HYPERGLYCAEMIA IN THE CRITICALLY ILL PATIENT

IDEAL TREATMENT AND IMPACT OF THE CONDITION

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

In the Discipline of
Acute Care Medicine
Adelaide Medical School
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By

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Abstract

This thesis is composed of four distinct related chapters focusing on glycaemia in the intensive care unit (ICU), its treatment, and the impact of the condition. Specifically, this research concentrates on approaches to glycaemic management, enteral feeding, and implications of hyperglycaemia in the critically ill. The work submitted comprises three literature reviews and five original studies.

In the critically ill, delayed gastric emptying occurs frequently and is associated with adverse outcomes. Numerous techniques to quantify gastric emptying exist *(Chapter 1.2)*. Energy dense feeds are sometimes administered to patients with delayed gastric emptying based on the rationale that volume is a determinant of gastric emptying. However, in health, tight regulation of gastric emptying occurs via 'enterogastric feedback', such that it is calorie load (not volume) that is the major determinant of gastric emptying. An analysis of previously obtained data suggested energy dense feed is associated with a slower emptying rate than a standard feed, resulting in similar caloric delivery *(Chapter 1.3)*.

Hyperglycaemia occurs frequently during critical illness in patients with pre-existing diabetes. The current approach to treatment of glycaemia in this group is to treat them identical to patients without diabetes. This strategy may be flawed as observational data suggest that the impact of acute glycaemia on outcomes is dependent on premorbid glycaemia *(Chapter 2.2)*. To provide further information, a prospective sequential period pilot study was completed *(Chapter 2.3)*. This study suggests that a more liberal approach may reduce hypoglycaemia episodes and that further trials of more liberal glucose targets are warranted. The treatment of hyperglycaemia during critical illness (for both patients with and without pre-existing diabetes) requires administration of insulin; however, this is not without risk. Therefore a prospective, randomised, cross over study in critically ill patients to determine the effect of glucose-dependent insulinotropic polypeptide (GIP) was performed. The administration of GIP in pharmacological doses when compared to placebo did not effect glycaemia, glucose absorption or gastric emptying *(Chapter 2.4)*.

Stress hyperglycaemia occurs frequently in critically ill patients but is not generally considered a risk factor for subsequent glucose intolerance. A systematic review and

meta-analysis was conducted suggesting stress hyperglycaemia was associated with an increased risk of both prediabetes and diabetes *(Chapter 3.2)*. Subsequently, a prospective cohort study was performed confirming this signal and providing mechanistic information regarding the progression to prediabetes and diabetes *(Chapter 3.3)*.

Patients during and recovering from critical illness as well as ambulant patients with diabetes frequently experience episodes of hypoglycaemia. The counter-regulatory response to hypoglycaemia is to accelerate gastric emptying, increasing carbohydrate absorption from the small intestine. A study was performed to understand whether recurrent episodes of hypoglycaemia diminish the standard counter-regulatory response *(Chapter 4.2)*. In healthy volunteers, the acceleration of gastric emptying during acute hypoglycaemia in health did not appear to be affected by antecedent hypoglycaemia.

In summary, this program of work has contributed new and important information in the fields of diabetes management, feeding in the critical care setting, and the implications of stress hyperglycaemia 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 my name, 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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Palash Kar
7 January 2018

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Not that long ago, I could never imagine why people would willfully enter a doctoral programme. I believed many had to have been forced at gunpoint or perhaps others had early dementia. Yet here I am, four years after starting my thesis, which was undertaken not because my life was threatened, but due to an overwhelming desire to perform clinical research. A journey this long and arduous is not completed alone, and I have many important individuals to thank.

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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 but complementary chapters each with a brief narrative introduction followed by the manuscripts and ending with a narrative conclusion of the major findings and future directions.

In total the thesis comprises eight manuscripts; three reviews of the literature and five original clinical trials. At the time of submission of this body of work, seven of the manuscripts have been published or accepted for publication. The manuscript that comprises chapter 5.3 is currently under review with *Critical Care*. None of the manuscripts were solicited.

The eight manuscripts are presented in the style of the publication to which they were submitted, accounting for the variance in manuscript structure. For consistency, manuscripts are presented in UK English. The references for all eight publications are included in each respective manuscript and references for each chapter follow each section.

The publications are as follows:

Kar P, Jones KL, Horowitz M, Chapman MJ, Deane AM. **Measurement of gastric emptying in the critically ill.** *Clinical Nutrition*. 2015 Aug; 34(4):557-64
Relevant section in this thesis; Chapter 1.2

Kar P, Plummer MP, Chapman MJ, Cousins CE, Lange K, Horowitz M, Jones KL, Deane AM. **Energy-dense formulae may slow gastric emptying in the critically ill.** *Journal of Parenteral & Enteral Nutrition*. 2016 Sep; 40(7):1050-6
Relevant section in this thesis; Chapter 1.3

Kar P, Jones KL, Horowitz M, Deane AM. **Management of critically ill patients with type 2 diabetes: the need for personalised therapy.** *World Journal of Diabetes*. 2015 Jun; 6(5):693-706
Relevant section in this thesis; Chapter 2.2

Kar P, Plummer MP, Bellomo R, Jenkins AJ, Januszewski AS, Chapman MJ, Jones KL, Horowitz M and Deane AM. **Liberal Glycemic Control in Critically Ill Patients with Type-2 Diabetes: an Exploratory Study.** *Critical Care Medicine*. 2016 Sep; 44(9):1695-703
Relevant section in this thesis; Chapter 2.3

Kar P, Cousins CE, Annink CE, Jones KL, Chapman MJ, Meier JJ, Nauck MA, Horowitz M, Deane AM. **Effects of glucose-dependent insulinotropic polypeptide**

on gastric emptying, glycaemia and insulinaemia during critical illness: a prospective, double blind, randomised, crossover study. *Critical Care*. 2015 Jan; 19:20
Relevant section in this thesis; Chapter 2.4

Ali Abdelhamid Y[#], Kar P[#], Finnis ME, Phillips LK, Plummer MP, Shaw JE, Horowitz M and Deane AM. **Stress hyperglycaemia in critically ill patients and the subsequent risk of diabetes: a systematic review and meta-analysis.** *Critical Care*. 2016 Sep; 20(1):301
Joint first authors
Relevant section in this thesis; Chapter 3.2

Kar P, Plummer MP, Ali Abdelhamid Y, Giersch EJ, Summers MJ, Weinel L, Finnis ME, Phillips LK, Jones KL, Horowitz M, Deane AM. **Incident diabetes in survivors of critical illness and mechanisms underlying persistent glucose intolerance: a prospective cohort study.** [under review with *Critical Care*]
Relevant section in this thesis; Chapter 3.3

Kar P, Jones KL, Plummer MP, Ali Abdelhamid Y, Giersch EJ, Summers MJ, Hatzinikolas S, Heller S, Horowitz M, Deane AM. **Antecedent hypoglycemia does not attenuate the acceleration of gastric emptying by hypoglycemia.** *The Journal of Clinical Endocrinology & Metabolism*. 2017 Nov; 102 (11):3953-60
Relevant section in this thesis; Chapter 4.2

Chapter 1

Enteral feeding in the critical ill patient

1.1 INTRODUCTION

Enteral nutrition, usually via a tube inserted into the stomach, is part of standard care in the critically ill patient. During critical illness, gastric emptying is frequently markedly delayed, typically as a result of increased inhibitory small intestinal feedback, which attenuates delivery of nutrient. This manifests clinically as large gastric residual volumes and/or vomiting. Not only is gastric dysmotility prevalent in critical illness, with delayed gastric emptying in the critically ill reported to occur in up to 50% of mechanically ventilated patients, it is also associated with complications such as gastro-oesophageal reflux, pulmonary aspiration and glycaemic variability. The capacity to measure gastric emptying clinically has the potential, therefore, to improve patient outcomes.

The structured narrative review (chapter 1.2) focuses on the measurement of gastric emptying in the critically ill. In this review, the various methods are outlined, and categorised as direct, indirect and surrogate assessments. Additionally, the strength and limitations of these techniques are summarised. Finally, recommendations are made as to which gastric emptying test to use; including the ideal method that is dependent on the requirements of the investigator (clinical versus research). Despite associations between delayed gastric emptying and adverse outcomes, there are controversies regarding quantification of gastric emptying for both clinical and research purposes in the critically ill which are addressed in this review.

The management of delayed gastric emptying in the critically ill is of considerable interest to clinicians. Energy dense formulae (liquid nutrients containing >1kcal/ml) are frequently prescribed by clinicians to augment nutrient delivery for patients with delayed gastric emptying, despite a lack of compelling evidence to support this approach. Gastric emptying is the result of a complex interplay between the interrelated factors of small intestinal nutrient, vagal and gastrointestinal hormonal stimuli. In health, increasing the fat content of a meal slows gastric emptying. Given energy dense feeds increase calorie content by increasing fat and reducing the

component of carbohydrate, there is a plausible physiological rationale that energy-dense formulae will empty slower than standard feeds. The objective of the study outlined within chapter 1.3 was to test the null hypothesis that standard and energy dense feeds would empty at comparable rates in the critically ill patient.

1.1.1 *Objectives*

The objectives of the literature review and study that comprise this chapter were to (i) review the techniques available for the measurement of gastric emptying and (ii) evaluate the effect of high calorie, energy dense formula on gastric emptying in the critically ill patient.

1.2 LITERATURE REVIEW

Measurement of gastric emptying in the critically ill

Statement of Authorship

Title of paper	Measurement of gastric emptying in the critically ill
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Principal Author

Name of Principal Author (Candidate)	Dr Palash Kar		
Contribution to paper	Conceptualisation of work, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	85		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
- The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Karen Jones		
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Measurement of gastric emptying in the critically ill

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Abstract

Background and aims: Enteral nutrition is important in critically ill patients and is usually administered via a nasogastric tube. As gastric emptying is frequently delayed, and this compromises the delivery of nutrient, it is important that the emptying rate can be quantified.

Methods: A comprehensive search of MEDLINE/PubMed, of English articles, from inception to 1 July 2014. The following MeSH words and combination of terms were used: gastric emptying, scintigraphy, absorption method, 3-O-methylglucose, isotope breath tests, octanoic acid, ultrasound, magnetic resonance imaging, gastric residual volumes, critical illness and intensive care.

Results: A number of methods are available to measure gastric emptying and these broadly can be categorised as direct- or indirect-test and surrogate assessments. Direct tests necessitate visualisation of the stomach contents during emptying and are unaffected by liver or kidney metabolism. The most frequently used direct modality is scintigraphy, which remains the 'gold standard'. Indirect tests use a marker that is absorbed in the proximal small intestine, so that measurements of the marker, or its metabolite measured in plasma or breath, correlates with gastric emptying. These tests include drug and carbohydrate absorption and isotope breath tests. Gastric residual volumes (GRVs) are used frequently to quantify gastric emptying during nasogastric feeding, but these measurements may be inaccurate and should be regarded as a surrogate measurement. While the inherent limitations of GRVs make them less suitable for research purposes they are often the only technique that is available for clinicians at the bedside.

Conclusions: Each of the available techniques has its strength and limitations. Accordingly, the choice of gastric emptying test is dictated by the particular requirement(s) and expertise of the investigator or clinician.

Introduction

Enteral nutrition is part of standard care for critically ill patients and is most commonly delivered via the gastric route [1]. Frequently, however, nutritional targets are not achieved via the gastric route, particularly because of critical illness induced gastrointestinal dysmotility, which leads to slow gastric emptying [2]. While the prevalence and magnitude of delayed gastric emptying in the critically ill are inconsistently reported [3-5], possibly because these variables are dependent on the precision of the methodology used to measure gastric emptying, as well as the definitions of critical illness and/or delayed gastric emptying, there is no doubt that markedly delayed gastric emptying occurs frequently [4].

It is now also recognised that the impact of delayed emptying, and hence the potential indications for its measurement, are broader then had been appreciated. For example, in the critically ill patient, markedly slow gastric emptying may be associated with increased risk of gastro-oesophageal reflux and pulmonary aspiration [6]. The rate of gastric emptying is a major determinant of postprandial glycaemia and, in patients treated with insulin, major changes in gastric emptying rate may impair the coordination between nutrient absorption and insulin availability predisposing to glycaemic variability [7].

Gastroparesis is a relatively frequent condition in ambulant populations with epidemiological data estimating the incidence between 2.4 and 9.8 per 100,000 person-years [8]. Delayed gastric emptying is particularly prevalent in the critically ill and has been reported to occur in up to one-half of all mechanically ventilated patients, which is similar to the prevalence reported in ambulant patients with long-standing type 1 or type 2 diabetes [9, 10].

In the research setting, the measurement technique must be precise, whereas in routine clinical situations clinicians may be willing to trade off accuracy for other factors, such as cost, convenience, and invasiveness, so that they can have a ‘point-of-care’ test. Comprehensive evaluation of gastric motility requires the measurement of multiple parameters. These include intraluminal volume, flow and pressure, wall motion, and electrical activity. No single technique can measure all of these concurrently. Consequently, in research studies a complete assessment of gastric

function can only be obtained by the simultaneous use of different techniques. Additional information specific to measurement of gastric emptying in the ambulant setting is covered elsewhere [11-14].

Gastric emptying is determined by a number of intraluminal and extraluminal factors, which can be endogenous or exogenous, and mediate their effects via neural and/or hormonal pathways. The intraluminal factors that modulate gastric emptying include meal composition – caloric load, volume, temperature and nutrient type, the osmolarity of small intestinal contents and length and region of small intestine exposed to nutrient – and systemic (or extraluminal factors) such as glycaemia, posture, pain, gender and age [14]

The Intensive Care Unit presents some unique challenges when measuring physiological derangements. Patients are often sedated and attached to multiple lines, tubes and monitoring devices. Perhaps paradoxically, this may facilitate measurement, as these allow ready access to body fluids such as blood, urine, faeces, and expired gases. However, as patients are non-ambulatory, it is preferable that measurements are performed at the bedside, particularly due to the logistical difficulties in transportation of these patients [15]. There is also limited space around the patient due to the frequent presence of essential but bulky equipment, such as a ventilator and dialysis machines. Techniques must be adaptable, as studies may be terminated prematurely due to unexpected clinical requirements. Of particular concern is that the reliability of a test may be impaired in the critically ill patient when compared to a 'more controlled' research setting. This may reflect technical difficulties exclusive to the intensive care environment, or physiological derangements in the patient caused by their clinical condition or treatment. Ideally, all measurement techniques should be validated in critically ill patients before their widespread use. The primary aim of this narrative review is to provide an overview of the measurement of gastric emptying in the critically ill, the evidence for each method that may be used to quantify emptying and recommendations for use in the critical are setting.

Methods

We performed a comprehensive search of English language manuscripts, on MEDLINE/PubMed, from inception to 1 July 2014. We used both the following key words and combinations of these terms: gastric emptying, scintigraphy, absorption method, 3-O-methylglucose, isotope breath tests, octanoic acid, ultrasound, magnetic resonance imaging, gastric residual volumes, critical illness and intensive care. Additionally, references of extracted manuscripts were examined for additional studies that had not been identified during the initial search. Only studies of human cohorts were included. The methodology of each study was evaluated and studies that were of greater methodological quality and/or data published more recently were preferred. However data was sourced from older studies when appropriate (due to insufficient recent data).

Measurement of gastric emptying in the critically ill

Formal measurement of gastric emptying is rarely performed in the critically ill other than for research purposes. A number of techniques are available, but all have potential limitations and particular difficulties for use in this population. Comparison of different methods of measurement of gastric emptying is limited by the practical inability to perform some measurements concurrently and differences in parameters acquired.

Changes in gastric volume over time can be quantified by techniques such as scintigraphy, fluoroscopy, ultrasound and MRI. Techniques such as labeled carbon breath tests and paracetamol or synthetic glucose absorption, require small intestinal absorption, as well as gastric emptying of the substrate. In the case of breath tests, metabolism of the substrate must also occur before excretion into expired air (Table 1).

Assessment of gastric emptying also requires careful definition of the variable to be measured. When ambulatory patients are being investigated for gastrointestinal symptoms, solid meals are generally considered to be a more reliable indicator of abnormal gastric emptying than nutrient liquids. However these are less applicable to the critically ill patient where liquid formulae are used for feeding. Because gastric emptying is frequently delayed in the critically ill and is dependent on the

composition of the nutrient (i.e. proportion of carbohydrate, lipid or protein) we favour the use of lower volumes of liquid nutrient that is representative of feeds that are clinically used (e.g. 100 ml of 1 kcal/ml containing between 50-65% carbohydrate, 20-40% lipid, 15-30% protein) [16]

In general, measurements of gastric emptying involve an ingested marker (in the mechanically-ventilated patient, the marker is infused via a nasogastric tube) that is monitored directly, via imaging, or indirectly, using blood or breath analysis. The 'ideal' technique for measurement of gastric emptying varies depending on the circumstances and priorities facing clinicians or researchers at any particular time. In addition, these patients frequently have delayed gastric emptying and, in some cases it is profoundly slow [2]. For this reason techniques that can measure gastric emptying over several hours are preferred. Finally, the function of other organs (e.g. liver and kidneys) may be impaired, and volume of distribution, metabolism and excretion of a marker may be altered in these patients [17]. This may influence the outcome of measurements obtained from indirect tests [18]. The advantages and disadvantages of the various measurements of gastric emptying are detailed below.

Direct Tests of Gastric Emptying

Direct tests require that the nutrient is 'visualised' during emptying and therefore the technique is unaffected by liver or kidney metabolism/excretion. Accordingly direct tests are generally more precise than indirect measurements.

Scintigraphy

Scintigraphy is regarded as the 'gold standard' technique to measure gastric emptying [19]. A substrate labelled with a radioactive marker is placed in the stomach. This 'meal' can be solid or liquid, and the liquid can be nutrient or non-nutrient. In the critically ill, liquid nutrient is the constituent of most interest. The disappearance of the radiolabelled meal component from the field of view is measured over time with either a fixed or mobile gamma camera, fitted with a large field-of-view collimator.

The choice of radioisotope is crucial to the success of the measurements. It should be non-toxic, impermeable to the gastric mucosa, and homogenously distributed throughout the 'meal' being evaluated. Markers are available to measure emptying of

specific meal components - solid, liquid or fat - but these must be bound tightly to that component. Simultaneous measurement of gastric emptying of solid and liquid meal components can be performed but requires the use of two radioisotopes. For single isotope studies, the radioisotope selected should ideally have a half-life of 4-12 hours, which allows adequate time for data acquisition without excessive radiation. Our experience is that the time period for data acquisition should be substantially longer than that used in health, because the emptying rate is so slow in the critically ill, and extended periods (of up to 6 hours) are required to quantify any emptying.

Radioisotopic data are then analysed using dedicated computer software. Using a computer display, a region-of-interest is drawn around the stomach, excluding the small intestine. This can be difficult when there is overlying bowel and represents a potential source of inaccuracy. Radioactive counts throughout the study are then measured within the region-of-interest and corrected subsequently for isotope decay and tissue attenuation (i.e. depth-related changes in activity), as both may substantially alter results [19]. In health, radioactivity may be corrected for tissue attenuation using factors derived from a lateral image or using the geometric mean of concurrent anteroposterior images [20]. In the critically ill, lateral images are difficult or impossible to attain and correction factors are frequently not performed. The left anterior oblique (LAO) position is a simple method for correcting for gamma ray attenuation and should be used in these patients [21]. In the case of dual isotope studies, a down-scatter correction should also be applied [20]. The maximum counts in the first 20 minutes of the study represents 100% retention of the meal [20]. Radioactivity in the region-of-interest is then plotted over time for each study frame, expressed as a % of the maximum (Figures 1 and 2a and Supplementary video file 1 and 2). Gastric emptying parameters may include the radioactivity over time (i.e. Area under the curve, AUC) or summarised as the time taken for 50% of the meal to empty (T50). Our experience is that complete or near-complete stasis occurs in some patients so that more than 50% of the meal remains in the stomach when data acquisition is ceased. As such, the T50 may not be the best parameter in the critically ill, and using dynamic imaging, the proportion of isotope residing in the stomach at a specific time points (e.g. 60 minutes post meal) may provide more accurate and reproducible data. When emptying of solids is measured, a lag phase is observed, corresponding to the time taken for intragastric redistribution and breakdown to particles 1mm in size prior

to transpyloric emptying. This is not relevant when measuring the emptying rate of liquid nutrient in critical illness. In addition to total gastric emptying, distribution of the meal within the stomach can be assessed. Comparison of results between different centres must be performed with care as meal properties and acquisition profiles may vary, affecting measurements. Normal ranges may therefore vary. Our preference is for a relatively low calorie nutrient liquid (e.g. 100-200 kcal) with dynamic images collected every 1-3 min with retention determined at 15-30 minutely intervals for between 4-6 hours per study.

It should be recognised that scintigraphy measures gastric emptying in terms of the meal volume remaining in the stomach over time relative to that originally instilled into the stomach or ingested (per cent of total tracer). The absolute volume of gastric contents and the rate at which this empties from the stomach cannot be measured with this technique because the volume of gastric secretions both present initially and produced during the study cannot be quantified [22].

The radiation dose for scintigraphic gastric emptying studies is dependent on the radioisotope used, the dose administered and the gastrointestinal transit time - a typical dose of 20MBq of 99mtechnetium labelled sulphur colloid (which is used to measure gastric emptying) is approximately 0.5mSv. This is comparable to the radiation exposure associated with a mammogram.

Several groups, including ours, have used scintigraphy to measure gastric emptying in critically ill patients (Table 2) [3, 5, 23-26]. The precision associated with this technique allows it to determine the effect of drugs that may have a small, but clinically important, gastrokinetic effect. However, in most centres scintigraphy involves transporting the patient to the nuclear medicine department, limiting its use, however, mobile cameras, when available, obviate the need to move a critically ill patient.

Indirect Tests of Gastric Emptying

Indirect tests use a marker that is not absorbed in the stomach, but is absorbed in the duodenum. Accordingly, the appearance of the marker in blood, or a metabolite excreted by the lungs or kidneys, correlates with the rate of gastric emptying.

Drug absorption (Paracetamol)

In health, plasma concentrations of an ingested drug that is not absorbed in the stomach, but is rapidly and freely absorbed by the small intestine, will reflect the gastric emptying rate [15]. Drug absorption tests have advantages as they are relatively simple to perform and, at least in the case of paracetamol, clinicians often have access to a laboratory that can quantify plasma concentrations.

The paracetamol absorption test has, until recently, been the most frequently used technique to measure gastric emptying in the critically ill [17, 27-36]. However, there have been substantial variations in the protocols used by various investigators (Table 3), including differences in the dose and form (tablets or solution) of paracetamol and the type of meal with which it was administered. There is also a lack of consistency in the calculated parameters used to report the results. The latter include the concentration at a specific time point, maximal concentration, time to reach maximal concentration, area under the curve, and the proportion of area under the curve at specific time points. In addition to the lack of standardisation, other issues limit the suitability of the paracetamol absorption test. Paracetamol is an effective and commonly prescribed analgesic in the critically ill. While the administration of paracetamol for the analgesic and/or temperature-lowering effects may be clinically desirable, the test requires a baseline paracetamol concentration of zero. Pharmacokinetics may also be affected during critical illness, and given the hepatic metabolism of paracetamol, its use for research purposes is contraindicated in patients with marked liver impairment, which occurs frequently in these patients [37]. Our opinion is that the paracetamol absorption test is limited and favour alternative techniques.

Carbohydrate absorption (3-O-methylglucose)

3-O-methylglucose (3-OMG) is a synthetic sugar that is impermeable to gastric mucosa but absorbed actively in the small intestine via the same co-transporters as glucose. The small intestinal absorption rates of 3-OMG and glucose are similar, but in contrast to glucose, 3-OMG is metabolically inert (i.e. it is not metabolised by intestinal mucosa or body tissues) and is excreted unchanged in the urine [38]. Accordingly, the appearance of 3-OMG in the circulation equals the rate of absorption

from the small intestine, with the majority of 3-OMG excreted in the 12 hours following a meal [38]. Unlike other carbohydrate absorption tests (e.g. d-xylose), abnormalities in small intestinal absorptive capacity must be substantial prior to any decrease in 3-OMG being detected [38]. These properties make 3-OMG a useful tool to estimate the absorption of glucose.

Our group has substantial experience with using plasma 3-OMG concentrations as a marker of gastric emptying and glucose absorption [5, 16, 39, 40]. A particular advantage of the carbohydrate absorption tests is that energy delivery is a focus of nutritional therapy, and tests such as 3-OMG measure the clinically important variable (i.e. nutrient absorption). Currently there is no commercially available assay to measure 3-OMG concentrations and high performance liquid chromatography is required. Accordingly the technique is expensive and is not widely available. Data are generally presented as area under the concentration curve [16, 37, 39].

Similar to paracetamol absorption, the limitations of the 3-OMG test include imprecision due to disordered pharmacokinetics in the critically ill. The volume of distribution of glucose however appears minimally affected by critical illness [41]. A further limitation is that small intestinal monosaccharide (3-OMG) absorption is impaired in the critically ill [37], due to mesenteric blood flow and molecular mechanisms [40, 42], which are factors distal to the pylorus. Accordingly, drugs may affect small intestinal motility thereby altering carbohydrate (3-OMG) absorption independent of any effect on gastric emptying [39]. Hence, while the 3-OMG test is a useful measure of a relatively important end-point, the technique is a somewhat imprecise measurement of gastric emptying per se.

Isotope breath tests

The measurement of gastric emptying with a breath test requires the ingestion of a 'standard meal', labelled with a stable isotope (e.g. 13C). The isotope is incorporated into a free fatty acid (e.g. octanoic acid or acetate), which is rapidly (and almost entirely) absorbed from the duodenum/small intestine, and then metabolised in the liver to 13CO2, which is excreted during exhalation [43]. In health gastric emptying is the rate-limiting step in this process, and pulmonary excretion of labelled CO2 is used to indicate the rate of gastric emptying of the meal. While 13C-acetate absorption and

metabolism is independent of the volume and caloric delivery of the test meal, measurement of the 'lag' before gastric emptying commences reflects a postgastric, dose-dependent delay in 13CO2 elimination [44]. 13CO2 is measured using an isotope ratio mass spectrometer. A greater cumulative dose of 13CO2 exhaled over time indicates increased gastric emptying (Figure 2b). Data are usually presented as the gastric emptying coefficient (GEC) and/or the gastric half-emptying time (t1/2). The GEC is a global index for the gastric emptying rate that accounts for the rate of appearance and disappearance of tracer in the breath, with the greater the value of GEC indicating a more rapid emptying rate. Gastric half-emptying time (t1/2) is the time to 50% of the total 13C recovered and is also reported. Unlike the scintigraphic T50, which reflects a directly measured amount (i.e. the time taken for half of the radioactivity to empty from the stomach), the t1/2 is a calculated measure. Moreover, 13CO2 is excreted via non-respiratory pathways (indeed it has been proposed that a three compartment model exists and a large proportion of 13CO2 is stored) [45, 46]. In our opinion, the t1/2 is less reliable than the GEC in the critically ill, as gastric emptying is markedly delayed in many patients, so that only small amounts of 13C are recovered during the measurement period, and calculations required to estimate t1/2 in these patients are prone to error.

We have extensive experience with this test (Table 4) [5, 16, 47-50] and it is a useful measurement of gastric emptying. Similar to other measures of gastric emptying that rely on small intestinal absorption, there may be factors that impair absorption which can adversely affect the result [19]. Indeed there may be factors within the small intestine that cause substantially different results in an individual patient when measuring gastric emptying using any of the indirect tests. A further concern is that CO2 production is variable between patients, with dynamic changes within patients during their acute illness. While the test appears to be relatively robust with acceptable intrasubject variability [29, 51] and provides a satisfactory correlation with scintigraphy (GEC; $r = -0.63$ to -0.74; $P < 0.0001$; t1/2; $r = 0.55$-0.66; $P < 0.001$) [25] in critically ill patients with normal to slow gastric emptying the relative imprecision of the test can be balanced by increasing the sample size. This technique is also particularly suited to evaluating changes in gastric emptying for example in response to therapy, but is less suitable for comparing gastric emptying between two patient groups. Its use is also limited in patients with marked gastroparesis.

Other Tests of Gastric Emptying

Other measures of gastric emptying are used less frequently in the critically ill patient, when compared to the tests described above.

Ultrasound

Conventional real-time ultrasound can be used to study antral contractility, gastric emptying, transpyloric flow, gastric configuration, intragastric distribution of meals, gastric accommodation and strain measurement of the gastric wall [52]. The advantages of ultrasound are that it is non-invasive, involves no radiation, and can be performed at the bedside.

Gastric emptying can be measured using both 2-dimensional (2D) or 3-dimensional (3D) ultrasound. Using 2D ultrasound gastric emptying is usually characterised by measurement of changes in antral cross sectional area or diameter over time [53]. These measurements correlate closely with emptying rates of a liquid or semi-solid 'meals', as determined by scintigraphy [54], however, in patients with gastroparesis, a higher proportion of the meal may be retained in the proximal stomach potentially increasing the likelihood of error [55]. 3D ultrasound is an accurate measurement of volume (including proximal volume) and this technique has been reported to closely match measurements obtained with scintigraphy [55].

In our experience, obtaining an acceptable 'window' can be very difficult due to 'patient factors' (subcutaneous tissue, interstitial oedema, air in the fundus) and 'liquid nutrient factors' (echo-dense feeds may obscure the view of the proximal stomach) (unpublished data). A further limitation is that 3D ultrasound requires a 'breath hold' and this is technically impossible in most spontaneously ventilating patients receiving invasive mechanical support unless paralysing drugs are administered. For these reasons we have been unable to obtain satisfactory images when piloting this technique (unpublished data). Accordingly, other techniques are currently preferred, but due to the potential advantages, further study using this technique is warranted.

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) has many advantages when measuring gastric emptying, such as image acquisition (involves no ionising radiation) and analysis (operator independent as interpretation does not involve manual tracing). MRI was first described as a technique to measure emptying liquids but, unlike scintigraphy, also allowed quantification of gastric secretions. MRI can detect changes in gastric emptying rate caused by pharmacotherapy [56]. A further advantage of MRI is that emptying of individual constituents of a liquid meal (i.e. lipid) may be measured in addition to that of the entire meal. Furthermore, MRI permits direct visualisation of gastric wall motion and morphological abnormalities.

The disadvantages of MRI relate to the expense and availability of the scanner itself. Moreover, transportation and the period within the MRI scanner for image acquisition may be associated with significant adverse events in the critically ill and, accordingly, the use of MRI in this group is impractical.

Surrogate Tests of Gastric Emptying

Surrogate markers are used in clinical practice and research to estimate gastric emptying.

Gastric Residual Volumes (GRVs)

Intermittent measurement of the gastric residual volume (GRV) during the infusion of enteral nutrition is a convenient clinical tool that is used widely as a surrogate indication of gastric emptying, 'success' of feeding, and, possibly, the risk of aspiration. Despite inclusion of GRV measurements in feeding protocols by ICUs worldwide, the utility and significance of GRVs remain controversial. In particular, GRVs are dependent on a number of factors such as the position of the tube, tube characteristics (such as tube type and number of openings), the operator performing the test, use of prokinetic drugs and calorie concentration of the liquid nutrient [57, 58]. Moreover, GRVs are usually measured 4-6 hourly and the significance of a single value may be uncertain. These factors have led to a lack of consensus for what is an acceptable threshold for GRV and the significance of a single GRV [57]. Our group has reported that a GRV $\geq$ 250mL in the preceding 24 hours was a relatively sensitive marker of delayed gastric emptying when measured using scintigraphy [5].

Additionally, patients with a GRV ≥ 250mL had delayed gastric emptying subsequently measured by scintigraphy [59]. In general, we favour commencing intragastric feeds at goal rate via a larger bore feeding tube with GRVs aspirated every 6 hours. Moreover, while large residual volumes may predict slow emptying, these may also identify patients at greater risk of complications such as vomiting or aspiration, but a recent study has challenged this notion [60]. Further studies are required to confirm this interesting observation. GRVs continue to be used clinically as this test is an easy, low risk, bedside surrogate measurement to identify patients at risk of slower gastric emptying. While it still needs to be clarified whether an increase in GRVs equates to clinically relevant adverse events, such as ventilator associated complications, it seems reasonable to use GRVs to select patients that have the capacity to benefit from gastrokinetic drugs or small intestinal feeding tubes.

Strengths and Limitations

This review provides a summary of current information related to gastric emptying in the critically ill. At our centre we have considerable experience with several of the methodologies described. While an asset, we recognise that the latter has the potential to bias our recommendations. Many of these studies involve small numbers of patients studied in highly specialised centres with sophisticated methodologies, which may compromise the generalisability of the outcomes.

Recommendations

The choice of gastric emptying test depends on the requirements of the investigator. For clinicians wanting a 'crude' but easy measurement to identify patients receiving nasogastric feeding with slow gastric emptying at the bedside, GRV remains a useful, albeit limited and controversial, test and pending further data we continue to use GRVs clinically to determine those patients who have the potential to benefit from interventions such as gastrokinetic drugs or small intestinal feeding tubes. We recommend a threshold of GRV ≥ 250mL to define slow gastric emptying to identify patients likely to have slow gastric emptying, but recognise this approach has hitherto not been translated into improved patient-centred outcomes.

However, in the research setting, particularly when studying smaller cohorts, GRVs are too imprecise. Indirect tests, such as carbohydrate absorption (3-OMG) or radio-

isotope breath tests (13CO2), are minimally invasive and have modest intrasubject variability. They are useful when studying the effect of an intervention thought to have a potent effect on gastric emptying in larger cohorts, particularly when researchers use a crossover design. However, when studying the effect of a less potent gastrokinetic drug and/or using a parallel design study, and a more precise measurement are required, direct tests, such as scintigraphy, are more suitable and remain the gold standard.

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Tables

Table 1: Methods of gastric emptying measurement in the critically ill

Type	Method
Direct	Scintigraphy
Indirect	Drug absorption Carbohydrate absorption (3-O-Methylglucose; 3-OMG) Isotope breath test
Other	Ultrasound MRI
Surrogate	Gastric Residual Volumes (GRVs)

Table 2: Scintigraphy as a measure of gastric emptying in the critically ill

* Denotes investigators from the same group; T50, scintigraphic half emptying time; Ret, intragastric content retention

Study Primary Investigator (year)	Dose administered (MBq)	Radionuclide used	Scintigraphy time after administration (min)	Outcomes reported
Ott (1991)	18.5-37	^{99m}Tc-diethylene-triamine-pentaacetic acid (DTPA)	20 – 60 mins	T_{50}
Spapen (1995)	20	^{99m}Tc-sulphur colloid	120 mins	T_{50}
Kao (1998)	18.5	^{99m}Tc-phytate	30 mins	T_{50}
Nguyen (2008)*	20	^{99m}Tc-sulphur colloid	240 mins	Ret
Chapman (2009)*	20	^{99m}Tc-sulphur colloid	240mins	Ret
Chapman (2011)*	20	^{99m}Tc-sulphur colloid	240mins	T_{50}, Ret

Table 3: Paracetamol absorption as a measure of gastric emptying in the critically ill

A lack of standardisation is apparent between investigators using the paracetamol absorption test to measure gastric emptying in the critically ill. The dose of paracetamol administered has ranged from 975 to 1600 mg, and some investigators administered the liquid suspension paracetamol, whereas others use dissolved tablets. There is also a lack of consistency across studies in terms of the timing of measurements of plasma concentrations and outcomes reported.

*,^ Denotes investigators from the same group; C_{max}, peak concentration of paracetamol; T_{max} time to peak concentration; AUC area under curve

Study Primary Investigator (year)	**Dose administered (mg)**	**Formulation of paracetamol administered S = Soluble L = Liquid**	**Plasma sampled at time after administration (min)**	**Outcomes reported**
Heyland (1996)	1600	L	30, 60, 90, 120	AUC_{120}
Tarling * (1997)	1000	S	5, 10, 15, 30, 45, 60, 90, 120	AUC_{60}
Goldhill * (1997)	1000	S	5, 10, 15, 30, 45, 60, 90, 120	C_{max}, T_{max} and AUC_{60}
McArthur (1995)	1000	S	5,10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180	C_{max}, T_{max} and AUC_{30} AUC_{180}
Cohen (2000)	1000	L	15, 30, 45, 60	AUC_{60}
Jooste (1999)	1500	S	15, 30, 45, 60, 90, 120	C_{max}, AUC_{120}
MacLaren ^ (2000)	1000	L	7 samples between 0 and 720	T_{max} AUC_{120}
MacLaren ^ (2008)	975	L	15, 30, 45, 60, 90, 120, 180, 240, 360	C_{max}, T_{max} and C_{60} AUC_{60}
Lucey (2003)	1500	L	15, 30, 45, 60, 90, 120	AUC_{120}
Marino (2003)	1000	S	15, 30, 45, 60, 90, 120	AUC_{120}
Tamion (2003)	1000	S	10, 20, 30, 60, 90, 120, 180	C_{max}, T_{max} and AUC_{60} AUC_{180}

Table 4: Breath test as a measure of gastric emptying in the critically ill

* Denotes investigators from the same group; GEC, gastric emptying coefficient; $t_{1/2}$ gastric half-emptying time

Study Primary Investigator (year)	Dose administered	Type of isotope used	Breath sampled at time after administration (min)	Outcomes reported
Ritz (2001)*	100 μL	13C-octanoic acid	Hr 0-1: 5 minutely Hr 1-4: 15 minutely	GEC, $t_{1/2}$
Chapman (2003)*	100 μL	13C-octanoic acid	Hr 0-1: 5 minutely Hr 1-4: 15 minutely	GEC, $t_{1/2}$
Ritz (2005)*	100 μL	13C-octanoic acid	Hr 0-1: 5 minutely Hr 1-4: 15 minutely	GEC, $t_{1/2}$
Chapman (2005)*	100 μL	13C-octanoic acid	Hr 0-1: 5 minutely Hr 1-4: 15 minutely	GEC
Deane (2010)*	100 μL	13C-octanoic acid	Hr 0-1: 5 minutely Hr 1-4: 15 minutely Hr 4-5.5: 30 minutely	GEC
Chapman (2011)*	75 KBq	14C-octanoic acid	Hr 0-1: 10 minutely Hr 1-4: 15 minutely	GEC, $t_{1/2}$

Figures

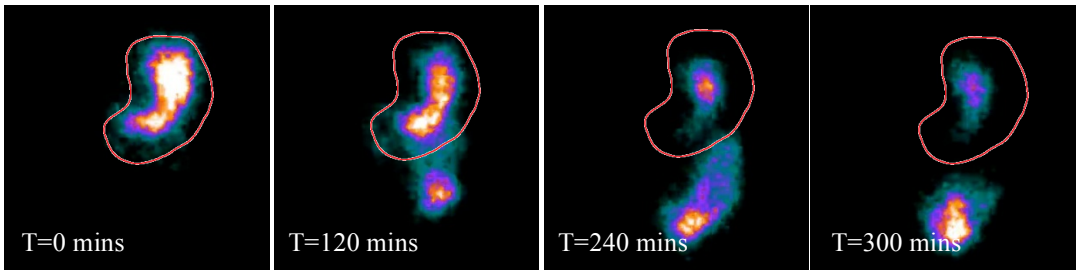

Fig 1a: Images in a patient with normal gastric emptying

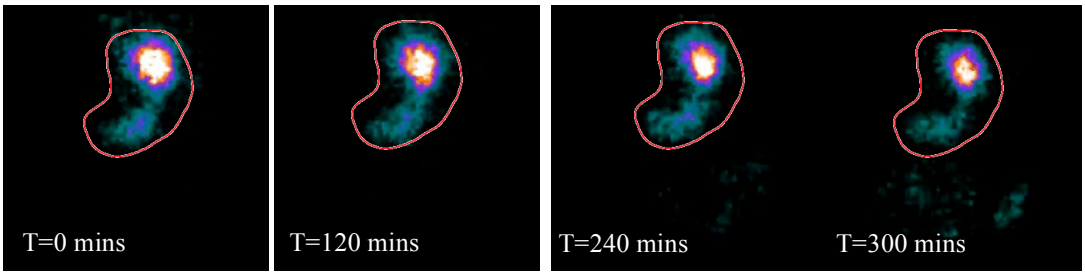

Fig 1b: Images in a patient with delayed gastric emptying

FIGURE 1: Normal and delayed gastric emptying of 100ml of liquid nutrient (2kcal/ml) measured in two critically ill patients (unpublished data) over 5 hours. On the first image after the meal is administered a region of interest (ROI) is drawn around the entire stomach contents. The images in 1a reveal a relatively 'normal' rate of gastric emptying in a patient. The radiolabelled meal progresses from the stomach into the small intestine across each image (with image acquisition taken at one hour intervals). Figure 1b shows delayed gastric emptying in the critically ill patient, with the radiolabelled being almost completed retained in the (proximal) stomach and radioisotope is only sparsely viewed distal to the pylorus.

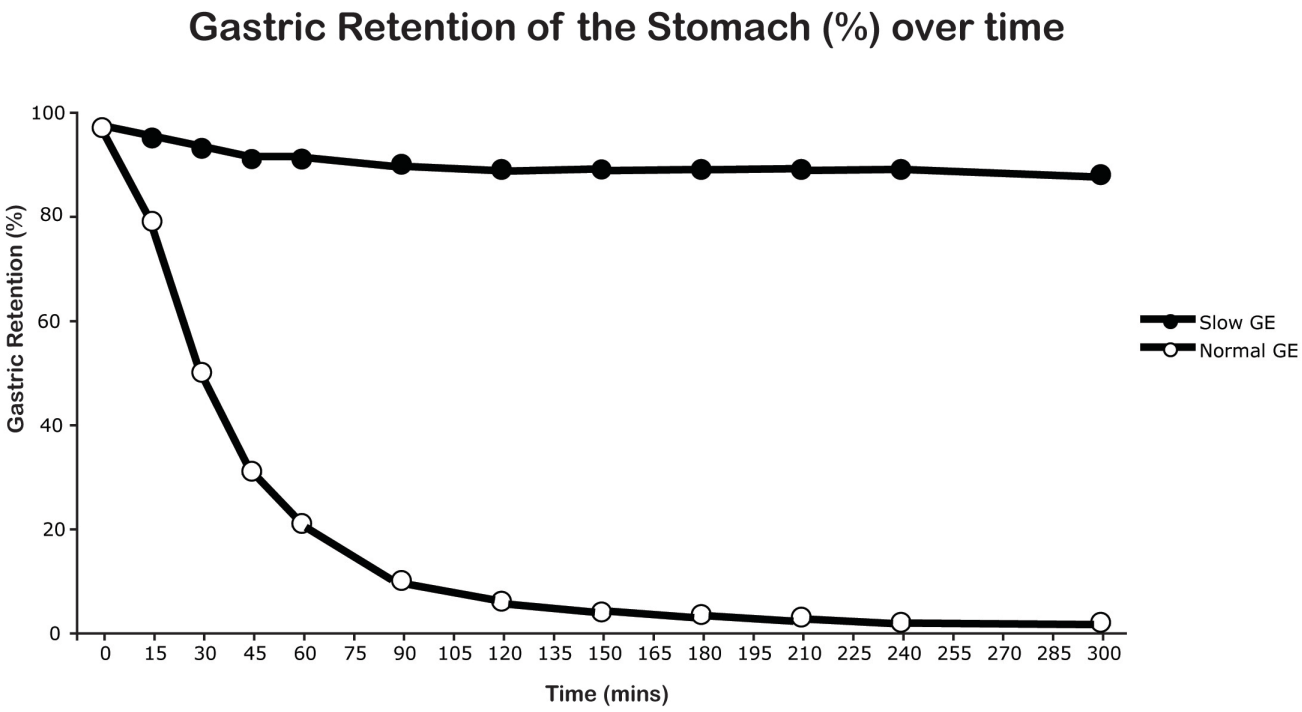

FIGURE 2a: Retention of gastric contents over time via scintigraphic technique.
Data from two critically ill patients after administration of 100ml of liquid nutrient (2kcal/ml) labelled with 20 MBq 99mTc- calcium phytate (unpublished data). A higher percentage of contents retained in the stomach over time indicate a slower rate of gastric emptying (solid circles). In a patient exhibiting 'normal' gastric emptying (open circle) a lesser percentage of the meal is retained over time.

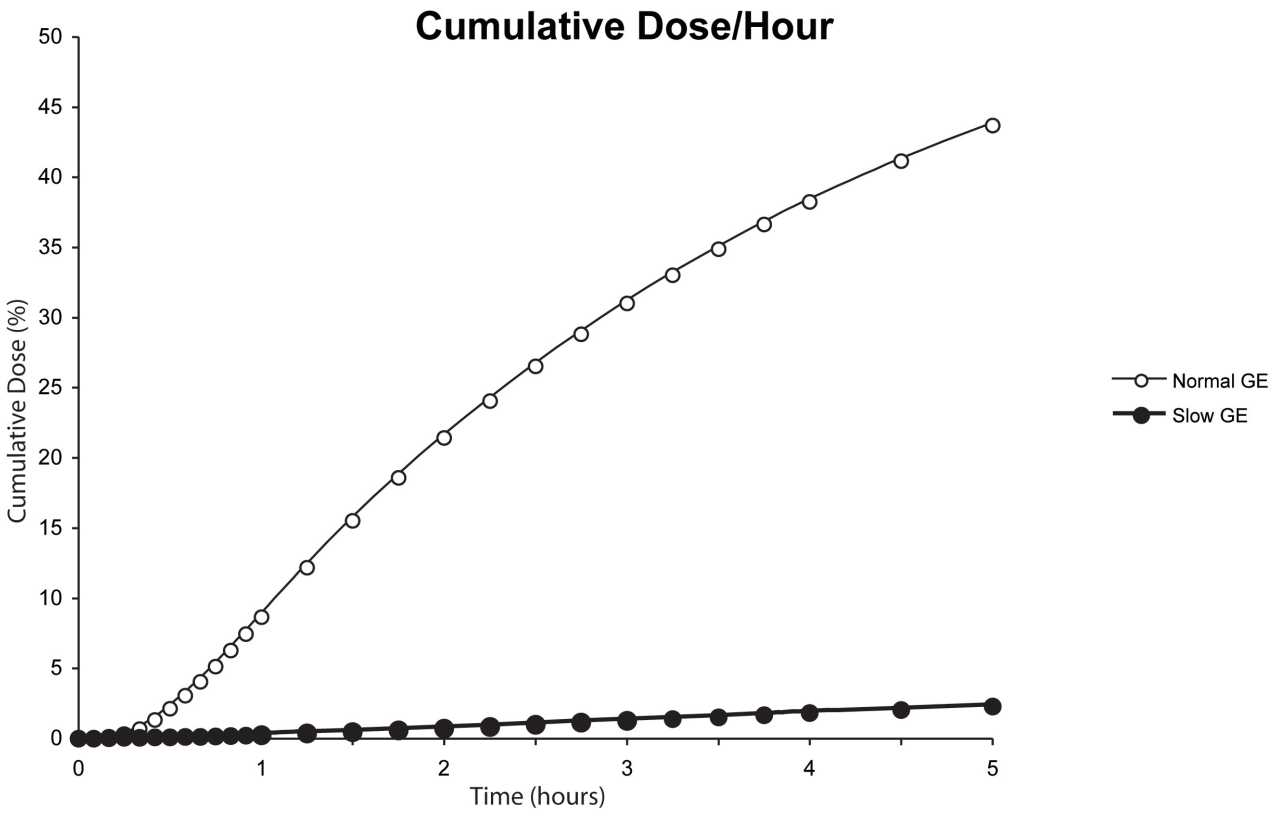

FIGURE 2b: Cumulative dose of $^{13}CO_2$ exhaled over time.

This figure displays data from the same two patients after administration of 100ml of liquid nutrient (2kcal/ml) labelled with 100 mcg of octanoic acid. The octanoic acid contains ^{13}C, which is absorbed by the small intestine and undergoes hepatic metabolism to $^{13}CO_2$, which is then exhaled. An increased amount of exhaled $^{13}CO_2$ indicates faster gastric emptying (open circles). Data from a patient with delayed gastric emptying (solid circles) displays a lesser cumulative dose/hour of CO2 exhaled. Due to the small change in cumulative dose from baseline in patients with very slow gastric emptying, this measure may be less precise when measuring markedly delayed gastric emptying.

