# Understanding the Relationship of Incretin Hormones, Gastric Emptying and the Glucoregulatory Responses to Nutrients

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## Thesis Abstract

Gastric emptying is a major determinant of postprandial glycaemia and varies substantially between individuals. There is a bi-directional relationship between gastric emptying and postprandial glycaemic excursions i.e. relatively more rapid gastric emptying increases postprandial glucose, but acute hyperglycaemia also slows gastric emptying. Glucose homeostasis is also mediated by the 'incretin' hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The release of the incretin hormones is stimulated by the arrival of nutrients in the small intestines and GLP-1, in particular, has a substantial effect to slow gastric emptying.

This thesis provides novel insights into the relationships between gastric emptying, incretin hormones and glucose homeostasis. In a study of 43 individuals with type 2 diabetes (T2D), gastric emptying was evaluated using the 'gold standard' technique of scintigraphy following a 75 g oral glucose tolerance test. I showed that in T2D, gastric emptying correlates with the plasma glucose levels at 30, 60 and 120 minutes but not 180 minutes. In a second study of 35 individuals with an intensive care unit admission complicated by stress hyperglycaemia, 3 months after discharge, gastric emptying was evaluated using the stable isotope breath test (a method of measurement of comparable sensitivity to scintigraphy) and found to correlate to plasma glucose at 30 and 60 minutes, but not 120 minutes. Furthermore, this cohort was found to have a high 1-hour plasma glucose which has been associated with an increased risk of progression to T2D and earlier mortality. This finding highlights the need for close follow-up of survivors of critical illness who had stress hyperglycaemia for the development of T2D.

In a study of 36 adults above 65 years of age without a history of diabetes, a biphasic pattern of the glucose response curve in an oral glucose tolerance test was found to correlate with GLP-1, but not GIP or gastric emptying (the latter measured by stable isotope breath test). This has provided insights into how the glucose response curve can be a marker of dysglycaemia. Liquids with greater nutrient density are known to be emptied more slowly. Therefore, in a

study of 8 healthy adults, I evaluated the effect of different types of beer (low carbohydrate, low alcohol and full strength) on gastric emptying and found that there was no substantial effect.

Bariatric surgery is known to be associated with markedly accelerated gastric emptying, particularly of liquids and a substantially greater GLP-1 response. To investigate if distension contributes to the GLP-1 response, I studied 8 healthy adults using the 'gold standard' barostat technique and I found that gastric distension was not associated with an increase in plasma GLP-1. In a metanalysis of 12 studies that have evaluated the GLP-1 response in post-bariatric surgery (Roux-en-Y gastric bypass) hypoglycaemia, I found that this response is greater in individuals that have experienced reactive hypoglycaemia, supporting GLP-1 antagonism as a rational therapeutic approach for this condition.

These studies have advanced our knowledge in gastric emptying and incretin physiology with the high potential to change current clinical practice and inform future studies.

## Format of the Thesis

This thesis consists of 9 chapters adapted from one review and six original manuscripts (chapters 2 – 8). The review and the original manuscripts have all been published in peer-reviewed journals and are presented in its original publication format. The first chapter is a general introduction to introduce the themes of the thesis and the last chapter summarizes the findings from all studies.

## 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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Ryan Joseph Jalleh

21 January 2024

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## Publications

**Publications related to work presented in this thesis:**

1. **Jalleh RJ**, Jones KL, Nauck M, Horowitz M. Accurate measurements of gastric emptying and gastrointestinal symptoms in the evaluation of glucagon-like peptide-1 receptor agonists. Ann Intern Med. 2023; Epub ahead of print. DOI: 10.7326/M23-2019.
2. **Jalleh RJ**, Marathe CS, Trahair LG, Jones KL, Horowitz M. A biphasic glucose response during an oral glucose tolerance test is associated with greater plasma insulin and GLP-1 responses and a reduction in 1-hour glucose but does not relate to the rate of gastric emptying in healthy, older adults. Nutrients. 2023;15(18):3889.
3. **Jalleh RJ**, Umapathysivam MM, Plummer MP, Deane A, Jones KL, Horowitz M. Postprandial plasma GLP-1 levels are elevated in individuals with postprandial hypoglycaemia following Roux-en-Y gastric bypass - a systematic review. Rev Endocr Metab Disord. 2023; doi: 10.1007/s11154-023-09823-3. Epub ahead of print.
4. **Jalleh RJ**, Trahair LG, Wu T, Standfield S, Feinle-Bisset C, Rayner CK, Horowitz M, Jones KL. Effect of gastric distension with concurrent small intestinal saline or glucose infusion on incretin hormone secretion in healthy individuals: A randomized, controlled, crossover study. Diabetes Obes Metab. 2023;25(7):1849-1854.
5. **Jalleh RJ**, Jones KL, Rayner CK, Marathe CS, Wu T, Horowitz M. Normal and disordered gastric emptying in diabetes: recent insights into (patho)physiology, management and impact on glycaemic control. Diabetologia. 2022;65(12):1981-93.
6. **Jalleh RJ**, Xie C, Deane AM, Plummer MP, Jones KL, Horowitz M, Kar P. One-hour plasma glucose level after a 75 g oral glucose load and its relationship to gastric emptying in survivors of critical illness and stress hyperglycaemia. Crit Care Resusc. 2022;24(3):268-71.

7. **Jalleh RJ,** Wu T, Jones KL, Rayner CK, Horowitz M, Marathe CS. Relationships of glucose, GLP-1 and insulin secretion with gastric emptying after a 75 g glucose load in type 2 diabetes. J Clin Endocrinol Metab. 2022;107(9):3850-6.
8. Stevens JE, **Jalleh RJ**, Trahair LG, Marathe CS, Horowitz M, Jones KL. Comparative effects of low-carbohydrate, full-strength and low-alcohol beer on gastric emptying, alcohol absorption, glycaemia and insulinaemia in health. Br J Clin Pharmacol. 2022;88(7):3421-7.

**Research presentations arising from this thesis:**

1. Abstract "Biphasic glucose curves during oral glucose tolerance tests are associated with increased insulin and GLP-1 secretion, but not gastric emptying rate, in adults without diabetes" oral presentation at the Australasian Diabetes Congress in Adelaide (Aug 2023)
2. Abstract "Incretin hormone and glucoregulatory responses to nutrients in individuals with and without post-bariatric hypoglycaemia – a systematic review" oral presentation at the European Association for the Study of Diabetes 58th annual meeting in Sweden (Sep 2022) and Australasian Diabetes Congress in Brisbane (Aug 2022)

Presentations at the Australasian Diabetes Congress Virtual Conference (Aug 2021)

3. Abstract "The relationship of the insulin secretory response and gastric emptying of a 75 g oral glucose load in type 2 diabetes" (Oral)
4. Abstract "The relationship of the insulin secretory response and gastric emptying of a 75 g oral glucose load in survivors of critical illness and stress-induced hyperglycaemia" (Poster)

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1. European Association for the Study of Diabetes Travel Grant 2022 ($1,400 EUR) for abstract “Incretin hormone and glucoregulatory responses to nutrients in individuals with and without post-bariatric hypoglycaemia – a systematic review”

2. Finalist of Australasian Diabetes Congress Young Investigator Award at the Australasian Diabetes Congress 2021 for abstract “The relationship of the insulin secretory response and gastric emptying of a 75 g oral glucose load in type 2 diabetes”

**Other publications produced during candidature:**

1. **Jalleh RJ**, Jones KL, Horowitz M. Diagnosing and managing diabetic gastroparesis. Endocrinology Today 2023; 12(3): 32-35.
2. Xie C, **Jalleh RJ**, Watson LE, Huang W, Sun Y, Jones KL, Horowitz M, Rayner CK, Wu T. Determinants of blood glucose concentrations following a high carbohydrate meal in type 2 diabetes: A multiple linear regression analysis. Diabetes Res Clin Pract. 2023;198:110606.
3. **Jalleh RJ**, Rayner CK, Jones KL, Horowitz M. Glucagon-like peptide-1 receptor agonists, weight loss, and gastric emptying: have I gut news for you. Obesity (Silver Spring). 2022;30(8):1533-4.
4. **Jalleh RJ**, Rayner CK, Jones KL, Horowitz M. Isseki nicho (one stone, two birds): a dual incretin receptor agonist for type 2 diabetes. Lancet Diabetes Endocrinol. 2022;10(9):610-1.
5. **Jalleh RJ**, Jones KL, Horowitz M. Gastroparesis and its implications for glycaemic management. Diabetes Management Journal. Aug 2022:13-14.
6. **Jalleh RJ,** Marathe CS, Jones KL, Horowitz M, Rayner CK. Digesting the pathogenesis of diabetic gastroparesis. J Diabetes Complications. 2021;35(10):107992.

7. **Jalleh R**, Torpy DJ. The emerging role of copeptin. Clin Biochem Rev. 2021;42(1):17-25.

## Abbreviations

| | |
|---|---|
| 5-HT | 5-Hydroxytryptamine |
| AMP | Adenosine monophosphate |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| AUC | Area under curve |
| BD | Twice daily |
| BMI | Body mass index |
| CFRD | Cystic fibrosis-related diabetes |
| CV | Coefficient of variation |
| D2 | Dopamine-2 |
| DPP-4 | Dipeptidyl peptidase-4 |
| EMA | European Medicines Agency |
| FDA | Food and Drug Administration |
| FGF-19 | Fibroblast growth factor 19 |
| FS | Full strength |
| FXR | Farsenoid X receptor |
| GCRC | Gastroparesis Clinical Research Consortium |
| GE | Gastric emptying |
| GIP | Glucose dependent insulinotropic polypeptide |
| GLP-1 | Glucagon-like peptide-1 |

| | |
|---|---|
| GLP-1RA | Glucagon-like peptide-1 receptor agonist |
| GLUT-2 | Glucose transporter 2 |
| G-POEM | Gastric per-oral endoscopic pyloromyotomy |
| HbA1c | Glycated haemoglobin |
| HOMA-IR | Homeostatic Model Assessment for Insulin Resistance |
| ICC | Interstitial cells of Cajal |
| IGT | Impaired glucose tolerance |
| ITLC | Instant thin layer chromatography |
| LA | Low alcohol |
| LC | Low carbohydrate |
| MDP | Minimum distending pressure |
| MMC | Migrating motor complex |
| MOOSE | Meta-analysis Of Observational Studies in Epidemiology |
| NOS | Nitric oxide synthase |
| OGTT | Oral glucose tolerance test |
| PBH | Post-bariatric surgery hypoglycaemia |
| PYY | Peptide tyrosine tyrosine |
| QTc | Corrected QT interval |
| QW | Once weekly |
| REML | Restricted maximum likelihood |
| RYGB | Roux-en-Y gastric bypass |

| | |
|---|---|
| SD | Standard deviation |
| SEM | Standard error of the mean |
| SGLT-2 | Sodium glucose co-transporter-2 |
| T1D | Type 1 diabetes |
| T2D | Type 2 diabetes |
| T50 | Time taken for 50% of gastric emptying |

# Chapter 1. General introduction.

This thesis focusses on the relationships between gastric emptying, incretin hormone secretion and glycaemia following oral nutrients. This introductory chapter provides relevant background information in relation to our current knowledge of gastric emptying and an overview of the themes explored in this thesis.

## *1.1 Physiology of gastric emptying and small intestinal motility*

Normal gastric emptying is mediated by a complex interplay between the extrinsic and enteric nervous systems, as well as neurohormonal pathways (Phillips et al., 2015). The interstitial cells of Cajal (ICCs), also known as the pacemaker cells of the gut, are located within the myenteric plexus and generate contractions within the antrum and pylorus, with differing patterns depending on whether the individual is in an inter-digestive (fasting) or postprandial state. Inter-digestive gastric and small intestinal motility, also called the "migrating motor complex" (MMC), is cyclical with a quiescent phase lasting ~40 minutes (phase I), intermittent contractions of ~50 minutes duration (phase II), and regular contractions at a rate of three per minute in the stomach and 10 – 12 per minute in the small intestine, lasting 5 – 10 minutes (phase III) (Marathe et al., 2011). Once food enters the stomach, the MMC is replaced by a postprandial motor pattern - the tone of the proximal stomach decreases to accommodate a greater volume of food without a significant increase in intragastric pressure and in the distal stomach, contractions pulverize digestible solid contents against a closed pylorus until they are reduced to a size of 1 – 2 mm (Marathe et al., 2011). Nutrients are retained in the stomach for a variable duration depending on both the composition and macronutrient content of a meal. For digestible solids, there is an initial lag phase of 20 – 40 minutes before emptying commences while solids are ground into small particles followed by an emptying phase at an approximately linear rate. For low nutrient liquids, there is minimal or no lag phase and gastric emptying occurs in an exponential, volume-dependent pattern. However, this becomes increasingly linear as the nutrient density increases, so that high nutrient liquids empty at a

comparable rate to solids with the exception of the lag phase (Phillips et al., 2015). Larger, non-digestible solids are emptied from the stomach after digestible solids and liquids, during phase III of the inter-digestive period (Phillips et al., 2015). Postprandially, the cyclical MMCs of the small intestines are also replaced by irregular contractions of variable amplitudes to deliver nutrients toward the caecum (Camilleri and Vassallo, 1991).

### *1.2 Regulation of gastric emptying*

There is substantial inter-individual variation in the overall rate of gastric emptying of nutrients that varies from 1 – 4 kcal/min (Horowitz et al., 1989a, Horowitz et al., 1991) with much less intra-individual variation in health (Nair et al., 2009). Aging appears to have a modest effect to slow gastric emptying (Pham et al., 2020) and gastric emptying in women appears to be slower than that in men (Datz et al., 1987) – in premenopausal women, possibly related to hormonal changes of the menstrual cycle. The rate of gastric emptying is also influenced by ethnicity with Han Chinese, Mexican Americans and American Indians, groups predisposed to type 2 diabetes (T2D) having more rapid gastric emptying than individuals of European descent (Phillips, 2006, Wang et al., 2020). There is a bi-directional relationship between gastric emptying and the postprandial rise in blood glucose. Relatively more rapid gastric emptying results in greater postprandial glycaemic excursions, accounting for up to 35% of the variance in peak glucose in individuals with and without diabetes (Horowitz et al., 1993, Jones et al., 1996). As well as being a determinant, gastric emptying is also influenced by acute changes in glycaemia (Jalleh et al., 2019). Acute hyperglycaemia (blood glucose concentration 16 – 20 mmol/L) slows both solid and liquid gastric emptying compared with euglycaemia (blood glucose concentration 5 – 8 mmol/L) (Fraser et al., 1990, Samsom et al., 1997) associated with antral hypomotility and abnormalities of gastric myoelectrical activity (Jebbink et al., 1994). However, modest, gradual elevations that occur on a day-to-day basis in individuals with type 1 diabetes (T1D) may have a lesser, or no influence on gastric emptying (Aigner et al., 2021). In contrast, acute insulin-induced hypoglycaemia (blood glucose concentration 2 – 2.6 mmol/L) is associated with a

substantial acceleration of gastric emptying in individuals with and without diabetes, with more marked hypoglycaemia having a greater effect (Russo et al., 2005, Murthy et al., 2021).

Optimal measurement of gastric emptying is essential for both clinical and research purposes and these methods are discussed further in Appendix 1 (Jalleh et al., 2023a). The relationship between gastric emptying and glycaemia is of particular importance in the management of diabetes (where gastric emptying is frequently abnormal) and this is discussed in further detail in Chapter 2.

### *1.3 Incretin hormones*

The two 'incretin hormones', glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are gut hormones that augment postprandial insulin secretion in a glucose-dependent manner. Thus, when glucose is administered orally, there is a much greater insulin secretory response compared with an isoglycaemic intravenous infusion, the so-called "incretin effect" (Nauck et al., 1986). The incretin effect accounts for up to 65% of the overall insulin secretory response to oral glucose (Nauck et al., 1986). In health, the incretin effect is predominantly mediated by GIP, however, in T2D, the capacity of GIP to stimulate insulin is markedly substantially reduced (Nauck and Meier, 2016). In contrast, GLP-1 retains substantial insulinotropic activity in T2D, although it is reduced when compared to healthy individuals (Kjems et al., 2003).

#### *1.3.1 Glucagon-like peptide-1 physiology*

GLP-1 is secreted by the enteroendocrine L cells found throughout the small and large intestine, but in greatest density within the distal small bowel (Baggio and Drucker, 2007). GLP-1 secretion is stimulated by the presence of nutrients (carbohydrate, protein or lipid) in the small intestines (Steinert et al., 2017). The consumption of glucose or other carbohydrates results in peak GLP-1 levels after 15 – 30 minutes, followed by a gradual decline to baseline after 3 – 4 hours, but there is a slower, more sustained rise in GLP-1 following oral intake of protein or fat (Steinert et al., 2017). In experimental models, the magnitude of the GLP-1 response is

dependent on the rate of delivery of nutrients. When glucose is infused intraduodenally at rates of 1 – 2 kcal/min, there is minimal stimulation of GLP-1, however, there is a substantial response at infusion rates of 3 – 4 kcal/min in individuals with and without T2D (Marathe et al., 2014, Pilichiewicz et al., 2007). The GLP-1 response is greater when nutrients are present at the distal, rather than proximal, small intestine. This concept is supported by studies showing (i) increased GLP-1 response to intrajejunal compared with intraduodenal glucose (Wu et al., 2015) and (ii) slowing small intestinal transit with hyoscine decreases the GLP-1 response to glucose (Chaikomin et al., 2007). GLP-1 has multiple effects on glucose homeostasis, including increasing insulin synthesis, increasing sensitivity of glucose-resistant β cells of the pancreas, stimulating β cell proliferation and inhibiting β cell apoptosis (Campbell and Drucker, 2013). While gastric emptying is a major determinant of the early (30 – 60 min) postprandial rise in glucose, in the "late phase" (90 – 180 min), increasing GLP-1 attenuates postprandial glucose rise (Xie et al., 2023) in T2D.

GLP-1 is an 'enterogastrone' as well as an incretin (Horowitz and Nauck, 2006). Acute intravenous infusion of GLP-1 slows gastric emptying substantially in individuals with and without type 2 diabetes (Nauck et al., 1997, Meier et al., 2003, Little et al., 2006). The GLP-1R antagonist, exendin(9-39) accelerates gastric emptying in health indicating that endogenous GLP-1 also slows gastric emptying (Deane et al., 2010b). The effect of GLP-1 to slow gastric emptying is mediated via vagal pathways to reduce antral and duodenal motility, as well as increased pyloric tone (Steinert et al., 2017, Schirra et al., 2002). The effect of GLP-1 together with peptide YY (another gut hormone co-secreted by L cells) to slow gastric emptying is referred to as the "ileal brake", a feedback mechanism relevant to reduce food intake and increase satiety (Camilleri, 2019).

Both 'short'- and 'long-acting' GLP-1 receptor agonists (RAs), used in the management of T2D and obesity, slow gastric emptying which is described in further detail in Chapter 2.

In addition to the effects of GLP-1 on the gut, GLP-1 receptors are expressed throughout the brain, including regions associated with appetite control, such as the hypothalamus and the area postrema, a region associated with aversive effects such as nausea (Farr et al., 2016). The use of functional MRI has shown diminished responses in appetite- and reward-related brain regions after intravenous administration of the GLP-1RA exenatide in individuals with obesity and T2D that correlates with reduced food intake (van Bloemendaal et al., 2014). I have shown (Jalleh et al., 2020) that acute administration of GLP-1RA lixisenatide reduces energy intake in the absence of nausea and this reduction is not related to the magnitude of slowing of gastric emptying or changes in intragastric meal distribution. This suggests that the effect of GLP-1RA in appetite reduction is predominantly centrally mediated.

*1.3.2 Glucose dependent insulinotropic polypeptide*

GIP is secreted from the enteroendocrine K cells located primarily in the duodenum and proximal jejunum (Campbell and Drucker, 2013). As with GLP-1, it is stimulated by nutrients entering the small intestine. GIP increases insulin secretion in a glucose dependent manner, increases insulin sensitivity, stimulates β cell proliferation and inhibits β cell apoptosis, however, unlike GLP-1, it stimulates, rather than suppresses, glucagon secretion (Campbell and Drucker, 2013). In experimental models, GIP secretion increases, concordant with the rate of intraduodenal glucose infusion i.e. more rapid infusion of glucose, leads to a greater GIP response (Pilichiewicz et al., 2007) even at slower rates of 1 – 2 kcal/min.

In contrast to GLP-1, GIP has no effect on gastric emptying (Meier et al., 2004).

In rodent models, GIP receptors have been identified in brain regions involved in the regulation of appetite and satiety (Fukuda, 2021) suggesting a role in modulating energy intake, similar to GLP-1. However, paradoxically, recent studies using both GIP receptor agonism and antagonism appear to be associated with reductions in body weight (Campbell, 2021).

### *1.4 Insulin*

Insulin is secreted from the β cells in the pancreatic islets in response to hyperglycaemia. Glucose enters the β cells through the Glucose Transporter 2 (GLUT2) channel on the cell membrane. Following entry, it is phosphorylated into glucose-6-phosphate and then metabolized further to ultimately generate adenosine triphosphate (ATP). ATP-sensitive potassium channels close in response to this, resulting in depolarization of the cell membrane, influx of calcium ions and the release of insulin (Ojha et al., 2019). Insulin lowers blood glucose levels by increasing glucose uptake in peripheral tissues, suppressing gluconeogenesis and promoting the synthesis of glycogen, proteins and lipids (Ojha et al., 2019). The pancreatic β cells have both GLP-1 and GIP receptors that are coupled to G-proteins. Upon stimulation, there is the intracellular production of cyclic adenosine monophosphate (AMP), which in, turn stimulates protein kinase A to augment insulin secretion (Ding et al., 1997). However, the incretin hormones do not initiate an insulin secretory response in the absence of hyperglycaemia as membrane depolarization from hyperglycaemia is required for insulin release (Gromada et al., 1998). Accordingly, both incretin hormones and their agonists are associated with minimal or no risk of hypoglycaemia – major deleterious outcome of insulin treatment for diabetes. While insulin may influence gastric emptying through modifying blood glucose levels, hyperinsulinaemia per se does not appear to have an effect on gastric emptying (Kong et al., 1999).

### *1.5 Glucagon*

Glucagon is secreted from the α cells of the pancreatic islets, but the underlying mechanism remains contentious. It has been suggested that activation of voltage-gated sodium channels and voltage dependent calcium channels in response to low glucose drives glucagon exocytosis, however, paracrine factors released by neighboring β and δ cells may also be important (Ojha et al., 2019). Glucagon potentiates glycogenolysis, gluconeogenesis and fatty acid breakdown to increase blood glucose levels, accordingly, it is an important counter-regulatory hormone in

hypoglycaemia. In addition to regulating glucose metabolism, glucagon also increases the resting metabolic rate in healthy adults (Nair, 1987).

## *1.6 Thesis aims*

### *1.6.1 Theme 1 – relationships between gastric emptying, incretin hormones and the rise in glucose following nutrients in type 2 diabetes and stress-induced hyperglycaemia*

Chapter 2 (Jalleh et al., 2022a) of the thesis is a literature review of what is known regarding normal and disordered gastric emptying in type 2 diabetes, with a particular emphasis on the bidirectional relationship between gastric emptying and glycaemia, as well as the effects of diabetes therapies on gastric emptying. While it is known that gastric emptying influences the initial glycaemic response to an oral glucose tolerance test (OGTT), the impact of gastric emptying on insulin secretion and at glucose levels 120 min (the latter used diagnostically) following the OGTT was not clear. This research question was evaluated in Chapter 3 (Jalleh et al., 2022b).

It has been reported that a 1 hour plasma glucose following a 75 g oral glucose greater than 8.6 mmol/L is a robust predictor of progression to type 2 diabetes, performing better than either the 2 hour plasma glucose or HbA1c (Bergman et al., 2016). In Chapter 3, I showed that gastric emptying correlates with the rise in glucose following an oral glucose tolerance test in individuals with T2D. In Chapter 4 (Jalleh et al., 2022c), I explore this relationship in individuals who have had stress hyperglycaemia following critical illness with a particular focus on the relationship between the 1 hour plasma glucose and gastric emptying.

### *1.6.2 Theme 2 – relationships between gastric emptying, incretin hormones and the rise in glucose following nutrient intake in health*

Chapter 5 (Jalleh et al., 2023b) evaluates the relationship of gastric emptying in older adults without diabetes with incretin hormones and the pattern of the glucose response curve following a 75 g oral glucose tolerance test. The pattern of the glucose response curve can be classified

into three major categories: (i) an incessantly rising pattern, (ii) a monophasic pattern and (iii) a biphasic pattern. The incessantly rising pattern is associated with the greatest risk of disordered glucose metabolism, the monophasic curve is also associated with impaired glucose tolerance and reduced insulin sensitivity/secretion, albeit to a lesser extent than the former and the biphasic curve, in contrast, is associated with better glucose tolerance and beta cell function (Arslanian et al., 2019). Given the relationship between gastric emptying and glycaemia as described in Chapter 3, in Chapter 5, I explored this relationship with the pattern of the glucose response following an OGTT.

As nutrient density is known to affect the rate of gastric emptying, in Chapter 6 (Stevens et al., 2022), I evaluated the effects of different strengths of beer (low carbohydrate, low alcohol and full strength beer) on gastric emptying and glycaemia in individuals without diabetes.

### *1.6.3 Theme 3 – understanding the effects of bariatric surgery on incretin hormones and postprandial glucose*

Bariatric surgery, especially the Roux-en-Y gastric bypass (RYGB), is associated with markedly accelerated gastric (pouch) emptying and a substantially increased GLP-1 response (Nguyen et al., 2014). In rodent models, gastric distension is associated with a greater GLP-1 response (Natochin et al., 2018, Ohbayashi et al., 2021). Following RYGB, the gastric volume is substantially reduced and, accordingly, consumption of even small amounts of food results in gastric pouch distension. In Chapter 7 (Jalleh et al., 2023c), I evaluated whether gastric distension is associated with increased GLP-1 and GIP responses in healthy adults, a potential mechanism to explain the marked rise in GLP-1 seen following RYGB (Holst et al., 2018). The beneficial effect of RYGB on glucose metabolism is substantial and many individuals undergo remission of type 2 diabetes following the procedure (Chang et al., 2014). However, following RYGB, post-prandial hypoglycaemia (post bariatric surgery hypoglycaemia) occurs in ~25% of individuals (Capristo et al., 2018, Brix et al., 2019). To evaluate whether individuals who experience post-prandial hypoglycaemia following RYGB surgery have a greater GLP-1

response compared with individuals who have had RYGB surgery, but do not experience post-prandial hypoglycaemia, in Chapter 8 (Jalleh et al., 2023d), I conducted a PROSPERO registered systematic review.

**Chapter 2. Normal and disordered gastric emptying in diabetes: recent insights into (patho)physiology, management and impact on glycaemic control.**

# Statement of Authorship

| | |
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| Name of Principal Author (Candidate) | Ryan Joseph Jalleh |
| Contribution to the Paper | I was responsible for the conception of this article, performing the literature review and writing the 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. |

| | | |
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| Signature: | | Date: 24/11/23 |

**Co-Author Contributions**

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

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

| | | |
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| Name of Co-Author | Karen L Jones | |
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| Signature: | | Date: 24/11/23 |

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| Contribution to the Paper | Prof Rayner has expertise in gastroenterology and provided feedback and mentorship in preparing the manuscript. | |
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| Contribution to the Paper | Dr Marathe has expertise in endocrinology and provided feedback on the manuscript | |
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| Name of Co-Author | Tongzhi Wu | |
| Contribution to the Paper | A/Prof Wu has expertise in endocrinology and provided feedback on the manuscript | |
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| Contribution to the Paper | Prof Horowitz is the primary supervisor for my PhD and therefore provided supervision and mentorship in the design of the manuscript and in preparing the manuscript for publication | |
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### *2.1 Abstract*

Gastric emptying is a major determinant of postprandial glycaemia, accounting for ~35% of variance in peak glucose in both health and type 2 diabetes. Gastric emptying is frequently disordered in diabetes (both abnormally delayed and accelerated). Delayed gastric emptying, i.e. diabetic gastroparesis, may be linked to upper gastrointestinal symptoms for which current treatment remains suboptimal; pharmacological acceleration of delayed emptying is only weakly associated with symptom improvement. Accordingly, the relationship between symptoms and delayed gastric emptying is not simply 'cause-and-effect'. In insulin-treated patients, disordered gastric emptying, even when not associated with gastrointestinal symptoms, can cause a mismatch between the onset of insulin action and the availability of absorbed carbohydrate, leading to suboptimal glycaemic control. In patients with type 2 diabetes, interventions that slow gastric emptying, e.g. glucagon-like peptide-1 receptor agonists, reduce postprandial glycaemia. This review focusses on recent insights into the impact of gastric emptying on postprandial glycaemia, effects of diabetes therapy on gastric emptying and management of disordered gastric emptying in diabetes. In view of the broad relevance of gastric emptying to diabetes management, it is important that future clinical trials evaluating novel therapies which may affect gastric emptying quantify the latter with an appropriate technique, such as scintigraphy or a stable isotope breath test.

*"There is nothing like looking, if you want to find something. You certainly usually find something, if you look, but it is not always quite the something you were after."* ***—J.R.R. Tolkien,*** The Hobbit

### *2.2 Introduction*

Gastric emptying (GE) is now appreciated to be central to the pathophysiology and rational management of diabetes (type 1, 2 and pancreatogenic). This relatively recent recognition represents a paradigm shift where the outcomes of methodologically sound research in humans, with and without diabetes, have in many cases refuted long-established dogma. Gastroparesis is defined by abnormally delayed GE in the absence of mechanical obstruction. Once thought to be relatively rare – a belief influenced by the outcome of epidemiological studies that defined gastroparesis based on upper gastrointestinal symptoms without measuring GE, it is now recognised that GE is abnormally slow in 30 – 50% of individuals with long-standing, complicated, type 1 and type 2 diabetes (T1D and T2D) (Horowitz et al., 1991, Horowitz et al., 1989a, Rayner et al., 2021). It is also appreciated that gastroparesis has clinical implications beyond that of symptoms, including malnutrition, glycaemic instability, and impaired absorption of orally administered drugs. By contrast, in uncomplicated T2D, GE is often accelerated (Watson et al., 2019, Xie et al., 2021a). Acute changes in blood glucose affect GE – which is slowed during hyperglycaemia and accelerated during hypoglycaemia (Phillips et al., 2015). A more recent recognition is that GE, even when normal, is a major, and hitherto generally underappreciated, determinant of the magnitude of the postprandial rise in blood glucose which, in many individuals with diabetes, predominates over fasting glycaemia in determining average glycaemic control as assessed by HbA1c, and, accordingly, represents a specific therapeutic target (Marathe et al., 2015, Phillips et al., 2015). In the broadest sense, the action of glucose-lowering therapy, particularly insulin, should ideally be coordinated closely with the rate of delivery of dietary carbohydrate, and subsequent absorption from the small intestine.

This review focusses on these advances in knowledge, including their implications for more personalised and effective management of diabetes.

### *2.3 Pathophysiology of diabetic gastroparesis*

In both health and diabetes, GE of solid and liquid nutrients exhibits a wide inter-individual, but much lesser intra-individual, variation (Horowitz et al., 1989a, Horowitz et al., 1991, Watson et al., 2019).

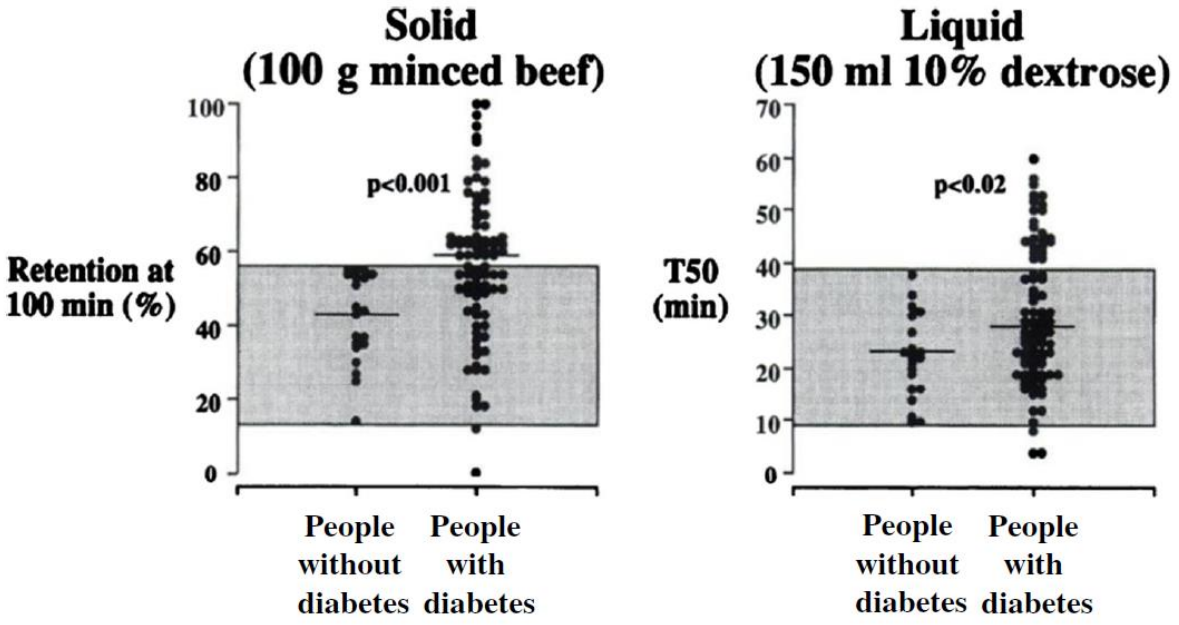


**Figure 2.1** Gastric emptying of solid (100 g minced beef, percent retention at 100 min) and liquid (150 ml 10% dextrose, half-emptying time [T50]) nutrients in 86 participants with long-standing diabetes (66 with type 1 diabetes, 20 with type 2 diabetes) and 20 healthy participants. Horizontal lines represent median values. The range of gastric emptying rates in the healthy participants is represented by the shaded area. Adapted from Jones et al (Jones et al., 1995) © SNMMI.

While predominantly a pulsatile, rather than continuous, phenomenon, GE of most nutrients approximates an overall linear pattern (in the case of solids following an initial lag phase of 20 – 30 min), ranging between 1 – 4 kcal/min in healthy individuals (Collins et al., 1983, Phillips

et al., 2015, Jones et al., 1995). Accordingly, in many individuals, whether healthy or with diabetes, a 75 g oral glucose load (~300 kcal), as in an oral glucose tolerance test (OGTT) will not have emptied completely from the stomach at 120 min (as this would require an emptying rate ≥ 2.5 kcal/min) (Marathe et al., 2015).

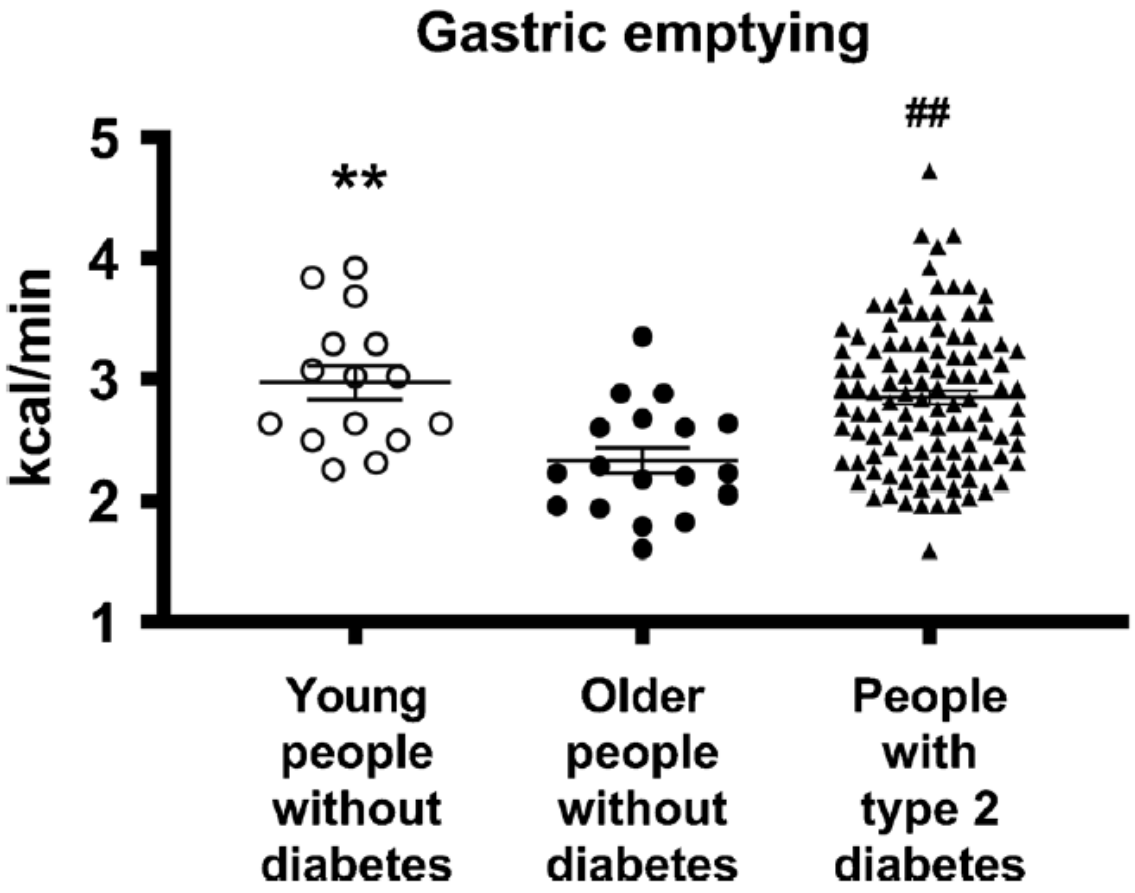


**Figure 2.2** Gastric emptying of a mashed potato meal in people with type 2 diabetes (n=111), and in young (n=15) and older (n=18) control participants without diabetes, as assessed by 13C-octanoic acid breath test and expressed as kcal/min. **p<0.01 for young vs older participants without diabetes, ##p<0.01 for people with type 2 diabetes vs older control participants without diabetes. Unpaired t test used to determine statistical difference. Adapted from Watson et al (Watson et al., 2019) by permission of Oxford University Press on behalf of the Endocrine Society. © The Endocrine Society.

In some ethnic populations (Han Chinese, Mexican Americans and American Indians) predisposed to T2D, GE appears to be more rapid than in Caucasians (Wang et al., 2020, Phillips, 2006).

Within the enteric nervous system, the interstitial cells of Cajal (ICC) act as pacemakers and, accordingly, play a major role in control of gastrointestinal motor function (Grover et al., 2011). It is not widely appreciated, however, that in healthy individuals, GE of nutrients is regulated primarily by inhibitory neuro-hormonal feedback arising from their interaction with the small intestine, rather than 'intragastric' mechanisms, and that both the length and region of small intestine exposed to nutrients modulate this feedback (Marathe et al., 2014, Zhang et al., 2019). Recent studies by the Gastroparesis Clinical Research Consortium (GCRC) in the US, where full thickness gastric biopsies have been obtained in individuals with refractory gastroparesis, have yielded important insights relating to the pathophysiology of diabetic gastroparesis (Grover et al., 2011). The latter is now recognised to be both complex and heterogeneous (Phillips et al., 2015). While there are abnormalities in vagal innervation, contrary to expectation, the relationship of delayed GE with autonomic dysfunction, as assessed by cardiovascular reflex tests, is weak (Horowitz et al., 1989a, Horowitz et al., 1991). Loss or damage to the ICCs appears to be a dominant abnormality (Phillips et al., 2015, Grover et al., 2011), where altered immune function with a shift from M2 to M1 macrophage expression and impaired regulation of haem-oxygenase-1 leading to increased oxidative stress, may be responsible (Grover et al., 2011, Phillips et al., 2015). Other common abnormalities include reductions in intrinsic nerves and inhibitory neurons expressing nitric oxide synthase (NOS) (Phillips et al., 2015, Goyal, 2021). Not surprisingly, gastroduodenal motor and sensory dysfunctions in gastroparesis are also highly variable; frequent abnormalities include impaired relaxation of the proximal stomach, reduced antral contractility and abnormalities of the gastric electrical rhythm (Phillips et al., 2015).

### *2.4 Evaluation of gastric emptying*

In the evaluation of gastric emptying, drugs that affect GE should be withheld where possible and to exclude mechanical obstruction, upper gastrointestinal endoscopy must be performed routinely (Schol et al., 2021, Phillips et al., 2015). Scintigraphy remains the 'gold-standard' technique for quantifying GE and allows measurement of both solid and liquid emptying, potentially concurrently, although frequently only solid emptying is assessed. Current guidelines recommend a test meal of eggs, toast, jam and water with only the solid component (eggs) radiolabelled (Schol et al., 2021, Abell et al., 2008). It should, however, be appreciated that the relationship of solid and liquid emptying is relatively weak (Horowitz et al., 1991). Recommendations to standardise the methodology for scintigraphy include ensuring blood glucose concentrations are <15 mmol/L during measurement (Abell et al., 2008). Limitations of scintigraphy include the need for expensive, specialised equipment and exposure to radiation. A more recently developed, and acceptable, alternative to scintigraphy is the stable isotope breath test, which is now well-validated, simple to perform and avoids exposure to ionising radiation (Trahair et al., 2022). Moreover, isotope breath test data can be adjusted, using the so-called 'Wagner-Nelson' method, to yield values comparable to scintigraphy (Trahair et al., 2022). The paracetamol absorption test (i.e., evaluation of the plasma kinetics of an oral paracetamol dose) is, unfortunately, still used widely to measure GE, particularly by the pharmaceutical industry. However, this technique has substantial limitations including uncertainty as to which component of a meal the paracetamol is emptying with, and the inability to measure GE of solids (Horowitz et al., 2020, Bartholome et al., 2015). Accordingly, in our opinion, this method should not be used in isolation for either clinical or research purposes, particularly given the availability of the isotope breath test. Similar considerations apply to the widespread assessment of gastrointestinal symptoms using participant self-report, which is known to be unreliable, rather than validated questionnaires which are now mandated by regulatory authorities in studies of drug therapy for functional gastrointestinal diseases (Du et

al., 2018). Evaluation of GE using the wireless motility capsule is a newer technique; however, the indigestible capsule tends to be emptied later than nutrient liquids and solids, and it may be insensitive for detecting abnormally rapid GE (Phillips et al., 2015, Rayner et al., 2021). Other newer techniques for measuring GE, including magnetic resonance imaging and three-dimensional ultrasound, remain primarily in the research domain (Phillips et al., 2015, Rayner et al., 2021).

### *2.5 Impact of gastric emptying on glycaemia*

GE is a major determinant of postprandial glycaemia in both health and diabetes, accounting for ~35% of the variance in peak glucose, and both the timing and significance of this relationship are impacted by glucose tolerance status (Phillips et al., 2015). In healthy individuals, relatively more rapid GE is associated with a greater glycaemic response in a 75 g oral glucose tolerance test (OGTT) at 30 min, but not 60 min, and the relationship is inverse at 120 min, presumably reflecting effective glucose counter-regulation (Marathe et al., 2015, Horowitz et al., 1993). In contrast, in individuals with impaired glucose tolerance (IGT), relatively more rapid GE is associated with a greater glycaemic response at 30 min and 60 min, but not 120 min, and in T2D there is a further 'shift to the right' so that the glycaemic response is related directly to GE both at 60 min and 120 min (Marathe et al., 2015, Jalleh et al., 2022b). These observations have implications for use of the OGTT to diagnose T2D, IGT (i.e. 120 min plasma glucose), gestational diabetes and an understanding of the determinants of the blood glucose at 60 min which may be the strongest predictor of future T2D (Bergman et al., 2018). Given the relationship of glycaemia with GE, the use of continuous blood glucose monitoring has the potential to predict disordered GE. It should also be appreciated that, in contrast to longstanding complicated T1D and T2D where GE is frequently delayed (Horowitz et al., 1991, Horowitz et al., 1989a, Rayner et al., 2021), T2D of short duration, and uncomplicated T1D in adolescents, are associated with abnormally rapid GE (Xie et al., 2021a, Perano et al., 2015).

In T1D, it has been suggested that this acceleration reflects the reduction in human islet amyloid pancreatic peptide secretion (Vinik et al., 2008). Older individuals with T2D tend to have slower GE compared with the young, consistent with the modest slowing of GE observed with healthy ageing (Watson et al., 2019). Rapid GE may also be associated with upper gastrointestinal symptoms, which cannot be discriminated from those associated with gastroparesis (Kuwelker et al., 2020).

In many cases, the magnitude of the delay in GE in individuals with diabetes and gastroparesis is modest (Horowitz et al., 1989a, Horowitz et al., 1991). The rate of GE also tends to remain relatively stable for up to 25 years (Chang et al., 2012). Acute changes in blood glucose affect GE – hyperglycaemia induced by intravenous glucose slows emptying, although there is recent evidence that this may not apply to spontaneous elevations in blood glucose (Aigner et al., 2021). In contrast to hyperglycaemia, acute insulin-induced hypoglycaemia (blood glucose ~2.0 – 2.6 mmol/L) robustly accelerates GE in T1D, even in individuals with cardiovascular autonomic neuropathy and gastroparesis (Marathe et al., 2019) (Figure 3). This is likely to represent an important counter-regulatory mechanism and warrants further study (Marathe et al., 2019).

In individuals with T1D or T2D, who are managed with insulin, 'carbohydrate counting' is used widely to determine insulin type and dosage; however, this approach fails to account for the substantial variation between individuals in the rate of delivery of carbohydrate to the small intestine. Accordingly, the choice and dosage of rapid-acting insulin should be considered carefully in individuals with disordered GE to optimise compatibility with the altered rate of absorption of carbohydrate – for example, if an ultra-rapid acting insulin tends to cause postprandial hypoglycaemia, an insulin with a slower onset of action may avoid this. In this context, GE should have major implications (which remain to be evaluated) for algorithms relating to the optimal use in T1D of insulin pumps linked to real time subcutaneous glucose monitoring systems.

