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TITLE PAGE

Title

Endogenous amylin and glucagon-like peptide-1 concentrations are not associated with the rate of gastric emptying in the critically ill.

Short Title

Amylin, GLP-1 and gastric emptying in ICU.

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ABSTRACT (≤ 250 words – Currently 250)

Background:

In health, the hormones amylin and glucagon-like peptide-1 (GLP-1) slow gastric emptying (GE) and modulate glycaemia. The aims of this study were to determine amylin and GLP-1 concentrations in the critically ill and their relationship with GE, glucose absorption and glycaemia.

Methods:

In fasted critically ill and healthy subjects (n=26 and 23 respectively) liquid nutrient, containing 100mg ^{13}C-sodium octanoate and 3g 3-O-methlyglucose (3-OMG), was administered via a nasogastric tube. Amylin, GLP-1, glucose and 3-OMG concentrations were measured in blood samples taken during fasting, and 30 and 60min after the 'meal'. Breath samples were taken to determine GE (Gastric Emptying Coefficient; GEC). Intolerance to intragastric feeding was defined as a gastric residual volume of ≥250ml and/or vomiting in the 24hours prior to the study.

Results:

Although GE was slower [GEC critically ill: 2.8±0.9 vs. health: 3.4±0.2; P=0.002], fasting blood glucose was higher [7.0±1.9 vs. 5.7±0.2mmol/l; P=0.005] and overall glucose absorption was reduced in critically ill patients (3-OMG) [9.4±8.0 vs. 17.7±4.9mmol/l.60min; P<0.001], there were no differences in fasting or postprandial amylin concentrations. Furthermore, although fasting [1.7(0.4-7.2) vs. 0.7(0.3-32.0)pmol/l; P=0.04] and postprandial [3.0(0.4-8.5) vs. 0.8(0.4-34.3)pmol/l; P=0.02] GLP-1 concentrations were increased in the critically ill and were greater in feed intolerant when compared with those tolerating feed [3.7(0.4-7.2) vs. 1.2(0.7-

4.6)pmol/l; P=0.02], there were no relationships between GE and fasting amylin or GLP-1 concentrations.

Conclusion:

In the critically ill, fasting GLP-1, but not amylin, concentrations are elevated and associated with feed intolerance. Neither amylin nor GLP-1 appears to substantially influence the rate of GE.

Key Words

Gastric Emptying, Glucagon Like Peptide-1, Amylin, Critical Illness, Enteral Nutrition

INTRODUCTION

In the critically ill, markedly delayed gastric emptying occurs frequently, which impairs delivery of intragastric nutrition[1,2]. The pathophysiology of delayed gastric emptying in the critically ill is multi-factorial but reflects, at least in part, a heightened feedback response to the presence of nutrients in the small intestine[3]. In health, hormones secreted from the stomach and small intestine play a pivotal role in the regulation of gastric emptying[4-7]. For example, cholecystokinin (CCK) and peptide YY (PYY) potently slow gastric emptying, while ghrelin may accelerate it[8]. Our group has reported that fasting and postprandial concentrations of CCK and PYY are elevated in the critically ill, particularly in patients intolerant to intragastric feed[4]. Conversely, the rate of gastric emptying is a determinant of CCK and PYY secretion in the critically ill, as it is in health[5,9,10]. Hence, there is evidence of disordered gastrointestinal hormone secretion in the critically ill, which may contribute to delayed gastric emptying.

Amylin is a 37-amino acid polypeptide co-secreted with insulin[11]. In humans, exogenous administration of amylin and the amylin analogue, pramlintide, slows gastric emptying substantially[11]. Furthermore, at least in animal models, amylin appears to be a more potent physiological regulator of gastric emptying than other enterogastrones, including CCK and glucagon-like peptide-1 (GLP-1)[12]. Using gastric residual volumes (GRVs) to determine tolerance to intragastric feed, Mayer and colleagues reported that fasting plasma amylin concentrations were much higher in critically ill children who were intolerant of intragastric feed, when compared to the feed-tolerant [13], suggesting that endogenous amylin may contribute to slow gastric emptying in the critically ill. However, there is no information about fasting or nutrient stimulated amylin concentrations in adult critically ill patients.