1.3 MANUSCRIPT

Energy-Dense Formulae May Slow Gastric Emptying in the Critically Ill

Statement of Authorship

Title of paper	Energy-Dense Formulae May Slow Gastric Emptying in the Critically Ill
Publication status	Published
Publication details	Kar P, Plummer MP, Chapman MJ, Cousins CE, Lange K, Horowitz M, Jones KL, Deane AM. **Energy-Dense Formulae May Slow Gastric Emptying in the Critically Ill**. Journal of Parenteral & Enteral Nutrition. 2016 Sep; 40(7):1050-6

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Contribution to paper	Conceptualisation of work, its realisation and its documentation. Collected and interpreted data and wrote manuscript.		
Overall percentage (%)	80		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/10/16

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
- The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to paper	Data collection		
Signature		Date	11/10/16

Name of co-author	Ms Kylie Lange		
Contribution to paper	Statistical analysis, interpretation of data and drafting the manuscript.		
Signature		Date	07/10/16

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Contribution to paper	Revision of the manuscript for important intellectual content.		
Signature		Date	01/10/16

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Contribution to paper	Data analysis, key intellectual input and manuscript evaluation		
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Contribution to paper	Conceptualisation of work, obtaining funding, supervision and manuscript evaluation		
Signature		Date	01/10/16

Full title

Energy-dense formulae may slow gastric emptying in the critically ill

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Abstract

Background: Enteral feed intolerance occurs frequently in critically ill patients and can be associated with adverse outcomes. 'Energy dense formulae' (i.e. > 1 kcal/ml) are often prescribed to critically ill patients to reduce administered volume and are presumed to maintain or increase calorie delivery. The aim of this study was to compare gastric emptying of standard and energy dense formulae in critically ill patients.

Materials and Methods: In a retrospective comparison of two studies, data from two groups of patients that received a radiolabelled 100 ml 'meal' containing either standard (1 kcal/ml) or concentrated (energy dense formulae; 2 kcal/ml) calories were analysed. Gastric emptying was measured using a scintigraphic technique. Radioisotope data were collected for 4 hours and gastric emptying quantified. Data are mean (SE) or median [IQR] as appropriate.

Results: 40 patients were studied (n = 18 energy dense formulae, 22 standard). Groups were well matched in terms of demographics. However, patients in the energy dense formulae group were studied earlier in their ICU admission (P=0.02) and had a greater proportion requiring inotropes (P=0.002). Similar amount of calories emptied out of the stomach per unit time (P=0.57), but in patients receiving energy dense formulae a greater volume of 'meal' was retained in the stomach (P=0.045) consistent with slower gastric emptying.

Conclusions: In critically ill patients the administration of the same volume of a concentrated enteral nutrition formula may not result in the delivery of more calories to the small intestine over time because gastric emptying is slowed.

Background

Administration of liquid nutrient via the enteral route is part of standard care of the critically ill [1-3]. However this may be compromised by delayed gastric emptying, identified clinically by large gastric residual volumes or vomiting, which occurs frequently in the critically ill and is associated with adverse outcomes [4-8].

Polymeric formulae vary in concentration (calories per unit volume) but, in general, contain between 1 and 2 kcal/ml [9, 10]. The use of liquid nutrient containing more calories per unit volume (i.e. > 1 kcal/ml), so-called energy-dense formulae, has been suggested as a method to augment nutrient delivery in certain patient groups [11, 12], however evidence to support its use is inconsistent [13, 14]. Despite this uncertainty, observational data suggest that energy dense formulae are prescribed in up to one-third of patients receiving enteral nutrition [15].

The effect of calorie concentration on gastric emptying has hitherto not been evaluated in critically ill patients. In health, gastric emptying is tightly regulated primarily by enterogastric feedback. Nutrients stimulate receptors on the small intestinal mucosa and slow gastric emptying via neuro-hormonal feedback mechanisms [16]. Mediators include small intestinal hormones such as cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which cause fundal relaxation, decreased antral contractions and increased pyloric tone resulting in slowed gastric emptying [16]. This feedback loop prevents excessing dumping of nutrients into the small intestine, resulting in a constant rate of calorie delivery of approximately 1-4 kcal/min [17].

In health, the secretion of these small intestinal hormones is dependent on both the calorie and macronutrient content of the meal. As such, increasing the proportion of fat also results in a greater delay in gastric emptying [18-21]. This variation in the rate of macronutrient emptying from the stomach is mediated by the aforementioned hormones. The magnitude of increase in CCK and PYY secretion is markedly greater in the presence of fat in the small intestine when compared to an isocaloric load of carbohydrate [22]. GLP-1 secretion appears to be comparable in the presence of both carbohydrate and lipids[23]. In addition, endogenous CCK potently slows gastric emptying [24, 25], whereas the effects of GLP-1 may not be as marked [17, 26-28].

It is recognised relatively recently that delayed gastric emptying in the critically ill patient may be due to up-regulation of these feedback mechanisms. When polymeric feed is infused directly into the small intestine at 1 kcal/min, substantial gastric and pyloric motility changes occur that markedly slow gastric emptying [29]. These motility effects are not observed in health at this low rate of calorie delivery (1 kcal/min), suggesting that critically ill patients may be hypersensitive to small intestinal caloric stimulation. Furthermore, fasting and nutrient stimulated plasma concentrations of CCK, PYY and GLP-1 are increased in the critically ill when compared to healthy controls [16, 30, 31] and are greater in feed intolerant than in feed tolerant critically ill patients [32]. Accordingly, it is likely that energy dense formulae may be emptied slower than standard feeds, but this has never been formally quantified.

Energy dense formulae are frequently prescribed without an understanding of how this affects calorie delivery to the small intestine. Based on our understanding of the exaggerated enterogastric feedback response in critical illness, it is plausible that the gastric emptying of energy dense formulae will be markedly slowed. How this affects calorie delivery to the small intestine is uncertain. The aim of this study was to evaluate the effect of calorie concentration on the rate of gastric emptying in terms of volume and calories in critically ill patients.

Materials and Methods

We analysed data from two studies [33,34]. In both studies patients were eligible for inclusion if aged ≥ 18 years and either receiving, or suitable to receive, enteral feeding. Exclusion criteria included pregnancy, administration of gastrokinetic drugs in the preceding 24 hours and previous surgery on the oesophagus, stomach or duodenum. Both protocols were approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and studies were performed according to the National Health and Medical Research Council guidelines for the conduct of research on unconscious patients. Written, informed consent for each patient was obtained from the patient's surrogate decision maker.

For each study day, patients were fasted for 4 hours (Figure 1). At time = -5min, the nasogastric tube placement within the stomach was confirmed via aspiration and the 'meal' administered over 5 minutes. The 'meal' consisted of 100 ml of either a mixed nutrient liquid that was representative of standard liquid feed, i.e. 1 kcal/ml (Ensure®, Abbott Nutrition, Botany, NSW, Australia, containing carbohydrate 68%, fat 13%, protein 19%, osmolality 590 mOsm/kg H2O) or a 2 kcal/ml energy dense formulae (TwoCal®, Abbott Nutrition, containing carbohydrate 43%, fat 40%, and protein 17%, osmolality 690 mOsm/kg H2O). Each study protocol dictated the type of formulae given. Each 'meal' labelled with 99mtechnetium sulphur-colloid allowed the measurement of gastric emptying using scintigraphy. The scintigraphic measurements were performed over four hours (0 to 240 minutes). Blood glucose levels were taken every 30 minutes during the course of the study.

Scintigraphy remains the most accurate method to quantify gastric emptying in the critically ill [6]. Scintigraphic data were captured with the patient positioned supine using a mobile gamma camera with three-minute dynamic frame acquisition. Radioisotopic data were corrected for subject movement and radionuclide decay. A region of interest was drawn around the total stomach, with gastric emptying curves generated over time and the intragastric retention at 15 minute intervals calculated. In addition, from these data the kcal emptied into the duodenum was calculated. Data were analysed by the same individual (KLJ) blinded to the study conditions.

Statistical Analysis

Data are mean (SE) or median [IQR] as appropriate. Demographic data were analysed using independent samples t-tests. Total areas under the curve from baseline to 240 minutes (AUC_{240}) were calculated for blood glucose, gastric emptying and calorie delivery using the trapezoidal rule. Additionally, independent samples t-tests were used to evaluate the differences between groups in gastric retention and in blood glucose (at baseline, at 60 min, and AUC_{240}). ICU length of stay, day of admission and gastric residual volumes used non-parametric Mann-Whitney tests for analysis. For nominal data (sex, tolerating feeding, insulin use, diabetes history, opiate use and inotrope use) chi-square tests were used. Kcal of feed, diabetes history, BMI, catecholamine use and opiate use were used as independent variables in a multiple linear regression analysis of gastric emptying.

Sample size was determined by scintigraphic data available from the previous studies. Feed intolerance was defined when at least one gastric residual volume was ≥250 ml in the 48 hours preceding enrolment [35]. Statistical analyses were performed using SPSS (Version 22) with statistical significance set at $P<0.05$.

Results

Forty patients were studied with 22 and 18 patients receiving standard and energy dense formulae respectively. Demographic and patient outcome details are summarized in table 1. Demographic data were normally distributed while ICU length of stay, day of admission and gastric residual volumes exhibited positively skewed distributions. The two groups were well matched with respect to age, gender, body mass index (BMI), acute physiological and chronic health evaluation severity of illness scores (APACHE II score) and history of diabetes. The groups were also comparable in length of admission in ICU, the number being fed in the previous 24 hours prior to the study and, of those being fed, how many were 'tolerating' feeds. Insulin use, sedative use (propofol and/or midazolam) and opiate use (including the equivalence dose in the preceding 24 hours) during the study days were also similar. All patients tolerated the ingestion of their 100ml "meal". Patients in the energy dense formula group were studied earlier in their ICU admission ($P=0.02$) and in this group, a higher proportion were received inotropes ($P=0.002$).

Blood glucose concentrations

Baseline blood glucose concentrations were similar in both groups (Figure 2). There was a small rise in blood glucose concentration after the meal in both groups, with this rise delayed in the energy dense formulae group, however there was no statistical difference in blood glucose concentrations at T=60 or in glycemic excursion during the postprandial period (Figure 2) between the groups.

Gastric emptying (scintigraphy)

Over 4 hours a larger volume of the energy dense formula was retained in the stomach when compared to the standard 1 kcal/ml feed ($P=0.045$; Figure 3). Moreover, there was no difference in the rate of calorie delivery into the small intestine (Figure 4). Following linear regression analysis, opiate use ($P=0.02$) and calorie content of feed

(P=0.05) were independently associated with increased retention of gastric content, suggesting increased opiate administration and increased calorie content of feed are associated with slower emptying.

Discussion

While feeding patients enterally is standard of care, this is frequently limited by slow gastric emptying and feed intolerance. Concentrated formulae are given to patients frequently for two main reasons – to increase calorie delivery in patients who are not tolerating standard feeds and to deliver calories while restricting fluid volume. These data suggest that if an energy dense formula is administered, the volume of a concentrated formulae is emptied more slowly from the stomach such that calorie delivery to the small intestine is maintained. After gastric infusion of 100 ml of nutrient, calorie delivery peaked at about 1 kcal/minute and was unaffected by calorie concentration. These data suggest that the administration of an energy dense formulae above a certain threshold may not increase energy delivery.

Our study suggests that in the critically ill patient, as in health, higher calorie liquids are emptied from the stomach slower than isocaloric feeds such that delivery into the small intestine is dependent primarily on calories rather than volume. Whilst a preliminary observation, which requires further validation, our finding is clinically important as it suggesting that administering EDFs to patients with enteral feed intolerance will simply prolong gastric emptying and exacerbate feed intolerance. In contrast a recently published multicentre feasibility study that enrolled 112 critically ill patients [12] suggested that a more concentrated formula (1.5 kcal/ml) when compared to standard formulae (1 kcal/ml), was not associated with adverse gastrointestinal effects (particularly feed intolerance). The difference between the latter study and our current observation may be due to either the greater caloric content or the precision of the measurement (scintigraphy vs. clinical feed intolerance) used in our current study.

There is a physiological basis to explain our observation [36]. As outlined, in health, gastric emptying of nutrient reflects the absorptive capacity of the small intestine and the rate is therefore regulated by the caloric load, with emptying occurring at a rate of ~ 1-4 kcal/min [37]. Deceleration of gastric emptying reflects a decrease in propulsive

force and/or increased resistance to flow, and occurs as nutrient stimulates small intestinal receptors leading to rapid, and persistent, motor changes within the proximal and distal stomach. This feedback regulation occurs via neural and hormonal mediators [38, 39].

In the critically ill this entero-gastric feedback is potentiated. For example our group has previously noted when liquid nutrient was infused into the small intestinal at 1 kcal/min, antral wave frequency is markedly reduced with corresponding increases in pyloric tone and the number of isolated pyloric pressure waves [29]. These motility changes retard trans-pyloric flow and slow gastric emptying. Given that critically ill patients appear to be 'hypersensitive' to the presence of nutrient in the small intestine we hypothesized that increasing caloric load for a given volume would slow gastric emptying.

In addition to calorie load macronutrient content is an important regulator of emptying rate. In our study, the proportion of fat was greater in the energy dense formulae (40% fat - with 8% medium chain triglycerides and 32% long chain triglycerides) compared to standard feed (22% fat – with all long chain triglycerides), and this is likely to have contributed to the slower gastric emptying observed. Similar observations have been made in ambulant cohorts. For example, Akrabawi and colleagues quantified gastric emptying using scintigraphy in 36 patients with chronic obstructive pulmonary disease (COPD) and reported gastric emptying was markedly slower following a 'high' fat meal (55% fat – all long chain triglycerides) when compared to a 'moderate' isocaloric fat meal (41% fat – with 30% medium chain triglycerides and 11% long chain triglycerides) [40].

The slower gastric emptying that we observed with the energy dense formula is likely to be mediated via humoral mechanisms [41]. CCK is secreted from the proximal small intestine in response to nutrient, with lipid having a pronounced effect and secretion substantially greater in response to long chain triglycerides when compared to medium chain triglycerides [42]. In critically ill patients, fasting plasma CCK concentrations appear to be approximately twice those of healthy controls, and nutrient-stimulated CCK concentrations are some 1.5-fold greater [30]. Furthermore, fasting plasma CCK concentrations are greater in critically ill patients with delayed,

when compared to those with normal, gastric emptying [30, 32]. Peptide YY (PYY) is secreted predominantly from the colon and rectum and like CCK, is most potently stimulated by fat [43]. Nematy and colleagues reported fasting PYY concentrations were increased almost threefold in the acute phase of critical illness, when compared to health [44]. In a separate study, fasting plasma PYY concentrations were substantially increased in those with delayed gastric emptying [31]. These humoral data are consistent with the concept that critically ill patients are hypersensitive to small intestinal nutrient. The presence of unabsorbed fat, carbohydrate and/or amino acid in the small intestine or colon also stimulates secretion of GLP-1 [43] and, in health, GLP-1 is a physiological mediator that slows gastric emptying [17].

The presence of hyperosmotic solutions in the small intestine also slows gastric emptying - in effect allowing greater time for dilution of the meal in the stomach [45]. The alteration in motor activity with increasing osmolarity results in an increase in frequency and amplitude of duodenal contractions leading to an increase in duodenal resistance to transpyloric flow mediated via the enteric nervous system [46]. The osmolality of the standard feed and the energy dense formulae differed (590 vs. 690 mOsm/kg H2O), and it is possible that the minor variation in osmolality also contributed to the marked changes in gastric emptying rate we observed.

Interestingly, there appeared to be a more rapid increase in blood glucose in patients receiving 1 kcal/ml. While the difference as not statistically significant it may reflect subtle differences in the absorption of glucose and lipid [47, 48] and/or secretion of the incretin hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) [49]. Another finding of this study was that the emptying of volume was slower using the more energy dense formula, however the emptying of calories was the same. It would seem that, as in health, the calorie delivery to the small intestine in critical illness is tightly controlled and is independent of volume. We can therefore speculate that our observations would have been similar were the volume reduced in patients receiving energy dense feeds.

These data support the concept that the average rate of gastric emptying in the critically ill is approximately 1 kcal/min, which is substantially less than in health. Our previous studies indicate that this slower rate of emptying reflects an increase in

inhibitory feedback arising from the small intestine [32, 50] and that prokinetic drugs have the capacity to accelerate gastric emptying substantially [50].

The major limitation of our study is the retrospective design. The two groups of patients were not recruited concurrently and were not randomly allocated the study 'meal', introducing the possibility of bias and confounding variables (such as severity of illness) [51], which could potentially influence the rate of gastric emptying. Retrospective cohort studies can, however, provide important information about nutritional therapy to the critically ill [52] and our observation is novel and is supported by knowledge of gastrointestinal motility in health and critical illness. Moreover, because our observation is clinically relevant it emphasizes that further study to establish the effect of energy dense formulae on gastric emptying in the critically ill is required.

A further limitation relates to the acute period of observation during the study. While it should be recognized that it is challenging to study critically ill patients for prolonged periods, further evaluation of patients over an extended time course would be of interest.

There are many factors that influence the rate of gastric emptying during critical illness. Gender and age can affect gastric emptying, but these were well matched in this study. Exogenous drugs also have the capacity to modify gastric emptying and catecholamines were more frequently given to patients in the energy dense formula group. Dopamine administration slows gastric emptying [53], however dopamine was not administered to any of the patients in our study. In a study of 132 patients at our centre we did not detect any association between administration of norepinephrine and/or epinephrine and delayed gastric emptying [54]. Accordingly, any effect of catecholamines is likely to be minor. Opiate drugs may also affect gastric emptying [54, 55], and this was confirmed in this study, however the use of these drugs was comparable between the groups.

Patients in the energy dense formula group were studied earlier in their admission to ICU and this is an additional potential confounder. It is plausible that gastric emptying is slower early on in the ICU stay when the patient is likely to be sicker, but

repeated measurements of gastric emptying during ICU stay to determine the natural history of this disorder have not been performed. However, the clinical manifestation of delayed gastric emptying - feed-intolerance (as evidenced by large gastric residual volumes, vomiting, diarrhoea, abdominal distension or subject discomfort) - arises in up to a third of ICU patients and can occur at any time within the first 2 weeks of ICU admission [5]. Additionally, in observational data from our center, no relationship was observed between time since admission to ICU and development of delayed gastric emptying [54]. Finally, while we speculate that small intestinal hormones may have mediated the slower gastric emptying observed with energy dense formulae, plasma was not available and therefore we could not measure hormone concentrations. Information regarding the hormones mentioned (CCK, PYY and GLP-1) would have given further understanding behind the possibility of there being a neurohumoral cause of the delayed gastric emptying.

Conclusion

While a prospective study randomizing patients to energy dense formulae and standard feeds is required to prove that gastric emptying is dependent on calorie and not volume load, our study is hypothesis generating, providing preliminary evidence that prescribing energy dense formulae - of at least 2 kcal/ml concentration - has the capacity to affect gastric motor function. We recommend that clinicians be mindful of the effects of macronutrient type and calorie load when prescribing feeds and remain alert for indicators of delayed gastric emptying (e.g. large gastric residual volumes) when prescribing energy dense formulae to critically ill patients, particularly those with pre-existing feed-intolerance. In conclusion, the use of energy dense formulae in the critically ill does not appear to increase calorie delivery to the small intestine, and is associated with prolonged gastric emptying. Further evaluation of this phenomenon is warranted.

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Tables

Table 1: Demographic and patient data for standard and energy dense formula groups. BMI = Body Mass Index. APACHE = Acute Physiology and Chronic Health Evaluation, §Tolerating feed defined as 6 hourly gastric residual volumes <250ml in the previous 24 hours prior to study commencement, * = Propofol/Midazolam. Data are mean (SE) or median [IQR] as appropriate.

Characteristics	1 kcal (n=22)	2 kcal (n=18)	P
Age (years); mean (SE)	60 (3)	55 (4)	.31
Male; n (%)	16 (73%)	11 (61%)	.44
Height (m); mean (SE)	1.71 (0.02)	1.73 (0.02)	.60
Weight (kg); mean (SE)	79 (4)	88 (4)	.12
BMI (kg/m^2); mean (SE)	26.7 (1.0)	29.2 (1.2)	.12
APACHE II at admission; mean (SE)	19 (2)	22 (2)	.29
History of diabetes; n (%)	4/22 (18%)	1/18 (6%)	.23
Length of ICU stay (day); median [IQR]	22.5 [12.0 – 30.3]	13.5 [10.0 – 21.8]	.09
Day in ICU when studied; median [IQR]	9.5 [2.8 – 17.0]	3.0 [2.0 – 5.3]	.02
Gastric aspirate in previous 24 hours (ml); median [IQR]	110 [18 – 251]	200 [25 – 680]	.21
Patients fed in previous 24 hours; n (%)	18/22 (82%)	16/18 (89%)	.53
Tolerated feeding§; n (%)	15/18 (83%)	13/16 (81%)	.87
Insulin use; n (%)	11/22 (50%)	7/18 (39%)	.48
Opiate use; n (%)	14/22 (64%)	12/18 (67%)	.84
Morphine equivalence dose in preceding 24 hrs (mg/hr); median [IQR]	8.0 [2.0 – 20.0]	4.5 [2.3 – 10.0]	.32
Vasoconstrictor/Inotrope use; n (%)	4/22 (18%)	12/18 (67%)	.002
Sedative* use; n (%)	12/22 (55%)	15/18 (83%)	.053
Diagnosis category			
Trauma	6	4	
Neurology	4	5	
Sepsis	4	3	
Respiratory	4	2	
Cardiovascular	1	2	
Other	3	2	

Figures

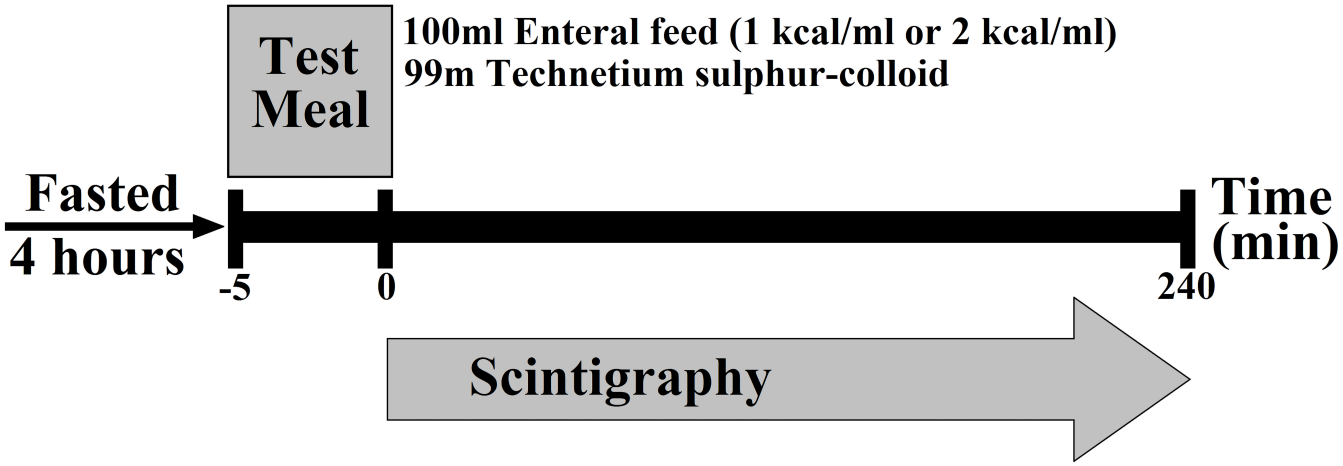

Figure 1: Protocol of the study

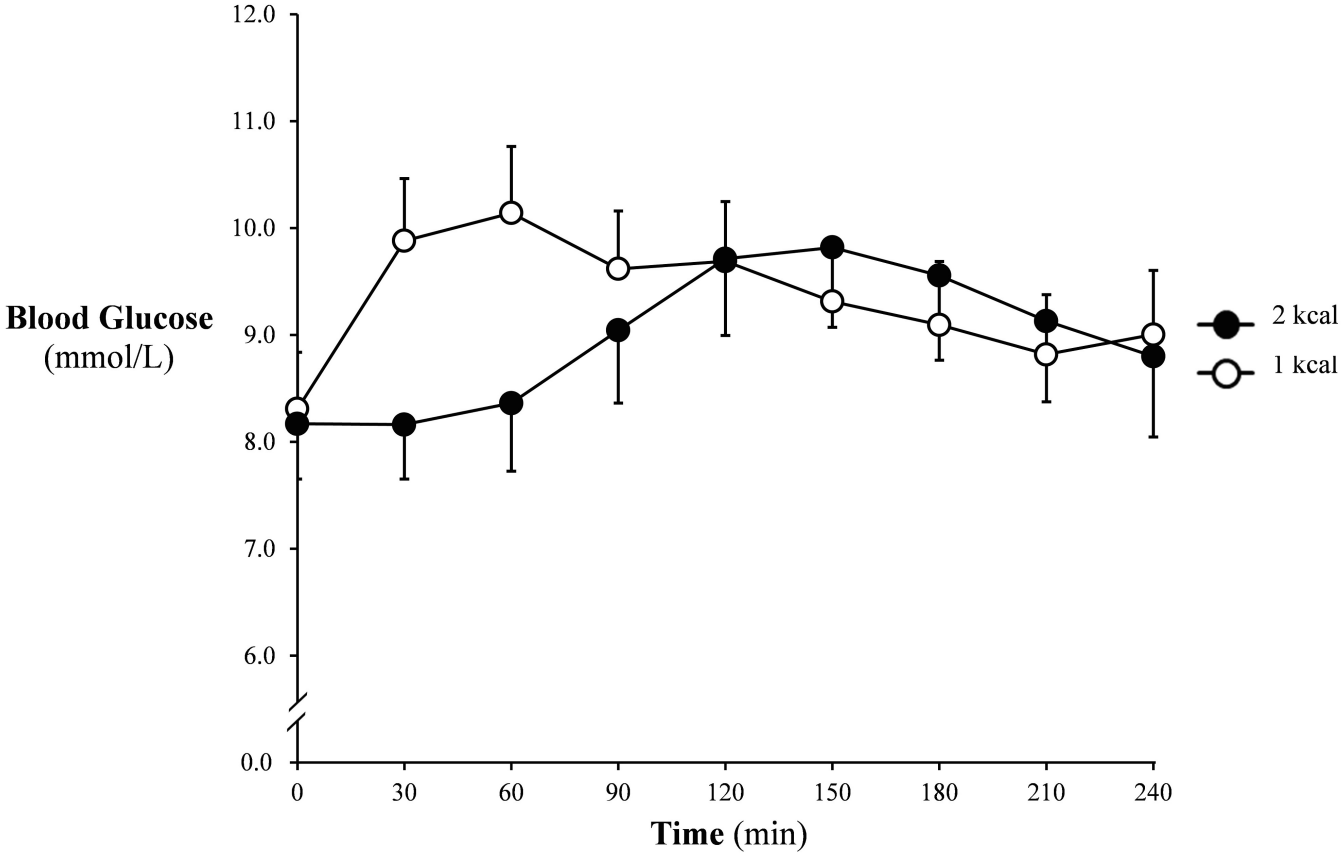

Figure 2: Blood glucose concentrations: Energy dense formula (2kcal/ml) vs. Standard (1kcal/ml). There was no difference in baseline blood glucose (8.4 (0.5) vs. 8.3 (0.5) mmol/l; P=0.90), glucose concentration at T=60 (8.6 (0.7) vs. 10.1 (0.6) mmol/l, 0=0.11) or glycemic excursion during the postprandial period. Glucose AUC240 2209 (158) vs. 2256 (128) mmol/l.240min; P=0.81. Data = Mean (SE).

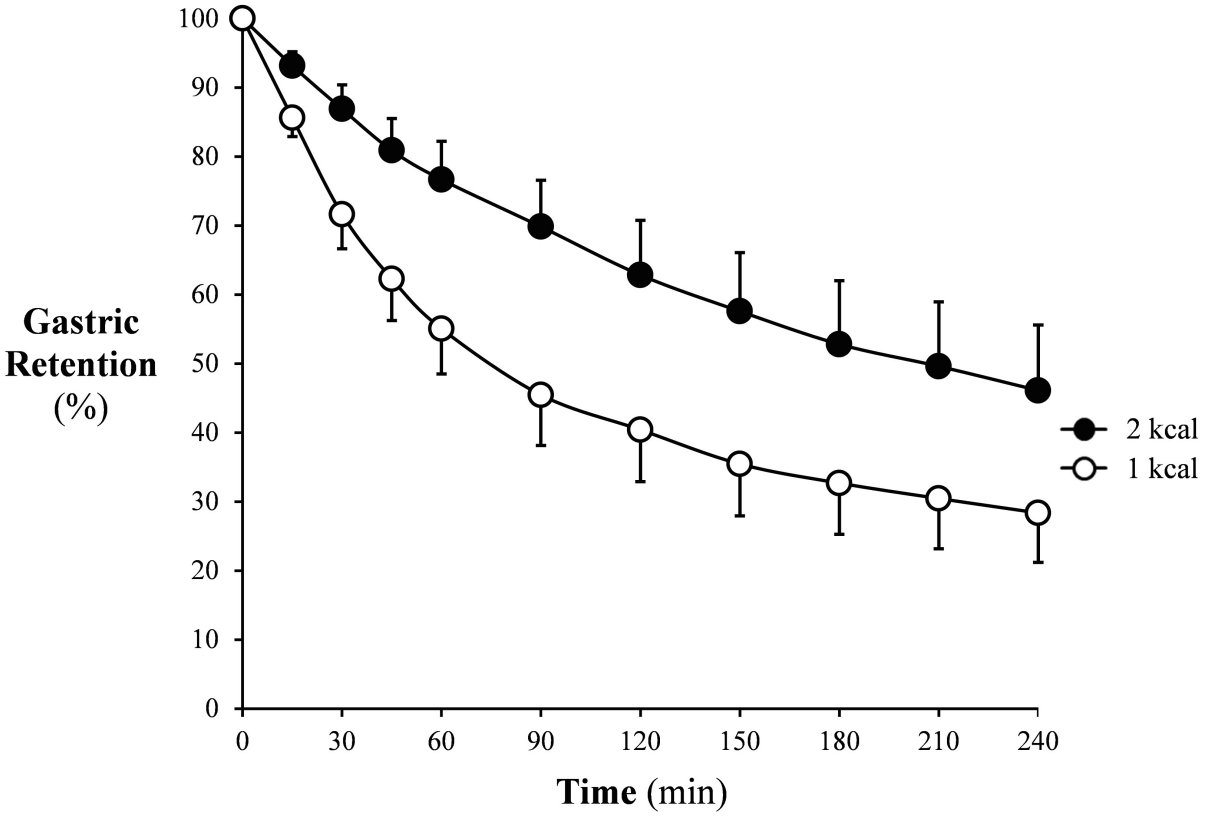

Figure 3: Gastric Retention. Gastric retention (%) of test meal (100ml) over 240 mins measured with scintigraphy in patients receiving energy dense formula (filled circles) and 1kcal/ml feed (open circles). Gastric retention AUC240 15,861 (1,616) vs. 11,240 (1,517) %.240min; P=0.045. Data = Mean (SE).

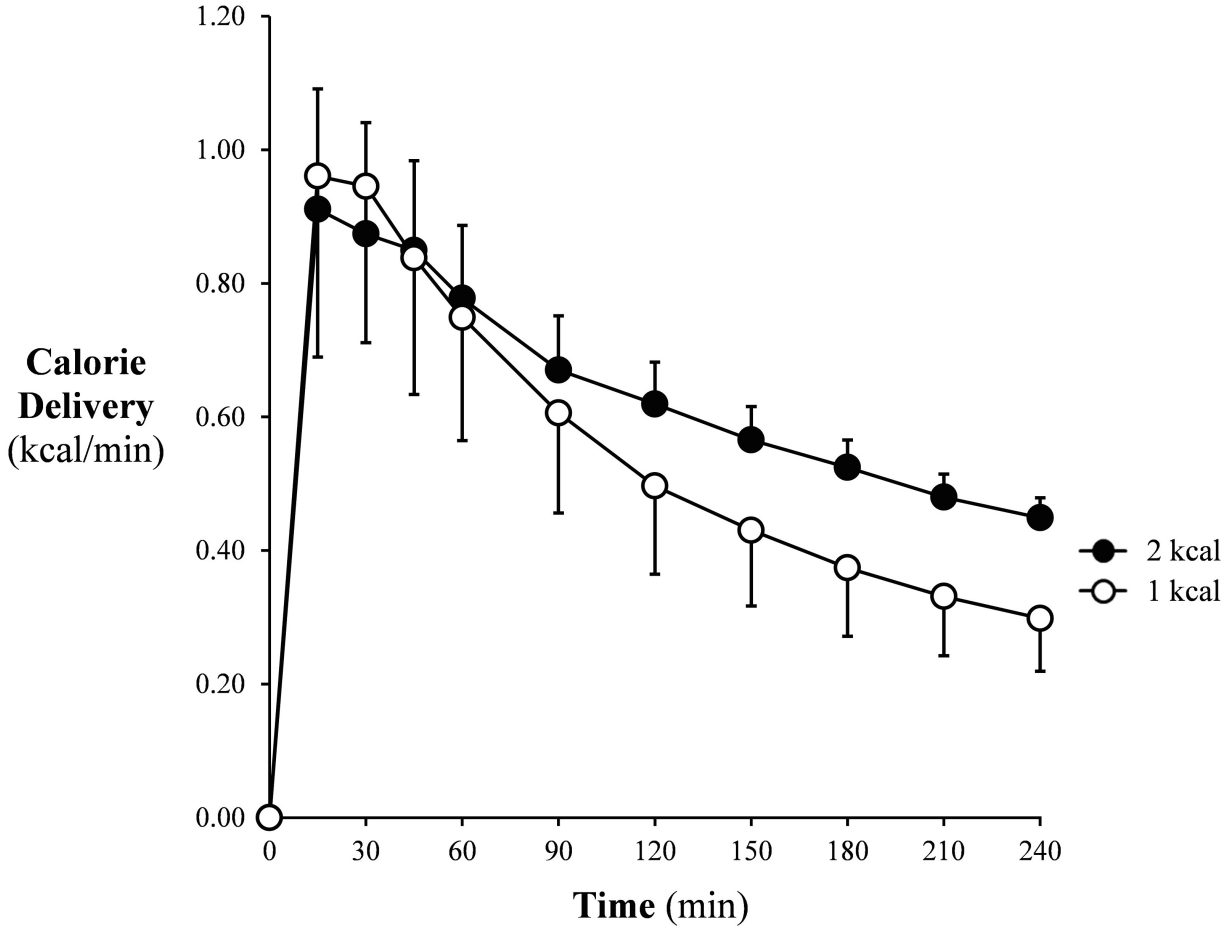

Figure 4: Calories delivered. The amount of calories per unit time were similar in patients receiving energy dense formulae (filled circles) and standard feeds (open circles). Calorie delivery AUC240 150 (32) vs. 130 (17) kcal/min.240min; P=0.57. Data = Mean (SE).

1.4 CONCLUSIONS

1.4.1 *Introduction*

Given the prevalence, associations with adverse outcomes and controversy surrounding the measurement of gastric dysmotility in the critically ill, and the use of energy dense formula as a treatment strategy for these patients, it was important that both these topics were investigated.

1.4.2 *Contribution of the work described in this thesis to the understanding of gastric dysmotility and its measurement in the critical care setting*

Enteral nutrition is part of standard care for patients admitted to the Intensive Care Unit [1]. As a result of delayed gastric emptying, nutritional targets are often unmet during enteral feeding [2, 3]. Accordingly, the measurement of gastric emptying in this group of patients is of particular interest. The literature review reported in Chapter 1.2 provides the rationale that the choice of gastric emptying test is dictated by the needs of either the clinician or researcher. In the clinical setting, the use of surrogate, or indirect measures of gastric emptying may be more appropriate, as they are generally easy for patients to tolerate, as well as relatively simple and cost-effective to administer. Currently, the use of gastric residual volumes is widespread, but its use may not translate into improved outcomes for patients [4, 5]. In the research setting, direct tests may be more suitable given their increased precision.

1.4.3 *Contribution of the work described in this thesis to the understanding of the effect of high calorie, energy dense feeds on gastric emptying in the critically ill patient*

Energy dense formulae are commonly administered to critically ill patients, with observational data suggesting that up to one-third of patients are being prescribed this type of nutrition [6]. The study presented in Chapter 1.3 is the first to evaluate the effect of energy dense formula (2 kcal/ml) on gastric emptying in the critical care setting using a direct technique (scintigraphy). The data suggest that administration of energy dense formula results in a greater volume of ‘meal’ being retained in the stomach. The amount of calories per unit time that entered the small intestine was similar, contrary to the concept that the rate of nutrient delivery to the small intestine will be increased with this approach. When compared to standard feeds, energy dense formulae contain a greater percentage of fat concentrations, which may contribute to

slower gastric emptying. In health, the interaction of nutrient in the small intestine stimulates feedback mechanisms, thereby, slowing gastric emptying via neural and hormonal mediators. These mechanisms appear to be potentiated in critically ill patients. Given the widespread use of energy dense feeds, clinicians should be vigilant for indicators of delayed gastric emptying, when prescribing energy-dense formulae, in critically ill patients

1.5 FUTURE DIRECTIONS

1.5.1 *Prospective trials to determine the effect of high calorie, energy dense feeds on gastric emptying in the critically ill patient*

The findings reported in chapter 1.3 should be viewed as hypothesis generating. Retrospective cohort studies have inherent limitations, with the possibility of bias and confounders affecting the results, however the study provides important preliminary data to indicate that administration of energy dense feeds affect gastric emptying, which, ironically, is the condition that clinicians are usually trying to treat when they prescribe these formulae. This novel finding can be explained physiologically and is highly clinically relevant given the use and importance of enteral feeding in the critical care setting. Based on these findings, a prospective cross over study comparing energy dense formula to standard feeds in critically ill patients is warranted.

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Chapter 2:

Glycaemia in the critical ill patient

2.1 INTRODUCTION

Critically ill patients frequently have co-existing type 2 diabetes. In these patients glycaemic control often deteriorates. This is usually attributable due to the pathophysiological response to acute illness and/or the treatments involved. Despite the prevalence of type 2 diabetes increasing worldwide, the optimal management of glycaemia in this group of patients during critical illness is contentious. Hyperglycaemia also frequently occurs in the critically ill without pre-existing diabetes and is associated with adverse outcomes, including increased mortality. Current guidelines recommend the treatment of hyperglycaemia with the administration of insulin to target blood glucose concentrations < 10 mmol/l, which *per se* increases the risks of both hypoglycaemia and glycaemic variability. Defining the ideal glycaemic targets and optimal management of altered glycaemia has the potential to improve patient outcomes.

Chapter 2.2 contains a narrative review that focuses on critically ill patients with type 2 diabetes; specifically, the prevalence of type 2 diabetes in the critically ill, as well as the evidence for harm of using the standard approach for management of glycaemia and the potential rationale for 'personalised' therapy in this group. The precision of prevalence estimations is limited, however, as some patients will be unaware of their diagnosis. The prevalence of known diabetes in hospitalised patients and patients admitted to Intensive Care Units is described, as well as the estimated prevalence of unrecognised diabetes in these groups. Additionally, the rationale for harm from hyperglycaemia, hypoglycaemia and glycaemic variability is also reviewed. Finally, the rationale for personalised therapy is discussed briefly, which is underpinned by recent observational data which indicates that previous chronic hyperglycaemia may be protective against acute hyperglycaemia during acute illness.

The management of glycaemia in patients with type 2 diabetes in the Intensive Care Unit is often challenging and not without risk. Insulin induced hypoglycaemia and glycaemic variability are both associated with adverse outcomes in the critically ill.

Increasing observational data suggests critically ill patients with pre-existing diabetes are more tolerant of acute hyperglycaemia, particularly those with chronic hyperglycemia, as evident by increased concentrations of glycated haemoglobin on admission – such that there are associations of increased survival benefit with mean blood glucose concentrations > 10 mmol/L. The objective of the study detailed in Chapter 2.3 was to determine whether a more liberal glucose concentration target in critically ill patients with pre-existing type 2 diabetes attenuated hypoglycemia and glycemic variability. Secondary objectives were to evaluate the biologic effects of liberal glucose targets, as assessed by plasma concentrations of recognised biomarkers of inflammation, glucose turnover, and oxidative stress.

The study described in Chapter 2.4 evaluates the use of a potential novel treatment for hyperglycaemia in the critically ill. The 'incretin' effect describes the greater insulinotropic effect to an oral/enteral glucose load compared with an intravenous glucose load that results in a comparable blood glucose response. The 'incretin effect' is mediated by two hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are secreted from the small intestine in response to nutrient exposure. In health, ambulant patients with diabetes and the critically ill, GLP-1 has been shown to stimulate insulin, suppress glucagon secretion and slow gastric emptying. GIP is also insulinotropic, however in contrast to GLP-1, at least in health, it stimulates glucagon secretion and, if anything, accelerates gastric emptying. In type 2 diabetes, the insulotropic effect of GIP is markedly attenuated, at least in part as a result of hyperglycaemia. However, GIP has never been evaluated in the critically ill. The objective of this study was to determine the effects of exogenous GIP on glycaemia, gastric emptying, glucose absorption, and insulin secretion during enteral nutrition in patients with acute critical illness-associated hyperglycaemia.

2.1.1 *Objectives*

The objectives of the literature review and studies included in this chapter were to (i) describe the prevalence of type 2 diabetes in the critically ill, as well as the potential deleterious effect of the 'standard' approach to glycaemic control and the potential rationale for 'personalised' therapy in this group (ii) evaluate the effect of more liberal glycaemic targets when compared to standard care in critically ill patients with

type 2 diabetes and (iii) determine the acute effects of GIP as a potential approach to the management of hyperglycaemia in critically ill patients without pre-existing diabetes.

2.2 LITERATURE REVIEW

Management of critically ill patients with type 2 diabetes: the need for personalised therapy

Statement of Authorship

Title of paper	Management of critically ill patients with type 2 diabetes: the need for personalised therapy
Publication status	Published
Publication details	Kar P, Jones KL, Horowitz M, Deane AM. **Management of critically ill patients with type 2 diabetes: the need for personalised therapy,** World Journal of Diabetes. 2015 Jun; 10;6(5):693-706

Principal Author

Name of Principal Author (Candidate)	Dr Palash Kar		
Contribution to paper	Conception and design of manuscript, acquiring and interpretation of data and drafting and revising the manuscript for final submission		
Overall percentage (%)	85		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/07/15

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
- The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Karen Jones		
Contribution to paper	Evaluated and edited the manuscript		
Signature		Date	01/07/15

Name of co-author	Professor Michael Horowitz		
Contribution to paper	Evaluated and edited the manuscript		
Signature		Date	11/07/15

Name of co-author	Associate Professor Adam Deane		
Contribution to paper	Conceptualisation of work, supervision and manuscript evaluation		
Signature		Date	01/07/15

Full title

Management of critically ill patients with type 2 diabetes: the need for personalised therapy

Running title

Managing ICU patients with type 2 diabetes

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Author contributions

PK was involved in conception and design of manuscript, acquiring and interpretation of data and drafting and revising the manuscript for final submission. KLJ and MH co-supervised PK and were involved in conception, design and coordination of the manuscript along with drafting and revising the manuscript. AMD supervised PK, and was involved in conception and design of manuscript, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission. All authors read and approved the final manuscript.

Keywords

Diabetes; Critically ill; Intensive Care; Management; Personalised therapy

Core tip

With diabetes increasing in prevalence, the optimal management of glycaemia in critically ill patients with pre-existing diabetes remains unknown. Recent data has highlighted therapeutic uncertainties specific to these patients with suggestions that targeted blood glucose concentrations may benefit from consideration of a patient's premorbid glucose state. In patients with uncontrolled type 2 diabetes, it may be safer to target blood glucose concentrations between 10-14 mmol/l, however definitive studies of critically ill patients with poorly controlled diabetes are required. In contrast, in patients with CIAH, or those with well-controlled diabetes (HbA1c < 7.0) have data supporting a more conservative target (6-10 mmol/l).

Conflict-of-interest statement

The authors declare there are no non-financial competing interests. M.H. has participated in advisory boards and/or symposia for Novo/Nordisk, Sanofi-aventis, Novartis, Eli-Lily, Boehringer Ingelheim, AstraZeneca, Satiogen and Meyer Nutraceuticals

Abstract

Critical illness in patients with pre-existing diabetes frequently causes deterioration in glycaemic control. Despite the prevalence of diabetes in patients admitted to hospital and intensive care units, the ideal management of hyperglycaemia in these groups is uncertain. There are data that suggest that acute hyperglycaemia in critically ill patients without diabetes is associated with increased mortality and morbidity. Exogenous insulin to keep blood glucose concentrations < 10 mmol/l is accepted as standard of care in this group. However, preliminary data have recently been reported that suggest that chronic hyperglycaemia may result in conditioning, which protects these patients against damage mediated by acute hyperglycaemia. Furthermore, acute glucose-lowering to < 10 mmol/l in patients with diabetes with inadequate glycaemic control prior to their critical illness appears to have the capacity to cause harm. This review focuses on glycaemic control in critically ill patients with type 2 diabetes, the potential for harm from glucose-lowering and the rationale for personalised therapy.

Introduction

Patients with diabetes mellitus may develop an acute severe illness that necessitates a level of care that can only be provided within an Intensive Care Unit (ICU) [1]. In the majority of critically ill patients with pre-existing diabetes, the pathophysiological response to the acute illness or injury, and/or the treatments involved, may lead to deterioration in glycaemic control. Despite the high and increasing prevalence of diabetes (both within the community and in the critically ill), the optimal management of glycaemia in critically ill patients with pre-existing diabetes remains unknown. However, recent data has highlighted the therapeutic uncertainties specific to these patients.

The majority of critically ill patients with diabetes have type 2 diabetes [2]. The limited information relating to patients with type 1 diabetes precludes speculation as to whether management of glycaemia in this group should be different from that in type 2 diabetes. Accordingly, this review focuses on critically ill patients with type 2 diabetes addressing issues including prevalence, potential rationale for harm and evidence for personalised therapy.

Prevalence

In the community type 2 diabetes occurs frequently with global health expenditure estimated at US $376 billion in 2010, which is expected to rise to US $490 billion by 2030 due to increasing prevalence [3, 4]. In Australia it is estimated over the last 15 years, the prevalence has increased from 8.5 % to 12.0% [5]. There is a substantial variation in the prevalence of diabetes between countries, peaking in Nauru (31%) [6]. Factors relating to the increase in prevalence include increasing obesity, increasing age and racial region. A limitation in estimating prevalence is that many patients remain unaware of their diagnosis. For example, the estimated prevalence in the USA is 13% of the population, of which 40% is unrecognised or undiagnosed [7].

Diagnosis of diabetes

The prevalence of recognised and unrecognised diabetes varies according to the definitions used, as well as the location and the populations studied. The current diagnostic criteria used by the American Diabetes Association (ADA) involves one of the following; an HbA1c $\geq$ 6.5, a fasting glucose $\geq$ 7 mmol/L, a 2 hour post glucose

tolerance test following a 75 gram oral glucose load of ≥ 11.1 mmol/L, or a random blood glucose ≥ 11.1 mmol/L with symptoms of hyperglycaemia [8]. These criteria were ratified by the World Health Organization (WHO) in 2011 [9].

Given each test (HbA1c, fasting, postprandial or random blood glucose) reflects different physiological phenomena, different populations may be diagnosed when using each criterion [10, 11]. Each diagnostic test has advantages and disadvantages. Both the fasting glucose and 2 hour post glucose tolerance test are established standards, relatively rapid and easy to perform, and predict microvascular complications. However, these tests are subject to day-to-day variability, require patients to fast and only reflect glucose homeostasis at a single point in time [12]. HbA1c is convenient (with no fasting required), can predict microvascular complications, is a better predictor of macrovascular disease (than fasting glucose or 2 hour post glucose tolerance test) and has low day-to-day variability [8, 12]. Additionally, as the physiological responses to acute illness cause deterioration in glycaemia, estimating glucose control prior to the acute illness – using markers such as HbA1c – to accurately determine which patients have unrecognised diabetes and which patients have 'stress hyperglycaemia' is possible [13]. Weaknesses include variations amongst ethnic groups and age, may be misrepresentative in certain medical conditions (such as certain forms of anaemia and haemoglobinopathies) and the need for a validated, standardised assay [12].