Because GE regulates the entry of nutrients into the small intestine, it modulates the secretion of the two incretin hormones, glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (Nauck et al., 2021). The insulinotropic effect of GIP, the dominant incretin in healthy individuals, is markedly attenuated in T2D, whereas that of GLP-1 is relatively preserved (Nauck et al., 2021). Experimental models have demonstrated a prompt, and load-related, stimulation of GIP in response to direct intraduodenal infusion of glucose (Phillips et al., 2015). In contrast, there is minimal stimulation of GLP-1 when glucose is infused intraduodenally at rates of 1 – 2 kcal/min, but a substantial response at 3 – 4 kcal/min in both health and T2D (Marathe et al., 2014, Pilichiewicz et al., 2007). It has recently been shown that there is a direct relationship between the rate of GE of oral glucose and the stimulation of plasma GLP-1 in response to intraduodenal glucose (Xie et al., 2021b), indicating that the rate of GE in a given individual is modulated by the GLP-1 response to small intestinal nutrients. Studies using a specific antagonist of GLP-1, exendin 9-39, have demonstrated that endogenous GLP-1 slows GE in healthy individuals (Deane et al., 2010b).

There is little information about the impact of GE on glycaemia in the large number of individuals with pancreatogenic diabetes. In the most studied form, cystic fibrosis-related diabetes (CFRD), small intestinal inhibitory feedback from the products of fat digestion is attenuated as a result of pancreatic exocrine insufficiency, GE is often abnormally rapid and the secretion of both GLP-1 and GIP attenuated, favouring an increase in the postprandial glycaemic excursion (Perano et al., 2014). Management of exocrine pancreatic insufficiency and associated malabsorption with enzyme supplementation slows GE and augments the incretin response, to reduce postprandial glucose (Perano et al., 2014). In patients who have undergone partial pancreatectomy, particularly proximal resection, there is often a transient post-operative delay in gastric emptying which may result in a reduction in the glycaemic response to an OGTT (Menge et al., 2009).

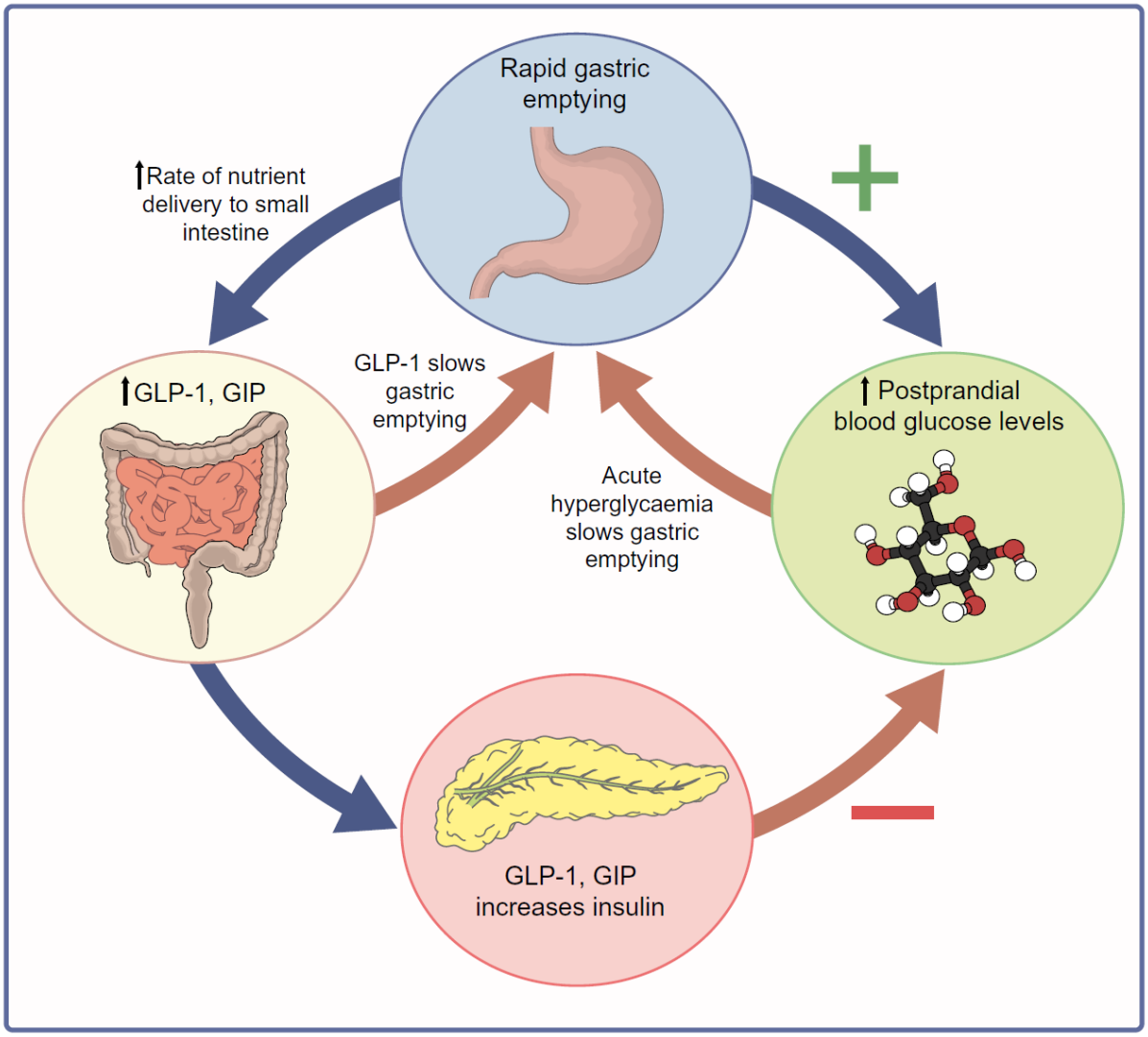


**Figure 2.3** Schema of the interdependent relationships of gastric emptying, incretin hormones and blood glucose. Gastric emptying is both a determinant of, and determined by, blood glucose. © Diabetologia.

### *2.6 Effect of diabetes therapies on gastric emptying*

A number of drugs used in the management of diabetes slow GE, which contributes to their capacity to reduce postprandial glycaemia.

#### *2.6.1 GLP-1 receptor agonists*

GLP-1 receptor agonists (RAs) are used widely in the management of T2D, and increasingly in obesity, and can be classified as either 'short-' or 'long-acting' based on their plasma half-lives.

The short-acting GLP-1RAs, exenatide BD (Linnebjerg et al., 2008) and lixisenatide (Jones et al., 2019, Rayner et al., 2020, Meier et al., 2015, Lorenz et al., 2013), have a robust, albeit variable, effect to slow GE. The magnitude of this deceleration is dependent on the baseline rate of GE (i.e. individuals with gastroparesis may potentially not exhibit further slowing, although this remains to be determined) and predictive of the magnitude of postprandial glucose lowering (Linnebjerg et al., 2008). The implication is that improvement in glycaemic control by short-acting GLP-1RAs, as assessed by HbA1c in T2D, may be dependent on the baseline rate of GE, particularly in individuals in whom fasting glycaemia is relatively well controlled. The effects of long-acting GLP-1RAs (i.e. exenatide QW, liraglutide, dulaglutide, semaglutide [subcutaneous and oral] and tirzepatide [a dual GIP/GLP-1RA]) on GE have, for the main part, been evaluated with the suboptimal paracetamol test (Horowitz et al., 2020, Dahl et al., 2021, Hjerpsted et al., 2018, Urva et al., 2020, Drucker et al., 2008, Barrington et al., 2011), rather than a more accurate technique (Jones et al., 2020). It has also been suggested that long-acting GLP-1RAs do not have an effect to slow GE with longer-term use because of 'tachyphylaxis' induced by sustained exposure of GLP-1 receptors. However, in humans, the slowing of GE by intravenous GLP-1 is diminished over 24h, but the effect is still significant (Umapathysivam et al., 2014). While the slowing of GE appears to be less with long-acting GLP-1RAs compared to short-acting GLP-1RAs, substantial and durable slowing of GE clearly persists with both liraglutide (Halawi et al., 2017) and exenatide QW (Jones et al., 2020), two long-acting GLP-1RAs for which GE has been quantified using a methodologically sound technique. In the latter case, the reduction in postprandial glucose has been shown to be related to the extent of this delay (Jones et al., 2020). Accordingly, it appears that both short- and long-acting GLP-1RAs slow GE.

| GLP-1RA | Dose and duration | Health condition | Method of gastric emptying measurement | Effect on gastric emptying |
|---|---|---|---|---|
| Short-acting | | | | |
| Exenatide BD | | | | |
| Drucker et al [42] | 5 μg sc BD for 14 weeks | T2D (*n*=50) | Paracetamol absorption | Exenatide vs placebo: + |
| Linnebjerg et al [34] | 5 or 10 μg sc BD for 5 days | T2D (*n*=7) | Scintigraphy | Exenatide vs placebo: +++ |
| Lixisenatide | | | | |
| Jones et al [35] | 10 μg sc daily (single dose) | T2D (*n*=15)<br>Healthy individuals (*n*=15) | Scintigraphy | Baseline vs lixisenatide: +++ (in health and T2D) |
| Lorenz et al [38] | 5 μg sc daily initially, increased by 2.5 μg every 5 days up to 20 μg sc daily (total duration 28 days) | T2D (*n*=43) | Stable isotope breath test | Baseline vs day 28 lixisenatide: +++ |
| Rayner et al [36] | 5 μg sc daily initially, increased to 10 μg sc daily after 7 days and then 20 μg sc daily after 14 days (total duration 56 days) | T2D (*n*=30) | Scintigraphy | Baseline vs day 56 lixisenatide: +++ |
| Long-acting | | | | |
| Dulaglutide | | | | |
| Barrington et al [43] | 5 and 8 mg sc weekly for 5 weeks | T2D (*n*=15) | Paracetamol absorption | Dulaglutide vs placebo: ++ |
| Exenatide QW | | | | |
| Drucker et al [42] | 2 mg sc weekly for 14 weeks | T2D (*n*=24) | Paracetamol absorption | Exenatide vs placebo: – |
| Jones et al [44] | 2 mg sc weekly for 8 weeks | Obesity (*n*=32) | Scintigraphy | Exenatide vs placebo: + |
| Liraglutide | | | | |
| Halawi et al [46] | 3 mg sc daily for 16 weeks | Obesity (*n*=40) | Scintigraphy | Liraglutide vs placebo: ++ |
| Meier et al [37] | 0.6 mg sc daily initially, increasing by 0.6 mg sc each week to either 1.2 or 1.8 mg sc daily (total duration 8 weeks) | T2D (*n*=94) | Stable isotope breath test | Baseline vs week 8: liraglutide: ++ |
| Semaglutide (subcutaneous) | | | | |
| Hjerpsted et al [40] | 0.25 mg sc weekly for 4 weeks, then 0.5 mg for 4 weeks, then 1 mg sc weekly (total duration 12 weeks) | Obesity (*n*=30) | Paracetamol absorption | Semaglutide vs placebo: ++ |
| Semaglutide (oral) | | | | |
| Dahl et al [39] | 3 mg daily for 4 weeks, then 7 mg daily for 4 weeks, then 14 mg daily for 3 days | T2D (*n*=15) | Paracetamol absorption | Semaglutide vs placebo: ++ |
| Tirzepatide (Dual GLP-1 and GIP receptor agonist) | | | | |
| Urva et al [41] | Tirzepatide 0.5, 1.5, 4.5, 5, 10 or 15 mg sc weekly (total duration 4 weeks) | T2D (*n*=53)<br>Healthy individuals (*n*=33) | Paracetamol absorption | Baseline vs week 4 tirzepatide: ++ |

Only the most relevant studies are included

sc subcutaneous; T2D, type 2 diabetes; –, no effect on GE; +, gastric emptying delayed by <25% compared with baseline/placebo; ++, gastric emptying delayed by 25–50% compared with baseline/placebo; +++, gastric emptying delayed by >50% compared with baseline/placebo

**Table 2.1** Effects of GLP-1RAs on gastric emptying in humans (Drucker et al., 2008, Linnebjerg et al., 2008, Jones et al., 2020, Lorenz et al., 2013, Rayner et al., 2020, Barrington et al., 2011, Jones et al., 2019, Halawi et al., 2017, Meier et al., 2015, Hjerpsted et al., 2018, Dahl et al., 2021, Urva et al., 2020). © Diabetologia.

Moreover, the European Medicines Agency considers that 'delayed GE' is an adverse effect of liraglutide . What remains to be clarified are the magnitude, duration and dose-relationship of

these effects – issues that can only be resolved if GE is measured in clinical trials using an appropriate method. Of note, studies that have shown that slowing of GE reduces postprandial blood glucose have generally not discriminated effects on GE from those on small intestinal motility/transit. The potential for GLP-1RAs to inhibit small intestinal transit may represent a substantial contribution to postprandial glucose lowering (Thazhath et al., 2016).

The insights from such studies are intuitively likely to facilitate much more personalised use of GLP-1RAs and have a number of other implications, including the timing of cessation of long-acting GLP-1RAs prior to endoscopy or surgery, where there are recent anecdotal reports of retained gastric contents despite 'appropriate' periods of fasting (Kalas et al., 2021). The effect of GLP-1RAs on GE may also be of relevance to their potential use in T1D. In addition to suppressing glucagon, the slowing of GE may attenuate the counter-regulatory acceleration of GE by hypoglycaemia (Horowitz et al., 2006), which could increase the risk of hypoglycaemic, as observed in a trial with liraglutide (Mathieu et al., 2016). Because GE was not measured concurrently in this trial, this potential mechanism cannot be confirmed. While it has been suggested that the frequent gastrointestinal adverse effects (which may compromise longer-term usage) and appetite suppression induced by GLP-1RAs are related to slowing of GE, this may not be the case (Jalleh et al., 2020). Another potential example of 'targeted' use of GLP-1RAs is CFRD, where abnormally rapid GE can be slowed using the short-acting GLP-1RA, exenatide BD, to reduce the postprandial glycaemic excursion (Geyer et al., 2019).

A substantial (>20mmHg) fall in blood pressure after meals ('postprandial hypotension') occurs in up to 40% of individuals with longstanding, complicated diabetes (more common than orthostatic hypotension), and is associated with more rapid GE (Jones et al., 2019). The slowing of GE by GLP-1RAs may attenuate the postprandial fall in blood pressure and could prove useful in the management of postprandial hypotension, which currently lacks an effective treatment (Jones et al., 2019).

### *2.6.2 DPP-4 inhibitors*

In contrast to GLP-1RAs, DPP-4 inhibitors have minimal, if any, effect on GE in T2D, although their postprandial glucose-lowering may be greater in individuals in whom GE is relatively faster (Stevens et al., 2020). The absence of an effect on GE is likely to reflect the much lesser stimulation of GLP-1 receptors than with GLP-1RAs; moreover, DPP-4 inhibition prevents the conversion of inactive PYY1-36 to the active 3-36 form which slows GE (Witte et al., 2009).

### *2.6.3 Other antidiabetic medications*

Metformin, when delivered intraduodenally, slows GE modestly in T2D in the absence of nausea (Borg et al., 2020). The mechanism(s) may reflect greater stimulation of GLP-1 from the distal gut resulting from a reduction in proximal intestinal glucose absorption, inhibition of bile acid resorption, and/or alterations in the gut microbiota (Borg et al., 2020). Pramlintide, an amylin analogue, slows GE substantially, which contributes to its marked effect to lower postprandial glucose (Phillips et al., 2015). Acarbose, an alpha-glucosidase inhibitor which delays carbohydrate absorption, may increase exposure of the L-cells in the distal gut to nutrients to enhance GLP-1 secretion. In some experimental paradigms, acarbose has been shown to slow GE, although this was not evident with a mixed meal in T2D (Hucking et al., 2005). While the effect of sodium-glucose co-transporter-2 (SGLT-2) inhibitors on GE has not been evaluated to our knowledge, there is no reason to anticipate a significant effect. Lastly, while insulin may influence GE through modulating blood glucose levels, hyperinsulinaemia *per se* does not appear to affect GE (Kong et al., 1999).

#### *2.6.4 Effects of weight loss procedures for obesity on gastric/pouch emptying*

GE of nutrient liquids is markedly accelerated following Roux-en-Y gastric bypass (Nguyen et al., 2014) and sleeve gastrectomy (Sista et al., 2017) leading to a supraphysiologic GLP-1 response that attenuates the rise in blood glucose and, in some cases, subsequent hypoglycaemia (Patti and Goldfine, 2010). GE of solids following gastric bypass may be delayed or accelerated

– emptying is influenced by concurrently ingested liquids (Horowitz et al., 1982) and may relate to weight loss (Deden et al., 2017).

Fluid-filled (but not air-filled) intragastric balloons slow GE of solids substantially (Vargas et al., 2020) probably because air-filled balloons float to the fundus of the stomach, whereas fluid-filled balloons reside in the antrum and impair grinding and emptying of solids.

### *2.7 Management of gastroparesis associated with upper gastrointestinal symptoms*

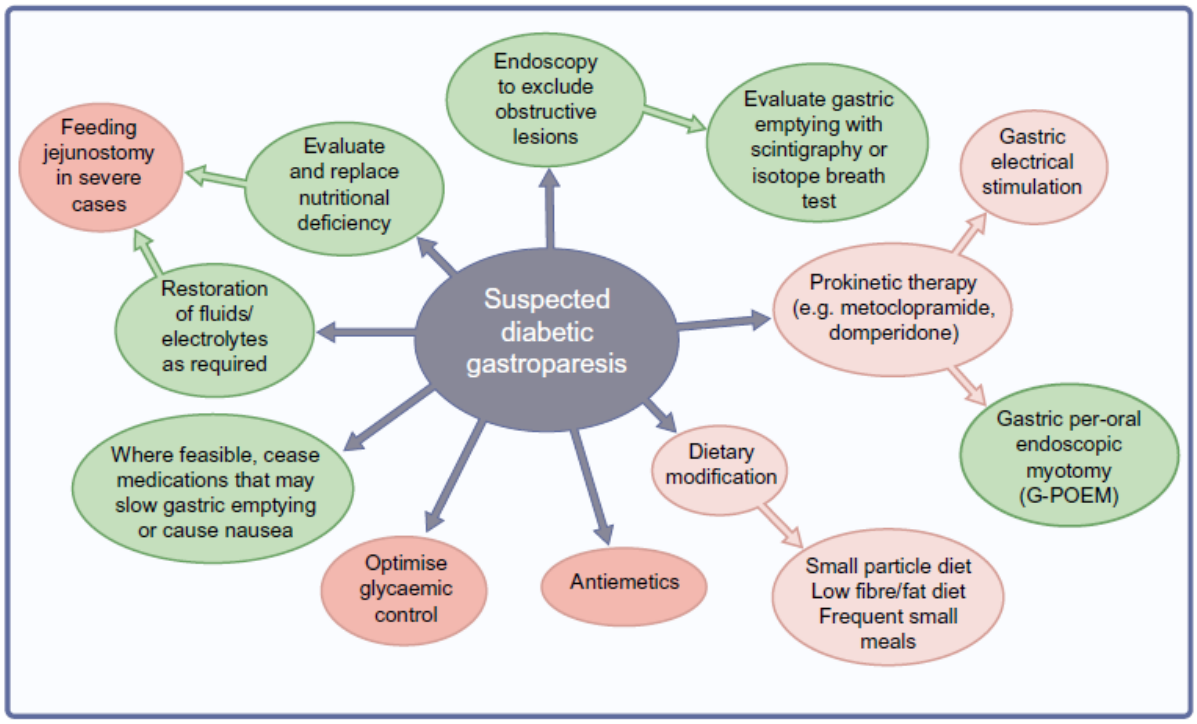


**Figure 2.4** Recommended management of suspected diabetic gastroparesis. Green, standard clinical practice/approaches supported by controlled trials; light pink, approaches supported by limited evidence; dark pink, approaches that may have a role but remain to be evaluated adequately. © Diabetologia.

Although it represents a substantial health burden, treatments for symptomatic gastroparesis remain limited and suboptimal. Progress has been compromised by two longstanding assumptions which have proved to be incorrect: (i) that delay in GE is the cause of symptoms, rather than being a marker of underlying dysfunction of gastroduodenal control mechanisms

and (ii) that making the stomach pump more effectively would inevitably lead to symptom improvement. A further limitation is that much of the current evidence is derived from poorly designed trials. For these reasons, while the heterogeneous nature of the motor/sensory dysfunctions in diabetic gastroparesis has implications for the development of more effective therapy, treatment should be targeted primarily at the relief of specific, bothersome symptoms.

### *2.7.1 Non-pharmacological management*

Dietary recommendations e.g. consumption of frequent, small meals and avoidance of high fat foods, while intuitively logical, remain to be evaluated adequately (Limketkai et al., 2020). Numerous drugs slow GE, but ceasing them may not prove feasible. Optimising glycaemic control is often recommended – in addition to slowing GE, acute hyperglycaemia may be associated with increased upper gastrointestinal 'sensitivity' (Phillips et al., 2015) – but this is often challenging and the rationale for the approach is hitherto only supported by uncontrolled studies. The effect of improvement in chronic glycaemic control (HbA1c) remains uncertain with an absence of controlled studies, although a prospective study reported normalisation of delayed GE, measured with scintigraphy, following improved glycaemic control (Laway et al., 2013). A small trial in individuals with T1D and gastroparesis found that hybrid closed loop insulin pump therapy improved mean percentage time that blood glucose was in range (3.9 – 10 mmol/L) from 26% to 58.4%, however neither gastrointestinal symptoms nor GE were evaluated (Daly et al., 2021). Nutritional support (e.g. feeding into the small intestine) may be necessary when there are severe, refractory symptoms associated with dehydration or malnutrition, but again, the evidence base for this approach is limited (Limketkai et al., 2020).

### *2.7.2 Pharmacological management*

Pharmacological treatment has focussed on prokinetic medications, but, as discussed, it is now appreciated that the relationship of symptomatic improvement to acceleration of GE is, at best, modest (Tack et al., 2021).

### *2.7.3 Dopamine receptor antagonists*

Metoclopramide, a dopamine-2 (D2) receptor antagonist, is the only pharmacologic treatment currently approved by the US Food and Drug Administration (FDA) for diabetic gastroparesis, but it has not been approved for this indication by the European Medicines Agency (EMA). Oral metoclopramide has been used for decades, but recently, a nasal formulation, which may be absorbed more reliably, has been shown to accelerate GE, and provide symptomatic relief in females (but not males) with diabetic gastroparesis (Gajendran et al., 2021). Based on anecdotal reports, subcutaneous metoclopramide may be effective in treating vomiting episodes. Other D2 receptor antagonists, including domperidone, may improve symptoms to a comparable extent to metoclopramide (Tendulkar et al., 2022). Tardive dyskinesia (more common with metoclopramide than domperidone) and QTc prolongation with the potential for arrhythmia are important concerns, although the risk of tardive dyskinesia may be as low as 0.1% per 1,000 patient years with metoclopramide (Al-Saffar et al., 2019). Nonetheless, metoclopramide carries a black box warning that chronic use (>12 weeks) may result in irreversible tardive dyskinesia (Schol et al., 2021). A newer dopamine D2/D3 receptor antagonist, trazpiroben (TAK-906), developed to have reduced cardiovascular and neurologic adverse effects, failed to show symptom improvement (Kuo et al., 2021b).

### *2.7.4 5-HT4 receptor agonists*

Prucalopride, a selective 5-hydroxytryptamine (5-HT)4 receptor agonist, was effective in improving symptoms in a cohort with predominantly idiopathic gastroparesis, but despite

accelerating GE in a diabetes-specific group, did not improve symptoms (Camilleri and Atieh, 2021). Velusetrag, a selective 5-HT4 receptor agonist, accelerates GE in diabetic gastroparesis, but effects on symptoms were not evaluated (Kuo et al., 2021a). For other 5-HT4 agonists, including clebopride, cinitapride and mosapride, there is relatively weak evidence to support efficacy (Camilleri and Atieh, 2021) and cisapride, which appeared to be effective at relieving symptoms, was withdrawn from the market in 2000 due to concerns about cardiovascular safety (Camilleri and Atieh, 2021).

*2.7.5 Motilin receptor agonists*

The macrolide antibiotics, erythromycin and azithromycin, are also motilin-receptor agonists and, at least in the short term, accelerate GE, although their effect on symptoms is uncertain (Camilleri and Atieh, 2021). There are also concerns regarding potential drug-interactions, and a reduction in long-term efficacy due to tachyphylaxis (Camilleri and Atieh, 2021). Mitemcinal, a newer motilin receptor agonist, was reported to improve symptoms in diabetic gastroparesis only in the subgroup of patients with BMI < 25 kg/m$^2$ and HbA1c < 86 mmol/mol (10%), for uncertain reasons (McCallum et al., 2007).

*2.7.6 Other therapies for gastroparesis*

Medications used to treat chronic abdominal pain, such as pregabalin and gabapentin, have not been formally evaluated in diabetic gastroparesis. The tricyclic antidepressant, nortriptyline, failed to improve symptoms in idiopathic gastroparesis (Parkman et al., 2013). Numerous classes of medication used to treat nausea, including 5HT-3 antagonists, phenothiazines, cannabinoids and H1 receptor antagonists, such as diphenhydramine, have not been evaluated adequately in the context of diabetic gastroparesis, but may have a place in its management (Rayner et al., 2021).

*2.7.7 Novel pharmacological targets*

The neurokinin-1 receptor antagonist, tradipitant, was reported to improve nausea in gastroparesis, but the study was underpowered to show a benefit in the subgroup with diabetes (Carlin et al., 2021). Sepiapterin, an essential cofactor of NOS, has been reported to improve gastric accommodation, but not symptoms, in women with diabetic gastroparesis (Abell et al., 2021). Relamorelin, a ghrelin agonist, led to both symptomatic improvement and acceleration of GE in three studies, but the subsequent phase 3 trial was terminated prematurely, apparently for commercial reasons (Hong et al., 2020).

*2.7.8 Endoscopic, surgical, and other therapies*

A number of invasive therapies have been used in individuals with refractory diabetic gastroparesis, but none can currently be considered to have an established place in management. The choice of therapy is, accordingly, empirical.

Gastric per-oral endoscopic pyloromyotomy (G-POEM) involves tunnelling through the submucosa to cut the pyloric sphincter. In a recent sham-controlled, randomised clinical trial of patients with refractory gastroparesis (41% had diabetes), G-POEM achieved symptomatic improvement in 71% of participants compared with 22% following the sham procedure (Martinek et al., 2022). GE, measured by scintigraphy, was accelerated after G-POEM but there was no relationship of symptom improvement with GE. In another recent larger but uncontrolled, prospective study in gastroparesis (Vosoughi et al., 2022) at 12 months, symptoms improved in 56% of participants who underwent G-POEM and GE, measured by scintigraphy, normalised in 47% with a moderate correlation between symptom improvement and GE acceleration (Vosoughi et al., 2022). These observations suggest that G-POEM may have a place in the management of individuals with refractory symptomatic gastroparesis.

The delivery of low energy, high frequency pulses to the antrum (gastric electrical stimulation) is effective in the treatment of vomiting in individuals with and without diabetes, regardless of

whether they had gastroparesis, as shown in a randomised, crossover study (Ducrotte et al., 2020). GE was not accelerated, nor was there an improvement in overall quality of life (Ducrotte et al., 2020).

Open-label studies initially suggested a benefit of intrapyloric injection of botulinum toxin; however, subsequent sham-controlled trials in gastroparesis of various aetiologies failed to demonstrate improvement in either symptoms or GE (Friedenberg et al., 2008). Likewise, a systematic review of 32 studies failed to support acupuncture for management of symptomatic gastroparesis (Kim et al., 2018).

There is anecdotal evidence that pancreatic transplantation may be effective in managing diabetic gastroparesis (Gaber et al., 1991); the effect of islet cell transplantation has not been reported. Roux-en-Y gastric bypass surgery (Papasavas et al., 2014), sleeve gastrectomy (Alicuben et al., 2021) and gastrojejunostomy (McCarty and Rustagi, 2015) have also been reported to improve symptoms in refractory gastroparesis, but in view of the small number of cases and the absence of controlled trials, further evaluation is required.

### *2.8 Conclusions*

The seminal importance of GE in diabetes is now established and recent insights have impacted on clinical practice. Clarification of a number of important, unresolved concepts will be dependent on the outcomes of future targeted research. The relative lack of progress in the management of gastroparesis associated with gastrointestinal symptoms reflects, in part, the pursuit of overly simplistic concepts and the fundamental importance of defining pathophysiology for the rational development of novel and effective therapy. The latter must be evaluated in well-designed clinical trials, which is itself challenging. In relation to postprandial glycaemic control, the clinical relevance of GE would be defined much more clearly if its measurement (e.g., by breath test) was incorporated widely in clinical trials where postprandial glycaemic excursions represent a major endpoint. Mechanisms should not be ignored when they

can be assessed both safely and simply. The words of Tolkien are relevant to our quest to understand diabetic gastroparesis – the latter will inevitably prove elusive unless we look carefully and with an open mind.

**Chapter 3. Relationships of glucose, GLP-1 and insulin secretion with gastric emptying after a 75 g glucose load in type 2 diabetes.**

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Relationships of glucose, GLP-1 and insulin secretion with gastric emptying after a 75 g glucose load in type 2 diabetes |
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**Principal Author**

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| Name of Principal Author (Candidate) | Ryan Joseph Jalleh | |
| Contribution to the Paper | I was responsible for the design of the study, data collection, data analysis and writing the 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: 24/11/23 |

**Co-Author Contributions**

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

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

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| Name of Co-Author | Tongzhi Wu | |
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| Signature: | | Date: 26/11/23 |

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| Contribution to the Paper | Prof Jones is a co-supervisor of my PhD and provided feedback and mentorship in preparing the manuscript | |
| Signature: | | Date: 24/11/23 |

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| Contribution to the Paper | Prof Rayner has expertise in gastroenterology and provided feedback on the manuscript | |
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| Contribution to the Paper | Prof Horowitz is my primary supervisor and provided supervision and mentorship in the design of study and preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

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### *3.1 Abstract*

*Context:* The relationships of gastric emptying (GE) with the glycemic response at 120min, GLP-1 and insulin secretion following a glucose load in type 2 diabetes (T2D) are uncertain.

*Objective:* We have evaluated the relationship of plasma glucose, GLP-1 and insulin secretion with GE of a 75 g oral glucose load in T2D.

*Design:* Single-center, cross-sectional, post hoc analysis.

*Setting:* Institutional research center.

*Participants:* 43 individuals with T2D age 65.6±1.1 years, HbA1c 7.2±1.0%, median duration of diabetes 5 years, managed by diet and/or metformin.

*Intervention:* Participants consumed the glucose drink radiolabelled with $^{99m}$Tc-sulphur colloid following an overnight fast. GE (scintigraphy), plasma glucose, GLP-1, insulin and C-peptide were measured between t=0-180min.

*Main outcome measures:* The relationships of the plasma glucose at 120 min, plasma GLP-1 and insulin secretion (calculated by $\Delta insulin_{0\text{-}30}/\Delta glucose_{0\text{-}30}$ and $\Delta C\text{-}peptide_{0\text{-}30}/\Delta glucose_{0\text{-}30}$ ) with the rate of GE (scintigraphy) were evaluated.

*Results:* There were positive relationships of plasma glucose at 30min ($r=0.56, P<0.001$), 60min ($r=0.57, P<0.001$) and 120min ($r=0.51, P<0.001$) but not at 180min ($r=0.13, P=0.38$), with GE. The 120min plasma glucose and GE correlated weakly in multiple regression models adjusting for age, GLP-1 and insulin secretion ($P=0.04$ and $P=0.06$). There was no relationship of plasma GLP-1 with GE. Multiple linear regression analysis indicated that there was no significant effect of GE on insulin secretion.

*Conclusion:* In T2D, while insulin secretion is the dominant determinant of the 120min plasma glucose, GE also correlates. Given the relevance to interpreting the results of an oral glucose

tolerance test, this relationship should be evaluated further. There appears to be no direct effect of GE on either GLP-1 or insulin secretion.

### *3.2 Background*

Gastric emptying (GE) of solid and liquid nutrients exhibits a wide inter-individual variation in health approximating a linear rate (in the case of solids, following an initial lag phase) ranging between ~1-4 kcal/min (Jones et al., 2001). This variation is even greater in type 2 diabetes (T2D) because of the high prevalence of both accelerated (usually individuals with well controlled diabetes) (Watson et al., 2019) and delayed (usually individuals with poorly controlled, complicated, diabetes) GE (Bharucha et al., 2015). GE is now appreciated as a major determinant of postprandial glycemia in health, as well as in T2D, accounting for up to 35% of the variance in the initial rise in glucose (Marathe et al., 2013). That certain ethnic groups with a greater propensity for type 2 diabetes have relatively more rapid GE supports the concept that GE may be a determinant of the risk of diabetes (Phillips, 2006).

An understanding of the relationship of glycemia with GE is of potential relevance to the use of the 120 min blood glucose in an oral glucose tolerance test (OGTT) to diagnose impaired glucose tolerance and T2D (Petersmann et al., 2019). The OGTT, despite longstanding concerns about its reproducibility, remains the 'gold-standard' diagnostic technique, for this purpose, including for the diagnosis of gestational diabetes (Bogdanet et al., 2020). Diabetes is classified by OGTT when fasting plasma glucose is ≥7.0 mmol/L or 120 min plasma glucose is ≥11.1 mmol/L (Petersmann et al., 2019). We have reported that there is an inverse relationship between the 120 min glucose and GE in health and postulated that this reflects a robust, earlier insulin response and intact insulin sensitivity (Marathe et al., 2015, Horowitz et al., 1993). This relationship appears to 'shift to the right' along the spectrum from health to impaired glucose tolerance to T2D, so that the inverse relationship between 120 min glucose and GE is not evident in individuals with impaired glucose tolerance (Marathe et al., 2015, Trahair et al., 2014). In T2D, we reported that the relationship of glycaemia with GE tended to be direct, rather than inverse, but this was not statistically significant, possibly reflecting the modest number (n=16) of participants studied (Marathe et al., 2015). Given the relevance of this

relationship, particularly to the use of the OGTT, the impact of gastric emptying on the 120 min glucose in T2D requires clarification. More recently, it has been suggested that the 60 min blood glucose in an OGTT may be a better predictor of dysglycemia compared to the 120 min glucose (Bergman, 2021); interestingly, this value is known to be related directly to the rate of gastric emptying in individuals with impaired glucose tolerance and T2D, but not in health (Marathe et al., 2015).

The impact of gastric emptying on insulin secretion in T2D is uncertain (Marathe et al., 2017). The latter is known to be influenced by the release of the 'incretin' hormones, glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP), following exposure of the small intestine to nutrients (Campbell and Drucker, 2013). In T2D, GLP-1 is of particular relevance given the marked impairment in the insulinotropic capacity of GIP (Nauck et al., 1993). In our studies which have evaluated the effects of direct intraduodenal infusion of glucose at rates spanning the normal range for GE in health and T2D, GIP secretion was shown to increase in proportion to the rate of nutrient delivery, whereas stimulation of GLP-1 was only evident at a higher threshold (3-4 kcal/min) (Ma et al., 2012, Pilichiewicz et al., 2007). Information relating to the impact of the rate of GE of glucose on plasma GLP-1 is inconsistent (Wishart et al., 1998); this may reflect the fact that endogenous GLP-1 itself slows GE (Deane et al., 2010b). It has been suggested that in T2D, a sustained increase in beta cell workload from exposure to persistent hyperglycemia may lead to reduced beta cell mass and function (Saisho, 2014). Accordingly, in T2D, relatively faster GE resulting in higher and more sustained postprandial glycemic fluxes, may have the potential to increase beta cell workload and, lead to beta cell exhaustion, with a consequent reduction in insulin secretory capacity.

Direct measurement of insulin secretion is usually impractical given the requirement for catheterisation of the hepatic portal vein (Song et al., 2000) and indirect methods use mathematical models to analyse the glucose, C-peptide and insulin data (Van Cauter et al., 1992). The ratio of the change in plasma insulin ("insulinogenic index") or C-peptide

concentration to the change in plasma glucose at 30 minutes during an OGTT are frequently used surrogate markers of insulin secretion (Hannon et al., 2018). In a previous study in health, we reported an inverse relationship of the insulinogenic index with GE i.e. when gastric emptying was relatively faster, the insulinogenic index was less (Marathe et al., 2017).

The primary aims of this study were to address two unresolved issues: the relationships of gastric emptying of a 75 g oral glucose load with (i) blood glucose at 120 minutes and (ii) insulin secretion, in T2D.

### *3.3 Methods*

Data were derived from two reported studies (Jones et al., 2019, Rayner et al., 2020).

*Participants*

43 Caucasians (14 female, 29 male) with T2D age 65.6 ± 1.1 years, HbA1c 7.2 ± 1.0%, BMI 31.6 ± 0.7 kg/$m^2$ median duration of known diabetes 5 years, managed by diet and/or metformin only (38 of 43 were taking metformin) were recruited. All participants had type 2 diabetes, diagnosed by either oral glucose tolerance test and/or measurement of HbA1c (Petersmann et al., 2019). Metformin was withheld for 48 hours prior to each study. None had a history of gastrointestinal disease, previous gastrointestinal surgery, epilepsy, significant alcohol intake (>20 g alcohol/day) significant cardiac, respiratory, hepatic and/or renal disease.

*Study protocol*

Participants fasted overnight (14 hours for solids, 12 hours for liquids). On arrival, an intravenous cannula was inserted into an antecubital vein for blood sampling. While seated in front of a gamma camera, participants consumed a 75 g glucose drink (280.5 kcal) dissolved in water (total volume 300 ml) radiolabelled with 20 MBq $^{99m}$Tc-phytate colloid (Radpharm Scientific, Belconnen, ACT) within 5 minutes. Completion of consumption of the drink was designated as t=0. Radioisotopic data were acquired for 180 minutes and corrected for γ-ray

attenuation, subject movement and radionuclide decay (Collins et al., 1983). GE data were acquired in 1-min frames for the first 60 minutes followed by 3 minute frames until t=180 minutes. GE was calculated as the amount of energy emptied (kcal/min) between 0 and 120 minutes. Plasma glucose (measured using hexokinase technique (Wu et al., 2016b)), total plasma GLP-1(RRID:AB_2757816, GLPIT-36HK, Millipore, Billerica, MA), C-peptide (RRID:AB_2750847, ELISA, 10-1136-01, Mercodia, Uppsala, Sweden), and plasma insulin (RRID:AB_2877672, ELISA, Diagnostics 10-1113, Mercodia, Uppsala, Sweden) were measured at 30, 60, 120 and 180 min. Insulin secretion was calculated as $\Delta insulin_{0\text{-}30}/\Delta glucose_{0\text{-}30}$ and $\Delta C\text{-}peptide_{0\text{-}30}/\Delta glucose_{0\text{-}30}$ (Hannon et al., 2018). The software used for the statistical analysis was SPSS (IBM) v27 and all analyses were supervised by a professional biostatistician.

*Statistical analysis*

Total areas under the curve (AUCs) between t= 0 – 180 min were calculated for GLP-1 and glucose using the trapezoidal rule. Participants were subdivided into two groups: those with 120 min plasma glucose ≥11.1 mmol/L and those with a lesser glycemic response and differences in mean gastric emptying rate were compared using Student's t test.

Multiple linear regression models were used to evaluate the primary outcomes, i.e. the relationship of (i) the 120 min plasma glucose and (ii) insulin secretion, with GE. In the former, age, GLP-1 AUC and insulin secretion were the independent variables, and in the latter, age, glucose AUC and GLP-1 AUC. Direct effects of GE on the 120 min glucose and insulin secretion were estimated by the standardized partial regression coefficient for GE (β). Scatterplots of all independent variables and covariates against GE indicated that linearity was appropriate for all relationships. Because the residuals from the insulin secretion model showed evidence of non-constant variance, values were log transformed for analysis (natural log, ln). Linear regression models were used to evaluate the relationships between glycemic responses (at t= 30, 60 and 180 min) and gastric emptying.

Each study was independently evaluated for these associations and homogeneity between studies was observed. A P value <0.05 was considered significant in all analyses. Data are presented as mean ± SEM. The software used for the statistical analysis was SPSS (IBM) v27 and all analyses were supervised by a professional biostatistician.

### *3.4 Results*

All participants tolerated the study well and in all cases, GE (1.85 ± 0.04 kcal/min) approximated a linear pattern from 0 – 120 min.

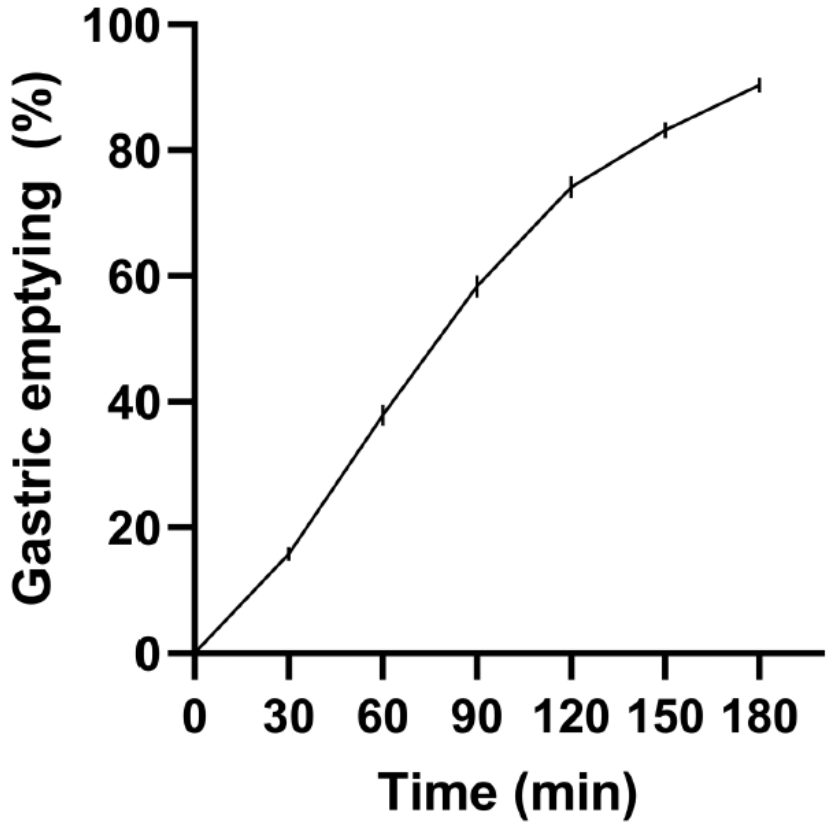


**Figure 3.1** Gastric emptying (expressed as percentage of glucose load emptied) over time in type 2 diabetes (n = 43). Data are mean ± SE of the mean.

Fasting glucose was 7.4 ± 0.2 mmol/L and blood glucose at 120 min 15.7 ± 0.6 mmol/L. Plasma glucose correlated positively and linearly with gastric emptying at 30 min (r= 0.56, P<0.001), 60 min (r= 0.57, P<0.001) and 120 min (r= 0.51, P<0.001), but not 180 min (r= 0.13, P=0.38).

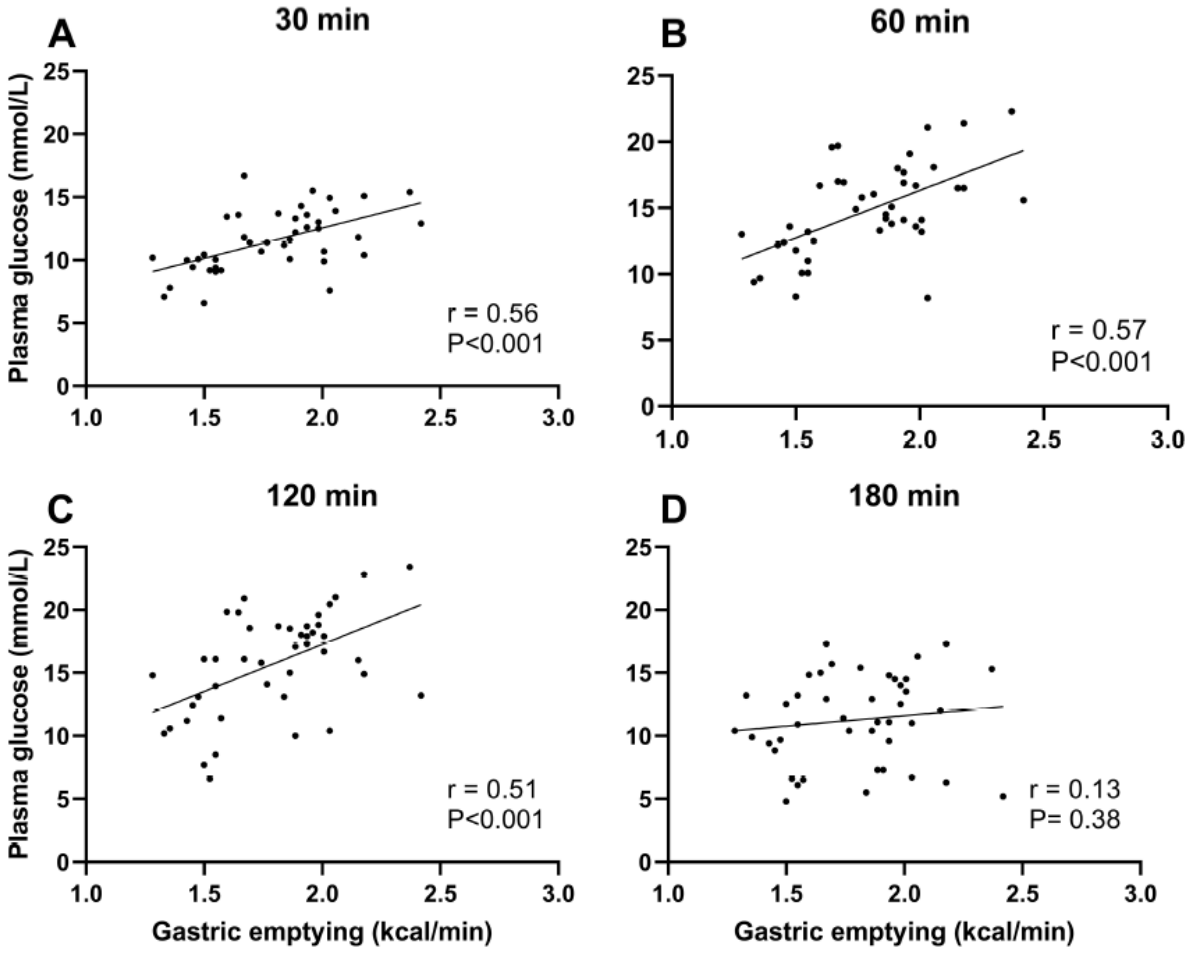


**Figure 3.2** Relationship of plasma glucose at (A) 30, (B) 60, (C) 120, and (D) 180 minutes after a 75-g oral glucose load and gastric emptying (kcal/min) in type 2 diabetes (n = 43).