GLP-1 is secreted from intestinal L-cells, predominantly from the ileum and colon, in response to intraluminal nutrient, stimulating glucose-dependent insulin secretion, and suppressing glucagon [3]. In health and type 2 diabetes, the rate of nutrient delivery into the small intestine is a pivotal determinant of the magnitude of endogenous GLP-1 secretion[9]. In health, endogenous GLP-1 slows gastric emptying[6], while exogenous GLP-1 at pharmacological doses slows gastric emptying substantially in health, type 2 diabetes and critical illness, leading to a reduction in postprandial glycaemic excursions[14,15]. The magnitude of the effects are dependent on the baseline rate of gastric emptying, such that GLP-1 has little, if any effect when gastric emptying is already delayed[14-16]. However, there is no information in adult critically ill patients about fasting or nutrient stimulated plasma GLP-1 concentrations and their relationship to gastric emptying.

In health and diabetes, there is a complex relationship between upper gastrointestinal function and glycaemic control, such that postprandial glycaemia is both a determinant of, and determined by, gastric emptying[17,18]. Pre-prandial blood glucose concentrations influence gastric emptying in the critically ill, as is the case in diabetes[17], while glucose absorption is reduced in these patients compared to health and is dependent on the rate of gastric emptying[19]. Glycaemic control appears to be a pivotal determinant of outcome in the critically ill, with marked hyperglycaemia and hypoglycaemia associated with adverse outcomes including increased mortality and hospital length of stay[20,21]. However, the relationship between endogenous secretion of GLP-1 and glycaemia in the critically ill is poorly defined[22].

The primary objectives of this study were to quantify fasting and postprandial concentrations of amylin and GLP-1 in critically ill adult patients, and to determine whether there is a relationship between these concentrations and the rate of gastric emptying. As gastric emptying affects postprandial blood glucose concentrations which are, in turn, affected by small intestinal glucose absorption, secondary objectives were to quantify blood glucose and glucose absorption.

METHODS

Subjects

Critically ill patients were eligible for inclusion if aged $\geq$ 17 years, mechanically ventilated and receiving enteral nutrition. Exclusion criteria included pregnancy, pre-existing diabetes and previous surgery on the oesophagus, stomach or duodenum. In patients receiving an insulin infusion (Actrapid; Novo-Nordisk, Denmark), this was ceased 3 hours prior to the commencement of the study. Healthy subjects were used as a comparator. In this group, exclusion criteria included pregnancy or breastfeeding, diabetes, contraindication to nasogastric feeding tube placement, previous gastrointestinal surgery, use of medications known to affect gastrointestinal motility, and current or previous gastrointestinal disease or major dysfunction. Critically ill patients were recruited between October 2008 and February 2010. A proportion of the data in this group (gastric emptying) have been reported previously as they provided baseline values in the placebo arm of a previous study[14]. Healthy subjects were studied between March 2010 and April 2011, and none of their data have previously been reported.

Study Protocol

Subjects were fasted for at least six hours. Following this a fasting blood sample, was obtained and a nutrient liquid test 'meal' of 100 ml Ensure® (Abbott, Victoria, Australia) (64% carbohydrate, 21% lipid, 13% protein, 1.06 kcal/ml) including 100mg[13]C-sodium octanoate (Cambridge Isotope Laboratories, Andover, Mass., USA) and 3g of 3-O-methyglucose (3-OMG) (Sigma-Aldrich, Castle Hill, NSW, Australia) was administered over 5 min via a nasogastric tube with the end of the infusion designated as t=0 min. The nasogastric tube was already in situ in critically ill patient,

while in healthy subjects a 12F x 91cm nasogastric tube (Flexiflo, Abbott, OH, USA) was inserted into the stomach via an anaesthetised nostril, using topical Co-phenylcaine Forte spray (ENT technologies Pty Ltd, Victoria, Australia) and Lignocaine gel 2% (Orion Laboratories, Pty Ltd Western Australia, Australia). Correct nasogastric tube positioning was verified by measuring pH of aspirates and auscultation of air boluses.