Prevalence of diabetes in hospitalised patients

Compared to the general population, the prevalence of diabetes in hospitalised adult patients (i.e. admitted to general wards) is considered to be greater. Depending on the population, estimates range from between 11-35% of all patients (Table 1).

Numerous studies in the critically ill have evaluated the prevalence of glucose intolerance (Table 1). However, a limitation of the studies reported is that investigators were unable to identify those patients who had so-called 'stress hyperglycaemia' (or critical illness associated hyperglycaemia (CIAH) - the condition of acute glucose intolerance that is confined to the period of critical illness) and those who have unrecognised diabetes. Several studies use either fasting blood glucose (≥ 7

mmol/L) and/or random glucose concentrations (≥ 11.1 mmol/L) for diagnosis of diabetes [14-16].

Investigators have also measured glycated haemoglobin (HbA1c) on admission to identify hospitalised patients with unrecognised diabetes. A prospective observational study of 695 patients in Boston, Massachusetts [17], selected a cutoff HbA1c of > 6.5% to diagnose diabetes, with 19% of patients having diabetes previously diagnosed and 5% having undiagnosed diabetes. Another study of 971 patients admitted to the general medical ward of an urban hospital located in the Bronx, New York [18] - which may be assumed to admit a larger cohort of lower-income patients - 35% were known to have diabetes, and 16% undiagnosed diabetes, using an HbA1c ≥ 6.5.

In summary, the prevalence of diabetes in hospitalised patients varies according to geography. In the developed world, diabetes is more prevalent amongst lower socioeconomic groups [19-21]. Furthermore, diabetes is a risk factor for certain diseases (e.g. cardiovascular disease) and prevalence will be greater if a specific population (e.g. patients presenting with myocardial ischaemia) is studied [22].

Prevalence of diabetes in patients admitted to intensive care units

The prevalence of diabetes in patients admitted to the ICU is estimated to be between 12-40% (Table 2). Similar to the prevalence in hospitalised patients, the wide range reflects the definitions used and the population studied. Multiple single centre observational studies from the USA [23-25] report prevalence between 13 and 21%, therefore it is likely that the true prevalence is close to this range. More recently, Falciglia and colleagues undertook a retrospective cohort study across 173 ICUs in the USA and reported that 30% of the 259 040 patients had a history of diabetes according to ICD-9 codes [26].

A single centre, observational study from London, UK [27], found 16% of patients had a history of diabetes. A retrospective observational study of 4 946 patients admitted to one of two hospitals in Melbourne and Sydney, Australia [28], reported 15% had diabetes. While a single, mixed medical/surgical ICU from Amsterdam, The Netherlands [29], found 12% of 5 961 patients admitted had a history of diabetes.

These data indicate that the prevalence in other developed countries may be similar to, or slightly less than, the USA.

Data from international studies are consistent with this concept. Stegenga and colleagues utilised data collected as part of a randomised interventional study [30] to evaluate whether diabetes affects the outcome of sepsis in patients admitted to one of 164 ICUs across 11 countries [31] and reported that 23% had pre-existing diabetes. In retrospective observational data derived from 44 964 patients admitted to one of 23 ICUs worldwide [32], 29% had a history of diabetes documented in their medical records, but the prevalence varied substantially according to geography. For example, in an ICU from Geelong, Australia, the prevalence was 14%, while in a hospital <100 km away (Melbourne) it was 24%, whereas patients admitted to Tampa Bay, USA, the prevalence was 39%.

The prevalence of diabetes in the critically ill varies across studies. Multiple observational studies estimate the prevalence at 12-30% [23-29, 31-35]. However, these studies have significant limitations. Most importantly, the prevalence may be under represented due to diabetes that is either unrecognised or not documented.

A number of interventional studies have also reported diabetes prevalence in ICU patients (Table 2). Two prospective, randomised, controlled studies of surgical and medical ICU patients admitted into the ICU in Leuven, Belgium, compared an intensive insulin therapy (ITT, blood glucose level 4.4-6.1 mmol/L) versus conventional treatment (insulin started if the blood glucose was > 12 mmol/L and maintained between 10-11.1 mmol/L)[36, 37]. These studies reported diabetes at 13% and 17% respectively.

Other interventional studies include single centre [38, 39] and mutlicentre trials [40-42], with the largest being in 2009, the NICE-SUGAR (Normoglycaemia in Intensive Care Evaluation–Survival Using Glucose Algorithm Regulation) study. This was conducted across 42 ICUs throughout Australia, New Zealand and Canada [41], and noted 20% of its 6 029 patients with a history of diabetes, with the majority (92%) having type 2 diabetes.

It should be recognised that there are limitations to using data from these interventional studies. Inclusion into these studies usually requires hyperglycaemia and therefore leads to selection bias, which artificially increases any estimate of prevalence. The interventional trials estimated ICU prevalence at 13-40% [36-42].

Prevalence of unrecognised diabetes

Patients may have diabetes that is unrecognised prior to admission [2]. This may not represent 'stress hyperglycaemia' or CIAH – as the hyperglycaemia is chronic rather than acute. Unrecognised diabetes is important as it not only impacts on estimations for the actual prevalence of the condition, but, as a growing body of evidence suggests, chronic glucose control may have implications on optimal acute glucose ranges in the critically ill.

Hospital and ICU prevalence of unrecognised diabetes can be estimated from the studies mentioned (Table 1 and 2) along with other studies cited below (Table 3). Hospital prevalence is estimated to be between 5-16% [16-18, 43] and ICU prevalence between 6-14% [34, 44]. The prevalence in patients with ischaemic heart disease (e.g. presenting with acute myocardial infarction) appears to be higher [45, 46].

In two European studies, patients with an acute myocardial infarct and without a history of diabetes subsequently underwent an oral glucose tolerance test (OGTT) to diagnose diabetes [45, 46]. The prevalence of diabetes was found to be over 30% at discharge, and between 25-31% at 3 months. In London (UK), Emergency Department patients were screened for diabetes via fasting blood glucose [47] and it was reported that 3% patients had unrecognised diabetes.

We recently performed a single centre observational study in a mixed medical/surgical ICU in Adelaide, Australia, and separated patients with diabetes (either known or unrecognised) and CIAH using HbA1c to accurately estimate the prevalence of each condition [34]. Of 1 000 consecutively admitted ICU patients, 22% had known diabetes (5% were type 1) and 6% had unrecognised diabetes (HbA1c $\geq$ 6.5%). The absence of previously diagnosed diabetes was confirmed by a phone call to the patient's usual local medical officer (general practitioner).

Subsequently, Hoang and colleagues also estimated the prevalence of undiagnosed diabetes in a prospective, observational study in a single medical ICU [44]. All patients with hyperglycaemia and those with known diabetes underwent measurement of HbA1c with diabetes defined as an HbA1c ≥ 6.5%. 66% of the 299 patients enrolled into the study had a history of diabetes. Of the remaining 102 hyperglycaemic patients without diabetes, 14% had an HbA1c ≥ 6.5%.

In summary the prevalence of undiagnosed diabetes is difficult to determine, and as previously noted, depends on the definitions used and the location of the patient population. Current 'best estimate', albeit on limited data from single centres, suggest that the prevalence of undiagnosed diabetes is either similar to, or slightly greater than, the background prevalence in the community.

Rationale for harm from hyperglycaemia, hypoglycaemia and glycaemic variability

Hyperglycaemia

Hyperglycaemia in type 2 diabetes reflects the outcome of factors affecting both insulin secretion, with β-cell dysfunction resulting in a relative insulin deficiency, and insulin resistance as a result of both environmental and genetic factors [48, 49]. However, the pathogenesis of hyperglycaemia in the critically ill patient, either with CIAH, or in those with pre-existing diabetes and experiencing a deterioration in their glucose control, is complex and poorly understood [2]. Patient predisposition (including insulin resistance and β-cell function), the underlying illness (which can result in catecholamine release, stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, and the release of inflammatory cytokines) and the management involved (including glucocorticoids, vasopressors and nutrition) appear to be of major relevance [1].

The activation of the HPA axis and the sympathetic system cause the 'stress' response. In the majority of patients 'stress' hormones (including cortisol and catecholamines) markedly increase. In addition, the underlying illness may stimulate the production of cytokines (such as TNF-α, IL-1 and IL-6)[1, 50]. These three components (HPA axis, sympathetic system and cytokine release) lead to excessive

gluconeogenesis, glycogenolysis and insulin resistance, thereby augmenting stress hyperglycaemia [50]. Glucagon is the major modulator of gluconeogenesis and may be stimulated by TNF- α, however cortisol and adrenaline (epinephrine) are also likely to contribute [1, 51, 52].

Insulin resistance is thought to occur due to a number of pathways. Glucose enters cells via plasma membrane glucose transporters (GLUTs), which are down regulated in times of stress, possibly due to the presence of TNF-α and IL-1 [50]. Diminished glucose uptake by peripheral tissue may occur due to high cortisol and adrenaline (epinephrine) concentrations [1, 53]. As discussed, acute illness results in increased level of cytokines, which exacerbates hyperglycaemia and stimulates inflammation and oxidative stress [1].

It should be considered that acute hyperglycaemia may represent a 'protective' physiological response of the host during periods of stress [50]. An acute rise in glycaemia may facilitate glucose delivery at critical times and promote anti-apoptotic pathways, protecting against cell death [50]. While uncontrolled acute hyperglycaemia is clearly harmful, the threshold at which harm occurs in the critically ill patient remains to be determined [2]. The majority of studies that have evaluated this issue have enrolled heterogenous cohorts - and patients with diabetes only comprised a small proportion of the sample evaluated. Based on recent data it is increasingly likely that the glucose threshold in a patient with diabetes, particularly those with chronic hyperglycaemia, will differ from that in a patient who is naïve to hyperglycaemia. A patient with poorly controlled diabetes, i.e. with a history of high blood glucose levels and consequently high HbA1c, will be more tolerant of hyperglycaemia but susceptible to the adverse effects of hypoglycaemia (see below), such that the thresholds for both variables are greater than a patient who is naïve to hyperglycaemia – either those with well controlled diabetes or those with CIAH.

Multiple studies have examined the effects of hyperglycaemia on morbidity and mortality in the ICU population with inconsistent and controversial outcomes. Moreover, the majority of these studies have not categorised patients into those with chronic hyperglycaemia or acute glucose intolerance.

There are numerous observational studies (Table 4). In 2005, a case controlled study of 7 285 ICU patients reported that in individuals without known diabetes, mortality was increased when blood glucose levels were > 8 mmol/L but this signal was absent in patients with diabetes [35]. Overall, mortality was significantly greater in patients without diabetes when compared to patients with diabetes. A retrospective study of 2 713 patients admitted into ICU [23] reported an association between mortality and hyperglycaemia in patients without a history of diabetes in the cardiac, cardiothoracic, and neurosurgical intensive care units. In an audit of 5 365 ICU patients evaluated before and after implementation of an intensive glucose control policy [24], mortality was increased in patients with hyperglycaemia who were not known to have diabetes when compared to those with diabetes. In 2008, Egi and colleagues reported a retrospective study of 4 946 patients in which ICU mortality increased with increasing mean blood glucose level in patients without diabetes but this signal of harm was absent in those with pre-existing diabetes [28].

A retrospective cohort study of 259 040 ICU admissions also reported an association between mortality and hyperglycaemia, with the relationship far stronger in patients without a diagnosis of diabetes when compared to those with pre-existing diabetes [26]. A retrospective analysis of a previous study [30] included 830 patients admitted with severe sepsis (defined as sepsis associated with acute organ dysfunction) [31], and reported that hyperglycaemia was predictive of subsequent death in those patients not known to have diabetes. Additionally, a multicentre retrospective study of 44 964 patients divided into 2 cohorts (with and without known diabetes) [32], reported increased mortality with higher mean blood glucose concentrations (≥ 7.8 mmol/L) when compared to blood glucose concentrations 4.4-7.8 mmol/L in patients without diabetes. In contrast, patients with diabetes were more likely to die when mean blood glucose concentrations were between 4.4-6.1 mmol/L when compared to patients with greater blood glucose concentrations (6.2-10 mmol/L).

A number of interventional studies have evaluated the relationship between chronic and acute hyperglycaemia and outcomes (Table 5). In a pooled analysis of studies conducted in a single centre in Leuven, intensive insulin therapy (ITT, aiming for blood glucose concentrations between 4.4-6.1 mmol/l) was reported to reduce mortality and morbidity in patients without a diagnosis of diabetes, but this was not

the case in patients with diabetes, if anything, there was a trend for harm with intensive insulin therapy in patients with diabetes such that mortality was non-significantly greater at a lower mean blood glucose range (< 6.1 mmol/L, 26.2% vs. 6.1-8.3 mmol/L, 21.2% vs. >8.3mmol/L, 21.6%, P=0.4) [54].

Subsequently, a number of interventional, randomised, controlled trials, containing patients with diabetes, comparing ITT to more conventional glucose targets have been published [38-42]. A trial of 523 mixed (medical and surgical) ICU patients [39] reported no survival benefit in patients with diabetes with ITT, but ITT was associated with an increased prevalence of hypoglycaemia. The Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) study assigned 537 ICU patients with severe sepsis to either ITT or more conventional glucose targets while receiving either 10% pentastarch or a modified Ringers lactate in a two-by-two factorial study [38]. The study was suspended at interim analysis for safety reasons with ITT being associated with increases in episodes of severe hypoglycaemia and adverse events. De La Rosa and colleagues also evaluated ITT in 504 ICU patients [42] (61 with diabetes) and there was no mortality or morbidity benefit observed, but an associated increased risk of hypoglycaemia, when administering ITT.

In 2009, the NICE-SUGAR study compared ITT with conventional glucose control in 6 029 ICU patients and established that the observations from the initial Leuven studies regarding ITT were not generalisable outside that specialised institution [41]. However, amongst the 1 211 patients with pre-existing diabetes in the NICE-SUGAR study the administration of ITT did not appear more harmful than in patients without diabetes. The Glucontrol study [40], an international, multicentre trial involving over 1 000 ICU patients was stopped early due to protocol violations, and it was, accordingly, underpowered. However, there was no evidence to suggest any benefit with ITT and data in patients with diabetes were not specifically described.

Recently a number of studies have attempted to measure chronic glycaemia as a dynamic (HbA1c), rather than a binary, variable (i.e. presence of diabetes - yes/no) (Table 6). Egi and colleagues performed a retrospective observational study of 415 patients with diabetes (from two Australian ICUs) in whom glycated haemoglobin (HbA1c) had been measured within 3 months of their critical illness and evaluated

how this measure of pre-existing glycaemia impacted on the interaction between acute glycaemia and mortality [55]. It was reported that in patients with elevated preadmission HbA1c levels (> 7%) the number of deaths were significantly fewer when blood glucose concentrations were > 10mmol/L.

Consistent with this observation, we recently measured HbA1c on admission and glucose concentrations for the first 48 hours of ICU admission [34] and observed that acute peak glucose concentrations were associated with increased mortality only in patients with adequate premorbid glycaemic control (defined as HbA1c < 7%), but not in patients with chronic hyperglycaemia (defined as an HbA1c ≥ 7%). This finding was also supported by Hoang and colleagues [44] who assessed the prevalence of undiagnosed diabetes (i.e. HbA1c ≥ 6.5%) among those with hyperglycaemia in a medical ICU. Patients with an HbA1c ≥ 6.5% were found to have significantly lower mortality compared to those with an HbA1c < 6.5% (11.7% vs 19.3%, P=0.038), despite having greater glucose concentrations.

In summary the outcomes of the largest and most generalisable randomised study are consistent with the concept that the optimal glucose concentrations in unselected critically ill patients are between 6-10 mmol/l [41]. However, observational data, post-hoc analysis of interventional studies and studies measuring chronic glycaemia as a dynamic variable suggest that patients with pre-existing diabetes may warrant higher targets. Indeed, there is increasing data suggesting that targets should be personalised depending on both diabetic status and recent glycaemic control.

Hypoglycaemia

In most cases, treatment of hyperglycaemia in the critically ill involves the use of insulin, which is associated with increased risks of both hypoglycaemia and glycaemic variability [56]. The severity of illness may also result in a hypoglycaemia and therefore it is important to be circumspect when attributing mortality to hypoglycaemia [57]. Additionally, hypoglycaemia may have adverse biological effects including an increase in systemic inflammatory response, impairment of the sympathetic nervous system, inhibition of the biological response to stress, along with cerebral vasodilation and neural damage [2, 58]. Experimentally, the use of insulin and consequent hypoglycaemia may be associated with hypotension, vasodilation, and

reduced autonomic responses to subsequent hypoglycaemic episodes [58]. Furthermore, critically ill patients may be more prone to the effects of hypoglycaemia itself, which may include cardiac arrest, seizure and coma [59].

Studies examining the effects of hypoglycaemia in critically ill patients with pre-existing diabetes are limited. Interventional studies describing this relationship have been summarised (Table 6). Of note, post hoc analysis of the NICE-SUGAR data indicate that intensive insulin therapy increases episodes of moderate (2.3-3.9 mmol/L) and severe (≤ 2.2 mmol/L) hypoglycaemia, both of which are associated with increased risk of death [56]. This relationship was similar among patients with and without a diagnosis of diabetes.

In addition to these studies, there are a number of observational studies that have evaluated this association (Table 7). A retrospective database review of 408 ICU patients (102 index cases, 306 controls) published in 2007 [60] reported that a history of diabetes was associated with severe hypoglycaemia and that a single hypoglycaemic episode was associated with an increased risk of mortality (compared with those without an episode of severe hypoglycaemia). Egi and colleagues [61] reported mild or moderate hypoglycaemia was associated with mortality in critically ill patients - with mortality substantially increasing according to severity of hypoglycaemia – and patients with diabetes were more likely to suffer from insulin-associated hypoglycaemia.

The blood glucose threshold that adverse events occur may be greater in patients with pre-existing diabetes. In a retrospective multi-centre observational study [32] increased mortality was reported in 12 880 patients with pre-existing diabetes who had mean glucose concentrations between 4.4-6.2 mmol/L. While the investigators were not able to differentiate between patients with well-controlled or poorly-controlled diabetes, these data support the concept that the threshold for ‘hypoglycaemia’ may be increased in critically ill patients with diabetes when compared to non diabetic patients. For example, if a patient typically has blood glucose concentrations above 10 mmol/L, and, in hospital, insulin is administered to achieve blood glucose concentration of ≈ 6 mmol/L, this may result in a ‘relative’ hypoglycaemia.

Glycaemic Variability

Glycaemic variability (GV) describes the fluctuations in blood glucose concentrations, as marked fluctuations may be associated with multiple adverse effects such as apoptosis, cytokine production and increased markers of oxidative stress [59]. Oxidative stress markers have been shown to increase with glucose fluctuations [62, 63]. GV may be assessed by a number of methods. Techniques to quantify variability are reviewed elsewhere [64].

Multiple studies in the critically ill have established as an association with poor outcomes and GV [44, 65-71], however the evidence in patients with pre-existing diabetes is limited and inconsistent (Table 8). In 2006, Egi and colleagues published a retrospective, electronic database analysis of 7 049 ICU patients in 4 centres around Australia, using standard deviation as a marker of glucose variability, and focusing on the association of blood glucose variability and mortality [65]. Both mean and standard deviation of blood glucose were independently associated with mortality.

A retrospective, single center cohort study of patients admitted with sepsis reported that GV was also independently associated with increased mortality and importantly, that this was independent of hypoglycaemia and the presence of diabetes [66]. Another retrospective study of 3 252 patients reported that increased GV was associated with mortality [67] and diabetes was associated with greater GV. A prospective, observational study of 42 patients used non-lineal dynamics to measure glycaemia in time series [69]. Patients underwent continuous glucose monitoring system measuring interstitial glucose concentrations every 5 minutes for 48 hours. The authors reported greater variability was associated with increasing mortality, even in patients with diabetes. However, given the small cohort, these results must be treated with caution.

Other studies have reported no relationship between mortality and GV in patients with diabetes. A retrospective, observational study of 4 084 critically ill patients (942 with known diabetes) [68] reported that GV was associated with mortality in patients without diabetes, but not in patients with diabetes. More recently in the study by Hoang and colleagues [44] of 299 patients there was no association between GV and

mortality in their entire cohort, however the group with diabetes (128 patients) had a lower rate of mortality despite having a higher GV. Additionally, a retrospective analysis of 2 782 ICU patients, comparing different GV indices and mean glucose concentrations to predict mortality and ICU acquired infections [70] reported that while GV was associated with infections and mortality in patients without pre-existing diabetes, in those with diabetes GV was greater but was not associated with either mortality or infection.

In summary, there is a strong relationship between GV and mortality in critically ill patients that has been confirmed in multiple studies. However, with respect to patients with diabetes, data are inconsistent. This may be due a number of factors, including small numbers studied resulting in lack of power, or that patients with chronic hyperglycaemia are protected somewhat by glycaemic excursions during acute illness. Research is warranted to further understand whether GV is harmful in patients with pre-existing diabetes.

Rationale for personalised therapy and that the harm from each of these domains may vary according to pre-existing physiology

Diabetes is known to be associated with a large burden of illness in the outpatient setting and is associated with increased mortality [72]. Paradoxically, as discussed, multiple studies exist suggesting that acute hyperglycaema in critically ill patients without diabetes (i.e. patients with CIAH) is associated with increased mortality and morbidity when compared to those with known diabetes [73]. There is growing evidence that chronic hyperglycaemia may lead to cellular conditioning, and that in fact, may be protective against acute hyperglycaemia mediated damage during an episode critical illness [1]. These outcomes suggest that current target glucose levels in patients naïve to hyperglycaemia, or those suffering from CIAH, may be harmful to those with chronic hyperglycaemia or poorly controlled diabetes.

Conclusion

This review articulates the need for further research to be done to identify the ideal glucose targets in critically ill patient with pre-existing diabetes. Not only does hyperglycaemia occur frequently in this group, but, recent data suggests that targeted

blood glucose concentrations may benefit from consideration of a patient's premorbid glucose state.

Our recommendations are to avoid treating patients with diabetes as a homogenous group. Treatment of the critically ill patient with type 2 diabetes should be personalised to their internal milieu. There is preliminary evidence suggesting that higher blood glucose concentrations (e.g. up to 14 mmol/l) in patients with uncontrolled type 2 diabetes may not be harmful. For this reason it may be safer to target blood glucose concentrations between 10-14 mmol/l in this group. However, definitive studies of critically ill patients with poorly controlled diabetes are required before this approach is incorporated into clinical practice. In contrast, in patients with CIAH, or those with well-controlled diabetes (HbA1c < 7.0), a more conservative target (6-10 mmol/l) is supported by considerable data.

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Tables

Table 1: Prevalence of diabetes in hospital population (chronological order)

Author	Year	R-D (%)	UR-D (%)	Total study patients	Location	Diabetes diagnosed by	Unrecognised diabetes diagnosed by
Umpierrez *et al.* [14]	2002	495 (26%)	223* (12%)	1 886	Atlanta, USA	• Admission history	• Fasting blood glucose ≥ 7 mmol/L • Random blood glucose ≥ 11.1 mmol/L x 2
Wallymahmed *et al.* [15]	2005	126 (11%)	13* (1%)	1 129	Liverpool, UK	• Admission history • Hospital records	• Random blood glucose ≥ 11.1 mmol/L
Wexler *et al*. [17]	2008	136 (19%)	33 (5%)	695	Boston, USA	• Admission history • Hospital records	• HbA1c > 6.5
Mazurek *et al.* [18]	2010	342 (35%)	152 (16%)	971	New York, USA	• Admission history • Hospital records • Medication review	• HbA1c ≥ 6.5
Feldman-Billard *et al.* [16]	2013	355 (17%)	156* (7%)	2 141	Multicentre (France)	• Admission history	• Fasting blood glucose ≥ 7 mmol/L

*May include patients with stress hyperglycaemia/critical illness associated hyperglycaemia (CIAH)

R-D = Recognised Diabetes, UR-D = Unrecognised Diabetes

Table 2: Prevalence of diabetes in the ICU population (chronological order)

Author	Year	Study type	R-D (%)	UR-D (%)	Total study patients	Location	Recognised DM Diagnosis	Unrecognised diabetes diagnosed by
Van den Berghe *et al.* [36]	2001	Interv	204 (13%)	N/A	1 548	Leuven, Belgium	• Admission history	• N/A
Finney *et al.* [27]	2003	Observ	86 (16%)	N/A	523	London, UK	• Unknown	• N/A
Whitcomb *et al.* [23]	2005	Observ	574 (21%)	395* (15%)	2 713	Baltimore, USA	• Admission history	• Hyperglycaemia without a history of DM
Van den Berghe *et al.* [37]	2006	Interv	203 (17%)	N/A	1 200	Leuven, Belgium	• Admission history	• N/A
Krinsely [24]	2006	Observ	1110 (21%)	N/A	5 365	Stamford, USA	• Hospital records (ICD-9 codes) for the first 2 years then all available info	• N/A
Egi *et al.* [28]	2008	Observ	728 (15%)	N/A	4 946	Multicentre (Australia)	• Hospital records	• N/A
Treggiari *et al.* [25]	2008	Observ	1361 (13%)	N/A	10 456	Seattle, USA	• Hospital records	• N/A
Arabi *et al.* [39]	2008	Interv	208 (40%)	N/A	523	Riyadh, Saudi Arabia	• Admission history • Hospital records	• N/A
Bronkhurst *et al.* [38]	2008	Interv	163 (30%)	N/A	537	Multicentre (Germany)	• Unknown	• N/A
Del La Rosa *et al.* [42]	2008	Interv	61 (12%)	N/A	504	Medellin, Colombia	• Admission history	• N/A
Finfer *et al.* [41]	2009	Interv	1211 (20%)	N/A	6 029	Multicentre (Australia, NZ, Canada)	• Admission history	• N/A
Preiser *et al.* [40]	2009	Interv	203 (19%)	N/A	1078	Multicentre (Europe)	• Admission history	• N/A
Falciglia *et al.* [26]	2009	Observ	77 850 (30%)	N/A	259 040	Multicentre (USA)	• Hospital records (ICD-9 codes)	• N/A
Stegenga *et al.* [31]	2010	Observ	188 (23%)	N/A	830	Multicentre (Worldwide)	• Admission history	• N/A

Hermanides *et al.* [29]	2010	Observ	699 (12%)	N/A	5 961	Amsterdam, The Netherlands	• Hospital records (computerised system)	• N/A
Krinsely *et al.* [33]	2011	Observ	669 (21%)	N/A	3 263	Multicentre (USA, Europe)	• Hospital records (ICU clinical database)	• N/A
Krinsley *et al.* [32]	2013	Observ	12 880 (29%)	N/A	44 964	Multicentre (Worldwide)	• Admission history	• N/A
Plummer *et al.* [34]	2014	Observ	220 (22%)	55 (6%)	1 000	Adelaide, Australia	• Admission history • Phone call to GP • HbA1c ≥ 6.5	• HbA1c ≥ 6.5 without a history of DM

*May include patients with stress hyperglycaemia/critical illness associated hyperglycaemia (CIAH)

Interv = Interventional, Observ = Observational, R-D = Recognised Diabetes, UR-D = Unrecognised Diabetes, NZ = New Zealand

Table 3: Prevalence of undiagnosed diabetes in the hospital population (chronological order)

Author	Year	Diagnosis	UR-D (%)	Total study patients	Location	Patient population
Norhammer *et al.* [45]	2002	OGTT	51 (31%) at discharge 36 (25%) at 3 months	164 144	Multicentre (Sweden)	• Post AMI, Hospital/ICU
George *et al.* [47]	2005	Fasting blood glucose ≥ 7 mmol/L	13 (3%)	427	London, UK	• Emergency Department
Wexler *et al*. [17]	2008	HbA1c > 6.5	33 (5%)	695	Boston, USA	• Hospital
Lankisch *et al.* [46]	2008	OGTT	31 (32%) at discharge 19 (31%) at 3 months	96 62	Wuppertal, Germany	• Post AMI, Hospital/ICU
Mazurek *et al.* [18]	2010	HbA1c ≥ 6.5	152 (16%)	971	New York, USA	• Hospital
Feldman-Billard *et al.* [16]	2013	Fasting blood glucose ≥ 7 mmol/L	156 (7%)	2141	Multicentre (France)	• Hospital
Plummer *et al.* [34]	2014	HbA1c ≥ 6.5	55 (6%)	1000	Adelaide, Australia	• ICU
Hoang *et al.* [44]	2014	HbA1c ≥ 6.5	14 (14%)	102	New Haven, USA	• Medical ICU
Ochoa *et al.* [43]	2014	HbA1c ≥ 6.5	8 (9%)	92	Abilene, USA	• Hospital

UR-D = Unrecognised Diabetes, OGTT = Oral Glucose Tolerance Test, AMI = Acute Myocardial Infarction

Table 4: Observational studies (diabetes as a binary variable) and outcomes related to hyperglycaemia (chronological order)

Author	Year	Study pts	Study point	Patients without diabetes	Patients with diabetes	Overall message
Rady *et al.* [35]	2005	7 285	Glycaemia vs hospital mortality	• Inc mortality with blood glucose > 8 mmol/L	• Inc mortality with blood glucose > 11.1 mmol/L	• Mortality inc in non diabetics (10%) compared to diabetics (6%), (P<0.01)
Whitcomb *et al.* [23]	2005	2 713	Admission hyperglycaemia (> 11.1 mmol/L) vs in-hospital mortality	• Admission hyperglycaemia associated with inc mortality in CICU, CTICU and NSICU	• Admission hyperglycaemia not associated with mortality	• Mortality inc in non diabetics (10%) compared to diabetics (5%), (P<0.05)
Krinsely [24]	2006	5 365	Pre ITT and post ITT vs hospital mortality	• Dec mortality risk with mean blood glucose 3.9-6.7 mmol/L • Inc mortality risk with mean blood glucose > 7.8 mmol/L • Mortality drop 19% (pre-ITT) to 14% (post-ITT), P<0.01	• Dec mortality risk with mean blood glucose 3.9-5.5 mmol/L • Inc mortality risk with mean blood glucose > 10.0 mmol/L • No statistically significant change in mortality pre and post ITT	• Non-diabetics: 4.5-fold inc in mortality from lowest mean blood glucose, 3.9-5.5 mmol/L (9%) to highest, > 10mmol/L (40%) • Diabetics: 2-fold inc in mortality from lowest mean blood glucose, 3.9-5.5 mmol/L (13%) to highest, > 10mmol/L (26%)
Egi *et al.* [28]	2008	4 896	Glycaemia vs mortality	• Inc risk of ICU mortality with hyperglycaemia – with non survivors spending more time with blood glucose > 8.0 mmol/L	• No association with hyperglycaemia and ICU mortality • Lower OR of death at all levels of hyperglycaemia.	• Diabetic patients: lower ICU mortality (P=0.02) • No difference in hospital mortality between groups (P=0.3)
Falciglia *et al.* [26]	2009	259 040	Glycaemia vs mortality	• 5-fold inc in mortality from lowest mean blood glucose, 3.9-6.1 mmol/L (8%) to highest, > 16.7 mmol/L (41%)	• 2-fold inc in mortality from lowest mean blood glucose, 3.9-6.1 mmol/L (6%) to highest, > 16.7 mmol/L (11%)	• Hyperglycaemia associated with inc mortality in diabetics and non diabetics • Mortality greater for hyperglycemic non diabetics patients
Stegenga *et al*. [31]	2010	830	DM vs outcomes of sepsis	• Admission hyperglycaemia (> 11.1 mmol/L) associated with inc 28 and 90 day mortality (P<0.03)	• Admission hyperglycaemia had no effect on diabetic mortaltity	• Diabetes did not influence mortality in sepsis
Krinsley *et al.* [32]	2013	44 964	Hyperglycaemia, hypoglycaemia, and glycemic variability vs mortality (and how DM effects this)	• Inc mortality with higher mean blood glucose (≥ 7.8 mmol/L) • Dec mortality with lower blood glucose (4.4-7.8 mmol/L)	• Inc mortality with mean blood glucose between 4.4-6.1 mmol/L • Dec mortality when blood glucose were higher (6.2-10 mmol/L).	• Hyperglycaemia, hypoglycaemia, and increased glycemic variability are independently associated with mortality in ICU patients • Diabetic status tempers these relations

Inc = Increased, Dec = Decreased, CICU = Cardiac Intensive Care Unit, CTICU = Cardiothoracic Intensive Care Unit, NSICU = Neurosurgical Intensive Care Unit, ITT = Intensive insulin therapy

Table 5: Interventional studies (diabetes as a binary variable) and outcomes related to hyperglycaemia (chronological order)

Author	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Van den Berghe *et al.** [54]	2006	2 748	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (insulin if blood glucose > 12 then target 10-11.1 mmol/L) on mortality	• Reduced mortality and morbidity with ITT	• No survival benefit with ITT • Higher rates of hypoglycaemia	• Hosp mortality 19% (38/200) of the DM patients in conventional arm • Hosp mortality 23% (48/207) of the DM patients in the ITT arm
Arabi *et al.* [39]	2008	523	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 10-11.1 mmol/L) on ICU mortality	• Mortality: ITT (14%) vs CIT (14%) – no significant difference (P=0.2)	• Mortality: ITT (13%) vs CIT (20%) – no significant difference (P=0.3)	• No significant difference in ICU mortality between IIT and CIT (P=0.3)
Brunkhorst *et al*. [38]	2008	537	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 10-11.1 mmol/L) on mortality	• 28 day mortality: ITT 25% vs CIT 23% (P=0.8) • 90 day mortality: ITT 40% vs CIT 32% (P=0.2)	• 28 day mortality: ITT 25% vs CIT 32% (P=0.3) • 90 day mortality: ITT 40% vs CIT 42% (P=0.9)	• No mortality benefit with ITT vs CIT • Stopped early due to safety risk
Del La Rosa *et al.* [42]	2008	504	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 10-11.1 mmol/L) on morbidity and mortality	• ICU mortality ITT 37% vs CIT 32% (no significance)^ • In-hospital mortality: ITT 40% vs CIT 39% (no significance)^	• Mortality: ITT (38%) vs CIT (31%) – no significant difference	• No difference in ICU mortality, 28 day mortality or ICU infections • Increased hypoglycaemia in ITT
Finfer *et al.* [41]	2009	6 029	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose <10 mmol/L) on mortality	• Mortality: ITT (27%) vs CIT (24%) – no significant difference	• Mortality: ITT (32%) vs CIT (28%) – no significant difference	• ITT arm – inc 90 day mortality • No difference in those with and without DM (P = 0.60)
Preiser *et al.* [40]	2009	1 078	ITT (blood glucose 4.4-6.1 mmol/L) vs CIT (blood glucose 7.8-10 mmol/L) on mortality	• ICU mortality ITT 17% vs CIT 15% (P=0.4)^ • Hospital mortality: ITT 23% vs CIT 19% (P=0.1)^	• Not described	• Stopped early due to protocol violations

*Contains pooled data from the 2001 (surgical) and 2006 (medical) study

^Mortality of total patients (includes non-diabetic and diabetic patients)

ITT = Intensive insulin therapy, CIT = Conventional insulin therapy, Inc = Increased, Dec = Decrease

Table 6: Observational studies that have recorded chronic glycaemia as a dynamic variable (chronological order)

Author	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Egi *et al.* [55]	2011	415	Does pre-existing hyperglycaemia modulate the association between glycemia and outcome in ICU patients with DM	• N/A	• Patients with elevated preadmission HbA1c levels (>7%) showed a mortality benefit when mean ICU glucose concentrations were > 10 mmol/L	• Relationship between HbA1c and mortality changed according to the levels of time-weighted average of blood glucose concentrations
Plummer *et al.* [34]	2014	1 000	Prevalence of CIAH and recognized/unrecognized DM in ICU and to evaluate the premorbid glycaemia on the association between acute hyperglycaemia and mortality	• 50% had CIAH • Risk of death inc by 20% for each increase in acute glycaemia of 1 mmol/l	• Well controlled DM (HbA1c <6%) and adequately controlled (DM 6-7%) – risk of death as per non diabetic patient • HbA1c ≥ 7% (insufficiently controlled DM) had no significance between mortality and acute glycaemia	• 22% had recognised DM • 6% had unrecognised diabetes
Hoang *et al.* [44]	2014	299	Prevalence of unrecognized DM amongst those with CIAH and the association between baseline glycaemia and mortality	• 102 (34%) had no history of DM • 14/102 (14%) had unrecognized DM (diagnosed with HbA1c ≥ 6.5)	• 197 (66%) had a history of DM	• Lower HbA1c had inc mortality (in this population of CIAH patients) despite lower median glucose values and less glucose variability • Mortality in HbA1c < 6.5 (19%) vs HbA1c ≥ 6.5 (12%), P = 0.04

Inc = Increased, Dec = Decreased

Table 7: Observational studies and outcomes related to hypoglycaemia (chronological order)

Author	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Krinsley and Grover [60]	2007	408	Risk factors for developing hypoglycaemia in ICU and outcomes	• Severe hypoglycaemia associated with septic shock. Renal insufficiency, mechanical ventilation, illness severity and use of ITT	• Associated with inc risk of severe hypoglycaemia (P<0.01) • DM had no association with mortality	• Mortality in severe hypoglycaemia cohort 56% vs control cohort 40%, P<0.01
Egi *et al.* [61]	2010	4 946	Hypoglycaemia vs risk of death in critically ill patients	• Mild or moderate hypoglycaemia was associated with mortality in critically ill patients • Mortality increases as severity of hypoglycaemia increases	• Diabetic patients more likely to suffer from insulin-associated hypoglycaemia	• 22% of total patients had one episode of hypoglycaemia • Hospital mortality: hypoglycaemic cohort 37% vs control cohort 20%, P<0.01
Krinsely *et al*. [33]	2011	6 240*	Mild hypoglycaemia (blood glucose level < 3.9 mmol/L) vs risk of mortality in critically ill patients.	• Mild hypoglycaemia was associated with a significantly increased risk of mortality	• The association between hypoglycaemia and mortality was independent of diabetic status	• Inc severity of hypoglycaemia was associated with inc risk of mortality • Hypoglycemic patients had higher mortality regardless of diagnostic category and ICU LOS
Krinsley *et al*. [32]	2013	44 964	Hyperglycaemia, hypoglycaemia, and glycemic variability vs mortality (and how DM effects this)	• Inc mortality with higher mean blood glucose (≥ 7.8 mmol/L) • Dec mortality with lower blood glucose (4.4-7.8 mmol/L)	• Inc mortality with mean blood glucose between 4.4-6.1 mmol/L • Dec mortality when blood glucose were higher (6.2-10 mmol/L).	• Hyperglycaemia, hypoglycaemia, and increased glycemic variability are independently associated with mortality in ICU patients • Diabetic status tempers these relations

*Contains partial data from one prospective RCT (Glucontrol trial) and complete data from two observational cohorts (USA and The Netherlands)

Inc = Increased, Dec = Decrease, LOS = Length of stay

Table 8: Observational and interventional studies and outcomes related to glycaemic variability (chronological order)

Author	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Egi *et al*. [65]	2006	7 049	GV (measured by SD and %CV) vs mortality (hospital and ICU)	• Both mean and GV of blood glucose were significantly and independently associated with ICU and hospital mortality • GV was a stronger predictor of ICU mortality than mean glucose concentration	• Inc mortality when comparing highest and lowest glucose SD • No other significant relation with blood glucose (SD and mean) and ICU/hospital mortality • Logistic regression: DM associated with decrease OR for ICU mortality	• The mean±SD of blood glucose: Survivors 1.7±1.3 mmol/L vs Non survivors 2.3±1.6 mmol/L ($P < 0.001$) • Post logistic regression analysis, both mean and SD of blood glucose were significantly associated with ICU and hospital
Ali *et al.* [66]	2008	1 246	GV vs hospital mortality in septic ICU patients	• GV is independently associated with hospital mortality in sepsis	• Mortality rise remained even after adjusting for a diagnosis of diabetes	• Higher observed mortality with increasing levels of variability • Higher odds of hospital mortality with lower mean blood glucose + high GV or higher mean blood glucose + lower GV
Krinsely [67]	2008	3 252	GV vs mortality in ICU patients	• Inc GV conferred a strong independent risk of mortality	• Multivariable regression analysis demonstrated that diabetes had an independent positive correlation to SD	• Amount of GV had a significant effect on mortality - eg patients with mean blood glucose 3.9-5.5 mmol/L mortality: Lowest GV 6% while high GV 30%
Krinsely [68]	2009	4 084	Impact of DM or its absence on GV as a risk factor for mortality	• Low GV was associated with increased survival • High GV was associated with increased mortality	• Higher measures of GV • No association between GV and mortality among diabetics	• Attempts to minimize GV may have a significant beneficial impact on outcomes of critically ill patients without diabetes
Lundelin *et al*. [69]	2010	42	Glycemic dynamics (measured via non-lineal dynamics) vs mortality in ICU patients	• Loss of complexity (therefore higher variability) in glycaemia time series is associated with higher mortality	• This association persisted in diabetics • No difference in DFA (detrended fluctuation analysis a measure of complexity) between DM and nondiabetics	• In critically ill patients, there is a difference in the complexity of the glycaemic profile between survivors and nonsurvivors • Loss of complexity correlates with higher mortality.
Meyfroidt *et al.* [71]*	2010	2 748	Blood glucose signal characteristics vs hospital mortality,	• GV was independently associated with hospital mortality	• Increased mortality was seen in both diabetics and non diabetic patients.	• Increased glucose amplitude variation was associated with mortality, irrespective of blood glucose level

Hoang *et al.* [44]	2014	299	Prevalance of unrecognized DM amongst those with CIAH and the association between baseline glycaemia and mortality	• 102 (34%) had no history of DM • 14/102 (14%) had unrecognized DM (diagnosed with HbA1c ≥ 6.5)	• 197 (66%) had a history of DM	• Lower HbA1c had inc mortality (in this population of CIAH patients) despite lower median glucose values and less glucose variability • Mortality in HbA1c < 6.5 (19%) vs HbA1c ≥ 6.5 (12%), P = 0.04
Donati *et al.* [70]	2014	2 782	GV and mean BGLs vs mortality and intensive care unit-acquired infections	• High GV is associated with higher risk of ICU acquired infection and mortality	• Diabetic patients had higher mean BGL and GV • No change in mortality or infections	• Mean BGL was not associated with infections and mortality

*Interventional study data – pooled from the Leuven trials

GV = Glycaemic variability, SD = Standard deviation, CV% = Coefficient of variation, Inc = Increased, Dec = Decreased, OR = Odds ratio

2.3 MANUSCRIPT

Liberal Glycemic Control in Critically Ill patients with Type-2 Diabetes: an Exploratory Study

Statement of Authorship

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Contribution to paper	Conceptualisation of work, its realisation and its documentation. Collected and interpreted data and wrote manuscript.		
Overall percentage (%)	75		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Liberal Glycemic Control in Critically Ill patients with Type-2 Diabetes: an Exploratory Study

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Reprints

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Key words

Diabetes, critical illness, intensive care, glucose, hypoglycaemia

Abstract

Objective: The optimal blood glucose target in critically ill patients with pre-existing diabetes and chronic hyperglycemia is unknown. In such patients, we aimed to determine whether a 'liberal' approach to glycemic control would reduce hypoglycemia and glycemic variability and appear safe.

Design: Prospective, open-label, sequential-period exploratory study.

Setting: Medical-surgical intensive care unit.

Patients: During sequential six-month periods we studied 83 patients with pre-existing type 2 diabetes and chronic hyperglycemia (HbA1c ≥7.0% at ICU admission).

Intervention: During the 'standard care' period, 52 patients received insulin to treat blood glucose concentrations >10mmol/l whereas during the 'liberal' period, 31 patients received insulin to treat blood glucose concentrations >14mmol/l.

Measurements and Main Results: Time-weighted mean glucose concentrations and the number and duration of moderate (<4.0mmol/l) and severe (≤2.2mmol/l) hypoglycemic episodes were recorded, with moderate and severe hypoglycemic episodes grouped together. Glycemic variability was assessed by calculating the coefficient of variability for each patient. Safety was evaluated using clinical outcomes and plasma concentrations of markers of inflammation, glucose-turnover and oxidative stress. Mean glucose ($TWglucose_{day0\text{-}7}$ standard care: 9.3 (1.8) vs. liberal: 10.3 (2.1) mmol/l; P=0.02) and nadir blood glucose (4.4 (1.5) vs. 5.5 (1.6) mmol/l; P<0.01) were increased during the liberal period. There was a signal towards reduced risk of moderate-severe hypoglycemia (RR: liberal compared to standard care: 0.47 (95% CI (0.19, 1.13): P=0.09). Ten patients (19%) during the standard period and one patient (3%) during the liberal period had recurrent episodes of moderate-severe hypoglycemia. Liberal therapy reduced glycemic variability (CV: 33.2 (12.9) vs. 23.8 (7.7)%; P< 0.01). Biomarker data and clinical outcomes were similar.

Conclusions: In critically ill patients with type 2 diabetes and chronic hyperglycaemia, liberal glycemic control appears to attenuate glycemic variability and may reduce the incidence of moderate-severe hypoglycemia.

Trial Registration: Australian New Zealand Clinical Trials Registry (ACTRN12612001274864)

Introduction

Type 2 diabetes is a frequent pre-existing medical condition in patients admitted to the Intensive Care Unit (ICU) [1] with approximately 50% of these patients having chronically increased blood glucose concentrations, defined as a glycated hemoglobin (HbA1c) ≥7% (53mmol/mol) [2].

Recent observational data indicate that patients with diabetes are generally tolerant of acute hyperglycemia during ICU admission and that, in particular, those with 'chronic hyperglycemia' appear to have a survival benefit when blood glucose concentrations are >10mmol/l [2-5].

In the critically ill both the magnitude of hypoglycemia and recurrent episodes of hypoglycemia are strongly associated with adverse outcomes [6]. Moreover, in patients with chronic hyperglycemia the physiological stress response to hypoglycemia may occur at blood glucose concentrations considered 'normal' in healthy individuals [7]. In addition, greater glycemic variability is associated with increased mortality in the critically ill [8, 9]. Finally, it is intuitively plausible that, by adjusting glucose thresholds to allow more liberal blood glucose concentration targets, the frequency and severity of insulin-induced hypoglycemia could be attenuated and glycemic variability diminished [6, 8].

The rationale to treat hyperglycemia in critically ill patients is to prevent or attenuate acute complications and, ultimately, increase survival [10]. However, exploratory or proof-of-concept studies do not have sufficient power to precisely establish effects on such patient centered outcomes [11]. For this reason plasma concentrations of biomarkers can be used as surrogate safety markers to detect an early signal for harm [11]. Relevant to glycemia, biomarkers of inflammation, glucose turnover and oxidative stress are all affected by glucose concentrations and are also associated with poor outcomes [12, 13].

Because observational data suggest that there may be benefit in liberal glycemic targets during critical illness based on chronic blood glucose control but current guidelines, including those from the Society of Critical Care Medicine, strongly recommend that blood glucose concentrations >10mmol/l should instead be treated

regardless of pre-existing glycemia [14, 15], we performed an exploratory prospective interventional study in critically ill patients with diabetes and chronic hyperglycemia.

Our objectives were to test the hypotheses that liberal glucose concentration targets attenuate hypoglycemia and glycemic variability, while also evaluating whether liberal glucose targets have adverse biological effects as assessed by plasma concentrations of recognized markers of inflammation, glucose-turnover and oxidative stress.

Materials and Methods

We performed an exploratory, prospective, open-label, before-and-after study over sequential six-month periods at the Royal Adelaide Hospital ICU. All patients within the study had their HbA1c measured on admission to the ICU. The first study period, 'standard care', was conducted between August 2012 and February 2013 (spring and summer) and was followed by a 'washout' period of six weeks. The second study period, 'liberal care', was conducted between April 2013 and October 2013 (autumn and winter). During each study period, consecutive patients were screened twice per week-day (9am and 4pm) with patients admitted outside of these times screened at the next available opportunity.