In the multiple regression analysis, where age, GLP-1 AUC and insulin secretion ($insulin_{0\text{-}30}$/ $\Delta glucose_{0\text{-}30}$ and $C\text{-}peptide_{0\text{-}30}$/ $\Delta glucose_{0\text{-}30}$) were used as covariates, the 120 min plasma glucose correlated positively with GE ($\beta$=0.28, P=0.04) when $C\text{-}peptide_{0\text{-}30}$/ $\Delta glucose_{0\text{-}30}$ was used as the measure of insulin secretion, but not significantly with $insulin_{0\text{-}30}$/ $\Delta glucose_{0\text{-}30}$ ($\beta$=0.27, P=0.06).

| Model[a] | Unstandardized b | Coefficients SE | Standardized coefficients, beta | Significance | 95% CI lower bound | 95% CI upper bound |
|---|---|---|---|---|---|---|
| 1 (Constant) | 2.245 | 3.588 | | 0.535 | −5.001 | 9.491 |
| Gastric emptying | 7.512 | 1.979 | 0.510 | <0.001 | 3.515 | 11.508 |
| 2 (Constant) | 7.777 | 6.485 | | 0.238 | −5.351 | 20.904 |
| Gastric emptying | 3.953 | 2.063 | 0.268 | 0.063 | −0.224 | 8.130 |
| Age, years | 0.054 | 0.076 | 0.097 | 0.479 | −0.099 | 0.208 |
| GLP1.AUC180.min | −0.19 | 0.057 | −0.042 | 0.742 | −0.134 | 0.097 |
| $\Delta Insulin_{0-30}/\Delta glucose_{0-30}$ | −0.097 | 0.031 | −0.449 | 0.004 | −0.160 | −0.034 |
| 3 (Constant) | 9.093 | 6.401 | | 0.164 | −3.864 | 22.050 |
| Gastric emptying | 4.182 | 1.980 | 0.284 | 0.041 | 0.175 | 8.190 |
| Age, years | 0.048 | 0.074 | 0.086 | 0.521 | −0.102 | 0.198 |
| GLP1.AUC180.min | −0.050 | 0.056 | −0.110 | 0.381 | −0.164 | 0.064 |
| $\Delta C\text{-peptide}_{0-30}/\Delta glucose_{0-30}$ | −0.022 | 0.007 | −0.471 | 0.001 | −0.036 | −0.009 |

[a]Outcomes of 3 models are provided. In each model, gastric emptying is the first independent variable (Model 1). Model 2 GLP1.AUC180.min: Area under the curve for the concentration of plasma GLP-1 from 0-180 min. includes the other 3 covariates (age, GLP-1, $\Delta insulin_{0-30}/\Delta glucose_{0-30}$), and in Model 3, the covariates are age, GLP-1 and $\Delta C\text{-peptide}_{0-30}/\Delta glucose_{0-30}$.

**Table 3.1** Multiple regression analysis using the 120-minute blood glucose level as the dependent variable.

Fasting glucose was ≥7.0 mmol/L in 25/43 (58.1%) and at 120 min, plasma glucose was ≥11.1 mmol/L in 35/43 (81.4%). For the subgroup with the 120 min plasma glucose ≥11.1 mmol/L (n=35), GE tended to be faster compared to the subgroup with a 120 min plasma glucose <11.1 mmol/L 1.90 ± 0.05 vs 1.65 ± 0.10 kcal/min (P=0.06).

Baseline fasting GLP-1 was 20.6 ± 1.3 pmol/L, rose to 34.3 ± 2.1 pmol/L at 30 min and, then gradually decreased to 31.3 ± 2.0 pmol/L at 60 min, 22.9 ± 1.3 pmol/L at 120 min and 16.3 ± 1.0 pmol/L at 180 min. There was no significant relationship between GLP-1 and gastric emptying at 30 (r= -0.21, P=NS), 60 (r= -0.09, P=NS), 120 (r= -0.13, P=NS) or 180 min (r= -0.27, P=NS).

*Relationship of insulin secretion with gastric emptying*

The ANOVA test for the model was statistically significant, indicating that there was a relationship between the combinations of GE, age and GLP-1 AUC with insulin secretion (P<0.001). The addition of the three covariates significantly improved the model fit, compared

to GE alone (P<0.001). In the final model, the direct effects for GE, age, glucose AUC and GLP-1 AUC were all negative. The effect for glucose AUC was significant (β=-0.59, P<0.001), while the direct effect of GE on insulin secretion was not (β=-0.13, P=0.39).

| Model[a] | Unstandardized b | Coefficients SE | Standardized coefficients, beta | Significance | 95% CI lower bound | 95% CI upper bound |
|---|---|---|---|---|---|---|
| 1 (Constant) | 5.231 | 0.787 | | <0.001 | 3.642 | 6.821 |
| Gastric emptying | −1.357 | 0.434 | −0.439 | 0.003 | −2.234 | −0.480 |
| 2 (Constant) | 6.303 | 1.088 | | <0.001 | 4.100 | 8.506 |
| Gastric emptying | −0.389 | 0.443 | −0.126 | 0.386 | −1.287 | 0.509 |
| Age, years | −0.007 | 0.016 | −0.064 | 0.632 | −0.039 | 0.024 |
| Gluc.AUC180.min | −0.164 | 0.041 | −0.588 | <0.001 | −0.247 | −0.081 |
| GLP1.AUC180.min | −0.004 | 0.012 | −0.041 | 0.746 | −0.028 | 0.020 |

[a]Model 1 includes gastric emptying as the first independent variable. Model 2 includes the other three covariates (age, glucose, glucagon-like peptide-1). GLP1.AUC180.min: Area under the curve for the concentration of plasma GLP-1 from 0-180 min.

**Table 3.2** Multiple regression analysis using Δinsulin0-30/Δglucose0-30 as the dependent variable.

*ΔC-peptide$_{0-30}$/Δglucose$_{0-30}$*

The ANOVA test for the overall model was significant (P<0.001) and the addition of the three covariates improved the model fit compared to GE alone (P<0.001). In the final model, the direct effects for GE, age, glucose AUC and GLP-1 AUC were all negative. The effect for glucose AUC was significant (β=-0.53, P=0.001) while the direct effect of GE on insulin secretion was not (β=-0.07, P=0.63).

| Model[a] | Unstandardized b | Coefficients SE | Standardized coefficients, beta | Significance | 95% CI lower bound | 95% CI upper bound |
|---|---|---|---|---|---|---|
| 1 (Constant) | 322.704 | 82.056 | | <0.001 | 156.987 | 488.420 |
| Gastric emptying | −111.198 | 45.262 | −0.358 | 0.018 | −202.606 | −19.791 |
| 2 (Constant) | 557.810 | 110.909 | | <0.001 | 333.285 | 782.334 |
| Gastric emptying | −22.019 | 45.197 | −0.071 | 0.629 | −113.516 | 69.477 |
| Age, years | −2.366 | 1.583 | −0.201 | 0.143 | −5.571 | 0.839 |
| Gluc.AUC180.min | −14.795 | 4.192 | −0.528 | 0.001 | −23.281 | −6.309 |
| GLP1.AUC180.min | −1.564 | 1.210 | −0.163 | 0.204 | −4.014 | 0.886 |

[a]Model 1 includes gastric emptying as the first independent variable. Model 2 includes the other three covariates (age, glucose, glucagon-like peptide-1).

**Table 3.3** Multiple regression analysis using ΔC-peptide0-30/Δglucose0-30 as the dependent variable.

### *3.5 Discussion*

We have shown that in type 2 diabetes, there is a positive relationship between glycemia at 30, 60 and, in particular, at 120 minutes following a 75 g oral glucose load, but no significant relationship at any time of the plasma GLP-1 or insulin secretory responses with GE. The correlation between glycemia at 120 min with GE is weaker in multiple regression analysis when age, GLP-1 and insulin secretion are covariates.

Consistent with previous reports (Marathe et al., 2015, Watson et al., 2019), the plasma glucose concentration following a 75 g oral glucose load was related directly to gastric emptying at 30 and 60 min in T2D. In the study by Marathe et al (Marathe et al., 2015), there was also a trend for a relationship between glycemia and GE at 120 min, but this did not achieve statistical significance likely due to the modest sample size. In health it has been well established that the relationship of the blood glucose response to a 75 g oral glucose load and GE is direct at 30 min, not significant at 60 min and inverse, rather than direct, at 120 min (Marathe et al., 2015, Horowitz et al., 1993), presumably reflecting effective glucose counter-regulation, particularly because (although it does not appear to be widely appreciated) in the majority of individuals, GE is not completed at that time (i.e. an emptying rate ≥2.5 kcal/min would be required, which is not usually the case (Phillips et al., 2015)). In contrast, at 180 min, GE of a 75 g oral glucose load would be complete in the majority of individuals, which may account for the absence of a relationship with glycemia at that time.

GE is currently not considered in the diagnosis of T2D using the OGTT, including in gestational diabetes where it remains the main diagnostic method (Bogdanet et al., 2020). Our study indicates that this may represent a limitation i.e. the blood glucose at 120 min appears to be dependent on the rate of emptying, which is highly variable between individuals. Moreover, in those in whom the 120 min blood glucose was not diagnostic of diabetes (18.6%) there was a trend for GE to be slower (P=0.06). This issue, accordingly, warrants further evaluation. It is, however, clear that the timing and direction of the relationship of the glycemic response to oral

glucose with GE is dependent on glucose tolerance, demonstrating a 'shift to the right' of the direct relationship with progressive impairment in glucose tolerance (Marathe et al., 2015, Trahair et al., 2014). The impact of GE on glycemia after a 75 g glucose load is also much more sustained in T2D than in health i.e. there were positive relationships between 30 – 120 min, whereas in health, the relationships were either non-significant or negative after 30 min. While this is intuitively, likely to also be the case after carbohydrate-containing meals, further studies are indicated to investigate this. The importance of glucose tolerance is reflected in outcomes of the multiple regression analysis where insulin secretion correlates, not surprisingly, more strongly with the 120 min plasma glucose then GE, indicating that it is the dominant determinant of the 120 min plasma glucose, a concept that is widely appreciated. However, our observations also support a lesser appreciated, albeit weaker, relationship of GE with glycemia at 120 min. This relationship implies that concomitant use of medications that affect gastric emptying, such as opioids, anticholinergics and GLP-1 receptor agonists may affect the OGTT result.

The absence of a relationship between plasma GLP-1 and GE following oral glucose was clear-cut, consistent with our previous study that found no relationship between GLP-1 and GE following a semi-solid meal (Xie et al., 2021b). Intuitively, relatively faster GE may be anticipated to result in more rapid delivery of nutrients to the distal small intestine to stimulate secretion of GLP-1 (Marathe et al., 2013). Moreover, as discussed, our previous studies using direct infusion indicates that the GLP-1 secretion in health (Pilichiewicz et al., 2007) and type 2 diabetes (Ma et al., 2012) is dependent on the rate of intraduodenal glucose delivery. There are several potential explanations for the differences observed. Firstly, in none of the current participants was GE faster than 3 kcal/min and in our intraduodenal glucose studies, a sustained rise in plasma GLP-1 was only evident when glucose was delivered at > 3 kcal/min (Ma et al., 2012). Secondly, more rapid GE may not translate to more rapid delivery of glucose to the distal, as opposed to the proximal, small intestine, possibly as a result of the, so-called 'ileal

brake' (Van Citters and Lin, 2006) where increased glucose delivery to the distal small intestine slows GE, probably by stimulating GLP-1 and PYY (Holst, 2007). Thirdly, studies using the specific GLP-1 antagonist, exendin 9-39, have established that endogenous GLP-1 is a physiological modulator of GE of glucose in health (Deane et al., 2010b, Plummer et al., 2014b) – by infusing glucose directly into the small intestine, this mechanism is 'bypassed'.

Our multiple regression analysis indicated that there was no direct relationship between insulin secretion and GE in T2D after adjusting for glucose and GLP-1. We had hypothesized that individuals with relatively faster GE may have a relative reduction in their insulin secretory response if the greater post-prandial glycemic excursions leading to beta cell 'stress', but this was not supported by our findings. A potential explanation is that our cohort comprised individuals with relatively well-controlled T2D and, therefore, insulin secretory function was relatively preserved.

Strengths of our study include the use of scintigraphy, the 'gold-standard' technique to measure GE and a participant population with similar baseline characteristics. Limitations are that the study population was entirely Caucasian, managed with lifestyle modification and/or metformin monotherapy and had uncomplicated T2D with reasonable chronic glycemic control as assessed by glycated hemoglobin. Of necessity, we employed surrogate, rather than direct, markers to estimate the insulin secretory response and - accordingly, our observations in this area should be viewed circumspectly. While our study failed to show an independent relationship between insulin secretion and GE, the design was cross-sectional and a prospective study to clarify this issue would be of interest.

### *3.6 Conclusion*

We conclude that in T2D, while insulin secretion is a dominant determinant of the glycemic response, relatively faster GE is also associated with an increased glycemic response at 30, 60

and, in particular, at 120 minutes following a 75 g oral glucose load, while GLP-1 and insulin secretion do not appear to correlate with GE. These findings should prompt more detailed evaluation of the impact of GE on the diagnostic accuracy of the OGTT. A prospective study to clarify the relevance of gastric emptying to the risk of T2D would be of interest.

**Chapter 4. The 1-hour plasma glucose after a 75 g oral glucose load and its relationship to gastric emptying in survivors of critical illness and stress hyperglycaemia.**

# Statement of Authorship

| | |
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| Title of Paper | One-hour plasma glucose level after a 75 g oral glucose load and its relationship to gastric emptying in survivors of critical illness and stress hyperglycaemia |
| Publication Status | Published |
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| 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:

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

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| Contribution to the Paper | Dr Xie assisted in data analysis and in the preparation of the manuscript | |
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| Contribution to the Paper | Prof Horowitz is the primary supervisor of my PhD and provided supervision and mentorship in the study design and preparation of the manuscript | |
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*4.1 Abstract*

**Objective:** A 1-hour plasma glucose ≥8.6 mmol/L in a 75 g oral glucose tolerance test has been strongly associated with increased morbidity and mortality in outpatients without diabetes. Our primary aim was to evaluate the 1-hour plasma glucose in a 75 g glucose tolerance test in survivors of critical illness with stress hyperglycaemia at 3 months post-ICU discharge with the secondary aims to evaluate the 2-hour plasma glucose, HbA1c and gastric emptying.

**Design:** Post hoc analysis of a cohort study.

**Setting:** Single centre, tertiary-referral, mixed medical-surgical ICU.

**Participants:** Consecutively admitted patients who developed stress hyperglycaemia and survived to hospital discharge were eligible.

**Interventions:** Participants returned at three months post-ICU discharge and underwent a 75 g oral glucose tolerance test.

**Main outcome measures:** 1-hour and 2-hour post load plasma glucose, HbA1c and assessment of gastric emptying via an isotope breath test.

**Results:** Thirty-five patients (12 females, mean age 58.5 years [SD 10.5], HbA1c 37.4 mmol/mol [SD 7.0]) attended the follow up. In 32/35 patients (91%) the 1-hour post load plasma glucose was ≥8.6 mmol/L. There was an inverse correlation between the plasma glucose at 1-hour ($r^2$=0.21, P=0.006), but no correlation between the 2-hour glucose ($r^2$=0.006, P=0.63) and gastric emptying T50.

**Conclusion:** Glucose intolerance, when defined as 1-hour glucose ≥8.6 mmol/L following a 75 g oral glucose load, persists at 3 months in the majority of survivors of stress hyperglycaemia and is dependent on the rate of gastric emptying. Longitudinal studies to characterize mechanisms underlying dysglycaemia and progression to diabetes in individuals with stress hyperglycaemia are indicated.

### *4.2 Introduction*

Stress hyperglycaemia describes impaired glucose tolerance in individuals without diabetes that resolves following resolution of their acute illness (Dungan et al., 2009). Glucose tolerance is traditionally evaluated by a 75 g oral glucose tolerance test (OGTT) - a 2-hour plasma glucose ≥7.8 mmol/L is defined as impaired glucose tolerance, and a 2-hour plasma glucose ≥11.1 mmol/L as diabetes . More recently, a 1-hour plasma glucose ≥8.6 mmol/L following a 75 g OGTT has been shown to be a robust predictor of incident type 2 diabetes in outpatients and outperforms the 2-hour plasma glucose and HbA1c (Bergman, 2021, Bergman et al., 2018, Peddinti et al., 2019). Furthermore, a 1-hour plasma glucose ≥8.6 mmol/L is strongly associated with increased diabetes-related morbidity and mortality in outpatients (Bergman, 2021, Bergman et al., 2018).

Gastric emptying is a major determinant of the glycaemic response to an OGTT in health, impaired glucose tolerance and type 2 diabetes (Marathe et al., 2015). However, the timing and direction of these relationships are influenced by glucose tolerance status (Marathe et al., 2015). In health, the relationship of plasma glucose with gastric emptying is direct at 30 min, not significant at 60 min and inverse at 120 min whereas in individuals with impaired glucose tolerance and type 2 diabetes, there is a direct relationship at 60 min (Marathe et al., 2015). The relationship of the 1-hour plasma glucose with gastric emptying has not been evaluated in survivors of critical illness with stress hyperglycaemia. We hypothesised that individuals the 1-hour plasma glucose post glucose load would be greater in individuals with relatively faster gastric emptying.

The primary aim of this study was to assess the 1-hour plasma glucose in survivors of critical illness who had stress hyperglycaemia detected during their ICU admission at three months following ICU discharge. Secondary aims were to measure the 2-hour plasma glucose, HbA1c and relationships of the 1 and 2-hour plasma glucose with gastric emptying.

### *4.3 Methods*

This is a post hoc analysis of a prospective, single centre cohort study (Kar et al., 2019). Forty patients without diabetes, who were admitted to ICU and were identified as having stress hyperglycaemia as a complication during their admission, were recruited. Stress hyperglycaemia was defined as an admission HbA1c less than or equal to 47.5 mmol/mol (≤6.5%) and subsequent blood glucose concentrations greater than 11.1 mmol/L (200 mg/dL) on two or more occasions within a 24-hour period or the commencement of insulin for glucose greater than 11.1 mmol/L (200 mg/dL). Thirty-five patients (87.5%) returned at 3 months for a 75 g OGTT where the drink was radiolabelled with 100 mg of $^{13}$C-octanoic acid. Breath samples were acquired prior to ingestion of the glucose load, every 5 minutes for the first hour, and then every 15 minutes for the subsequent 2 hours. For the purposes of this analysis, a 1-hour plasma glucose ≥8.6 mmol/L (measured using the hexokinase technique (Neese, 1982)) was considered indicative of glucose intolerance (Bergman et al., 2018) and either a HbA1c ≥47.5 mmol/mol, fasting glucose ≥7.0 mmol/L or 2-hour plasma glucose ≥11.1 mmol/L was considered incident diabetes (Kar et al., 2019). $CO_2$ concentration and the percentage of $^{13}CO_2$ were measured in each sample with an isotope ratio mass spectrometer (ABCA model 20\20; Europa Scientific, Crewe, United Kingdom) (Deane et al., 2010a). The gastric emptying half-time (T50) was derived from these data and adjusted with the Wagner-Nelson method (Wagner and Nelson, 1964). The gastric emptying rate in kcal/min was derived by calculating the ratio of half the total caloric content of the glucose drink to the T50. Differences in mean 1-hour plasma glucose and gastric emptying between the subgroups with or without incident diabetes were evaluated with an unpaired t-test and the relationship between 1-hour plasma glucose and T50 was evaluated via linear regression. A P value <0.05 was considered significant. Data are presented as means ± SD.

### *4.4 Results*

At the 3-month follow up, the participants were 58.5 ± 10.5 years old, with a HbA1c of 37.4 ± 7.0 mmol/mol, weight of 80 ± 20.9 kg and Body Mass Index of 27 ± 6.3 kg/$m^2$. The mean 1-hour plasma glucose was 11.3 ± 2.6 mmol/L and 32 of 35 participants (91.4%) had glucose intolerance (1-hour plasma glucose ≥8.6 mmol/L). There was no significant difference in the 1-hour plasma glucose in those diagnosed with (HbA1c ≥47.5 mmol/mol, fasting glucose ≥7.0 mmol/L or 2-hour plasma glucose ≥11.1 mmol/L) (13/35) and without (22/35) (12.0 ± 3.2 mmol/L vs. 10.9 ± 2.2 mmol/L; P=0.26) diabetes. The mean gastric emptying T50 was 128.2 ± 52.5 min (corresponding to a gastric emptying rate of 1.2 kcal/min) and there was an inverse relationship between plasma glucose at 1-hour and gastric emptying T50 ($r^2$ = 0.21, P=0.006) indicating that individuals in whom gastric emptying was relatively faster had a greater 1-hour plasma glucose. There was no relationship between plasma glucose at 2-hour and gastric emptying T50 ($r^2$ = 0.006, P=0.63). The was no difference between gastric emptying T50 in those with and without diabetes; (128.5 ± 40.3 min vs. 127.5 ± 58.8 min; P=0.96).

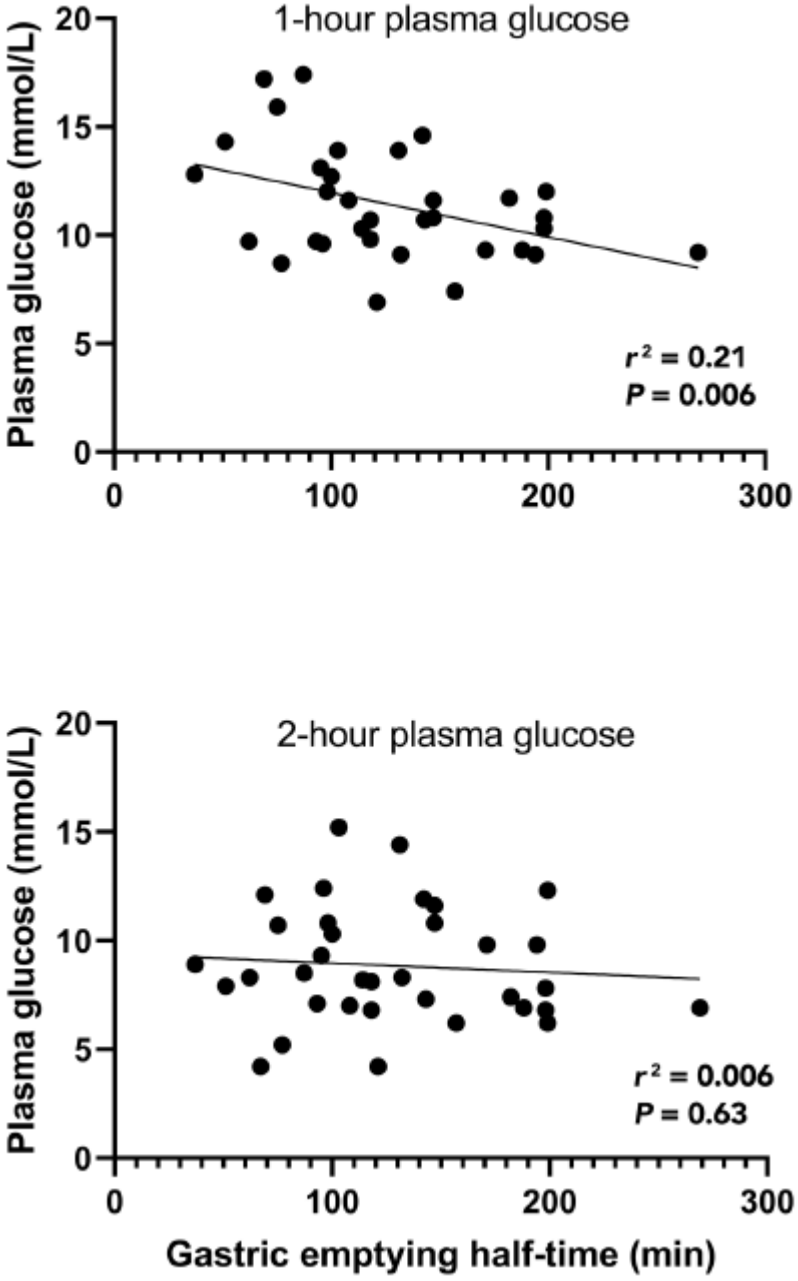


**Figure 4.1** Relationship of 1-hour and 2-hour plasma glucose level following a 75 g oral glucose load and gastric emptying half-time in survivors of critical illness with stress hyperglycaemia ($n$ = 35).

### *4.5 Discussion*

The major observation is that the vast majority of this cohort (>90%) of survivors of critical illness with stress hyperglycaemia, studied 3 months after ICU discharge, had glucose

intolerance, when defined as a 1-hour plasma glucose ≥8.6 mmol/L. There is compelling evidence that a 1-hour plasma glucose ≥8.6 mmol/L is associated with mortality and morbidity in general practice, and is a robust predictor of progression to type 2 diabetes (Bergman et al., 2018). Early identification of individuals with glucose intolerance is important, as it provides the opportunity to intervene and thereby reduce the risk of progression to overt diabetes (Paulweber et al., 2010). Currently, there are no formal guidelines regarding follow-up of patients who have been diagnosed with stress hyperglycaemia during ICU admission. In comparison, gestational diabetes, a condition that is associated with transient dysglycaemia, is routinely followed up with an OGTT postpartum (Thayer et al., 2020). As individuals with stress hyperglycaemia, which may comprise up to 50% of all ICU admissions, (Plummer et al., 2014a) are likely to be at high risk of future complications of diabetes and increased mortality, we believe that further evaluation is required to determine the effectiveness of screening programs and interventions (Paulweber et al., 2010).

The pathogenesis of stress hyperglycaemia is multifactorial, driven by increased glucose production, relative insulin insufficiency, temporary insulin resistance and iatrogenic therapies such as steroids and catecholamines (Bar-Or et al., 2019). Our findings suggest that there is a hangover effect from acute illness resulting in persistent dysglycaemia despite recovery from the acute stressor. Further research is required to delineate whether this is due to a reduction in β-cell secretory capacity, insulin resistance or both.

In health and type 2 diabetes, gastric emptying, which exhibits a substantial inter-, but much lower, intra-individual variation is a primary determinant of the glycaemic response to oral glucose and carbohydrate-containing meals (Marathe et al., 2015). We have demonstrated (in survivors of stress hyperglycaemia) a similar relationship with faster gastric emptying associated with a greater glycaemic response post glucose load. Intuitively, this is due to increased glucose delivery to the small intestine and more rapid absorption. This cohort had gastric emptying rates similar to that seen in health (1 – 4 kcal/min) (Marathe et al., 2015) and

there was no difference in gastric emptying between the subgroups (incident diabetes vs no diabetes). In health, there is a direct relationship of glycaemia at 30 min, but not 60 min with gastric emptying during an OGTT and the relation with the 120 min glucose is inverse, rather than direct. In contrast, in impaired glucose tolerance, there is a 'shift to the right' with a direct relationship of glycaemia and gastric emptying at 30 and 60 min, but not 120 min (Marathe et al., 2015). In type 2 diabetes, there is a further shift with a direct relationship at 30 and 60 min and a trend for a direct relationship at 120 min (Marathe et al., 2015). This 'shift to the right' likely is a result of glucose rises being more sustained in the setting of declining glucose counter-regulation. Accordingly, the demonstration of a relationship at 60 min in individuals with prior stress hyperglycaemia is indicative of a sustained glycaemic rise, presumably reflecting ineffective glucose counter-regulation (Marathe et al., 2015).

The limitations of this study include the relatively small sample size. We were also unable to analyse for differences in gastric emptying between the subgroup with and without an elevated 1-hour plasma glucose as only three patients had normal glucose tolerance. Gastric emptying was measured using the $^{13}$C-octanoic acid breath test, rather than the "gold standard" scintigraphy but this limitation was mitigated by using the Wagner-Nelson method (Wagner and Nelson, 1964), known to make the data comparable to scintigraphy (Trahair et al., 2018). The study was designed post-hoc and the findings need to, accordingly, be confirmed by dedicated, prospective studies, ideally assessing the risk of incident diabetes and its complications in this cohort.

### *4.6 Conclusion*

The majority of survivors of critical illness with stress hyperglycaemia have a 1-hour plasma glucose consistent with glucose intolerance at 3-months post ICU discharge. Prospective studies are required to evaluate the long-term risk of diabetes or its complications in this cohort and determine if early intervention may prevent progression to diabetes.

**Chapter 5. A biphasic glucose response during an oral glucose tolerance test is associated with greater plasma insulin and GLP-1 responses and a reduction in 1-hour plasma glucose but does not relate to the rate of gastric emptying in healthy, older adults.**

# Statement of Authorship

| | |
|---|---|
| Title of Paper | A biphasic glucose response during an oral glucose tolerance test is associated with greater plasma insulin and GLP-1 responses and a reduction in 1-hour glucose but does not relate to the rate of gastric emptying in healthy, older adults. |
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| 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:

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

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| Contribution to the Paper | Prof Horowitz is my primary supervisor and provided supervision and mentorship with preparation of the manuscript | |
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*5.1 Abstract*

**Background**: The pattern of the plasma glucose response curve during an oral glucose tolerance test (OGTT) is of prognostic significance with "biphasic" when compared with "monophasic" patterns being associated with greater insulin sensitivity/secretion and a reduced risk of progression to diabetes. The relationships of the glucose response curves with gastric emptying and incretin hormone secretion are not known.

**Methods**: Thirty-six adults (age > 65 years) without known diabetes consumed a 300 mL drink containing 75 g glucose and 150 mg $C^{13}$-acetate at baseline and follow-up after 5.8 ± 0.1 years. Plasma glucose, glucagon-like peptide-1 (GLP-1), glucose independent insulinotropic polypeptide (GIP) and insulin were measured, and participants classified according to the pattern of their glucose response. Gastric emptying was measured on breath samples (stable isotope breath test). Results: At baseline, 22 participants had a "monophasic" and 14 a "biphasic" glucose response. The 1 h plasma glucose response curve was greater and the GLP-1 $AUC_{0–120}$ min and insulin secretion lower in the monophasic group. There were no differences in gastric emptying, GIP or insulin sensitivity. At the follow-up, the 1 h glucose response curve was greater again, while GLP-1 $AUC_{0–120}$ min was lower in the monophasic group.

**Conclusions**: A biphasic curve is associated with a higher 60 min glucose response curve and increases in GLP-1, but no difference in either GIP or gastric emptying.

### *5.2 Introduction*

It is not widely appreciated that in addition to the diagnostic implications of baseline, 60 min and 120 min plasma glucose levels (Bergman et al., 2016), the shape of the glucose response curve during an oral glucose tolerance test (OGTT) provides useful insights into insulin secretion and sensitivity, even in individuals with normal glucose tolerance (Manco et al., 2017, Kim et al., 2012, Arslanian et al., 2019, Ismail et al., 2018, Tura et al., 2011). The shape has been classified according to whether it exhibits either (i) an incessant rise in glucose, (ii) a monophasic curve, (iii) a biphasic curve and (iv) a more complex curve (Arslanian et al., 2019). The incessant/progressive rise in glucose appears to be associated with the greatest risk of dysglycaemia and incident diabetes (Arslanian et al., 2019). A monophasic curve, as evaluated in Latino youths (Kim et al., 2012), youths with obesity (Arslanian et al., 2019) and autoantibody-positive relatives of people with type 1 diabetes (Ismail et al., 2018), is associated with impaired glucose tolerance, reduced insulin sensitivity and secretion, and an increased risk of future diabetes when compared with biphasic, or more complex, curves. In contrast, increasing complexity of the shape (i.e. greater numbers of glucose peaks) is associated with better glucose tolerance and beta cell function (Tura et al., 2011). While these associations are well-established, the mechanisms accounting for these differences in glucose patterns and their implications for glucose tolerance remain poorly defined (Manco et al., 2017). Specifically, there is no information about the potential roles of the rate of gastric emptying (GE) and the incretin hormones.

GE for which there is a substantial inter- (~1 – 4 kcal/min), but lesser intra-, individual variation, is now recognized to be a major determinant of postprandial glucose, accounting for ~35% of the post-prandial blood glucose response (Marathe et al., 2015). In some racial groups predisposed to the development of type 2 diabetes (T2D), gastric emptying is accelerated (Wang et al., 2020, Phillips, 2006). Moreover, uncomplicated T2D, in contrast to longstanding, complicated T2D, is associated with more rapid, rather than delayed, GE (Watson et al., 2019).

Glucagon-like peptide-1 (GLP-1) and glucose independent insulinotropic polypeptide (GIP), released from the small intestine, increase insulin sensitivity and secretion and, in the case of GLP-1, suppress glucagon (Drucker and Nauck, 2006). An increase in small intestinal glucose delivery, as when gastric emptying is more rapid, is associated with greater GIP and GLP-1 secretion. Studies in which glucose has been delivered directly into the small intestine indicate that the patterns of response differs (Pilichiewicz et al., 2007). The rate of small intestinal glucose delivery correlates linearly with a rise in GIP, whereas the GLP-1 response is minimal at lower rates of glucose delivery (1 – 2 kcal/min), but substantial at rates of 3 – 4 kcal/min (Pilichiewicz et al., 2007). In older adults without diabetes, we have shown that glucose-stimulated GLP-1 and GIP concentrations correlate, even after a period of ~6 years, but 'healthy' aging is associated with modest reductions in fasting GLP-1 and GIP, as well as glucose-stimulated GLP-1 (Pham et al., 2019). In addition to its glucose dependent insulinotropic and glucagonostatic properties, GLP-1 also plays a physiological role to slow GE (Deane et al., 2010b) and the rate of GE in both health and T2D appears to be determined in part by the GLP-1 response to intestinal nutrients. GIP, in contrast, has no effect on GE (Meier et al., 2004).

We have now performed, in older individuals (>65 years) without known diabetes, a cross-sectional, longitudinal evaluation of the association of the shape of the glucose response curve with GLP-1 and GIP secretion, and GE as well as insulin secretion and sensitivity. We hypothesized that a relatively more rapid rate of GE would be associated with a monophasic rather than a biphasic curve reflecting the relatively faster influx of glucose into the small intestine. We also hypothesized that plasma GLP-1 and GIP concentrations would be greater in the monophasic group in this cohort as a compensatory response to the glycaemic excursion. A follow up study after a minimum of 4 years was conducted to evaluate for changes in the glucose response curve, incretin secretion and insulin secretion/sensitivity.

### *5.3 Materials and Methods*

*Participants*

Information relating to the relationship of blood pressure to the rate of gastric emptying of a glucose drink in this cohort of older individuals has been reported (Pham et al., 2020). At the time of the initial study, participants were 65 – 90 years old, without a history of diabetes. No participant was taking medication known to influence gastric emptying and smoking (which may slow GE (Johnson et al., 1991)) was prohibited on the morning of the studies. Individuals with a history of significant cardiac, respiratory, gastrointestinal, renal or hepatic disease, previous gastrointestinal surgery (apart from appendicectomy or cholecystectomy), or with an alcohol consumption (>20g per day), were excluded.

Following the initial study, participants were invited to attend a follow-up study after a mean interval of 5.8 ± 0.1 (SEM) years.

All participants gave informed consent for their participation. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital.

*Protocol*

The protocol at the initial and follow up studies was identical. Participants presented at 8.30am after an overnight fast (14 hours for solids and 12 hours for liquids) when an intravenous cannula was inserted into the antecubital vein to facilitate blood sampling. A drink containing 75 g of glucose and 150 mg of 13C-acetate (Cambridge Isotope Laboratories, Tewksbury, MA, USA), made up to 300 ml with water at room temperature was then consumed within 5 minutes - t = 0 was defined as the time of completion of the drink.

*Biochemical measurements*

Plasma glucose (hexokinase method), insulin (RRID: AB_2877672, ELISA, Diagnostics 10-1113, Mercodia, Uppsala, Sweden), GLP-1 (RRID: AB_2757816, GLPIT-36HK, Millipore, Billerica, MA, USA) and GIP (RRID: AB_518352, In-house assay, Peninsula Laboratories, CA, USA, cat. no T-4052 rabbit anti-GIP [human] antiserum) were measured at baseline and t=15, 30, 45, 60, 90 and 120 min.

*Insulin secretion, sensitivity and oral disposition index*

Insulin secretion was estimated using the "insulinogenic index" of $\Delta insulin_{0\text{-}30}/\Delta glucose_{0\text{-}30}$, insulin sensitivity using 1/fasting insulin and the oral disposition index (oDI) calculated using the product of insulin secretion and insulin sensitivity i.e. 1/fasting insulin x $\Delta insulin_{0\text{-}30}/\Delta glucose_{0\text{-}30}$ (Utzschneider et al., 2009).

*Gastric emptying*

Exhaled breath samples were collected before ingestion of the drink (t = -3 min) and then every 5 minutes for the first hour (commencing at t = 5 min) followed by every 15 min for the next 3 hours. The $^{13}CO_2$ concentration in the breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK), and the gastric 50% emptying time (T50) calculated (Trahair et al., 2022). Wagner-Nelson analysis was utilized to generate a gastric emptying curve from the percentage of $^{13}CO_2$ measured in breath samples and the gastric emptying rate (kcal/min) calculated (Trahair et al., 2022).

*Statistical analysis*

Glucose tolerance was classified according to The Expert Committee on Diagnosis and Classification of Diabetes Mellitus definitions (American Diabetes Association 2010): Impaired fasting glucose was defined as fasting plasma glucose 5.6 – 6.9 mmol/L, impaired

glucose tolerance as a 2 hour value post OGTT of 7.8 – 11.0 mmol/L and type 2 diabetes (T2D) as fasting glucose ≥ 7.0 mmol/L or 2 hour plasma glucose of ≥11.1 mmol/L post OGTT. Total areas under the curve (AUCs) between t = 0 and 120 min were calculated using the trapezoidal rule. Participants were subdivided into groups according to their plasma glucose response – either an incessantly rising glucose, a monophasic response (a gradual increase in plasma glucose to a peak followed by a subsequent decline), a biphasic response (a gradual increase in plasma glucose to a peak, followed by a fall of ≥0.25 mmol/L, and then a second rise of ≥0.25 mmol/L within 2 hours) (Arslanian et al., 2019) or a more complex pattern (Tura et al., 2011). If a better fit of normal distribution was obtained by log transformation (log 10), this was performed before the statistical analysis. Normality was confirmed with a Shapiro-Wilk test. Differences were analysed using an unpaired Student's t test and shown as means ± SEM. A p-value of less than 0.05 was considered a significant difference.

### *5.4 Results*

Participant characteristics are summarized in the table below.

*Baseline measurements*

| | **Monophasic (n=22)** | **Biphasic (n=14)** | **P-value** |
|---|---|---|---|
| **Age (years)** | 77.3 ± 0.7 | 69.2 ± 0.9 | <0.001* |
| **Height (m)** | 1.68 ± 0.02 | 1.67 ± 0.03 | 0.94 |
| **Weight (kg)** | 72.0 ± 2.9 | 73.4 ± 3.4 | 0.77 |
| **Body mass index (kg/m$^2$)** | 25.6 ± 0.7 | 26.0 ± 0.6 | 0.62 |

*statistically significant difference

**Table 5.1** Participant characteristics at baseline analysed using an unpaired Student's t-test. Data are mean ± SEM.

*Plasma glucose*

Forty-one participants (17 women, 24 men) were recruited: 19 (46%) had normal fasting glucose and normal glucose tolerance, 2 (5%) had impaired fasting glucose, 12 (29%) had

impaired glucose tolerance, 3 (7%) had both impaired fasting glucose and impaired glucose tolerance, 5 (12%) had undiagnosed type 2 diabetes- in these 5 participants, their general practitioners were notified of the results and they were excluded from the analysis. Of the remaining 36 individuals (who all attended the following visit), 22 participants (61%) had a "monophasic" and 14 (39%) a "biphasic" glucose response. None had either an incessantly rising pattern, triphasic pattern or a more complex pattern. The monophasic group was modestly older (P<0.001). While fasting glucose was not different in both groups (P=0.67), the 1-hour post OGTT plasma glucose was greater in the monophasic vs biphasic group 9.5 ± 0.5 mmol/L vs 8.0 ± 0.5 mmol/L (P=0.04).

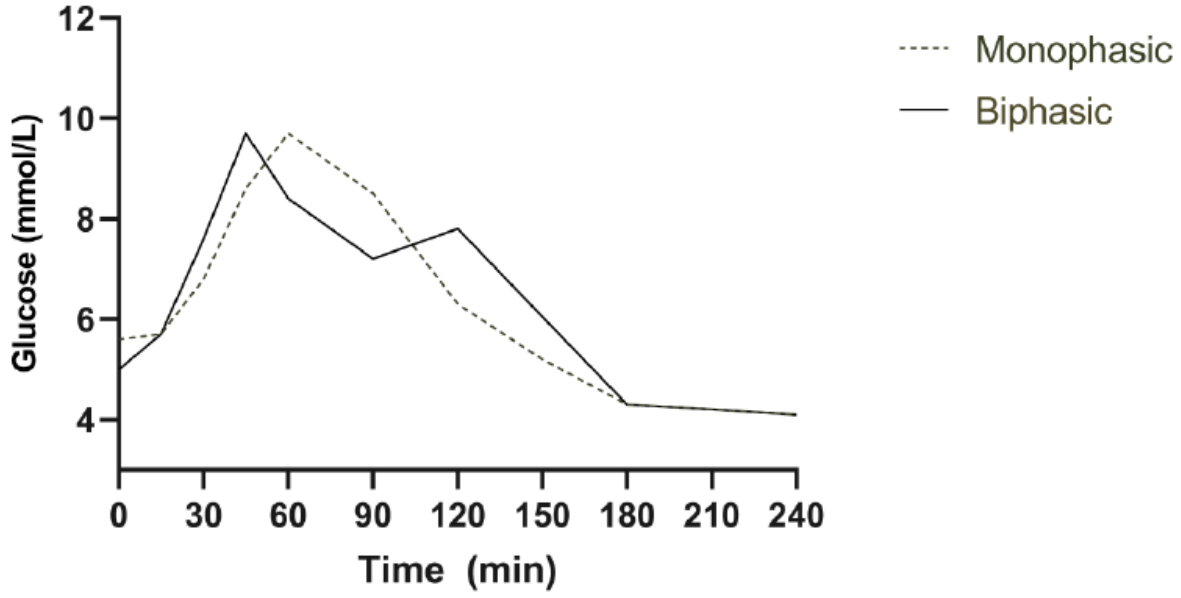


**Figure 5.1** Monophasic (dotted line) and biphasic (solid line) glucose response curves following a 75 g oral glucose tolerance test.

*Insulin secretion and sensitivity*

In the monophasic, there was an approximate two-fold reduction in insulin secretion compared to the biphasic (10.4 ± 1.1 vs 20.9 ± 4.3, P=0.03) group. There were no differences in either insulin sensitivity or the oral disposition index between the two groups.

| | | Monophasic (n = 22) | Biphasic (n = 14) | *p* Value |
|---|---|---|---|---|
| Glucose (mmol/L) | Fasting | 5.8 ± 0.9 | 5.7 ± 1.0 | 0.67 |
| | 1 h | 9.5 ± 0.5 | 8.0 ± 0.5 | 0.04 * |
| | 2 h | 7.5 ± 0.4 | 7.3 ± 0.4 | 0.66 |
| | Peak | 10.6 ± 0.4 | 9.6 ± 0.3 | 0.01 * |
| | $AUC_{0-60}$ | 522 ± 15 | 472 ± 16 | 0.04 * |
| | $AUC_{0-120}$ | 1032 ± 43 | 920 ± 37 | 0.08 |
| Insulin (pmol/L) | Fasting | 3.8 ± 0.4 | 4.9 ± 0.6 | 0.13 |
| | 1 h | 54.5 ± 5.4 | 67.2 ± 13.7 | 0.40 |
| | 2 h | 44.9 ± 5.5 | 54.1 ± 9.1 | 0.36 |
| | Peak | 63.2 ± 7.0 | 75.7 ± 13.3 | 0.37 |
| | $AUC_{0-60}$ | 1942 ± 211 | 2712 ± 488 | 0.17 |
| | $AUC_{0-120}$ | 3568 ± 352 | 4522 ± 859 | 0.32 |
| | Insulin secretion | 10.4 ± 1.1 | 20.9 ± 4.3 | 0.03 * |
| | Insulin sensitivity | 0.32 ± 0.04 | 0.24 ± 0.02 | 0.14 |
| | Oral disposition index | 3.2 ± 0.4 | 4.5 ± 0.8 | 0.13 |
| GLP-1 (pmol/L) | Fasting | 20.8 ± 1.2 | 21.3 ± 1.5 | 0.82 |
| | 1 h | 30.7 ± 1.8 | 33.3 ± 2.4 | 0.37 |
| | 2 h | 24.3 ± 1.4 | 30.3 ± 2.3 | 0.02 * |
| | Peak | 36.2 ± 2.1 | 49.4 ± 4.8 | 0.007 * |
| | $AUC_{0-60}$ | 1846 ± 97 | 2290 ± 171 | 0.02 * |
| | $AUC_{0-120}$ | 3421 ± 165 | 4190 ± 288 | 0.02 * |
| GIP (pmol/L) | Fasting | 20.0 ± 1.7 | 19.5 ± 1.8 | 0.83 |
| | 1 h | 52.1 ± 3.8 | 49.2 ± 3.7 | 0.60 |
| | 2 h | 52.7 ± 3.9 | 53.8 ± 3.5 | 0.84 |
| | Peak | 56.4 ± 4.1 | 55.7 ± 3.7 | 0.91 |
| | $AUC_{0-60}$ | 2668 ± 189 | 2436 ± 148 | 0.39 |
| | $AUC_{0-120}$ | 5863 ± 424 | 5546 ± 354 | 0.60 |

* statistically significant difference.

**Table 5.2** Participant glucose, insulin and incretin concentrations at the initial study analysed using an unpaired Student's t-test. Data are mean ± SEM.

*Plasma GLP-1 and GIP*

In the monophasic group both peak GLP-1 (P=0.007) and the GLP-1 $AUC_{0\text{-}120min}$ OGTT (P=0.02) were less. In contrast there were no differences in plasma GIP between the two groups.

