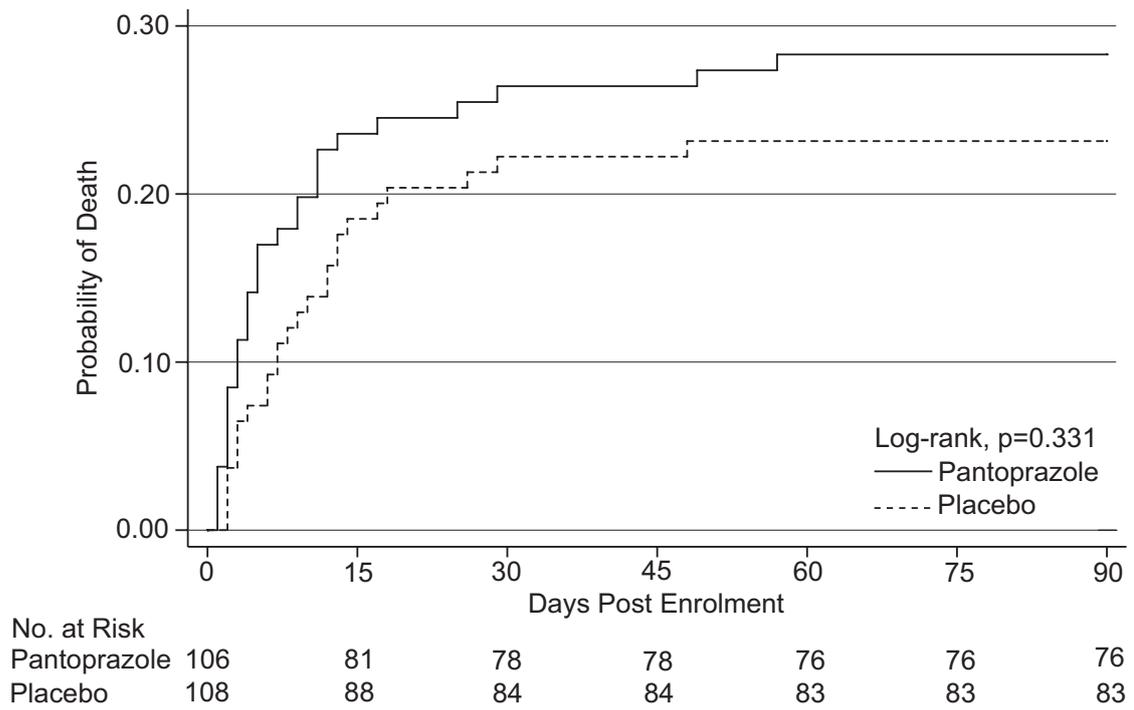


Fig 3. Kaplan-Meier estimates for the probability of death (Log-rank; $P=0.33$)

CHAPTER 4

4.4 CONCLUSIONS

4.4.1 Introduction

The objectives of the study that comprises this chapter were to evaluate (i) benefit or harm of prophylactic administration of the proton pump inhibitor, pantoprazole, in enterally fed, mechanically ventilated critically ill patients and (ii) establish estimates of the event rates of clinically significant gastrointestinal bleeding and infective complications in these patients receiving and not receiving pantoprazole as prophylaxis.

4.4.2 Contribution of the work described in this thesis to the understanding of the role for prophylactic proton pump inhibitor administration in the critically ill.

Routine administration of stress ulcer prophylaxis is standard practice endorsed by international guidelines despite minimal evidence of benefit and mechanistic plausibility that it may cause harm. The randomised, double-blind, placebo controlled parallel group study reported in chapter 4.3 was the largest cohort studied that compared outcomes when critically ill patients were randomised to prophylactic pantoprazole, or placebo and the first to assess outcomes with investigators blinded to treatment. These data indicate that the prophylactic administration of pantoprazole was neither superior nor inferior to placebo on the outcomes of clinically significant gastrointestinal bleeding, infective ventilator-associated complications or pneumonia and *Clostridium difficile* infection. A secondary, but important, observation was that no patient in either arm of the study had clinically significant gastrointestinal bleeding. The lack of clinically important bleeding occurred despite the cohort of patients being a group at considerable risk of stress related mucosal damage; all patients were mechanically ventilated, the majority required inotropic or vasoactive support and approximately one third had coagulation dysfunction. Together these data suggest that with current intensive care practice, including early enteral nutrition, significant gastrointestinal bleeding occurs rarely, and accordingly, routine stress ulcer prophylaxis with acid-suppressive medication may well not be warranted, even in moderate risk patients. Finally, infective complication rates were low in both cohorts with no overtly harmful effect attributable to pantoprazole administration. Out of the

216 patients recruited for the study only three were diagnosed with an infective ventilator associated complication and one with *Clostridium difficile* infection.

4.5 FUTURE DIRECTIONS

4.5 Future directions

4.5.1 Large multi-centre trials to determine the safety and efficacy of routine stress ulcer prophylaxis in the critically ill.