Data were obtained from patients aged ≥18 years who were admitted to the ICU with chronic hyperglycemia (admission HbA1c ≥7%) and who subsequently had a blood glucose concentration >10mmol/l during their ICU admission. Patients with type 1 diabetes, patients having cardiac surgery and patients readmitted to the ICU were excluded. The glucose targets were ceased if patients were discharged from ICU, if the treating physician chose to administer insulin subcutaneously rather than intravenously, or if treatment goals were altered to focus solely on comfort care.

During the initial six-month standard care period, insulin (Actrapid, Novo Nordisk) was commenced when blood glucose concentrations were >10mmol/l and adjusted according to the pre-existing local protocol with the aim of maintaining blood glucose concentrations between 6-10mmol/l (Supplemental figure 1A). During the second six-month liberal period, the protocol was adjusted to facilitate greater glucose concentrations, such that insulin was commenced when blood glucose concentrations

were >14mmol/l and adjusted to maintain blood glucose concentrations between 10-14mmol/l (Supplemental figure 1B). Arterial blood glucose was measured one to four-hourly according to preceding glucose values (Supplemental figures 1A and 1B).

Glycemia was evaluated using time-weighted mean glucose, hypoglycemia - with the number and recurrence of moderate-severe hypoglycemic episodes of particular interest – and glycemic variability. Where we measured clinical outcomes - mortality, time to discharge alive, ventilator-free days and days of inotrope administration – because of the relatively small size of the cohort studied, outcomes from clinical data were unlikely to be meaningful. We also measured plasma concentrations of several biomarkers that had the capacity to be affected by the intervention and, accordingly had the potential to identify biological signals of harm, if they existed. We quantified acute phase inflammation using interleukin-6 (IL-6) and high sensitivity C-reactive protein (hs-CRP), glucose turnover using 1,5 anhydroglucitol and oxidative stress using plasma isoprostanes [13, 16, 17].

A delayed opt out consent process was approved by the local Human Research Ethics Committee.

Data analysis

Blood glucose

We measured blood glucose concentrations at the bedside using portable glucometers (FreeStyle Optium H) or via blood gas analyzer (ABL800 Flex; Radiometer Medical) [18]. To limit surveillance bias we calculated time-weighted mean glucose concentrations as described [19]. We defined severe hypoglycaemia (≤2.2mmol/l) and moderate hypoglycemia (2.3-3.9mmol/l) as per standard definitions [6, 20, 21]. Based on harm observed during the NICE-SUGAR study when blood glucose <6.1mmol/l was targeted, we also introduce the term ‘relative hypoglycemia’ to discriminate values between 4.0-6.0mmol/l. We anticipated severe hypoglycemia would be rare and so accordingly grouped moderate and severe hypoglycemia for inferential statistics. Additionally, targeting blood glucose concentrations <6.1mmol/l has been proven to cause harm [22] and therefore we also recorded relative hypoglycemia episodes (4.1-6.0mmol/l). A unique hypoglycemic episode was recorded for each episode below the described threshold until the next measurement above this level,

with the duration of hypoglycemia between these time points calculated from patient charts. Glycemic variability was also evaluated with the standard deviation and coefficient of variability calculated for each patient [8].

Glycated hemoglobin (HbA1c)

We obtained blood on admission and this was analyzed at a central laboratory at ~1100h Monday-Friday using high performance liquid chromatography to quantify HbA1c [23].

Plasma Biomarkers

We collected blood on day 0, 1, 2, 4, and 7 for the measurement of plasma biomarkers. Day 0 plasma samples were taken once we were aware that the patient's HbA1c was ≥7% and before glucose thresholds were altered. Blood samples were collected into chilled ethylenediaminetetraacetic acid tubes, separated by centrifugation (3200rpm for 15minutes at 4°C) within 30minutes of collection and then stored at -70°C until assayed [24].

i) Inflammation (IL-6 and hs-CRP)

We measured plasma IL-6 using a Human IL-6 Quantikine ELISA Kit (R&D Systems), with assay sensitivity 0.039pg/mL and intra and inter coefficient of variation of 4.6% and 6.3% respectively. Where appropriate, samples for IL-6 assays were diluted 20x with phosphate-buffered saline. We measured hs-CRP using a high-sensitivity immunoassay (Roche Diagnostics) with assay lower limit of detection 0.1mg/mL and intra- and inter-assay coefficient of variations of 0.9% and 6.3% respectively.

ii) Acute glycemia (1,5 anhydroglucitol)

We measured 1,5 anhydroglucitol using the glycomark test (Glycomark Incorporated) implemented on the chemical autoanalyzer Cobas Integra 400+ (Roche Diagnostics) with assay sensitivity 0.2ug/mL and intra- and inter-assay coefficient of variations of 2.7% and 4.4% respectively.

iii) Oxidative stress (isoprostanes)

Isoprostane concentration was determined using a gas chromatography–mass spectrometry method [25]. The assay sensitivity was <100pmol/mL and intra- and inter-assay coefficient of variations of 3.4% and 5.4% respectively.

Demographic and baseline data

We recorded demographic data including age, admission diagnosis, severity of illness scores and Charlson comorbidity index. Clinical parameters of interest included mechanical ventilation, administration of inotrope/vasoconstrictor drugs, as well as serum creatinine, blood lactate and ratio of arterial oxygen partial pressure to fractional inspired oxygen (P:F ratio) for the first 24 hours. Calories administered, with amount and route of admission noted, and when the only source of nutrient was oral intake calories were recorded as 0.

Patient outcomes

Clinical outcomes of interest included ICU and hospital mortality. At day 90 we contacted patients or their family doctor (general practitioners) to determine survival. We also collected information related to whether death occurred while patients were receiving life-sustaining treatments or if care had shifted to palliation.

Statistical analysis

Based on our previous observational data [2] we estimated between 100 and 120 patients would be admitted and eligible for the study over a 12-month period and would be sufficient to identify whether there were signals to benefit or harm; i.e. reduction in moderate/severe hypoglycemic episodes and glycemic variability and an absence of harm with biomarker data and/or clinical outcomes .

Data are presented as n (%, 95% CI), mean (SD) or median [IQR] as appropriate. Demographic and time weighted glucose data were analyzed using independent samples t-tests, Chi-square, Fisher's exact and Mann-Whitney tests as appropriate. Due to the small number of hypoglycemic episodes, particularly during the liberal period, when appropriate these data are presented as summary data without inferential statistics. We report mortality data prior to ICU and hospital discharge but only analyzed mortality at day 90. ICU and hospital lengths of stay were analyzed as time

until discharge alive from ICU/hospital with death included as a competing event [26] and censored as a length of stay equal to the highest length of stay amongst survivors plus one day. These data were then analyzed using Kaplan-Meier analyses and log-rank tests. Differences between periods in mortality were adjusted for APACHE II score and Charlson comorbidity index using logistic regression. Statistical significance for benefit was set at $P<0.05$.

Results

We studied 52 patients for 4047 hours of observation during the standard care period and 31 patients for 3244 hours during the liberal target period (Figure 1). Baseline characteristics of the groups were comparable (Table 1).

Blood glucose and insulin concentrations

Peak blood glucose concentrations were similar between the groups (Table 2) but time-weighted blood glucose concentrations were greater during the liberal period (Figure 2A).

The nadir recorded blood glucose was less during the standard care period (Table 2). Blood glucose concentrations were in the moderate-severe hypoglycemic range for 61 hours during the standard care period and 12 hours during the liberal period. Eighteen patients (35% (23, 48)%) had an episode of moderate-severe hypoglycemia (13 patients had moderate hypoglycemia and 5 patients severe hypoglycemia) during the standard care period and five patients (16% (7, 33)%) during the liberal period (4 moderate and 1 patient severe). The relative risk of a moderate-severe episode during the liberal target period when compared to standard care was 0.47 (0.19, 1.13): $P=0.09$.

Ten patients had recurrent moderate-severe hypoglycemic episodes during standard care, whereas only one patient had a recurrent episode of moderate-severe hypoglycemia on liberal care. Of patients with moderate-severe hypoglycemic episodes, blood glucose concentrations were sufficiently low to be quantified as severe in four patients in the standard group and one patient during liberal care.

Relative hypoglycemia (i.e. 4.1-6.0mmol/l) occurred more frequently in the standard period (46 (89%) vs. 20 (65%) patients; P=0.01). There were also more patients in the standard period sustaining recurrent relative hypoglycemic events when compared to liberal period (34 (66%) vs. 12 (39%) patients; P=0.02).

Glycemic variability decreased during liberal care, with reduction in both coefficient of variability (33.2 (12.9) vs. 23.8 (7.7)%; P<0.01) and glucose standard deviation (3.1 (1.3) vs. 2.5 (0.9); P=0.04).

Insulin was administered to all patients except one during the liberal period. The rate of insulin administration was similar between groups (Figure 2B).

Biomarkers

Plasma concentrations of all biomarkers were similar at baseline and over the course of the study (Figure 3A-D).

Processes of care

The median number of ventilator free days and days of inotrope/vasoconstrictor administration were comparable. Calorie delivery per day was similar between groups (Table 2).

Patient outcomes

Patient outcomes were similar between the groups during the study period (Supplemental File 2).

Discussion

Key findings

We performed a prospective, open-label, before-and-after exploratory study to evaluate the effect of liberal glycemic control in critically ill patients with pre-existing type 2 diabetes and chronic hyperglycemia. We found that the liberal approach was associated with reduced hypoglycemia and glycemic variability. In addition, we observed no signal for harm via relevant biomarkers or clinical outcomes and that insulin administration was comparable between groups.

Comparison with previous studies in the critically ill

While observational studies have reported associations between hyperglycemia and adverse outcomes in patients with normal glucose tolerance prior to their critical illness, the strength of this relationship appears to be either markedly attenuated or abolished in patients with pre-existing diabetes [1, 27, 28]. Meanwhile inferences from interventional studies are limited for patients with chronic hyperglycemia as investigations so far have enrolled patients regardless of pre-existing diabetes and these studies were never powered to detect differences in the subgroup of patients with diabetes [22, 29, 30]. Furthermore, the interpretation of data from these previous studies is limited as diabetes is usually reported as a binary phenomenon when in fact optimal acute glucose targets in patients with diabetes may well be more nuanced than simple dichotomous categorization, with the presence of chronic glycemia being more revealing than simply a diagnosis of diabetes per se [2, 3, 5]. Accordingly, there is an absence of data evaluating more bespoke approaches to glycemic targets during critical illness that are based on chronic glycemia.

The incidence of hypoglycemia in our study (35% during the standard care period and 16% during the liberal period) may initially appear high. However, for unselected patients allocated to conventional treatment in the NICE-SUGAR study (6-10 mmol/l) the incidence of hypoglycemia was ~16% and yet insulin was only administered to < 70% of patients [6]. Furthermore, it is established that prior diabetes and insulin use are independent risk factor for developing hypoglycemia [6, 31-34] Similarly, glycemic variability is increased in those with a diagnosis of diabetes, with coefficients of variations of ~30% consistently reported in this group [4, 34-38]. Given that we chose to study a group of 'high-risk' patients, i.e. patients who not only had diabetes but were also chronically hyperglycemic, many of whom also required considerable amounts of exogenous insulin (insulin was administered to all except one patient, at a mean dose of 2 U/h) we believe that the incidence of hypoglycemia and glycemic variability during our standard care period may well reflect 'real world' practice in this group of patients [4]. However, safety and effects on clinical outcomes need to be evaluated in larger clinical trials before this strategy is widely adopted.

Comparison with previous studies in outpatients and physiological investigations

Data from phase III studies in outpatient populations suggest that reducing the risk of hypoglycemia will lead to an improvement in patient centered outcomes if studies are powered to determine these effects. For example, the Action to Control Cardiovascular Risk in Diabetes study, a large international multi-center randomized controlled trial of ambulatory patients with type 2 diabetes reported that rapid glucose-lowering strategies increase mortality [39], probably via an increased risk of hypoglycemia [40].

Moreover, physiological studies have evaluated that chronic hyperglycemia induces adaptive changes that leave patients particularly vulnerable to 'relative hypoglycemia' at blood glucose concentrations that would be otherwise considered normal in health [41], such that counter-regulatory hormonal responses are stimulated at greater blood glucose concentrations [7]. For these reasons the signal toward less moderate-severe and mild hypoglycemia we observed also identifies that liberal therapies have the potential to improve patient centered outcomes.

Finally, in health, patients with diabetes and critical illness an episode of hypoglycemia predisposes to further episodes of hypoglycemia and also harm from hypoglycemia [20, 42]. These antecedent episodes of hypoglycemia attenuate endogenous adrenaline secretion and diminish neurogenic symptoms during subsequent hypoglycemic episodes [43], thereby blunting the physiological response to 'normalize' glycemia. Accordingly, a reduction in recurrent hypoglycemic episodes is likely to be of considerable benefit.

Significance of study findings

Our study is the first to study a cohort of patients based on pre-existing chronic hyperglycemia and to prospectively allocate them to different acute blood glucose targets. Our data provide confirmation of feasibility and biological and safety. We also provide preliminary evidence, albeit from a relatively small sample, of a potent effect to reduce the risk of hypoglycemia and attenuate glycemic variability. However, due to the exploratory study design, the absence of adverse clinical outcomes does not establish that liberal therapy is safe. It is, accordingly, appropriate to emphasize that we do not recommend changes to practice. Additional studies are

essential to validate our observations and to test whether such liberal targets improve patient outcomes.

Limitations

There are limitations to our study. The intervention was not blinded, but previous important studies of glycemic control have also utilized open-label design [22, 29, 30, 44]. Patients were not randomized and the before-and-after design increases risks of seasonal variation and secular changes. However, the liberal period occurred during the winter when excess deaths in Australian patients with diabetes have been reported [45]. Due to the exploratory study design an absence of adverse clinical outcomes does not establish that liberal therapy is absolutely safe. To increase our confidence in clinical outcomes quantified we also measured plasma concentrations of several biomarkers. We selected biomarkers that had the capacity to be affected by the intervention and had the capacity to identify biological signals of harm, if this existed. While these also showed no difference between the groups, after day 2 biomarkers were obtained in fewer than 25% of patients. Hence, speculation regarding signal/s after this time point should, therefore, be circumspect. Our observations were also obtained during intermittent blood glucose monitoring, measured using both glucometer and blood gas analyser. It is possible that other strategies, such as exclusive use of blood gas analyser, continuous glucose monitoring [46, 47] or incretin-based regimens [48], could similarly reduce hypoglycemia and glycemic variability without the need to increase peak blood glucose concentrations. Finally, we used laboratory based HbA1c. This test was performed once per week-day at a central laboratory and this resulted in a delay in the initiation of the intervention.

Future studies

Additional studies conducted at other centers are required to validate our observations and to test whether such liberal targets improve patient outcomes. We suggest that the use of point of care tests for HbA1c may be particularly useful to operationalize these targets in subsequent studies and that validation of admission HbA1 would also be important.

Conclusions

We conducted a single-center before-and-after study comparing standard glucose targets to more liberal targets in critically ill patients with pre-existing type 2 diabetes and chronic hyperglycemia. We found that a liberal approach attenuated glycemic variability, may reduce risk of insulin-induced moderate-severe hypoglycemia, and showed no biological biomarkers signal of concern. Based on these data further studies of this approach are justified.

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Tables

Table 1: Demographic and baseline data. Data are n (%), mean (SD) or median [IQR]

	Standard period Insulin commenced when blood glucose ≥10 mmol/l (n=52)	**Liberal period Insulin commenced when blood glucose ≥14 mmol/l (n=31)**	**P value**
Age (years) (mean (SD))	63.7 (14.5)	62.8 (11.5)	.76
Male (n(%))	30 (58%)	18 (58%)	.97
HbA1c (%) (mean (SD))	8.6 (1.5)	8.7 (1.4)	.57
APACHE-II at admission (mean (SD))	20.4 (7.1)	19.9 (6.4)	.72
APACHE-III at admission (mean (SD))	74.7 (26.9)	72.9 (22.6)	.77
SAPS II score (mean (SD))	40.2 (15.5)	43.3 (13.3)	.35
Charlson Comorbidity Index (mean (SD))[1]	4.4 (1.8)	4.3 (1.5)	.82
Emergency patient (n(%))	44 (85%)	29 (94%)	.31
Medical patient (n(%))	32 (62%)	14 (45%)	.15
Indigenous patient (n(%))	4 (8%)	2 (7%)	1.0
Not known to have diabetes (n(%))	5 (10%)	4 (13%)	.72
ICU admission source			
• Emergency department	23 (44%)	7 (23%)	.22
• Theatre	15 (29%)	14 (45%)	
• Ward	10 (19%)	8 (26%)	
• Hospital transfer	4 (8%)	2 (7%)	
Hospital Admission source			
• Home	31 (60%)	21 (68%)	.46
• Other hospital	21 (40%)	10 (32%)	
Admission Category			
• Cardiovascular/Cardiothoracic surgery	10	3	
• Neurology/Neurosurgery	9	4	
• Sepsis	7	2	
• Respiratory	7	4	
• Trauma	4	4	
• Other	15	14	
Serum creatinine on admission, umol/L (med [IQR])	122 [56.3 - 185.3]	101 [43.8 – 171.0]	.21
Blood lactate on admission, mmol/L (med [IQR])	1.6 [1.0 - 2.7]	1.2 [0.9 - 2.4]	.40
Ventilated Y/N (n(%)) [2]	33/51 (65%)	25/30 (83%)	.07
P:F ratio on admission (med [IQR]) [3]	239 [103 - 366]	280 [110 - 385]	.46

[1]Charlson comorbidity index (standard care n = 47 and liberal targets n = 29 with patients aged > 80 years excluded.

[2]Invasive mechanical ventilation during admission

[3]P:F (ratio of arterial oxygen partial pressure in mmHg to fractional inspired oxygen) was only recorded from patients who were receiving any ventilator support

Table 2: Processes of care

	Standard period Insulin commenced when blood glucose ≥10 mmol/l (n=52)	**Liberal period Insulin commenced when blood glucose ≥14 mmol/l (n=31)**	**P value**
Blood glucose levels			
• Nadir (mmol/l) (mean (SD))	4.4 (1.5)	5.5 (1.6)	<.01
• Maximum (mmol/l) (mean (SD))	15.8 (3.5)	16.2 (3.9)	.60
Worst P:F ratio in first 24 hours (med [IQR])[1]	163 [71 - 213]	222 [83 - 283]	.08
Inotrope use Y/N (n(%))	29/51 (57%)	18/30 (60%)	.78
• Days of inotrope use (med [IQR])	2.0 [1.5 – 3.5]	2.0 [1.8 – 4.0]	.64
Worst lactate in first 24 hours (med [IQR])	1.9 [0.9 – 4.1]	1.9 [0.9 – 3.0]	.54
Mean lactate in first 24 hours (med [IQR])	1.5 [0.7 – 2.3]	1.3 [0.8 – 2.2]	.63
Nutrition			
• Total enteral feeding days	111	98	
• Total fasting days	45	36	
• Calories per day (med [IQR])	1098 [476 – 1477]	1373 [945 – 1536]	.24

[1]P:F (ratio of arterial oxygen partial pressure in mmHg to fractional inspired oxygen) was only recorded from patients who were receiving any ventilator support

Figures

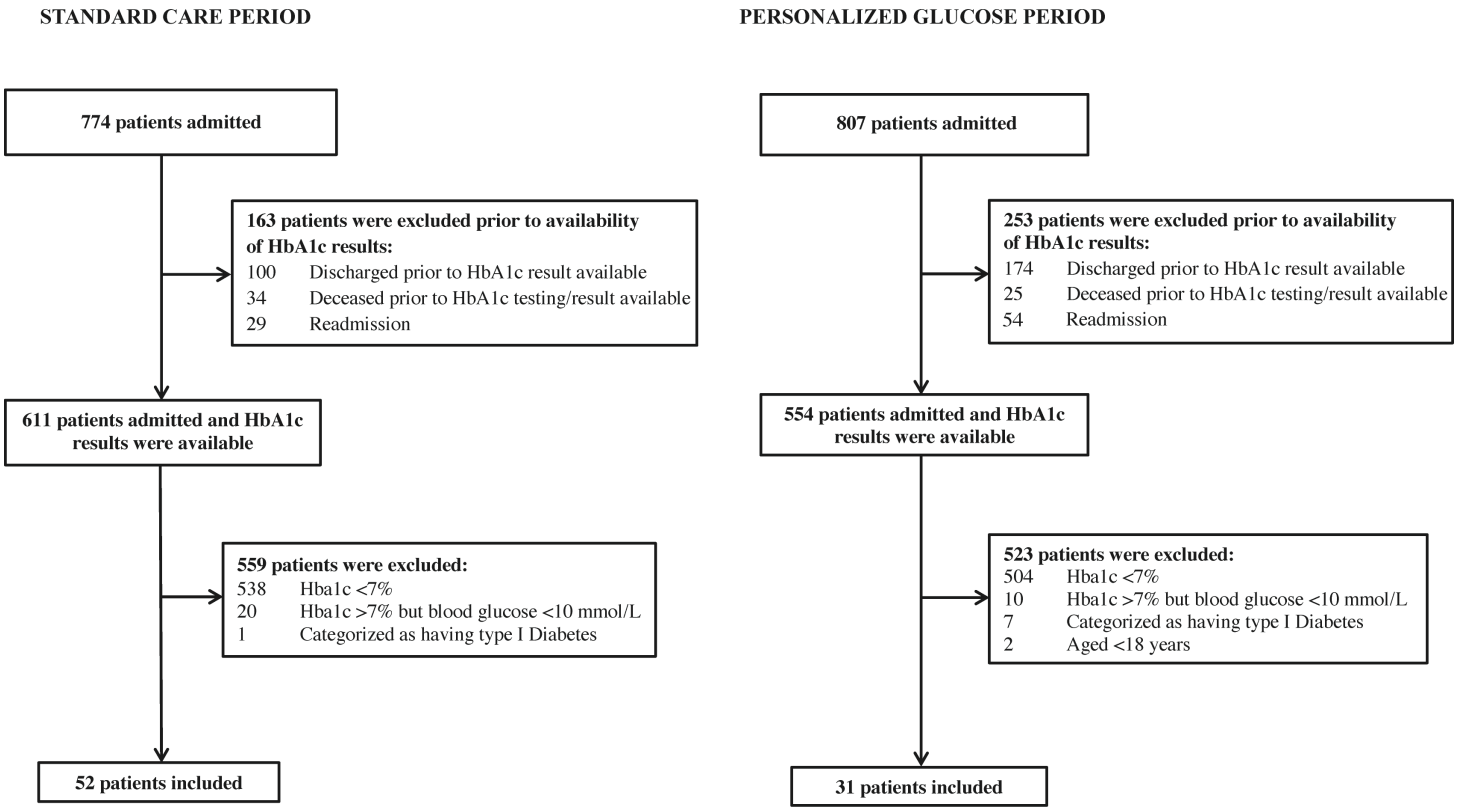
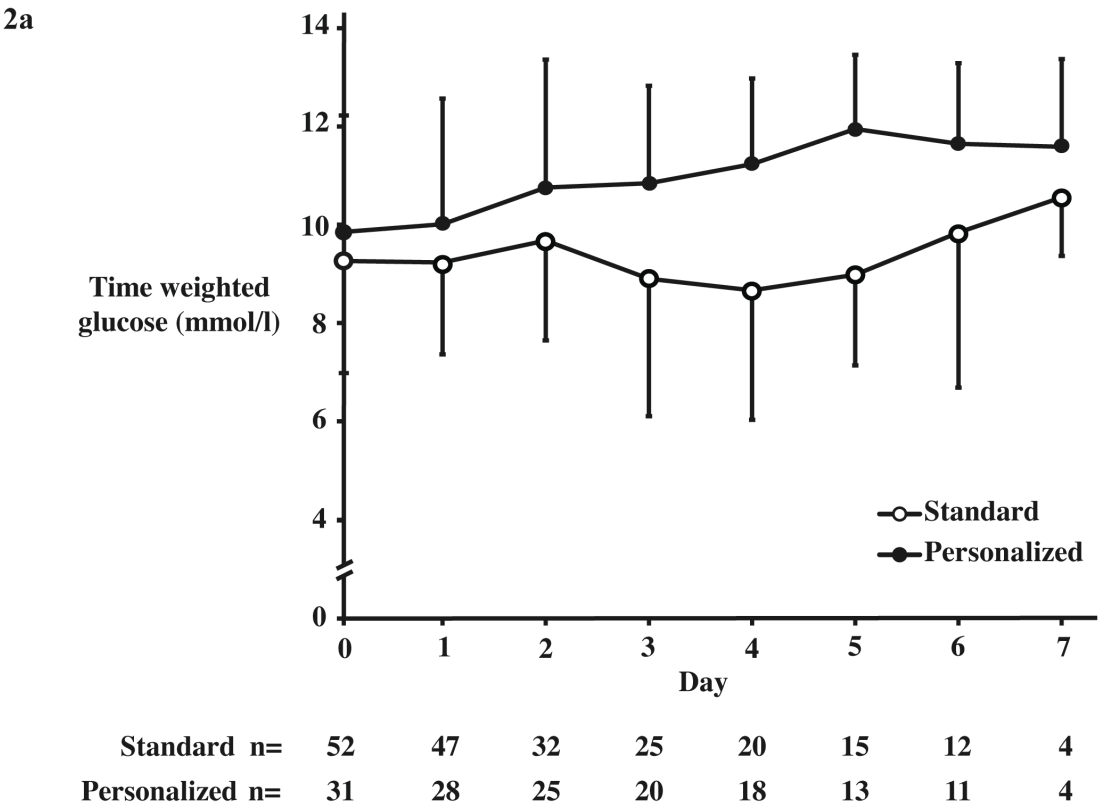


Figure 1: Assessment and inclusion into study of patients during the standard care (August 2012 – February 2013) and personlized glucose (April 2013 – October 2013) periods.



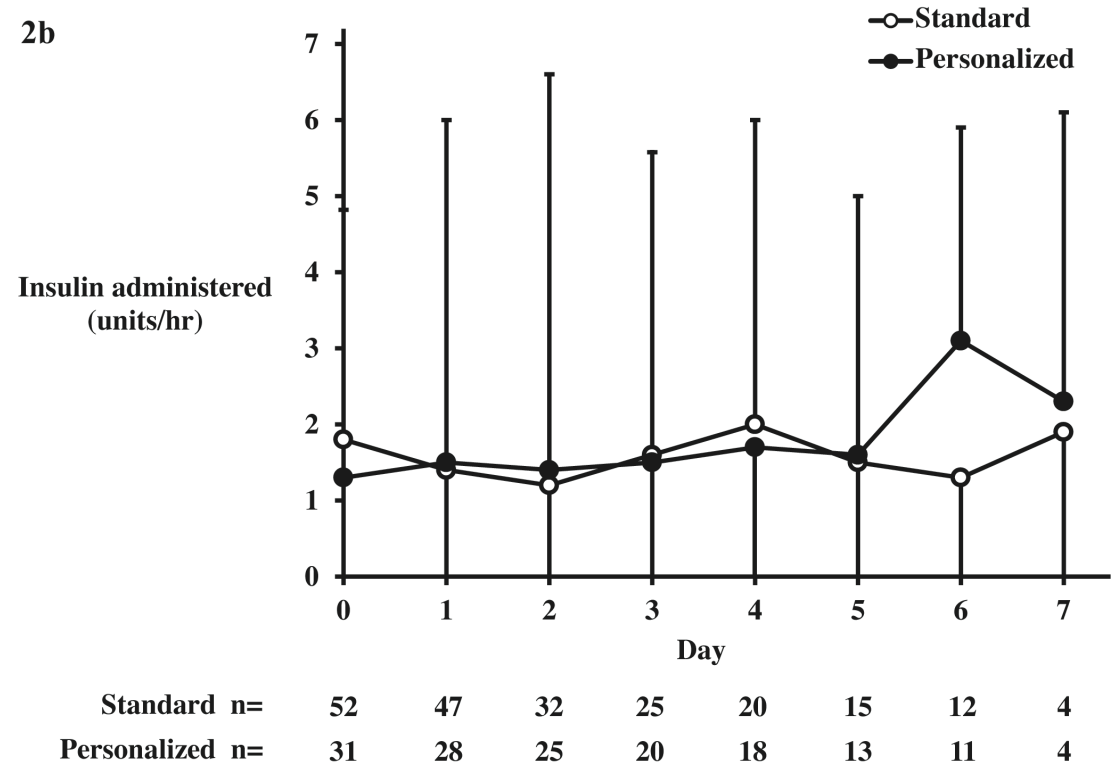


Figure 2: Daily time-weighted blood glucose (data are mean (SD)) and insulin (data are median [IQR]) administered.

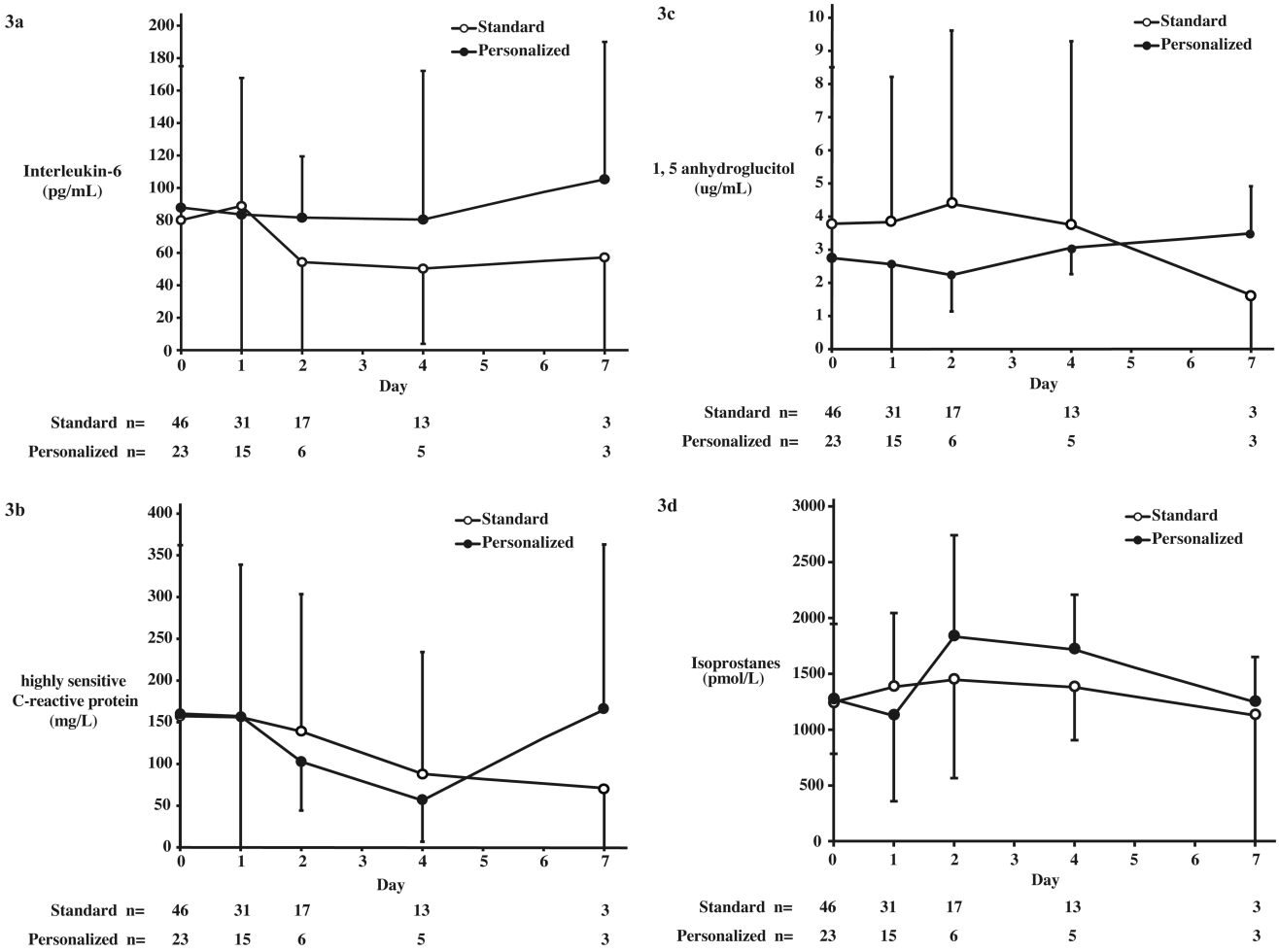


Figure 3: Plasma biomarker concentration at baseline and during the first week of ICU admission: A - interleukin-6; B - highly sensitive C-reactive protein; C 1,5 anhydroglucitol; and D isoprostanes. Data are median [IQR].

Supplemental File 1

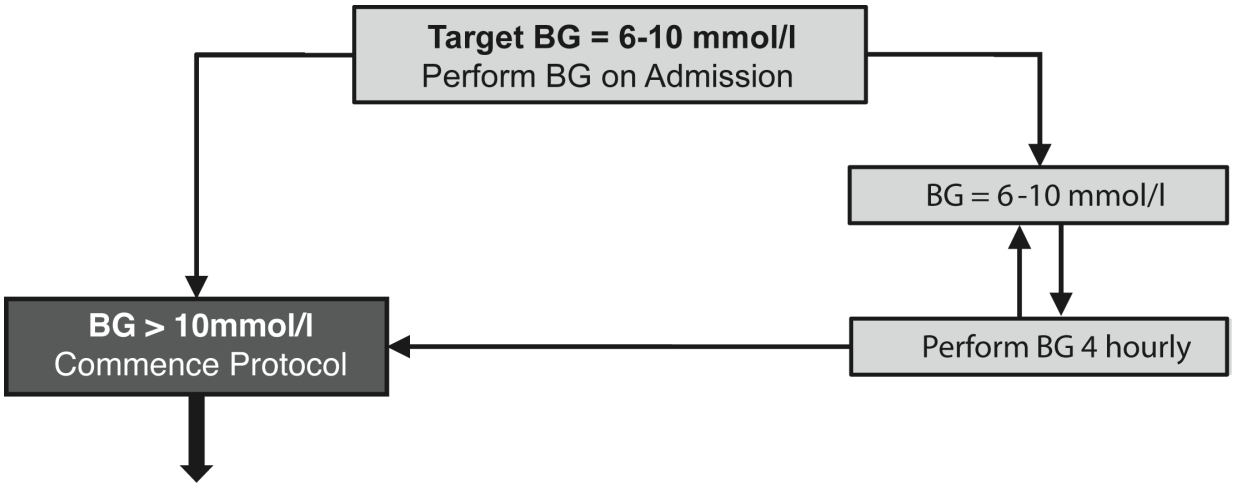


Table: Insulin Infusion Protocol

BG	Bolus	Starting Infusion	Subsequent infusion	Repeat BG
mmol/l	Units IV	Units/hr	Units/hour	Hours
>15	2	2	Increase by 1	1
10.1 - 14.9	1	1	Increase 1	1
8 - 10	0	0	If BG dropping continue current rate static or rising increase by 0.5	1
5 - 7.9	0	0	Continue current rate If BG dropping for 2 consecutive hrs decrease rate by 0.5	1 (2hrly if BG stable for 6hrs)
3.5 - 4.9	0	0	Cease	1 (4hrly if off insulin >6)
<3.5	0	0	Call medical officer	1

Supplemental Figure 1A: Blood glucose (BG) management in the ICU (Standard)

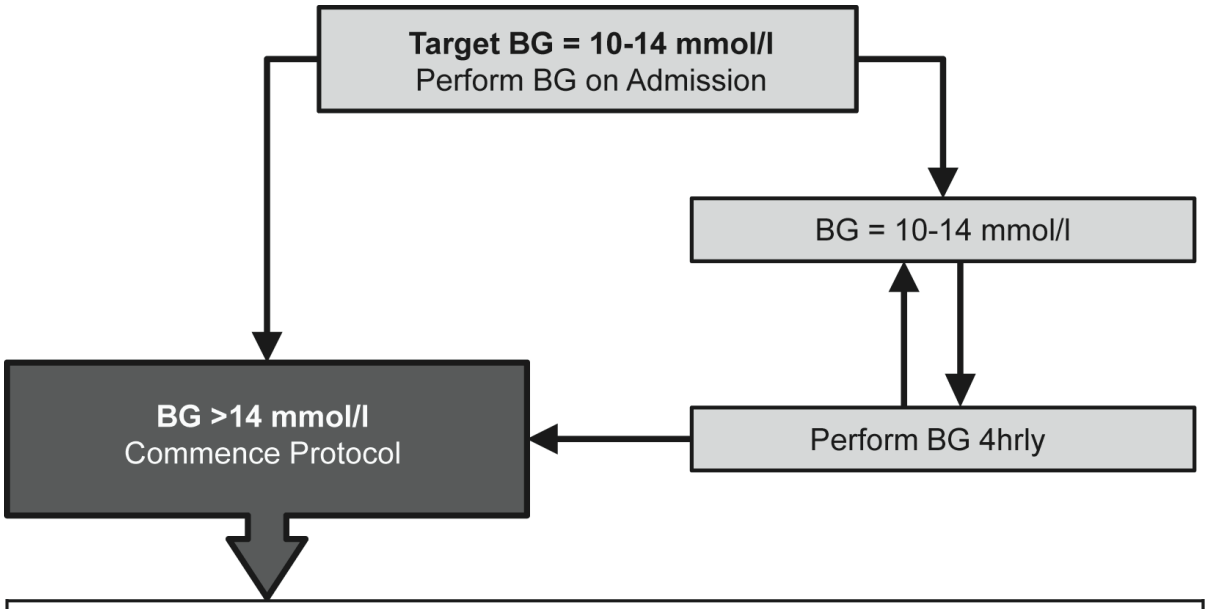


Table: Insulin Infusion Protocol

BG	Bolus	Starting Infusion	Subsequent infusion	Repeat BG
mmol/l	Units IV	Units/hr	Units/hour	Hours
>20	6	BPIR or 3 u/h	Increase by 2	1
>16	4	BPIR - 0.5 or 2 u/h	Increase by 1	1
>14	2	BPIR - 1 or 1 u/h	Increase by 1	1
10 - 14	0	BPIR - 1.5	Continue current rate	1
8 - 9.9	0	0	Drop by 1	1
6 - 7.9	0	0	Drop by 2	1
3.5 - 5.9	0	0	Cease	1
<3.5	0	0	Call medical officer	1

Supplemental Figure 1B: Liberal blood glucose management in ICU (Intervention). Baseline premorbid insulin requirements (BPIR) is for patients with diabetes who are previously receiving insulin and is the total insulin that the patient is receiving prior to their acute illness divided by 24 (hours). If patient is not receiving insulin prior to ICU, then commence as per protocol.

2.4 MANUSCRIPT

Effects of glucose-dependent insulinotropic polypeptide on gastric emptying, glycaemia and insulinaemia during critical illness: a prospective, double blind, randomised, crossover study

Statement of Authorship

Title of paper	Effects of glucose-dependent insulinotropic polypeptide on gastric emptying, glycaemia and insulinaemia during critical illness: a prospective, double blind, randomised, crossover study
Publication status	Published
Publication details	Kar P, Cousins CE, Annink CE, Jones KL, Chapman MJ, Meier JJ, Nauck MA, Horowitz M, Deane AM. **Effects of glucose-dependent insulinotropic polypeptide on gastric emptying, glycaemia and insulinaemia during critical illness: a prospective, double blind, randomised, crossover study**. Critical Care. 2015 Jan; 19:20

Principal Author

Name of Principal Author (Candidate)	Dr Palash Kar
Contribution to paper	Conception and design of the study, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission
Overall percentage (%)	75
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this

	paper.		
Signature		Date	04/02/15

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
- The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of co-author	Ms Caroline Cousins		
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Contribution to paper	Acquisition of data and formatting and revising the manuscript		
Signature		Date	14/02/15

Name of co-author	Professor Karen Jones		
Contribution to paper	Design of the study, analysis of the scintigraphic data and revising the manuscript		
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Contribution to paper	Conception, design and coordination of the study along with drafting and revising the manuscript.		
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Signature		Date	04/02/15

Full title

Effects of glucose-dependent insulinotropic polypeptide on gastric emptying, glycaemia and insulinaemia during critical illness: a prospective, double blind, randomised, crossover study

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Competing interests

The authors declare there are no non-financial competing interests.

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 and Sanofi-Aventis.

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

Authors' contributions

PK was involved in conception and design of study, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission. CEC and CEA made significant contributions to the acquisition of data and formatting and revising the manuscript. KLJ was involved in design of the study, analysis of all the scintigraphic data and revising the manuscript. MJC and MH were

involved in conception, design and coordination of the study along with drafting and revising the manuscript. JJM and MN helped conceive the study and assisted in revising the manuscript. AMD supervised PK, and was involved in conception and design of study, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission. All authors read and approved the final manuscript.

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Abstract

Introduction: Insulin is used to treat hyperglycaemia in critically ill patients but can cause hypoglycaemia, which is associated with poorer outcomes. In health glucose-dependent insulinotropic polypeptide (GIP) is a potent glucose-lowering peptide that does not cause hypoglycaemia. The objectives of this study were to determine the effects of exogenous GIP infusion on blood glucose concentrations, glucose absorption, insulinaemia and gastric emptying in critically ill patients without known diabetes.

Methods: Twenty ventilated patients (Median age 61 range [22 – 79] years, APACHE II 21.5 [17-26], BMI 28 [21-40] kg/m2) without known diabetes were studied on two consecutive days in a randomised, double blind, placebo controlled, cross over fashion. Intravenous GIP (4pmol/kg/min) or placebo (0.9% saline) was infused between T=-60 to 300min. At T0, 100ml of liquid nutrient (2kcal/ml) and containing 3-O-Methylglucose (3-OMG), 100mcg of Octanoic acid and 20 MBq Tc-99m Calcium Phytate, was administered via a nasogastric tube. Blood glucose and serum 3-OMG (an index of glucose absorption) concentrations were measured. Gastric emptying, insulin and glucagon levels and plasma GIP concentrations were measured.

Results: While administration of GIP increased plasma GIP concentrations three- to four-fold (T=-60 23.9 (16.5-36.7) vs. T=0 84.2 (65.3-111.1); P<0.001) and plasma glucagon (iAUC300 4217 (1891 – 7715) vs. 1232 (293 – 4545) pg/ml.300min; P=0.04), there were no effects on postprandial blood glucose (AUC300 2843 (2568-3338) vs. 2819 (2550-3497) mmol/L.300min; P=0.86), gastric emptying (AUC300 15611 (10993-18062) vs. 15660 (9694-22618) %.300min; P=0.61), glucose absorption (AUC300 50.6 (22.3-74.2) vs. 64.3 (9.9-96.3) mmol/L.300min; P=0.62) or plasma insulin (AUC300 3945 (2280-6731) vs. 3479 (2316-6081) mU/L.300min; P=0.76).

Conclusions: In contrast to its profound insulinotropic effect in health, the administration of GIP at pharmacological doses does not appear to affect glycaemia, gastric emptying, glucose absorption or insulinaemia in the critically ill patient.

Introduction

Hyperglycaemia frequently occurs in the critically ill patient, is exacerbated by feeding, and is associated with adverse outcomes [1, 2]. Outcomes appear particularly poor in patients without pre-existing diabetes, which accounts for the majority of critically ill patients with hyperglycaemia [1, 3-6]. When blood glucose concentrations are elevated, current guidelines recommend administering exogenous insulin, which is associated with substantial risks of hypoglycaemia and perturbations in blood glucose [4, 7, 8]. Both hypoglycaemia and glycaemic variability may be more harmful than hyperglycaemia [9-11]. Accordingly, in hyperglycaemic critically ill patients who are not known to have diabetes there is a compelling rationale to maintain blood glucose within a narrow range that does not cause hypoglycaemia and limits blood glucose variability [4, 12].

The 'incretin' effect refers to the much greater insulinotropic response to an oral/enteral, when compared to an intravenous, glucose load. The incretin effect is accounted for by incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are secreted from the small intestine in response to nutrient exposure [4]. GLP-1 stimulates insulin and suppresses glucagon secretion [13]. GIP is also insulinotropic but, in contrast, may stimulate glucagon secretion, particularly at a lower blood glucose [14]. Importantly, the effects of GLP-1 and GIP are glucose-dependent, so that exogenous administration of GLP-1 and/or GIP, even at pharmacological doses, does not cause hypoglycaemia [14]. For this reason there is considerable interest in their potential use in the management of hyperglycaemia in the critically ill patient [4, 15].

Our group has reported that exogenous GLP-1 retains its potent glucose-lowering effect in the critically ill during enteral feeding as it stimulates insulin secretion and slows gastric emptying [16-18]. Slower gastric emptying may however be undesirable, particularly in relation to the potential to exacerbate gastroesophageal reflux [17] and compromise enteral feeding [19, 20].

In health, 'physiological' doses of GIP (~1 pmol/kg/min) are well tolerated and 'pharmacological' doses (≥ 1.5 pmol/kg/min) may accelerate gastric emptying [21], with even greater doses (~4 pmol/kg/min) having potent insulinotropic effects [22-

24]. Additionally, GIP may promote weight gain via increased glucose absorption and/or a trophic effect on adipose tissue [25].

The effects of GIP on insulin and glucagon are affected acutely by perturbations in glycaemia. For example at 'normal' (6-10 mmol/l) and 'low' (≈2.5 mmol/l) blood glucose concentrations exogenous GIP stimulates glucagon secretion and has negligible effects on insulin secretion, whereas at elevated blood (≥12.0 mmol/l) glucose concentrations GIP appears to have no effect on glucagon secretion and is profoundly insulinotropic [22, 26]. Given that GIP has a bi-directional glucose-dependent effect on glucagon secretion and has been reported to have a 'stabilizing' effect on glycaemia in patients with type 2 diabetes [27], exogenous GIP could potentially reduce glycaemic variability in this cohort.

Our group has reported that in the critically ill, GIP at a dose considered slightly above postprandial physiological concentrations (2 pmol/kg/min) when administered with another potent insulinotropic hormone, GLP-1, does not have an additive glucose-lowering effect [20]. However the effects of GIP when administered as a sole agent at doses that are pharmacological in this group are unknown. Given that GIP may have a more favourable effect profile on gastric emptying and glucose absorption it is important to determine the effects of GIP in the critically ill patient.

We hypothesize that exogenous GIP will lower fasting and nutrient-stimulated glycaemia by stimulating insulin secretion, while modestly accelerating gastric emptying, and increasing the rate of glucose absorption. The objectives of this study were to determine the acute effects of exogenous GIP (4pmol/kg/min) on glycaemia, gastric emptying, glucose absorption, and insulin secretion during enteral nutrition in patients with acute critical illness-associated hyperglycaemia.

Methods

Subjects

Critically ill patients without known diabetes, and with blood glucose concentration(s) >7.1mmol/L when fasting and/or >10mmol/L during enteral feeding, and who were expected to remain mechanically ventilated via a tracheal tube for at least 48 hours, were studied between April and December 2012. All patients had an arterial catheter

in situ, which is routine care for ventilated patients within our unit, and this was used for blood sampling. Patients were excluded due to pregnancy, anaemia (haemoglobin <80g/L), age (<18 years), contraindication to enteral feeding, previous surgery on the small intestine, or any gastrointestinal surgery during their then current hospital admission.