*Gastric emptying*

Gastric emptying was comparable in the two groups whether expressed as a caloric rate (monophasic 1.15 ± 0.04 vs biphasic 1.10 ± 0.05 kcal/min, P=0.45) or as the gastric 50% emptying time (T50) (135 ± 6 min vs 141 ± 8 min, P=0.52).

*Follow-up*

At follow-up, 6 (17%) with an initial monophasic had a biphasic response and 8 (22%) with a biphasic had a monophasic response; the remaining 22 (61%) had the same response curve.

There was a modest reduction in fasting glucose (0.2 ± 0.1 mmol/L, P=0.036) at the follow-up visit, but no difference in 1h/2h plasma glucose, glucose peaks or AUCs. As with the initial visit, a monophasic glucose pattern was associated with a higher 1-hour plasma glucose (P=0.01) but not 2-hour (P=0.78) or fasting glucose (P=0.09). There was, again, no difference in GIP, while GLP-1 was less in the monophasic compared with the biphasic group. There were no differences in insulin secretion (P=0.20), sensitivity (P=0.44) and the oral disposition index (P=0.15).

| | | Monophasic (n = 24) | Biphasic (n = 12) | *p* Value |
|---|---|---|---|---|
| Glucose (mmol/L) | Fasting conc. | 5.6 ± 0.1 | 5.4 ± 0.2 | 0.09 |
| | 1 h | 10.0 ± 0.4 | 7.9 ± 0.3 | 0.01 * |
| | 2 h | 7.4 ± 0.4 | 7.6 ± 0.4 | 0.78 |
| | Peak | 10.6 ± 0.3 | 9.2 ± 0.4 | 0.02 * |
| | $AUC_{0-60}$ | 498 ± 13 | 457 ± 14 | 0.06 |
| | $AUC_{0-120}$ | 1027 ± 32 | 936 ± 39 | 0.10 |
| Insulin (pmol/L) | Fasting | 5.2 ± 0.5 | 4.9 ± 0.8 | 0.76 |
| | 1 h | 76.4 ± 10.2 | 50.6 ± 11.2 | 0.11 |
| | 2 h | 72.0 ± 12.3 | 52.1 ± 8.5 | 0.19 |
| | Peak | 101.4 ± 14.9 | 80.3 ± 13.3 | 0.42 |
| | $AUC_{0-60}$ | 2500 ± 300 | 2676 ± 587 | 0.79 |
| | $AUC_{0-120}$ | 7472 ± 1044 | 5920 ± 923 | 0.27 |
| | Insulin secretion | 12.4 ± 1.6 | 17.4 ± 3.4 | 0.20 |
| | Insulin sensitivity | 0.23 ± 0.02 | 0.28 ± 0.07 | 0.44 |
| | Oral disposition index | 2.7 ± 0.4 | 3.9 ± 0.7 | 0.15 |
| GLP-1 (pmol/L) | Fasting | 14.6 ± 0.9 | 16.8 ± 1.2 | 0.16 |
| | 1 h | 25.5 ± 1.4 | 31.7 ± 3.1 | 0.04 * |
| | 2 h | 20.2 ± 1.3 | 25.2 ± 2.3 | 0.06 |
| | Peak | 30.9 ± 1.8 | 44.4 ± 4.7 | 0.003 * |
| | $AUC_{0-60}$ | 1422 ± 73 | 2036 ± 188 | 0.001 * |
| | $AUC_{0-120}$ | 2759 ± 116 | 3837 ± 335 | 0.001 * |
| GIP (pmol/L) | Fasting | 16.4 ± 1.2 | 18.3 ± 1.5 | 0.36 |
| | 1 h | 49.1 ± 2.6 | 49.3 ± 4.3 | 0.97 |
| | 2 h | 49.0 ± 3.0 | 53.6 ± 3.7 | 0.36 |
| | Peak | 53.0 ± 3.0 | 56.7 ± 4.0 | 0.47 |
| | $AUC_{0-60}$ | 2384 ± 127 | 2534 ± 189 | 0.51 |
| | $AUC_{0-120}$ | 5365 ± 298 | 5662 ± 426 | 0.57 |

* statistically significant difference.

**Table 5.3** Participant glucose, insulin and incretin concentrations at the follow-up study analysed using an unpaired Student's t-test. Data are mean ± SEM.

Gastric emptying, again, was not significantly different in the monophasic vs biphasic group. The gastric 50% emptying time was 104 ± 10 min vs 131 ± 17 min (P=0.16) and the caloric rate 1.78 ± 0.16 vs 1.36 ± 0.16 kcal/min (P=0.11).

### *5.5 Discussion*

In this cross-sectional and longitudinal study, a biphasic glucose response to an oral glucose tolerance test was associated with a reduction in the 60 min glucose, increases in insulin secretion and plasma GLP-1, but not differences in insulin sensitivity or plasma GIP or gastric emptying at both baseline and follow-up when compared with a monophasic response. These observations suggest that an increased GLP-1 response may be central to the reduced risk of dysglycaemia known to be associated with biphasic, compared to monophasic glucose responses (Arslanian et al., 2019, Manco et al., 2017). The absence of an incessantly rising pattern in our cohort is not surprising given that this pattern is associated with the most severe impairments in glucose metabolism where individuals would have been likely to be excluded from our study due to a diagnosis of T2D. The monophasic group were older than the biphasic group but this could be explained by older age being associated with reduced insulin secretion (Chang and Halter, 2003) and therefore, monophasic responses.

The demonstrated association of a biphasic glucose curve with a greater GLP-1 response is novel and also provides an explanation for the observed increase in insulin secretion and reduction in 1 hour glucose. We would speculate that the greater GLP-1 response in individuals with biphasic curves reflects an intrinsic increase in GLP-1 secretion, particularly as there was no difference in GE (Xie et al., 2021b). In a prior study (Kim et al., 2016) in youths with obesity, neither GIP or GLP-1 responses differed significantly in biphasic or monophasic cohorts, however, the GLP-1 response to oral glucose is known to be reduced in individuals with obesity which may account for this difference in observations (Faerch et al., 2015). We suggest that the elevated GLP-1 response accounts for the observed increases in insulin secretion and reduction in blood glucose during the OGTT in healthy, older individuals with biphasic curves.

That the pattern of the glucose response curve changed in 39% of individuals after 5 years of follow up is consistent with the only longitudinal study (Manco et al., 2017) in which follow-up was for 3 years. While the mechanisms underlying the change in this glucose response curve

are unknown, our study suggests that it is most unlikely to be related to either gastric emptying or incretin hormone secretion. Factors that warrant evaluation include changes in the gut microbiota that have been associated with altered glucose homeostasis (Sharma and Tripathi, 2019). In addition, it should be appreciated that the oral glucose tolerance test is associated with some intra-individual variability with the 1-hour blood glucose typically varying by 1.9 mmol/L which may have led to an altered glucose response curve (McDonald et al., 1965).

The observation that the biphasic curve is associated with a reduction in 1-hour glucose is of potential importance given that an elevated 1-hour glucose post-OGTT (≥8.6 mmol/L) is now recognized as a strong predictor of future type 2 diabetes, outperforming both the 2-hour glucose and HbA1c (Peddinti et al., 2019). In the monophasic group, mean 1-hour glucose was ≥8.6 mmol/L at both baseline and follow up whereas it was <8.6 mmol/L in the biphasic group. It would, accordingly, be of interest to know how the shape of the glucose curve compares with an elevated 1-hour glucose as a predictor of future type 2 diabetes and whether in individuals with an elevated 1-hour glucose, GLP-1 secretion is reduced. Furthermore, it would be of clinical relevance to evaluate, in dedicated studies, if use of GLP-1 receptor agonists could improve dysglycaemia in individuals with a monophasic response.

Our study is the first to evaluate the shape of the glucose curve longitudinally, concurrently with measurements of incretin hormones and GE. Furthermore, Wagner-Nelson analysis was used to evaluate GE as this method enables isotope breath tests more comparable to the "gold-standard" of scintigraphy (Trahair et al., 2022).

*Limitations*

We had a modest sample size and used surrogate markers of insulin secretion and sensitivity rather than the hyperinsulinaemic, euglycaemic clamp study (DeFronzo et al., 1979). We also did not measure the OGTT repeatedly or measure glucagon, glucose absorption or the changes in gut microbiota.

### *5.6 Conclusion*

In summary, a biphasic plasma glucose curve following a 75 g oral glucose tolerance test is associated with a reduction in 60 min glucose and increases in GLP-1, but no difference in GIP or gastric emptying when compared to a monophasic response in older individuals without diabetes.

**Chapter 6. Comparative effects of low-carbohydrate, full-strength and low-alcohol beer on gastric emptying, alcohol absorption, glycaemia and insulinaemia in health.**

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Comparative effects of low-carbohydrate, full-strength and low-alcohol beer on gastric emptying, alcohol absorption, glycaemia and insulinaemia in health. |
| Publication Status | Published |
| Publication Details | STEVENS, J. E., JALLEH, R. J., TRAHAIR, L. G., MARATHE, C. S., HOROWITZ, M. & JONES, K. L. 2022. Comparative effects of low-carbohydrate, full-strength and low-alcohol beer on gastric emptying, alcohol absorption, glycaemia and insulinaemia in health. *Br J Clin Pharmacol*, 88(7):3421-7. |

**Principal Author**

| | |
|---|---|
| Name of Principal Author (Candidate) | Ryan Joseph Jalleh |
| Contribution to the Paper | I was responsible for the literature review, data analysis and writing the manuscript |
| Overall percentage (%) | 40% |
| 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 second author of this paper and a major contributor to the publication of this study. | |
| Signature: | | Date: 24/11/23 |

**Co-Author Contributions**

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

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

| | | |
|---|---|---|
| Name of Co-Author | Julie E Stevens | |
| Contribution to the Paper | Dr Stevens contributed to the study design, data collection, data analysis and writing of the manuscript | |
| Signature: | | Date: 27/11/23 |

| | | |
|---|---|---|
| Name of Co-Author | Laurence G Trahair | |
| Contribution to the Paper | Dr Trahair has expertise in gastric emptying studies and contributed with data collection and analysis. | |
| Signature: | | Date: 25/11/23 |

| | | |
|---|---|---|
| Name of Co-Author | Chinmay S Marathe | |
| Contribution to the Paper | Dr Marathe has expertise in endocrinology and provided feedback on the manuscript | |
| Signature: | | Date: 26/11/23 |

| | | |
|---|---|---|
| Name of Co-Author | Michael Horowitz | |
| Contribution to the Paper | Prof Horowitz is my primary supervisor and provided supervision and mentorship in the preparation of this manuscript | |
| Signature: | | Date: 24/11/23 |

| | | |
|---|---|---|
| Name of Co-Author | Karen L Jones | |
| Contribution to the Paper | Prof Jones is co-supervisor and provided supervision with study design, data collection, data analysis and in the preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

*6.1 Abstract*

**Aim:** To evaluate the comparative effects of low carbohydrate (LC), full-strength (FS), and low-alcohol (LA) beer on gastric emptying (GE), ethanol absorption, glycemia and insulinemia in health.

**Methods:** Eight subjects (4M, 4F; age: 20.4±0.4 yr; BMI 22.7±0.4 kg/m2) had concurrent measurements of GE, plasma ethanol, blood glucose and plasma insulin for 180min on 3 separate occasions after ingesting 600mL of (i) FS beer (5.0% w/v, 246 kcal, 19.2 g carbohydrate), (ii) LC beer (4.6% w/v, 180 kcal, 5.4 g carbohydrate) and (iii) LA beer (2.6% w/v, 162 kcal, 17.4 g carbohydrate) labeled with 20 MBq 99mTc-calcium phytate, in random order.

**Results:** There was no difference in the gastric 50% emptying time (T50) (FS: 89.0 ± 13.5 min vs LC: 79.5 ± 12.9 min vs LA: 74.6 ± 12.4 min; P=0.39). Plasma ethanol was less after LA than LC (P<0.001) and FS (P<0.001), with no difference between LC and FS (P=1.0). There was an inverse relationship between plasma ethanol at 15min and GE after LA (r=-0.87, P<0.01) and a trend for inverse relationships after LC (r=-0.67, P=0.07) and FS (r=-0.69, P=0.06). The AUC 0-180min for blood glucose was greater for LA than LC (P<0.001), with no difference between LA and FS (P=0.40) or LC and FS (P=1.0).

**Conclusion:** In healthy young subjects, GE of FS, LC, and LA, beer is comparable and a determinant of the plasma ethanol response.

### *6.2 Introduction*

Low-carbohydrate ("low-carb") beer has become increasingly popular since its introduction. Consumption is popular, particularly amongst the tertiary-educated, as well as men and women approaching middle age. In the latter group, usage may reflect, in part, the presence of disorders including type 2 diabetes and cardiovascular disease, or as an attempt to "bust the beer gut" (Miller et al., 2010). There is a frequent perception that low-carb beer represents a 'healthy' alternative to full-strength beer because its caloric content is lower.

While low-carb beers contain ~0.9 g carbohydrate per 100 mL (compared with ~3 g carbohydrate per 100 mL in full-strength beer), there is little difference in the total caloric load, because, at least in most cases, there is no difference in alcohol content, which represents a major contributor to the total caloric load, compared with full-strength beers. Low-carb beers pose a potential health risk in those who ingest them in the belief that they contain less alcohol (and, hence, ingest more) or that they confer health benefits such as weight loss and/or lowered glycemia, as in patients with type 2 diabetes. In contrast, "light" (low-alcohol) beer represents a more appropriate alternative with respect to minimizing both energy intake and the amount of alcohol ingested.

As with the majority of drugs, alcohol is absorbed predominantly from the small intestine rather than the stomach, in part reflecting the greater surface area for absorption provided by small intestine (Nimmo, 1976, Prescott et al., 1979, Holt, 1981). Metabolism by gastric alcohol dehydrogenase is small (Oneta et al., 1998). We (Chaikomin et al., 2006, Horowitz et al., 1989b, Wu et al., 2006) and others (Kechagias et al., 1999) have demonstrated that the rate of alcohol absorption is highly dependent on the rate of gastric emptying. The latter is known to exhibit a substantial inter-, but much lower intra-individual variation in health. Gastric emptying of nutrients, including alcohol, is tightly regulated at an overall rate of ~1-4 kcal/min, primarily as a result of feedback inhibition generated by the interaction of nutrients with the small intestine (Brener et al., 1983, Lin et al., 1989). The rate of gastric emptying is also a major determinant

of the postprandial elevation in blood glucose in both health (Horowitz et al., 1993) and type 2 diabetes (Jones et al., 1996), accounting for ~30% of the variance in peak plasma glucose after oral carbohydrate (Horowitz et al., 1993). Accordingly, when gastric emptying is relatively faster, the initial rise in blood glucose is greater (Horowitz et al., 1993). Not surprisingly, pharmacological (Chaikomin et al., 2006, Nimmo, 1976) and dietary(Horowitz et al., 1989b, Russo et al., 2003) interventions that modify (delay or accelerate) gastric emptying influence both alcohol absorption (Holt, 1981) and postprandial glycemia (Marathe et al., 2013). Dietary and pharmacological strategies that slow gastric emptying are now used widely in both the prevention and management of type 2 diabetes (Ma et al., 2009). Substitution of artificial sweeteners for regular mixers (containing sucrose) accelerates gastric emptying and increases peak blood alcohol concentrations in healthy males (Wu et al., 2006). Surprisingly, the comparative effects of low-carb, full-strength and low-alcohol beer on gastric emptying, alcohol absorption and glycemia have not been evaluated, which was the purpose of the current study.

### *6.3 Materials and methods*

#### *Subjects*

Eight healthy subjects (4 male, 4 female), mean age 20.4 ± 0.4 years (range 19 – 22 years), mean body mass index (BMI) 22.7 ± 0.4 kg/m$^2$ (range 20.9 – 24.1 kg/m$^2$), were recruited by advertisement. None had a history of significant disease including, diabetes, alcohol intake >20 g daily, drug use or smoking >10 cigarettes/day. No subject was pregnant, breastfeeding, or was taking any medication known to influence gastrointestinal function. The number of participants recruited was based on power calculations derived from our previous work (Chaikomin et al., 2006, Wu et al., 2006).

#### *Protocol*

Each subject was studied on three occasions in a randomized, double-blind fashion, with each study day separated by a minimum of one week. On each study day, the subject attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry at the Royal Adelaide Hospital at 0830h after fasting from solids for 14 hours, liquids for 12 hours, and abstaining from alcohol for at least 72 hours and tobacco for 12 hours. On arrival, the subject was seated in front of a gamma camera and an IV cannula inserted into an antecubital vein for blood sampling. They then consumed 600 mL of either (i) full-strength beer (5.0 % w/v alcohol, 246 kcal, 19.2 g carbohydrate;), (ii) low-carbohydrate beer (4.6 % w/v alcohol, 180 kcal, 5.4 g carbohydrate), or (iii) low-alcohol beer (2.6 % w/v alcohol, 162 kcal, 17.4 g carbohydrate) (Hahn Brewing Company, Sydney, NSW, Australia) each radiolabeled with 20 MBq $^{99m}$Tc-calcium phytate (Radpharm Scientific, Belconnen, ACT, Australia), within 10 min. $^{99m}$Tc-calcium phytate is a colloid that has been used widely as a marker of liquid gastric emptying (Jalleh et al., 2020, Rayner et al., 2020, Chapple et al., 2021). Another colloid ($^{99m}$Tc-sulfur colloid) was tested to determine the stability of the radiolabel in the presence of acetic acid (to mimic the gastric environment) with and without alcohol. *In vitro* experiments utilising instant thin layer chromatography (ITLC) demonstrated at least 98% labeling over a 3 hour period, indicative of little, or no, degradation over time (*data not shown*). The beers were in their carbonated form when ingested and the time of drink completion was defined as t= 0 min. Blood samples and radioisotopic gastric emptying data were collected for 180 min following drink completion. At t= 180 min, the cannula was removed, and the subject was offered a meal prior to leaving the laboratory.

The protocol was approved by the Human Research Ethics Committees of the Royal Adelaide Hospital, the University of South Australia and the University of Adelaide, and each subject provided written, informed consent prior to their inclusion. All experiments were carried out in accordance with the Declaration of Helsinki.

***Measurements***

*Gastric emptying*

Radioisotopic data were acquired for 180 min following consumption of the drink (60 sec frames between t= 0–60 min, then 180 sec frames from t= 60–180 min). Data were corrected for subject movement, radionuclide decay and γ-ray attenuation (Gentilcore et al., 2005). A region-of-interest was drawn around the total stomach and gastric emptying curves (expressed as percentage retention over time) derived. The amount of the drink remaining in the stomach at 15 min intervals between t= 0 – 180 min, as well as the 50% gastric emptying time (T50) (Gentilcore et al., 2005), were calculated.

*Plasma ethanol, blood glucose and serum insulin*

Venous blood samples were obtained immediately prior to the ingestion of the drink (at t= -10 min) and then at t= 0, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min. Samples were separated by centrifugation at 3200 rpm for 15 min at 4˚C within 10 min of collection and stored at -70° for subsequent analysis.

Plasma ethanol was measured prior to the ingestion of the drink (at t= -10 min) and then at t= 15, 30, 60, 120 and 180 min by spectrophotometric enzymatic assay (ADVIA 2400; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). The intra-assay coefficient of variation (CV) of this technique is 0.9% with limits of determination ranging from 0.01 to 0.6 g/dL.

Blood glucose concentrations (mmol/L) were determined at all time points using the glucose oxidase method with a 2300 Stat Plus glucose analyser. 25 μL of whole blood was diluted in 600 μL of buffer solution and placed in direct contact with a glucose oxidase impregnated membrane for sampling (Nowotny et al., 2012). The intra- and inter-assay CV of this technique are 2% and 6.6%, respectively (Astles et al., 1996).

Serum insulin (mU/L) was measured prior to the ingestion of the drink (at t= -10 min) and then at t= 15, 30, 45, 60, 90, 120, 150 and 180 min by ELISA immunoassay (Diagnostics 10-1113,

Mercodia, Uppsala, Sweden). The sensitivity of the assay was 1.0 mU/L and intra- and inter-assay CVs were 2.1% and 5.3%, respectively (Trahair et al., 2012).

***Statistical analysis***

All variables were analysed as absolute values. Areas under the curve between t= 0 – 180 min were calculated for all variables using the trapezoidal rule. Changes in blood glucose, serum insulin and plasma ethanol over time (t= 0 – 180 min) were assessed with one-way repeated measures ANOVA. For all variables, differences between the conditions were assessed with two-way repeated measures ANOVA with treatment and time as factors, and post hoc comparisons were adjusted using Bonferroni correction. Differences in AUCs for all variables, as well as baseline blood glucose and serum insulin, were assessed with two-way repeated measures ANOVA. Relationships between the variables were assessed with Pearson's correlation. A P value <0.05 was considered significant in all analyses. Data are presented as mean ± SEM.

***6.4 Results***

The studies were all well tolerated.

***Gastric emptying***

GE of beer approximated a linear pattern for the first ~120 min. There was no treatment x time effect for total gastric emptying (P= 0.46), nor any difference in the AUCs for the three conditions (P= 0.29). There was also no difference in T50 between the three conditions (low-alcohol (LA) vs low-carbo (LC) vs full-strength (FS): 74.6 ± 12.4 min vs 79.5 ± 12.9 min vs 89.0 ± 13.5 min; P= 0.39), although the mean T50 was higher for FS.

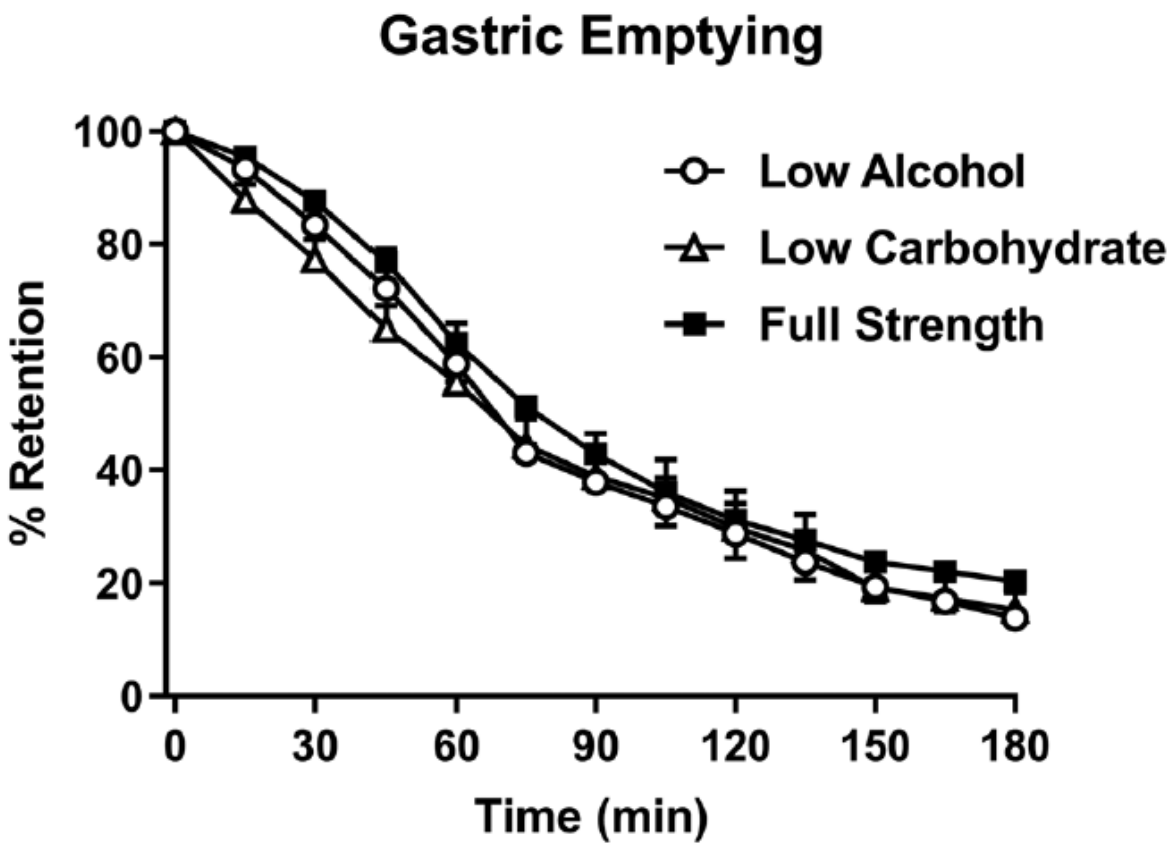


**Figure 6.1** Gastric emptying (GE) of low-alcohol (LA, open circles), low-carbohydrate (LC, open triangles) and full-strength (FS, closed squares) beers (mean ± SEM; n = 8).

***Plasma ethanol***

There was an increase in plasma ethanol during all three conditions (P< 0.001 for all). There were treatment (P< 0.001), time (P< 0.001) and treatment x time (P< 0.001) effects *(post-hoc comparisons are shown in the figure).* There was a difference (P< 0.001) between the AUC for all three conditions, so that the AUC for LA was lower than LC (P< 0.001) and FS (P< 0.001), with no difference between LC and FS (P= 1.0).

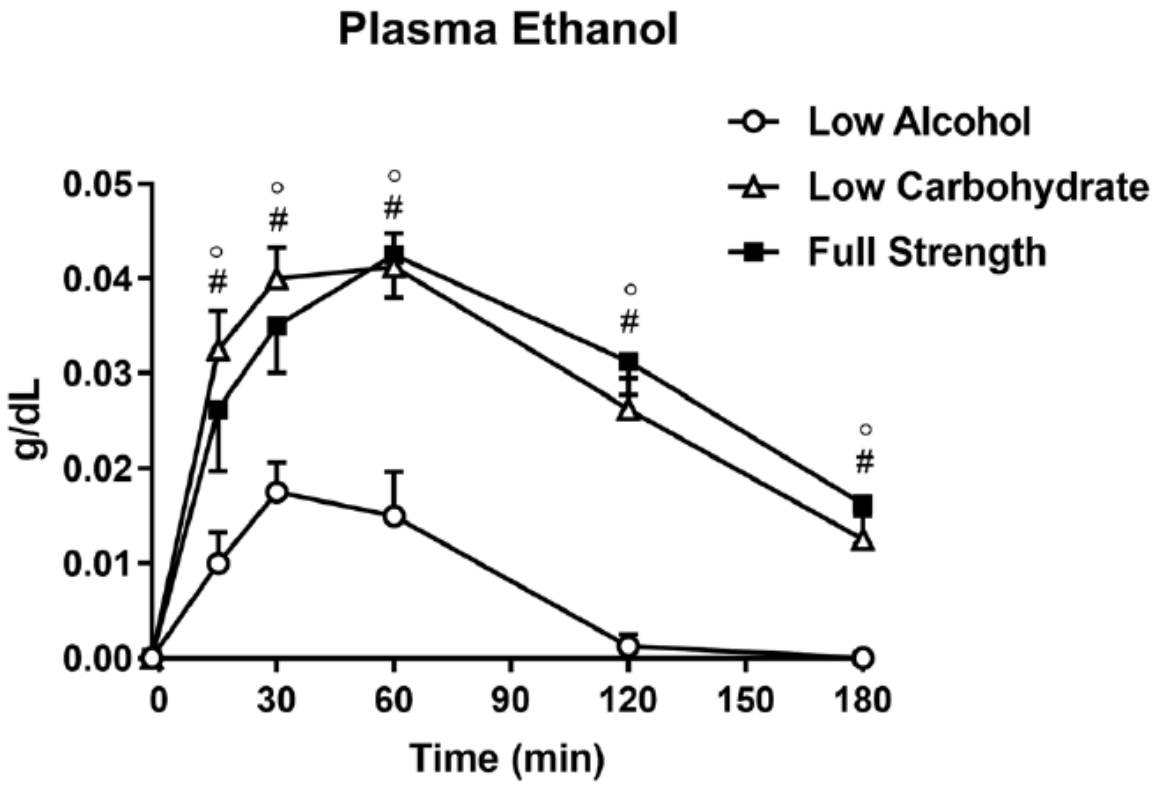


**Figure 6.2** Plasma ethanol following low-alcohol (LA, open circles), low-carbohydrate (LC, open triangles) and full-strength (FS, closed squares) beers. ○ $P < 0.05$ LA vs LC, # $P < 0.05$ LA vs FS (mean ± SEM; n = 8)

***Relationships between plasma ethanol and gastric emptying***

Partial correlation coefficients (adjusted for sex, age and BMI) between plasma ethanol level and gastric emptying T50 were significant at 15 (r=-0.56, P= 0.01), 30 (r = -0.58, P< 0.01) and 60 min (r= -0.48, P= 0.03), but not 120 min (r=-0.46, P=NS). There was a strong, inverse relationship between the plasma ethanol response at t= 15 min during the LA condition and the T50 (r= -0.87, P< 0.01) and trends during the LC (r= -0.67, P= 0.07) and FS (r= -0.69, P= 0.06) conditions with an inverse relationship at t= 30 min (r= -0.74, P< 0.05) for FS but not for LA and LC.

***Blood Glucose***

There was no difference in baseline blood glucose (P= 0.82) and an increase in blood glucose during all three conditions (P< 0.01 for all) **(Figure 6.3A)**. There were treatment (P< 0.001),

time (P< 0.001) and treatment x time (P< 0.001) effects *(post-hoc comparisons are shown on the figure)*. There was a difference (P< 0.001) between the AUCs for all three conditions, so that the AUC for low alcohol (LA) was greater than low carbohydrate (LC) (P< 0.01), and full strength (FS) (P< 0.05). While mean levels were lower for LC than FS, this difference was not significant (P= 0.11).

***Insulin***

There was no difference in baseline plasma insulin (P= 0.11) and an increase in insulin during all three conditions (P< 0.001 for all) **(Figure 6.3B)**. There were treatment (P< 0.01), time (P< 0.001) and treatment x time (P< 0.001) effects *(post-hoc comparisons are shown in the figure)*. There was a difference (P< 0.05) between the AUCs (0 – 180 min) for all three conditions, so that for LA, the AUC was greater than LC (P< 0.05), with no differences between LA and FS (P= 0.35) or LC and FS (P= 0.40).

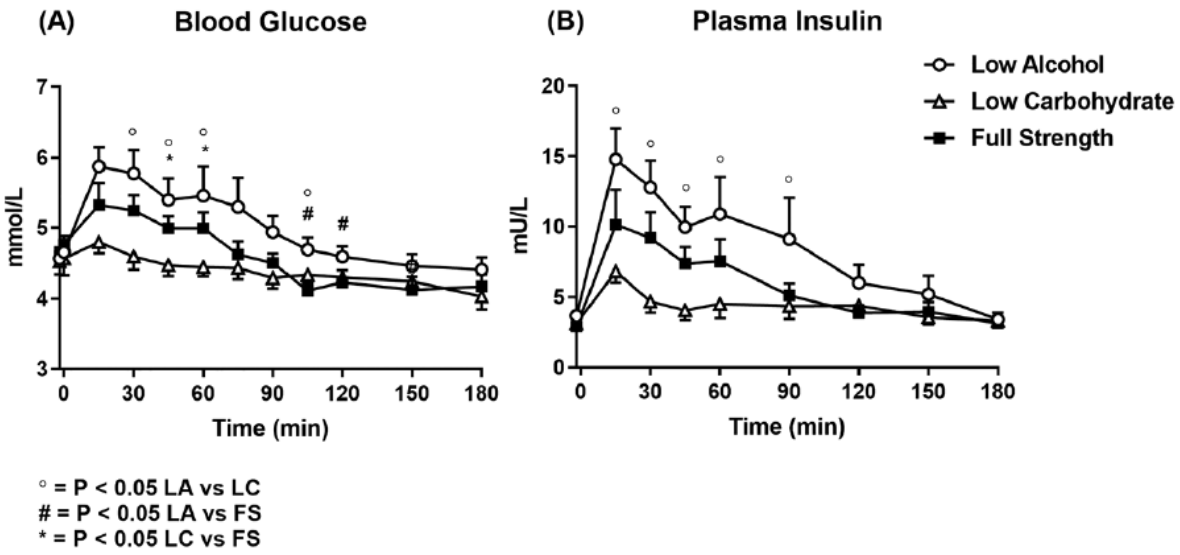


**Figure 6.3** Blood glucose (A) and plasma insulin (B) responses following low-alcohol (LA, open circles), low-carbohydrate (LC, open triangles) and full-strength (FS, closed squares) beers. ◦ P < .05 LA vs LC, # P < .05 LA vs FS, * LC vs FS (mean ± SEM; n = 8).

### *6.5 Discussion*

We evaluated the effects of different types of beer on gastric emptying, plasma ethanol, glycemia and plasma insulin response. Scintigraphy, which is recognised as the 'gold standard' technique, was used to measure gastric emptying (Abell et al., 2008). There was no difference in gastric emptying of low-alcohol, low-carb or full-strength although the mean T50 with full-strength, which had the most calories, was numerically greater. Predictably, the plasma alcohol response was least with low-alcohol and there was no difference between full-strength and low-carb. An inverse relationship between the plasma ethanol response and the rate of gastric emptying was evident for low-alcohol, with similar trends for low-carb and full-strength. The rise in blood glucose was modest, but was greater, with low-alcohol than full-strength and low-carb.

The effect of alcohol and alcohol-containing beverages on gastric emptying are contentious. Some studies report a relative delay in gastric emptying caused by beer or alcohol (Franke et al., 2004, Jian et al., 1986, Mushambi et al., 1993), others observed accelerated gastric emptying of beer compared with an equivalent ethanol solution (Pfeiffer et al., 1992, Schwartz et al., 1996) and one showed no change in gastric emptying (Charles et al., 1994). We did not observe any difference in gastric emptying between full-strength , low-carb and low-alcohol beer although the mean gastric emptying rate of full-strength was (non-significantly) slowest which is likely to reflect its higher caloric density (Hunt and Stubbs, 1975). The beers were carbonated when ingested so as to closely mimic the 'real world' setting as possible. Carbonation of liquids has been reported to have little, or no, effect on gastric emptying (Lau and Henry, 2017, Zachwieja et al., 1991). Consistent with prior observations (Schwartz et al., 1996, Horowitz et al., 1989b, Hebbard et al., 1995), there was a relationship between the plasma ethanol response and the rate of gastric emptying. The substantial inter-individual variation (Horowitz and Dent, 1991) of gastric emptying is not widely appreciated, nor the fact that the rise in blood alcohol occurs earlier when gastric emptying is relatively more rapid, both of which have potential

medico-legal implications. Gastric emptying is markedly accelerated after some forms of gastric surgery including Roux-en-Y gastric bypass that is used widely in the management of obesity (Nguyen et al., 2014) which would effect the rate of alcohol absorption. Furthermore, addition of artificial sweeteners to "low-carb" alcoholic beverages may have a marked effect on gastric emptying and, hence, ethanol absorption (Wu et al., 2006). Consumption of low-alcohol predictably resulted in reduced plasma ethanol concentrations compared with full-strength and low-carb.

The relationship between ethanol and glycemia is complex given that acute ethanol ingestion may inhibit gluconeogenesis (Krebs, 1968), but also inhibits peripheral glucose uptake (Yki-Jarvinen and Nikkila, 1985). In our study, the consumption of low-alcohol led to a greater increase in plasma glucose and insulin compared with full-strength and low-carb. This finding is unexpected given that low-alcohol had the least calories and it has been reported that alcoholic beer results in a greater increase in plasma glucose and insulin compared to non-alcoholic beer (Hatonen et al., 2012). A possible explanation is that the effect of alcohol to inhibit gluconeogenesis is greater than the rise in glycemia from absorption of the drink. The observed rises in blood glucose after the drinks were predictably modest - whether this effect is evident in individuals with insulin resistance and/or type 2 diabetes, where the rise in blood glucose would be anticipated to be greater, warrants evaluation.

Limitations of our study include the small sample size (this is likely to account for the observation that emptying of full-strength beer was not significantly slower) and the evaluation of young participants only. Aging is associated with a modest slowing of gastric emptying (Pham et al., 2020). While only one volume of alcohol containing beverage was evaluated, there is no reason to expect a difference with high nutrient liquids. The alcohol-containing beverages were also ingested without a meal – we have demonstrated that in this situation, liquids are still emptied before solids, but their emptying is slowed (Horowitz et al., 1989b).

### *6.6 Conclusion*

We conclude that in healthy, young individuals, gastric emptying of low-carbohydrate, low-alcohol and full-strength beer is comparable and a determinant of the plasma ethanol response.

**Chapter 7. Effect of gastric distension with concurrent small intestinal saline or glucose infusion on incretin hormone secretion in healthy individuals: A randomized, controlled, crossover study.**

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Effect of gastric distension with concurrent small intestinal saline or glucose infusion on incretin hormone secretion in healthy individuals: A randomized, controlled, crossover study |
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| Publication Details | JALLEH, R. J., TRAHAIR, L. G., WU, T., STANDFIELD, S., FEINLE-BISSET, C., RAYNER, C. K., HOROWITZ, M. & JONES, K. L. 2023b. Effect of gastric distension with concurrent small intestinal saline or glucose infusion on incretin hormone secretion in healthy individuals: A randomized, controlled, crossover study. *Diabetes Obes Metab,* 25(7):1849-54. |

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| Name of Principal Author (Candidate) | Ryan Joseph Jalleh | |
| Contribution to the Paper | I was responsible for study design, data collection, data analysis and writing of the 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: 24/11/23 |

**Co-Author Contributions**

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

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

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| Name of Co-Author | Laurence G Trahair | |
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| Contribution to the Paper | Prof Horowitz is my primary supervisor and provided supervision and mentorship in the study design, conduct and preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

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|---|---|---|
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| Contribution to the Paper | Prof Jones is a co-supervisor of my PhD and provided feedback and mentorship in preparing the manuscript. | |
| Signature: | | Date: 24/11/23 |

### *7.1 Abstract*

Aims

There is increasing interest in the use of intragastric balloons as a weight loss procedure, however, the underlying mechanism(s) remain unclear. In rodents, gastric distension has recently been shown to stimulate the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1) substantially, but the effect of gastric distension on GLP-1 and the other incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), in humans is not known. We conducted a randomized, controlled, crossover study to evaluate the effect of gastric distension, induced using a gastric 'barostat' on incretin hormones in healthy individuals.

Materials and methods

Eight healthy participants (2 female, 6 male, mean age 69.3±1.2 years, and body mass index 23.5±0.8 kg/m$^2$) were each studied on four occasions when they received an intraduodenal infusion of either (i) 0.9% saline or (ii) glucose delivered at a rate of 3 kcal/min both with, and without, an intragastric balloon with the pressure set to 8 mmHg above the intragastric minimum distending pressure.

Results

Following intraduodenal saline or glucose infusion, there was no difference in plasma GLP-1 with or without gastric distension (P=1.00 for both saline and glucose infusions). There was also no difference in plasma GIP with or without gastric distension (P=1.00 for saline infusion and P=0.99 for glucose infusion).

Conclusion

We conclude that gastric distension, either alone or during small intestinal glucose exposure, does not stimulate incretin hormone secretion significantly in healthy humans.

### *7.2 Introduction*

The use of intragastric balloons as a therapy for obesity was initially explored in the mid-1980s, but following the negative outcome of a sham-controlled trial and evidence of substantial complications, was not evaluated further (Lindor et al., 1987). Decades later, interest in this therapy for obesity has re-emerged (Vantanasiri et al., 2020, Abu Dayyeh et al., 2021). Newer balloon designs are safer (Vantanasiri et al., 2020, Abu Dayyeh et al., 2021), and may be 'procedureless' (i.e. swallowable) (Vantanasiri et al., 2020) or adjustable (Abu Dayyeh et al., 2021), while the reversibility of this therapy is an advantage over metabolic surgery. Furthermore, the use of an intragastric balloon may also be an effective 'bridging therapy' for people with obesity where urgent weight reduction is indicated to reduce operative risk (Loo et al., 2022). However, the fundamental question of how gastric distension induces weight loss remains unknown. Increased secretion of incretin hormones, in particular glucagon-like peptide-1 (GLP-1), is thought to be a key mediator for weight loss and remission of type 2 diabetes following metabolic surgery, and represents a potential mechanism for the effectiveness of intragastric balloons (Al-Najim et al., 2018, Larraufie et al., 2019). The concept that non-nutrient gastric distension could stimulate GLP-1 has recently been demonstrated in rodent studies. Natochin et al (Natochin et al., 2018) reported that intragastric administration of water and sodium chloride stimulated plasma GLP-1 markedly within 15 min, as did gastric balloon distension in anesthetized rats. In unanesthetized mice, Ohbayashi et al (Ohbayashi et al., 2021) reported that gastric distension with a pectin-containing carbonated solution resulted in a load-dependent, sustained, substantial stimulation of portal concentrations of GLP-1. It has been suggested that effects on vagal function may underlie the stimulation of GLP-1. There is no information about the effect of gastric distension on the secretion of the other incretin hormone, glucose dependent insulinotropic polypeptide (GIP) in animals.

In contrast to the robust evidence of a major effect of gastric distension on GLP-1 secretion in rodents, it is not known whether gastric distension affects either GLP-1 and/or GIP secretion in

humans. That Steinert et al (Steinert et al., 2012) observed that intragastric infusion of glucose stimulated plasma GLP-1 more than an equivalent amount of intraduodenally infused glucose in healthy individuals, suggests that gastric signalling may potentiate nutrient-induced GLP-1 secretion.

As incretin hormones, GIP and GLP-1 have a major role in postprandial glucose homeostasis (Wu et al., 2016a). GIP is secreted predominantly by K-cells in the proximal small intestine and GLP-1 by L-cells in the distal small intestine and colon (Zhang et al., 2019). GIP and GLP-1 are released in response to all three macronutrients (Wu et al., 2010) following nutrient entry into the small intestine and subsequent digestion, and the stimulation of GLP-1 in turn inhibits subsequent gastric emptying (Deane et al., 2010b), suppresses appetite and reduces energy intake (Bergmann et al., 2019). In contrast to GLP-1, GIP neither slows gastric emptying (Meier et al., 2004) nor appears to reduces energy intake in humans (Bergmann et al., 2019), however, a co-agonist of both GLP-1 and GIP, tirzepatide, has recently been shown to promote greater weight loss than GLP-1 mono-agonists (Frias et al., 2018). Accordingly, stimulation of incretin hormones, perhaps particularly GLP-1, represents an important mechanism for inducing weight loss. Nutrient pre-loads of glucose, protein or fat stimulate the secretion of endogenous incretins, but at the cost of additional energy intake (Wu et al., 2012, Gentilcore et al., 2006, Ma et al., 2009). A non-nutritive strategy to enhance incretin secretion would be highly desirable.

The purpose of this study was to evaluate the effects of gastric distension, using a gastric barostat, on the secretion of GIP and GLP-1 in the presence and absence of small intestinal nutrients in healthy individuals. While gastric distension may potentially have a different effect on incretin hormones in individuals with obesity, we considered that an understanding of normal physiology represented the appropriate initial step.

### *7.3 Materials and Methods*

#### ***Participants***

Eight healthy older participants (2 female and 6 male, mean age 69.3 ± 1.2 years, body mass index (BMI) 23.5 ± 0.8 kg/m$^2$) were recruited. All participants were non-smokers and none had a history of cardiac, respiratory, gastrointestinal (or prior gastrointestinal surgery), hepatic or renal disease. None had a history of diabetes, epilepsy or intake of >20g alcohol/day. No participant took medication known to influence gastrointestinal function. This is a secondary analysis and data from this cohort relating to the effects of gastric distension and small intestinal nutrients on blood pressure and superior mesenteric artery blood flow have been reported (Vanis et al., 2011).

***Protocol***

Participants were studied on four occasions in a randomized order following an overnight fast (10h for solids, 8h for liquids), separated by at least 3 days. Upon arrival at the laboratory at the Royal Adelaide Hospital, a silicone-rubber, multilumen nasoduodenal catheter (Dentsleeve International, Mui Scientific, Mississauga, Canada) was inserted into the stomach via an anaesthetized nostril and allowed to pass into the duodenum by peristalsis. The catheter comprised an infusion channel (internal diameter ~1 mm) located ~10 cm distal to the pylorus with two other channels, located 2.5cm either side of the pylorus, perfused continuously with 0.9% saline, allowing continuous measurement of the transmucosal potential difference from the antral (-40 mV) and duodenal (0 mV) channels to ensure correct positioning of the catheter (Heddle et al., 1988, Trahair et al., 2012). An intravenous cannula was positioned in the antecubital fossa of one arm for blood sampling.

On two of the four study days, the participant also swallowed a single-lumen polyvinyl orogastric catheter (external diameter ~4 mm) (Tygon tubing, Saint Gobain Performance Plastics, Akron, OH, USA) equipped with a thin, flaccid polyethylene bag (capacity 1200 ml) that was tightly wrapped around the distal end. The proximal end of the catheter was connected to a gastric barostat (Distender Series II, G&J Electronics, Willowdale, ON, Canada). The barostat bag was inflated by 1mmHg every 5min to determine the intragastric minimum

distending pressure (MDP), which represents the minimum pressure required to overcome the intraabdominal pressure and is defined as the pressure to achieve a volume >30 ml in the bag (Feinle et al., 2000, Vanis et al., 2011). The stomach was then distended using a stepwise increase in intragastric pressure by 2mmHg increments every 3min, in four steps, to achieve a distension of 8 mmHg above MDP (Whitehead and Delvaux, 1997). Between t=0–60 min subjects received an intraduodenal infusion of either 1) 0.9% saline, 2) 25% glucose at 3 kcal/min, 3) 0.9% saline with intrabag pressure set to 8 mmHg above MDP or 4) glucose at 3kcal/min with intrabag pressure set to 8 mmHg above MDP in a computer-generated randomized order. At this rate of glucose infusion, there would be substantial stimulation of incretin hormones (Schirra et al., 1996). Participants and investigators were blinded to whether saline or glucose was infused. At t=60 min the barostat bag was deflated and between t=60–120 min 0.9% saline was infused intraduodenally (Trahair et al., 2012, Vanis et al., 2011). Intraduodenal infusions were performed at a rate of 3 ml/min.

***Data collection***

Venous blood samples (~18 ml) were obtained at 15-min intervals between t=0–120 min. Blood samples were centrifuged at 3200 rpm for 15 min and plasma or serum was separated and stored at -70°C for subsequent analysis.

***Ethics***

The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and performed in accordance with the Declaration of Helsinki. Each subject provided written, informed consent.