Arterial blood samples in critically ill patients and venous blood samples in healthy subjects were obtained during fasting and at 30 and 60 min following the test meal. Blood samples were collected into chilled ethylenediaminetetraacetic acid (EDTA) tubes containing 2000KIU aprotinin (Trasylol, Bayer Healthcare, NSW, Australia) for measurement of plasma total amylin, or 40ul dipeptyl-peptidase-4 (DPP-4) inhibitor (Millipore, Billerica, MA, USA) for the measurement of plasma active GLP-1. For the measurement of serum 3-OMG, blood samples were collected into 5mL serum tubes. Plasma/serum were separated by centrifugation (3,200 rpm for 15 min at 4 degree Celsius) within 30 min and subsequently stored at -70 degrees Celsius until assayed.

Breath samples were collected at intervals (5 min for the first hour, 15 min for the subsequent 3 hours, and every 30 min until t = 330 min) from the expiratory limb of the ventilator tubing using an airway adapter (Smiths Medical, WI, USA) and venoject holder containing a needle (VenojectLuer Adapter; Terumo Corporation, Tokyo, Japan) in critically ill patients[23].This technique allowed reliable filling of Exetainer (Labco Limited, Buckinghamshire, UK) for measurement of exhaled $^{13}CO_2$. In healthy subjects, subjects exhaled completely through a straw into the Exetainer.

The protocols were approved by the Human Research Ethics Committee of the Royal Adelaide Hospital (RAH Protocol No: 061229c and 100208) and performed according to the National Health and Medical Research Council guidelines for the conduct of research on unconscious patients. Written, informed consent was obtained from healthy subjects directly, and from the next of kin for critically ill patients.

Measurements

Gastric emptying

Breath samples were analysed to determine the percentage of ^{13}C recovered per hour, plotted over time. The area under the recovery curve was used to calculate the gastric emptying coefficient (GEC), which is a global index of the gastric emptying rate that accounts for the rate of appearance and disappearance of tracer in the breath. Greater GEC values indicate the emptying rate is more rapid[23].

Measurement of blood glucose, plasma amylin and GLP-1 concentrations and glucose absorption

Blood glucose was measured at the bedside with a portable glucometer (Medisense Optimum, Abbott, IL, USA). Plasma total amylin was measured using the Milliplex kit, with an assay sensitivity of 13 pg/mL, and CVs were <5 % within, and <8 % between, assays. Plasma active GLP-1 was measured using an enzyme-linked immunosorbent assay (ELISA) (Epitope Diagnostics, CA, USA). The assay sensitivity and intra and inter run CV were 0.4pmol/l, and <4 % and <6 % respectively. Serum 3-OMG was used as an index of glucose absorption, measured using liquid chromatography/mass spectroscopy, with an assay sensitivity of 0.0103 mmol/l. 3-OMG is a monosaccharide

absorbed from the small intestine via the same transporters as glucose, however is not metabolised, providing an accurate measure of glucose absorption[24,25].

Statistical Analysis

Data are presented as mean ± SD or median (range) as appropriate. Depending on data distribution, within-subject comparisons were made using Student's t-test or Wilcoxon Signed Ranks tests and between-subject comparisons using Mann-Whitney U tests. Relationships were evaluated using Spearman correlation. The null hypothesis was rejected at a P value ≤0.05. Statistical analyses were performed using SPSS (Version 17.0; SPSS, IL, USA).

Based on the study by Mayer and colleagues[13] it was decided, a priori to classify patients as tolerant or intolerant to intragastric feed and perform subgroup analysis. Intolerance to intragastric feed was defined as a GRV of ≥250 mL and/or vomiting during feeding within the 24 hours prior to the study [26]. One patient had not commenced enteral feeding prior to the study and, therefore, feed tolerance could not be classified. This patient was omitted from sub group analysis. As blood glucose concentrations were predictably different at baseline between critically ill patients and healthy subjects, these data are reported as change from baseline. The incremental area under the curve (iAUC) for blood glucose and area under the curve (AUC) for serum 3-OMG concentrations after the meal were calculated using the trapezoidal rule.

RESULTS

Subjects

Twenty-six critically ill patients [20M:6F; Age 51 (20-85) years; BMI 26 (17-36) kg/m^2; APACHE II score on admission 22 (11-38); serum creatinine>120 umol/l 4/26 (15%); 15 tolerating intragastric feed, 10 intolerant to intragastric feed, 1 fasted from admission] and twenty-three healthy subjects [19M:4F; Age 34(18-88) years; BMI 24 (18-30) kg/m^2] were studied.