Protocol

This was a prospective, randomised, double-blind, crossover study. Patients were studied on two consecutive days, on which they received intravenous (IV) GIP (4pmol/kg/min) or placebo (0.9% saline) at the commencement of the study period (T-60) (Fig.1). Patients were fasted for four hours and exogenous insulin (Actrapid) was ceased two hours prior to each study. Patient weight was measured using bed scales (MPWS, A&D Medical, Australia). Synthetic GIP (Bachem, Weil am Rhein, Germany) was reconstituted by the Royal Adelaide Hospital Department of Pharmacy in 0.9% saline. The Department of Pharmacy was also responsible for computer generated randomisation. While study drugs appeared identical, treatment blinding was ensured by the use of plastic coverings over all solutions. Study drugs were delivered through low absorbance tubing (Verasafe, Carefusion, California, USA) to prevent protein binding [16-18]. The randomisation schedule was kept in a locked facility within the Department of Pharmacy and the investigators had no access to the schedule during the study period. All solutions were given via a central venous catheter at 1mL/min using an infusion pump (Alaris, Cardinal Health, NSW, Australia). Sixty minutes after the study drug was commenced (i.e. at T0), a liquid nutrient 'meal' was administered via nasogastric tube over 5 minutes. The 'meal' contained 100 ml of TwoCal® (Abbott Nutrition, NSW, Australia, 2 kcal/ml), a mixed nutrient liquid containing carbohydrate (43%), fat (40%), and protein (17%), as well as 3g of 3-O-Methyglucose (3-OMG, Sigma-Aldrich, NSW, Australia) dissolved in 5ml water, 100 mcg of Octanoic acid (Sigma-Aldrich, Sydney, NSW, Australia), and 20 MBq Tc-99m Calcium Phytate (Radpharm Scientific, ACT, Australia). Patients were studied for 360 minutes (from T-60 to T300) in total during each study period.

This study was approved by the Research Ethics Committee of the Royal Adelaide Hospital and the protocol was registered with the Australian New Zealand Clinical

Trials Registry (ACTRN number 12612000488808). Patients were unconscious when enrolled and consent was therefore obtained from and signed by their next-of-kin.

Data collection

Arterial blood samples (5mL) were collected immediately prior to administration of the study drug (T-60) and the intragastric meal (T0), and at 15-minutely intervals from T0 to T60, and then at 30-minute intervals until T300, for measurements of serum 3-OMG and blood glucose concentrations. Samples for measurement of serum insulin were collected at T-60, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240 and 300 min, for serum glucagon at T-60, 0, 30, 60, 120, 180, 240 and 300 min and for plasma GIP at T-60, 0, 60, 120 and 300 min. Bloods were stored in ice at all times. Serum was separated by centrifugation within thirty minutes of completion of the study (3200 rpm for 15 mins at 4 degree Celsius) and then stored at -70C degrees until assayed. Expiratory breath samples were collected as previously described [17]. Left anterior oblique (45o) images were acquired using a mobile gamma camera (Digirad, California, USA) in 3-minute dynamic frames from T0-T300 minutes with patients positioned supine [28].

Blood glucose, glucose absorption and insulin, glucagon and glucose-dependent insulinotropic polypeptide

Blood glucose concentrations were measured and recorded immediately, by the investigators, using a blood gas analyser (ABL800 FLEX, Radiometer, Copenhagen, Denmark) [20]. The monosaccharide, 3-OMG, is absorbed from the small intestine via the same transporters as glucose, but is not metabolised [28, 29], and measurement of serum 3-OMG concentrations provides an accurate measure of glucose absorption in health and the critically ill [28, 29]. Serum 3-OMG concentrations were measured using liquid chromatography/mass spectroscopy, with an assay sensitivity of 0.0103mmol/l [17]. When the baseline (T-60) serum concentrations of 3-OMG on day 2 were greater than the assay sensitivity (i.e. fasting serum 3-OMG concentration >0.0103 mmol/l), the concentration at T-60 was referenced as zero for subsequent analysis [28].

Serum insulin was measured by enzyme-linked immunosorbent assay (ELISA) (10-1113, Mercodia, Uppsala, Sweden), which had an inter-assay coefficient of variation

(CV) of 5.4% and intra-assay CV of 2.7 [20]. Serum glucagon was measured via radioimmunoassay (RIA) (GL-32K, Millipore, Billerica, MA). Minimum detectable limit was 20 pg/ml, with inter-assay CV of 6.1% and intra-assay CV of 4.1 % [18]. Plasma total GIP was measured by radioimmunoassay (Perkin Elmer, Boston, MA, USA), with an inter-assay CV of 8.3% and intra-assay CV of 6.3 [20].

Glycated haemoglobin

HbA1c was determined using high performance liquid chromatography (HPLC) [20]. Unrecognised diabetes was defined as an HbA1c > 6.5% (48 mmol/mol) in patients with no history of diabetes [6].

Gastric emptying

Gastric emptying was measured using two different techniques: 1) Scintigraphy – although the 'gold-standard' the technique requires the availability of both a mobile gamma camera and a trained nuclear medicine technologist, and these could not be guaranteed to be available on every study day; and 2) Radioisotope (13C–Octanoic breath test) – which was available for every study day.

Gastric scintigraphy requires mixing of a radioisotope (20 MBq Tc-99m Calcium Phytate) with a 'meal' that is administered via nasogastric tube. A gamma camera then records images of the labelled meal, which indicates the percentage of the 'meal' remaining within the stomach at any time point. The greater percentage retained within the stomach the slower gastric emptying. Scintigraphic data were analysed by a nuclear medicine technologist (KLJ) blinded to the study conditions. Radioisotopic data were corrected for subject movement and radionuclide decay. A region of interest was drawn around the total stomach, gastric emptying curves generated over time and intragastric retention derived at 15 minute intervals from T=0-300 minutes.

The 13C–Octanoic breath test was performed as described [17, 28]. Data were expressed as the Gastric Emptying Coefficient (GEC), a global measure of gastric emptying, with a higher number indicative of more rapid emptying [17].

Statistical Analysis

Sample size was based on calculations that 20 patients would provide 80% power, at two-sided α-level of 0.05, to detect a minimum difference in 'postprandial' glycaemia (glucose levels in the blood) of 290 mmol/L.300min between groups, which was predefined as clinically significant, and was based on the within-patient standard deviation of glycaemia as mmol/L.300min [17].

While differences between GIP and placebo were distributed normally, most of the raw data were skewed. Accordingly, all data are presented as median [range] or (25th – 75th percentile), unless specified otherwise. Significance was determined using non-parametric Wilcoxon Signed-Ranks tests. Serum 3-OMG (glucose absorption), plasma insulin and blood glucose concentrations are presented as areas under the concentration curve (AUC), and calculated using the trapezoidal rule. Relative glucagon response was measured by the incremental area under the curve (iAUC) using the trapezoidal rule. The absolute glucagon change from baseline was used to remove intra-subject variation in baseline levels. As the maximal effects of gastric emptying were anticipated to occur in the first 60 min after the 'meal', this period was also chosen a priori for analyses. All reported P values are two-sided, with the 0.05 level selected to determine significance. When significant, multiple comparisons were adjusted for using the Bonferroni-Holm procedure. Data were evaluated for potential carry over and/or period effects by including the order variable in repeated measures analysis of variance (RM-ANOVA), however, there were no order-by-treatment interactions. Between subject Pearson correlations were calculated on each study visit separately between initial rate of gastric emptying (% gastric retention at T=60 mins as determined using scintigraphy) and each of glycaemia, insulin and 3-OMG absorption (the delta from 0-60 mins for each). Scatterplots were examined to assess the linearity of the relationship and Pearson's correlation was considered appropriate in each case. Steiger's Z2* test for difference between two dependent correlations was used to compare the correlations between the same outcomes between the two visits. Statistical analyses were performed using SPSS (Version 18.0). An independent professional biostatistician had access to all data and verified these analyses.

Results

Twenty-four patients were enrolled and no adverse effects (vomiting, hypoglycaemia, seizure or rash) were observed with the study drug. Blood results were also reviewed with no unexpected changes to haemoglobin, platelets, liver function tests and electrolytes. Four patients failed to complete both study days due to tracheal extubation (2 patients), withdrawn consent (1 patient) and migration of the feeding tube into the small intestine (1 patient). Data from these patients were not included in the analyses. Demographic details for patients completing the study are summarised in table 1. One patient was diagnosed with unrecognised diabetes with an HbA1c of 9.1%. Peak fasting and peak postprandial glucose concentrations, along with administered medications, sedation score and temperature were also recorded (Table 1).

Blood glucose, glucose absorption and hormones

Baseline blood glucose concentrations were similar on both days (T=-60: GIP 7.5 (6.5 – 9.5) vs. control 7.6 (7.0 – 9.4) mmol/L; P=0.68). GIP had no effect on blood glucose before the 'meal' (at T=0: 8.1 (9.6 – 9.0) vs. 7.8 (6.8 – 9.0) mmol/L; P=0.53). There was a rise in blood glucose concentration after the meal (Fig. 2A), peaking between 60-90 minutes, but GIP had no effect on either peak glucose concentrations (9.4 (8.3 – 11.9) vs. 9.8 (8.4 – 11.8) mmol/L; P = 0.73) or the overall glycaemic response (AUC300 2843 (2568-3338) vs. 2819 (2550-3497) mmol/L.300min; P=0.86). Data were similar when the patient with unrecognised diabetes was excluded (AUC300 2991 (2469-3639) vs. 2781 (2578-3738) mmol/L.300min P = 0.74). Glucose absorption was unaffected by GIP administration (AUC300 50.6 (22.3-74.2) vs. 64.3 (9.9-96.3) mmol/L.300min; P = 0.62, Fig. 2B).

Insulin concentrations were similar at baseline on both study days (T=-60: 7.9 (4.8 – 12.0) vs. 6.4 (2.9 – 13.5) mU/L; P = 0.75). There was a postprandial rise in insulin concentrations peaking between 60-90 minutes. Overall insulin response was not affected by GIP (AUC300 3945 (2280-6731) vs. 3479 (2316-6081) mU/L.300min; P=0.76, Fig. 2C). Plasma GIP concentrations were comparable at baseline (T=-60: 23.9 – (16.5 – 36.7) vs. 23.0 (15.6 – 41.9) pmol/L; P = 0.96) and the exogenous GIP infusion resulted in a three- to four-fold increase above physiological concentrations (P<0.001, Fig. 2D).

Glucagon concentrations were also similar at baseline (T=-60: 104.5 (85.1 – 236.6) vs. 115.7 (85.8 – 287.6) pg/ml; P = 0.37) and prior to the meal (T=0: 128.5 (99.4 – 290.8) vs. 112.5 (82.8 – 292.9) pg/ml;P = 0.08). However the postprandial increment was significantly increased with GIP as compared to control (iAUC300 4217 (1891 – 7715) vs. 1232 (293 – 4545) pg/ml.300min; P=0.04, Fig. 2E).

Gastric emptying

Paired scintigraphic data were collected in 18 patients and breath test data were available for all patients. Using scintigraphy 100% of the meal remained in the stomach at T=300min in one patient on both study days and in two other patients during either GIP or placebo, indicative of markedly delayed gastric emptying.

GIP had no effect on intragastric retention at 60 min after the meal (T=60: 80 (66 – 89) vs. 84 (60 – 96)%; P = 0.88), at the study end (T=300: 26 (10 – 63) vs. 37 (7 -92) %; P = 0.33), or the 'overall' gastric emptying rate as determined using scintigraphy and breath test techniques (Fig. 3A & 3B).

Relationships

The change in blood glucose was related to gastric emptying, with the more rapid the emptying the greater the glycaemic excursion during placebo (r=0.85; P < 0.01) and GIP (r=0.48; P = 0.04), with the correlation significantly stronger during placebo (z = 2.1; P = 0.04). There was a close relationship between 3-OMG concentrations (glucose absorption) and gastric emptying during both placebo and GIP (Fig. 4). However the relationship was significantly stronger during placebo day (z = 3.1, P < 0.01). Relatively more rapid gastric emptying was also associated with increased insulin secretion during placebo (r=0.48; P = 0.04) and GIP (r = 0.47; P < 0.05) with no difference between placebo and GIP (z = 0.02, P = 0.98).

Discussion

This study indicates that in patients with critical illness associated hyperglycaemia acute intravenous administration of GIP at pharmacological doses has no insulinotropic activity, does not reduce elevated blood glucose concentrations and does cause a significant post prandial rise in glucagon.

The mechanism(s) underlying the absence of a glucose-lowering effect of GIP are uncertain. Based on the known effects of GIP in ambulant populations it is likely that the preceding acute glycaemic disturbance associated with critical illness is important. Chronic hyperglycaemia has been shown to profoundly diminish the insulinotropic effect of GIP, i.e. the insulinotropic effect is almost abolished in patients with longstanding hyperglycaemia [24, 30, 31]. In patients with type 2 diabetes, four weeks of intensive insulin therapy aiming for 'near-normal' glycaemia partially re-established the insulinotropic properties of GIP [32]. While the duration of normo- or hyperglycaemia required to modify the response to GIP in humans remains to be determined, in cell cultures as little as 24 hours exposure to glucose concentrations >11 mmol/L leads to a substantial down regulation of GIP receptors on β-cells [33]. However, with respect to this study, in the absence of data from patients without hyperglycaemia, this hypothesis is difficult to prove.

The critically ill patients in this study were studied relatively early in their admission and the objective prior to the intervention was to restrict glucose concentrations to <10 mmol/L while in ICU. These features are consistent with the concept that the magnitude and duration of hyperglycaemia required to attenuate the insulinotropic effect of GIP in humans is relatively modest. While hyperglycaemia may possibly be an important modulator, the possibility that the response to GIP is caused by critical illness per se cannot be excluded. Increased secretion of cytokines and other counter-regulatory hormones are prominent features of critical illness-associated hyperglycaemia [1, 4] and it is possible these cytokines down regulates responsiveness to GIP in the critically ill independent of hyperglycaemia.

Other reasons for the lack of glucose lowering effect may be due to the effect of GIP on glucagon. Exogenous GIP is known to be glucagonotropic at 'normal' and 'low' blood glucose concentrations, therefore the rise in levels of glucagon within this study may have contributed to the absence of blood glucose lowering.

It has been reported in healthy subjects that exogenous GIP attenuates postprandial glycaemia while mildly accelerating gastric emptying [21], but in this study gastric emptying was unaffected by GIP during critical illness. A possible explanation is that

the acceleration of gastric emptying observed in the former study may have resulted from the insulinotropic effects of GIP, which, by lowering blood glucose concentrations, had a mild gastrokinetic effect, given systemic glycaemia is a major determinant of emptying rate [34]. However, blood glucose concentrations were unaffected in the study population and therefore it was somewhat expected that the effect of GIP on gastric emptying rate would be marginal.

The effect of GIP on nutrient absorption in the critically ill was of particular interest. Glucose absorption is markedly diminished in the critically ill patient and down regulation of the sodium-glucose co-transporter (SGLT-1) appears to be pivotal [29, 35]. In isolated mice jejunum, GIP increases glucose transport across the lumen, via up regulation of SGLT-1 [25]. In this study glucose absorption did not appear to be affected by GIP. However, an effect of GIP on nutrient absorption cannot be completely dismissed because nutrient was delivered into the stomach and small intestinal nutrient absorption can only be accurately measured when nutrient is delivered distal to the pylorus [35]. That the relationship between glucose absorption and gastric emptying was weaker during GIP suggests that factors distal to the pylorus may be relevant. Furthermore, the study period was relatively short and may have been insufficient to detect any effect on of SGLT-1 expression and subsequent functional (absorptive) outcomes.

A particular strength of this study is that the cohort had features consistent with acutely impaired glucose tolerance, although one patient was subsequently shown to have unrecognised type 2 diabetes. In addition, median blood glucose concentrations were ~8 mmol/L, which should have been sufficient to stimulate an insulinotropic effect of GIP [13]. For these reasons it is likely that the lack of effect observed represents a true observation.

There are however limitations to this study. Only a single dose of GIP was tested, and it cannot be assumed that at greater doses glycaemia will remain unaffected. However, GIP administered at 4 pmol/kg/min has substantial biological effects in both health and patients with diabetes, consistent with the concept that this amount reflects a potent pharmacological dose [21, 22, 24, 31, 36, 37], and even at half the dose administered in this study (2 pmol/kg/min) it is reported that GIP accelerates

gastric emptying [21], suggesting that the dose chosen was sufficient to have a pharmacological effect. Additionally, insulin levels were measured as opposed to C-peptide, which may be a better marker of endogenous insulin production, as C-peptide analysis was cost prohibitive.

In the current study a profound three to four fold increase in plasma GIP concentrations were evident. This increase in plasma concentration is similar to previous studies where a significant effect has been shown with GIP administration in both health [21] and patients with diabetes [37]. While some studies using a similar dose have reported greater increases in plasma GIP concentrations [24, 31] this may well be explained by the different assay techniques. Nonetheless, there is the possibility that achieving greater GIP concentrations may affect glycaemia differently.

The number of patients studied was relatively few, such that it may be underpowered to show a difference in insulin and 3-OMG concentrations. There was also substantial heterogeneity between patients in regards to their diagnosis and the duration of ICU admission. However, the capacity of GLP-1 to affect glycaemia has been observed using smaller cohorts [16, 38]. The exposure to exogenous GIP was relatively short (6 hours) and it remains possible, albeit intuitively unlikely, that more prolonged exposure to GIP would reveal an insulinotropic effect.

The patients within this study had only moderate hyperglycaemia. There is a possibility that clamping blood glucose at a higher concentration (e.g 12 mmol/L) may have lead to administration of GIP at 4 pmol/kg/min causing a greater insulinotropic effect. Finally, synthetic GIP currently remains an expensive product, which limits its use to proof-of-principle studies.

Along with these limitations, other factors may influence blood glucose levels such as catecholamine infusions, corticosteroid use, depth of sedation and body temperature. These variables are common within the critical care population, and may be confounders with respect to this study.

Several different groups have evaluated the effects of synthetic GLP-1, or its agonists, on glycaemia in the critically ill with- and without- antecedent type 2 diabetes and there is consistent evidence that GLP-1 has a prominent glucose-lowering effect [16-18, 39]. When evaluating this study with our previous observation, that GIP (2 pmol/kg/min) had no additive insulinotropic effect in the critically ill patient when administered in combination with GLP-1, it appears that future studies should focus on the use of GLP-1 or its agonists rather than GIP. These observations should not however be extrapolated to the potential use of dipeptidyl peptidase-4 (DPP-4) inhibitors (which inhibits the enzyme that inactivates GIP and GLP-1) to treat hyperglycaemia in the critically ill patient, as the efficacy of DPP-4 inhibitors may result in part from increases in intestinal and portal blood GLP-1 and GIP concentrations [40].

Conclusions

In critically ill patients an acute infusion of GIP at 4 pmol/kg/min had no effect on glycaemia, gastric emptying, glucose absorption, insulin or glucagon secretion. Because the magnitude and duration of hyperglycaemia required to attenuate the insulinotropic effect of GIP appears to be relatively modest future evaluation of the use of incretin-based approaches in the critically ill should focus on GLP-1 and its agonists.

Key messages

- In health glucose-dependent insulinotropic polypeptide (GIP) is a potent insulinotropic hormone leading to glucose-lowering
- In the critically ill patient the effects of GIP are not apparent

List of abbreviations

3-OMG: 3-O-Methylglucose

ACTRN: Australian and New Zealand Clinical Trials Number

AUC: Area Under the Curve

CV: Coefficient of Variation

DPP-4: Dipeptidyl Peptidase-4

ELISA: Enzyme-Linked Immunosorbent assay

GEC: Gastric Emptying Coefficient

GIP: Glucose-dependent Insulinotropic Polypeptide

GLP-1: Glucagon-like Peptide 1

HbA1c: Glycated Haemoglobin

HPLC: High Performance Liquid Chromatography

IV: Intravenous

MBq: Megabecquerel

RM-ANOVA: Repeated Measures Analysis of Variance

SGLT-1: Sodium-Glucose co-transporter 1

TC: Technetium

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Tables

Table 1: Patient characteristics. Data are presented as median (range), APACHE (Acute Physiology and Chronic Health Evaluation), Richmond Agitation Sedation Scale (RASS); n = 20. *Patients were on multiple medications during the course of the study

Age (years)	62 (22 – 79)
Sex	Male: 12 Female: 8
Body Mass Index (kg/m^2)	28 (21 – 40)
APACHE II (score)	21.5 (17 – 26)
Length of ICU Admission prior to study day 1 (days)	3.0 (1 – 16)
Glycated hemoglobin (HbA1c)	
%	5.9 (5.3 – 9.1)
mmol/mol	40.5 (34 – 76)
Calories delivered in previous 24 hours (kcal)	885 (0 – 1680)
Feed Tolerant (patients)	14
Blood Glucose Concentration (mmol/l)	
Peak Fasting	9.5 (6.6 – 14.2)
Peak Post Prandial	10.7 (7.9 – 17.9)
Medications*	
Catecholamines	10
Noradrenalin	10
Adrenalin	1
Opiates	11
Fentanyl	10
Oxycodone	1
Sedatives	16
Propofol	14
Midazolam	3
Ketamine	1
Dexmedetomidine	1
Insulin	8
Peak dose (units/hr)	5.5 (2.5 – 10.5)
Dose in previous 24 hours (units)	42.4 (15.0-117.0)
Corticosteroid	7
Hydrocortisone dose (or equivalent) on study day (mg/day)	200 (50 – 1000)
RASS Sedation Score	-4 (-2 – -5)
Body temperature (^oC)	37.0 (31.7 – 38.5)
Diagnosis Category	
Neurology	6
Trauma	4

Respiratory	3
Sepsis	2
Cardiovascular	2
Other	3

Figures

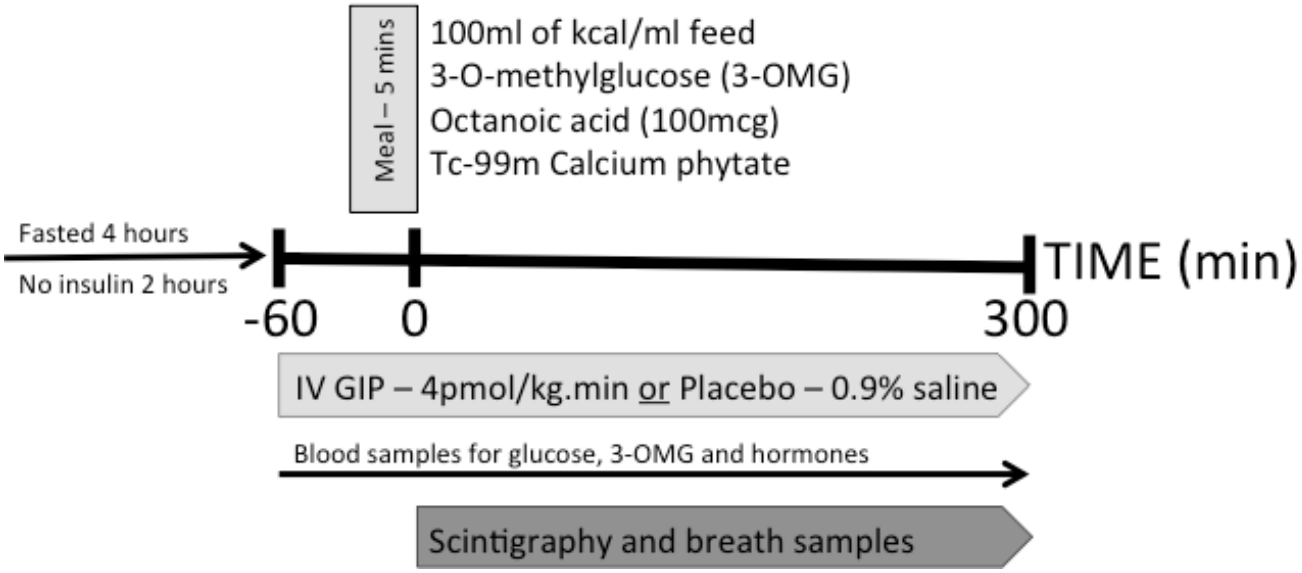

Figure 1: Protocol of the study

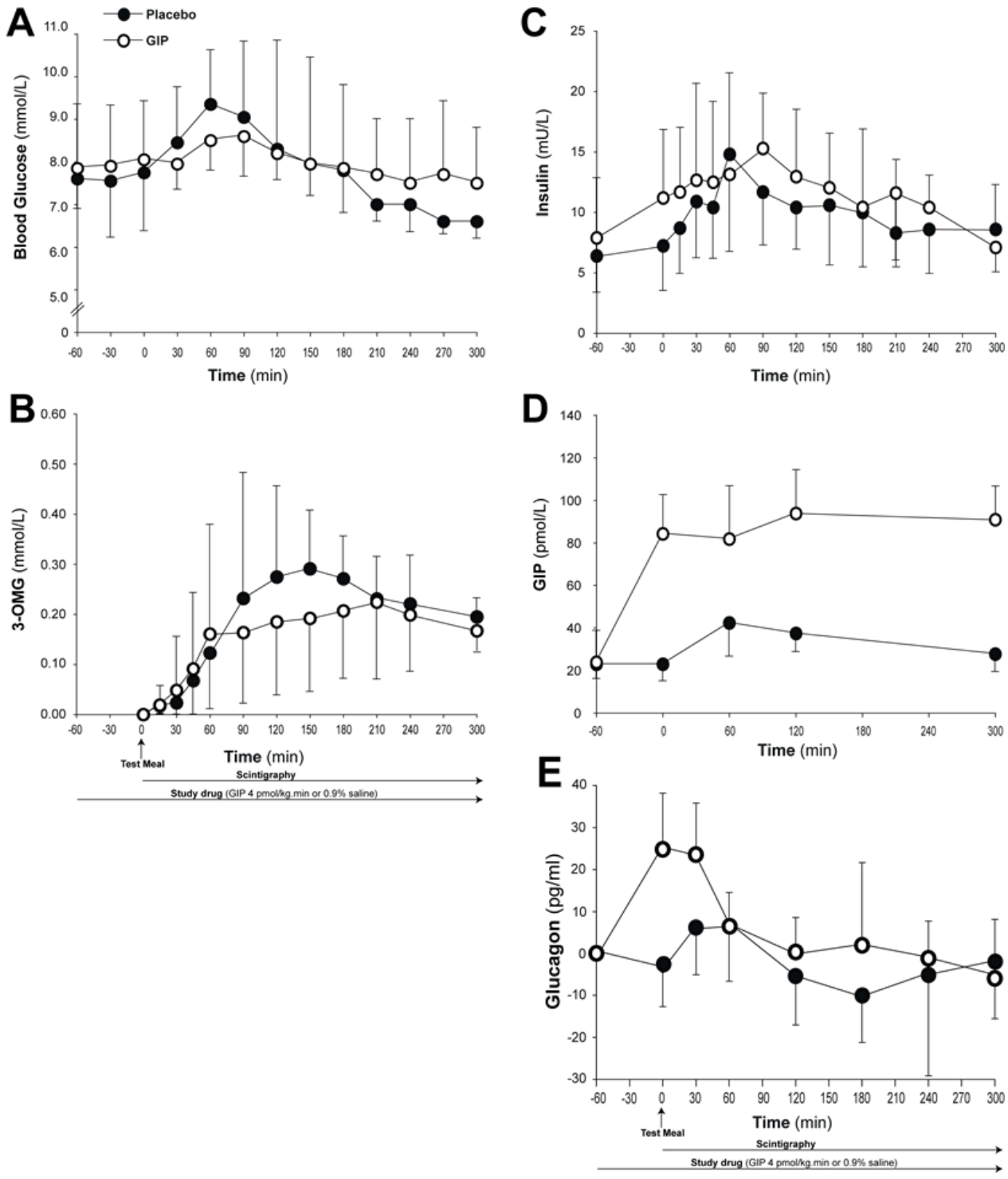

Figure 2: The effects of glucose-dependent insulinotropic polypeptide (GIP) (4 pmol/kg/min) on: A. Glycaemia ($AUC_{-60-300}$: GIP: 2843 (2468 – 3639) vs. control: 2819 (2578 – 3788) mmol/L.300min; P = 0.86); B. Glucose absorption (Serum 3-O-Methylglucose) (AUC_{0-300}: 50.6 (22.3 – 74.2) vs. 64.3 (9.9 – 96.3) mmol/L.300min; P = 0.62). C. Insulin concentrations ($AUC_{-60-300}$: 3945 (2280 – 6731) vs. 3479 (2499–

5658) mU/L.300 min; P = 0.76). D. GIP concentrations (* P < 0.001; Bonferroni-Holm correction for all time points) E. Glucagon concentrations ($iAUC_{-60-300}$ 4217 (1891 – 7715) vs. 1232 (293 – 4545) pg/ml.300min; P=0.04).

Data are median (25^{th} – 75^{th} percentile); analysed using Wilcoxon Signed Rank Test; n = 20.

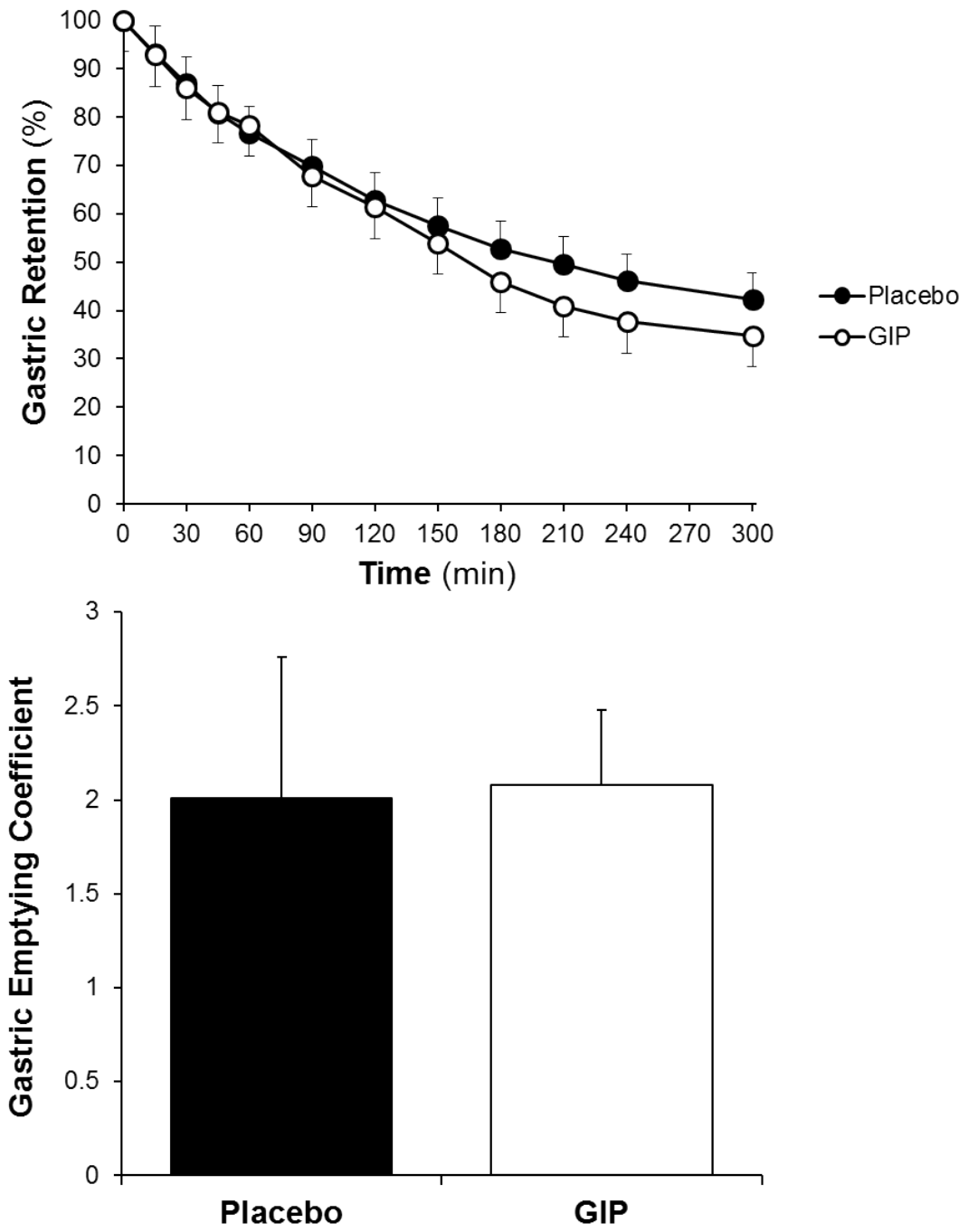

Figure 3: Gastric Emptying. The effect of glucose-dependent insulinotropic polypeptide (GIP) on gastric emptying as measured using: A. Retention of gastric contents over time (scintigraphic technique) ($AUC_{0\text{-}300}$: GIP: 15611 (10993 – 18062) vs. placebo: 15660 (9694 – 22618) %.300 min; P =0.61; n = 18); and B. Gastric Emptying Coefficient (GEC) (labelled breath test) (GEC: 1.98 (1.60 – 2.50) vs. 2.01 (1.14 – 2.81); P = 0.99; n = 20).

Data are median (25^{th} – 75^{th} percentile); analysed using Wilcoxon Signed Rank Test.

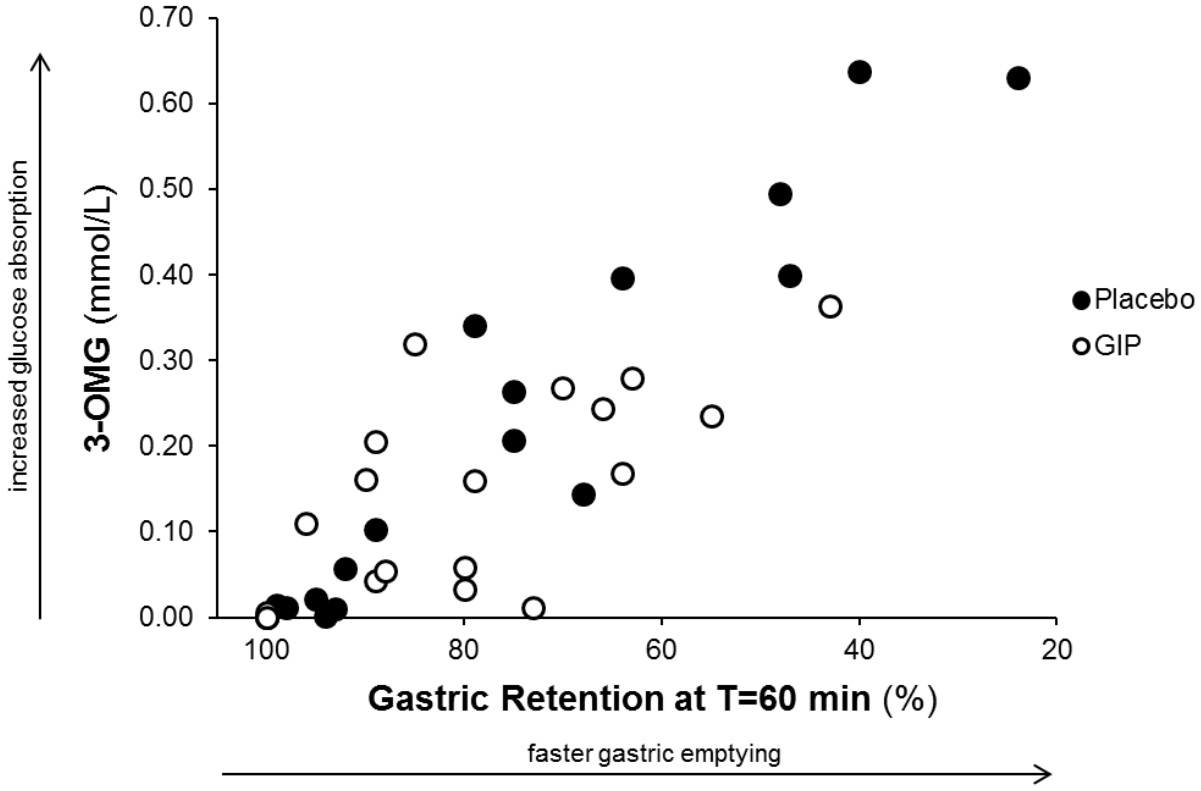

Figure 4: Relationship between glucose absorption and gastric emptying. The relationship between 3-OMG concentrations (glucose absorption) and gastric emptying (retention at T=60; scintigraphy) during GIP ($r=0.66$; $P < 0.01$) and placebo ($r=0.95$; $P <0.01$).

Data are analysed between subject using Pearson correlations; n = 18

2.5 CONCLUSIONS

2.5.1 *Introduction*

At the community level, there is a high and, in many countries, increasing prevalence of type 2 diabetes, which is intuitively likely to result in an increasing prevalence of critically ill patients with pre-existing type 2 diabetes. During critical illness disorders of glucose metabolism, i.e. hyperglycaemia, hypoglycaemia and glycaemic variability, are associated with adverse outcomes, including increased mortality. Accordingly, management of glycaemia in all critically ill patients, both those with and without pre-existing type 2 diabetes, is important.

2.5.2 *Contribution of the work described in this thesis to the understanding of the prevalence of both recognsied and unrecognised diabetes in the hospital and critical care setting*

The prevalence of diabetes is rising worldwide, with an estimated increase from 8.5% to 12.0% in Australia over the last 15 years [1]. The prevalence of recognised and unrecognised diabetes is dependent on the definitions used and the population studied. The diagnostic criteria used by the American Diabetes Association (ADA) and the World Health Organization (WHO) outline four criteria (glycated haemoglobin, fasting blood glucose, post prandial blood glucose and random blood glucose), which reflect different physiological phenomena [2, 3]. The literature review in Chapter 2.2 provides summary data regarding the prevalence of type 2 diabetes in both the hospital and critical care settings. Additionally, it highlights the lack of information relating to unrecognised diabetes in both the hospital and intensive care settings. Given the inconsistent diagnosis of unrecognised diabetes, the prevalence is likely to be even higher then stated.

2.5.3 *Contribution of the work described in this thesis to the understanding of the effect of liberal glucose targets in treating type 2 diabetes in the critical care setting*

While the optimal glucose concentration during critical illness is unknown, management of hyperglycaemia, particularly in those with pre-existing diabetes, is challenging. The literature review in Chapter 2.2 outlines observational data, post-hoc analysis of interventional studies and studies measuring chronic glycaemia as a dynamic variable which suggest that patients with pre-existing diabetes may warrant

higher blood glucose targets than patients with previously 'normal' glucose metabolism. The work outlined in Chapter 2.3 was the first to study a cohort of patients and prospectively allocate different blood glucose targets based on pre-existing chronic hyperglycaemia. It provides preliminary evidence, albeit with the limitations of a small cohort studied, of the feasibility and safety of this approach.

2.5.4 *Contribution of the work described in this thesis to the understanding of the effect of Glucose-dependent Insulinotropic Polypeptide (GIP) in treating hyperglycaemia in the critically ill patient*

Incretin based therapies (GLP-1 agonists and DPP-4 inhibitors) have recently been incorporated into standard care for ambulant patients with type 2 diabetes [4]. Incretin based therapies have been used in the critical care setting, with several studies evaluating the use of Glucagon-like peptide-1 (GLP-1), or its agonists [5-9]. However, the effect of exogenous GIP in this population was unknown. The rationale for investigating this peptide is that GIP may have a more favourable effect profile when compared to GLP-1. The study outlined in Chapter 2.4 was the first to examine the effects of exogenous GIP during critical illness. GIP had no effect on glycaemia, gastric emptying or glucose absorption in critically ill patients, arguing against its potential use.

2.6 FUTURE DIRECTIONS

2.6.1 *Prospective trials to determine the effect of liberal glucose targets in treating type 2 diabetes in the critical care setting*

The outcomes reported in Chapter 2.3 provide preliminary evidence that targeted blood glucose targets in critical care patients with diabetes are feasible and safe, with a reduction in glycaemic variability and hypoglycaemia. The study of Di Muzio, et al. using a similar study methodology, provided supportive observations [10], and the two studies were published as companion papers in the same issue of *Critical Care Medicine*. Based on these encouraging data, the Student is a Chief Investigator on the trial, Liberal glUcose Control in critically Ill patient with pre-existing type 2 Diabetes (LUCID): a phase IIB multi-centre randomised control trial, that has commenced enrolment and should be completed by December 2018 (Australian New Zealand Clinical Trials Registry Number 12616001135404).

This trial is a new collaboration between 12 Intensive Care Units across Australia and New Zealand, with the Royal Adelaide Hospital being the lead site and coordinating centre of this multi-centre trial. This study will evaluate the physiological and clinical impact of a 'liberal' approach to glucose control (commencing insulin for a blood glucose ≥ 14 mmol/L and titrating to blood glucose 10-14 mmol/L), when compared to that of 'standard' care (commencing insulin for a blood glucose ≥ 10 mmol/L and titrating to blood glucose 6-10 mmol/L) in 450 patients with type-2 diabetes mellitus who are admitted to the intensive care unit. Outcomes will examine whether a 'liberal' protocol i) reduces incident hypoglycaemia, ii) reduces relative hypoglycaemia, recurrent hypoglycaemia and glycaemic variability and iii) improves patient centred outcomes such as 90-day all-cause mortality, 28-day ICU free days and 60-day hospital free days - with no signal of harm.

2.6.2 *Prospective trials to determine the effect of Glucose-dependent Insulinotropic Polypeptide (GIP) in treating hyperglycaemia in the critically ill patient*

Chapter 2.4 provides data that will inform future trials. An acute infusion GIP at 4 pmol/kg/minute had no effect on glycaemia or gastric empting in the critically ill patient. As such, future studies to further evaluate incretin based therapy should use GIP in greater doses or focus on GLP-1 and its agonists.

2.6.3 *The effects of other incretin based therapy in the management of patients with type 2 diabetes*

Recent data on the use of GLP-1 agonists in patients with type 2 diabetes who were at high cardiovascular risk, show that use of either liraglutide or semaglutide significantly reduced the rates of major adverse cardiovascular events (including death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke) [11,12]. Despite both drugs resulting in higher mean levels of serum amylase and lipase, the rates of pancreatitis were similar to placebo, suggesting noninferiority of the GLP-1 agonist. Until now, the use of GLP-1 (or its agonists) in the critical care setting has been limited to small studies [4]. With the prevalence of type 2 diabetes and the use of incretin therapy increasing, a large the study of their use and potential benefits in the critical care setting is warranted.

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Chapter 3:

Implications of critical illness for type 2 diabetes

3.1 INTRODUCTION

Stress hyperglycaemia describes critical illness associated transient hyperglycaemia that resolves with resolution of the critical illness. Typically it is a state of temporary insulin resistance and concomitant relative insulin deficiency. Given that stress hyperglycaemia resolves, it has not been thought to contribute to adverse effects on long term. However, other conditions of temporary glucose intolerance, such as gestational diabetes, can predict the development of diabetes. Early identification and treatment may result in a delay or reduce associated complications. Additionally, the impact of stress hyperglycemia on the risk of incident diabetes for survivors of critical illness remains unclear.

The systematic review and meta-analysis contained within chapter 3.2 evaluates the longitudinal risk of developing prediabetes and diabetes in critically ill patients with stress hyperglycemia. Observational studies of adult intensive care patients suffering stress hyperglycaemia and reporting incident diabetes or prediabetes were reviewed. Despite the statistical and clinical heterogeneity of the included studies, stress hyperglycaemia was found to be associated with an increased risk of both prediabetes and diabetes. Additionally, the clinical significance of these findings and the potential mechanisms are also addressed in this review.

The pathophysiology of stress hyperglycemia is inadequately defined. As previously noted, it is thought to signify both a temporary insulin resistance and an inadequate insulin secretory response to the degree of hyperglycemia, however previous work on this topic have been unable to generate insights into the mechanisms underlying progressive glucose intolerance. Furthermore, the rate of gastric emptying may also be an important risk factor for the development of prediabetes and diabetes given that gastric emptying is a critical determinant of absorption and postprandial glycaemia. The objectives of the study outlined in chapter 3.3 were to determine incident diabetes and prevalent prediabetes in survivors of critical illness with stress hyperglycemia and

the mechanisms underlying progression from temporary glucose intolerance through to prediabetes and diabetes.

3.1.1 *Objectives*

The objectives of the review and study that comprise this chapter were to (i) review previous observational data and evaluate the longitudinal risk of developing prediabetes and diabetes in critically ill patients with stress hyperglycemia (ii) prospectively determine incident diabetes and prevalent prediabetes in survivors of critical illness with stress hyperglycemia and (iii) explore the mechanisms underlying progression from critical illness related temporary glucose intolerance to diabetes and prediabetes should such a progression exist.

3.2 LITERATURE REVIEW

Stress Hyperglycemia in Critically Ill Patients and the Subsequent Risk of Diabetes: A Systematic Review and Meta-Analysis

Statement of Authorship

Title of paper	Stress Hyperglycemia in Critically Ill Patients and the Subsequent Risk of Diabetes: A Systematic Review and Meta-Analysis
Publication status	Published
Publication details	Ali Abdelhamid Y*, Kar P*, Finnis M, Phillips LK, Plummer MP, Shaw JE, Horowitz M, Deane AM. **Stress Hyperglycemia in Critically Ill Patients and the Subsequent Risk of Diabetes: A Systematic Review and Meta-Analysis**. Critical Care. 2016 Sep; 20(1):301-9 *Joint first authors

Principal Author

Name of Principal Author (Candidate)	Dr Palash Kar
Contribution to paper	Conceptualisation of work, assessed and screened all articles, extracted data and wrote manuscript
Overall percentage (%)	45 (Joint first author)
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature		Date	01/10/16

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
- The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr Yasmine Ali Abdelhamid		
Contribution to paper	Joint first author, assessed and screened all articles, extracted data and wrote manuscript		
Signature		Date	01/10/16

Name of co-author	Dr Mark Finnis		
Contribution to paper	Statistical analysis and edited the manuscript		
Signature		Date	08/10/16

Name of co-author	Dr Liza Phillips		
Contribution to paper	Evaluated and edited the manuscript		
Signature		Date	09/10/16

Name of co-author	Dr Mark Plummer		
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Signature		Date	01/10/16

Name of co-author	Associate Professor Jonathan Shaw		
Contribution to paper	Evaluated and edited the manuscript		
Signature		Date	21/10/16

Name of co-author	Professor Michael Horowitz		
Contribution to paper	Evaluated and edited the manuscript		
Signature		Date	01/10/16

Name of co-author	Associate Professor Adam Deane		
Contribution to paper	Conceptualisation of work, supervision and manuscript evaluation		
Signature		Date	01/10/16

Full title

Stress Hyperglycemia in Critically Ill Patients and the Subsequent Risk of Diabetes: A Systematic Review and Meta-Analysis

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Keywords

Critical care, Blood glucose, Hyperglycemia, Type 2 diabetes mellitus, Prediabetes, Meta-analysis

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Authors' Contributions

YA and PK were responsible for study concept and design, conducting the literature review, data collection, interpretation of the data, drafting the manuscript and approving the final version to be published.

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

LKP was responsible for interpretation of the data, revision of the manuscript for important intellectual content and approving the final version to be published.

MPP, JES and MH were responsible for revision of the manuscript for important intellectual content and approving the final version to be published.

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

YA, PK and AMD are guarantors of this work and take responsibility for the integrity of these data and the contents of the manuscript.

Ethics approval and consent to participate

The study did not need ethics approval because it was a retrospective analysis of anonymous data

Consent for publication

Not applicable

Availability of data and material

All data generated and analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors report no potential financial or non-financial competing interests relevant to this paper.

Abstract

Background: Hyperglycemia occurs frequently in critically ill patients without diabetes. We conducted a systematic review and meta-analysis to evaluate whether this 'stress hyperglycemia' identifies survivors of critical illness at increased risk of subsequently developing diabetes.

Methods: We searched Medline and Embase from inception to February 2016. We included observational studies evaluating adults admitted to the Intensive Care Unit (ICU) who developed stress hyperglycemia if they reported incident diabetes or prediabetes diagnosed ≥3 months after hospital discharge. Two reviewers independently screened titles and abstracts of identified studies and evaluated full text of relevant studies. Data were extracted using predefined data fields and risk of bias assessed using the Newcastle-Ottawa Quality Assessment Scale. Pooled odds ratio (OR) with 95% confidence interval (CI) for the occurrence of diabetes was calculated using a random-effects model.

Results: Four cohort studies provided 2923 participants, including 698 with stress hyperglycemia and 131 cases of newly diagnosed diabetes. Stress hyperglycemia was associated with increased risk of incident diabetes (OR 3.48; 95% CI 2.02-5.98; I2 = 36.5%). Studies differed with regard to definitions of stress hyperglycemia, follow-up and cohorts studied.

Conclusions: Stress hyperglycemia during ICU admission is associated with increased risk of incident diabetes. The strength of this association remains uncertain because of statistical and clinical heterogeneity among the included studies.