***Laboratory methods***

***Blood Glucose***

Blood glucose concentrations (mmol/L) were determined immediately using a portable blood glucose meter (Precision QID System, Abbott Laboratories, Medisense Products, Bedford, MA, USA).

***GLP-1***

Plasma total GLP-1 (pmol/L) was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). The minimum detectable limit was 3 pmol/L, and the intra- and inter-assay CV were 4.2% and 10.5%, respectively (Trahair et al., 2012).

***GIP***

Plasma total GIP (pmol/L) was measured by radioimmunoassay with modifications of a published method (Wishart et al., 1992). The minimum detectable limit was 2 pmol/L, and the intra- and inter-assay CV were 6.1% and 15.4%, respectively (Trahair et al., 2012).

***Statistical Analysis***

The area under the curve (AUC) was calculated for all variables using the trapezoidal rule. Basal values and AUCs were compared using one-factor repeated-measures ANOVA. Variables were also analysed using two-factor repeated measures ANOVA, with treatment and time as factors. Where significance was revealed by ANOVAs, post hoc comparisons adjusted by Bonferroni-Holm's correction, were performed. All analyses were performed using SPSS 17.0.0 (SPSS Inc, Chicago, IL, USA). Data are presented as mean values ± SEM. A P value $< 0.05$ was considered significant.

***7.4 Results***

The studies were well tolerated and completed by all participants. There were no differences in baseline concentrations of blood glucose (P=0.78), plasma GIP (P=0.59) or plasma GLP-1 (P=0.98) between the 4 study days.

***Blood Glucose***

There was a predictable increase in blood glucose during glucose and glucose + distension ($P<0.001$ for both), and no change during either saline or saline + distension ($P=0.55$ and $P=0.48$, respectively). There was a treatment effect ($P< 0.001$) for the AUC for blood glucose, so that blood glucose was greater during glucose compared with saline ($P<0.001$) and saline + distension ($P<0.001$), and greater during glucose + distension compared with saline ($P<0.005$) and saline + distension ($P<0.005$), with no difference between glucose and glucose + distension ($P=1.00$) or between saline and saline + distension ($P=1.00$). (Figure 7.1A)

***GIP***

There was a prompt and sustained increase in GIP during glucose and glucose + distension ($P<0.001$ for both), and no change during saline and saline + distension ($P=0.59$ and $P=0.18$, respectively). There was a treatment effect ($P<0.001$) for the AUC for GIP, so that GIP was greater during glucose compared with saline ($P<0.001$) and saline + distension ($P<0.001$), and greater during glucose + distension compared with saline ($P<0.005$) and saline + distension ($P<0.005$), with no difference between glucose and glucose + distension ($P=0.99$) or between saline and saline + distension ($P=1.00$). (Figure 7.1B)

***GLP-1***

There was a gradual and sustained increase in GLP-1 during glucose and glucose + distension ($P<0.001$ and $P<0.05$, respectively), and no change during saline and saline with distension ($P=0.67$ and $P=0.25$, respectively). There was a treatment effect ($P<0.001$) for the AUC for GLP-1, so that GLP-1 was greater during glucose compared with saline ($P<0.001$) and saline + distension ($P<0.005$), but not glucose + distension ($P=1.00$). There was no difference between saline and saline + distension ($P=1.00$) or between glucose + distension and saline + distension ($P=0.12$). (Figure 7.1C)

***Intrabag volumes***

At baseline, the intrabag volume was 443 ± 80 ml during glucose + distension and 410 ± 80 ml for saline + distension and at 60 min, intrabag volume was 790 ± 71 ml for glucose + distension and 637 ± 80 ml for saline + distension.

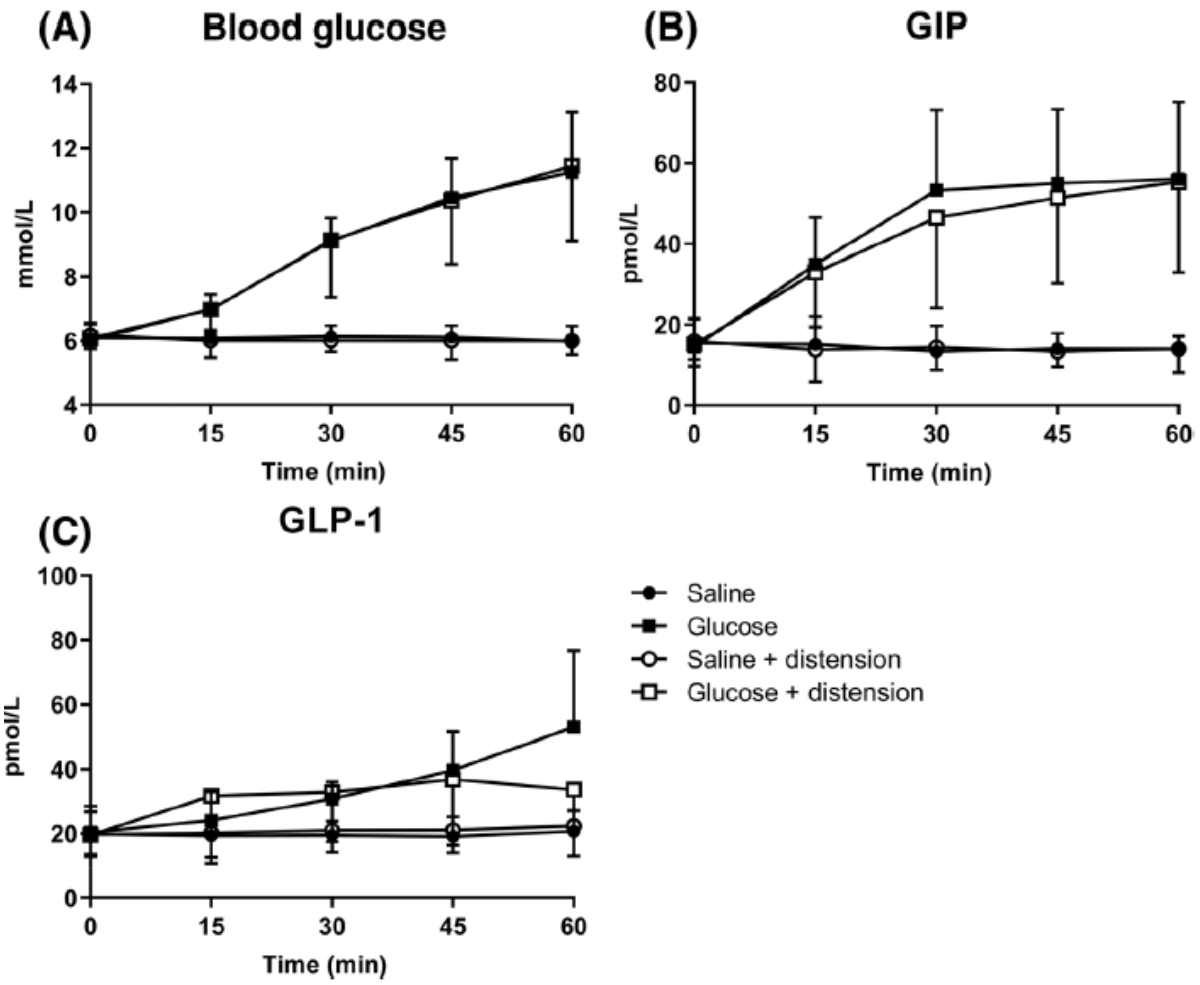


**Figure 7.1** Effects of intraduodenal saline (●, ○) and glucose at 3 kcal/min (■, □) with (○, □) and without (●, ■) gastric distension achieved by a barostat set at 8 mmHg above minimum distending pressure on A, Blood glucose, B, Plasma glucose dependent insulinotropic polypeptide (GIP), and C, Glucagon-like peptide-1 (GLP-1) in eight healthy participants. Data are mean ± standard error.

| | ID saline | ID glucose | ID saline and distension | ID glucose and distension |
|---|---|---|---|---|
| Glucose tAUC (mmol/L x min) | 366 ± 7 | 530 ± 17 | 362 ± 9 | 528 ± 29 |
| GIP tAUC (pmol/L x min) | 870 ± 110 | 2680 ± 330 | 850 ± 120 | 2490 ± 360 |
| GLP-1 tAUC (pmol/L x min) | 1170 ± 120 | 1970 ± 170 | 1250 ± 160 | 1920 ± 300 |

**Table 7.1** Total area under the curve (tAUC) from 0 to 60 minutes for glucose (mmol/L x min), glucose-dependent insulinotropic polypeptide (GIP) (pmol/L x min) and glucagon-like peptide-1 (GLP-1) (pmol/L x min) following intraduodenal (ID) saline, glucose, saline with balloon distension and glucose with balloon distension (n = 8). Data are mean values ± SEM.

### *7.5 Discussion*

Our study indicates that, unlike rodents (Natochin et al., 2018, Ohbayashi et al., 2021), gastric distension, with or without small intestinal glucose exposure, does not appear to be a stimulus for either GIP or GLP-1 secretion in humans. The study was conducted under carefully controlled experimental settings to determine whether there was an effect of gastric distension on GIP or GLP-1 secretion. Intraduodenal infusion of glucose or saline was used to avoid the confounding effect of variable gastric emptying (Horowitz et al., 1993), and glucose was administered at a rate that mimics that of gastric emptying (Pilichiewicz et al., 2007) and is known to elicit substantial GLP-1 and GIP responses (Schirra et al., 1996). It is, accordingly, unlikely that our observations would have been modified with a higher rate of nutrient delivery. Our study used the barostat technique, a method accepted as the 'gold standard' for controlled gastric distension (Ang, 2011), and yielded distension volumes of ~700 ml at the given pressure, which was associated with a sensation of fullness (Rayner et al., 2000). For comparison, intragastric balloons with volumes of 550 ml are effective for inducing weight loss (Machytka et al., 2017), and for adjustable intragastric balloons, volumes are initiated at 400 – 550 ml and do not exceed 850 ml (Abu Dayyeh et al., 2021). It should be appreciated that the air-filled barostat balloon distends predominantly the proximal, rather than distal, stomach. Distension of the antrum may be of particular importance in satiation (Sturm et al., 2004) and intragastric balloons positioned in the antrum have been associated with greater weight loss (Papavramidis et al., 2018). However, traditionally, intragastric balloons have been inserted proximally in the fundus (Salmi et al., 2020, Genco et al., 2005), although they may migrate to the antrum (Papavramidis et al., 2018).

The reason(s) for the apparent discrepancy in the effect of gastric distension on GLP-1 secretion between rodents and humans is uncertain. In response to nutrients, the effect of GLP-1 to increase insulin secretion is evident in both rodents (Wang et al., 1995) and humans (Nauck and Meier, 2016). Similarly, GIP has been considered to have a major insulinotropic effect in rodents (Tseng et al., 1999), a concept recently consolidated by studies using antagonists to GIP ($GIP_{3-30}NH_2$) in humans (Gasbjerg et al., 2020). A notable difference between rodent and human incretin physiology is that nutrient-stimulated GLP-1 secretion is reduced in rodents following subdiaphragmatic vagotomy, while direct vagal stimulation increases GLP-1 secretion (Rocca and Brubaker, 1999), indicating that GLP-1 secretion in rodents is modulated by the vagus nerve. In contrast, humans who have had a truncal vagotomy with pyloroplasty display an increase, rather than a decrease, in nutrient-stimulated GLP-1 and GIP secretion, although more rapid gastric emptying as a consequence of the pyloroplasty may well represent a confounder (Plamboeck et al., 2013).

Future studies investigating the effect of gastric distension on incretin hormone secretion in individuals with obesity and/or type 2 diabetes would be of interest.

*Limitations*

Our proof-of-concept pilot study had a small sample size, in part because of its technical complexity and may be underpowered to identify small differences in the effect of gastric distension on GLP-1 or GIP secretion. Our healthy participants were older (i.e. >65 years), but any reductions in incretin hormone secretion with age are modest, and the stimulation of both GLP-1 and GIP by intraduodenal glucose remained substantial (Pham et al., 2019). We also cannot exclude the possibility that exposure of the stomach to nutrients (i.e. nutritive distension) in the course of normal meal ingestion may influence incretin secretion (Steinert et al., 2012).

### *7.6 Conclusion*

In healthy, older adults, gastric distension did not stimulate GIP or GLP-1 secretion either in the absence or presence of small intestinal nutrient exposure.

**Chapter 8. Postprandial plasma GLP-1 levels are elevated in individuals with postprandial hypoglycaemia following Roux-en-Y gastric bypass – a systematic review.**

# Statement of Authorship

| | |
|---|---|
| Title of Paper | Postprandial plasma GLP-1 levels are elevated in individuals with postprandial hypoglycaemia following Roux-en-Y gastric bypass - a systematic review. |
| Publication Status | Published |
| Publication Details | JALLEH, R. J., UMAPATHYSIVAM, M. M., PLUMMER, M. P., DEANE, A., JONES, K. L. & HOROWITZ, M. 2023c. Postprandial plasma GLP-1 levels are elevated in individuals with postprandial hypoglycaemia following Roux-en-Y gastric bypass - a systematic review. *Rev Endocr Metab Disord*. Epub ahead of print. DOI: 10.1007/s11154-023-09823-3. |

**Principal Author**

| | |
|---|---|
| Name of Principal Author (Candidate) | Ryan Joseph Jalleh |
| Contribution to the Paper | I was responsible for the study design, prospective registration of the protocol, data collection, data analysis and preparation of the 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. | |
| Signature: | | Date: 24/11/23 |

**Co-Author Contributions**

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

i. The candidate's state contribution to the publication is accurate (as detailed above);

ii. Permission is granted for the candidate in including the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution

| | | |
|---|---|---|
| Name of Co-Author | Mahesh M Umapathysivam | |
| Contribution to the Paper | Dr Umapathysivam contributed to data collection, data analysis and preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

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|---|---|---|
| Name of Co-Author | Mark Philip Plummer | |
| Contribution to the Paper | Dr Plummer provided expertise in the conduct and design of systematic reviews and provided feedback on preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

| | | |
|---|---|---|
| Name of Co-Author | Adam Deane | |
| Contribution to the Paper | A/Prof Deane provided expertise in the conduct and design of systematic reviews and provided feedback on preparation of the manuscript | |
| Signature: | | Date: 26/11/23 |

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|---|---|---|
| Name of Co-Author | Karen L Jones | |
| Contribution to the Paper | Prof Jones is a co-supervisor of my PhD and provided supervision and mentorship in the preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

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| Name of Co-Author | Michael Horowitz | |
| Contribution to the Paper | Prof Horowitz is my primary supervisor and provided supervision and mentorship in the preparation of the manuscript | |
| Signature: | | Date: 24/11/23 |

*8.1 Abstract*

**Background and aims:** Bariatric surgery is the most effective treatment in individuals with obesity to achieve remission of type 2 diabetes. Post-bariatric surgery hypoglycaemia occurs frequently, and management remains suboptimal, because of a poor understanding of the underlying pathophysiology. The glucoregulatory hormone responses to nutrients in individuals with and without post-bariatric surgery hypoglycaemia have not been systematically examined.

**Materials and methods:** The study protocol was prospectively registered with PROSPERO. PubMed, EMBASE, Web of Science and the Cochrane databases were searched for publications between January 1990 and November 2021 using MeSH terms related to post-bariatric surgery hypoglycaemia. Studies were included if they evaluated individuals with post-bariatric surgery hypoglycaemia and included measurements of plasma glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), insulin, C-peptide and/or glucagon concentrations following an ingested nutrient load. Glycated haemoglobin ($HbA_{1c}$) was also evaluated. A random-effects meta-analysis was performed, and Hedges' *g* (standardised mean difference) and 95% confidence intervals were reported for all outcomes where sufficient studies were available. The $\tau^2$ estimate and $I^2$ statistic were used as tests for heterogeneity and a funnel plot with the Egger regression-based test was used to evaluate for publication bias.

**Results:** From 377 identified publications, 12 were included in the analysis. In all 12 studies, the type of bariatric surgery was Roux-en-Y gastric bypass (RYGB). Comparing individuals with and without post-bariatric surgery hypoglycaemia following an ingested nutrient load, the standardised mean difference in peak GLP-1 was 0.57 (95% CI, 0.32, 0.82), peak GIP 0.05 (-0.26, 0.36), peak insulin 0.84 (0.44, 1.23), peak C-peptide 0.69 (0.28, 1.1) and peak glucagon 0.05 (-0.26, 0.36). $HbA_{1c}$ was less in individuals with hypoglycaemia -0.40 (-0.67, -0.12). There was no evidence of substantial heterogeneity in any outcome except for peak insulin: $\tau^2 = 0.2$, $I^2 = 54.3$. No publication bias was evident.

**Conclusion:** Following RYGB, postprandial peak plasma GLP-1, insulin and C-peptide concentrations are greater in individuals with post-bariatric surgery hypoglycaemia, while $HbA_{1c}$ is less. These observations support the concept that antagonism of GLP-1 would prove beneficial in the management of individuals with hypoglycaemia following RYGB.

PROSPERO Registration Number: CRD42021287515

### *8.2 Introduction*

*Impact of bariatric surgery on obesity, type 2 diabetes and glucagon-like peptide-1 secretion*

Bariatric surgery is the most effective treatment in the management of morbid obesity (Buchwald et al., 2009). Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy are frequently performed procedures and, in general, have favorable short- and longer-term outcomes, including the remission of type 2 diabetes (Chang et al., 2014). The exaggerated post-prandial secretion of glucagon-like peptide-1 (GLP-1) and consequent increase in insulin secretion/sensitivity is thought to be central to this effect (Holst et al., 2018). The increase in GLP-1 is multifactorial and likely to reflect more rapid gastric pouch emptying and increased delivery of nutrients to the GLP-1-secreting L-cells in the distal small intestine, changes in the bile acid profile to stimulate GLP-1 release mediated via the G-protein-coupled bile acid receptor and alterations in the gut microbiota leading to stimulation of GLP-1 secretion via metabolites (van Olst et al., 2020, Nguyen et al., 2014). An improvement in glucose tolerance is evident within a few days after surgery and precedes weight loss (Holst et al., 2018). Although it is well-recognised that nutrient-induced GLP-1 secretion is greatly increased following some forms of bariatric surgery, it is not clear if individuals who experience hypoglycaemia post-bariatric surgery have a greater GLP-1 response than individuals without hypoglycaemia.

*Prevalence of post-bariatric surgery hypoglycaemia*

Post-bariatric surgery hypoglycaemia (PBH) is a complication of metabolic surgery and defined as having a low blood glucose level (<3.0 mmol/L) associated with autonomic or neuroglycopenic symptoms, although there is no consensus for the threshold glucose level (Salehi et al., 2018). It occurs in about a quarter of patients following RYGB, characteristically 1 – 3 hours following a meal (Capristo et al., 2018, Brix et al., 2019) and has also been described following sleeve gastrectomy (Capristo et al., 2018). PBH causes substantial morbidity (Emous et al., 2017, Capristo et al., 2018). and, in extreme cases, is life-threatening (Marsk et al., 2010). The role of GLP-1 in PBH remains unclear with inconsistent observations (Vidal et al., 2009,

Tharakan et al., 2017a). Clarification of this is important as GLP-1 receptor antagonists are being evaluated as a potential therapy in early clinical studies (Craig et al., 2021), where paradoxically, GLP-1 receptor agonists have also been suggested to have benefit in PBH (Abrahamsson et al., 2013). There is currently no accepted standard medical treatment for PBH. Accordingly, this study has systematically reviewed the literature to evaluate if nutrient-induced peak GLP-1 concentrations are greater in individuals with PBH compared with individuals who have had bariatric surgery but do not have PBH.

### *8.3 Methods*

#### Study Design and Registration

This systematic review and meta-analysis of cohort and case-control studies was designed in accordance with the latest methodological guidance (Deeks et al., 2001, Moons et al., 2014), and was reported in compliance with the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (Stroup et al., 2000). Protocol details were prospectively registered on PROSPERO (CRD42021287515); there were no major protocol deviations.

#### Eligibility Criteria

We included original research studies that reported a prognostic association between bariatric surgery (Roux-en-Y gastric bypass, sleeve gastrectomy or single anastomosis gastric bypass) and hypoglycaemia. We excluded abstracts and conference presentations, case reports, case series, editorials, expert opinions, publications with incompletely reported data, studies published in language other than English and non-human studies.

#### Search Strategy

We searched PubMed, EMBASE, Web of Science and Cochrane Database of Systematic Reviews from inception to 27 Nov 2021. Our search strategy included a comprehensive set of relevant search terms (Supplemental 1) and was designed with the support of a professional librarian, experienced in systematic reviews (Geersing et al., 2012).

**Study Selection**

Two authors (R.J.J. and M.M.U.) independently screened titles and abstracts for potentially relevant studies. The full texts of shortlisted studies were extracted and were assessed against eligibility criteria independently and in duplicate. A third author (M.P.P.) adjudicated any disagreements. We also reviewed the reference and citation lists of included studies for additional potentially relevant studies.

**Data Extraction and Management**

Two authors (R.J.J. and M.M.U.) independently used standardised spreadsheets to extract data from included studies. Where reported, the following were recorded: study design, population baseline characteristics, operative details, diabetes status at the time of the study, test meal contents and definition of hypoglycaemia. The primary outcome was post-bariatric surgery nutrient stimulated peak plasma levels of GLP-1 in individuals with and without hypoglycaemia. Secondary outcomes included (i) post-bariatric surgery nutrient stimulated plasma levels of glucose dependent insulinotropic polypeptide (GIP), insulin and C-peptide in patients with and without hypoglycaemia, (ii) HbA1c, (iii) hypoglycaemic counter-regulatory hormones (cortisol, glucagon, adrenaline, noradrenaline) and (iv) gastric emptying data.

**Risk of bias (quality) assessment**

The same authors (R.J.J. and M.M.U.) independently assessed the risk of bias using the Newcastle-Ottawa Scale (NOS). Disagreements in assessment were discussed and consensus obtained.

**Statistical Analysis and Data Synthesis**

In studies where outcomes of interest were presented in tables, but not reported in numerical form, the corresponding authors were contacted via email to request this information. No responses were received. The software PlotDigitizer.exe (Huwaldt JA. Plot Digitizer. Version

2.6.9, Free Software Foundation 2020) was used to extract peak concentrations of relevant enteropancreatic hormones and standardized deviations. We tabulated weighted mean differences (reported as Hedges' *g*) and 95% confidence interval from each study and generated summary estimates using random effects modelling (Dettori et al., 2022). We performed separate meta-analyses for each outcome where reporting was sufficient across studies; otherwise, we performed qualitative analyses. Random-effects meta-analysis was used to account for potential between-study heterogeneity, and REML (restricted maximum likelihood) was used for the random effect estimation.-Statistical heterogeneity is reported as the $I^2$ statistic and $\tau^2$ estimate (Deeks et al., 2001). Where there were fewer than 10 included studies reporting on an outcome, publication bias was unable to be formally assessed . All data analyses were performed in consultation with a professional biostatistician using STATA (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC).

**Search Results**

The search returned 376 results. No additional citations were identified from secondary searching of reference lists. After de-duplication, 334 studies underwent title and abstract screening. 104 potentially relevant studies underwent full-text review, from which 12 studies were included in this review. (Figure 8.1). The majority of these 104 studies described an increase in GLP-1 following RYGB (which has been well established) but did not determine whether GLP-1 secretion is increased to a greater extent in individuals with PBH and were, therefore, excluded.

*8.4 Results*

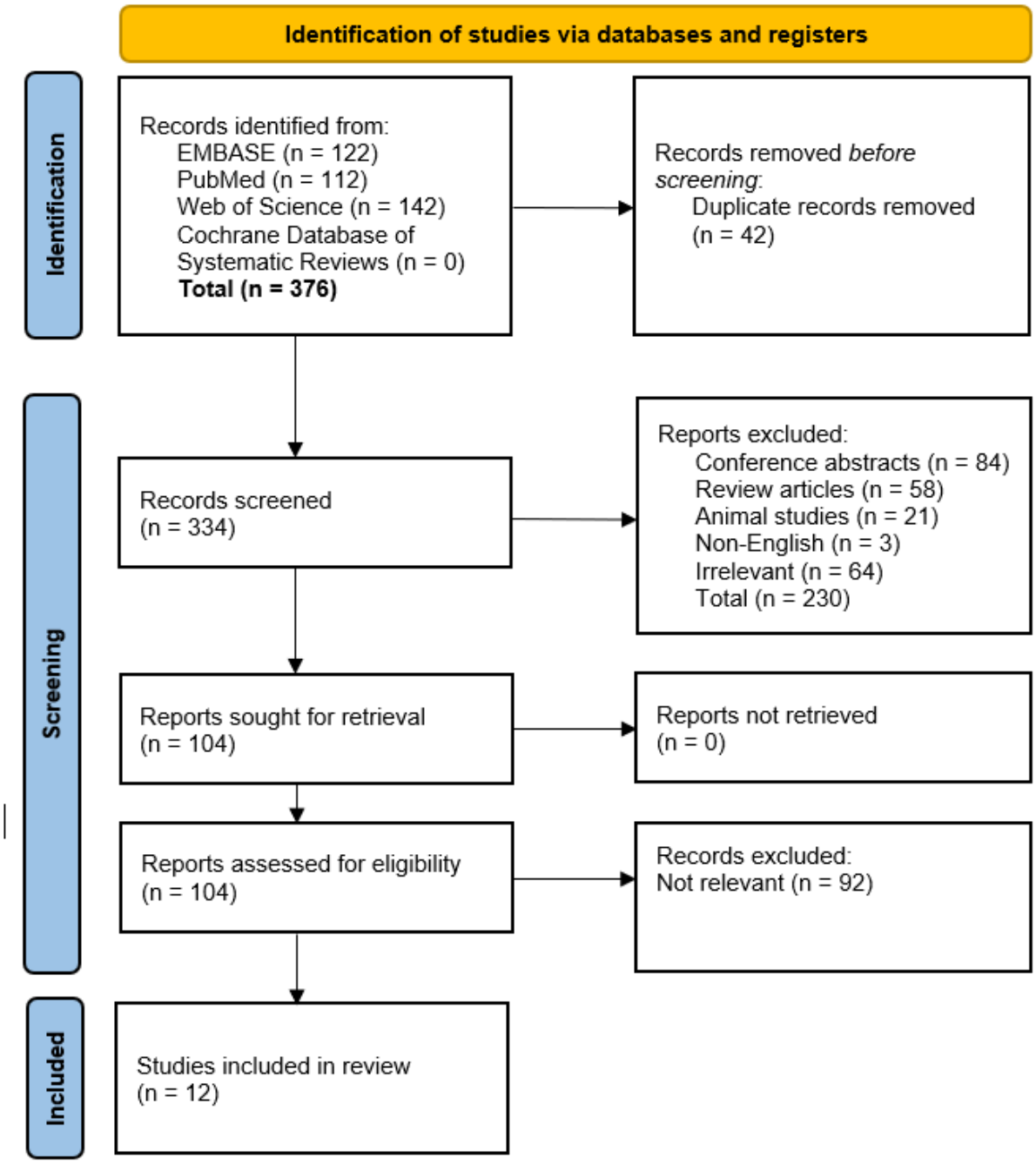


**Figure 8.1** Identification of studies via databases and registers

**Description of Included Studies**

Twelve studies involving 324 participants post-bariatric surgery were included. Detailed characteristics of included studies are presented in Table 8.1.

| Study | Design | Sample size | Surgery | Age (years ± SEM) | Sex (M/F) | Diabetes (diabetes/remission/no prior diabetes) | Definition of hypoglycaemia | Test meal | Outcome on incretin hormones |
|---|---|---|---|---|---|---|---|---|---|
| Goldfine | Case-control | 21 | RYGB | 48 ± 3 | 4/17 | 0/0/21 | Prior documented severe hypoglycaemia (severe neuroglycopenic symptoms attributable to hypoglycaemia requiring bystander assistance) | Ensure 240 ml (40 g carbohydrate, 6 g fat, 9 g protein) | Higher fasting and postprandial GLP-1 but lower postprandial GIP in hypoglycaemia group. |
| Guarino | Cohort | 35 | RYGB | 52 ± 2 | 9/24 | 9/26/0 | Plasma glucose ≤ 3.3 mmol/L following a standard meal with autonomic/neuroglycopenic symptoms | 75 g glucose drink | Comparable postprandial GLP-1 response. GIP not measured. |
| Kellogg | Cohort | 14 | RYGB | 46 ± 3 | 2/10 (2 not reported) | 1/0/13 | Plasma glucose < 3.9 mmol/L following a high carbohydrate meal with autonomic/neuroglycopenic symptoms | High carbohydrate meal (405 kcal, 79% carbohydrate, 11% fat, 10% protein) | Higher postprandial insulin following high carbohydrate meal but GLP-1 and GIP not measured. |
| Laurenius | Case-control | 16 | RYGB | 47 ± 2 | 5/11 | 0/1/15 | Prior documented severe hypoglycaemia | Liquid mixed meal (99.5 g carbohydrate, 0.1 g fat, 5.7 g protein) | Comparable postprandial GLP-1 response. GIP not measured. |
| Lobato | Cohort | 23 | RYGB | 43 ± 3 | 4/19 | 0/3/20 | Plasma glucose ≤ 3.05 mmol/L following a standard liquid mixed meal with autonomic/neuroglycopenic symptoms | Fresubin 200ml, (300 kcal, 50% carbohydrate, 35% fat, 15% protein) | Higher peak postprandial GLP-1 but no difference in GIP. |
| Poitou | Cohort | 20 | RYGB | 44 ± 3 | 4/16 | N/A | Plasma glucose ≤ 3 mmol/L following a standard liquid mixed meal with autonomic/neuroglycopenic symptoms | Fresubin 400 ml (800 kcal, 45% carbohydrate, 35% fat, 20% protein) | Comparable postprandial GLP-1 and GIP responses. |

| Study | Design | Sample size | Surgery | Age (years ± SEM) | Sex (M/F) | Diabetes (diabetes/remission/no prior diabetes) | Definition of hypoglycaemia | Test meal | Outcome on incretin hormones |
|---|---|---|---|---|---|---|---|---|---|
| Salehi 2014a | Case-control | 65 | RYGB | 48 ± 2 | 10/55 | 16/16/33 | History of autonomic/ neuroglycopenic symptoms following a meal | Ensure Plus, 237 ml (350 kcal, 57% carbohydrate, 28% fat, 15% protein) | Comparable postprandial GLP-1 and GIP responses. |
| Salehi 2014b | Case-control | 16 | RYGB | 46 ± 3 | 4/12 | 4/4/8 | Plasma glucose < 2.8 mmol/L following a standard liquid mixed meal with neuroglycopenic symptoms | Ensure Plus, 237 ml (350 kcal, 57% carbohydrate, 28% fat, 15% protein | Trend towards a higher postprandial GLP-1 in the hypoglycaemia group but comparable GIP responses. |
| Salehi 2019 | Case-control | 14 | RYGB | 45 ± 3 | 3/11 | 1/1/12 | Plasma glucose < 2.8 mmol/L following a standard liquid mixed meal with neuroglycopenic symptoms | Ensure Plus, 237 ml (350 kcal, 57% carbohydrate, 28% fat, 15% protein | Higher postprandial GLP-1 in the hypoglycaemia group but comparable GIP response. |
| Soeby | Case-control | 26 | RYGB | 43 ± 2 | 5/21 | 0/3/23 | Plasma glucose ≤ 3.5 mmol/L following a 50 g glucose drink with autonomic/ neuroglycopenic symptoms | Fresubin 200 ml, (300 kcal, 50% carbohydrate, 35% fat, 15% protein) | Comparable postprandial GLP-1 and GIP responses. |
| Tharakan | Cohort | 28 | RYGB | 46 ± 2 | 9/19 | N/A | Prior history of hypoglycaemia fulfilling Whipple's triad | Ensure Plus | Higher postprandial GLP-1 in the hypoglycaemia group but comparable GIP response. |
| Vaurs | Cohort | 46 | RYGB | 43 ± 3 | N/A | N/A | Plasma glucose < 2.8 mmol/L following a 75 g glucose drink with autonomic/ neuroglycopenic symptoms | 75 g glucose drink | Comparable postprandial GLP-1 response. GIP not measured. |

**Table 8.1** Detailed characteristics of included studies (n=12)

### Methodological quality

The studies included were observational in design and of small sample sizes associated with large confidence intervals. The studies included were mostly at a low risk of bias as assessed by the Newcastle-Ottawa scale. Results are summarized in Table 8.2 below.

| Study | Selection (Maximum: ★★★★) | Comparability (Maximum: ★★) | Outcome (Maximum: ★★★) | Total |
|---|---|---|---|---|
| Goldfine | ★★★ | ★★ | ★★★ | 8/9 |
| Guarino | ★★★★ | ★★ | ★★★ | 9/9 |
| Kellogg | ★★★ | ★★ | ★★★ | 8/9 |
| Laurenius | ★★★★ | ★★ | ★★★ | 9/9 |
| Lobato | ★★★ | ★★ | ★★★ | 8/9 |
| Poitou | ★★★★ | ★ | ★★★ | 8/9 |
| Salehi 2014a | ★★★★ | ★★ | ★★★ | 9/9 |
| Salehi 2014b | ★★★ | ★★ | ★★★ | 8/9 |
| Salehi 2019 | ★★★★ | ★★ | ★★★ | 9/9 |
| Soeby | ★★★ | ★★ | ★★★ | 8/9 |
| Tharakan | ★★ | ★★ | ★★★ | 7/9 |
| Vaurs | ★★★★ | ★★ | ★★★ | 9/9 |

**Table 8.2** Risk of bias assessment by Newcastle-Ottawa scale. Studies with total score 8 or greater (n = 11/12) are considered to be at low risk of bias.

**Outcomes**

*Peak postprandial GLP-1*

In ten studies (Goldfine et al., 2007, Guarino et al., 2019, Laurenius et al., 2014, Lobato et al., 2020, Poitou et al., 2018, Salehi et al., 2014, Salehi et al., 2019, Soeby et al., 2020, Tharakan et al., 2017b) (involving 264 individuals), there was an increase in peak postprandial GLP-1 in those with PBH compared to those without; Hedges' *g* 0.57 (95% CI 0.32, 0.82) reported this outcome for both PBH (n = 132) and non-PBH (n = 132) cohorts (Figure 8.2). There was no evidence of between-study heterogeneity ($\tau^2$=0.01, $I^2$= 3.36). Only two studies (Guarino et al., 2019, Salehi et al., 2014) reported the outcome for GLP-1 AUC in both PBH and non-PBH

cohorts, hence a meta-analysis for this outcome was not performed. The *p* value for Egger's test was 0.306. There was no evidence of bias in the funnel plot. (Figure 8.3)

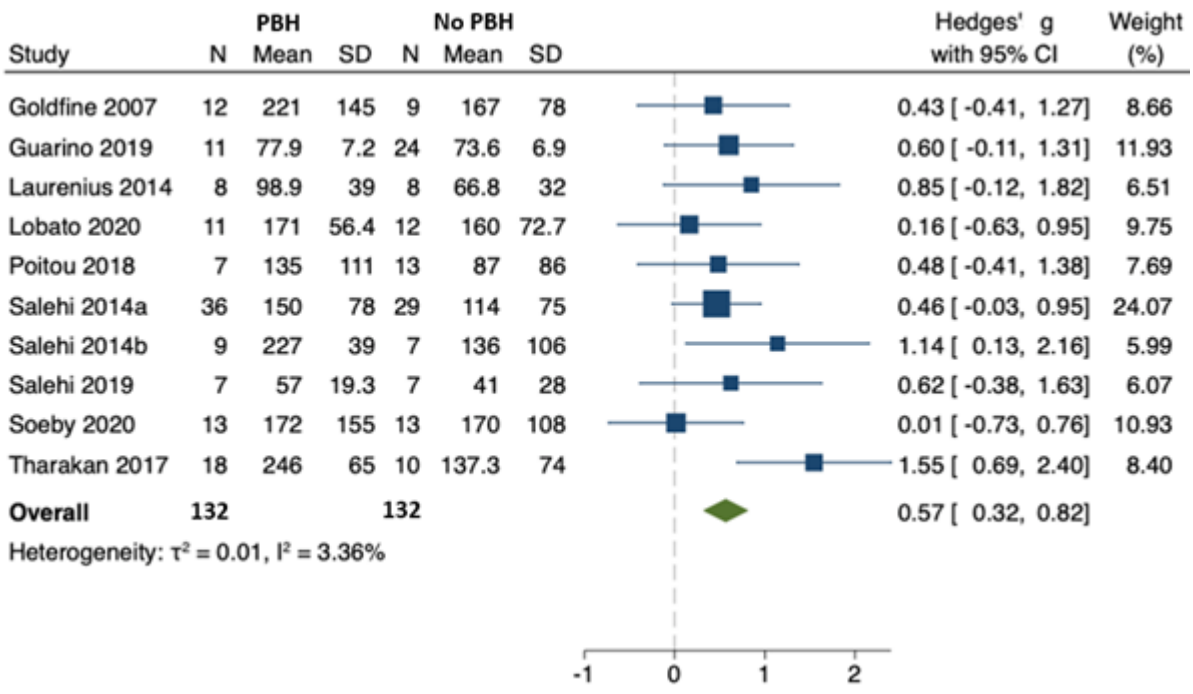


**Figure 8.2** Peak postprandial GLP-1 concentration in those with PBH (n = 132) compared to those without (n = 132).

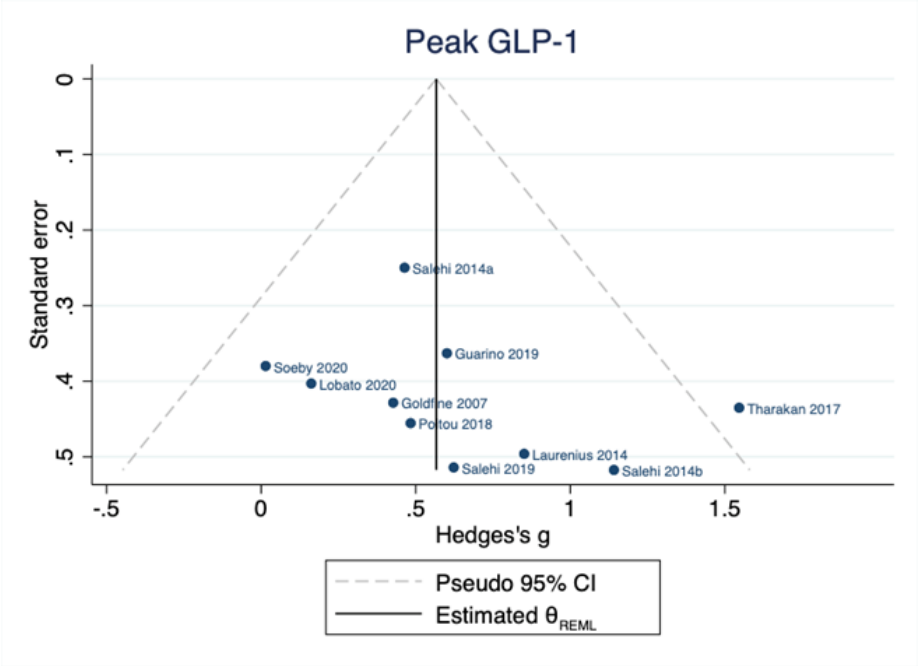


**Figure 8.3** Funnel plot for peak GLP-1 publication bias

*Peak postprandial GIP*

In 7 studies, involving 193 individuals, there was no difference between postprandial GIP in those with (n = 106) and without (n = 87) PBH (Goldfine et al., 2007, Lobato et al., 2020, Salehi et al., 2014, Salehi et al., 2019, Soeby et al., 2020, Tharakan et al., 2017b). (Figure 8.4) Hedges' $g$ was 0.05 (95% CI -0.26, 0.36) and there was no evidence of between-study heterogeneity ($\tau^2$=0.03, $I^2$= 16.82). No study reported GIP AUC. As there were fewer than ten studies, we were unable to formally assess for publication bias.

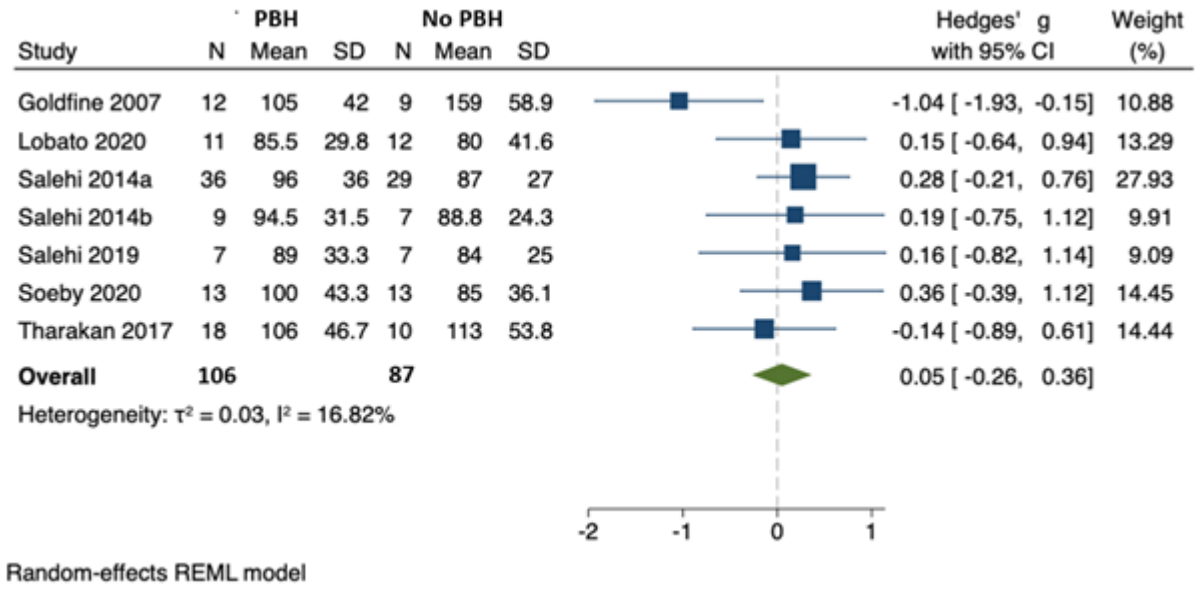


**Figure 8.4** Peak postprandial GIP concentration in those with PBH (n = 106) compared to those without (n = 87).

*Peak postprandial insulin*

In 10 studies (involving 269 individuals), there was an increase in peak postprandial insulin in those with PBH (n = 151) compared to those without (n = 118) (Goldfine et al., 2007, Lobato et al., 2020, Salehi et al., 2014, Salehi et al., 2019, Soeby et al., 2020, Tharakan et al., 2017b, Kellogg et al., 2008, Laurenius et al., 2014, Vaurs et al., 2016). Hedges' $g$ was 0.84 (95% CI 0.44, 1.23), however, there was some indication of between-study heterogeneity ($\tau^2$=0.20, $I^2$= 54.25, p=0.03). One study (Laurenius et al., 2014) reported the means, but not standard deviations, of the insulin AUC. This study was not included in the meta-analysis. (Figure 8.5)

The *p* value for Egger's test was 0.632. There was no evidence of bias in the funnel plot. (Figure 8.6)

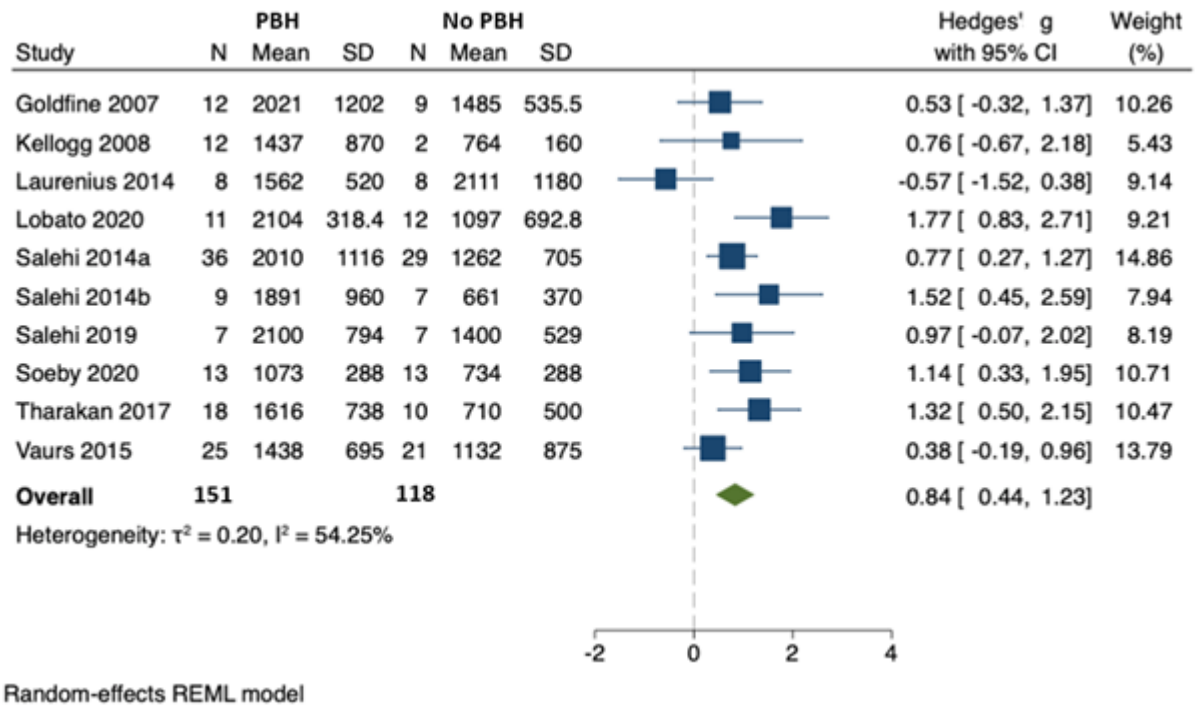


**Figure 8.5** Peak postprandial insulin concentration in those with PBH (n = 132) compared to those without (n = 132).

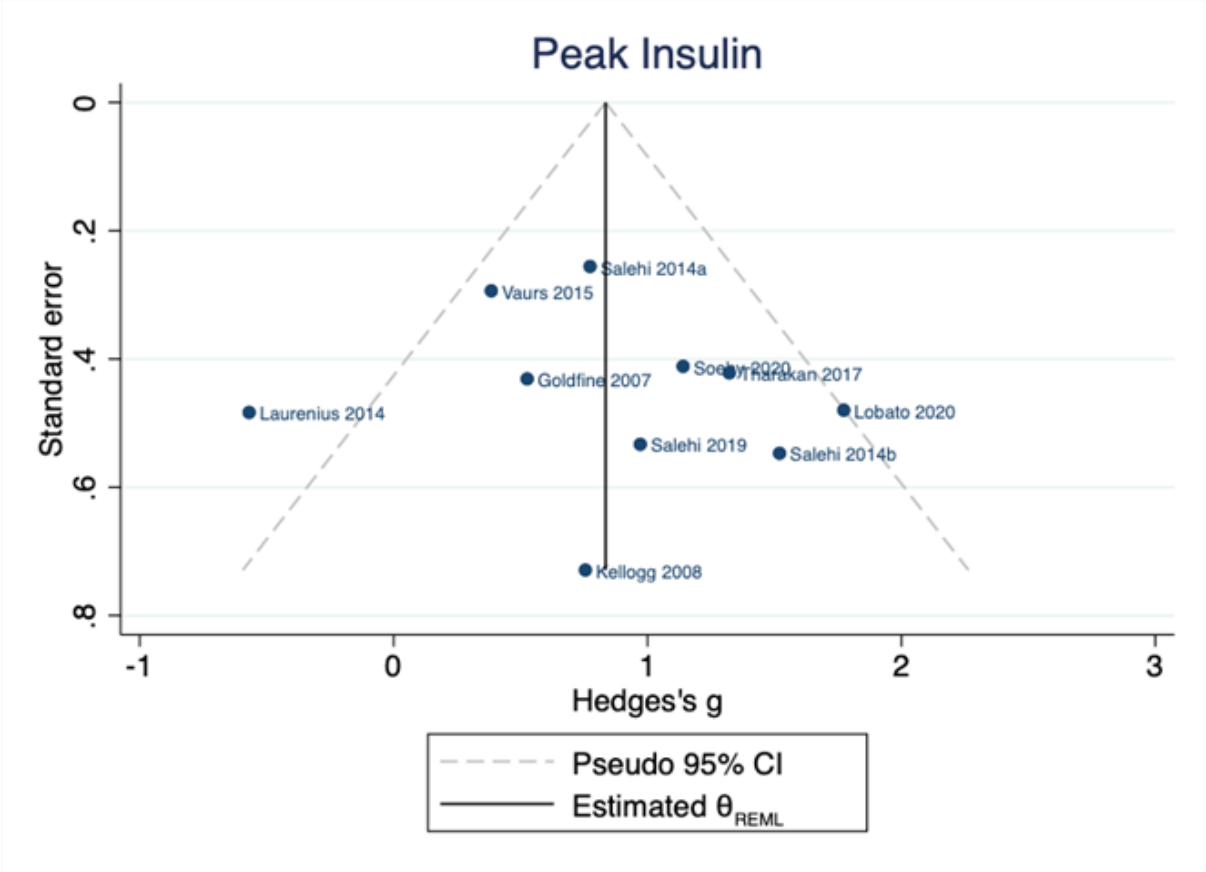


**Figure 8.6** Funnel plot for peak insulin publication bias.

*Peak post-prandial C-peptide*

In 6 studies (involving 191 individuals), there was an increase in peak postprandial C-peptide in those with PBH (n = 100) compared to those without (n = 91) (Goldfine et al., 2007, Lobato et al., 2020, Poitou et al., 2018, Salehi et al., 2014, Vaurs et al., 2016). Hedges' *g* was 0.69 (95% CI 0.28, 1.10) and there was no evidence of between-study heterogeneity ($\tau^2$=0.11, $I^2$= 44.47) (Figure 8.7). No study reported the C-peptide AUC. As there were fewer than ten studies, we were unable to formally assess for publication bias.