Gastric Emptying

When compared to health, critical illness was associated with slower gastric emptying [GEC: critically ill: 2.8 ± 0.9 vs. health: 3.4 ± 0.2; P=0.002] (figure 1a). Gastric emptying was slower in patients classified as intolerant to intragastric feed [GEC: 3.1 ± 0.8 in patients tolerating intragastric feed vs. 2.3 ± 1.0 in the intolerant; P=0.04] (figure 1b).

Plasma amylin concentrations

During fasting, amylin concentrations were similar in critically ill and healthy subjects [critically ill: 12.0 (1.8-124.6) pmol/l vs. health: 7.8 (4.1-18.7) pmol/l; P=0.31] respectively. Fasting amylin concentrations were similar in patients tolerant and intolerant to intragastric feed [Feed tolerant: 15.4 (1.8-58.5) pmol/l vs. Feed intolerant: 10.2 (3.8-124.6) pmol/l; P=0.37]. Amylin concentrations were also comparable in critical illness and health both 30 minutes [critically ill: 12.8 (2.2-121.4) pmol/l vs. health: 13.8 (7.4-30.0) pmol/l; P=0.89] and 60 minutes [critically ill: 30.0 (7.2-191.3) pmol/l vs. health: 13.4 (6.7-36.2) pmol/l; P=0.11] following the meal. Amylin

concentrations increased significantly postprandially in the critically ill and healthy subjects respectively [P=0.002 and P<0.001].

Plasma Glucagon-like Peptide-1 concentrations

In critical ill patients, GLP-1 concentrations were increased when compared to health during fasting [critically ill: 1.7 (0.4-7.2) vs. health: 0.7 (0.3-32.0) pmol/l; P=0.04], and 30 minutes after the meal [critically ill: 3.0 (0.4-8.5) vs. health: 0.8 (0.4-34.3) pmol/l; P=0.02], but were similar at 60 minutes [critically ill: 1.1 (0.4-8.2) vs. health: 0.7 (0.4-35.7) pmol/l; P=0.55]. Fasting GLP-1 concentrations were greater in patients intolerant to intragastric feed [Feed intolerant: 3.7 (0.4-7.2) pmol/l vs. Feed tolerant: 1.2 (0.7-4.6) pmol/l; P=0.02] (figure 2). GLP-1 concentrations increased postprandially in both the critically ill and healthy subjects [P=0.002 and P=0.03].

Blood glucose concentrations

Fasting blood glucose concentrations were greater in critical illness than health [critically ill: 7.0 ± 1.9 vs. health: 5.7 ± 0.8 mmol/l; P=0.005]. The increment in blood glucose concentrations following the 'meal' was greater in healthy subjects than in the critically ill [iAUC; critically ill: 38.6 ± 63.3 mmol/l.min vs. health: 71.3± 38.0 mmol/l.min; P=0.04] (figure 3).

Glucose absorption

Glucose absorption was also reduced in critical illness compared to health [3-OMG AUC; critically ill: 9.4 ± 8.0 mmol/l.min vs. health: 17.7 ± 4.9 mmol/l.min; P<0.001] (figure 4), as was the peak 3-OMG concentration [critically ill: 0.27 ± 0.24 mmol/l vs. health: 0.48 ± 0.12 mmol/l; P<0.001].

Relationships of glucose, amylin, GLP-1 and 3-OMG concentrations with gastric emptying

In the critically ill, there was no association between gastric emptying (GEC) and either fasting amylin concentrations [rho=0.145; P=0.49] or fasting GLP-1 concentrations [rho=-0.10; P=0.66]. There were however strong relationships between gastric emptying (GEC) and glycaemia (iAUC) [rho=0.54; P=0.006], gastric emptying and glucose absorption (AUC for 3-OMG) [rho=0.73; P<0.001], and between glycaemia (iAUC) and AUC for 3-OMG [rho=0.59; P=0.002].