Background

'Stress hyperglycemia' is defined as a blood glucose concentration that, in health, would lead to a diagnosis of diabetes [1-3] and represents a state of temporary insulin resistance and concomitant relative insulin deficiency [4, 5]. While stress hyperglycemia is associated with greater illness severity and short-term mortality [2, 6, 7], it typically resolves, at least acutely, following recovery [8]. For this reason stress hyperglycemia has traditionally not been considered to have an adverse impact on long-term health. It is plausible, however, that critical illness uncovers latent insulin resistance and/or impaired pancreatic β-cell function, such that stress hyperglycemia identifies patients at risk of subsequently developing diabetes [9].

Transient hyperglycemia occurring in other contexts of physiological 'stress', such as pregnancy, is known to predict the development of diabetes [10-12]. Postpartum screening programs for women with gestational diabetes allow early identification of type 2 diabetes to delay or reduce the associated complications [13-15].

The impact of stress hyperglycemia on the risk of incident diabetes for survivors of critical illness remains unclear. We, therefore, performed a systematic review and meta-analysis of observational studies to evaluate the longitudinal risk of developing diabetes in critically ill patients with stress hyperglycemia. Our secondary objective was to evaluate the impact of stress hyperglycemia on the risk of prediabetes (impaired fasting glucose and/or impaired glucose tolerance).

Methods

We performed this meta-analysis in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) statement [16]. Methods and inclusion criteria were specified and documented in advance (Additional File 1).

Eligibility Criteria

Eligible studies met the following criteria: a) study design: retrospective or prospective controlled (case-control or controlled cohort); b) study population: adult patients (aged ≥ 18 years) admitted to an ICU; c) exposure: stress hyperglycemia, with normoglycemia during ICU admission as the reference exposure; and d)

outcomes: development of diabetes or prediabetes diagnosed ≥3 months after ICU discharge. Studies that reported diagnosis of diabetes only at ICU admission or shortly after ICU discharge (within 3 months) were excluded as they were deemed to be reporting rates of established, but previously undiagnosed, diabetes [2]. Studies that reported outcomes from acutely ill patients not admitted to an ICU were excluded. In studies with overlapping samples, we only included the largest study to avoid duplication of data. We only considered studies reported in English. No date or publication status restrictions were imposed.

Data Sources and Searches

A librarian and two reviewers (Y.A. and P.K.) searched Medline and Embase (from inception to February 2016). Searches included synonyms and combinations of the following terms: critical illness, intensive care, hyperglycemia, glucose, insulin, type 2 diabetes and prediabetes. Terms were truncated in order to capture variable terminology. The full search strategies are provided (Additional File 2). We applied no language restrictions during the searches. We also reviewed reference lists of retrieved papers to identify other potentially eligible studies not captured in the primary search.

Study Selection

Two reviewers independently screened titles and abstracts of all identified studies. Relevant studies were independently evaluated in full text for eligibility. Disagreements were resolved by consensus or by consultation with a third reviewer. In order to avoid duplications from several reports of the same study, a comparison was conducted across studies when needed, checking for authors, study location, sample sizes and outcomes.

Quality Assessment

Two reviewers independently assessed methodological quality using the 8-item Newcastle-Ottawa Quality Assessment Scale (NOS) [17]. Risk of bias was assigned based upon the number of NOS items deemed inadequate for each study: low risk of bias (0-1 item); medium risk of bias (2-3 items); high risk of bias (>3 items); very high risk of bias (no description of methods). Studies judged to be at high or very high risk of bias were to be excluded from the meta-analysis.

Data Extraction

Two reviewers independently extracted data from included studies using a standardized data collection form. Extracted information included: study characteristics (author, publication year, country, design, sample size); participant characteristics (age, sex, diagnosis, illness severity, mortality, body mass index [BMI], family history of diabetes, steroid use, and nutrition delivery); definition of stress hyperglycemia and method of detection; methods to exclude pre-existing undiagnosed diabetes; definitions of diabetes and prediabetes; methods to diagnose diabetes or prediabetes; duration of follow-up; odds ratios (ORs) for the development of diabetes and/or prediabetes with corresponding 95% confidence intervals (CIs); and any statistical adjustment performed for the competing risk of death.

Supplementary files were also examined for the purposes of data extraction. When necessary, we contacted authors of the included studies for additional information.

Data Synthesis and Statistical Analysis

OR (95% CI) was used as the measure of association between stress hyperglycemia and development of diabetes or prediabetes across the studies. We used the Cochran Q statistic ($p<0.1$) and the I^2 index to investigate the possibility of statistical heterogeneity [18]. Meta-analysis was performed using a random effects model and a pooled OR with 95% CI was calculated. We elected *a priori* to perform an additional subgroup analysis of studies that excluded patients with pre-existing unrecognised diabetes via the use of glycated haemoglobin (HbA1c) on ICU admission [19]. As there were only a small number of studies graphic representation of publication bias was not performed [20]. Analyses were performed using STATA, version 14.1 (Stata Corp).

Results

Study Selection

Our search yielded 2389 non-duplicate citations. 2331 were discarded (on the basis of title and abstract) because they did not meet inclusion criteria. Five additional records were identified from reference lists of relevant retrieved articles, with 63 articles evaluated in full text. Of these, 18 were not controlled studies; 23 did not assess a relevant outcome; 12 were not conducted in an ICU setting; two were duplicate

reports; two were not in English; and one did not include data on inpatient blood glucose levels. One conference abstract was excluded because it reported solely on patients after coronary artery bypass graft surgery, and was not deemed representative of the majority of patients admitted to ICU due to the elective nature of the surgery and its association with a short length of ICU stay. After these exclusions, four cohort studies remained and were included in the analysis (Figure 1). Because of the overlapping duration of recruitment periods for two studies from one centre [21, 22], the primary author was contacted and confirmed that each cohort contained different study participants.

Study Characteristics and Risk of Bias within Studies

Characteristics of the included studies [21-24] are summarised (Table 1). Three single centre studies recalled patients after ICU discharge to test for diabetes or prediabetes with an oral glucose tolerance test (OGTT) [21, 22, 24]. Additionally, one study performed HbA1c testing at ICU admission and 8 months after discharge, but this was not performed in all enrolled patients [24]. One study was a multi-centre database-record linkage study evaluating the risk of diabetes in patients with stress hyperglycemia who were emergency admissions to hospital [23]. Only the subgroup of patients admitted to ICU in this study was included.

In total, 2923 ICU survivors from four studies were included. Illness severity was inconsistently reported. Only one study reported ventilation rates and provided illness severity scores [24]. Three studies defined stress hyperglycemia as ≥7.8 mmol/l. The database linkage study used a higher threshold (≥11.1 mmol/l) [23]. The relationship between the timing of blood glucose measurement and the delivery of nutrition was not reported in any study. Three studies [21, 22, 24] defined diabetes and prediabetes according to published consensus criteria for plasma glucose and HbA1c [19]. The database linkage study [23] determined incident diabetes following registration with the national register.

Risk of bias within included studies is presented (Table 2). Three studies [21, 22, 24] were deemed to be at risk of incomplete outcome data due to the number and limited description of patients lost to follow-up. One study provided no description as to whether missing outcome data were equal across the stress hyperglycemia and

normoglycemia cohorts [24]. In general, stress hyperglycemia and normoglycemia cohorts were comparable in terms of age, sex and, when reported, nutrient delivery. However, when reported, the stress hyperglycemia cohorts had a higher BMI, more frequent family history of diabetes and greater illness severity. No data on the specific characteristics of the subgroup of patients admitted to ICU in the database linkage study [23] were provided. Finally, each study employed different methods to identify patients with pre-existing undiagnosed diabetes (Table 1). No study was deemed at overall high or very high risk of bias and, therefore, all four studies were included in the meta-analysis.

Stress Hyperglycemia and the Risk of Diabetes

Among the 2923 participants, 698 (23.9%) experienced stress hyperglycemia and 131 (4.5%) cases of incident diabetes were detected during follow-up. Stress hyperglycemia was associated with an increased risk of developing diabetes in survivors of critical illness, with low-moderate degree of heterogeneity between studies (Figure 2A).

No studies measured HbA1c levels on ICU admission for the majority of patients and so we were unable to perform our pre-specified subgroup analysis. We were unable to undertake further subgroup analyses to examine the effect of age, sex and diagnosis due to the small number of events and inconsistent reporting of this information.

Stress Hyperglycemia and the Risk of Prediabetes

Three studies [21, 22, 24] reported risk of developing prediabetes, defined according to the same criteria [19]. Among the 2923 participants, 221 (7.6%) cases of prediabetes were detected during follow-up. Stress hyperglycemia was associated with increased risk of developing prediabetes in survivors of critical illness, with moderate degree of heterogeneity between studies (Figure 2B).

Discussion

Main Findings

We undertook the first meta-analysis to examine the impact of stress hyperglycemia in survivors of critical illness. Our findings suggest that stress hyperglycemia identifies patients at increased risk for incident diabetes. In addition, stress

hyperglycemia also identified patients at increased risk of developing prediabetes, a well-accepted risk factor for type 2 diabetes with an annual conversion rate in ambulatory subjects of 5-10% [25]. Our observations are consistent with outcomes of other studies performed in non-ICU settings including patients following stroke [26], myocardial infarction [27, 28] and pneumonia [29] where comparable rates of incident diabetes following stress hyperglycemia were observed.

Clinical Implications

Our findings have substantial clinical significance. There usually exists a protracted period of time between the development of diabetes and its diagnosis, with microvascular complications often established at the time of diagnosis [30]. If stress hyperglycemia during critical illness identifies a population at risk of diabetes, an opportunity exists for early diagnosis and intervention to prevent long-term complications of diabetes. Readily available and cost-effective strategies, such as the use of metformin and lifestyle interventions including weight loss and exercise, exist to reduce progression to diabetes in at-risk populations. These strategies have been demonstrated to be effective in patients with prediabetes and in women with prior gestational diabetes [15, 31-33].

While general population screening programs for type 2 diabetes are not always cost-effective [34], targeted screening of high-risk groups, as is the case in gestational diabetes, improves health outcomes [35]. Our meta-analysis suggests that the risk of diabetes in ICU survivors with stress hyperglycemia is similar to the risk in women with gestational diabetes over comparable observation periods [10, 12]. Furthermore, survivors of critical illness often experience long-term physical problems [36-38], and therefore may have unique capacity to benefit from screening programs to identify prediabetes or diabetes.

Potential Mechanisms

Failure of pancreatic β-cells to meet insulin secretory demand in the face of diminished insulin sensitivity is fundamental to the pathogenesis of type 2 diabetes [39]. Several mechanisms appear to underlie stress hyperglycemia during critical illness including increased release of counter-regulatory hormones, altered insulin

receptor signalling due to inflammation, pancreatic β-cell inhibition and interventions such as administration of glucocorticoids or parenteral nutrition [1, 8, 40]. However, studies included in our meta-analysis also reported that in patients with stress hyperglycemia there was more often a family history of diabetes and higher BMI, suggesting that well accepted risk factors for diabetes also contribute to development of stress hyperglycemia. Mechanistically, it is highly plausible that one or more pre-existing disorders of insulin sensitivity and/or production result in predisposition to stress hyperglycemia during critical illness and may lead to subsequent development of diabetes.

We also speculate that additional mechanisms may be implicated in the progression to diabetes in survivors of critical illness. These include the reduction in physical activity post-ICU [37] and autonomic dysfunction, which affects more than half of ICU patients [41].

Strengths and Limitations

Strengths of our meta-analysis include the structured search, complete retrieval of the identified research and validated methods in accordance with the MOOSE statement. Included cohort studies were of reasonable methodological quality, particularly given the logistical challenges involved in studying these cohorts, and almost 3000 patients were included.

However, our study has limitations. We only included studies in English. We were also unable to exclude publication bias and negative studies may be missing, potentially resulting in overestimation of the effect size. Our meta-analysis reflects data from only four studies, which limits our certainty in the results [42]. In addition, along with moderate statistical heterogeneity, we observed considerable clinical heterogeneity between the studies, e.g. definitions of stress hyperglycemia, methods of outcome assessment and duration of follow-up differed.

Conceptually, stress hyperglycemia is defined by a glucose concentration normally indicative of diabetes (i.e. random blood glucose ≥11.1 mmol/l). However a strict definition has not been consistently applied, and whether a single elevated reading is sufficient, or documentation of more than one episode of hyperglycemia is required,

has yet to be established. Given that there was no corresponding data identifying that blood glucose concentrations were fasting or postprandial, three studies [21, 22, 24] used a relatively low threshold for stress hyperglycemia (≥7.8 mmol/l), which could underestimate the risk of diabetes. Conversely, the study which utilised a threshold of ≥11.1 mmol/l [23] only required a single elevated reading, which may not be sufficiently specific to identify risk because transient disturbances in blood glucose can occur during critical illness following administration of catecholamines or corticosteroids. Furthermore only two studies specifically excluded patients who received corticosteroids [21, 22].

Overall, the small number of incident events (diabetes) in our meta-analysis means that our point estimates have greater uncertainty [43] and our ability to assess the effects of age, sex and diagnosis on risk of diabetes is limited. In addition, some patients with undiagnosed diabetes may not have been recognised at baseline and could have been misclassified as incident diabetes cases. These patients would have been more likely categorised in the stress hyperglycemia group and this differential misclassification could bias toward inflating the estimates of risk for incident diabetes. Only one study formally tested all patients with an OGTT to exclude pre-existing diabetes [21]. However, gastric emptying is delayed during critical illness [44] and gastric emptying is a major determinant of oral glucose tolerance in health and diabetes [44, 45]. This has implications for the interpretation of the OGTT, such that identification of unrecognised diabetes using the OGTT in critically ill patients is uncertain. None of the studies measured HbA1c on admission for the majority of patients. HbA1c is a validated tool for the diagnosis of previously unrecognised diabetes in hospitalised and critically ill patients [46-48] and consensus guidelines now recommend the measurement of HbA1c in all hospitalised patients with hyperglycemia [49].

Individual study results were also likely influenced by management of missing data. Most studies had high rates of withdrawal and limited descriptions were provided of patients lost to follow-up. It is plausible that patients lost to follow-up were those who experienced greater illness severity and subsequent impaired mobility. These patients may have a higher risk of disturbed glucose metabolism and the true incidence of diabetes may have been underestimated. It is also possible that patients who develop

hyperglycemia during ICU admission are likely to receive more intense screening for diabetes after hospital discharge than those who remained normoglycemic throughout ICU admission [49]. Furthermore, in one study the duration of follow-up was short (8 months) and risk of incident diabetes may increase with period of observation [24]. Across the four studies included in our meta-analysis, the odds ratio for incident diabetes was observed to increase with increasing duration of follow-up. Only one study performed statistical adjustment for the competing risk of death [23].

There are also limitations to the generalisability of individual study results: information about illness severity is absent in most studies; only a small subset of patients was admitted to ICU in the large multi-centre study [23]; two single-centre studies [21, 22] included a high proportion of patients presenting with myocardial ischaemia; and one study reported high rates of parenteral nutrition administration [21]. We restricted our search to studies of patients admitted to ICU and our results may not reflect outcomes from acutely ill patients not admitted to ICU. Furthermore, the two studies that demonstrated the strongest relationship between stress hyperglycemia and subsequent incident diabetes [21, 22] were conducted in the same centre and this is a limitation of our findings. However, it is important to note that these studies had the longest duration of follow-up and were the only studies to recall patients regularly after ICU discharge and formally test for diabetes.

Implications for Research

Our meta-analysis supports the concept that stress hyperglycemia is a risk factor for incident diabetes in survivors of critical illness. A multi-centre, prospective cohort study with a follow-up period of several years would be required to precisely quantify this risk. Such a study should define stress hyperglycemia based upon repeated blood glucose measurements and in relation to nutrient delivery, as well as utilise routine measurement of HbA1c to exclude undiagnosed diabetes at baseline. Furthermore, studies which evaluate mechanisms underlying progressive glucose intolerance are required because such understanding is critical to guide intervention.

Conclusion

Stress hyperglycemia during ICU admission is associated with increased risk for incident diabetes. The strength of this relationship should be interpreted with caution because of statistical and clinical heterogeneity among included studies.

List of abbreviations

ICU: Intensive care unit

OR: Odds ratio

CI: Confidence interval

MOOSE: Meta-analysis of observational studies in epidemiology

NOS: Newcastle-Ottawa quality assessment scale

BMI: Body mass index

HbA1c: Glycated haemoglobin

OGTT: Oral glucose tolerance test

PC: Prospective cohort

RC: Retrospective cohort

SH: Stress hyperglycemia

IQR: Interquartile range

BG: Blood glucose

EN: Enteral nutrition

PN: Parenteral nutrition

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Table 1. Summary of included studies evaluating subsequent risk of diabetes in critically ill patients with stress hyperglycemia

First author, Year [Reference]	Study design, Location and Recruitment period	Follow-up duration	Participants	Recruitment: Number (Normal/SH); Male (%); Age in years, median (IQR)	Follow-up: Number completing, Normal (%) SH (%)	SH definition	Nutrition	Number of new cases of diabetes: Normal (%), SH (%)	Methods to: i) diagnose incident diabetes and ii) exclude baseline diabetes
Gornik, 2010 [21]	Single centre, PC, Croatia Jul 1998 – Jun 2004	5 years	Medical patients with no history of steroid use, pancreatitis, disturbed glucose metabolism or other endocrine disorder admitted to ICU.	1029 (669 / 360); Male 55%; Age, Normal 58 (19-86), SH 59 (22-87)	591 Normal 398 (67%), SH 193 (33%)	Venous BG in ICU > 7.7 mmol/l; measured twice per day with point-of-care blood gas analyser	EN & PN	47: Normal 14 (4%), SH 33 (17%)	i) Annual OGTT for 5 years[a] ii) History; OGTT 4-6 weeks after discharge
Gornik, 2010 [22]	Single centre, PC, Croatia Jan 2000 – Dec 2002	5 years	Patients admitted to ICU with sepsis, acute coronary syndrome and acute heart failure with no history of disturbed glucose metabolism or steroid use.	258 (168 / 90); Male 54%; Age, Normal 57 (48-65), SH 60 (49-65)	166 Normal 115 (69%), SH 51 (31%)	Random venous BG in ICU > 7.7 mmol/l on at least two occasions	Not stated	12: Normal 4 (3%), SH 8 (16%)	i) OGTT: follow up at least 5 years but frequency not specified[a] ii) History; absence of hyperglycemia before discharge
McAllister, 2014 [23]	Multi-centre, RC, Scotland Dec 2004 – Nov 2008	3 years	Patients aged ≥ 30 years with an emergency admission to hospital between 2004-2008.[b]	1828;[b] sex & age not specified for ICU subgroup	1828 Normal 1620 (89%), SH 208 (11%)[b]	Admission BG (first BG taken within 2 days of admission) ≥ 11.1 mmol/l	Not stated	48: Normal 37 (2%), SH 11 (5%)[b]	i) Record of new diagnosis in national register[c] between 31 days & 3 years after discharge ii) Record in national register[c] prior to admission or within 30 days of discharge; admission BG > 20 mmol/l
Van Ackerbroeck, 2015 [24]	Single centre, PC, Belgium Sep 2011 – Mar 2013	8 months	Patients aged 18-85 years admitted to a medical-surgical ICU for ≥ 48 hours. Patients with pancreatitis, known disturbed glucose metabolism and those using glucose-lowering drugs excluded.	385[d]; Male 66%; Age, Normal 56 (18-82), SH 62 (20-88)	338 Normal 92 (27%), SH 246 (73%)	Arterial BG > 140 mg/dl (> 7.8 mmol/l); measured using on-site blood gas analyser	EN & PN	24: Normal 4 (4%), SH 20 (8%)	i) OGTT +/- HbA1c 8 months after ICU admission[a] ii) History; medication review; +/- HbA1c [e]

PC prospective cohort, RC retrospective cohort, ICU intensive care unit, SH stress hyperglycemia, IQR inter-quartile range, BG blood glucose, EN enteral nutrition, PN parenteral nutrition, OGTT oral glucose tolerance test, HbA1c glycated haemoglobin

[a] Diabetes defined according to American Diabetes Association criteria: fasting plasma glucose ≥ 7.0 mmol/l or two-hour plasma glucose ≥ 11.1 mmol/l during a 75g OGTT performed as described by the World Health Organisation or HbA1c ≥ 6.5% (48 mmol/mol) [19].

[b] Only the subgroup of 1828 patients admitted to ICU is included in the analysis. The total number of patients included in the study is 86 634.

[c] Scottish Care Information – Diabetes Collaboration is a national register including >99% of people with diabetes in Scotland.

[d] Number of patients with normoglycemia and stress hyperglycemia in original cohort not stated.

[e] Admission HbA1c measured only in 45% of study population. HbA1c ≥ 6.5% (48 mmol/mol) considered diagnostic of diabetes.

Table 2. Risk of bias within included studies assessed by the Newcastle-Ottawa Quality Assessment Scale.

Study	**Selection** (maximum score 4★)				**Comparability of Cohorts** (maximum score 2★)	**Outcome** (maximum score 3★)			**Total Score & Risk of Bias**
	Representativeness of the exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest not present at start of study		Assessment of outcome	Duration of follow-up	Adequacy of follow-up	
Gornik, 2010 [21]	★	★	★	★	★	★	★	-	7, medium risk of bias
Gornik, 2010 [22]	★	★	★	-	★	★	★	-	6, medium risk of bias
McAllister, 2014 [23]	-	★	★	★	-	★	★	★	6, medium risk of bias
Van Ackerbroeck, 2015 [24]	★	★	★	★	★	★	-	-	6, medium risk of bias

Figures

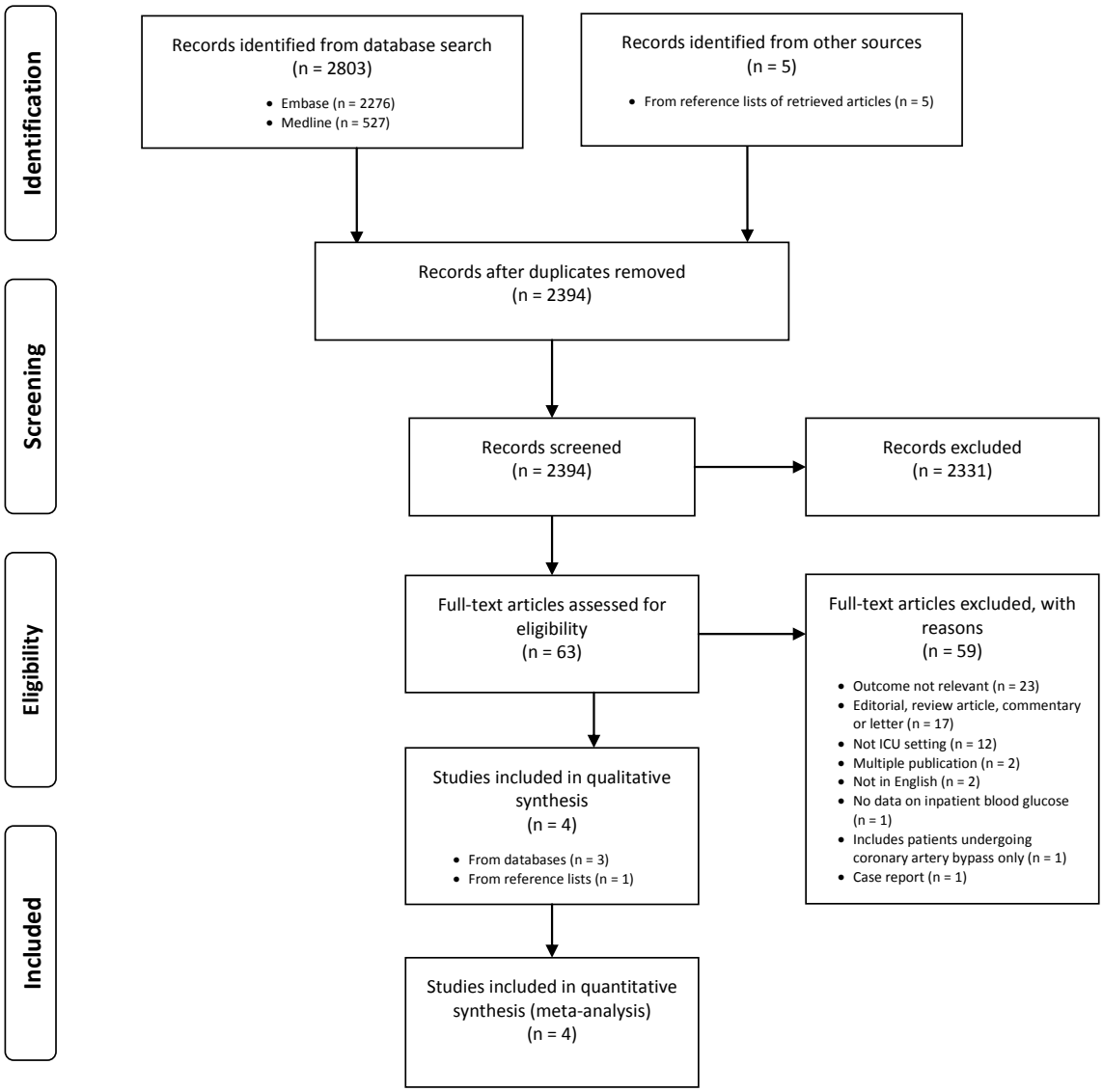

Figure 1: Flow diagram for selection of studies, ICU Intensive care unit

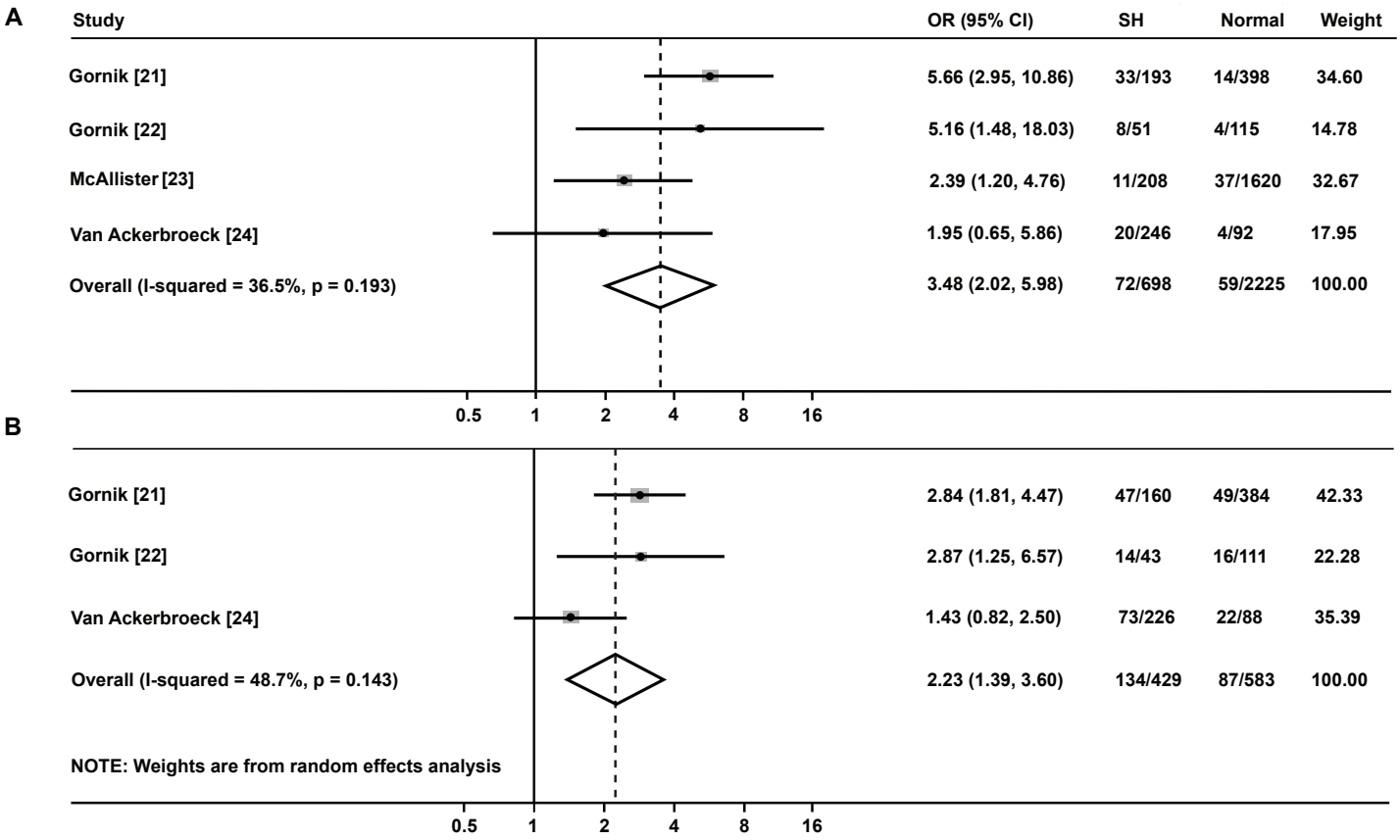

Figure 2a: Forest plot showing the risk of diabetes in critically ill adult patients with stress hyperglycaemia. 2b: Forest plot showing the risk of prediabetes in critically ill adult patients with stress hyperglycaemia. SH stress hyperglycaemia. Prediabetes was defined according to American Diabetes Association criteria: fasting plasma glucose 5.6–6.9 mmol/L (impaired fasting glucose), or 2-h plasma glucose during 75-g oral glucose tolerance test 7.8–11.0 mmol/L (impaired glucose tolerance), or glycated haemoglobin 5.7–6.4 % (39–46 mmol/mol) [19]

3.3 MANUSCRIPT

Incident diabetes in survivors of critical illness and mechanisms underlying persistent glucose intolerance: a prospective cohort study

Statement of Authorship

Title of paper	Incident diabetes in survivors of critical illness and mechanisms underlying persistent glucose intolerance: a prospective cohort study
Publication status	Submitted (Critical Care, November 2017)
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Principal Author

Name of Principal Author (Candidate)	Dr Palash Kar		
Contribution to paper	Conceptualisation of work, its realisation and its documentation. Collected and interpreted data and wrote manuscript.		
Overall percentage (%)	85		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/11/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
- The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of co-author	Dr Mark Plummer		
Contribution to paper	Assisted study design, data collection and revision of the manuscript for important intellectual content.		
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Full title

Incident diabetes in survivors of critical illness and mechanisms underlying persistent glucose intolerance: a prospective cohort study

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The author reports no conflicts of interest in this work

Clinical trial registration:

Australian New Zealand Clinical Trial Registry (ACTRN12614000449639)
Registered 1 May 2014

Abstract

Background: Stress-hyperglycaemia occurs frequently in critically ill patients and may be a risk factor for subsequent diabetes. The aims of this study were to determine incident diabetes and prevalent prediabetes in survivors of critical illness experiencing stress hyperglycaemia and to explore underlying mechanisms.

Methods: This was a prospective, single centre, cohort study. Consecutively admitted patients without diabetes who developed stress hyperglycaemia and survived to hospital discharge were eligible. On admission to ICU, HbA1c was measured. Participants returned at 3 and 12 months after ICU admission and underwent HbA1c testing and an oral glucose tolerance test (OGTT). Blood was also collected for hormone concentrations, while gastric emptying was measured via an isotope breath test. β-cell function was modeled using standard techniques.

Results: Consent was obtained from 40 patients (mean age 58 (standard deviation 10) years, HbA1c 36.8 (4.9) mmol/mol) with 35 attending the 3-month and 26 the 12-month visits. At 3 months, 13 (37%) had diabetes and 15 (43%) prediabetes. At 12 months, 7 (27%) participants had diabetes, while 11 (42%) had prediabetes. Mean HbA1c increased from baseline during the study: +0.7 (-1.2, 2.5) mmol/mol at 3 months and +3.3 (0.98, 5.59) mmol/mol at 12 months (P=0.02). Gastric emptying was not significantly different across groups at either 3 or 12 months.

Conclusions: Diabetes and pre-diabetes occur frequently in survivors of ICU experiencing stress hyperglycaemia. Based on the incidence observed in this cohort, structured screening and intervention programs appear warranted.

Trial Registration: Registered with the Australian New Zealand Clinical Trial Registry (ACTRN12614000449639). Registered 1 May 2014.

Introduction

Conditions of temporary glucose intolerance, such as gestational diabetes, identify those with an increased risk of developing prediabetes and type 2 diabetes (T2DM) [1]. As readily available and cost-effective interventions to prevent the progression from prediabetes to diabetes exist, timely identification of individuals with persistent glucose intolerance may facilitate earlier management, with a consequent reduction in complications associated with undiagnosed and prolonged hyperglycaemia [2, 3].

'Stress-hyperglycaemia' describes the phenomenon of glucose intolerance occurring during critical illness; it is generally presumed to normalize as critical illness resolves. It can be defined as blood glucose concentrations that, in health, would lead to a diagnosis of diabetes and yet occurs in critically ill patients without diabetes [4, 5]. Depending on the blood glucose threshold and population studied, stress hyperglycaemia may occur in up to 50% of critically ill patients [6, 7]. While the pathophysiology of stress hyperglycaemia is inadequately defined, it is believed to reflect both temporary insulin resistance and an inadequate insulin secretory response to hyperglycaemia [8, 9]. It is possible stress hyperglycaemia unmasks patients at risk of metabolic conditions that may be associated with long term adverse outcomes.

There is evidence that stress hyperglycaemia may be a risk factor for incident type 2 diabetes [10-15]. However, interpretation of prior work in this area is compromised by the failure to consistently capture glycated haemoglobin (HbA1c) on admission and information relating to the magnitude, time course or management of hyperglycaemia during critical illness [16]. Furthermore, there is no information about the mechanisms underlying glucose intolerance, which is pivotal to targeting potential interventions effectively. While a number of complex environmental and genetic interactions modify the risk of type 2 diabetes, both reduced insulin sensitivity and relative insulin deficiency are important precursors to type 2 diabetes [17]. These can be investigated via modeling of β-cell function, using insulin and glucose data obtained from an oral glucose tolerance test (OGTT), to provide a comprehensive description of pancreatic β-cell function [18].

Numerous factors predispose a person to type 2 diabetes, including insulin resistance, relative insulin deficiency and, through direct glucose toxicity to β-cells,

hyperglycaemic excursions [19, 20, 24]. Post-prandial glycaemic excursions are critically related to the absorption of carbohydrate in the small intestine and, therefore, the rate of gastric emptying. This is seen both in health and in patients with diabetes [21-23]. Accelerated gastric emptying has been shown to be a predictor of incident diabetes in other populations [20, 25]. Gastric dysmotility occurs frequently in the critically ill but there is limited data describing gastric emptying as patients recover [26]. Persistent gastric dysmotility may therefore be important to persistent glucose intolerance [27].

The primary aim of this study was to determine incident diabetes and prevalent prediabetes in survivors of critical illness suffering stress hyperglycaemia. Secondary outcomes were to explore potential mechanisms underlying progression from temporary glucose intolerance through to prediabetes and diabetes should such a progression exist.

Materials and Methods

This was a prospective, single centre, cohort study of patients surviving admission to a tertiary-referral, mixed medical-surgical intensive care unit (ICU) over a 30-month period, between April 2014 and October 2016.

Study participants

Consecutively admitted patients who developed stress hyperglycaemia and survived to hospital discharge were eligible. During the study period all patients had HbA1c measured on admission to ICU. Patients with stress hyperglycaemia were defined as those without a previous diagnosis of diabetes, who had an admission HbA1c ≤ 47.5 mmol/mol (≤ 6.5%) and subsequently recorded blood glucose concentrations >11.1 mmol/l (200 mg/dl) on two or more occasions within a 24 hour period or who had insulin commenced for blood glucose >11.1 mmol/l (200 mg/dl). Exclusion criteria included pregnancy, age <30 and >70 years on admission, chronic and acute pancreatitis, inability to give informed consent, poor prognosis and those living >100 km from the Royal Adelaide Hospital. The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and prospectively registered with the Australian New Zealand Clinical Trial Registry (ACTRN12614000449639). Written informed consent was obtained from all patients.

Methods

Baseline data, including patient demographics, relevant past medical history, blood glucose, insulin administered during ICU admission (censored at day 7) and ICU length of stay, were extracted from medical records. Study participants were invited to return to the ICU research facility at 3 and 12 months after ICU discharge.

Protocol

Participants attended the hospital after an overnight fast. On arrival, an intravenous catheter was inserted into the antecubital vein of one arm for blood sampling. Participants were placed in a semi-recumbent position for the duration of the study. At the conclusion of the study period, participants were given a meal. Studies also included a demographic questionnaire (including alcohol and tobacco consumption, physical activity and current medications), basic examination (including height, weight, hip/waist circumference, heart rate and blood pressure) and an oral glucose tolerance test (OGTT).

Oral glucose tolerance test (OGTT)

After an overnight fast, participants consumed a drink consisting of 300mL water containing 75g glucose (Glucaid 75g/300ml, Thermo Fisher, Adelaide, Australia). Venous blood samples for glucose measurement were taken immediately prior to the drink, at T=0 minutes, then every 30 minutes for 180 minutes [28]. Glucose was measured using a portable glucose meter (Optium Xceed; Abbott Laboratories, Bedford, MA, USA).

Definition of incident diabetes

Using HbA1c and OGTT data (fasting and 2 hour glucose), each participant was classified, according to the World Health Organisation (WHO) criteria [29], as having either normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance or type 2 diabetes (Supplemental Table 1). Patients were then divided into three categories (normal glucose tolerance, prediabetes and T2DM) with those having impaired fasting glucose or impaired glucose tolerance jointly defined as having prediabetes [30]. Sixty-minute blood glucose during OGTT was also recorded, given that this may be the strongest predictor of future incident T2DM [31, 32]. Participants

with incident diabetes during the study were informed of their diagnosis and a letter, outlining the test results, was sent to their General Practitioner (i.e. Family Doctor or Primary Care Physician). Those diagnosed with diabetes at 3-months remained in the study and were invited back for a repeat OGTT at 12 months.

Measurements of blood glucose and plasma hormones

Blood, for hormone concentrations, was collected into chilled tubes at baseline (T=0 minutes) and then every 30 minutes for 180 minutes. Serum insulin and C-peptide were measured on samples collected into 5ml Z Serum Separator Clot Activator tubes while glucagon was collected into 4ml K3 Ethylenediaminetetraacetic acid (EDTA) tubes. Serum was separated by centrifugation within 30 minutes of collection (4,500 rpm for 15 minutes at 4°C) and then stored at –70°C until assayed. Serum insulin was measured by enzyme-linked immunosorbent assay (10-1113; Mercodia, Uppsala, Sweden); the sensitivity of the assay was 1.0 mU/L and the coefficient of variation 2.9% within and 6.7% between assays [33]. C-peptide was measured by enzyme-linked immunosorbent assay (10-1136-01, Mercodia, Uppsala, Sweden); the sensitivity of the assay was 15 pmol/L and the coefficient of variation 3.7% within, and 7.7% between assays. Serum glucagon was measured via radioimmunoassay (GL-32 K; Millipore, Billerica, MA, USA); the sensitivity of the assay was 20 pg/mL and the coefficient of variation 3.1% within and 7.2% between assays. Blood for HbA1c was collected into 4ml K3 EDTA tubes and determined using high performance liquid chromatography [33].

Evaluation of beta cell function and insulin sensitivity

β-cell function modeling was performed as described by Mari and Ferranini [18].

B cell modelling

β-cell function modeling was performed using insulin, C-peptide and glucose data obtained during the OGTT. The insulin secretory response was calculated using the Insulinogenic Index, measured as the ratio of change of insulin/change in glucose over the first 30 minutes of the test [34]. Insulin sensitivity was calculated using the Oral Glucose Insulin Sensitivity method [35-37].

Measurement of gastric emptying (isotope breath test)

The OGTT liquid drink was labelled with 100mg of ^{13}C-octanoic acid. Breath samples were taken prior to ingestion, at T=0 minutes, 5 minutely for the first hour after the drink and subsequently every 15 minutes for the remaining 2 hours. The concentration of CO_2 and the percentage of $^{13}CO_2$ was measured in each sample with an isotope ratio mass spectrometer (Europa Scientific, ABCA model 20\20, Crewe UK) [38]. The concentration of $^{13}CO_2$ in each breath sample was plotted over time and the area under the resulting curves used to calculate the gastric emptying coefficient (GEC), providing a continuous variable with a larger value representing a more rapid emptying rate [33].

Statistical analysis

Sample size was determined pragmatically, based upon the number of eligible patients during the planned 30-month screening window. Summary statistics are presented as number (%), mean (standard deviation, SD) or median [interquartile range, IQR] as indicated. Comparisons between subgroups were performed by t-test, Chi-squared or rank-sum tests as indicated.

Concentration-time profiles for blood glucose, serum insulin, C-peptide and glucagon were analysed as their respective areas under the curve (AUC) from T=0 to T=180 minutes, with analysis across groups (normal, prediabetes, diabetes) performed by linear regression, with results presented as the marginal group means (95% confidence interval, 95%CI) and associated P-value. The impact of gastric emptying, the Insulinogenic Index and Oral Glucose Insulin Sensitivity measures on the AUC values for glucose and insulin was assessed via multivariable linear regression, assessing for interaction effects across groups. Longitudinal data changes across time were analysed by paired t-tests or generalized estimating equations, with results presented as the point estimate (95%CI) and associated P-value. Analysis was performed with Stata MP/15.0 software and a two-sided P-value of 0.05 was used to indicate statistical significance.

Results

During the study period, 5424 patients were admitted to the ICU with 5358 being excluded for one or more reasons; 2299 had blood glucose concentrations <11.1

mmol/l, 1532 were aged <30 or >70 years, 1150 were previously diagnosed with diabetes and 106 had a screening HbA1c > 47.5 mmol/mol (>6.5%). Of the 66 eligible patients, 40 agreed to participate (Figure 1).

Study visit details

During the study period, 35 and 26 participants attended the 3 and 12-month study visits respectively; with 21 attending both visits (Figure 1). Study days were well tolerated. The study was stopped in one participant due to hypoglycaemia at T=150 minutes (blood glucose 2.7 mmol/l). Baseline characteristics are provided (Table 1). The features of participants at each study visit are summarised in Table 2.

Glucose metabolism during critical illness

During the first 7 days of ICU admission, time weighted mean glucose peaked during day 0 and was lowest on day 7. Insulin was administered to 29 of the participants, with most of those needing insulin within the first 72 hours.

Incident diabetes and prevalent prediabetes

At 3 months, 13/35 (37%) and at 12 months, 7/26 (27%) of participants had incident diabetes. The proportions of participants fulfilling individual diagnostic criteria at each visit are provided in Table 3. Of the 21 participants who attended both 3 and 12 month visits, 8 (38%) at 3 months and 6 (29%) at 12 months had incident diabetes (Supplemental Table 2). Of the 8 participants classified as having diabetes at 3 months, 3 remained in this category at 12 months, while 4 were reclassified as having prediabetes, and 1 recorded normal glucose tolerance. Analysis of the 60 min blood glucose level, at 3 months, as a single predictor of subsequent diabetes was not superior to other time points in the OGTT.

At 3 months, 15 (43%) and at 12 months, 11 (42%) participants had prediabetes. The proportions fulfilling individual diagnostic criteria at each visit are provided in Table 3. Of the 21 participants attending both 3 and 12 month visits, 10 (48%) and 9 (43%) respectively had prediabetes (Supplemental Table 2). Only 3 (14%) and 6 (29%) participants respectively recorded normal glucose tolerance, with only 2 (10%) being normal at both visits.

Pooling pre-diabetes and diabetes at 12 months, no associations were found between baseline or 3 month characteristics and the development of abnormal glucose tolerance.

Glycated haemoglobin

Glycated haemoglobin data are provided in Figure 2a. At 3 months, 4 (11%) and at 12 months, 2 (8%) participants met the criteria for diabetes, with no participants positive at both visits. Mean HbA1c increased over time, being +0.7 (-1.2, 2.5) mmol/mol at 3 months and +3.3 (0.98, 5.59) mmol/mol at 12 months, P=0.02 (Figure 2b).

Response to OGTT

The plasma glucose-time profiles following the OGTT followed a typical early ascent, slower descent pattern (Supplemental Figure 1) with the AUC being greater in the group that were diagnosed with diabetes both at 3 months, P=0.002, and at 12 months, P<0.001 (Figure 3a). Insulin secretion profiles showed a phase delay with a slower rise compared to glucose, however these were not significantly different across groups at either visit (Supplemental Figure 2). The C-peptide concentration-time profiles mirrored serum insulin levels, with similar profiles at 3 and 12 months (Supplemental Figure 3). The correlation between C-peptide and insulin AUCs at 3 and 12 months were $R^2 = 0.75$ and $R^2 = 0.83$ respectively (Supplemental Figure 4).

In univariate analysis, the Insulinogenic Index was not significantly different across groups at either 3 or 12 months (P>0.6 for both). Differences in Oral Glucose Insulin Sensitivity were non-significant at 3 months, but were consistent with reduced insulin sensitivity in the diabetes group at 12 months (Figure 3b). When added to a multivariate regression model for plasma glucose AUC, including diabetes group, both Insulinogenic Index and Oral Glucose Insulin Sensitivity were independently and negatively associated with glucose AUC, i.e. a higher Insulinogenic Index or Oral Glucose Insulin Sensitivity were associated with lower AUCs (P≤0.001 for both). At 3 months there was no interaction effect of either measure with the group with diabetes, however, at 12 months the negative association was stronger in the group with diabetes, P<0.02, (Supplemental Figure 4).

The gastric emptying coefficient was not significantly different across diabetes groups at either 3 or 12 months. Modelling the glucose AUC at 3 and 12-months, there was no relationship between AUC and the gastric emptying coefficient; further, including diabetes group in the model, there was no interaction effect between the GEC and diabetes group, and in the main-effects only model the diabetes group-effect persisted, P=0.003 and P=0.001 at 3 and 12 months respectively.

Discussion

This study suggests that stress hyperglycaemia in the critically ill patient identifies survivors at risk of subsequent pre-diabetes and diabetes. Given that glucose metabolism appears to be normal in less than one third of patients at follow up, and that HbA1c deteriorated over time, these data provide further evidence that patients with stress hyperglycaemia may benefit from surveillance to detect and treat incident diabetes.

In a recently published systematic review and meta-analysis 10 percent of ICU survivors with stress hyperglycaemia were diagnosed with diabetes and almost 30 percent prediabetes [14]. These point estimates were less than this current prospective study; however, the studies included in the meta-analysis used inconsistent definitions for stress hyperglycaemia, with three studies using blood glucose > 7.7 mmol/l (>140 mg/dl) [10, 11, 13] and one an admission glucose of > 11.1 mmol/l (>200 mg/dl) [12]. The current study was more conservative in defining stress hyperglycaemia, i.e. blood glucose concentrations >11.1 mmol/l (200 mg/dl) on two or more occasions within 24 hours or the commencement of insulin for blood glucose >11.1 mmol/l (>200 mg/dl), and such an approach may have identified a cohort with more profound acute glucose disturbance and possible greater risk of subsequent diabetes.

This study is the first to evaluate mechanisms that may contribute to diabetes in critically ill patients with stress hyperglycaemia. While the rate of gastric emptying did not impact on the subsequent incidence of diabetes, the plasma glucose-time profiles displayed the expected separation across subgroups, with both insulin secretion (as Insulinogenic Index) and insulin sensitivity (as Oral Glucose Insulin Sensitivity) independently associated with diabetes. These data suggest that both β-

cell secretory capacity and insulin resistance contribute to the deterioration in glucose metabolism.