The findings reported in chapter 4.3 should be viewed as exploratory, but emphasise the need to challenge the widely practiced, and guideline endorsed routine prescription of prophylactic proton pump inhibitors to mechanically ventilated critically ill patients. The infrequency of the major outcomes of clinically significant bleeding and infective complications within the relatively small sample size may have resulted in the study being underpowered to detect small, but clinically important, harmful effects of pantoprazole, particularly as the adjusted point estimates were for harm in the pantoprazole group, albeit without reaching statistical significance. With current practice, including an emphasis on early enteral nutrition, it appears that clinically significant bleeding occurs infrequently. Moreover, based on the observations from this exploratory study that bleeding episodes without pantoprazole are rare, any benefit of pantoprazole to reduce episodes of clinically significant bleeding is likely to be minimal. Based on the infrequency of bleeding and the signal for potential harm from routine pantoprazole administration, a large, multi-centre non-inferiority study would be a logical and important approach to definitively inform clinical practice.

APPENDIX A

PRESENTATIONS AT NATIONAL OR INTERNATIONAL MEETINGS

Dr Plummer presented the studies that comprised his doctoral programme as oral or poster presentations at the following national and international meetings, run by learned societies of intensive care medicine and diabetes:

2013

Australia and New Zealand Intensive Care Society Annual Scientific Meeting (Hobart) Australian Diabetes Society Annual Scientific Meeting (Sydney)

European Association for the Study of Diabetes Annual Scientific Meeting (Barcelona)

European Society of Intensive Care Medicine Annual Scientific Meeting (Paris)

2014

Australia and New Zealand Intensive Care Society Annual Scientific Meeting (Melbourne)

European Society of Intensive Care Medicine Annual Scientific Meeting (Barcelona)

United Kingdom Intensive Care Society State of the Art Meeting (London)

2015

Australia and New Zealand Intensive Care Society Annual Scientific Meeting (Auckland)

American Diabetes Association Annual Scientific Meeting (Boston)

Australian Diabetes Society Annual Scientific Meeting (Adelaide)

European Society of Intensive Care Medicine Annual Scientific Meeting (Berlin)

APPENDIX B

PRIZES AWARDED DURING CANDIDATURE

During his doctoral programme Dr Plummer was awarded several prizes, which are listed below:

2013

Australian Diabetes Society President's Prize – Clinical Young Investigator Award for the best presentation by a young researcher at the Australian Diabetes Society Annual Scientific Meeting.

Finalist, Matt Spence Medal - Australia and New Zealand Intensive Care Society Annual Scientific Meeting.

2014

Matt Spence Medal for the best registrar presentation at the Australia and New Zealand Intensive Care Society Annual Scientific Meeting.

NIMMO Prize for the best full-time research at the Royal Adelaide Hospital

Florey Medical Research Foundation Prize presented for outstanding research presented at the University of Adelaide Florey International Postgraduate Research Conference

2015

Finalist, Best Medical Paper – Australia and New Zealand Intensive Care Society Annual Scientific Meeting

Finalist, Australian Diabetes Society President's Prize – Clinical Young Investigator Award

APPENDIX C

GRANTS AWARDED DURING CANDIDATURE

2014

Royal Adelaide Hospital Research Foundation, Clinical Project Grant

Plummer MP, Deane AM, Chapman MJ, Rayner C. The effect of routine pantoprazole administration when compared to placebo on gastrointestinal bleeding, ventilator-associated pneumonia and *Clostridium difficile* infection in enterally-fed mechanically ventilated critically ill patients: A prospective randomised study.

Value \$49,139

Intensive Care Foundation Inaugural Trainee Grant

Plummer MP, Deane AM, Chapman MJ, Horowitz M, Jones K. Gallbladder motility in critical illness.

Value \$5000

National Health and Medical Research Council Grant Postgraduate Scholarship

Plummer MP. Patient safety and therapeutic implications of a new glucose lowering agent for type 2 diabetes and high blood glucose states in the critically ill.

APP1075657

Value \$84, 904.67

2015

Royal Adelaide Hospital Research Foundation, Clinical Project Grant

Plummer MP, Deane AM, Chapman MJ, Jones KL, Hausken T. The effect of critical illness on gallbladder motility, lipid absorption and plasma cholecystokinin concentrations.

Value \$49,565

Maurice Sando Foundation Grant

Plummer MP, Deane AM, Finnis ME, Shaw JE, Moodie S, Biradar V. A state-wide study to determine whether hyperglycaemia during critical illness identifies survivors at risk of subsequently developing diabetes.

Value \$10,000

Diabetes Australia Research Trust Grant

Philips LK, Horowitz M, Shaw JE, Deane AM, **Plummer MP**. Glycaemia in the critically ill – dysglycaemia as a predictor for incident type 2 diabetes and the effect of pre-existing type 2 diabetes on outcomes in survivors of critical illness.

Value \$59,915