Similarly, in the healthy subjects, there was no association between gastric emptying (GEC) and either fasting amylin concentrations [rho= -0.13; P=0.56] or fasting GLP-1 concentrations [rho=-0.28; P=0.10]. In health, there was also no relationship between gastric emptying and either glycaemia or glucose absorption, nor was there a relationship between glycaemia and glucose absorption (data not shown).

DISCUSSION

This study was performed primarily to gain insights into whether elevated concentrations of amylin and GLP-1 have a potential role in the delayed gastric emptying that occurs frequently in adult critically ill patients. In the cohort studied, gastric emptying was much slower than in health, as would be predicted. Novel findings are that in the critically ill, fasting GLP-1 concentrations were greater than in healthy subjects, particularly in patients intolerant to intragastric feed, while fasting amylin concentrations were comparable between critical illness and health. However, the rate of gastric emptying was not related to fasting concentrations of either amylin or GLP-1, suggesting that they are not major determinants of gastric emptying in this group. Consistent with previous findings, the rate of glucose absorption was reduced[25] and fasting blood glucose concentrations greater in critically ill patients when compared to health[25].

The observation that amylin concentrations did not appear to be a determinant of feed tolerance in this adult cohort differs from a previous report in critically ill children, in which increased serum amylin concentrations were associated with delayed gastric emptying[13]. However the latter study may have had methodological limitations including a small sample size and a heterogeneous cohort, including a wide age range[13]. While our cohort was not large, a substantial difference in amylin levels would appear unlikely to be found with even greater numbers. It is also possible that different factors influence gastric motility in critically ill children and adults.

Potential limitations of the current study should be recognised. Metabolism and/or excretion of hormones is often reduced in the critically ill – especially in patients with

renal failure – and the use of plasma concentrations as an index of secretion in this group has inherent inaccuracies[27]. For this reason, we measured concentrations of the active form of GLP-1 (GLP-1 amide 7-36), as opposed to total GLP-1 which includes both the active form and the inactive form GLP-1 9-36. Furthermore GLP-1 9-36 has no effect on gastric emptying[28] and measurement of total GLP-1 would accordingly also have compromised evaluation of the association with gastric emptying. It is somewhat surprising that there was no relationship between GLP-1 and gastric emptying, as intolerance to intragastric feed was defined by the presence of large gastric residual volumes, which is known to relate to the rate of gastric emptying as measured by scintigraphy[1] and fasting GLP-1 concentrations were elevated in feed intolerant patients. Indeed, fasting GLP-1 concentrations in feed intolerant patients were almost three-fold greater than in patients tolerating intragastric feed and almost five-fold the concentrations observed in health. However, the radioisotope breath test used to measure gastric emptying, while validated in the critical care, is less precise than the 'gold-standard' of scintigraphy[29]. In addition, the assigning of patients to a feed tolerance status by dichotomizing on the basis of GRVs, may have inaccuracies[30]. However, the use of a GRV of ≥250ml as an indication of feed intolerance is a clinical measure used by the centre at which the study was performed to indicate the requirement for therapies to treat failed intragastric feeding i.e. prokinetic therapy or small intestinal feeding catheters, and hence is of clinical significance to critically ill patients.

The cohort studied was a heterogeneous sample of critically ill patients, and other non-hormonal factors may have contributed to delayed gastric emptying, such as opiate administration[31]. Moreover, we have reported that pharmacological doses of GLP-1, the

peptide that profoundly slows gastric emptying in health, may not have an effect when gastric emptying is already delayed[14]. For these reasons we cannot exclude the possibility that GLP-1 may play a role in the regulation of gastric emptying in the critically ill, with an effect on glucose absorption and postprandial glycaemia. We have previously demonstrated, using the specific GLP-1 antagonist exendin 9-39 amide, that endogenous GLP-1 slows GE in health, thereby attenuating postprandial glycaemia[6]. However, there are substantial logistical difficulties with undertaking a study using exendin 9-39 in the critically ill and there is no currently available amylin antagonist. Accordingly, although the current study may have failed to show an effect of GLP-1 and/or amylin on gastric emptying because of type II error, we suggest that this study is the best currently available evidence.

