RELATIONSHIPS OF GASTRIC EMPTYING WITH GLYCAEMIA, INSULIN SECRETION AND THE INCRETIN EFFECT IN HEALTH AND TYPE 2 DIABETES

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THESIS ABSTRACT

This thesis focuses on the inter-dependent relationships between gastric emptying, incretin hormones (GIP and GLP-1) and postprandial glycaemic and insulinaemic responses in health and type 2 diabetes.

Key themes relate to:

1) Evaluation of the relationships of ‘early’ and ‘late’ glycaemic responses with gastric emptying in subjects with normal glucose tolerance, impaired glucose tolerance and type 2 diabetes.

2) Evaluation of the relationships of the insulin secretory response and the oral disposition index with gastric emptying in subjects with normal glucose tolerance.

3) Utilisation of intraduodenal glucose infusions to assess the impact of gastric emptying on: a) the incretin effect, gastrointestinal glucose disposal and the glucagon response, b) the oral disposition index, c) the secretion of incretin hormone secretory pattern in Caucasian compared with Han Chinese subjects and d) postprandial blood pressure and heart rate in type 2 diabetes.

Gastric emptying, which regulates the entry of nutrients into the small intestine, is a major determinant of the ‘initial’ glycaemic response (i.e. at 30 min) following an oral glucose tolerance test in health, as well as in type 2 diabetes such that if gastric emptying is more rapid, the initial blood glucose levels are greater. However, the relationships of the 60 min blood glucose (a known predictor of type 2 diabetes) and 120 min blood glucose (used for diagnosis) with gastric emptying during oral glucose tolerance tests have not been studied. My study explored the relationships of 30 min, 60 min and 120 min blood glucose with gastric emptying (measured scintigraphically – the ‘gold standard’ method) in participants with normal glucose tolerance, impaired glucose tolerance and type 2 diabetes.
The relationship between the insulin secretory response (calculated as the ratio of change in insulin at 30 min to the change in glucose at 30 represented as $\Delta I_{0,30} / \Delta G_{0,30}$) and insulin sensitivity (calculated as the reciprocal of fasting insulin and represented as $1/\text{fasting insulin}$) during an oral glucose tolerance test is hyperbolic in subjects with normal glucose tolerance such that their product, referred to as the ‘oral disposition index’ ($\Delta I_{0,30} / \Delta G_{0,30} \times 1/\text{fasting insulin}$) is always constant. This implies that as long as the pancreatic beta cells are able to compensate adequately (by up-regulating insulin secretion) for any reduction in insulin sensitivity, the oral disposition index remains constant so that the individual has a ‘normal glucose tolerance’. It is, therefore, the failure to compensate fully for the reduction in insulin sensitivity (resulting in a lower oral disposition index) that leads to development of impaired glucose tolerance and type 2 diabetes. Oral disposition index is widely used as a predictor of type 2 diabetes. While the relationship of the early glycaemic response ($\Delta G_{0,30}$) with gastric emptying has been characterised, the relationships of the early insulin response ($\Delta I_{0,30} / \Delta G_{0,30}$) and the oral disposition index with gastric emptying are uncertain. My study explored these relationships in subjects with normal glucose tolerance.

There is a wide inter-individual, but relatively little intra-individual variation in the overall rate of gastric emptying (between 1-4 kcal/min in health); this range is even wider in diabetes as a substantial proportion of patients have gastroparesis (i.e. delayed gastric emptying) while in some gastric emptying is abnormally accelerated. This has profound implications for control of glycaemia in diabetes, as even minor variations in the rate of entry of nutrients into the small intestine may be associated with substantial changes in postprandial glycaemic and insulinaemic responses. The incretin hormones, GIP and GLP-1, located in the gut and stimulated by exposure of nutrients to the intestine, play a major role in postprandial glucose metabolism accounting for up to 50% of the post-meal insulin response in health. The incretin hormones are responsible for the so-called ‘incretin effect’ – the amplified insulin secretory response following oral, compared with intravenous, glucose. The incretin effect is known to
be attenuated in type 2 diabetes. The impact of gut in glucose disposal can also be described by the so-called ‘gastrointestinal glucose disposal’ (GIGD). GIGD, the amount of glucose required by intravenous infusion to ‘copy’ the glucose excursions after the oral load, was calculated as follows: if 25g intravenous glucose is required to copy a 75g oral glucose load, the GIGD amounts to $100 \times \frac{(75 - 25)}{75} = 66\%$. GIGD is also reduced in type 2 diabetes.