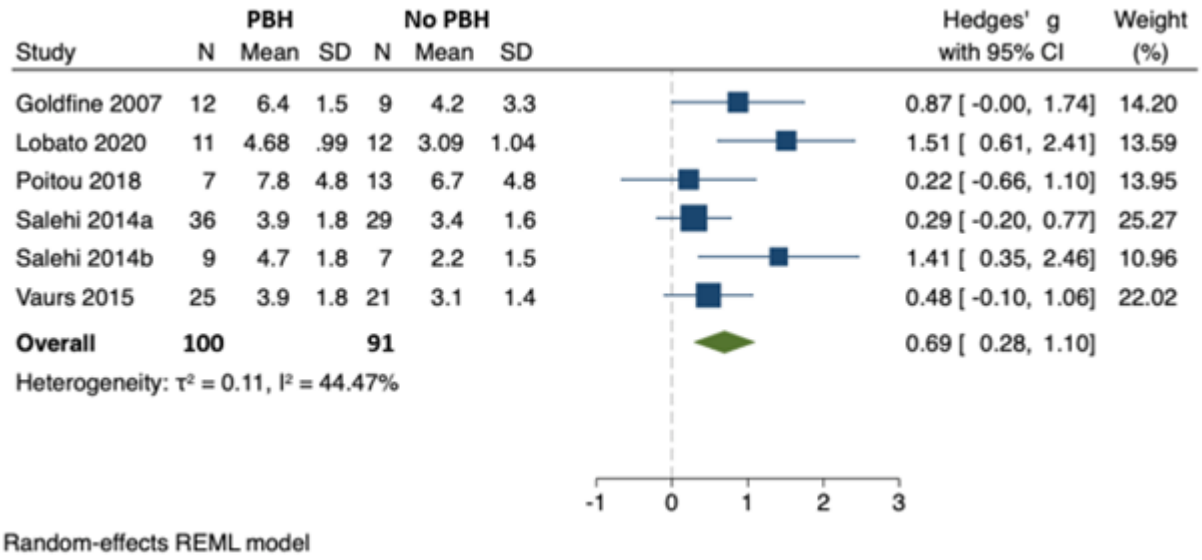


**Figure 8.7** Peak postprandial C-peptide concentration in those with PBH (n = 100) compared to those without (n = 91).

*Peak glucagon*

In 10 studies, involving 264 individuals, there was no difference between postprandial glucagon in those with (n = 132) and without (n = 132) PBH (Goldfine et al., 2007, Guarino et al., 2019, Laurenius et al., 2014, Lobato et al., 2020, Poitou et al., 2018, Salehi et al., 2014, Salehi et al., 2019, Soeby et al., 2020, Tharakan et al., 2017b). Hedges' *g* was 0.05 (95% CI -0.26, 0.36) and there was no evidence of substantial between-study heterogeneity ($\tau^2$=0.09, $I^2$= 35.53) (Figure 8.8). No study reported the glucagon AUC. The p value for Egger's test was 0.015. There was no evidence of bias in the funnel plot. (Figure 8.9)

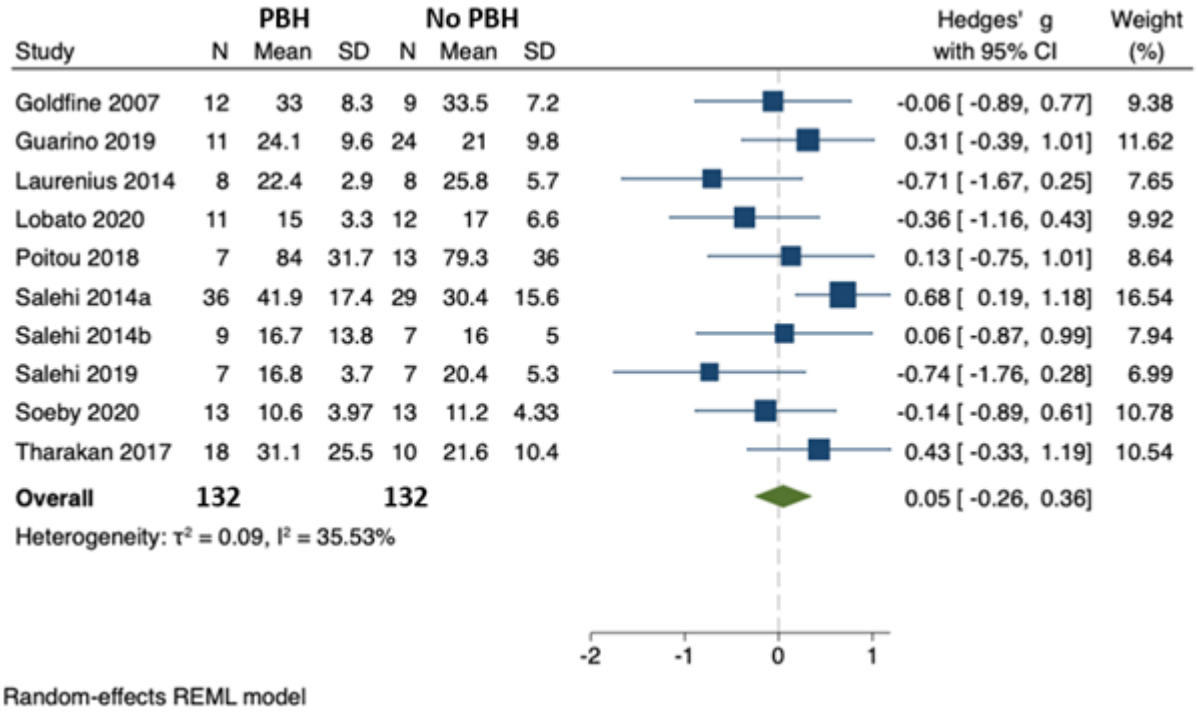


**Figure 8.8** Peak postprandial glucagon concentration in those with PBH (n = 132) compared to those without (n = 132).

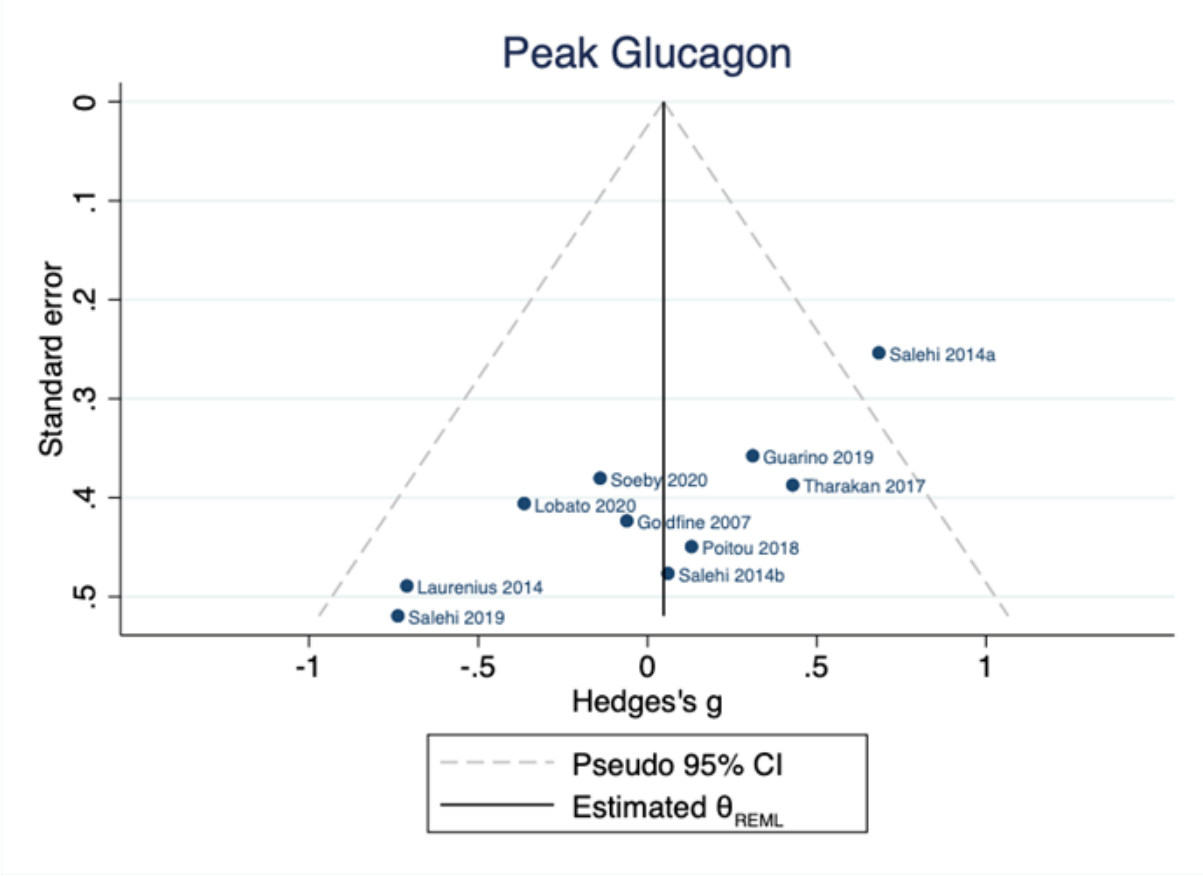


**Figure 8.9** Funnel plot for peak glucagon publication bias.

*HbA1c*

In 7 studies, involving 208 individuals, HbA1c was lower in individuals with PBH (n = 104) compared with those without PBH (n = 104) (Goldfine et al., 2007, Guarino et al., 2019, Lobato et al., 2020, Poitou et al., 2018, Salehi et al., 2014, Tharakan et al., 2017b). Hedges' *g* was -0.40 (95% CI -0.67, -0.12) and there was no evidence of between-study heterogeneity ($\tau^2$=0.00, $I^2$= 0.00). (Figure 8.10) As there were fewer than ten studies, we were unable to formally assess for publication bias.

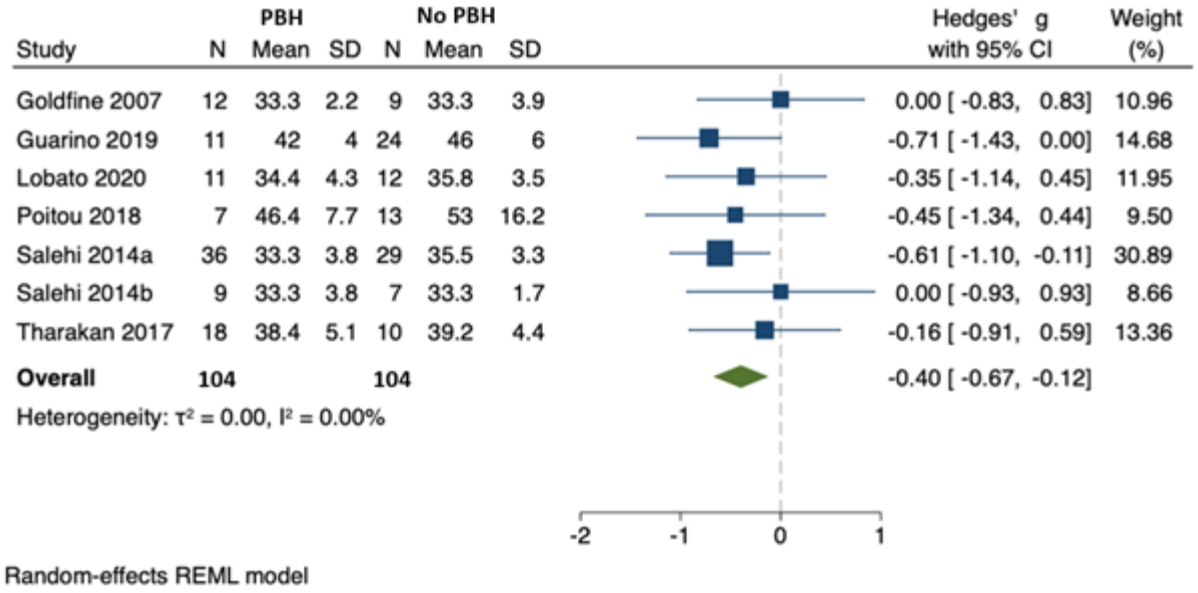


**Figure 8.10** HbA1c in those with PBH (n = 104) compared to those without (n = 104).

*Other parameters*

The studies were screened for other outcomes, including gastric emptying data (not reported in any study), catecholamines (not reported in any study), cortisol (not reported in any study) insulin secretion (reported in 1 study), disposition index (reported in 1 study) and HOMA-IR (not reported in any study), for which meta-analyses were, accordingly, unable to be performed.

### *8.5 Discussion*

This is the first systematic review to evaluate associations between nutrient-stimulated enterohormones in individuals with postprandial hypoglycaemia following RYGB. Individuals

who had post-bariatric surgery hypoglycaemia had greater postprandial GLP-1 and insulin concentrations, but GIP responses were not different, when compared with individuals who had the same type of bariatric surgery but who did not develop hypoglycaemia. Furthermore, in individuals with post-bariatric surgery hypoglycaemia, mean HbA1c was less. There was no substantial between-study heterogeneity in the outcomes analysed apart from peak insulin, where there was a strong association with PBH. The observed heterogeneity is likely due to reflect different frequencies of diabetes and remission as summarized in Table 1. Based on the funnel plots and Egger's test, there was no evidence of publication bias, apart from the analysis of peak glucagon where the funnel plot was skewed to the right by the largest study (Salehi et al., 2014). Overall, the pooled results from all studies indicated that there was no association between peak glucagon and PBH. The majority of the studies (11/12) were well designed with a low risk of bias, as evaluated with the Newcastle-Ottawa Scale. Pre-specified sensitivity analyses of low risk of bias studies (included in Supplementary material) did not alter the results.

This meta-analysis of all studies has made it evident that a greater peak GLP-1 response is associated with PBH following RYGB while peak GIP is not. The latter is not surprising given that GIP-secreting K-cells are predominantly found in the duodenum/proximal small intestine which is bypassed following RYGB. There was only one study which found that a greater postprandial peak in GIP was associated with a lower risk of hypoglycaemia (Goldfine et al., 2007). It should be appreciated that this study was published much earlier (2007) than the other studies, and the difference in GIP may be related to variations in surgical technique such as the length of proximal intestine bypassed, however, this information was not provided. Both peak insulin and C-peptide were predictably strongly associated with PBH, consistent with the concept that PBH is driven by exaggerated endogenous insulin secretion. In contrast, there was no evidence of an association between peak glucagon and PBH. We had hypothesized that, reflecting the capacity of GLP-1 to suppress glucagon, that a lower peak glucagon may be

associated with PBH. It is possible that PBH is mainly driven by the exaggerated GLP-1 and insulin response rather than suppression of glucagon. As glucagon is released as a counter-regulatory response to hypoglycaemia (Marathe et al., 2019), this may over-ride the suppressive effect of GLP-1 which occurs in a glucose-dependent manner. A lower HbA1c was associated with an increased risk of PBH. This may be explained by either the effect of hypoglycaemia to lower mean blood glucose or a protective effect against hypoglycaemia due to greater insulin resistance associated with higher HbA1c.

The underlying cause of PBH remains unclear, however, there are several putative mechanisms including increased beta cell mass (Service et al., 2005), increased insulin-independent glucose uptake (Patti et al., 2015), increased secretion of GLP-1 (Craig et al., 2017), altered bile acid metabolism (van den Broek et al., 2021), altered gut microbiota (Zhou et al., 2020) and increased fibroblast growth factor-19 (Mulla et al., 2019). Although GLP-1RA therapy in T2D without concomitant administration of insulin is not associated with hypoglycaemia, there are multiple physiologic changes that occur following RYGB that could explain why hypoglycaemia may occur in this group. RYGB is associated with gastric pouch emptying rates of up to 100 kcal/min (Nguyen et al., 2014) compared with gastric emptying rates of 1 – 4 kcal/min in health, which may lead to a transient marked postprandial glycaemic excursion (Phillips et al., 2015). The rapid and increased delivery of nutrients to the small intestine is thought to underlie the supraphysiologic levels of GLP-1 (Nguyen et al., 2014, Hutch and Sandoval, 2017). GLP-1 has glucose-dependent effects to augment insulin and inhibit glucagon secretion (Drucker, 2018), with a consequent marked glucose-lowering effect. RYGB also alters bile acid composition and the activation of intestinal farnesoid X receptor (FXR) by bile acids increases fibroblast growth factor-19 (FGF-19), an intestinally derived hormone, which reduces hepatic glucose production and increases peripheral glucose disposal independent of insulin (Wang et al., 2019, Mulla et al., 2019). Interestingly, increased FGF-19 levels have been associated with PBH; moreover, the progressive increase in FGF-19 levels over time

corresponds to the time course of PBH (Mulla et al., 2019). Changes to the gut microbiota occur also within 3 months post-RYGB, are sustained in the longer-term (Tremaroli et al., 2015), and have been associated with lower postprandial glucose levels in rodent models (Arora et al., 2017). While these changes are thought to be pivotal to remission of type 2 diabetes, it is likely that, in combination, they also contribute to PBH and in such individuals, we have now shown that GLP-1 responses are greater.

**Limitations**

Although all forms of bariatric surgery were included in the search criteria, only studies involving RYGB were identified. Thus, it is not known if there are similar associations between glucoregulatory hormones and PBH with other bariatric surgery procedures such as sleeve gastrectomy or the one anastomosis gastric bypass. Only English language articles were included, however, only 3 identified articles were non-English language. Only peak hormone levels were consistently reported among studies. Data were collected for area under the curve (AUC) hormone levels over time but due to varying time-frames and most studies not reporting this, a meta-analysis could not be performed. There is no standardized meal for the evaluation of PBH and accordingly, there was significant variation in the meals between studies. Similarly, there was varied definitions for hypoglycaemia between studies and we were unable to assess the relationship between severity of postprandial hypoglycaemia and GLP-1. Furthermore, we were unable to evaluate the temporal relationship between GLP-1 and hypoglycaemia/hyperinsulinaemia. The final number of studies included for GIP, C-peptide and HbA1c were too small to analyse for publication bias.

**Implications and areas for further evaluation**

The outcomes of this analysis support an association between the stimulation of GLP-1 with PBH. Early clinical trials have suggested that GLP-1 antagonism (Craig et al., 2017, Craig et al., 2018) may represent a novel therapy for PBH. In a proof-of-principle double-blinded,

placebo-controlled, crossover study, the intravenous infusion of exendin (9-39), a GLP-1 receptor antagonist, prevented hypoglycaemia in 8 out of 8 participants with PBH (Craig et al., 2017). Our findings support the development of larger-scale clinical trials to evaluate the potential role of GLP-1 antagonism in PBH management. Recent trials have also suggested a role for GLP-1 receptor agonist (RA) treatment as an adjunct to increase rates of type 2 diabetes remission (Miras et al., 2019). The effect of GLP-1RAs on PBH has not been established, however, two small, uncontrolled studies reported improvement of PBH following GLP-RA treatment (Abrahamsson et al., 2013, de Heide et al., 2023) but sample sizes were small (n= 5 and n= 13 respectively). Possible explanations to account for this paradox include slowing of intestinal transit, resulting in decreased stimulation of L-cells (Thazhath et al., 2016) and marked suppression of endogenous GLP-1 secretion (Kim et al., 2021).

Gastric emptying has been recognized as a major determinant of postprandial glycaemic excursions in individuals with and without type 2 diabetes (Jalleh et al., 2022a) but was not evaluated in any of the included studies. There is no information about the role of gastric emptying/small intestinal transit in the pathogenesis of PBH and this would be of considerable interest. Additionally, there is evidence that an acceleration of gastric emptying is important in the counter-regulation of hypoglycaemia (Murthy et al., 2021). Catecholamines are an important counter-regulatory mechanism in hypoglycaemia and this response may be blunted following RYGB (Abrahamsson et al., 2016). It is not known if individuals with PBH have a greater reduction in the catecholamine response to hypoglycaemia.

Finally, our findings suggest additional attention to PBH be given to individuals who have a lower HbA1c as they may be at greater risk.

### *8.6 Conclusion*

Following RYGB, nutrient-induced peak concentrations of GLP-1, insulin and C-peptide are greater, while HbA1c is less in individuals with post-bariatric surgery hypoglycaemia. These observations suggest that antagonism of GLP-1 represents a rational intervention to prevent

hypoglycaemia in patients who suffer from post-bariatric surgery hypoglycaemia after RYGB. Further evaluation of the role of gastric emptying and catecholamine response in this condition is warranted.

# Chapter 9: Conclusions

## *9.1 Summary of key findings of this thesis*

This thesis was undertaken to provide increased understanding of the relationships between gastric emptying, incretin hormone responses and glycaemic responses to nutrients. Gastric emptying has now been recognized to be of major relevance to the management of type 2 diabetes supported by studies conducted by my colleagues and I (Jalleh et al., 2022a) and others (Goyal et al., 2019). In the studies reported in this thesis, I have advanced knowledge in this field significantly.

### *9.1.1 Theme 1 – relationships between gastric emptying, incretin hormones and the rise in glucose following nutrients in type 2 diabetes and stress-induced hyperglycaemia*

In Chapter 3, I have shown that in individuals with type 2 diabetes, that relatively more rapid gastric emptying is associated with a greater rise in plasma glucose at 30, 60 and, in particular, at 120 minutes, but not 180 minutes after a 75 g oral glucose load. The finding of a correlation at 120 minutes is novel and important as this indicates that the oral glucose tolerance test, which uses a cut-off of a plasma glucose of ≥ 11.1 mmol/L at 120 minutes to diagnose diabetes mellitus (World Health Organization and International Diabetes Federation 2006), may be influenced by factors that alter gastric emptying, such as medications or comorbidity. There was no relationship between GLP-1 and the rate of gastric emptying on multiple regression analysis which may appear counter-intuitive as faster gastric emptying may be anticipated to result in more rapid delivery of nutrients to the distal small intestine to stimulate GLP-1. However, in prior studies involving intraduodenal administration of glucose (Ma et al., 2012), a sustained rise in GLP-1 was only evident when glucose was delivered at a rate of >3 kcal/min, but in all of the participants in this cohort, gastric emptying was less than 3 kcal/min. Furthermore, rapid gastric emptying may not translate to more rapid glucose delivery to the distal small intestine, possibly because endogenous GLP-1 slows gastric emptying. There was also no relationship between gastric emptying and insulin secretion. Chapter 4 advanced knowledge of the

relationships between gastric emptying and glycaemia in individuals without diabetes who had previous critical illness-induced stress hyperglycaemia. I found that in 32 out of 35 patients (91.4%) the 1-hour plasma glucose was ≥ 8.6 mmol/L at 3 months following ICU discharge. This observation is important as in individuals with impaired glucose tolerance, a 1-hour plasma glucose ≥ 8.6 mmol/L is a robust predictor of progression to type 2 diabetes and associated with increased morbidity and earlier mortality (Bergman et al., 2018).

*9.1.2 Theme 2 – relationships between gastric emptying, incretin hormones and the rise in glucose following nutrient intake in health*

Chapter 5 advances knowledge of the relationship between gastric emptying and glycaemia by evaluating the correlation of gastric emptying and the pattern of the glucose response following an oral glucose tolerance test in older adults without diabetes. This is the first study to evaluate the pattern of the glucose response curves in adults >65 years of age, where the prevalence of T2D is high . Furthermore, it is the first study to evaluate this pattern with gastric emptying and incretin hormone measurements concurrently. In the group with a biphasic pattern, the mean plasma glucose at 1 hour was less at 7.9 mmol/L compared with that in the monophasic group 10.0 mmol/L. Given a 1-hour plasma glucose ≥ 8.6 mmol/L is a predictor of future progression to type 2 diabetes is consistent with the understanding that a biphasic curve is associated with a reduced risk of dysglycaemia. There was no significant difference in the rate of gastric emptying or plasma GIP in the monophasic vs biphasic group, however, GLP-1 was significantly greater in the biphasic group. It is, accordingly, possible that individuals with a biphasic pattern may have a greater intrinsic GLP-1 response, which could account for the lower 1-hour plasma glucose and reduced propensity for future type 2 diabetes.

Given that liquids with higher nutrient densities emptying slower, I evaluated the gastric emptying rate of low-carbohydrate, low-alcohol and full-strength beer in Chapter 6. Differences in carbohydrate, or alcohol, content did not have a significant effect on the rate of gastric emptying. Low alcohol beer increased blood glucose compared with low carbohydrate and full

strength beer, but the increase was modest and is unlikely to be of clinical relevance in individuals without diabetes. The rise in glucose in individuals with diabetes would be anticipated to be greater and warrants evaluation.

### *9.1.3 Theme 3 – understanding the effects of bariatric surgery on incretin hormones and postprandial glucose*

In Chapter 7, I showed that gastric distension was not associated with an increased plasma GLP-1 or GIP response in healthy humans. This contrasts with animal studies (Ohbayashi et al., 2021, Natochin et al., 2018), where gastric distension has been associated with a substantial increase in GLP-1 secretion. This study used the 'barostat' technique, which is accepted as the 'gold standard' for controlled gastric distension (Ang, 2011) and is the first to investigate the impact of gastric distension on incretin hormone secretion in humans. This study has improved our understanding of the physiological changes that occur with procedures to facilitate weight loss such as sleeve gastrectomy or RYGB, where gastric volume is reduced surgically, and intragastric balloon insertion. The findings of my study indicate that distension is unlikely to be a contributing factor to the elevation of GLP-1 seen following bariatric surgery.

In Chapter 8, I showed that following a nutrient load, individuals who have had RYGB surgery and experience post-bariatric surgery hypoglycaemia have a greater plasma GLP-1 response than those who have had RYGB surgery, but do not experience hypoglycaemia. These findings support the use of GLP-1 receptor antagonist (avexitide) as a potential treatment for post-bariatric hypoglycaemia (Tan et al., 2020), which to date, has only been evaluated in a small study, but was found to be highly effective. This is important, given that currently, the treatment of this condition is limited and there is the lack of randomized controlled trials. I also showed, that post-bariatric surgery hypoglycaemia was associated with a hyperinsulinaemic response ,but not associated with differences in plasma GIP. In prior studies, gastric pouch emptying has been shown to be extremely rapid and up to 100 kcal/min (Nguyen et al., 2014). This may result in a marked rise in blood glucose following high carbohydrate meals and likely rapid delivery

of nutrients to the distal small bowel to stimulate GLP-1 secretion. The supraphysiologic response of GLP-1 combined with improved insulin sensitivity may result in a sharp decline in blood glucose and consequently, postprandial hypoglycaemia.

### *9.2 Clinical practice changes supported by findings of this thesis*

From my findings in Chapter 3, in interpreting results of a 75 g oral glucose tolerance test for the diagnosis of diabetes, factors that alter gastric emptying need to be considered. Medications that slow gastric emptying, such as opioids, may result in a lower plasma glucose and in contrast, medications that accelerate gastric emptying, such as some anti-emetics (metoclopramide) and erythromycin may raise plasma glucose. Consideration should be given to performing the test when not on such medications where feasible. This is especially relevant in gestational diabetes where the OGTT remains the main diagnostic method and for individuals where an early OGTT is indicated, many may be on anti-emetic medications for management of hyperemesis. Similarly, medical conditions that are associated with slow gastric emptying (e.g. Parkinson disease) or rapid gastric emptying (bariatric surgery) may affect the OGTT results. In the case of bariatric surgery, in particular, there is the suggestion that the OGTT should be avoided altogether due to the risk of post-bariatric surgery hypoglycaemia (Salehi et al., 2018). This is a novel and important finding that is not addressed in current guidelines for diagnosing diabetes (ElSayed et al., 2023).

My findings in Chapter 4 indicate that the majority of survivors of critical illness (>90%) who had stress hyperglycaemia during the admission continue to have dysglycaemia at 3 months post ICU discharge and are at high risk of progression to type 2 diabetes. Close follow up of this cohort for incident diabetes would be reasonable, although larger studies are required. In contrast, as reported in Chapter 8, individuals who have had a prior gastric bypass with a lower HbA1c had a increased association with post-bariatric surgery hypoglycaemia. Accordingly, in this cohort, clinicians should have a low threshold for evaluating symptoms of reactive hypoglycaemia. Guidelines are required in relation to the need for follow up of survivors of

critical illness associated stress hyperglycaemia and for the management of post-bariatric surgery hypoglycaemia.

### *9.3 Direction for future research*

The studies in this thesis have improved our understanding of the complex relationship between gastric emptying, incretin hormones and glucose levels. However, there remain substantial gaps in our knowledge. The effects of exogenous GLP-1 and GLP1-RAs to slow gastric emptying is variable and the precise reason for this is not known. It has been suggested that this may be related to genetic variation (Umapathysivam et al., 2023). Predicting which individuals may be prone to slow gastric emptying may assist with pre-operative management as a modified diet and longer duration of fasting may potentially be indicated to reduce the risk of aspiration in patients receiving GLP-1RA therapy associated with marked reductions in gastric emptying. Whether prokinetic agents discussed in Chapter 2 can accelerate GLP-1RA is another important research question, as this may allow residual gastric contents to be emptied in preparation for emergency surgery.

With continuous glucose monitoring being increasingly accessible, there is the opportunity to evaluate the shape of the glucose response curve in larger cohorts and in response to meals. If the pattern of the glucose response curve identified with continuous glucose monitoring (Chapter 5) can be used to estimate beta cell function and/or the risk of progression to future diabetes, this would represent a valuable biomarker. As survivors of critical illness with stress induced hyperglycaemia have a high risk of progressing to type 2 diabetes (Chapter 4), prospective studies are needed to further stratify their risk, potentially by evaluating the pattern of their glucose response curve and identifying strategies to reduce this risk.

In Chapter 7, I showed that there was no relationship between gastric distension and incretin hormone secretion. However, a non-nutritive method was used to cause distension. It remains to be determined whether distension with nutrients would stimulate GLP-1 secretion and if the response would be different in people with obesity or type 2 diabetes. Further insights into GLP-

1 physiology following bariatric surgery would be of value, as this appears to be a major factor in the pathophysiology of post-bariatric surgery hypoglycaemia. Larger prospective trials using GLP-1 antagonists to treat this condition are warranted given the very limited, and suboptimal, treatment currently available. Therapies that may slow gastric pouch emptying and/or small intestinal transit to diminish the GLP-1 response in this cohort also represent a potential novel approach to managing post-bariatric surgery hypoglycaemia.

My goal is to consolidate the findings reported in this thesis with post-doctoral studies. An improved characterization of gastric emptying and incretin physiology will, almost certainly, lead to more effective and personalized management of patients.

# Appendix 1. Accurate measurements of gastric emptying and gastrointestinal symptoms in the evaluation of glucagon-like peptide-1 receptor agonists

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# Accurate Measurements of Gastric Emptying and Gastrointestinal Symptoms in the Evaluation of Glucagon-like Peptide-1 Receptor Agonists

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The advent of glucagon-like peptide 1 (GLP-1) receptor agonists (RAs) and, recently, a GLP-1/glucose-dependent insulinotropic polypeptide (GIP) coagonist (tirzepatide), represents a paradigm shift in the management of type 2 diabetes and obesity. The use of these medications is likely to increase: An oral formulation of semaglutide is available, and small-molecule GLP-1 RAs (for example, orforglipron), as well as GLP-1/GIP/glucagon triple agonists (for example, retatrutide) are in late development. The impacts of GLP-1 RAs on glycemic control and body weight are variable, usually for uncertain reasons. Importantly, there is a lack of reliable information relating to their effects to slow gastric emptying and provoke gastrointestinal (GI) symptoms such as nausea and diarrhea, because these either have not been evaluated or have been assessed using suboptimal methods. Gastric emptying is a major determinant of postprandial glycemic increases (and, therefore, overall glycemic control), and GI symptoms affect the patients' tolerance of and adherence to GLP-1 RAs. The GLP-1 RAs exenatide twice daily and lixisenatide are short-acting (that is, exposure to relevant plasma concentrations is intermittent) and slow gastric emptying markedly and persistently. A diminished effect to delay gastric emptying after continuous exposure to GLP-1 (1) stimulated the belief that longer-acting GLP-1 RAs (administered once daily or once weekly)—most GLP-1 RAs used currently—would not slow gastric emptying with sustained use. However, liraglutide (2), exenatide once weekly (3), and subcutaneous semaglutide (4) are now recognized to have a durable effect to delay gastric emptying, sometimes markedly. With both short- and longer-acting GLP-1 RAs, the slowing of gastric emptying is greater when baseline emptying is relatively faster and predictive of postprandial glucose lowering (5). There are also ongoing misconceptions related to the diagnosis and clinical implications of gastroparesis (including GLP-1 RA–induced): 1) delayed gastric emptying and symptoms, such as nausea, correlate weakly (6); 2) suboptimal measurement techniques continue to be used widely to quantify gastric emptying and symptoms (7, 8); and 3) other manifestations of gastroparesis such as effects on glycemia and the pharmacokinetics of oral drugs are poorly appreciated (6).

Scintigraphic techniques (the use of radioisotopically labeled meals and a gamma camera) to measure gastric emptying were developed in the 1970s and, surprisingly, remain the gold standard. With the use of scintigraphy, it was shown that delayed gastric emptying occurred frequently (30% to 50% of cases) in patients with long-standing type 1 or type 2 diabetes, providing a plausible basis for upper GI symptoms, which were recognized to also occur frequently (6). Pharmacologically induced acceleration of delayed gastric emptying was used to manage symptoms, with several drugs developed for this purpose (for example, cisapride). It was then appreciated that marked delay in gastric emptying may occur in the absence of symptoms, whereas in persons with bothersome, chronic symptoms, gastric emptying is frequently normal (6). Unsurprisingly, the efficacy of prokinetic therapy for the management of GI symptoms in patients with diabetic gastroparesis has proven poor (6).

In the evaluation of GLP-1 RAs, gastric emptying and GI symptoms should be measured optimally. Scintigraphy is costly, involves exposure to radiation, and may not be readily available, but the stable isotope breath test can evaluate emptying of both solids and liquids without a radiation burden and is a validated alternative (7). This test involves consumption of a meal containing $^{13}$C-labeled fatty acids that are hydrolyzed after they leave the stomach, are rapidly absorbed, and are oxidized in the liver to yield $^{13}CO_2$ detected in breath using infrared spectrometry. Mathematical modeling generates gastric emptying curves (7). The acetaminophen absorption technique, used extensively in clinical trials, has substantial limitations (6) including poorly validated assumptions about its absorption kinetics and its unsuitability to measure gastric emptying of solids. Its use in isolation should be avoided. Other assessments of gastric sensorimotor function (for example, accommodation testing) are likely to be useful in profiling both positive and deleterious effects of GLP-1RAs.

In clinical trials of GLP-1 RAs, GI symptoms are usually evaluated solely by participant self-report, which is unreliable (for example, symptoms are frequently not reported) despite the availability of validated measures used in functional GI disorders (for example, functional dyspepsia, irritable bowel syndrome) and mandated by regulatory bodies concerned with these conditions (8). Examples are the Gastroparesis Cardinal Symptom Index (GCSI), a daily diary that quantifies the severity of 9 symptoms, including nausea and loss of appetite, and the Patient Assessment of Upper Gastrointestinal Disorder Symptom Severity Index (PAGI-SYM), which contains 20 items, has a 2-week recall, and encompasses socioeconomic and psychological domains (8). The development of an instrument specific for GI adverse effects of GLP-1 RAs is to be encouraged.

Diabetic gastroparesis may predispose to poor glycemic control and compromise the efficacy of drugs that require a rapid onset of action, such as L-dopa. It has been recently appreciated, based on anecdotal reports, that

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long-acting GLP-1 RAs may lead to the prolonged retention of gastric contents, evident at endoscopy or surgery (9), increasing the risk for aspiration. A statement from the American Society of Anesthesiologists suggests that short-acting GLP-1 RAs should be discontinued the day before, and longer-acting GLP-1 RAs a week before, an elective procedure (9). This guideline lacks a strong evidence base. Moreover, short-acting GLP-1 RAs may slow gastric emptying markedly in doses that are substantially less than those used for glucose lowering (6), so 1 day, or in the case of long-acting GLP-1 RA, 1 week, may be insufficient. Furthermore, retained gastric contents is not unusual in cases of diabetes, nor is it pathognomonic of gastroparesis, and gastric emptying of larger, nondigestible solids occurs in the interdigestive periods rather than after meals, highlighting the necessity for further evaluation.

Slowing of gastric emptying and the induction of GI symptoms are likely to be relevant to weight loss induced by GLP-1 RAs. An obesity subclassification of a "hungry gut," associated with relatively more rapid gastric emptying, has been proposed (10), in which strategies that slow gastric emptying are logical.

We conclude that the effects of GLP-1 RAs/coagonists on gastric emptying and GI symptoms have major implications, both positive and deleterious. In clinical trials, GI symptoms should be evaluated by validated instruments. Measurement of gastric emptying, using a precise technique, should be a mandatory component of approval packages for GLP-1 RAs. Implementation of these relatively simple changes is likely to explain some of the variability of the therapeutic response to GLP-1 RAs and to lead to their more personalized use.

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## References

1. Umapathysivam MM, Lee MY, Jones KL, et al. Comparative effects of prolonged and intermittent stimulation of the glucagon-like peptide 1 receptor on gastric emptying and glycemia. Diabetes. 2014; 63:785-790. [PMID: 24089511] doi:10.2337/db13-0893
2. Halawi H, Khemani D, Eckert D, et al. Effects of liraglutide on weight, satiation, and gastric functions in obesity: a randomised, placebo-controlled pilot trial. Lancet Gastroenterol Hepatol. 2017;2:890-899. [PMID: 28958851] doi:10.1016/S2468-1253(17)30285-6
3. Jones KL, Huynh LQ, Hatzinikolas S, et al. Exenatide once weekly slows gastric emptying of solids and liquids in healthy, overweight people at steady-state concentrations. Diabetes Obes Metab. 2020;22: 788-797. [PMID: 31903712] doi:10.1111/dom.13956
4. Jensterle M, Ferjan S, Lezaic L, et al. Semaglutide delays 4-hour gastric emptying in women with polycystic ovary syndrome and obesity. Diabetes Obes Metab. 2023;25:975-984. [PMID: 36511825] doi:10.1111/dom.14944
5. Linnebjerg H, Park S, Kothare PA, et al. Effect of exenatide on gastric emptying and relationship to postprandial glycemia in type 2 diabetes. Regul Pept. 2008;151:123-129. [PMID: 18675854] doi:10.1016/j.regpep.2008.07.003
6. Jalleh RJ, Jones KL, Rayner CK, et al. Normal and disordered gastric emptying in diabetes: recent insights into (patho)physiology, management and impact on glycaemic control. Diabetologia. 2022;65:1981-1993. [PMID: 36194250] doi:10.1007/s00125-022-05796-1
7. Trahair LG, Nauck MA, Wu T, et al. Measurement of gastric emptying using a 13C-octanoic acid breath test with Wagner-Nelson analysis and scintigraphy in type 2 diabetes. Exp Clin Endocrinol Diabetes. 2022;130:751-757. [PMID: 35231948] doi:10.1055/a-1784-6185
8. Du YT, Rayner CK, Jones KL, et al. Gastrointestinal symptoms in diabetes: prevalence, assessment, pathogenesis, and management. Diabetes Care. 2018;41:627-637. [PMID: 29463666] doi:10.2337/dc17-1536
9. American Society of Anesthesiologists. American Society of Anesthesiologists consensus-based guidance on preoperative management of patients (adults and children) on glucagon-like peptide-1 (GLP-1) receptor agonists. 29 June 2023. Accessed at www.asahq.org/about-asa/newsroom/news-releases/2023/06/american-society-of-anesthesiologists-consensus-based-guidance-on-preoperative on 18 Jul 2023.
10. Acosta A, Camilleri M, Abu Dayyeh B, et al. Selection of antiobesity medications based on phenotypes enhances weight loss: a pragmatic trial in an obesity clinic. Obesity (Silver Spring). 2021;29: 662-671. [PMID: 33759389] doi:10.1002/oby.23120

# Bibliography


ABELL, T. L., CAMILLERI, M., DONOHOE, K., HASLER, W. L., LIN, H. C., MAURER, A. H., MCCALLUM, R. W., NOWAK, T., NUSYNOWITZ, M. L., PARKMAN, H. P., SHREVE, P., SZARKA, L. A., SNAPE, W. J., JR., ZIESSMAN, H. A., AMERICAN, N., MOTILITY, S. & THE SOCIETY OF NUCLEAR, M. 2008. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *J Nucl Med Technol,* 36(1)**:**44-54.

ABELL, T. L., GARCIA, L. M., WIENER, G. J., WO, J. M., BULAT, R. S. & SMITH, N. 2021. Effect of Oral CNSA-001 (sepiapterin, PTC923) on gastric accommodation in women with diabetic gastroparesis: A randomized, placebo-controlled, Phase 2 trial. *J Diabetes Complications,* 35(9)**:**107961.

ABRAHAMSSON, N., BÖRJESSON, J. L., SUNDBOM, M., WIKLUND, U., KARLSSON, F. A. & ERIKSSON, J. W. 2016. Gastric bypass reduces symptoms and hormonal responses in hypoglycemia. *Diabetes,* 65(9)**:**2667-75.

ABRAHAMSSON, N., ENGSTRÖM, B. E., SUNDBOM, M. & KARLSSON, F. A. 2013. GLP1 analogs as treatment of postprandial hypoglycemia following gastric bypass surgery: a potential new indication? *Eur J Endocrinol,* 169(6)**:**885-9.

ABU DAYYEH, B. K., MASELLI, D. B., RAPAKA, B., LAVIN, T., NOAR, M., HUSSAN, H., CHAPMAN, C. G., POPOV, V., JIRAPINYO, P., ACOSTA, A., VARGAS, E. J., STORM, A. C., BAZERBACHI, F., RYOU, M., FRENCH, M., NORIA, S., MOLINA, D. & THOMPSON, C. C. 2021. Adjustable intragastric balloon for treatment of obesity: a multicentre, open-label, randomised clinical trial. *Lancet,* 398(10315)**:**1965-73.

AIGNER, L., BECKER, B., GERKEN, S., QUAST, D. R., MEIER, J. J. & NAUCK, M. A. 2021. Day-to-day variations in fasting plasma glucose do not influence gastric emptying in subjects with type 1 diabetes. *Diabetes Care,* 44(2)**:**479-88.

AL-NAJIM, W., DOCHERTY, N. G. & LE ROUX, C. W. 2018. Food intake and eating behavior after bariatric surgery. *Physiol Rev,* 98(3)**:**1113-41.

AL-SAFFAR, A., LENNERNAS, H. & HELLSTROM, P. M. 2019. Gastroparesis, metoclopramide, and tardive dyskinesia: Risk revisited. *Neurogastroenterol Motil,* 31(11)**:**13617.