In conclusion, neither the endogenous secretion of amylin, nor GLP-1, appears to be a major determinant of gastric emptying in the critically ill.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

REFERENCES

1.   Chapman MJ, Besanko LK, Burgstad CM, Fraser RJ, Bellon M, O'Connor S, Russo A, Jones KL, Lange K, Nguyen NQ, Bartholomeusz F, Chatterton B, Horowitz M. Gastric emptying of a liquid nutrient meal in the critically ill: relationship between scintigraphic and carbon breath test measurement. Gut 2011; 60: 1336-43.

2.   Heyland DK, Tougas G, King D, Cook DJ. Impaired gastric emptying in mechanically ventilated, critically ill patients. Intensive Care Med 1996; 22: 1339-44.

3.   Deane A, Chapman MJ, Fraser RJ, Horowitz M. Bench-to-bedside review: the gut as an endocrine organ in the critically ill. Crit Care 2010; 14: 24.

4.   Nguyen NQ, Fraser RJ, Chapman MJ, Bryant LK, Holloway RH, Vozzo R, Wishart J, Feinle-Bisset C, Horowitz M. Feed intolerance in critical illness is associated with increased basal and nutrient-stimulated plasma cholecystokinin concentrations. Crit Care Med 2007; 35: 82-8.

5.   Nguyen NQ, Fraser RJ, Bryant LK, Chapman MJ, Wishart J, Holloway RH, Butler R, Horowitz M. The relationship between gastric emptying, plasma cholecystokinin, and peptide YY in critically ill patients. Crit Care 2007; 11.

6.   Deane AM, Nguyen NQ, Stevens JE, Fraser RJ, Holloway RH, Besanko LK, Burgstad C, Jones KL, Chapman MJ, Rayner CK, Horowitz M. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab 2010; 95: 215-21.

7.   Crona D, MacLaren R. Gastrointestinal hormone concentrations associated with gastric feeding in critically ill patients. JPEN J Parenter Enteral Nutr 2012; 36: 189-96.

8. Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, Johnston C, Bloom SR, Emmanuel AV. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. Gut 2005; 54: 1693-8.

9. Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Smout AJ, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. Am J Physiol Endocrinol Metab 2007; 293: E743-53.

10. Deane AM, Rayner, C.K., Keeshan A., Cvijanovic, N., Marino, Z., Nguyen, N.Q, Chia, B., Summers, M.J., Sim, J.A., Van Beek T., Chapman, M.J., Horowitz, M., Young, R.L. Disordered regulation of intestinal glucose sensor, transporters and absorption in critically ill humans and mice. Crit Care Med 2013; In press.

11. Kong MF, King P, Macdonald IA, Stubbs TA, Perkins AC, Blackshaw PE, Moyses C, Tattersall RB. Infusion of pramlintide, a human amylin analogue, delays gastric emptying in men with IDDM. Diabetologia 1997; 40: 82-8.

12. Young AA, Gedulin BR, Rink TJ. Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7-36) NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. Metabolism 1996; 45: 1-3.

13. Mayer AP, Durward A, Turner C, Skellett S, Dalton N, Tibby SM, Murdoch IA. Amylin is associated with delayed gastric emptying in critically ill children. Intensive Care Med 2002; 28: 336-40.

14. Deane AM, Chapman MJ, Fraser RJ, Summers MJ, Zaknic AV, Storey JP, Jones KL, Rayner CK, Horowitz M. Effects of exogenous glucagon-like peptide-1 on gastric emptying and glucose absorption in the critically ill: relationship to glycemia. Crit Care Med 2010; 38: 1261-9.

15. Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab 2003; 88: 2719-25.

16. Linnebjerg H, Park S, Kothare PA, Trautmann ME, Mace K, Fineman M, Wilding I, Nauck M, Horowitz M. Effect of exenatide on gastric emptying and relationship to postprandial glycemia in type 2 diabetes. Regul Pept 2008; 151: 123-9.

17. Schvarcz E, Palmer M, Aman J, Horowitz M, Stridsberg M, Berne C. Physiological hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus. Gastroenterology 1997; 113: 60-6.

18. Hansen KB, Vilsboll T, Bagger JI, Holst JJ, Knop FK. Reduced glucose tolerance and insulin resistance induced by steroid treatment, relative physical inactivity, and high-calorie diet impairs the incretin effect in healthy subjects. J Clin Endocrinol Metab 2010; 95: 3309-17.