This study has limitations, including the potential for selection and misclassification bias. In an attempt to minimise these biases, consecutively admitted patients were screened and HbA1c measured on admission to exclude patients with undiagnosed type 2 diabetes. Analysis for baseline factors associated with an abnormal glucose tolerance test at 12 months was limited due to small study numbers. Outcomes from single centre studies should also be circumspectly extrapolated to other settings. It should also be noted that only survivors aged between 30 and 70 years old were eligible. The rationale for exclusion of younger survivors was based on evidence that incident diabetes is infrequent in those <30 years of age [39]. Survivors aged >70 years were excluded so that estimates of incidence were obtained in a group that has the potential to benefit from any intervention to delay or limit the progression to diabetes. It should also be recognised that pre-ICU data were not available and there was no matched cohort of hospitalised patients who did not require ICU admission. Accordingly, it is not possible to determine whether hyperglycaemia during ICU admission uncovers those with pre-existing impaired β-cell reserve and insulin resistance, or critical illness *per se* accelerates these abnormalities. It is also important to appreciate that while this study indicates that it may be possible to predict a cohort at risk of disordered glucose metabolism long-term, it did not test whether an intervention could prevent the development of diabetes. This is crucial, as an intervention that is proven to limit development of diabetes and improve patient-centred outcomes in this cohort, is likely to represent a prerequisite to the establishment of screening programs. There have been, however, a number of effective preventative strategies that have been shown to reduce the risk of incident diabetes in other at risk populations [2, 40]. Nonetheless, identifying that this cohort is at considerable risk provides a rationale to study potential interventions in this group.

Conclusions

In this single centre cohort study less than one third of patients with stress hyperglycaemia returned to having normal glucose metabolism at one year post ICU

discharge. Critically ill patients with stress hyperglycaemia represent a population at significant risk of pre-diabetes and incident diabetes.

Author Contributions

PK was involved in the conception and design of the study, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission. MPP was involved in the conception, design, data collection and revising the manuscript. YA was involved in data collection and revised the manuscript for important intellectual content. EJG, MJS and LW assisted with collecting data and reviewed the manuscript. MEF performed statistical analysis and reviewed the manuscript. LKP, KLJ and MH assisted in design of the study along with evaluation and revising of the manuscript. AMD supervised PK, and was involved in the conception and design of the study, analysis and interpretation of data, and drafting and revising the manuscript for final submission. All authors read and approved the final manuscript.

PK is the 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.

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Tables

Table 1: Demographic data for study participants at baseline.

Characteristics	N = 40
Age, mean (SD) years	58 (10)
Male, n (%)	27 (68%)
HbA1c, mean (SD) mmol/mol	36.8 (4.9)
APACHE II Score, median [IQR]	17 [15,25]
APACHE III Score, median [IQR]	66 [49, 86]
Length of stay - ICU, median [IQR] days	4.2 [1.8, 9.3]
Length of stay - hospital, median [IQR] days	13.9 [7.2, 24.8]
Blood glucose	
Peak first 24 hours, mean (SD) mmol/L	13.7 (4.8)
Nadir first 24 hours, mean (SD) mmol/L	6.1 (1.4)
Peak first 7 days, mean (SD) mmol/L	14.3 (4.4)
Nadir first 7 days, mean (SD) mmol/L	5.1 (1.2)
Time weighted mean, mean (SD) mmol/L	8.4 (1.1)
Insulin Treatment*	
Use, n (%)	29 (73%)
Insulin dose over the first 7 days, mean (SD) units/hr	0.9 (0.9)
Peak first 24 hours, mean (SD) units/hr	0.6 (1.0)
Peak first 7 days, mean (SD) units/hr	1.8 (1.8)
Vasopressor/Inotrope use, n (%)	29 (73%)
Diagnostic category, n (%)	
Cardiothoracic Surgery	12 (30%)
Cardiovascular	7 (17.5%)
Respiratory	7 (17.5%)
Neurology	6 (15%)
Gastrointestinal	3 (7.5%)
Sepsis	2 (5%)
Trauma	2 (5%)
Metabolic	1 (2.5%)
Family History of T2DM^, n (%)	
Father / Mother	5 (13%) / 3 (8%)
Brother / Sister	1 (3%) / 2 (5%)

*Figures reflect only those patients who received an insulin infusion.
^One patient had a positive family of diabetes in two family members (Mother and daughter)

Table 2: Demographic data for study participants at 3 and 6 months.

Characteristics	**3 Months**	**12 Months**	**(N) P-value[2]**
Number attending, n (%)[1]	35 (88%)	26 (65%)	
BMI, mean (SD) kg/m^2	27 (6.3)	30 (6.4)	(21) 0.03
Weight, mean (SD) kg	80 (21)	92 (23)	(21) 0.01
Height, mean (SD) m	1.72 (0.1)	1.75 (0.1)	
Physical attributes, mean (SD) cm			
Hip circumference	105 (8.8)	113 (11.4)	(20) 0.001
Waist circumference	104 (16.7)	109 (18.4)	(20) 0.26
Neck circumference	41 (4.4)	43 (5.3)	(20) 0.05
HbA1c, mean (SD) mmol/mol	37.4 (7.0)	40.3 (7.8)	(20) 0.06
Blood pressure, mean (SD) mmHg			
Systolic	131 (18)	123 (18)	(16) 0.22
Diastolic	77 (11)	81 (18)	(16) 0.36
Heart rate, mean (SD) beats per minute	75 (13)	72 (17)	(18) 0.29
Smoking			
Yes, n (%)	6 (17%)	8 (33%)	
Cigarettes per day[3], mean (SD)	11 (11)	12 (5)	
Alcohol			
Yes, n (%)	22 (63%)	15 (63%)	
Drinks per week[4], mean (SD)	8 (12)	5 (6)	
Physical Activity, mean (SD) hours per week			
Mild	4.4 (4.5)	6.9 (12.3)	
Moderate	1.0 (2.2)	1.2 (2.6)	
Heavy	0.1 (0.3)	0	
Medication use, n (%)			
Insulin	0	0	
Oral hypoglycaemic agent	0	1 (5%)	
Steroids	5 (14%)	2 (10%)	

1. Number (%) with reference to the enrolled, n=40; all other (%) are per attending group number.
2. P-values by paired t-test for (N) participants presenting at both 3 and 12-month visits.
3. Mean cigarettes per day calculated in those who admit to smoking.
4. Mean drinks per week calculated in those who admit to drinking.

Table 3: Incident diabetes and prevalent prediabetes at 3 and 12 months.

		3 months	12 months[2]
Number returning for visit, n		35	26
Incident Diabetes, n (%)		13 (37%)	7 (27%)
Single criterion			
HbA1c	≥ 47.5 mmol/mol	2 (6%)	-
Fasting Glucose	≥ 7.1 mmol/L	3 (9%)	-
2h OGTT	≥ 11.1 mmol/L	4 (11%)	3 (13%)
Two criteria			
HbA1c + Fasting Glucose		1 (3%)	-
HbA1c + 2h OGTT		1 (3%)	-
Fasting Glucose + 2h OGTT		2 (6%)	2 (8%)
Three criteria			
HbA1c + Fasting Glucose + 2h OGTT		-	2 (8%)
Prevalent Prediabetes, n (%)		15 (43%)	11 (42%)
Single criterion			
Fasting Glucose	= 6.1-6.9 mmol/L	3 (9%)	2 (8%)
2h OGTT	= 7.8-11.0 mmol/L	8 (23%)	7 (27%)
Two criteria			
Fasting Glucose + 2h OGTT		4 (3%)	2 (8%)

1. Participants meeting criteria for both prediabetes and incident diabetes were classified as having diabetes
2. Glycated haemoglobin was unable to be obtained for 2 patients at the 12-month study visit.

Figures

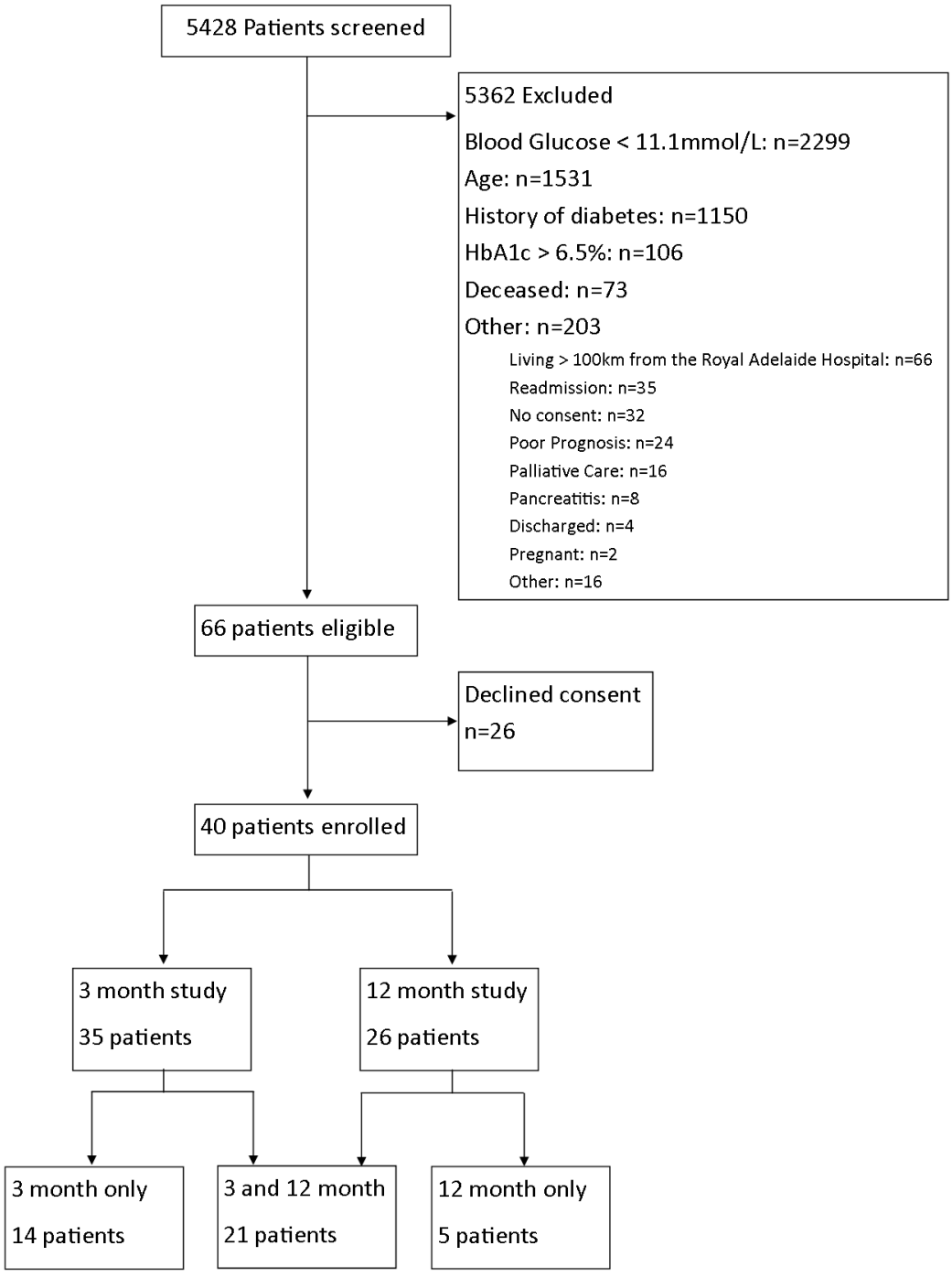


Figure 1: Consort diagram outlining assessment and inclusion of patients into the study

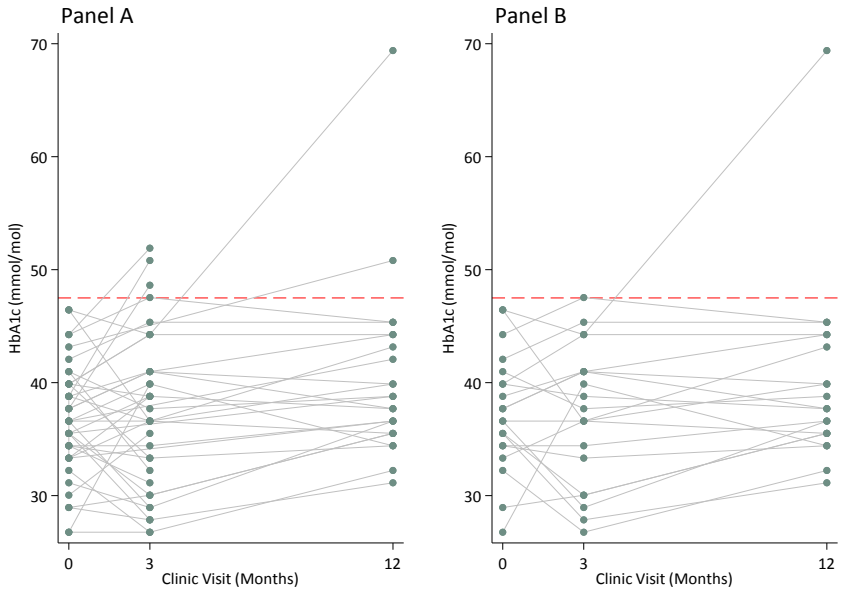


Figure 2a) Scatter plot of HbA1c versus clinic visit (months). Panel A showing all patients (n=35), Panel B those patients attending all visits (n=21). Dashed red line at cut-point, HbA1c = 47.5 mmol/mol (6.5%).

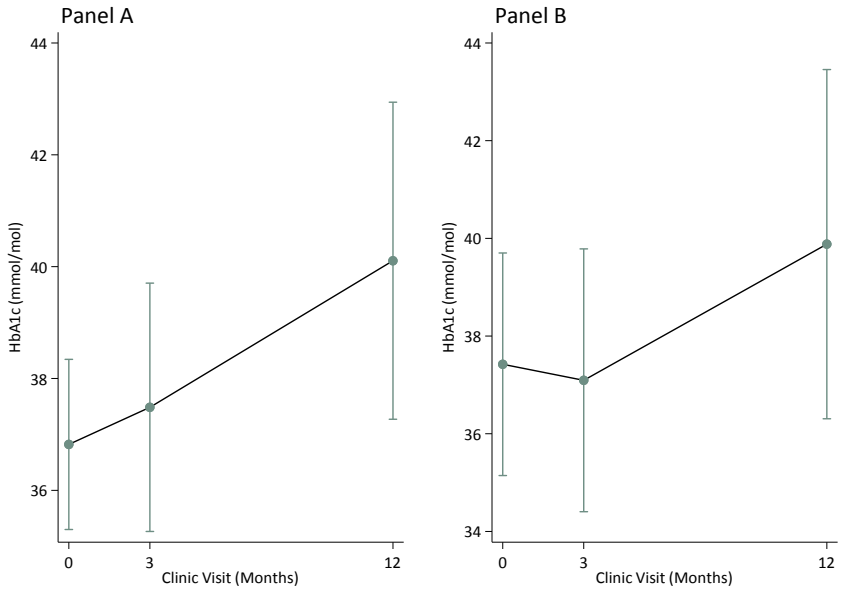


Figure 2b) Mean HbA1c (95%CI) versus study visit (months). Panel A showing all patients (n=35), Panel B those patients attending all visits (n=21).

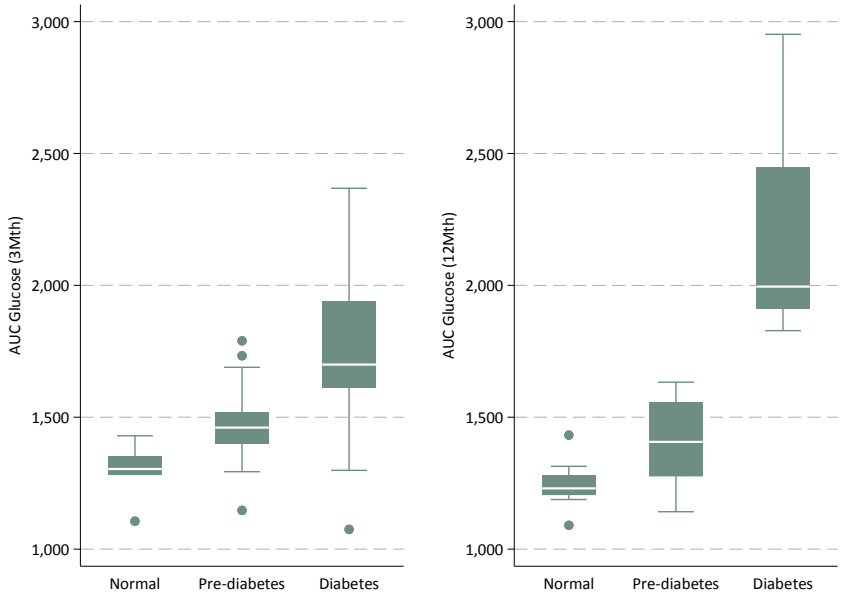


Figure 3a) Area under plasma concentration-time curves for glucose across groups (normal, prediabetes, diabetes) at 3 and 12-month visits following an oral glucose tolerance test.

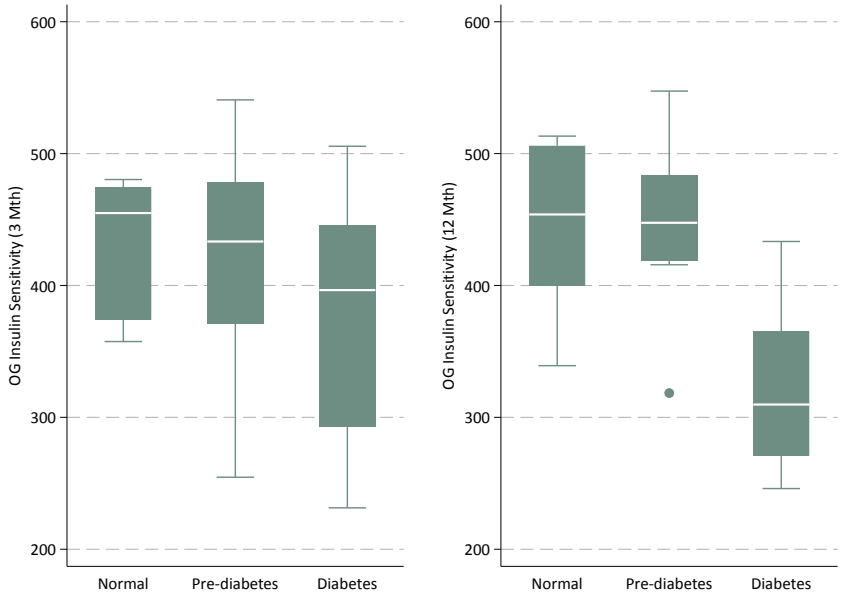


Figure 3b) Oral glucose insulin sensitivity across groups (normal, prediabetes, diabetes) at 3 and 12 month visits following an OGTT. Insulin sensitivity is reduced in the group with diabetes at 12 months (P=0.002).

Supplemental tables

Supplemental Table 1: Definitions of normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or type 2 diabetes. Participants were classified as having NGT, IFG, IGT or T2DM. Those with IFG or IGT were defined as having prediabetes.

Normal glucose tolerance (NGT)	Fasting blood glucose <6.1 mmol/l (110 mg/dl) and 2 hour post OGTT <7.8 mmol/L (140 mg/dl)
Impaired fasting glucose (IFG)	Fasting blood glucose between 6.1-6.9 mmol/l (110-125 mg/dl) inclusive
Impaired glucose tolerance (IGT)	Fasting blood glucose < 7 mmol/l (126 mg/dl) and 2 hour post OGTT blood glucose between 7.8-11.0 mmol/l (140-200 mg/l) inclusive
Type 2 diabetes (T2DM)	Fasting blood glucose ≥ 7 mmol/l (126 mg/dl), 2 hour post OGTT glucose ≥ 11.1 mmol/l (200 mg/dl) or HbA1c ≥ 48 mmol/mol

Supplemental Table 2: Incident diabetes and prevalent prediabetes at 3 and 12 months in patients returning for both study visit days (n=21)

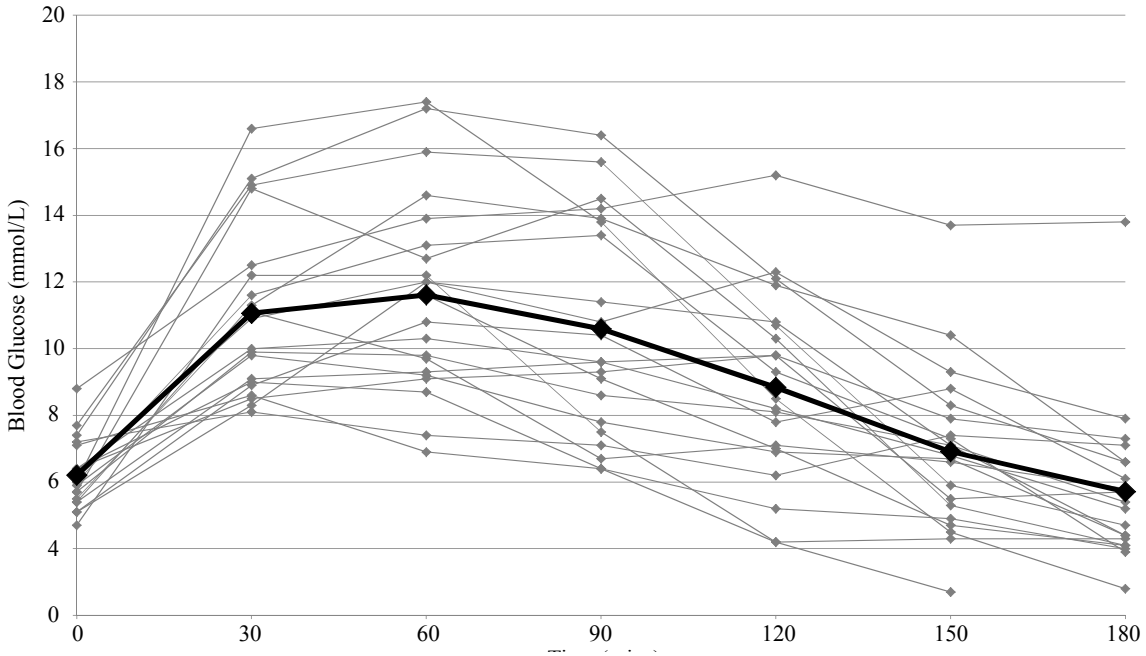
		3 months	12 months[2]
Incident Diabetes, n (%)		8 (38%)	6 (29%)
Single criterion			
HbA1c	≥ 47.5 mmol/mol	1 (5%)	-
Fasting Glucose	≥ 7.1 mmol/L	3 (14%)	-
2h OGTT	≥ 11.1 mmol/L	2 (10%)	3 (14%)
Two criteria			
HbA1c + Fasting Glucose		-	-
HbA1c + 2h OGTT		-	-
Fasting Glucose + 2h OGTT		2 (10%)	2 (10%)
Three criteria			
HbA1c + Fasting Glucose + 2h OGTT		-	1 (5%)
Prevalent Prediabetes, n (%)		10 (48%)	9 (43%)
Single criterion			
Fasting Glucose	= 6.1-6.9 mmol/L	2 (10%)	2 (10%)
2h OGTT	= 7.8-11.0 mmol/L	6 (29%)	6 (29%)
Two criteria			
Fasting Glucose + 2h OGTT		2 (10%)	1 (5%)

1. Participants meeting criteria for both prediabetes and incident diabetes were classified as having diabetes.
2. Glycated haemoglobin was unable to be obtained for 2 patients at the 12-month study visit.

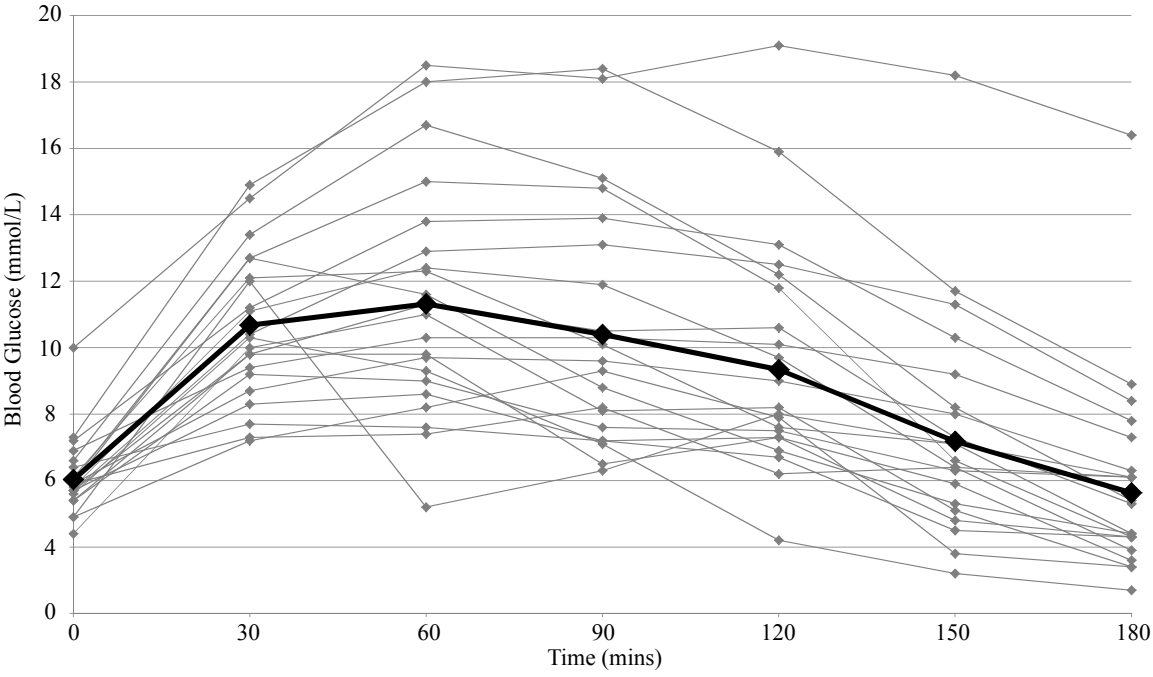
Supplemental Figures

Supplemental Figure 1: Blood glucose (mmol/L) in participants who attended both study days at a) 3 months (n=35) and b) 12 months (n=26). Grey lines are individual data, black line is mean.

a)

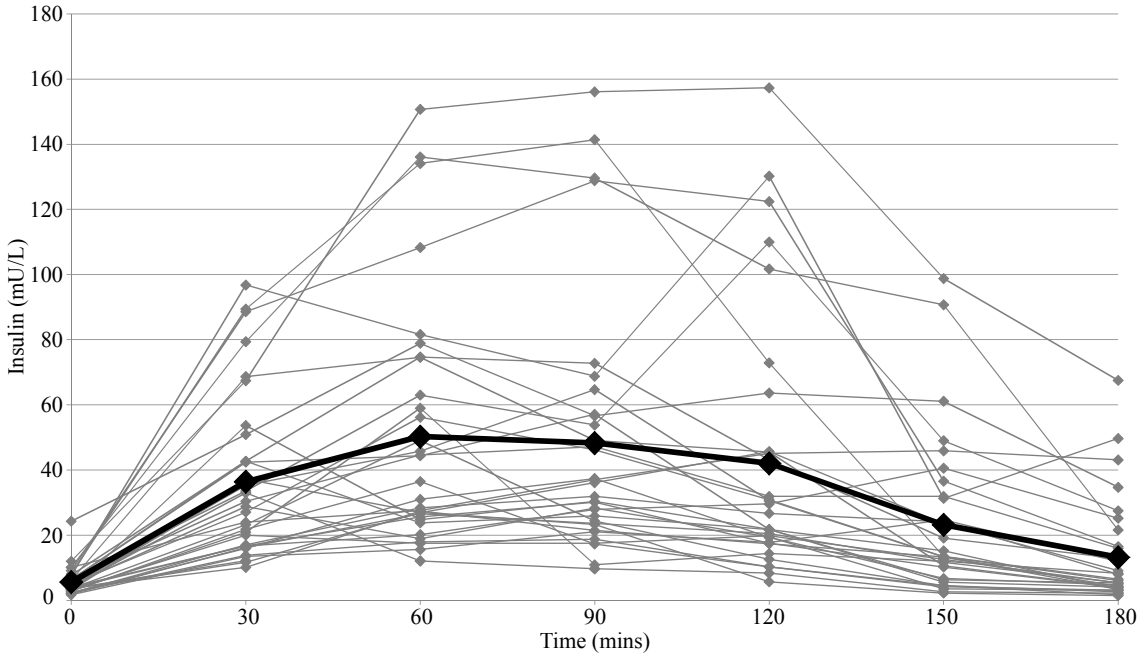


b)

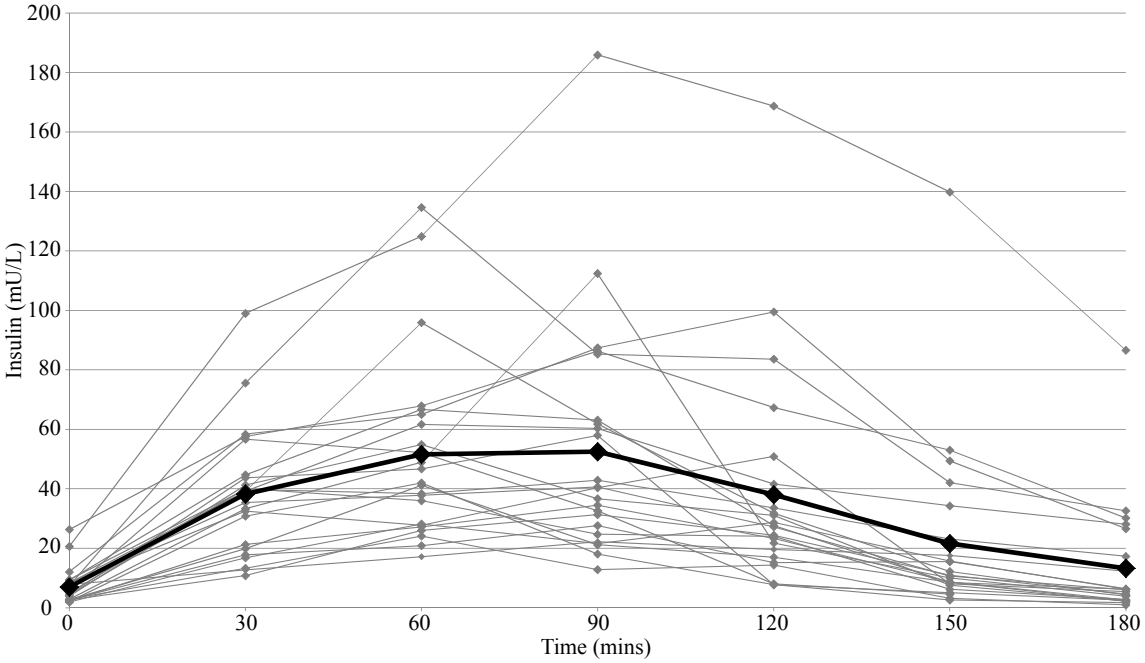


Supplemental Figure 2: Insulin (mU/L) in participants who attended both study days at a) 3 months (n=35) and b) 12 months (n=26). Grey lines are individual data, black line is mean.

a)

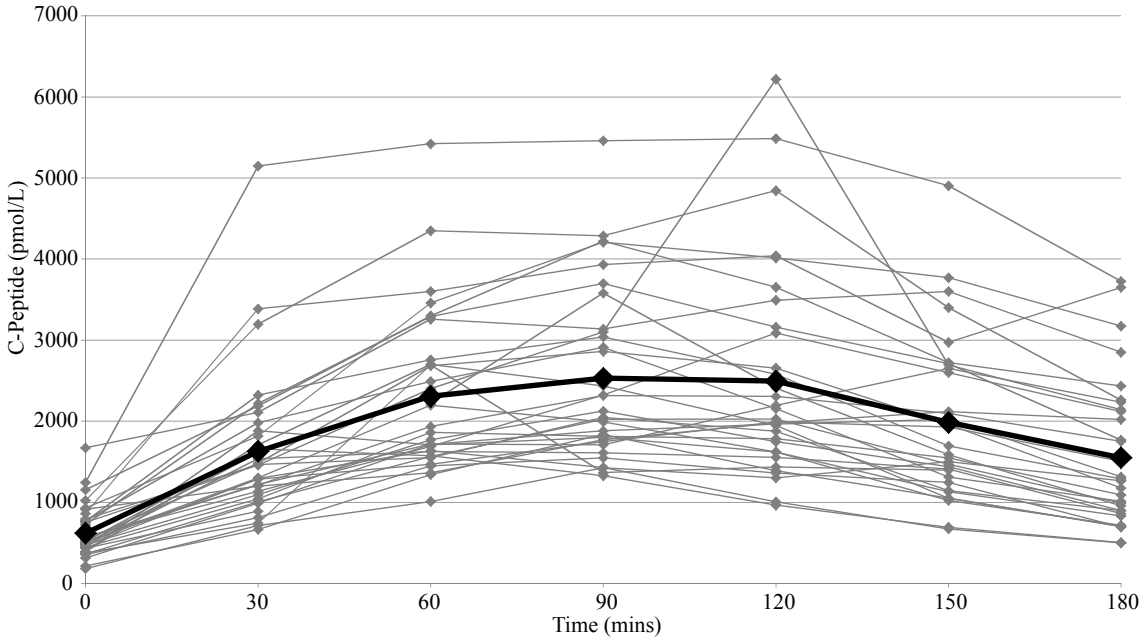


b)

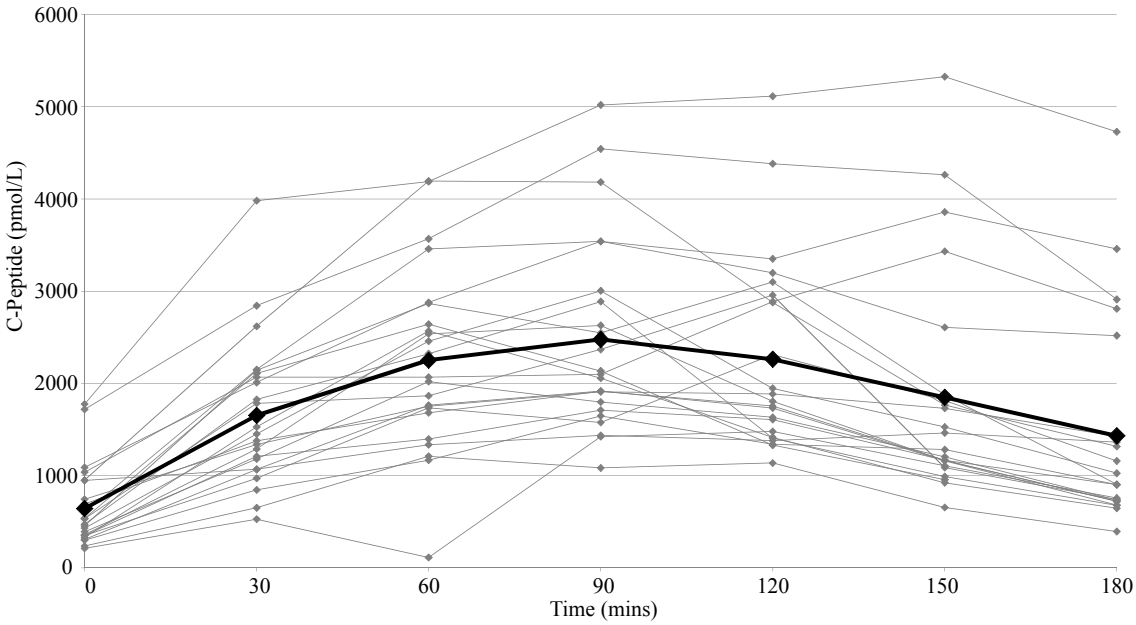


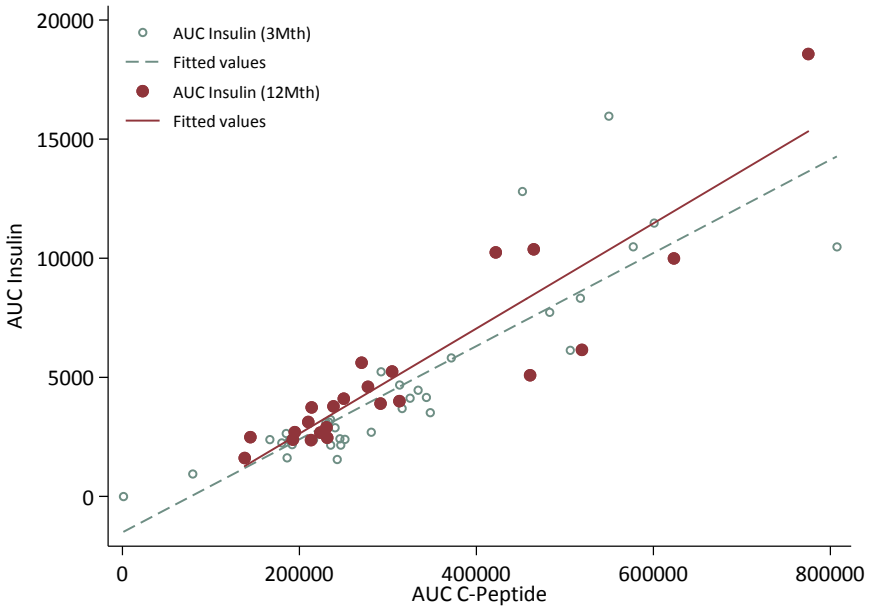
Supplemental Figure 3: C-peptide (pmol/L) in participants who attended both study days at a) 3 months (n=35) and b) 12 months (n=26). Grey lines are individual data, black line is mean.

a)

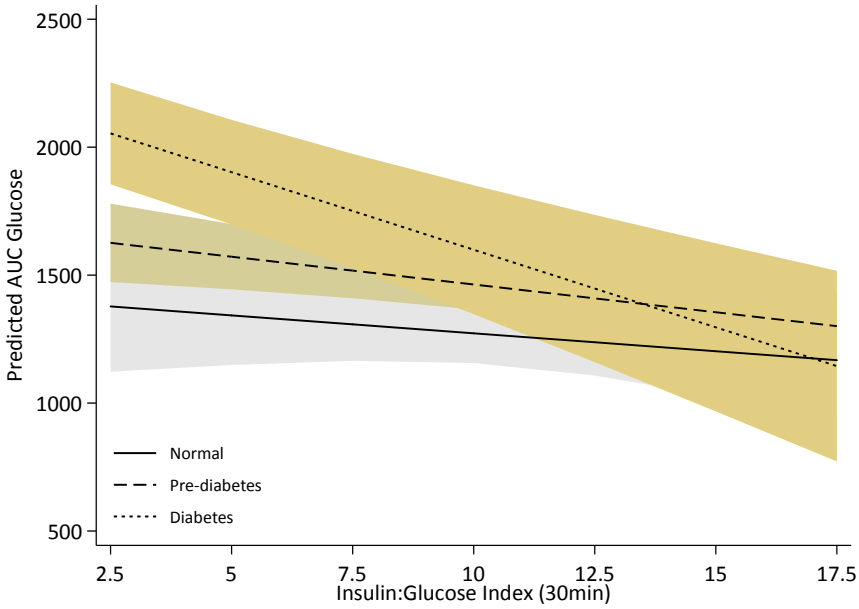


b)





Supplemental Figure 4: Association between Insulin AUC and C-Peptide AUC at 3 and 12 months, $R^2 = 0.75$ and $R^2 = 0.83$ respectively.



Supplemental Figure 5: Model predicted AUC glucose and 95% confidence intervals versus insulogenic index for sub-groups according to oral glucose tolerance test, plotted at the mean insulin sensitivity (OGIS) value.

3.4 CONCLUSIONS

3.4.1 *Introduction*

Stress hyperglycaemia describes a condition of glucose intolerance associated with acute illness [1]. Depending on the blood glucose threshold and studied population, stress hyperglycaemia has been noted in up to 50% of critically ill patients [2, 3]. Stress hyperglycaemia is a marker of illness severity, especially in critically ill patients without diabetes [2, 4]. Previously, other conditions of impaired glucose intolerance, such as gestational diabetes, had been considered a temporary disorder. This concept has recently been challenged, for example, gestational diabetes is now known to identify a substantially increased risk for prediabetes and type 2 diabetes in later life [5, 6]. Identifying those at increased risk of subsequent type 2 diabetes allows earlier interventions delaying progression of disease and subsequent complications [6, 7]. Prior to this thesis, stress hyperglycaemia had been inadequately studied and there remained considerable uncertainty as to whether stress hyperglycaemia identifies those at risk of prediabetes and diabetes. In addition, understanding the mechanisms underlying the progression to prediabetes and type 2 diabetes, had never been conducted and this information is essential to guide any future study of interventions in this group.

3.4.2 *Contribution of the work described in this thesis to the understanding of the effects of stress hypoglycaemia on the development of prediabetes and type 2 diabetes*

The work outlined in Chapter 3.2 is the first published systematic review and meta-analysis to evaluate relationships between stress hyperglycaemia and the development of prediabetes and type 2 diabetes [8]. Despite the four studies included in the analysis having considerable statistical and clinical heterogeneity, these findings suggest that stress hyperglycaemia identifies patients at increased risk for developing prediabetes and incident diabetes. This observation is consistent with other studies performed in the non intensive care setting [9-11]. In addition to this, a recent retrospective, data-linkage, cohort study of all adult critically ill patients surviving admission to a public hospital intensive care unit (ICU) in the state of South Australia over a 7 year period yielded a similar result to that observed in the meta-analysis [12]. The Student was a co-author on this publication but it is not included in this thesis.

The work outlined in Chapter 3.3 supports the concept that survivors of critical illness suffering stress hyperglycaemia are at risk of subsequent prediabetes and diabetes. Compared to previous data [13-16], this study used a more conservative definition for stress hyperglycaemia, thereby likely identifying a group at greater risk of developing subsequent diabetes.

3.4.3 *Contribution of the work described in this thesis to the understanding of stress hypoglycaemia and mechanisms behind the development of prediabetes and type 2 diabetes*

There is negligible information regarding the mechanisms underlying progression from stress hyperglycaemia to type 2 diabetes. Such understanding is critical to guide potential intervention/s. Chapter 3.3 contains the first work to evaluate mechanisms contributing to the development of diabetes in critically ill patients who suffered stress hyperglycaemia. It appears both insulin secretion (as Insulinogenic Index) and insulin sensitivity (as Oral Glucose Insulin Sensitivity) are independently associated with diabetes. These data suggest that both β-cell secretory capacity and insulin resistance contribute to the deterioration in glucose metabolism are factors leading to the development of diabetes, whereas gastric emptying is less likely to play a role.

3.5 FUTURE DIRECTIONS

3.5.1 *Prospective trials to determine the effect of stress hyperglycaemia and its mechanisms in treating type 2 diabetes in the critical care setting*

Chapters 3.2 and 3.3 provide evidence suggesting stress hyperglycaemia is a major risk factor for prediabetes and incident type 2 diabetes. A multi-centre, prospective cohort study with a follow-up period of several years is needed to precisely quantify this risk. Additionally, further trials studying strategies to delay the progression to diabetes are likely to be of value.

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Chapter 4:

Antecedent hypoglycaemia and gastric emptying

4.1 INTRODUCTION

Hypoglycaemia is a major cause of morbidity and increased mortality in critically ill patients, as well as patients with type 1 and (usually insulin-treated) type 2 diabetes. Hypoglycaemia is known to increase the risk of cardiovascular death in patients with type 1 diabetes, while bradycardia and atrial/ventricular ectopic beats occur more frequently during (both symptomatic and asymptomatic) hypoglycaemia in patients with type 2 diabetes. In health, and in patients with diabetes, hypoglycaemia causes an autonomic response reflected by increases in catecholamine and cortisol secretion. However, this response may be diminished when hypoglycaemia is preceded by even one episode of antecedent hypoglycaemia and associated with reduced patient recognition of hypoglycaemia.

Gastric emptying and glycaemia share a complex and bidirectional relationship. Gastric emptying is a major determinant of the postprandial glycaemic response, while acute changes in the blood glucose concentration markedly effect gastric emptying. Acute hypoglycaemia accelerates gastric emptying substantially, increasing the delivery of carbohydrate and the rate of glucose absorption, resulting in a subsequent rise in blood glucose. This acceleration of gastric emptying is likely to be important in the management of patients with insulin-induced hypoglycaemia because conventional treatment of hypoglycaemia (orally administered carbohydrate) is delivered to the stomach and, therefore, absorbed more rapidly, reducing both the time and nadir of hypoglycaemia. Given the significance, it is surprising that the rate of gastric emptying during hypoglycaemia has received little attention. If antecedent hypoglycaemia is shown to be associated with a reduced acceleration of gastric emptying, this would support the use of interventions other than oral carbohydrate for patients with recurrent hypoglycaemic episodes.

Acute hypoglycaemia also affects the cardiovascular system, with an increase in blood pressure and left ventricular ejection fraction resulting from sympathetic stimulation and counter regulatory hormone secretion. The observed changes in heart

rate are, however, inconsistent which is likely to reflect some parasympathetic activation. Additionally electrophysiological abnormalities may occur, increasing the risk of arrhythmia. Such counter-regulatory responses are likely to be very important during critical illness.

The study described in Chapter 4.2 evaluates the effects of antecedent hypoglycaemia on the gastric emptying and cardiac responses to subsequent hypoglycaemia.

4.1.1 *Objectives*

The objectives of the study reported within this chapter were to compare the effects of antecedent hypoglycaemia to an initial acute hypoglycaemic episode to determine whether there is attenuation of the (i) acceleration of gastric emptying and (ii) augmentation of cardiac fractional shortening.

4.2 MANUSCRIPT

Antecedent hypoglycemia does not attenuate the acceleration of gastric emptying by hypoglycemia

Statement of Authorship

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By signing the Statement of Authorship, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above);
- Permission is granted for the candidate to include the publication in the thesis; and
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Antecedent Hypoglycemia Does Not Attenuate the Acceleration of Gastric Emptying by Hypoglycemia

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Abstract

Context: Acute hypoglycemia accelerates gastric emptying and increases cardiac contractility. However, antecedent hypoglycemia attenuates counter-regulatory hormonal responses to subsequent hypoglycaemia.

Objective: To determine the effect of antecedent hypoglycemia on gastric and cardiac responses to subsequent hypoglycemia in health.

Design: A prospective, single-blind, randomised, cross over study

Patients: Ten healthy young men aged 18-35 years of age were studied for 36 hours on two occasions.

Interventions: Participants were randomly assigned to either antecedent hypoglycemia - three 45min periods of strict hypoglycemia (2.8mmol/L), or control - three 45min periods of strict euglycemia (6mmol/L) during the initial 12h period. Participants were monitored overnight and the following morning blood glucose clamped at 2.8mmol/L for 60min and then at 6mmol/L for 120min. At least 6 weeks later participants returned for the alternative intervention. Gastric emptying and cardiac fractional shortening were measured with scintigraphy and 2D echocardiography respectively on the morning of all 4 study days.

Results: A single, acute episode of hypoglycemia accelerated gastric emptying ($P=0.01$) and augmented fractional shortening ($P<0.01$). Gastric emptying was unaffected by antecedent hypoglycemia ($P=0.74$) whilst fractional shortening showed a trend to attenuation ($P=0.06$). The adrenaline response was diminished ($P<0.05$) by antecedent hypoglycemia

Conclusions: In health, the acceleration of gastric emptying during hypoglycemia is unaffected by antecedent hypoglycemia, whereas the increase in cardiac contractility may be attenuated.

Introduction

Hypoglycemia is associated with considerable morbidity, as well as an increased risk of death among ambulant and hospitalised patients with diabetes [1, 2]. In both health and diabetes, hypoglycemia triggers profound physiological responses, in part due to autonomic activation, including increases in catecholamine and cortisol secretion [3]. However, antecedent hypoglycaemia - even one episode, attenuates the catecholamine response and diminishes patient recognition to subsequent hypoglycemia [4]. This effect is also noted with an increased number and differing duration of antecedent hypoglycaemia [5-7].

The rate of gastric emptying is now recognised as a major determinant of the postprandial glycemic response in health and diabetes [8-10]. The relationship between gastric emptying and glycemia is complex and bidirectional, as acute changes in the blood glucose concentration also have major effects on the rate of gastric emptying [11-13]. In particular, in both healthy subjects and patients with type 1 diabetes, gastric emptying is accelerated markedly during acute hypoglycemia [11, 14, 15]. This acute response, known to be blocked by intravenous atropine [16] and attenuated by exogenous administration of GLP-1 [12], is an important counter regulatory mechanism leading to an increased delivery of carbohydrate to the small intestinal and a consequent prompt increment in blood glucose [17].