ALICUBEN, E. T., SAMAAN, J. S., HOUGHTON, C. C., SOFFER, E., LIPHAM, J. C. & SAMAKAR, K. 2021. Sleeve gastrectomy as a novel procedure for gastroparesis. *Am Surg,* 87(8)**:**1287-91.

AMERICAN DIABETES ASSOCIATION 2010. Diagnosis and classification of diabetes mellitus. *Diabetes Care,* 33 Suppl 1(Suppl 1)**:**S62-9.

ANG, D. 2011. Measurement of gastric accommodation: a reappraisal of conventional and emerging modalities. *Neurogastroenterol Motil,* 23(4)**:**287-91.

ARORA, T., SEYFRIED, F., DOCHERTY, N. G., TREMAROLI, V., LE ROUX, C. W., PERKINS, R. & BACKHED, F. 2017. Diabetes-associated microbiota in fa/fa rats is modified by Roux-en-Y gastric bypass. *ISME J,* 11(9)**:**2035-46.

ARSLANIAN, S., EL GHORMLI, L., YOUNG KIM, J., BACHA, F., CHAN, C., ISMAIL, H. M., LEVITT KATZ, L. E., LEVITSKY, L., TRYGGESTAD, J. B., WHITE, N. H. & GROUP, T. S. 2019. The Shape of the Glucose Response Curve During an Oral Glucose Tolerance Test: Forerunner of Heightened Glycemic Failure Rates and Accelerated Decline in beta-Cell Function in TODAY. *Diabetes Care,* 42(1)**:**164-72.

ASTLES, J. R., SEDOR, F. A. & TOFFALETTI, J. G. 1996. Evaluation of the YSI 2300 glucose analyzer: algorithm-corrected results are accurate and specific. *Clin Biochem,* 29(1)**:**27-31.

BAGGIO, L. L. & DRUCKER, D. J. 2007. Biology of incretins: GLP-1 and GIP. *Gastroenterology,* 132(6)**:**2131-57.

BAR-OR, D., RAEL, L. T., MADAYAG, R. M., BANTON, K. L., TANNER, A., 2ND, ACUNA, D. L., LIESER, M. J., MARSHALL, G. T., MAINS, C. W. & BRODY, E. 2019. Stress Hyperglycemia in Critically Ill Patients: Insight Into Possible Molecular Pathways. *Front Med (Lausanne),* 6**:**54.

BARRINGTON, P., CHIEN, J. Y., SHOWALTER, H. D., SCHNECK, K., CUI, S., TIBALDI, F., ELLIS, B. & HARDY, T. A. 2011. A 5-week study of the pharmacokinetics and pharmacodynamics of LY2189265, a

novel, long-acting glucagon-like peptide-1 analogue, in patients with type 2 diabetes. *Diabetes Obes Metab,* 13(5)**:**426-33.

BARTHOLOME, R., SALDEN, B., VROLIJK, M. F., TROOST, F. J., MASCLEE, A., BAST, A. & HAENEN, G. R. 2015. Paracetamol as a post prandial marker for gastric emptying, a food-drug interaction on absorption. *PLoS One,* 10(9)**:**0136618.

BERGMAN, M. 2021. The 1-hour plasma glucose: common link across the glycemic spectrum. *Front. Endocrinol.,* 12**:**752329.

BERGMAN, M., CHETRIT, A., ROTH, J., JAGANNATHAN, R., SEVICK, M. & DANKNER, R. 2016. One-hour post-load plasma glucose level during the OGTT predicts dysglycemia: Observations from the 24year follow-up of the Israel Study of Glucose Intolerance, Obesity and Hypertension. *Diabetes Res Clin Pract,* 120**:**221-8.

BERGMAN, M., JAGANNATHAN, R., BUYSSCHAERT, M., PAREEK, M., OLSEN, M. H., NILSSON, P. M., MEDINA, J. L., ROTH, J., CHETRIT, A., GROOP, L. & DANKNER, R. 2018. Lessons learned from the 1-hour post-load glucose level during OGTT: Current screening recommendations for dysglycaemia should be revised. *Diabetes Metab Res Rev,* 34(5)**:**2992.

BERGMANN, N. C., LUND, A., GASBJERG, L. S., MEESSEN, E. C. E., ANDERSEN, M. M., BERGMANN, S., HARTMANN, B., HOLST, J. J., JESSEN, L., CHRISTENSEN, M. B., VILSBOLL, T. & KNOP, F. K. 2019. Effects of combined GIP and GLP-1 infusion on energy intake, appetite and energy expenditure in overweight/obese individuals: a randomised, crossover study. *Diabetologia,* 62(4)**:**665-75.

BHARUCHA, A. E., KUDVA, Y., BASU, A., CAMILLERI, M., LOW, P. A., VELLA, A. & ZINSMEISTER, A. R. 2015. Relationship between glycemic control and gastric emptying in poorly controlled type 2 diabetes. *Clin Gastroenterol Hepatol,* 13(3)**:**466-76.

BOGDANET, D., O'SHEA, P., LYONS, C., SHAFAT, A. & DUNNE, F. 2020. The Oral Glucose Tolerance Test-Is It Time for a Change?-A Literature Review with an Emphasis on Pregnancy. *J Clin Med,* 9(11)**:**3451.

BORG, M. J., RAYNER, C. K., JONES, K. L., HOROWITZ, M., XIE, C. & WU, T. 2020. Gastrointestinal mechanisms underlying the cardiovascular effect of metformin. *Pharmaceuticals (Basel),* 13(11)**:**410.

BRENER, W., HENDRIX, T. R. & MCHUGH, P. R. 1983. Regulation of the gastric emptying of glucose. *Gastroenterology,* 85(1)**:**76-82.

BRIX, J. M., KOPP, H. P., HÖLLERL, F., SCHERNTHANER, G. H., LUDVIK, B. & SCHERNTHANER, G. 2019. Frequency of hypoglycaemia after different bariatric surgical procedures. *Obes Facts,* 12(4)**:**397-406.

BUCHWALD, H., ESTOK, R., FAHRBACH, K., BANEL, D., JENSEN, M. D., PORIES, W. J., BANTLE, J. P. & SLEDGE, I. 2009. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med,* 122(3)**:**248-56.

CAMILLERI, M. 2019. Gastrointestinal hormones and regulation of gastric emptying. *Curr Opin Endocrinol Diabetes Obes,* 26(1)**:**3-10.

CAMILLERI, M. & ATIEH, J. 2021. New developments in prokinetic therapy for gastric motility disorders. *Front Pharmacol,* 12**:**711500.

CAMILLERI, M. & VASSALLO, M. 1991. Small intestinal motility and transit in disease. *Baillieres Clin Gastroenterol,* 5(2)**:**431-51.

CAMPBELL, J. E. 2021. Targeting the GIPR for obesity: To agonize or antagonize? Potential mechanisms. *Mol Metab,* 46**:**101139.

CAMPBELL, J. E. & DRUCKER, D. J. 2013. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab,* 17(6)**:**819-37.

CAPRISTO, E., PANUNZI, S., DE GAETANO, A., SPUNTARELLI, V., BELLANTONE, R., GIUSTACCHINI, P., BIRKENFELD, A. L., AMIEL, S., BORNSTEIN, S. R., RAFFAELLI, M. & MINGRONE, G. 2018. Incidence of hypoglycemia after gastric bypass vs sleeve gastrectomy: a randomized trial. *J Clin Endocrinol Metab,* 103(6)**:**2136-46.

CARLIN, J. L., LIEBERMAN, V. R., DAHAL, A., KEEFE, M. S., XIAO, C., BIRZNIEKS, G., ABELL, T. L., LEMBO, A., PARKMAN, H. P. & POLYMEROPOULOS, M. H. 2021. Efficacy and safety of tradipitant in patients with diabetic and idiopathic gastroparesis in a randomized, placebo-controlled trial. *Gastroenterology,* 160(1)**:**76-87.

CHAIKOMIN, R., RUSSO, A., RAYNER, C. K., FEINLE-BISSET, C., O'DONOVAN, D. G., HOROWITZ, M. & JONES, K. L. 2006. Effects of lipase inhibition on gastric emptying and alcohol absorption in healthy subjects. *Br J Nutr,* 96(5)**:**883-7.

CHAIKOMIN, R., WU, K. L., DORAN, S., JONES, K. L., SMOUT, A. J., RENOOIJ, W., HOLLOWAY, R. H., MEYER, J. H., HOROWITZ, M. & RAYNER, C. K. 2007. Concurrent duodenal manometric and impedance recording to evaluate the effects of hyoscine on motility and flow events, glucose absorption, and incretin release. *Am J Physiol Gastrointest Liver Physiol,* 292(4)**:**G1099-104.

CHANG, A. M. & HALTER, J. B. 2003. Aging and insulin secretion. *Am J Physiol Endocrinol Metab,* 284(1)**:**E7-12.

CHANG, J., RUSSO, A., BOUND, M., RAYNER, C. K., JONES, K. L. & HOROWITZ, M. 2012. A 25-year longitudinal evaluation of gastric emptying in diabetes. *Diabetes Care,* 35(12)**:**2594-6.

CHANG, S. H., STOLL, C. R., SONG, J., VARELA, J. E., EAGON, C. J. & COLDITZ, G. A. 2014. The effectiveness and risks of bariatric surgery: an updated systematic review and meta-analysis, 2003-2012. *JAMA Surg,* 149(3)**:**275-87.

CHAPPLE, L. S., SUMMERS, M. J., WEINEL, L. M., ABDELHAMID, Y. A., KAR, P., HATZINIKOLAS, S., CALNAN, D., BILLS, M., LANGE, K., POOLE, A., O'CONNOR, S. N., HOROWITZ, M., JONES, K. L., DEANE, A. M. & CHAPMAN, M. J. 2021. Effects of Standard vs Energy-Dense Formulae on Gastric Retention, Energy Delivery, and Glycemia in Critically Ill Patients. *JPEN J Parenter Enteral Nutr,* 45(4)**:**710-9.

CHARLES, F., EVANS, D. F., CASTILLO, F. D. & WINGATE, D. L. 1994. Daytime ingestion of alcohol alters nighttime jejunal motility in man. *Dig Dis Sci,* 39(1)**:**51-8.

COLLINS, P. J., HOROWITZ, M., COOK, D. J., HARDING, P. E. & SHEARMAN, D. J. 1983. Gastric emptying in normal subjects--a reproducible technique using a single scintillation camera and computer system. *Gut,* 24(12)**:**1117-25.

CRAIG, C. M., LAWLER, H. M., LEE, C. J. E., TAN, M., DAVIS, D. B., TONG, J., GLODOWSKI, M., ROGOWITZ, E., KARAMAN, R., MCLAUGHLIN, T. L. & PORTER, L. 2021. PREVENT: a randomized, placebo-controlled crossover trial of avexitide for treatment of post-bariatric hypoglycemia. *J Clin Endocrinol Metab,* 106(8)**:**3235-48.

CRAIG, C. M., LIU, L. F., DEACON, C. F., HOLST, J. J. & MCLAUGHLIN, T. L. 2017. Critical role for GLP-1 in symptomatic post-bariatric hypoglycaemia. *Diabetologia,* 60(3)**:**531-40.

CRAIG, C. M., LIU, L. F., NGUYEN, T., PRICE, C., BINGHAM, J. & MCLAUGHLIN, T. L. 2018. Efficacy and pharmacokinetics of subcutaneous exendin (9-39) in patients with post-bariatric hypoglycaemia. *Diabetes Obes Metab,* 20(2)**:**352-61.

DAHL, K., BROOKS, A., ALMAZEDI, F., HOFF, S. T., BOSCHINI, C. & BAEKDAL, T. A. 2021. Oral semaglutide improves postprandial glucose and lipid metabolism, and delays gastric emptying, in subjects with type 2 diabetes. *Diabetes Obes Metab,* 23(7)**:**1594-603.

DALY, A., HARTNELL, S., BOUGHTON, C. K. & EVANS, M. 2021. Hybrid closed-loop to manage gastroparesis in people with type 1 diabetes: a case series. *J Diabetes Sci Technol,* 15(6)**:**1216-23.

DATZ, F. L., CHRISTIAN, P. E. & MOORE, J. 1987. Gender-related differences in gastric emptying. *J Nucl Med,* 28(7)**:**1204-7.

DE HEIDE, L. J. M., WOUDA, S. H. T., PETERS, V. J. T., OOSTERWERFF-SUIKER, M., GERDES, V. A., EMOUS, M. & VAN BEEK, A. P. 2023. Medical and surgical treatment of postbariatric hypoglycaemia: Retrospective data from daily practice. *Diabetes Obes Metab,* 25(3)**:**735-47.

DEANE, A. M., CHAPMAN, M. J., FRASER, R. J., SUMMERS, M. J., ZAKNIC, A. V., STOREY, J. P., JONES, K. L., RAYNER, C. K. & HOROWITZ, M. 2010a. Effects of exogenous glucagon-like peptide-1 on gastric emptying and glucose absorption in the critically ill: relationship to glycemia. *Crit Care Med,* 38(5)**:**1261-9.

DEANE, A. M., NGUYEN, N. Q., STEVENS, J. E., FRASER, R. J., HOLLOWAY, R. H., BESANKO, L. K., BURGSTAD, C., JONES, K. L., CHAPMAN, M. J., RAYNER, C. K. & HOROWITZ, M. 2010b. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. *J Clin Endocrinol Metab,* 95(1)**:**215-21.

DEDEN, L. N., COOIMAN, M. I., AARTS, E. O., JANSSEN, I. M. C., GOTTHARDT, M., HENDRICKX, B. W. & BERENDS, F. J. 2017. Gastric pouch emptying of solid food in patients with successful and

unsuccessful weight loss after Roux-en-Y gastric bypass surgery. *Surg Obes Relat Dis,* 13(11)**:**1840-6.

DEEKS, J. J., ALTMAN, D. G. & BRADBURN, M. J. 2001. Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. *In:* EGGER, M., SMITH, G. & ALTMAN, D. (eds.) *Systematic reviews in health care.* Wiley.

DEFRONZO, R. A., TOBIN, J. D. & ANDRES, R. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol,* 237(3)**:**E214-23.

DETTORI, J. R., NORVELL, D. C. & CHAPMAN, J. R. 2022. Fixed-Effect vs Random-Effects Models for Meta-Analysis: 3 Points to Consider. *Global Spine J,* 12(7)**:**1624-6.

DING, W. G., RENSTROM, E., RORSMAN, P., BUSCHARD, K. & GROMADA, J. 1997. Glucagon-like peptide I and glucose-dependent insulinotropic polypeptide stimulate Ca2+-induced secretion in rat alpha-cells by a protein kinase A-mediated mechanism. *Diabetes,* 46(5)**:**792-800.

DRUCKER, D. J. 2018. Mechanisms of action and therapeutic application of glucagon-like peptide-1. *Cell Metab,* 27(4)**:**740-56.

DRUCKER, D. J., BUSE, J. B., TAYLOR, K., KENDALL, D. M., TRAUTMANN, M., ZHUANG, D., PORTER, L. & GROUP, D.-S. 2008. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet,* 372(9645)**:**1240-50.

DRUCKER, D. J. & NAUCK, M. A. 2006. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet,* 368(9548)**:**1696-705.

DU, Y. T., RAYNER, C. K., JONES, K. L., TALLEY, N. J. & HOROWITZ, M. 2018. Gastrointestinal symptoms in diabetes: prevalence, assessment, pathogenesis, and management. *Diabetes Care,* 41(3)**:**627-37.

DUCROTTE, P., COFFIN, B., BONAZ, B., FONTAINE, S., BRULEY DES VARANNES, S., ZERBIB, F., CAIAZZO, R., GRIMAUD, J. C., MION, F., HADJADJ, S., VALENSI, P. E., VUITTON, L., CHARPENTIER, G., ROPERT, A., ALTWEGG, R., POUDEROUX, P., DORVAL, E., DAPOIGNY, M., DUBOC, H., BENHAMOU, P. Y., SCHMIDT, A., DONNADIEU, N., GOURCEROL, G., GUERCI, B. & GROUP, E. R. 2020. Gastric electrical stimulation reduces refractory vomiting in a randomized crossover trial. *Gastroenterology,* 158(3)**:**506-14.

DUNGAN, K. M., BRAITHWAITE, S. S. & PREISER, J. C. 2009. Stress hyperglycaemia. *Lancet,* 373(9677)**:**1798-807.

ELSAYED, N. A., ALEPPO, G., ARODA, V. R., BANNURU, R. R., BROWN, F. M., BRUEMMER, D., COLLINS, B. S., HILLIARD, M. E., ISAACS, D., JOHNSON, E. L., KAHAN, S., KHUNTI, K., LEON, J., LYONS, S. K., PERRY, M. L., PRAHALAD, P., PRATLEY, R. E., SELEY, J. J., STANTON, R. C., GABBAY, R. A. & ON BEHALF OF THE AMERICAN DIABETES, A. 2023. 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes-2023. *Diabetes Care,* 46(Suppl 1)**:**S19-S40.

EMOUS, M., WOLFFENBUTTEL, B. H. R., TOTTE, E. & VAN BEEK, A. P. 2017. The short- to mid-term symptom prevalence of dumping syndrome after primary gastric-bypass surgery and its impact on health-related quality of life. *Surg Obes Relat Dis,* 13(9)**:**1489-500.

EUROPEAN MEDICINES AGENCY *Liraglutide: Scientific conclusions and grounds for the variation to the terms of the marketing authorisation(s). In: Committee for Medicinal Products for Human Use.* [Online]. Available: https://www.ema.europa.eu/en/documents/scientific-conclusion/victoza-h-c-psusa-00001892-201812-epar-scientific-conclusions-grounds-recommending-variation-terms_en.pdf [Accessed 9 Apr 2022].

FAERCH, K., TOREKOV, S. S., VISTISEN, D., JOHANSEN, N. B., WITTE, D. R., JONSSON, A., PEDERSEN, O., HANSEN, T., LAURITZEN, T., SANDBAEK, A., HOLST, J. J. & JORGENSEN, M. E. 2015. GLP-1 Response to Oral Glucose Is Reduced in Prediabetes, Screen-Detected Type 2 Diabetes, and Obesity and Influenced by Sex: The ADDITION-PRO Study. *Diabetes,* 64(7)**:**2513-25.

FARR, O. M., SOFOPOULOS, M., TSOUKAS, M. A., DINCER, F., THAKKAR, B., SAHIN-EFE, A., FILIPPAIOS, A., BOWERS, J., SRNKA, A., GAVRIELI, A., KO, B. J., LIAKOU, C., KANYUCH, N., TSELENI-BALAFOUTA, S. & MANTZOROS, C. S. 2016. GLP-1 receptors exist in the parietal cortex, hypothalamus and medulla of human brains and the GLP-1 analogue liraglutide alters brain activity related to highly desirable food cues in individuals with diabetes: a crossover, randomised, placebo-controlled trial. *Diabetologia,* 59(5)**:**954-65.

FEINLE, C., GRUNDY, D., OTTO, B. & FRIED, M. 2000. Relationship between increasing duodenal lipid doses, gastric perception, and plasma hormone levels in humans. *Am J Physiol Regul Integr Comp Physiol,* 278(5)**:**R1217-23.

FRANKE, A., TEYSSEN, S., HARDER, H. & SINGER, M. V. 2004. Effect of ethanol and some alcoholic beverages on gastric emptying in humans. *Scand J Gastroenterol,* 39(7)**:**638-44.

FRASER, R. J., HOROWITZ, M., MADDOX, A. F., HARDING, P. E., CHATTERTON, B. E. & DENT, J. 1990. Hyperglycaemia slows gastric emptying in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia,* 33(11)**:**675-80.

FRIAS, J. P., NAUCK, M. A., VAN, J., KUTNER, M. E., CUI, X., BENSON, C., URVA, S., GIMENO, R. E., MILICEVIC, Z., ROBINS, D. & HAUPT, A. 2018. Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. *Lancet,* 392(10160)**:**2180-93.

FRIEDENBERG, F. K., PALIT, A., PARKMAN, H. P., HANLON, A. & NELSON, D. B. 2008. Botulinum toxin A for the treatment of delayed gastric emptying. *Am J Gastroenterol,* 103(2)**:**416-23.

FUKUDA, M. 2021. The Role of GIP Receptor in the CNS for the Pathogenesis of Obesity. *Diabetes,* 70(9)**:**1929-37.

GABER, A. O., OXLEY, D., KARAS, J., CARDOSO, S., HATHAWAY, D., SHOKOUH-AMIRI, M. H., JENSEN, S. L. & ABELL, T. L. 1991. Changes in gastric emptying in recipients of successful combined pancreas-kidney transplants. *Dig Dis,* 9(6)**:**437-43.

GAJENDRAN, M., SAROSIEK, I. & MCCALLUM, R. 2021. Metoclopramide nasal spray for management of symptoms of acute and recurrent diabetic gastroparesis in adults. *Expert Rev Endocrinol Metab,* 16(2)**:**25-35.

GASBJERG, L. S., HELSTED, M. M., HARTMANN, B., SPARRE-ULRICH, A. H., VEEDFALD, S., STENSEN, S., LANNG, A. R., BERGMANN, N. C., CHRISTENSEN, M. B., VILSBOLL, T., HOLST, J. J., ROSENKILDE, M. M. & KNOP, F. K. 2020. GIP and GLP-1 receptor antagonism during a meal in healthy individuals. *J Clin Endocrinol Metab,* 105(3)**:**175.

GEERSING, G. J., BOUWMEESTER, W., ZUITHOFF, P., SPIJKER, R., LEEFLANG, M. & MOONS, K. G. 2012. Search filters for finding prognostic and diagnostic prediction studies in Medline to enhance systematic reviews. *PLoS One,* 7(2)**:**32844.

GENCO, A., BRUNI, T., DOLDI, S. B., FORESTIERI, P., MARINO, M., BUSETTO, L., GIARDIELLO, C., ANGRISANI, L., PECCHIOLI, L., STORNELLI, P., PUGLISI, F., ALKILANI, M., NIGRI, A., DI LORENZO, N., FURBETTA, F., CASCARDO, A., CIPRIANO, M., LORENZO, M. & BASSO, N. 2005. BioEnterics intragastric balloon: the Italian experience with 2,515 patients. *Obes Surg,* 15(8)**:**1161-4.

GENTILCORE, D., BRYANT, B., WISHART, J. M., MORRIS, H. A., HOROWITZ, M. & JONES, K. L. 2005. Acarbose attenuates the hypotensive response to sucrose and slows gastric emptying in the elderly. *Am J Med,* 118(11)**:**1289.

GENTILCORE, D., CHAIKOMIN, R., JONES, K. L., RUSSO, A., FEINLE-BISSET, C., WISHART, J. M., RAYNER, C. K. & HOROWITZ, M. 2006. Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. *J Clin Endocrinol Metab,* 91(6)**:**2062-7.

GEYER, M. C., SULLIVAN, T., TAI, A., MORTON, J. M., EDWARDS, S., MARTIN, A. J., PERANO, S. J., GAGLIARDI, L., RAYNER, C. K., HOROWITZ, M. & COUPER, J. J. 2019. Exenatide corrects postprandial hyperglycaemia in young people with cystic fibrosis and impaired glucose tolerance: A randomized crossover trial. *Diabetes Obes Metab,* 21(3)**:**700-4.

GOLDFINE, A. B., MUN, E. C., DEVINE, E., BERNIER, R., BAZ-HECHT, M., JONES, D. B., SCHNEIDER, B. E., HOLST, J. J. & PATTI, M. E. 2007. Patients with neuroglycopenia after gastric bypass surgery have exaggerated incretin and insulin secretory responses to a mixed meal. *J Clin Endocrinol Metab,* 92(12)**:**4678-85.

GOYAL, R. K. 2021. Gastric emptying abnormalities in diabetes mellitus. *N Engl J Med,* 384(18)**:**1742-51.

GOYAL, R. K., CRISTOFARO, V. & SULLIVAN, M. P. 2019. Rapid gastric emptying in diabetes mellitus: Pathophysiology and clinical importance. *J Diabetes Complications,* 33(11)**:**107414.

GROMADA, J., HOLST, J. J. & RORSMAN, P. 1998. Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch,* 435(5)**:**583-94.

GROVER, M., FARRUGIA, G., LURKEN, M. S., BERNARD, C. E., FAUSSONE-PELLEGRINI, M. S., SMYRK, T. C., PARKMAN, H. P., ABELL, T. L., SNAPE, W. J., HASLER, W. L., UNALP-ARIDA, A., NGUYEN, L., KOCH, K. L., CALLES, J., LEE, L., TONASCIA, J., HAMILTON, F. A., PASRICHA, P. J. & CONSORTIUM, N. G. C. R. 2011. Cellular changes in diabetic and idiopathic gastroparesis. *Gastroenterology,* 140(5)**:**1575-85.

GUARINO, D., MORICONI, D., MARI, A., REBELOS, E., COLLIGIANI, D., BALDI, S., ANSELMINO, M., FERRANNINI, E. & NANNIPIERI, M. 2019. Postprandial hypoglycaemia after Roux-en-Y gastric bypass in individuals with type 2 diabetes. *Diabetologia,* 62(1)**:**178-86.

HALAWI, H., KHEMANI, D., ECKERT, D., O'NEILL, J., KADOUH, H., GROTHE, K., CLARK, M. M., BURTON, D. D., VELLA, A., ACOSTA, A., ZINSMEISTER, A. R. & CAMILLERI, M. 2017. Effects of liraglutide on weight, satiation, and gastric functions in obesity: a randomised, placebo-controlled pilot trial. *Lancet Gastroenterol Hepatol,* 2(12)**:**890-9.

HANNON, T. S., KAHN, S. E., UTZSCHNEIDER, K. M., BUCHANAN, T. A., NADEAU, K. J., ZEITLER, P. S., EHRMANN, D. A., ARSLANIAN, S. A., CAPRIO, S., EDELSTEIN, S. L., SAVAGE, P. J., MATHER, K. J. & CONSORTIUM, R. 2018. Review of methods for measuring beta-cell function: Design considerations from the Restoring Insulin Secretion (RISE) Consortium. *Diabetes Obes Metab,* 20(1)**:**14-24.

HATONEN, K. A., VIRTAMO, J., ERIKSSON, J. G., PERALA, M. M., SINKKO, H. K., LEIVISKA, J. & VALSTA, L. M. 2012. Modifying effects of alcohol on the postprandial glucose and insulin responses in healthy subjects. *Am J Clin Nutr,* 96(1)**:**44-9.

HEBBARD, G. S., SUN, W. M., BOCHNER, F. & HOROWITZ, M. 1995. Pharmacokinetic Considerations in Gastrointestinal Motor Disorders. *Clinical Pharmacokinetics,* 28(1)**:**41-66.

HEDDLE, R., DENT, J., TOOULI, J. & READ, N. W. 1988. Topography and measurement of pyloric pressure waves and tone in humans. *Am J Physiol,* 255(4 Pt 1)**:**G490-7.

HJERPSTED, J. B., FLINT, A., BROOKS, A., AXELSEN, M. B., KVIST, T. & BLUNDELL, J. 2018. Semaglutide improves postprandial glucose and lipid metabolism, and delays first-hour gastric emptying in subjects with obesity. *Diabetes Obes Metab,* 20(3)**:**610-9.

HOLST, J. J. 2007. The physiology of glucagon-like peptide 1. *Physiol Rev,* 87(4)**:**1409-39.

HOLST, J. J., MADSBAD, S., BOJSEN-MOLLER, K. N., SVANE, M. S., JORGENSEN, N. B., DIRKSEN, C. & MARTINUSSEN, C. 2018. Mechanisms in bariatric surgery: Gut hormones, diabetes resolution, and weight loss. *Surg Obes Relat Dis,* 14(5)**:**708-14.

HOLT, S. 1981. Observations on the relation between alcohol absorption and the rate of gastric emptying. *Can Med Assoc J,* 124(3)**:**267-77, 97.

HONG, S. W., CHUN, J., KIM, J., LEE, J., LEE, H. J., CHUNG, H., CHO, S. J., IM, J. P., KIM, S. G. & KIM, J. S. 2020. Efficacy and safety of ghrelin agonists in patients with diabetic gastroparesis: a systematic review and meta-analysis. *Gut Liver,* 14(5)**:**589-600.

HOROWITZ, M., COOK, D. J., COLLINS, P. J., HARDING, P. E., HOOPER, M. J., WALSH, J. F. & SHEARMAN, D. J. 1982. Measurement of gastric emptying after gastric bypass surgery using radionuclides. *Br J Surg,* 69(11)**:**655-7.

HOROWITZ, M. & DENT, J. 1991. Disordered gastric emptying: mechanical basis, assessment and treatment. *Baillieres Clin Gastroenterol,* 5(2)**:**371-407.

HOROWITZ, M., EDELBROEK, M. A., WISHART, J. M. & STRAATHOF, J. W. 1993. Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects. *Diabetologia,* 36(9)**:**857-62.

HOROWITZ, M., HARDING, P. E., MADDOX, A. F., WISHART, J. M., AKKERMANS, L. M., CHATTERTON, B. E. & SHEARMAN, D. J. 1989a. Gastric and oesophageal emptying in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia,* 32(3)**:**151-9.

HOROWITZ, M., JONES, K. L., RAYNER, C. K. & READ, N. W. 2006. 'Gastric' hypoglycaemia--an important concept in diabetes management. *Neurogastroenterol Motil,* 18(6)**:**405-7.

HOROWITZ, M., MADDOX, A., BOCHNER, M., WISHART, J., BRATASIUK, R., COLLINS, P. & SHEARMAN, D. 1989b. Relationships between gastric emptying of solid and caloric liquid meals and alcohol absorption. *Am J Physiol,* 257(2 Pt 1)**:**G291-8.

HOROWITZ, M., MADDOX, A. F., WISHART, J. M., HARDING, P. E., CHATTERTON, B. E. & SHEARMAN, D. J. 1991. Relationships between oesophageal transit and solid and liquid gastric emptying in diabetes mellitus. *Eur J Nucl Med,* 18(4)**:**229-34.

HOROWITZ, M. & NAUCK, M. A. 2006. To be or not to be--an incretin or enterogastrone? *Gut,* 55(2)**:**148-50.

HOROWITZ, M., RAYNER, C. K., MARATHE, C. S., WU, T. & JONES, K. L. 2020. Glucagon-like peptide-1 receptor agonists and the appropriate measurement of gastric emptying. *Diabetes Obes Metab,* 22(12)**:**2504-6.

HUCKING, K., KOSTIC, Z., POX, C., RITZEL, R., HOLST, J. J., SCHMIEGEL, W. & NAUCK, M. A. 2005. alpha-Glucosidase inhibition (acarbose) fails to enhance secretion of glucagon-like peptide 1 (7-36 amide) and to delay gastric emptying in Type 2 diabetic patients. *Diabet Med,* 22(4)**:**470-6.

HUNT, J. N. & STUBBS, D. F. 1975. The volume and energy content of meals as determinants of gastric emptying. *J Physiol,* 245(1)**:**209-25.

HUTCH, C. R. & SANDOVAL, D. 2017. The role of GLP-1 in the metabolic success of bariatric surgery. *Endocrinology,* 158(12)**:**4139-51.

ISMAIL, H. M., XU, P., LIBMAN, I. M., BECKER, D. J., MARKS, J. B., SKYLER, J. S., PALMER, J. P., SOSENKO, J. M. & TYPE 1 DIABETES TRIALNET STUDY, G. 2018. The shape of the glucose concentration curve during an oral glucose tolerance test predicts risk for type 1 diabetes. *Diabetologia,* 61(1)**:**84-92.

JALLEH, R., MARATHE, C. S., RAYNER, C. K., JONES, K. L. & HOROWITZ, M. 2019. Diabetic Gastroparesis and Glycaemic Control. *Curr Diab Rep,* 19(12)**:**153.

JALLEH, R., PHAM, H., MARATHE, C. S., WU, T., BUTTFIELD, M. D., HATZINIKOLAS, S., MALBERT, C. H., RIGDA, R. S., LANGE, K., TRAHAIR, L. G., FEINLE-BISSET, C., RAYNER, C. K., HOROWITZ, M. & JONES, K. L. 2020. Acute effects of lixisenatide on energy intake in healthy subjects and patients with type 2 diabetes: relationship to gastric emptying and intragastric distribution. *Nutrients,* 12(7)**:**1962.

JALLEH, R. J., JONES, K. L., NAUCK, M. & HOROWITZ, M. 2023a. Accurate measurements of gastric emptying and gastrointestinal symptoms in the evaluation of glucagon-like peptide-1 receptor agonists. *Ann Intern Med,* 176**:**1542-3.

JALLEH, R. J., JONES, K. L., RAYNER, C. K., MARATHE, C. S., WU, T. & HOROWITZ, M. 2022a. Normal and disordered gastric emptying in diabetes: recent insights into (patho)physiology, management and impact on glycaemic control. *Diabetologia,* 65(12)**:**1981-93.

JALLEH, R. J., MARATHE, C. S., TRAHAIR, L. G., JONES, K. L. & HOROWITZ, M. 2023b. A biphasic glucose response during an oral glucose tolerance test is associated with greater plasma insulin and GLP-1 responses and a reduction in 1-hour glucose but does not relate to the rate of gastric emptying in healthy, older adults. *Nutrients,* 15(18)**:**3889.

JALLEH, R. J., TRAHAIR, L. G., WU, T., STANDFIELD, S., FEINLE-BISSET, C., RAYNER, C. K., HOROWITZ, M. & JONES, K. L. 2023c. Effect of gastric distension with concurrent small intestinal saline or glucose infusion on incretin hormone secretion in healthy individuals: A randomized, controlled, crossover study. *Diabetes Obes Metab,* 25(7)**:**1849-54.

JALLEH, R. J., UMAPATHYSIVAM, M. M., PLUMMER, M. P., DEANE, A., JONES, K. L. & HOROWITZ, M. 2023d. Postprandial plasma GLP-1 levels are elevated in individuals with postprandial hypoglycaemia following Roux-en-Y gastric bypass - a systematic review. *Rev Endocr Metab Disord*. Epub ahead of print. DOI: 10.1007/s11154-023-09823-3.

JALLEH, R. J., WU, T., JONES, K. L., RAYNER, C. K., HOROWITZ, M. & MARATHE, C. S. 2022b. Relationships of glucose, GLP-1, and insulin secretion with gastric emptying after a 75-g glucose load in type 2 diabetes. *J Clin Endocrinol Metab,* 107(9)**:**3850-6.

JALLEH, R. J., XIE, C., DEANE, A. M., PLUMMER, M. P., JONES, K. L., HOROWITZ, M. & KAR, P. 2022c. One-hour plasma glucose level after a 75 g oral glucose load and its relationship to gastric emptying in survivors of critical illness and stress hyperglycaemia. *Crit Care Resusc,* 24(3)**:**268-71.

JEBBINK, R. J., SAMSOM, M., BRUIJS, P. P., BRAVENBOER, B., AKKERMANS, L. M., VANBERGE-HENEGOUWEN, G. P. & SMOUT, A. J. 1994. Hyperglycemia induces abnormalities of gastric

myoelectrical activity in patients with type I diabetes mellitus. *Gastroenterology,* 107(5)**:**1390-7.

JIAN, R., CORTOT, A., DUCROT, F., JOBIN, G., CHAYVIALLE, J. A. & MODIGLIANI, R. 1986. Effect of ethanol ingestion on postprandial gastric emptying and secretion, biliopancreatic secretions, and duodenal absorption in man. *Dig Dis Sci,* 31(6)**:**604-14.

JOHNSON, R. D., HOROWITZ, M., MADDOX, A. F., WISHART, J. M. & SHEARMAN, D. J. 1991. Cigarette smoking and rate of gastric emptying: effect on alcohol absorption. *BMJ,* 302(6767)**:**20-3.

JONES, K. L., HOROWITZ, M., CARNEY, B. I., WISHART, J. M., GUHA, S. & GREEN, L. 1996. Gastric emptying in early noninsulin-dependent diabetes mellitus. *J Nucl Med,* 37(10)**:**1643-8.

JONES, K. L., HOROWITZ, M., WISHART, M. J., MADDOX, A. F., HARDING, P. E. & CHATTERTON, B. E. 1995. Relationships between gastric emptying, intragastric meal distribution and blood glucose concentrations in diabetes mellitus. *J Nucl Med,* 36(12)**:**2220-8.

JONES, K. L., HUYNH, L. Q., HATZINIKOLAS, S., RIGDA, R. S., PHILLIPS, L. K., PHAM, H. T., MARATHE, C. S., WU, T., MALBERT, C. H., STEVENS, J. E., LANGE, K., RAYNER, C. K. & HOROWITZ, M. 2020. Exenatide once weekly slows gastric emptying of solids and liquids in healthy, overweight people at steady-state concentrations. *Diabetes Obes Metab,* 22(5)**:**788-97.

JONES, K. L., RIGDA, R. S., BUTTFIELD, M. D. M., HATZINIKOLAS, S., PHAM, H. T., MARATHE, C. S., WU, T., LANGE, K., TRAHAIR, L. G., RAYNER, C. K. & HOROWITZ, M. 2019. Effects of lixisenatide on postprandial blood pressure, gastric emptying and glycaemia in healthy people and people with type 2 diabetes. *Diabetes Obes Metab,* 21(5)**:**1158-67.

JONES, K. L., RUSSO, A., STEVENS, J. E., WISHART, J. M., BERRY, M. K. & HOROWITZ, M. 2001. Predictors of delayed gastric emptying in diabetes. *Diabetes Care,* 24(7)**:**1264-9.

KALAS, M. A., GALURA, G. M. & MCCALLUM, R. W. 2021. Medication-induced gastroparesis: a case report. *J Investig Med High Impact Case Rep,* 9**:**23247096211051919.

KAR, P., PLUMMER, M. P., ALI ABDELHAMID, Y., GIERSCH, E. J., SUMMERS, M. J., WEINEL, L. M., FINNIS, M. E., PHILLIPS, L. K., JONES, K. L., HOROWITZ, M. & DEANE, A. M. 2019. Incident Diabetes in Survivors of Critical Illness and Mechanisms Underlying Persistent Glucose Intolerance: A Prospective Cohort Study. *Crit Care Med,* 47(2)**:**103-11.

KECHAGIAS, S., JONSSON, K. A. & JONES, A. W. 1999. Impact of gastric emptying on the pharmacokinetics of ethanol as influenced by cisapride. *Br J Clin Pharmacol,* 48(5)**:**728-32.

KELLOGG, T. A., BANTLE, J. P., LESLIE, D. B., REDMOND, J. B., SLUSAREK, B., SWAN, T., BUCHWALD, H. & IKRAMUDDIN, S. 2008. Postgastric bypass hyperinsulinemic hypoglycemia syndrome: characterization and response to a modified diet. *Surg Obes Relat Dis,* 4(4)**:**492-9.

KIM, J. Y., COLETTA, D. K., MANDARINO, L. J. & SHAIBI, G. Q. 2012. Glucose response curve and type 2 diabetes risk in Latino adolescents. *Diabetes Care,* 35(9)**:**1925-30.

KIM, J. Y., MICHALISZYN, S. F., NASR, A., LEE, S., TFAYLI, H., HANNON, T., HUGHAN, K. S., BACHA, F. & ARSLANIAN, S. 2016. The Shape of the Glucose Response Curve During an Oral Glucose Tolerance Test Heralds Biomarkers of Type 2 Diabetes Risk in Obese Youth. *Diabetes Care,* 39(8)**:**1431-9.

KIM, K. H., LEE, M. S., CHOI, T. Y. & KIM, T. H. 2018. Acupuncture for symptomatic gastroparesis. *Cochrane Database Syst Rev,* 12**:**CD009676.

KIM, S. H., ABBASI, F., NACHMANOFF, C., STEFANAKIS, K., KUMAR, A., KALRA, B., SAVJANI, G. & MANTZOROS, C. S. 2021. Effect of the glucagon-like peptide-1 analogue liraglutide versus placebo treatment on circulating proglucagon-derived peptides that mediate improvements in body weight, insulin secretion and action: A randomized controlled trial. *Diabetes Obes Metab,* 23(2)**:**489-98.

KJEMS, L. L., HOLST, J. J., VOLUND, A. & MADSBAD, S. 2003. The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. *Diabetes,* 52(2)**:**380-6.

KONG, M. F., KING, P., MACDONALD, I. A., BLACKSHAW, P. E., HOROWITZ, M., PERKINS, A. C., ARMSTRONG, E., BUCHANAN, K. D. & TATTERSALL, R. B. 1999. Euglycaemic hyperinsulinaemia does not affect gastric emptying in type I and type II diabetes mellitus. *Diabetologia,* 42(3)**:**365-72.

KREBS, H. A. 1968. The effects of ethanol on the metabolic activities of the liver. *Adv Enzyme Regul,* 6**:**467-80.

KUO, B., BARNES, C. N., NGUYEN, D. D., SHAYWITZ, D., GRIMALDI, M., RENZULLI, C., CANAFAX, D. & PARKMAN, H. P. 2021a. Velusetrag accelerates gastric emptying in subjects with gastroparesis: a multicentre, double-blind, randomised, placebo-controlled, phase 2 study. *Aliment Pharmacol Ther,* 53(10)**:**1090-7.

KUO, B., SCIMIA, C., DUKES, G., ZHANG, W., GUPTA, S., CHEN, C., CHUANG, E. & CAMILLERI, M. 2021b. Randomised clinical trial: safety, pharmacokinetics and pharmacodynamics of trazpiroben (TAK-906), a dopamine D2 /D3 receptor antagonist, in patients with gastroparesis. *Aliment Pharmacol Ther,* 54(3)**:**267-80.

KUWELKER, S., MUTHYALA, A., O'CONNOR, M. & BHARUCHA, A. E. 2020. Clinical features and disturbances of gastrointestinal transit in patients with rapid gastric emptying. *Neurogastroenterol Motil,* 32(4)**:**13779.

LARRAUFIE, P., ROBERTS, G. P., MCGAVIGAN, A. K., KAY, R. G., LI, J., LEITER, A., MELVIN, A., BIGGS, E. K., RAVN, P., DAVY, K., HORNIGOLD, D. C., YEO, G. S. H., HARDWICK, R. H., REIMANN, F. & GRIBBLE, F. M. 2019. Important role of the GLP-1 axis for glucose homeostasis after bariatric surgery. *Cell Rep,* 26(6)**:**1399-408.

LAU, E. R. L. & HENRY, C. J. 2017. No influence of carbonation on glycemic response, gastric emptying, and satiety of sweetened drinks. *Nutrition,* 39-40**:**1-7.

LAURENIUS, A., WERLING, M., LE ROUX, C. W., FÄNDRIKS, L. & OLBERS, T. 2014. More symptoms but similar blood glucose curve after oral carbohydrate provocation in patients with a history of hypoglycemia-like symptoms compared to asymptomatic patients after Roux-en-Y gastric bypass. *Surg Obes Relat Dis,* 10(6)**:**1047-54.

LAWAY, B. A., MALIK, T. S., KHAN, S. H. & RATHER, T. A. 2013. Prevalence of abnormal gastric emptying in asymptomatic women with newly detected diabetes and its reversibility after glycemic control-a prospective case control study. *J Diabetes Complications,* 27(1)**:**78-81.

LIMKETKAI, B. N., LEBRETT, W., LIN, L. & SHAH, N. D. 2020. Nutritional approaches for gastroparesis. *Lancet Gastroenterol Hepatol,* 5(11)**:**1017-26.

LIN, H. C., DOTY, J. E., REEDY, T. J. & MEYER, J. H. 1989. Inhibition of gastric emptying by glucose depends on length of intestine exposed to nutrient. *Am J Physiol,* 256(2)**:**G404-11.

LINDOR, K. D., HUGHES, R. W., JR., ILSTRUP, D. M. & JENSEN, M. D. 1987. Intragastric balloons in comparison with standard therapy for obesity--a randomized, double-blind trial. *Mayo Clin Proc,* 62(11)**:**992-6.

LINNEBJERG, H., PARK, S., KOTHARE, P. A., TRAUTMANN, M. E., MACE, K., FINEMAN, M., WILDING, I., NAUCK, M. & HOROWITZ, M. 2008. Effect of exenatide on gastric emptying and relationship to postprandial glycemia in type 2 diabetes. *Regul Pept,* 151(1-3)**:**123-9.

LITTLE, T. J., PILICHIEWICZ, A. N., RUSSO, A., PHILLIPS, L., JONES, K. L., NAUCK, M. A., WISHART, J., HOROWITZ, M. & FEINLE-BISSET, C. 2006. Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. *J Clin Endocrinol Metab,* 91(5)**:**1916-23.

LOBATO, C. B., PEREIRA, S. S., GUIMARÃES, M., HARTMANN, B., WEWER ALBRECHTSEN, N. J., HILSTED, L., HOLST, J. J., NORA, M. & MONTEIRO, M. P. 2020. A potential role for endogenous glucagon in preventing post-bariatric hypoglycemia. *Front Endocrinol (Lausanne),* 11**:**608248.

LOO, J. H., LIM, Y. H., SEAH, H. L., CHONG, A. Z. Q. & TAY, K. V. 2022. Intragastric balloon as bridging therapy prior to bariatric surgery for patients with severe obesity (BMI >/= 50 kg/m(2)): a systematic review and meta-analysis. *Obes Surg,* 32(2)**:**489-502.

LORENZ, M., PFEIFFER, C., STEINSTRASSER, A., BECKER, R. H., RUTTEN, H., RUUS, P. & HOROWITZ, M. 2013. Effects of lixisenatide once daily on gastric emptying in type 2 diabetes--relationship to postprandial glycemia. *Regul Pept,* 185**:**1-8.

MA, J., PILICHIEWICZ, A. N., FEINLE-BISSET, C., WISHART, J. M., JONES, K. L., HOROWITZ, M. & RAYNER, C. K. 2012. Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes. *Diabet Med,* 29(5)**:**604-8.

MA, J., STEVENS, J. E., CUKIER, K., MADDOX, A. F., WISHART, J. M., JONES, K. L., CLIFTON, P. M., HOROWITZ, M. & RAYNER, C. K. 2009. Effects of a protein preload on gastric emptying,

glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. *Diabetes Care,* 32(9)**:**1600-2.

MACHYTKA, E., GAUR, S., CHUTTANI, R., BOJKOVA, M., KUPKA, T., BUZGA, M., GIANNAKOU, A., IOANNIS, K., MATHUS-VLIEGEN, E., LEVY, S. & RAFTOPOULOS, I. 2017. Elipse, the first procedureless gastric balloon for weight loss: a prospective, observational, open-label, multicenter study. *Endoscopy,* 49(2)**:**154-60.