19. Chapman MJ, Fraser RJ, Matthews G, Russo A, Bellon M, Besanko LK, Jones KL, Butler R, Chatterton B, Horowitz M. Glucose absorption and gastric emptying in critical illness. Crit Care 2009; 13: 27.

20. Egi M, Bellomo R, Stachowski E, French CJ, Hart GK, Hegarty C, Bailey M. Blood glucose concentration and outcome of critical illness: the impact of diabetes. Crit Care Med 2008; 36: 2249-55.

21. Deane AM, Horowitz M. Dysglycaemia in the critically ill - significance and management. Diabetes Obes Metab 2013; 1: 12078.

22. Llompart-Pou JA, Fernandez-de-Castillo AG, Burguera B, Perez-Barcena J, Marse P, Rodriguez-Yago M, Barcelo A, Raurich JM. Stress hyperglycaemia in critically ill patients: potential role of incretin hormones; a preliminary study. Nutr Hosp 2012; 27: 130-7.

23. Deane AM, Zaknic AV, Summers MJ, Chapman MJ, Lange K, Ritz MA, Davidson G, Horowitz M, Fraser RJ. Intrasubject variability of gastric emptying in the critically ill using a stable isotope breath test. Clin Nutr 2010; 29: 682-6.

24. Fordtran JS, Clodi PH, Soergel KH, Ingelfinger FJ. Sugar absorption tests, with special reference to 3-0-methyl-d-glucose and d-xylose. Ann Intern Med 1962; 57: 883-91.

25. Deane AM, Summers MJ, Zaknic AV, Chapman MJ, Di Bartolomeo AE, Bellon M, Maddox A, Russo A, Horowitz M, Fraser RJ. Glucose absorption and small intestinal transit in critical illness. Crit Care Med 2011; 39: 1282-8.

26. Deane A, Chapman MJ, Fraser RJ, Bryant LK, Burgstad C, Nguyen NQ. Mechanisms underlying feed intolerance in the critically ill: implications for treatment. World J Gastroenterol 2007; 13: 3909-17.

27. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. J Clin Endocrinol Metab 2000; 85: 3575-81.

28.   Meier JJ, Gethmann A, Nauck MA, Gotze O, Schmitz F, Deacon CF, Gallwitz B, Schmidt WE, Holst JJ. The glucagon-like peptide-1 metabolite GLP-1-(9-36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans. Am J Physiol Endocrinol Metab 2006; 290: 10.

29.   Nguyen NQ, Bryant LK, Burgstad CM, Chapman M, Deane A, Bellon M, Lange K, Bartholomeuz D, Horowitz M, Holloway RH, Fraser RJ. Gastric emptying measurement of liquid nutrients using the C-octanoate breath test in critically ill patients: a comparison with scintigraphy. Intensive Care Med 2013; 8: 8.

30.   Streiner DL. Breaking up is hard to do: the heartbreak of dichotomizing continuous data. Can J Psychiatry 2002; 47: 262-6.

31.   Reintam A, Parm P, Kitus R, Kern H, Starkopf J. Gastrointestinal symptoms in intensive care patients. Acta Anaesthesiol Scand 2009; 53: 318-24.

FIGURE LEGEND

Figure 1: a) Gastric emptying was slower in the critically ill; * indicates P=0.002. b) Gastric emptying was slower in patients intolerant to intragastric feed; ^ indicates P=0.04. Data are mean ± SD; comparisons calculated using Student's t-test.

Figure 2: In the critically ill fasting GLP-1 concentrations, were increased in patients classified as intolerant to intragastric feed when compared to feed tolerant patients: Individual patients fasting GLP-1 concentrations are displayed with black line representing the median; ** indicates P=0.02; calculated using Mann-Whitney U test.

Figure 3: Postprandial glycaemic increment above fasting was attenuated in the critically ill; Data are mean ± SD; Δ indicates P=0.02; calculated using Student's t-test.

Figure 4: Glucose absorption, as determined by serum 3-OMG concentrations, was reduced in critically ill patients in the period 60min following the intragastric meal; # indicates P=0.02; calculated using Student's t-test.