Acute hypoglycemia also has major effects on the cardiovascular system. Sympatho-adrenal activation and counter-regulatory hormonal secretion results in an increase in blood pressure and left ventricular ejection fraction; the latter changes are sustained for some time after resolution of hypoglycemia. However, changes in heart rate are inconsistent, probably because parasympathetic activation has the capacity to prevent much of the sympathetic associated increase [3, 18, 19]. Cardiac electrophysiological abnormalities may also occur, increasing the risk of arrhythmia [20].

No studies have evaluated whether antecedent hypoglycemia affects the rate of gastric emptying and cardiac responses to subsequent hypoglycemia. Because oral carbohydrate is the preferred treatment for the conscious individual with hypoglycemia [17], any attenuation of the protective response to accelerate gastric emptying may undermine management of the condition.

The primary and secondary hypotheses of this study were that compared to an initial hypoglycemic episode, antecedent hypoglycemia would i) attenuate the acceleration of gastric emptying and ii) attenuate the augmentation of cardiac fractional shortening.

Material and Methods

This was a prospective, single-blind, randomised, cross over study.

Study Participants

Healthy young men aged 18-35 years of age were recruited, with written informed consent obtained prior to their participation. A young, healthy, relatively homogeneous cohort was chosen given that advancing age is a risk factor for autonomic neuropathy, which can affect both gastric emptying and the response to hypoglycemia [21]. Exclusion criteria included a history of diabetes, HbA1c >6.0%, impaired renal function, use of medication known to effect gastrointestinal function, and previous stomach or small intestinal surgery. The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and prospectively registered with the Australian New Zealand Clinical Trial Registry (ACTRN12614000986673).

Protocol

Each participant attended the hospital after an overnight fast on two occasions for approximately 30 hours comprising a 'Day 1' and 'Day 2' on each occasion, separated by at least 6 weeks. The order of the two studies –'control' or 'antecedent hypoglycemia' – was randomized. Randomization was performed by the Department of Pharmacy at the Royal Adelaide Hospital. Allocation concealment was performed with study investigators notified of the intervention for participants on the morning of the study. Once enrolled, participants were unaware of the glycemic clamp that they had been allocated to on each study day (i.e. euglycemia or antecedent hypoglycemia). On arrival, an intravenous catheter was inserted into the antecubital veins of both arms for IV infusion of insulin/glucose and blood sampling, respectively. Each participant was given the same meals during both study periods [13]. The protocol is summarised in Figure 1.

Clamp techniques have been described [12]. In brief, insulin (Actrapid; Novo Nordisk Pharmaceuticals) was commenced at 125 mU/m^2 per minute, and then titrated to a maintenance rate of 40 mU/m^2 per minute over 10 minutes. During each clamp, 25% glucose was administered intravenously concurrently with insulin. Blood was sampled intravenously and the blood glucose concentration was measured using a portable glucose meter (Optium Xceed; Abbott Laboratories). During the longer 3 hour clamps, blood glucose was measured every 5 minutes for 90 minutes (ie during the 45 minute period of hypoglycaemia/euglycaemia, followed by 45 minutes of stabilization) then every 15 minutes for the remaining 90 minutes. During the shorter 60 minute clamps, blood glucose was measured every 5 minutes for 90 minutes (ie during the 60 minute clamp and for 30 minutes post clamp). Using these measurements, intravenous glucose infusion was varied to maintain the desired blood glucose target [12].

Control

During 'Day 1' of the control period (C1), each participant underwent three euglycemic clamps at a blood glucose of 6 mmol/L [12]. The initial clamp was three hours in duration, allowing measurement of gastric emptying (see below), with each subsequent clamp lasting 60 minutes. There were two-hour periods between each clamp, during which the clamp was ceased and blood glucose concentrations reflected endogenous concentrations (ie euglycemia). The following morning (control 'Day 2', C2), each participant underwent a hypoglycemic clamp with blood glucose stabilised at 2.8 mmol/L for 45 minutes, followed by a period of 15 minutes to return to euglycemia, then titration to 6 mmol/L for the remaining 2 hours (Figure 1). This hypoglycemic clamp was predefined as the 'control acute hypoglycemic clamp (C2)' as it followed 24 hours in hospital under controlled euglycemic study conditions.

Antecedent Hypoglycemia

During day 1 of the antecedent hypoglycemia period (AH1), each participant underwent three hypoglycemic clamps at a blood glucose of 2.8 mmol/L. For each clamp period at least 15 minutes was allowed to reach a blood glucose of 2.8 mmol/L, and then another 15 minutes to achieve euglycemia at the end of each clamp. The initial clamp consisted of strict hypoglycemia (2.8 mmol/L) for 45 minutes, followed

by a period of 15 minutes to return to euglycemia, and then 2 hours at 6 mmol/L (total clamp time: 3 hours). This initial hypoglycemic clamp allowed quantification of the effects of acute hypoglycemia without the confounder of study conditions or hospitalization for 24 hours. The following two clamps were each of 45 minutes in duration at 2.8 mmol/L, followed by 15 minutes to return to euglycemia (total clamp time: 60 minutes). There were two-hour periods of euglycaemia, with cessation of the insulin infusion, between each clamp. The following morning (antecedent hypoglycemia 'Day 2', AH2) each participant underwent a hypoglycemic clamp with blood glucose stabilised at 2.8 mmol/L for 45 minutes, followed by 15 minutes to return to euglycemia, and then maintained at 6 mmol/L for the subsequent 2 hours with measurement of gastric emptying. The period of strict hypoglycemia totalled 3 hours during the intervention period, which was based on previous studies that have established major effects of antecedent hypoglycemia with clamps lasting at least 30 minutes [7] and the total hypoglycemic time equaling 3 hours [5]. *A priori* we defined that the primary period of interest as data from the antecedent hypoglycemic clamp (AH2) when compared to data from control acute hypoglycemic clamp (C2), as the period of time in hospital, meals and exertion were standardized, with the only difference between the 2 periods being three antecedent hypoglycemic clamp periods in the previous 24 hours (Figure 1).

Gastric emptying

Gastric emptying was measured by scintigraphy during the first clamp of 'Day 1' and 'Day 2' of both the control (GE_{C1} and GE_{C2}) and antecedent hypoglycemia (GE_{AH1} and GE_{AH2}) periods. The test meal consisted of 100g of minced beef (25g protein, 21g fat, ~270Kcal) labelled with 20 MBq ^{99m}Technetium-sulphur colloid [22]. It was administered once the target blood glucose concentration was achieved. Radioisotopic data were acquired in dynamic mode every minute for 180 minutes using a gamma camera (DigiRad 2020tc, Gammasonics) with the patient lying in the semi-recumbent position. Data were corrected for subject movement, radionuclide decay and gamma ray attenuation [23]. Gastric emptying curves were derived for the total stomach and expressed as percent retention over time. Data were analysed by the same individual (KLJ) blinded to the study conditions.

Cardiac function

During each visit, a 4-chamber view echocardiography was performed using an ultrasound machine (SonoSite X-Porte, SonoSite Australasia Pty Limited), via parasternal long axis view by a single, unblinded, operator (PK) on day 1 and day 2 of both the control (FS_{C1} and FS_{C2}) and antecedent hypoglycemia (FS_{AH1} and FS_{AH2}) periods. Left ventricular end diastolic diameter (LV_{EDD}) and end systolic diameter (LV_{ESD}) were measured, with fractional shortening calculated via a standardised formula [(LV_{EDD}-LV_{ESD})/LV_{EDD} x 100] [24]. Echocardiography was performed at the commencement of the clamp, 15 minutes after reaching and maintaining the target blood glucose concentration and then 45 minutes after maintaining target blood glucose to quantify fractional shortening. Ejection fraction and stroke volume were calculated via the Teichholz formula [25].

Catecholamines and pancreatic polypeptide

Plasma samples were collected at the commencement of the clamp, 15 minutes after reaching the target blood glucose concentration and then 45 minutes after maintaining target blood glucose for the measurement of catecholamines (adrenaline and noradrenaline) and pancreatic polypeptide. Catecholamines samples were collected into chilled lithium heparin tubes containing 2mg of sodium metabisulphite (Sigma-Aldrich). Pancreatic polypeptide samples were collected into chilled ethylenediaminetetraacetic acid tubes. All samples were separated by centrifugation (3200rpm for 15 minutes at 4°C) within 30 minutes of collection and then stored at -70°C until assayed [26]. Catecholamine concentrations were measured via reverse phase isocratic High Performance Liquid Chromatography (HPLC) coupled with Electrochemical Detection (ECD). The sensitivity of the assay was 0.1 nmol/L with intra and inter run coefficient of variation <6% and <9% respectively. Pancreatic polypeptide was measured via enzyme-linked immunosorbent assay kit with assay sensitivity of 12.3 pg/ml and the coefficient of variation 9.5% within assays and 8.9% between assays.

Symptoms of hypoglycemia

Symptoms of hypoglycemia were recorded, using a previously described Likert scale [27], every 30 minutes (i.e. starting at clamp commencement, ending 45 minutes after achieving target blood glucose concentration), from 1 (none) to 7 (very severe), for

each symptom. These questionnaires were completed during the 3-hour clamp on 'Day 2' of each study period. Additionally, a visual analogue scale (VAS) was used quantify appetite and nausea [28].

Statistical analysis

The sample size was determined on the basis of mean and standard deviation of previous gastric emptying data (AUC over 0-180 minutes) [12]. Ten participants completing both study periods provided 80% power to detect a 63% difference in gastric emptying (quantified as AUC over 180 minutes) when comparing the predefined, primary outcome of a single episode of hypoglycemia (control day 2) versus antecedent hypoglycemia (intervention day 2) at a two-sided significance level of 0.05. Only data from participants completing both study days was retained and analysed. The primary outcome of gastric emptying (AUC from baseline to 180 minutes) was derived using the trapezoidal rule [12, 13, 29]. Catecholamine and pancreatic polypeptide levels were compared using incremental AUC ($iAUC_{0-60}$). Data were analysed using paired samples t-tests, Wilcoxon Signed Ranks tests or McNemar tests as appropriate. Data were also tested for period and carryover effects. To test the robustness of observations of the primary outcome during the longer time period (AUC_{0-180}) *a priori* secondary analyses of gastric emptying data were also performed for the time period of strict hypoglycemia (i.e. GE AUC_{0-45}) [12, 29]. Statistical analyses were performed using SPSS Version 22.0 with statistical significance set at $P<0.05$.

Results

Thirteen participants were enrolled with ten completing both study periods. All studies were well tolerated. Three participants did not return for the second visit – two because of personal reasons and one withdrew consent. Gastric emptying data from the 10 participants who completed both study periods were included (age 22.5 (3.1) years, BMI 23.8 (1.7) kg/m^2, HbA1c 5.2 (0.2) %). Echocardiography images were inadequate in one study participant and these data are limited to 9 participants.

Blood glucose

Blood glucose concentrations were clamped effectively at the desired hypoglycemic and euglycemic targets (Supplemental Figure 1).

Gastric emptying

Acute hypoglycemia accelerated gastric emptying substantially over the total measured gastric emptying period (Figure 2; $AUC_{0\text{-}180}$: GE_{C1} vs. GE_{C2}, P=0.01). Moreover, this acceleration was not affected by antecedent episodes of hypoglycemia (Figure 2; $AUC_{0\text{-}180}$: GE_{AH2} vs. GE_{C2}, P=0.74). When evaluating only the time period of strict hypoglycemia, there was no significant difference with either acute hypoglycemia when compared to normoglycemia (Figure 2; $AUC_{0\text{-}45}$: GE_{C1} vs. GE_{C2}, P=0.08) or antecedent episodes of hypoglycemia compared to first episode of hypoglycaemia (Figure 2; $AUC_{0\text{-}45}$: GE_{AH2} vs. GE_{C2}, P=0.85).

Cardiac function

When compared to euglycemia, acute hypoglycemia increased fractional shortening at the end of the strict hypoglycaemic period (Table 1 and Supplemental Figure 2; FS_{C2} vs. FS_{C1}, P<0.01). Mean fractional shortening was less after antecedent hypoglycemia, but the difference did not achieve the predefined level of significance (FS_{AH2} vs. FS_{C2}, P=0.06).

Acute hypoglycemia also increased the ejection fraction (EF_{C2} 65.8 (3.5) vs. EF_{C1} 54.3 (3.1) %, P<0.01) and stroke volume (SV_{C2} 70.4 (7.1) vs. SV_{C1} 62.4 (6.5) ml/beat, P=0.03). The increase in ejection fraction was attenuated by antecedent hypoglycemia (EF_{AH2} 58.6 (4.0) vs. EF_{C2} 65.8 (3.5) %, P=0.04) without any effect on stroke volume (SV_{AH2} 70.8 (8.0) vs. SV_{C2} 70.4 (7.1) ml, P=0.90).

Catecholamines and pancreatic polypeptide

There was a significant rise in adrenaline levels on both the control (P=0.01) and antecedent hypoglycemia days (P<0.01). However, when compared to acute hypoglycemia, antecedent episodes of hypoglycemia resulted in a smaller rise in the concentration of adrenaline (Figure 3; $iAUC_{60}$, AH2 vs. C2, P<0.05). No differences were observed with noradrenaline concentrations (P=0.61). When compared to baseline, pancreatic polypeptide concentrations increased during the postprandial phase of the control (P=0.02) and antecedent hypoglycaemia days (P<0.01); mean values were not significantly less after antecedent hypoglycemeia.

Neurological symptoms and hunger ratings

No significant differences were noted between the groups in autonomic and neuroglycopenic questionnaire scores (Supplemental Table 1). Patients reported greater hunger during C2 when compared to the AH2 (P=0.04; Supplemental Table 2). No other significant differences were noted.

Discussion

This study in healthy subjects confirms that acute hypoglycemia accelerates gastric emptying and that antecedent hypoglycemia attenuated the adrenaline response to subsequent hypoglycemia. The key finding is that the marked acceleration of gastric emptying rate is not modified by antecedent episodes of hypoglycemia. Accordingly, the primary hypothesis that acceleration of gastric emptying would diminish with antecedent hypoglycemia was rejected. The major secondary finding is that antecedent episodes of hypoglycemia may attenuate the increase in fractional shortening of the left ventricle in response to acute hypoglycemia.

The mechanisms underlying acceleration of gastric emptying by a single episode of acute hypoglycemia are poorly defined. In rats the acceleration of gastric emptying during hypoglycemia is suppressed by intravenous administration of fructose (a monosaccharide that does not cross the blood brain barrier), but suppression is eliminated by hepatic vagotomy [30]. These data suggest that hypoglycemia-induced acceleration of gastric emptying may be inhibited by signals from the periphery via increased calorie delivery to the liver. That this inhibition is abolished with hepatic vagotomy, suggests that the hepatic branch of the vagus nerve may modulate this effect on gastric emptying. Hypoglycemia also completely overrides the slowing of gastric emptying by exogenous amylin in rats [31]. Furthermore, glucose-sensitive neurons in rodents may compete with, or take precedence over, amylin receptors, which are both found in the area postrema and the nucleus tractus solitarius [32].

In humans, acute hypoglycemia accelerates the rate of gastric emptying [14] but cholinergic blockade with atropine inhibits this response [16]. The results from this study therefore suggest that vagal cholinergic mechanisms may be more important than adrenergic mechanism in modulating gastric emptying rate at differing blood glucose concentrations. However, this hypothesis is highly speculative, as no animal

or human studies have previously evaluated the effect of, and mechanisms underlying, antecedent hypoglycemia on gastric emptying or cardiac contractility. Previous data related to counter-regulatory hormones and gastric emptying suggest that exogenous, and endogenous, noradrenaline and adrenaline may slow gastric emptying via β-adrenoreceptor stimulation [33, 34], but the magnitude of effect is less than with cholinergic blockade, which may explain, at least in part, why the rate of gastric emptying during hypoglycemia did not appear to be affected by antecedent hypoglycemic episodes even though the adrenaline rise was diminished.

Consistent with the demonstrated effect on adrenaline concentrations, it appears the sympathetic nervous system is attenuated following repeated hypoglycemia. In contrast, pancreatic polypeptide, which may be indicative of the central parasympathetic response, was unaffected. However, pancreatic polypeptide may also be influenced by the rate of gastric emptying *per se*, possibly negating an effect of antecedent hypoglycemia [35]. It is also possible that antecedent hypoglycemia has differing effects on the sympathetic and parasympathetic nervous systems, consistent with the concept that blunted physiological responses to antecedent hypoglycemia occurs in a hierarchal order, as previously noted [7].

In contrast to gastric emptying, antecedent episodes of hypoglycemia attenuated the augmentation of fractional shortening and ejection fraction. The effect of antecedent hypoglycemia on cardiac function is not well studied. Adler, et al. explored its effect on cardiovascular autonomic function in humans and reported that baroreflex sensitivity and the sympathetic response to hypotensive stress are attenuated after antecedent episodes of hypoglycemia [36].

The diminished endogenous counter-regulatory hormones during antecedent hypoglycemic episodes [5, 7] may explain the observations related to fractional shortening. The mechanisms underlying the attenuation of counter-regulator hormone secretion in the setting of repeated hypoglycemia are uncertain [37], with possibilities including increased levels of cortisol [38], an increase in alternative sources of metabolic fuel (lactate and ketone bodies) [39] and changes in brain glucose uptake [40]. The potential mechanism(s) are described in detail elsewhere [41].

Strengths of the current study include novelty and the clinical relevance of the primary outcome, gastric emptying. The methodology ensured that the gastric emptying studies were performed after a prolonged period of stabilization and monitoring – thereby reducing bias and potential confounders. The blood glucose targets for hypoglycemia were based on previous studies [5, 6, 37, 42] and the insulin/glucose algorithm was established [12] and ensured precise glycemic clamping. Moreover the depth, duration and frequency of hypoglycemia, was chosen based on previous studies [7, 43, 44] and ensured a potent antecedent hypoglycemic stimulus. Scintigraphy was used to quantify gastric emptying, which is the gold standard for the measurement of gastric emptying and allows small, but clinically important differences to be detected using similar sample sizes [9, 13, 22], with analysis performed by an investigator blinded to the blood glucose concentration. Additionally, no significant period effects and carryover effects were evident.

There are, however, a number of limitations. This study was performed on healthy young men who do not normally experience hypoglycemia. Accordingly, relevance to older persons, patients with diabetes, those with autonomic neuropathy and taking medications that affect the gastrointestinal tract can only be inferred. Only a specific blood glucose concentration and defined period of hypoglycemia were tested [7]. Blood glucose was measured on venous samples using a portable glucometer for practical reasons, with inherent limitations in precision, but the differences in blood glucose between euglycemia and hypoglycemia were substantial. Furthermore, the acceleration in gastric emptying that occurred during hypoglycemia was marked. The methodology used to evaluate secondary outcomes, including the use of echocardiography to measure fractional shortening and ejection fraction, was chosen because the technique is minimally invasive, does not involve radiation and could, accordingly, be performed without compromising the assessment of the primary outcome. Nonetheless, echocardiography is operator-dependent which could potentially bias toward a positive result. Other changes in cardiovascular physiology are known to occur with hypoglycemia, as described elsewhere [3, 18, 19]. Unmeasured confounders must also be considered. The overnight stay in hospital may effect the regulation of gastrointestinal function [45, 46], but was chosen based on our previous study that demonstrated a positive result [13]. Symptoms which have been shown to be affected by antecedent hypoglycemia were not a major focus. Lastly,

given the relatively small number of study participants, a β-type error cannot be excluded – ie it is possible that antecedent hypoglycemia has a minor effect on gastric emptying that was not detected. The effects of antecedent hypoglycemia on fractional shortening 'borders on significance', which may represent an insufficient sample size but over-interpretation of P values should be avoided [47].

This study provides important inferences for clinical care. Hypoglycemia occurs repeatedly in a proportion of patients with diabetes, it may be accompanied by unawareness, and is causally associated with increased morbidity and mortality [48, 49]. The cornerstone of management involves the administration of an oral carbohydrate. The observations from this study suggests that, at least in health, the rate of gastric emptying is maintained in the face of repeated hypoglycemic events. This represents an important safety factor in treating recurrent events with oral carbohydrate.

In conclusion, the acceleration of gastric emptying during acute hypoglycemia in health does not appear to be affected by antecedent hypoglycemia, while the increase in cardiac contractility may be attenuated. Accordingly, if similar gastric responses are observed in individuals with type 1 and 2 diabetes, management of hypoglycemia with oral carbohydrate should remain effective in patients with recurrent hypoglycemia.

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Author Contributions

PK was involved in the conception and design of the study, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission. KLJ was involved in design of the study, analysis of the scintigraphic data and revising the manuscript. MPP was involved in the conception, design and coordination of the study along with acquiring data and revising the manuscript. EJG and MJS assisted with collecting data and reviewed the manuscript. SH collected the scintigraphic data and revised the manuscript. SH was involved in design of the study

and edited and redrafted the manuscript. MH helped conceive and design the study and assisted in revising the manuscript. AMD supervised PK, and was involved in the conception and design of the study, analysis and interpretation of data, and drafting and revising the manuscript for final submission. All authors read and approved the final manuscript.

PK is the 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.

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Tables

Table 1: Fractional shortening (FS) at the end of the period of strict hypoglycaemia during Control (Day 1, C1 and day 2, C2) and Antecedent Hypoglycemia (Day 1, AH1 and day 2, AH2) study periods. Data is mean (SD).

	FS (%)
Control Day 1, C1	28.4 (5.7)
Control Day 2, C2	36.8 (8.1)
Antecedent Hypoglycemia Day 1, AH1	33.9 (11.0)
Antecedent Hypoglycemia Day 2, AH2	31.7 (8.4)

Figures

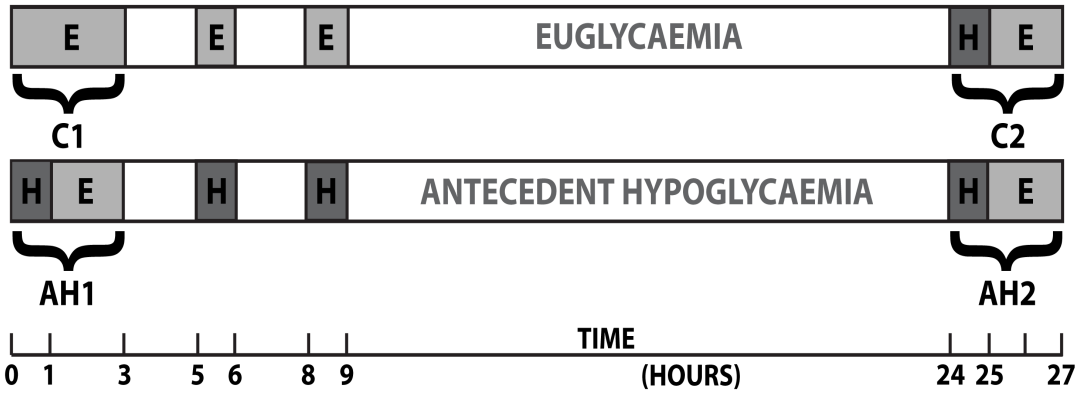


Figure 1: Protocol of the study outlining a) Euglycemia (Control) and b) Antecedent hypoglycemia interventions. To compare effects of antecedent hypoglycemia the primary comparison was between measurements made during hypoglycemia after 24 hours of euglycemia in hospital (C2) with measurements made during hypoglycemia after 3 periods of hypoglycemia in hospital in the preceding 24 hours (AH2)

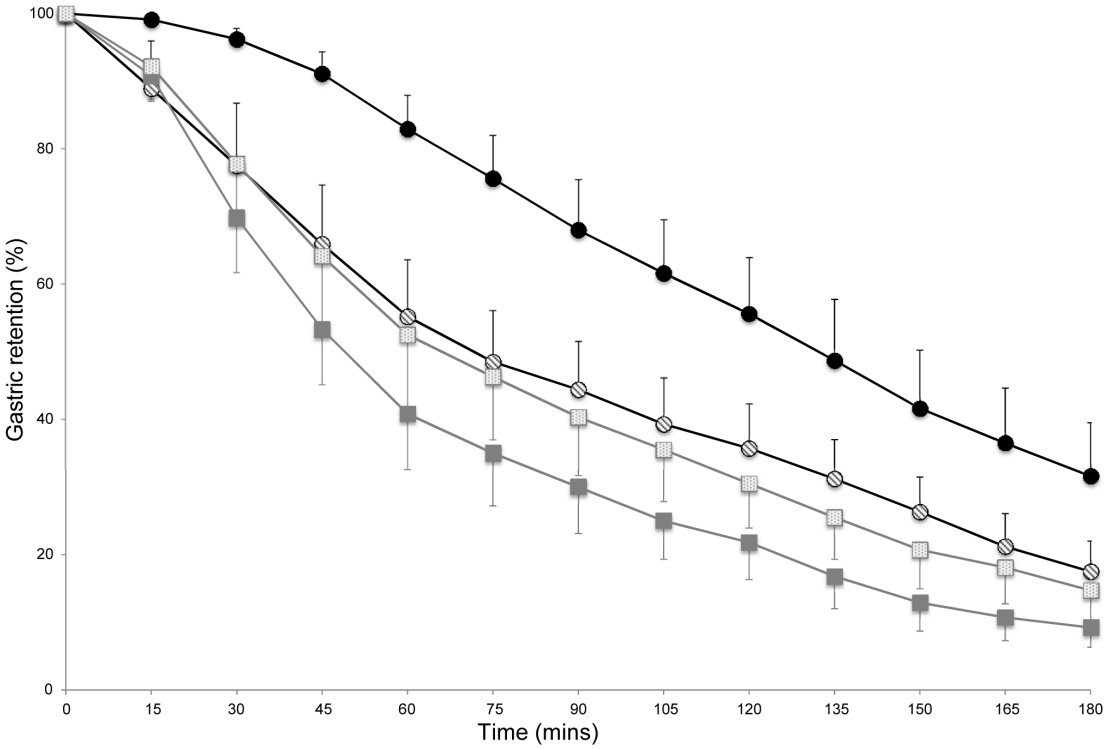


Figure 2: Gastric emptying curves during Control (C1 black circles, C2 striped circles) and Antecedent Hypoglycemia (AH1 dark grey squares, AH2 light grey squares) study periods. Control period underwent a clamp at 6 mmol/L for 180 minutes. Antecedent hypoglycaemia period underwent an initial clamp at 2.8 mmol/L for 45 minutes, followed by 15 minutes to achieve euglycemia, then another 120 minutes at 6 mmol/L (Total 180 minutes). Data are mean (SD).

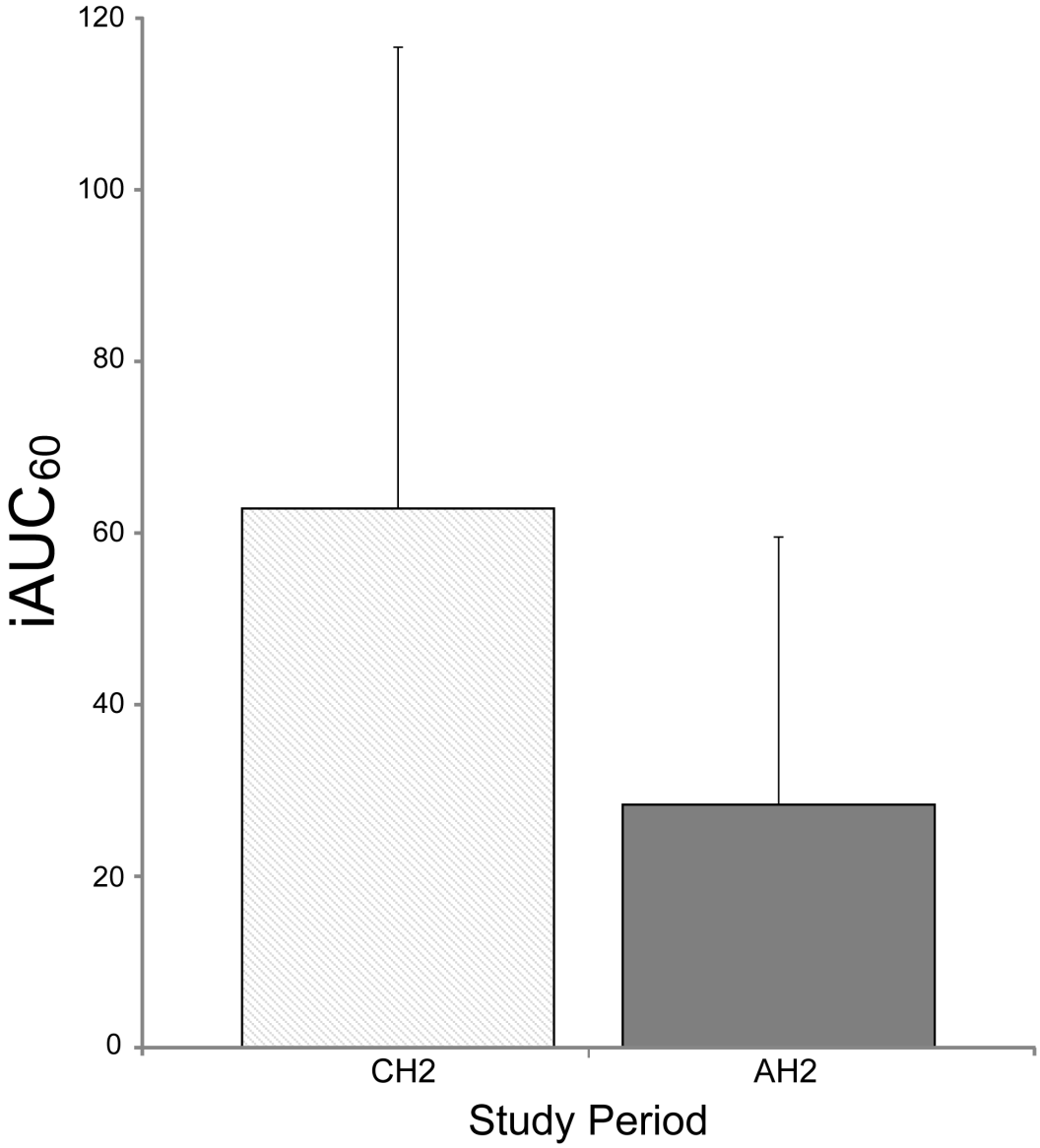


Figure 3: Plasma adrenaline $iAUC_{60}$ comparing day 2 of Control and Antecedent Hypoglycemia study periods. Data are mean (SD)

Supplemental Tables

Supplemental Table 1: Autonomic and neuroglycopaenic questionnaire scores. Figures represent the numbers of participants who felt the following symptoms during the second day of each study period. Analysed using the McNemar test and due to this, P values cannot be computed when the responses were positive.

	Control day 2, C2	Antecedent hypoglycemia day 2, AH2	P values
Odd behaviour	3	0	
Pounding heart	4	3	>0.99
Itching	0	0	
Drowsiness	6	7	>0.99
Difficulty speaking	1	1	>0.99
Shaking/Tremor	4	3	>0.99
Headache	2	0	
Hunger	10	9	
Clumsiness	3	2	>0.99
Incoordination	4	1	0.25
Sweating	5	3	0.63
Confusion	1	1	>0.99
Nausea	1	0	
Feel like sugar is low	9	9	>0.99

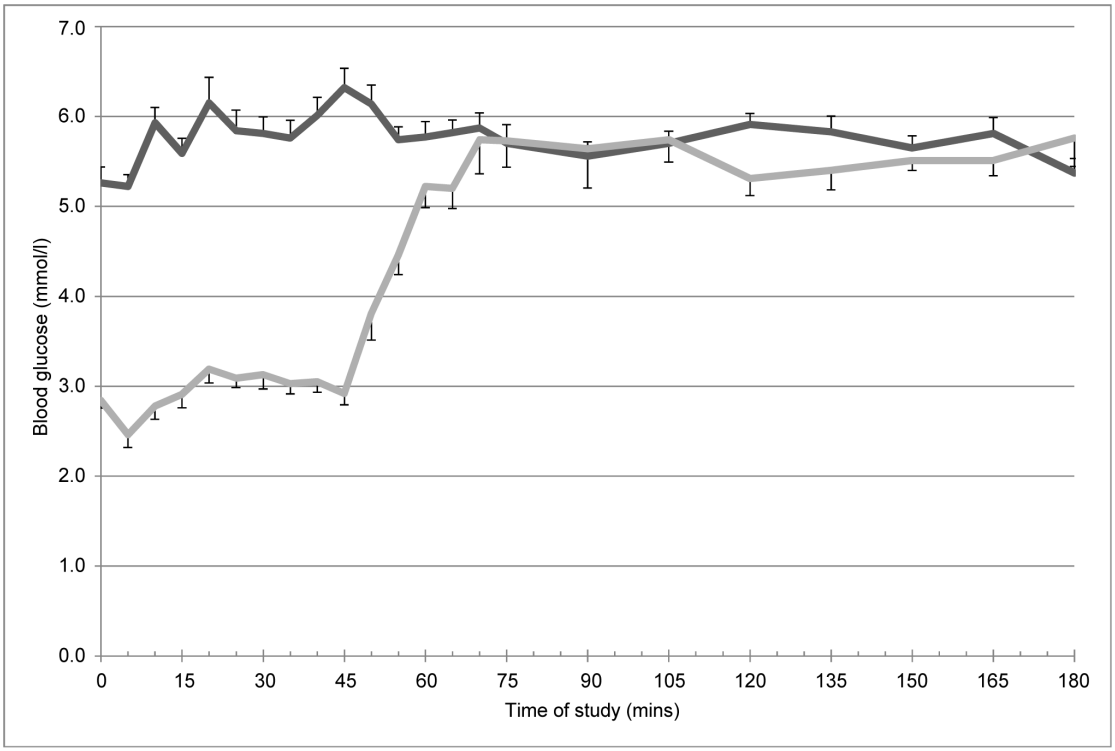
Supplemental Table 2: Appetite Visual Analogue Scale scores. Mean scores during the 3 hour clamp on Control day 2, C2, were compared to mean scores during the 3 hour clamp on Antecedent hypoglycemia day 2, AH2, and tabulated according to whether AH2 was higher, lower or equal to the score from C2. Data were analysed using the Wilcoxen Rank test.

	Higher	Lower	Equal	P value
I feel dizzy	3	1	6	0.47
I feel tired	2	4	4	0.92
How strong is your desire to eat	4	5	1	0.37
I feel hungry	1	8	1	0.04
Do you think you could eat	4	5	1	0.68
I feel sick	2	2	6	0.85
I feel full	5	2	3	0.31
I feel faint	2	4	4	0.67
I have indigestion	1	2	7	>0.99
I have a headache	1	2	7	>0.99
I feel thirsty	4	4	2	0.89
My chest hurts	1	2	7	>0.99
I feel weak	1	3	6	0.47
If given a meal, would you eat it	3	1	6	0.47

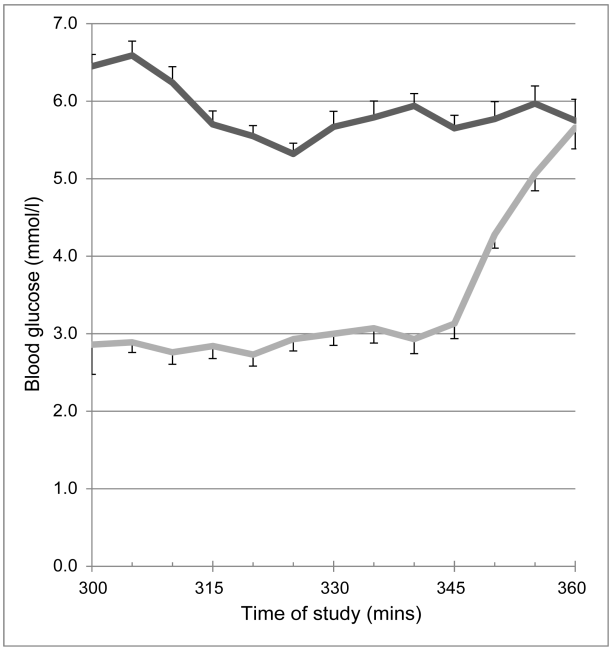
Supplemental Figures

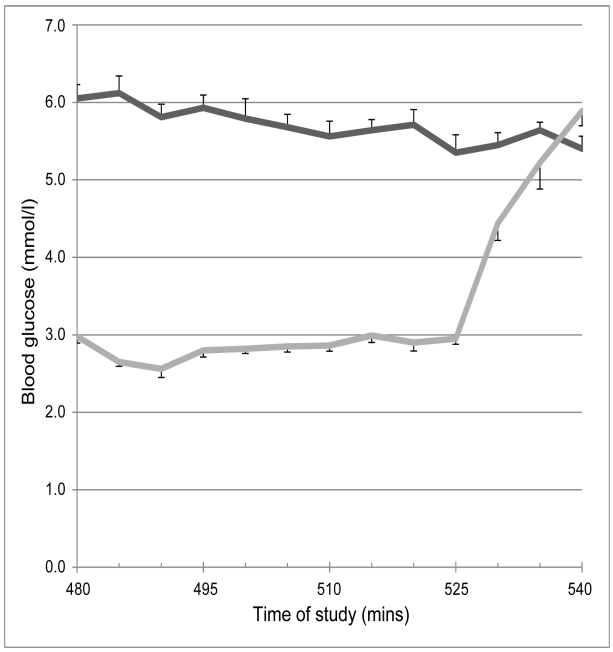
Supplemental Figure 1: Glycemia curves on day 1 and day 2 of Control (Dark grey) and Antecedent Hypoglycemia (Light grey) study periods. Data are mean (SD)

a) Clamp 1 (C1 vs AH1)

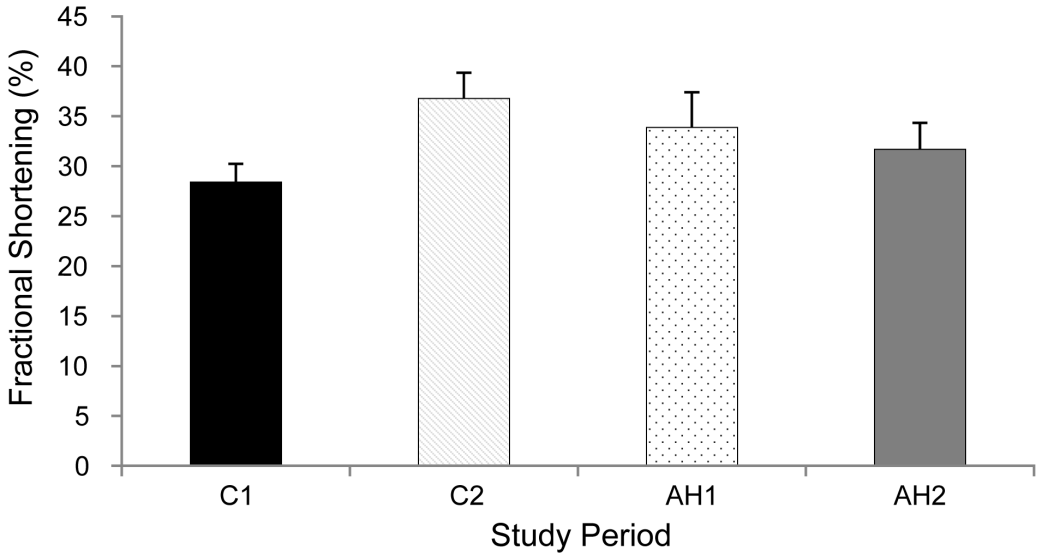


b) Clamp 2 and 3





c) Clamp 4 (C2 vs AH2)



Supplemental Figure 2: Cardiac fractional shortening on day 1 and day 2 of Control and Antecedent Hypoglycemia study periods. Data are mean (SD).

4.3 CONCLUSIONS

4.3.1 *Introduction*

Given the increasing prevalence of type 2 diabetes and clinical risks associated with hypoglycaemia, optimal management of this condition is particularly important [1, 2]. Compared to a single, acute episode of hypoglycaemia, antecedent hypoglycaemia results in a diminished catecholamine response [3]. There have not been any human studies which have assessed whether the gastric emptying and cardiac responses to subsequent hypoglycaemia are affected by antecedent hypoglycaemia. Acceleration of gastric emptying during hypoglycaemia is likely to be a very important counter-regulatory response to facilitate the standard treatment of hypoglycaemia. Accordingly investigating a process that may compromise successful treatment is important.

4.3.2 *Contribution of the work described in this thesis to the understanding of the effects of antecedent hypoglycaemia on gastric emptying*

Acute hypoglycaemia substantially accelerated gastric emptying, however, the acceleration was apparently unaffected by antecedent episodes of hypoglycaemia. The reasons for this are uncertain, but a possibility is that vagal cholinergic effects are more important than adrenergic mechanisms in modulating gastric emptying at differing blood glucose concentrations. The results from this study suggest that, in health, the rate of gastric emptying is maintained despite repeated hypoglycaemic events allowing effective management with oral carbohydrate.

4.3.3 *Contribution of the work described in this thesis to the understanding of the effects of antecedent hypoglycaemia on cardiac function*

When compared to acute hypoglycaemia, antecedent hypoglycaemia attenuated fractional shortening and the ejection fraction. Previous data relating to the effect of antecedent hypoglycaemia on cardiac function is very limited, with a single study by Adler, et al. noting baroreflex sensitivity and the sympathetic response to hypotensive stress are attenuated after antecedent episodes of hypoglycaemia [4].

4.3.4 *Contribution of the work described in this thesis to the understanding of the effects of antecedent hypoglycaemia on hormone release*

Compared to acute hypoglycaemia, antecedent hypoglycaemia was associated with a smaller rise in the concentration of adrenaline. No difference was noted with noradrenaline or pancreatic polypeptide concentrations. The reduced adrenalin response is consistent with previous data showing diminished counter-regulatory hormones during antecedent hypoglycaemic episodes and may account for the attenuation of cardiac findings in the study [3, 5].

4.4 FUTURE DIRECTIONS

4.4.1 *Future trials to determine the effects of antecedent hypoglycaemia in patients with diabetes*

The outcomes reported in Chapter 4.2 provide evidence that in health, three episodes of antecedent hypoglycaemia does not affect the rate of gastric emptying. If similar 'gastric' responses are observed in individuals with diabetes, many of whom have autonomic impairment, it would signify an important safety aspect in the management of hypoglycaemia. Additionally, cardiac responses to antecedent hypoglycaemia have not been evaluated in patients with diabetes.

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Appendix A

PRESENTATIONS AT NATIONAL OR INTERNATIONAL MEETINGS

The Student presented the studies completed during 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:

National Presentations

2016

Australian Diabetes Society Annual Scientific Meeting

Effects of antecedent hypoglycaemia on the gastric and cardiac responses to subsequent hypoglycaemia in health

Gold Coast, Australia

2015

Australian New Zealand Intensive Care Society Annual Scientific Meeting

Personalised glycaemic control in critically ill type-2 diabetic patients: an exploratory study

Auckland, New Zealand

Australian Diabetes Society Annual Scientific Meeting

Personalised glucose therapy: glucose targets in critically ill patients with pre-existing poorly controlled type 2 diabetes

Adelaide, Australia

2014

Australian New Zealand Intensive Care Society Annual Scientific Meeting

Energy dense feeds may worsen enteral feed-intolerance

Melbourne, Australia

International Presentations

2016

European Association for the Study of Diabetes - 52nd EASD Annual Meeting 2016

Effects of antecedent hypoglycaemia on the gastric and cardiac responses to subsequent hypoglycaemia in health
Munich, Germany

2015
European Society of Intensive Care Medicine - LIVES 2015, 28th Annual Congress
Personalised glucose therapy: glucose targets in critically ill patients with pre-existing poorly controlled type 2 diabetes
Berlin, Germany

2014
Society of Critical Care Medicine - 43rd Critical Care Congress
The effect of glucose-dependent insulinotropic polypeptide (GIP) in critically ill patients
San Francisco, USA

State of the Art Meeting 2014
Energy dense feeds may worsen enteral feed-intolerance
London, UK

State of the Art Meeting 2014
Liberal when compared to standard blood glucose targets in critically ill patients with pre-existing inadequately controlled type 2 diabetes
London, UK

Appendix B

PRIZES AWARDED DURING CANDIDATURE

2016

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

2015

Australian New Zealand Intensive Care Society Annual Scientific Meeting – Matt Spence Medal Winner for the for the best registrar presentation

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

Australian New Zealand Intensive Care Society / College of Intensive Care Medicine Continuing Education Meeting - Tub Worthley Travelling Scholarship Winner for the best registrar presentation

2014

Australian New Zealand Intensive Care Society Annual Scientific Meeting – Matt Spence Medal Finalist

Appendix C

GRANTS AWARDED DURING CANDIDATURE

2017

Deane A, Poole A, Kar P, Finnis M, Martensson J, Finfer S, Horowitz M, Bellomo R. **Liberal glUcose Control in critically Ill patient with pre-existing type 2 Diabetes (LUCID): a phase IIB multi-centre single-blinded parallel group randomised control trial.**
Intensive Care Foundation Grant
$25 000

Kar P, Deane AM, M Horowitz, Finfer S, Bellomo R, Maiden MJ, Finnis ME, Poole A. **Liberal glUcose Control in critically Ill patient with pre-existing type 2 Diabetes (LUCID): a phase IIB multi-centre single-blinded parallel group randomised control trial.**
2017 RAH Research Committee Clinical Project Grant
Royal Adelaide Hospital Research Committee
$49 950

Stevens J, Jones KL, Rayner C, Kar P, Deane AM, Horowitz M. **Acute hypoglycaemia and gastric emptying – 'dose-response' and impact on oral drug absorption.**
2017 RAH Research Committee Clinical Project Grant
Royal Adelaide Hospital Research Committee
$30 000

2016

Ali Abdelhamid Y, Jones K, Horowitz M, Kar P, Phillips L, Nguyen T, Deane AM. **Prevalence, mechanisms and impact of postprandial hypotension in elderly survivors of critical illness.**
2016 Royal Adelaide Hospital NHMRC 'Near Miss' Grant
Royal Adelaide Hospital Research Committee
$49 207

2015 - 2017

Kar P

AR Clarkson Scholarship

Royal Adelaide Hospital Research Committee

$300 000

Appendix D

OTHER PUBLICATIONS COMPLETED DURING CANDIDATURE

2018

Nguyen TAN, Abdelhamid YA, Weinal LM, Hatzinikolas S, Kar P, Summers MJ, Phillips LK, Horowitz M, Jones KJ, Deane AM. **Postprandial Hypotension in Older Survivors of Critical Illness.** *Journal of Critical Care.* [Accepted Jan 2018]

2017

Du YT, Kar P, Abdelhamid YA, Horowitz M, Deane AM. **Glycated haemoglobin is increased in critically ill patients with stress hyperglycaemia: implications for risk of diabetes in survivors of critical illness.** *Diabetes Research and Clinical Practice*. 2017 Nov;135:73-5 [Epub ahead of print]

Ali Abdelhamid Y, Plummer MP, Finnis ME, Birader V, Bihari S, Kar P, Moodie S, Horowitz M, Shaw JE, Phillips LK, Deane AM. **Long-term mortality of critically ill patients with diabetes who survive admission to Intensive Care.** *Critical Care and Resuscitation.* 2017 Dec;19(4):303-9

2016

Plummer MP, Finnis ME, Phillips LK, Kar P, Bihari S, Birader V, Moodie S, Horowitz M, Shaw JE, Deane AM. **Stress induced hyperglycemia and the subsequent risk of type 2 diabetes in survivors of critical illness.** *PLoS ONE*. 2016 Nov; 11(11):e0165923

Plummer MP, Finnis ME, Horsfall H, Ly M, Kar P, Ali Abdelhamid Y and Deane AM. **Prior exposure to hyperglycaemia attenuates the relationship between glycaemic variability during critical illness and mortality.** *Critical Care and Resuscitation*. 2016 Sep; 18(3):189-97

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*. 2016 Sep; 44(9):e790-6

Luethi N, Cioccari L, Tanaka A, Kar P, Giersch E, Deane AM, Mårtensson J and Bellomo R. ***Glycated hemoglobin (HbA1c) levels are not affected by critical illness.*** *Critical Care Medicine*. 2016 Sep; 44(9):1692-4

Plummer MP, Kar P, Cousins CE, Lange K, Chapman MJ, Nauck MA, Horowitz M, Meier JJ and Deane AM. **The insulinotropic effect of pulsatile compared with continuous intravenous delivery of GLP-1.** *Diabetologia*. 2016 May; 59(5):966-9