MANCO, M., NOLFE, G., PATAKY, Z., MONTI, L., PORCELLATI, F., GABRIEL, R., MITRAKOU, A. & MINGRONE, G. 2017. Shape of the OGTT glucose curve and risk of impaired glucose metabolism in the EGIR-RISC cohort. *Metabolism,* 70**:**42-50.

MARATHE, C. S., HOROWITZ, M., TRAHAIR, L. G., WISHART, J. M., BOUND, M., LANGE, K., RAYNER, C. K. & JONES, K. L. 2015. Relationships of early and late glycemic responses with gastric emptying during an oral glucose tolerance test. *J Clin Endocrinol Metab,* 100(9)**:**3565-71.

MARATHE, C. S., MARATHE, J. A., RAYNER, C. K., KAR, P., JONES, K. L. & HOROWITZ, M. 2019. Hypoglycaemia and gastric emptying. *Diabetes Obes Metab,* 21(3)**:**491-8.

MARATHE, C. S., RAYNER, C. K., BOUND, M., CHECKLIN, H., STANDFIELD, S., WISHART, J., LANGE, K., JONES, K. L. & HOROWITZ, M. 2014. Small intestinal glucose exposure determines the magnitude of the incretin effect in health and type 2 diabetes. *Diabetes,* 63(8)**:**2668-75.

MARATHE, C. S., RAYNER, C. K., JONES, K. L. & HOROWITZ, M. 2011. Effects of GLP-1 and incretin-based therapies on gastrointestinal motor function. *Exp Diabetes Res,* 2011**:**279530.

MARATHE, C. S., RAYNER, C. K., JONES, K. L. & HOROWITZ, M. 2013. Relationships between gastric emptying, postprandial glycemia, and incretin hormones. *Diabetes Care,* 36(5)**:**1396-405.

MARATHE, C. S., RAYNER, C. K., LANGE, K., BOUND, M., WISHART, J., JONES, K. L., KAHN, S. E. & HOROWITZ, M. 2017. Relationships of the early insulin secretory response and oral disposition index with gastric emptying in subjects with normal glucose tolerance. *Physiol Rep,* 5(4)**:**13122.

MARSK, R., JONAS, E., RASMUSSEN, F. & NASLUND, E. 2010. Nationwide cohort study of post-gastric bypass hypoglycaemia including 5,040 patients undergoing surgery for obesity in 1986-2006 in Sweden. *Diabetologia,* 53(11)**:**2307-11.

MARTINEK, J., HUSTAK, R., MARES, J., VACKOVA, Z., SPICAK, J., KIESLICHOVA, E., BUNCOVA, M., POHL, D., AMIN, S. & TACK, J. 2022. Endoscopic pyloromyotomy for the treatment of severe and refractory gastroparesis: a pilot, randomised, sham-controlled trial. *Gut,* 71(11)**:**2170-8.

MATHIEU, C., ZINMAN, B., HEMMINGSSON, J. U., WOO, V., COLMAN, P., CHRISTIANSEN, E., LINDER, M., BODE, B. & INVESTIGATORS, A. O. 2016. Efficacy and safety of liraglutide added to insulin treatment in type 1 diabetes: the ADJUNCT ONE treat-to-target randomized trial. *Diabetes Care,* 39(10)**:**1702-10.

MCCALLUM, R. W., CYNSHI, O. & US INVESTIGATIVE, T. 2007. Efficacy of mitemcinal, a motilin agonist, on gastrointestinal symptoms in patients with symptoms suggesting diabetic gastropathy: a randomized, multi-center, placebo-controlled trial. *Aliment Pharmacol Ther,* 26(1)**:**107-16.

MCCARTY, T. R. & RUSTAGI, T. 2015. Endoscopic treatment of gastroparesis. *World J Gastroenterol,* 21(22)**:**6842-9.

MCDONALD, G. W., FISHER, G. F. & BURNHAM, C. 1965. Reproducibility of the Oral Glucose Tolerance Test. *Diabetes,* 14**:**473-80.

MEIER, J. J., GALLWITZ, B., SALMEN, S., GOETZE, O., HOLST, J. J., SCHMIDT, W. E. & NAUCK, M. A. 2003. 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,* 88(6)**:**2719-25.

MEIER, J. J., GOETZE, O., ANSTIPP, J., HAGEMANN, D., HOLST, J. J., SCHMIDT, W. E., GALLWITZ, B. & NAUCK, M. A. 2004. Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. *Am J Physiol Endocrinol Metab,* 286(4)**:**E621-5.

MEIER, J. J., ROSENSTOCK, J., HINCELIN-MERY, A., ROY-DUVAL, C., DELFOLIE, A., COESTER, H. V., MENGE, B. A., FORST, T. & KAPITZA, C. 2015. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. *Diabetes Care,* 38(7)**:**1263-73.

MENGE, B. A., SCHRADER, H., BREUER, T. G., DABROWSKI, Y., UHL, W., SCHMIDT, W. E. & MEIER, J. J. 2009. Metabolic consequences of a 50% partial pancreatectomy in humans. *Diabetologia,* 52(2)**:**306-17.

MILLER, P. G., MCKENZIE, S. P., DE GROOT, F. P., DAVOREN, S. L. & LESLIE, E. R. 2010. The growing popularity of "low-carb" beers: good marketing or community health risk? *Med J Aust,* 192(4)**:**235.

MIRAS, A. D., PEREZ-PEVIDA, B., ALDHWAYAN, M., KAMOCKA, A., MCGLONE, E. R., AL-NAJIM, W., CHAHAL, H., BATTERHAM, R. L., MCGOWAN, B., KHAN, O., GREENER, V., AHMED, A. R., PETRIE, A., SCHOLTZ, S., BLOOM, S. R. & TAN, T. M. 2019. Adjunctive liraglutide treatment in patients with persistent or recurrent type 2 diabetes after metabolic surgery (GRAVITAS): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol,* 7(7)**:**549-59.

MOONS, K. G., DE GROOT, J. A., BOUWMEESTER, W., VERGOUWE, Y., MALLETT, S., ALTMAN, D. G., REITSMA, J. B. & COLLINS, G. S. 2014. Critical appraisal and data extraction for systematic reviews of prediction modelling studies: the CHARMS checklist. *PLoS Med,* 11(10)**:**1001744.

MULLA, C. M., GOLDFINE, A. B., DREYFUSS, J. M., HOUTEN, S., PAN, H., POBER, D. M., WEWER ALBRECHTSEN, N. J., SVANE, M. S., SCHMIDT, J. B., HOLST, J. J., CRAIG, C. M., MCLAUGHLIN, T. L. & PATTI, M. E. 2019. Plasma FGF-19 levels are increased in patients with post-bariatric hypoglycemia. *Obes Surg,* 29(7)**:**2092-9.

MURTHY, T. A., GRIVELL, J., HATZINIKOLAS, S., CHAPPLE, L. S., CHAPMAN, M. J., STEVENS, J. E., MALBERT, C. H., RAYNER, C. K., HOROWITZ, M., JONES, K. L. & MARATHE, C. S. 2021. Acceleration of gastric emptying by insulin-induced hypoglycemia is dependent on the degree of hypoglycemia. *J Clin Endocrinol Metab,* 106(2)**:**364-71.

MUSHAMBI, M. C., BAILEY, S. M., TROTTER, T. N., CHADD, G. D. & ROWBOTHAM, D. J. 1993. Effect of alcohol on gastric emptying in volunteers. *Br J Anaesth,* 71(5)**:**674-6.

NAIR, K. S. 1987. Hyperglucagonemia increases resting metabolic rate in man during insulin deficiency. *J Clin Endocrinol Metab,* 64(5)**:**896-901.

NAIR, N. S., BRENNAN, I. M., LITTLE, T. J., GENTILCORE, D., HAUSKEN, T., JONES, K. L., WISHART, J. M., HOROWITZ, M. & FEINLE-BISSET, C. 2009. Reproducibility of energy intake, gastric emptying, blood glucose, plasma insulin and cholecystokinin responses in healthy young males. *Br J Nutr,* 101(7)**:**1094-102.

NATOCHIN, Y. V., KUTINA, A. V., MARINA, A. S. & SHAKHMATOVA, E. I. 2018. Stimulus for glucagon-like peptide 1 secretion in rats. *Dokl Biol Sci,* 479(1)**:**57-9.

NAUCK, M. A., HEIMESAAT, M. M., ORSKOV, C., HOLST, J. J., EBERT, R. & CREUTZFELDT, W. 1993. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest,* 91(1)**:**301-7.

NAUCK, M. A., HOMBERGER, E., SIEGEL, E. G., ALLEN, R. C., EATON, R. P., EBERT, R. & CREUTZFELDT, W. 1986. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab,* 63(2)**:**492-8.

NAUCK, M. A. & MEIER, J. J. 2016. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *Lancet Diabetes Endocrinol,* 4(6)**:**525-36.

NAUCK, M. A., NIEDEREICHHOLZ, U., ETTLER, R., HOLST, J. J., ORSKOV, C., RITZEL, R. & SCHMIEGEL, W. H. 1997. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol,* 273(5)**:**E981-8.

NAUCK, M. A., QUAST, D. R., WEFERS, J. & PFEIFFER, A. F. H. 2021. The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: A pathophysiological update. *Diabetes Obes Metab,* 23 Suppl 3**:**5-29.

NEESE, J. W. 1982. Glucose, direct hexokinase method. Selected methods. *Clin Chem***:**9241-8.

NGUYEN, N. Q., DEBRECENI, T. L., BAMBRICK, J. E., BELLON, M., WISHART, J., STANDFIELD, S., RAYNER, C. K. & HOROWITZ, M. 2014. Rapid gastric and intestinal transit is a major determinant of changes in blood glucose, intestinal hormones, glucose absorption and postprandial symptoms after gastric bypass. *Obesity (Silver Spring),* 22(9)**:**2003-9.

NIMMO, W. S. 1976. Drugs, diseases and altered gastric emptying. *Clin Pharmacokinet,* 1(3)**:**189-203.

NOWOTNY, B., NOWOTNY, P. J., STRASSBURGER, K. & RODEN, M. 2012. Precision and accuracy of blood glucose measurements using three different instruments. *Diabet Med,* 29(2)**:**260-5.

OHBAYASHI, K., OYAMA, Y., YAMAGUCHI, C., ASANO, T., YADA, T. & IWASAKI, Y. 2021. Gastrointestinal distension by pectin-containing carbonated solution suppresses food intake and enhances glucose tolerance via GLP-1 secretion and vagal afferent activation. *Front Endocrinol (Lausanne),* 12**:**676869.

OJHA, A., OJHA, U., MOHAMMED, R., CHANDRASHEKAR, A. & OJHA, H. 2019. Current perspective on the role of insulin and glucagon in the pathogenesis and treatment of type 2 diabetes mellitus. *Clin Pharmacol,* 11**:**57-65.

ONETA, C. M., SIMANOWSKI, U. A., MARTINEZ, M., ALLALI-HASSANI, A., PARES, X., HOMANN, N., CONRADT, C., WALDHERR, R., FIEHN, W., COUTELLE, C. & SEITZ, H. K. 1998. First pass metabolism of ethanol is strikingly influenced by the speed of gastric emptying. *Gut,* 43(5)**:**612-9.

PAGE, M. J., HIGGINS, J. P. T. & STERNE, J. A. C. 2022. Chapter 13: Assessing risk of bias due to missing results in a synthesis. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane handbook for systematic reviews of interventions version 6.3 (updated February 2022). Cochrane; Available from www.training.cochrane.org/handbook.

PAPASAVAS, P. K., NG, J. S., STONE, A. M., AJAYI, O. A., MUDDASANI, K. P. & TISHLER, D. S. 2014. Gastric bypass surgery as treatment of recalcitrant gastroparesis. *Surg Obes Relat Dis,* 10(5)**:**795-9.

PAPAVRAMIDIS, T. S., STAVROU, G., PAPAKOSTAS, P., GROSOMANIDIS, V., KOKKOTA, S., MICHALOPOULOS, A., KOLIOS, G. & KOTZAMPASSI, K. 2018. Displacement of the intragastric balloon from the fundus to the antrum results in enhanced weight loss. *Obes Surg,* 28(8)**:**2374-8.

PARKMAN, H. P., VAN NATTA, M. L., ABELL, T. L., MCCALLUM, R. W., SAROSIEK, I., NGUYEN, L., SNAPE, W. J., KOCH, K. L., HASLER, W. L., FARRUGIA, G., LEE, L., UNALP-ARIDA, A., TONASCIA, J., HAMILTON, F. & PASRICHA, P. J. 2013. Effect of nortriptyline on symptoms of idiopathic gastroparesis: the NORIG randomized clinical trial. *JAMA,* 310(24)**:**2640-9.

PATTI, M. E. & GOLDFINE, A. B. 2010. Hypoglycaemia following gastric bypass surgery--diabetes remission in the extreme? *Diabetologia,* 53(11)**:**2276-9.

PATTI, M. E., LI, P. & GOLDFINE, A. B. 2015. Insulin response to oral stimuli and glucose effectiveness increased in neuroglycopenia following gastric bypass. *Obesity (Silver Spring),* 23(4)**:**798-807.

PAULWEBER, B., VALENSI, P., LINDSTROM, J., LALIC, N. M., GREAVES, C. J., MCKEE, M., KISSIMOVA-SKARBEK, K., LIATIS, S., COSSON, E., SZENDROEDI, J., SHEPPARD, K. E., CHARLESWORTH, K., FELTON, A. M., HALL, M., RISSANEN, A., TUOMILEHTO, J., SCHWARZ, P. E., RODEN, M., PAULWEBER, M., STADLMAYR, A., KEDENKO, L., KATSILAMBROS, N., MAKRILAKIS, K., KAMENOV, Z., EVANS, P., GILIS-JANUSZEWSKA, A., LALIC, K., JOTIC, A., DJORDEVIC, P., DIMITRIJEVIC-SRECKOVIC, V., HUHMER, U., KULZER, B., PUHL, S., LEE-BARKEY, Y. H., ALKERWI, A., ABRAHAM, C., HARDEMAN, W., ACOSTA, T., ADLER, M., ALKERWI, A., BARENGO, N., BARENGO, R., BOAVIDA, J. M., CHARLESWORTH, K., CHRISTOV, V., CLAUSSEN, B., COS, X., COSSON, E., DECEUKELIER, S., DIMITRIJEVIC-SRECKOVIC, V., DJORDJEVIC, P., EVANS, P., FELTON, A. M., FISCHER, M., GABRIEL-SANCHEZ, R., GILIS-JANUSZEWSKA, A., GOLDFRACHT, M., GOMEZ, J. L., GREAVES, C. J., HALL, M., HANDKE, U., HAUNER, H., HERBST, J., HERMANNS, N., HERREBRUGH, L., HUBER, C., HUHMER, U., HUTTUNEN, J., JOTIC, A., KAMENOV, Z., KARADENIZ, S., KATSILAMBROS, N., KHALANGOT, M., KISSIMOVA-SKARBEK, K., KOHLER, D., KOPP, V., KRONSBEIN, P., KULZER, B., KYNE-GRZEBALSKI, D., LALIC, K., LALIC, N., LANDGRAF, R., LEE-BARKEY, Y. H., LIATIS, S., LINDSTROM, J., MAKRILAKIS, K., MCINTOSH, C., MCKEE, M., MESQUITA, A. C., MISINA, D., MUYLLE, F., NEUMANN, A., PAIVA, A. C., PAJUNEN, P., PAULWEBER, B., PELTONEN, M., PERRENOUD, L., PFEIFFER, A., POLONEN, A., PUHL, S., et al. 2010. A European evidence-based guideline for the prevention of type 2 diabetes. *Horm Metab Res,* 42 Suppl 1**:**S3-36.

PEDDINTI, G., BERGMAN, M., TUOMI, T. & GROOP, L. 2019. 1-Hour Post-OGTT Glucose Improves the Early Prediction of Type 2 Diabetes by Clinical and Metabolic Markers. *J Clin Endocrinol Metab,* 104(4)**:**1131-40.

PERANO, S. J., COUPER, J. J., HOROWITZ, M., MARTIN, A. J., KRITAS, S., SULLIVAN, T. & RAYNER, C. K. 2014. Pancreatic enzyme supplementation improves the incretin hormone response and attenuates postprandial glycemia in adolescents with cystic fibrosis: a randomized crossover trial. *J Clin Endocrinol Metab,* 99(7)**:**2486-93.

PERANO, S. J., RAYNER, C. K., KRITAS, S., HOROWITZ, M., DONAGHUE, K., MPUNDU-KAAMBWA, C., GILES, L. & COUPER, J. J. 2015. Gastric emptying is more rapid in adolescents with type 1 diabetes and impacts on postprandial glycemia. *J Clin Endocrinol Metab,* 100(6)**:**2248-53.

PETERSMANN, A., MULLER-WIELAND, D., MULLER, U. A., LANDGRAF, R., NAUCK, M., FRECKMANN, G., HEINEMANN, L. & SCHLEICHER, E. 2019. Definition, Classification and Diagnosis of Diabetes Mellitus. *Exp Clin Endocrinol Diabetes,* 127(S 01)**:**S1-S7.

PFEIFFER, A., HOGL, B. & KAESS, H. 1992. Effect of ethanol and commonly ingested alcoholic beverages on gastric emptying and gastrointestinal transit. *Clin Investig,* 70(6)**:**487-91.

PHAM, H., MARATHE, C. S., PHILLIPS, L. K., TRAHAIR, L. G., HATZINIKOLAS, S., HUYNH, L., WU, T., NAUCK, M. A., RAYNER, C. K., HOROWITZ, M. & JONES, K. L. 2019. Longitudinal changes in fasting and glucose-stimulated GLP-1 and GIP in healthy older subjects. *J Clin Endocrinol Metab,* 104(12)**:**6201-6.

PHAM, H., PHILLIPS, L., TRAHAIR, L., HATZINIKOLAS, S., HOROWITZ, M. & JONES, K. L. 2020. Longitudinal changes in the blood pressure responses to, and gastric emptying of, an oral glucose load in healthy older subjects. *J Gerontol A Biol Sci Med Sci,* 75(2)**:**244-8.

PHILLIPS, L. K., DEANE, A. M., JONES, K. L., RAYNER, C. K. & HOROWITZ, M. 2015. Gastric emptying and glycaemia in health and diabetes mellitus. *Nat Rev Endocrinol,* 11(2)**:**112-28.

PHILLIPS, W. T. 2006. Gastric emptying in ethnic populations: possible relationship to development of diabetes and metabolic syndrome. *Ethn Dis,* 16(3)**:**682-92.

PILICHIEWICZ, A. N., CHAIKOMIN, R., BRENNAN, I. M., WISHART, J. M., RAYNER, C. K., JONES, K. L., SMOUT, A. J., HOROWITZ, M. & FEINLE-BISSET, C. 2007. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. *Am J Physiol Endocrinol Metab,* 293(3)**:**E743-53.

PLAMBOECK, A., VEEDFALD, S., DEACON, C. F., HARTMANN, B., WETTERGREN, A., SVENDSEN, L. B., MEISNER, S., HOVENDAL, C., KNOP, F. K., VILSBOLL, T. & HOLST, J. J. 2013. Characterisation of oral and i.v. glucose handling in truncally vagotomised subjects with pyloroplasty. *Eur J Endocrinol,* 169(2)**:**187-201.

PLUMMER, M. P., BELLOMO, R., COUSINS, C. E., ANNINK, C. E., SUNDARARAJAN, K., REDDI, B. A., RAJ, J. P., CHAPMAN, M. J., HOROWITZ, M. & DEANE, A. M. 2014a. Dysglycaemia in the critically ill and the interaction of chronic and acute glycaemia with mortality. *Intensive Care Med,* 40(7)**:**973-80.

PLUMMER, M. P., JONES, K. L., ANNINK, C. E., COUSINS, C. E., MEIER, J. J., CHAPMAN, M. J., HOROWITZ, M. & DEANE, A. M. 2014b. Glucagon-like peptide 1 attenuates the acceleration of gastric emptying induced by hypoglycemia in healthy subjects. *Diabetes Care,* 37(6)**:**1509-15.

POITOU, C., BOUAZIZ-AMAR, E., GENSER, L., OPPERT, J. M., LACORTE, J. M. & LE BEYEC, J. 2018. Fasting levels of glicentin are higher in Roux-en-Y gastric bypass patients exhibiting postprandial hypoglycemia during a meal test. *Surg Obes Relat Dis,* 14(7)**:**929-35.

PRESCOTT, L. F., ILLINGWORTH, R. N., CRITCHLEY, J. A., STEWART, M. J., ADAM, R. D. & PROUDFOOT, A. T. 1979. Intravenous N-acetylcystine: the treatment of choice for paracetamol poisoning. *Br Med J,* 2(6198)**:**1097-100.

RAYNER, C. K., JONES, K. L. & HOROWITZ, M. 2021. Chapter 18 - Diabetic gastroparesis. *In:* MCCALLUM, R. W. & PARKMAN, H. P. (eds.) *Gastroparesis.* Academic Press.

RAYNER, C. K., VERHAGEN, M. A., HEBBARD, G. S., DIMATTEO, A. C., DORAN, S. M. & HOROWITZ, M. 2000. Proximal gastric compliance and perception of distension in type 1 diabetes mellitus: effects of hyperglycemia. *Am J Gastroenterol,* 95(5)**:**1175-83.

RAYNER, C. K., WATSON, L. E., PHILLIPS, L. K., LANGE, K., BOUND, M. J., GRIVELL, J., WU, T., JONES, K. L., HOROWITZ, M., FERRANNINI, E., TRICO, D., FRASCERRA, S., MARI, A. & NATALI, A. 2020. Effects of Sustained Treatment With Lixisenatide on Gastric Emptying and Postprandial Glucose Metabolism in Type 2 Diabetes: A Randomized Controlled Trial. *Diabetes Care,* 43(8)**:**1813-21.

ROCCA, A. S. & BRUBAKER, P. L. 1999. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology,* 140(4)**:**1687-94.

RUSSO, A., STEVENS, J. E., CHEN, R., GENTILCORE, D., BURNET, R., HOROWITZ, M. & JONES, K. L. 2005. Insulin-induced hypoglycemia accelerates gastric emptying of solids and liquids in long-standing type 1 diabetes. *J Clin Endocrinol Metab,* 90(8)**:**4489-95.

RUSSO, A., STEVENS, J. E., WILSON, T., WELLS, F., TONKIN, A., HOROWITZ, M. & JONES, K. L. 2003. Guar attenuates fall in postprandial blood pressure and slows gastric emptying of oral glucose in type 2 diabetes. *Dig Dis Sci,* 48(7)**:**1221-9.

SAISHO, Y. 2014. Importance of Beta Cell Function for the Treatment of Type 2 Diabetes. *J Clin Med,* 3(3)**:**923-43.

SALEHI, M., GASTALDELLI, A. & D'ALESSIO, D. A. 2014. Altered islet function and insulin clearance cause hyperinsulinemia in gastric bypass patients with symptoms of postprandial hypoglycemia. *J Clin Endocrinol Metab,* 99(6)**:**2008-17.

SALEHI, M., GASTALDELLI, A. & D'ALESSIO, D. A. 2019. Role of vagal activation in postprandial glucose metabolism after gastric bypass in individuals with and without hypoglycaemia. *Diabetes Obes Metab,* 21(6)**:**1513-7.

SALEHI, M., VELLA, A., MCLAUGHLIN, T. & PATTI, M. E. 2018. Hypoglycemia after gastric bypass surgery: current concepts and controversies. *J Clin Endocrinol Metab,* 103(8)**:**2815-26.

SALMI, A., GRECO, F. & BELLERI, E. 2020. Ultrasound-guided insertion of the Elipse((R)) gastric balloon: technical details, learning curve, and perioperative outcome in 36 cases. *J Ultrasound,* 23(4)**:**593-7.

SAMSOM, M., AKKERMANS, L. M., JEBBINK, R. J., VAN ISSELT, H., VANBERGE-HENEGOUWEN, G. P. & SMOUT, A. J. 1997. Gastrointestinal motor mechanisms in hyperglycaemia induced delayed gastric emptying in type I diabetes mellitus. *Gut,* 40(5)**:**641-6.

SCHIRRA, J., KATSCHINSKI, M., WEIDMANN, C., SCHAFER, T., WANK, U., ARNOLD, R. & GOKE, B. 1996. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest,* 97(1)**:**92-103.

SCHIRRA, J., WANK, U., ARNOLD, R., GOKE, B. & KATSCHINSKI, M. 2002. Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans. *Gut,* 50(3)**:**341-8.

SCHOL, J., WAUTERS, L., DICKMAN, R., DRUG, V., MULAK, A., SERRA, J., ENCK, P., TACK, J. & GROUP, E. G. C. 2021. United European Gastroenterology (UEG) and European Society for Neurogastroenterology and Motility (ESNM) consensus on gastroparesis. *United European Gastroenterol J,* 9(7)**:**883-4.

SCHWARTZ, J. G., SALMAN, U. A., MCMAHAN, C. A. & PHILLIPS, W. T. 1996. Gastric emptying of beer in Mexican-Americans compared with non-Hispanic whites. *Metabolism,* 45(9)**:**1174-8.

SERVICE, G. J., THOMPSON, G. B., SERVICE, F. J., ANDREWS, J. C., COLLAZO-CLAVELL, M. L. & LLOYD, R. V. 2005. Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *N Engl J Med,* 353(3)**:**249-54.

SHARMA, S. & TRIPATHI, P. 2019. Gut microbiome and type 2 diabetes: where we are and where to go? *J Nutr Biochem,* 63**:**101-8.

SISTA, F., ABRUZZESE, V., CLEMENTI, M., CARANDINA, S., CECILIA, M. & AMICUCCI, G. 2017. The effect of sleeve gastrectomy on GLP-1 secretion and gastric emptying: a prospective study. *Surg Obes Relat Dis,* 13(1)**:**7-14.

SOEBY, M., NIELSEN, J. B., PEDERSEN, S. B., GRIBSHOLT, S. B., HOLST, J. J. & RICHELSEN, B. 2020. Relationship between biochemical and symptomatic hypoglycemia after RYGB. Responses to a mixed meal test: a case-control study. *Surg Obes Relat Dis,* 16(9)**:**1179-85.

SONG, S. H., MCINTYRE, S. S., SHAH, H., VELDHUIS, J. D., HAYES, P. C. & BUTLER, P. C. 2000. Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *J Clin Endocrinol Metab,* 85(12)**:**4491-9.

STEINERT, R. E., FEINLE-BISSET, C., ASARIAN, L., HOROWITZ, M., BEGLINGER, C. & GEARY, N. 2017. Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. *Physiol Rev,* 97(1)**:**411-63.

STEINERT, R. E., MEYER-GERSPACH, A. C. & BEGLINGER, C. 2012. The role of the stomach in the control of appetite and the secretion of satiation peptides. *Am J Physiol Endocrinol Metab,* 302(6)**:**E666-73.

STEVENS, J. E., BUTTFIELD, M., WU, T., HATZINIKOLAS, S., PHAM, H., LANGE, K., RAYNER, C. K., HOROWITZ, M. & JONES, K. L. 2020. Effects of sitagliptin on gastric emptying of, and the glycaemic and blood pressure responses to, a carbohydrate meal in type 2 diabetes. *Diabetes Obes Metab,* 22(1)**:**51-8.

STEVENS, J. E., JALLEH, R. J., TRAHAIR, L. G., MARATHE, C. S., HOROWITZ, M. & JONES, K. L. 2022. Comparative effects of low-carbohydrate, full-strength and low-alcohol beer on gastric emptying, alcohol absorption, glycaemia and insulinaemia in health. *Br J Clin Pharmacol,* 88(7)**:**3421-7.

STROUP, D. F., BERLIN, J. A., MORTON, S. C., OLKIN, I., WILLIAMSON, G. D., RENNIE, D., MOHER, D., BECKER, B. J., SIPE, T. A. & THACKER, S. B. 2000. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA,* 283(15)**:**2008-12.

STURM, K., PARKER, B., WISHART, J., FEINLE-BISSET, C., JONES, K. L., CHAPMAN, I. & HOROWITZ, M. 2004. Energy intake and appetite are related to antral area in healthy young and older subjects. *Am J Clin Nutr,* 80(3)**:**656-67.

TACK, J., SCHOL, J. & HOROWITZ, M. 2021. Gastroparesis: A Dead-end Street After All? *Gastroenterology,* 160(6)**:**1931-3.

TAN, M., LAMENDOLA, C., LUONG, R., MCLAUGHLIN, T. & CRAIG, C. 2020. Safety, efficacy and pharmacokinetics of repeat subcutaneous dosing of avexitide (exendin 9-39) for treatment of post-bariatric hypoglycaemia. *Diabetes Obes Metab,* 22(8)**:**1406-16.

TENDULKAR, P., KANT, R., RANA, S., YADAV, P., MIRZA, A. A. & AGARWAL, D. 2022. Efficacy of pro-kinetic agents in type 2 diabetes mellitus patients with gastroparesis using lactulose hydrogen breath testing: a randomized trial. *Cureus,* 14(1)**:**20990.

THARAKAN, G., BEHARY, P., ALBRECHTSEN, N. J. W., CHAHAL, H., KENKRE, J., MIRAS, A. D., AHMED, A. R., HOLST, J. J., BLOOM, S. R. & TAN, T. 2017a. Roles of increased glycaemic variability, GLP-1 and glucagon in hypoglycaemia after Roux-en-Y gastric bypass. *European Journal of Endocrinology,* 177(6)**:**455-64.

THARAKAN, G., BEHARY, P., WEWER ALBRECHTSEN, N. J., CHAHAL, H., KENKRE, J., MIRAS, A. D., AHMED, A. R., HOLST, J. J., BLOOM, S. R. & TAN, T. 2017b. Roles of increased glycaemic variability, GLP-1 and glucagon in hypoglycaemia after Roux-en-Y gastric bypass. *Eur J Endocrinol,* 177(6)**:**455-64.

THAYER, S. M., LO, J. O. & CAUGHEY, A. B. 2020. Gestational Diabetes: Importance of Follow-up Screening for the Benefit of Long-term Health. *Obstet Gynecol Clin North Am,* 47(3)**:**383-96.

THAZHATH, S. S., MARATHE, C. S., WU, T., CHANG, J., KHOO, J., KUO, P., CHECKLIN, H. L., BOUND, M. J., RIGDA, R. S., CROUCH, B., JONES, K. L., HOROWITZ, M. & RAYNER, C. K. 2016. The glucagon-like peptide 1 receptor agonist exenatide inhibits small intestinal motility, flow, transit, and absorption of glucose in healthy subjects and patients with type 2 diabetes: a randomized controlled trial. *Diabetes,* 65(1)**:**269-75.

TRAHAIR, L. G., HOROWITZ, M., MARATHE, C. S., LANGE, K., STANDFIELD, S., RAYNER, C. K. & JONES, K. L. 2014. Impact of gastric emptying to the glycemic and insulinemic responses to a 75-g oral glucose load in older subjects with normal and impaired glucose tolerance. *Physiol Rep,* 2(11)**:**12204.

TRAHAIR, L. G., HOROWITZ, M., RAYNER, C. K., GENTILCORE, D., LANGE, K., WISHART, J. M. & JONES, K. L. 2012. Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. *J Clin Endocrinol Metab,* 97(3)**:**844-51.

TRAHAIR, L. G., NAUCK, M. A., WU, T., BUTTFIELD, M. D. M., STEVENS, J. E., HATZINIKOLAS, S., RAYNER, C. K., MEIER, J., HOROWITZ, M. & JONES, K. L. 2018. Measurement of gastric emptying using scintigraphy and a C-13-octanoic acid breath test with Wagner-Nelson analysis in type 2 diabetes. *Diabetologia,* 61**:**S338-S9.

TRAHAIR, L. G., NAUCK, M. A., WU, T., STEVENS, J. E., BUTTFIELD, M. D., HATZINIKOLAS, S., PHAM, H., MEIER, J. J., RAYNER, C. K., HOROWITZ, M. & JONES, K. L. 2022. Measurement of gastric emptying using a 13C-octanoic acid breath test with Wagner-Nelson analysis and scintigraphy in type 2 diabetes. *Exp Clin Endocrinol Diabetes,* 130(11)**:**751-7.

TREMAROLI, V., KARLSSON, F., WERLING, M., STAHLMAN, M., KOVATCHEVA-DATCHARY, P., OLBERS, T., FANDRIKS, L., LE ROUX, C. W., NIELSEN, J. & BACKHED, F. 2015. Roux-en-Y Gastric Bypass and Vertical Banded Gastroplasty Induce Long-Term Changes on the Human Gut Microbiome Contributing to Fat Mass Regulation. *Cell Metab,* 22(2)**:**228-38.

TSENG, C. C., ZHANG, X. Y. & WOLFE, M. M. 1999. Effect of GIP and GLP-1 antagonists on insulin release in the rat. *Am J Physiol,* 276(6)**:**E1049-54.

TURA, A., MORBIDUCCI, U., SBRIGNADELLO, S., WINHOFER, Y., PACINI, G. & KAUTZKY-WILLER, A. 2011. Shape of glucose, insulin, C-peptide curves during a 3-h oral glucose tolerance test: any relationship with the degree of glucose tolerance? *Am J Physiol Regul Integr Comp Physiol,* 300(4)**:**R941-8.

UMAPATHYSIVAM, M. M., ARALDI, E., HASTOY, B., DAWED, A. Y., VATANDASLAR, H., SENGUPTA, S., KAUFMANN, A., THOMSEN, S., HARTMANN, B., JONSSON, A. E., KABAKCI, H., THAMAN, S., GRARUP, N., HAVE, C. T., FAERCH, K., GJESING, A. P., NAWAZ, S., CHEESEMAN, J., NEVILLE, M. J., PEDERSEN, O., WALKER, M., JENNISON, C., HATTERSLEY, A. T., HANSEN, T., KARPE, F., HOLST, J. J., JONES, A. G., RISTOW, M., MCCARTHY, M. I., PEARSON, E. R., STOFFEL, M. & GLOYN, A. L. 2023. Type 2 Diabetes risk alleles in Peptidyl-glycine Alpha-amidating Monooxygenase influence GLP-1 levels and response to GLP-1 Receptor Agonists. *medRxiv*. Epub ahead of print. DOI: 10.1101/2023.04.07.23288197.

UMAPATHYSIVAM, M. M., LEE, M. Y., JONES, K. L., ANNINK, C. E., COUSINS, C. E., TRAHAIR, L. G., RAYNER, C. K., CHAPMAN, M. J., NAUCK, M. A., HOROWITZ, M. & DEANE, A. M. 2014. Comparative effects of prolonged and intermittent stimulation of the glucagon-like peptide 1 receptor on gastric emptying and glycemia. *Diabetes,* 63(2)**:**785-90.

URVA, S., COSKUN, T., LOGHIN, C., CUI, X., BEEBE, E., O'FARRELL, L., BRIERE, D. A., BENSON, C., NAUCK, M. A. & HAUPT, A. 2020. The novel dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 (GLP-1) receptor agonist tirzepatide transiently delays gastric emptying similarly to selective long-acting GLP-1 receptor agonists. *Diabetes Obes Metab,* 22(10)**:**1886-91.

UTZSCHNEIDER, K. M., PRIGEON, R. L., FAULENBACH, M. V., TONG, J., CARR, D. B., BOYKO, E. J., LEONETTI, D. L., MCNEELY, M. J., FUJIMOTO, W. Y. & KAHN, S. E. 2009. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care,* 32(2)**:**335-41.

VAN BLOEMENDAAL, L., RG, I. J., TEN KULVE, J. S., BARKHOF, F., KONRAD, R. J., DRENT, M. L., VELTMAN, D. J. & DIAMANT, M. 2014. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes,* 63(12)**:**4186-96.

VAN CAUTER, E., MESTREZ, F., STURIS, J. & POLONSKY, K. S. 1992. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes,* 41(3)**:**368-77.

VAN CITTERS, G. W. & LIN, H. C. 2006. Ileal brake: neuropeptidergic control of intestinal transit. *Curr Gastroenterol Rep,* 8(5)**:**367-73.

VAN DEN BROEK, M., DE HEIDE, L. J. M., SIPS, F. L. P., KOEHORST, M., VAN ZUTPHEN, T., EMOUS, M., VAN FAASSEN, M., GROEN, A. K., VAN RIEL, N. A. W., DE BOER, J. F., VAN BEEK, A. P. & KUIPERS, F. 2021. Altered bile acid kinetics contribute to postprandial hypoglycaemia after Roux-en-Y gastric bypass surgery. *Int J Obes (Lond),* 45(3)**:**619-30.

VAN OLST, N., MEIRING, S., DE BRAUW, M., BERGMAN, J. J. G. H. M., NIEUWDORP, M., VAN DER PEET, D. L. & GERDES, V. E. A. 2020. Small intestinal physiology relevant to bariatric and metabolic endoscopic therapies: Incretins, bile acid signaling, and gut microbiome. *Techniques and Innovations in Gastrointestinal Endoscopy,* 22(3)**:**109-19.

VANIS, L., GENTILCORE, D., RAYNER, C. K., WISHART, J. M., HOROWITZ, M., FEINLE-BISSET, C. & JONES, K. L. 2011. Effects of small intestinal glucose load on blood pressure, splanchnic blood flow,

glycemia, and GLP-1 release in healthy older subjects. *Am J Physiol Regul Integr Comp Physiol,* 300(6)**:**R1524-31.

VANTANASIRI, K., MATAR, R., BERAN, A. & JARUVONGVANICH, V. 2020. The efficacy and safety of a procedureless gastric balloon for weight loss: a systematic review and meta-analysis. *Obes Surg,* 30(9)**:**3341-6.

VARGAS, E. J., BAZERBACHI, F., CALDERON, G., PROKOP, L. J., GOMEZ, V., MURAD, M. H., ACOSTA, A., CAMILLERI, M. & ABU DAYYEH, B. K. 2020. Changes in time of gastric emptying after surgical and endoscopic bariatrics and weight loss: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol,* 18(1)**:**57-68.

VAURS, C., BRUN, J. F., BERTRAND, M., BURCELIN, R., DU RIEU, M. C., ANDUZE, Y., HANAIRE, H. & RITZ, P. 2016. Post-prandial hypoglycemia results from a non-glucose-dependent inappropriate insulin secretion in Roux-en-Y gastric bypassed patients. *Metabolism,* 65(3)**:**18-26.

VICHEALTH NATIONAL COMMUNITY ATTITUDES SURVEY 2010. *Awareness and behaviours of low carb beer drinkers.* [Online]. Available: https://www.vichealth.vic.gov.au/~/media/ResourceCentre/PublicationsandResources/alcohol%20misuse/K-013_Low-carb-beer_FactSheet_FINAL.ashx [Accessed 14 Sept 2021].

VIDAL, J., NICOLAU, J., ROMERO, F., CASAMITJANA, R., MOMBLAN, D., CONGET, I., MOŔFNIGO, R. & LACY, A. M. 2009. Long-term effects of roux-en-y gastric bypass surgery on plasma glucagon-like peptide-1 and islet function in morbidly obese subjects. *Journal of Clinical Endocrinology and Metabolism,* 94(3)**:**884-91.

VINIK, A., NAKAVE, A. & CHUECOS MDEL, P. 2008. A break in the brake mechanism in diabetes: a cause of postprandial hyperglycemia. *Diabetes Care,* 31(12)**:**2410-3.

VOSOUGHI, K., ICHKHANIAN, Y., BENIAS, P., MILLER, L., AADAM, A. A., TRIGGS, J. R., LAW, R., HASLER, W., BOWERS, N., CHAVES, D., PONTE-NETO, A. M., DRAGANOV, P., YANG, D., EL HALABI, M., SANAEI, O., BREWER GUTIERREZ, O. I., BULAT, R. S., PANDOLFINO, J. & KHASHAB, M. 2022. Gastric per-oral endoscopic myotomy (G-POEM) for refractory gastroparesis: results from an international prospective trial. *Gut,* 71(1)**:**25-33.

WAGNER, J. G. & NELSON, E. 1964. Kinetic Analysis of Blood Levels and Urinary Excretion in the Absorptive Phase after Single Doses of Drug. *J Pharm Sci,* 53**:**1392-403.

WANG, W., CHENG, Z., WANG, Y., DAI, Y., ZHANG, X. & HU, S. 2019. Role of Bile Acids in Bariatric Surgery. *Front Physiol,* 10**:**374.

WANG, X., XIE, C., MARATHE, C. S., MALBERT, C. H., HOROWITZ, M., JONES, K. L., RAYNER, C. K., SUN, Z. & WU, T. 2020. Disparities in gastric emptying and postprandial glycaemia between Han Chinese and Caucasians with type 2 diabetes. *Diabetes Res Clin Pract,* 159**:**107951.

WANG, Z., WANG, R. M., OWJI, A. A., SMITH, D. M., GHATEI, M. A. & BLOOM, S. R. 1995. Glucagon-like peptide-1 is a physiological incretin in rat. *J Clin Invest,* 95(1)**:**417-21.

WATSON, L. E., XIE, C., WANG, X., LI, Z., PHILLIPS, L. K., SUN, Z., JONES, K. L., HOROWITZ, M., RAYNER, C. K. & WU, T. 2019. Gastric emptying in patients with well-controlled type 2 diabetes compared with young and older control subjects without diabetes. *J Clin Endocrinol Metab,* 104(8)**:**3311-9.

WHITEHEAD, W. E. & DELVAUX, M. 1997. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. The Working Team of Glaxo-Wellcome Research, UK. *Dig Dis Sci,* 42(2)**:**223-41.

WISHART, J., MORRIS, H. A. & HOROWITZ, M. 1992. Radioimmunoassay of gastric inhibitory polypeptide in plasma. *Clin Chem,* 38(10)**:**2156-7.

WISHART, J. M., HOROWITZ, M., MORRIS, H. A., JONES, K. L. & NAUCK, M. A. 1998. Relation between gastric emptying of glucose and plasma concentrations of glucagon-like peptide-1. *Peptides,* 19(6)**:**1049-53.

WITTE, A. B., GRYBACK, P., HOLST, J. J., HILSTED, L., HELLSTROM, P. M., JACOBSSON, H. & SCHMIDT, P. T. 2009. Differential effect of PYY1-36 and PYY3-36 on gastric emptying in man. *Regul Pept,* 158(1-3)**:**57-62.

WORLD HEALTH ORGANIZATION & INTERNATIONAL DIABETES FEDERATION 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of a WHO/IDF Consultation. Geneva (Switzerland): WHO Press.

WU, K. L., CHAIKOMIN, R., DORAN, S., JONES, K. L., HOROWITZ, M. & RAYNER, C. K. 2006. Artificially sweetened versus regular mixers increase gastric emptying and alcohol absorption. *Am J Med,* 119(9)**:**802-4.

WU, T., RAYNER, C. K. & HOROWITZ, M. 2016a. Incretins. *In:* HERZIG, S. (ed.) *Metabolic control.* Switzerland: Springer.

WU, T., RAYNER, C. K., JONES, K. & HOROWITZ, M. 2010. Dietary effects on incretin hormone secretion. *Vitam Horm,* 84**:**81-110.

WU, T., THAZHATH, S. S., MARATHE, C. S., BOUND, M. J., JONES, K. L., HOROWITZ, M. & RAYNER, C. K. 2015. Comparative effect of intraduodenal and intrajejunal glucose infusion on the gut-incretin axis response in healthy males. *Nutr Diabetes,* 5(5)**:**156.

WU, T., ZHANG, X., TRAHAIR, L. G., BOUND, M. J., LITTLE, T. J., DEACON, C. F., HOROWITZ, M., JONES, K. L. & RAYNER, C. K. 2016b. Small Intestinal Glucose Delivery Affects the Lowering of Blood Glucose by Acute Vildagliptin in Type 2 Diabetes. *J Clin Endocrinol Metab,* 101(12)**:**4769-78.

WU, T., ZHAO, B. R., BOUND, M. J., CHECKLIN, H. L., BELLON, M., LITTLE, T. J., YOUNG, R. L., JONES, K. L., HOROWITZ, M. & RAYNER, C. K. 2012. Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans. *Am J Clin Nutr,* 95(1)**:**78-83.

XIE, C., HUANG, W., WANG, X., TRAHAIR, L. G., PHAM, H. T., MARATHE, C. S., YOUNG, R. L., JONES, K. L., HOROWITZ, M., RAYNER, C. K. & WU, T. 2021a. Gastric emptying in health and type 2 diabetes: An evaluation using a 75 g oral glucose drink. *Diabetes Res Clin Pract,* 171**:**108610.

XIE, C., HUANG, W., WATSON, L. E., SOENEN, S., YOUNG, R. L., JONES, K. L., HOROWITZ, M., RAYNER, C. K. & WU, T. 2021b. Plasma GLP-1 response to oral and intraduodenal nutrients in health and type 2 diabetes - impact on gastric emptying. *J Clin Endocrinol Metab,* 107(4)**:**1643-52.

XIE, C., JALLEH, R. J., WATSON, L. E., HUANG, W., SUN, Y., JONES, K. L., HOROWITZ, M., RAYNER, C. K. & WU, T. 2023. Determinants of blood glucose concentrations following a high carbohydrate meal in type 2 diabetes: A multiple linear regression analysis. *Diabetes Res Clin Pract,* 198**:**110606.

YKI-JARVINEN, H. & NIKKILA, E. A. 1985. Ethanol decreases glucose utilization in healthy man. *J Clin Endocrinol Metab,* 61(5)**:**941-5.

ZACHWIEJA, J. J., COSTILL, D. L., WIDRICK, J. J., ANDERSON, D. E. & MCCONELL, G. K. 1991. Effects of drink carbonation on the gastric emptying characteristics of water and flavored water. *Int J Sport Nutr,* 1(1)**:**45-51.

ZHANG, X., YOUNG, R. L., BOUND, M., HU, S., JONES, K. L., HOROWITZ, M., RAYNER, C. K. & WU, T. 2019. Comparative effects of proximal and distal small intestinal glucose exposure on glycemia, incretin hormone secretion, and the incretin effect in health and type 2 diabetes. *Diabetes Care,* 42(4)**:**520-8.

ZHOU, L. Y., DENG, M. Q. & XIAO, X. H. 2020. Potential contribution of the gut microbiota to hypoglycemia after gastric bypass surgery. *Chin Med J (Engl),* 133(15)**:**1834-43.