Intraduodenal glucose infusions (via a naso-duodenal catheter) bypass the pylorus and allow glucose to be delivered directly into the small intestine at a pre-determined rate. This model has been employed to study the impact of gastric emptying on postprandial glycaemic and insulinaemic excursions. The outcome of these studies, in which glucose was infused at variable rates within the ‘physiological’ range of gastric emptying i.e. 1,2,3 and 4 kcal/min, indicate that the relationship between the rise in glycaemia and the rate of small intestinal glucose exposure is non-linear. While the glycaemic response was significantly greater in response to 2 kcal/min intraduodenal infusion than 1 kcal/min, increasing the infusion rate further (i.e. 3 and 4 kcal/min) only resulted in minimal, if any, further increase in blood glucose. Glucagon, the hormone produced by the $\alpha$ cells of pancreas, is suppressed following ingestion of glucose in health and thus an important determinant of postprandial blood glucose response, although glucagon suppression is impaired in type 2 diabetes. While GLP-1 suppresses glucagon, GIP does not and may, in fact, modestly elevate it. The effect of gastric emptying on glucagon responses in health and type 2 diabetes is not known. My study looked at the impact of variable duodenal glucose load on the incretin effect and GIGD as well as on glucagon responses in health as well as in type 2 diabetes.

There is evidence that East Asians secrete less insulin than Caucasians following oral glucose suggesting that impaired insulin secretion is fundamental to the pathogenesis of type 2 diabetes. However, information about the secretory patterns of GIP and GLP-1, dependent on duodenal glucose load in East Asians, is limited. My study evaluated the glycaemic,
insulinaemic and incretin hormone response to a duodenal glucose load in healthy Han Chinese men compared with healthy Caucasian men.

Postprandial hypotension (PPH), defined as a fall in systolic blood pressure ≥20mmHg after a meal, occurs frequently in diabetes and its management remains sub-optimal. As well as influencing postprandial glycaemia, gastric emptying also affects the postprandial hypotensive response in ‘healthy’ older subjects and type 2 patients, such that when GE is relatively more rapid, the magnitude of fall in systolic blood pressure is greater. In healthy older subjects, when gastric distension – which may influence blood pressure – is ‘bypassed’ by infusing glucose directly into the duodenum, the fall in systolic blood pressure is greater in response to 2 and 3 kcal/min than 1 kcal/min. It is not known whether duodenal glucose delivery influences blood pressure in type 2 patients. My study evaluated the effects of variations in the intraduodenal glucose load on blood pressure and heart rate in type 2 patients.
FORMAT OF THE THESIS

This thesis consists of chapters adapted from one review and six original manuscripts. The review and the original manuscripts have all been published (except chapter 3, which has been submitted for publication) in peer-reviewed journals. None of these articles were solicited by the journals. All published papers were submitted to appropriate diabetes journals, and underwent peer review by 2-4 reviewers with further revisions until the reviewers and editors were satisfied. Each study is reported “in full” as a chapter, to allow independent review with unavoidable repetition. The methodology is described individually in each chapter. All chapters (and published papers) report on data derived from three main clinical studies.
THESIS DECLARATION

I, Chinmay Marathe,

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Signed,

Date 14th February 2016
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RESEARCH PRESENTATIONS ARISING FROM THIS THESIS

Marathe CS, Jones KL, Horowitz M, Rayner CK.
Relationships of early insulin response and oral disposition index with gastric emptying during an oral glucose tolerance test in subjects with normal glucose tolerance.
*Diabetes UK Annual Conference, Glasgow, United Kingdom*
March 2016 - Oral presentation
(In Competition for Type 2 Diabetes Research Award)

Marathe CS, Rayner CK, Jones KL, Horowitz M.
Small intestinal glucose exposure is a determinant of the magnitude of the incretin effect in health and type 2 diabetes.
*Royal Australasian College of Physicians Annual Scientific Sessions, Cairns*
2015 – Oral presentation
(RACP Trainee Research Award for Excellence – South Australia)

Marathe CS, Horowitz M, Bound M, Lange K, Rayner CK, Jones KL.
Relationships of early and late glycaemic responses with gastric emptying during an oral glucose tolerance test.
*Australian Diabetes Society Annual Meeting, Adelaide, Australia*
2015 – Poster presentation

Marathe CS, Rayner CK, Jones KL, Horowitz M.
Ethnic variation in insulin and incretin responses to intraduodenal glucose in healthy humans.
*American Diabetes Association Annual Scientific Sessions, San Francisco*
2014 – Poster presentation
Marathe CS, Rayner CK, Jones KL, Horowitz M.
The magnitude of the incretin effect is dependent on the small intestinal glucose load in type 2 diabetes.

*American Diabetes Association Annual Scientific Sessions, Chicago*

2013 – *Poster presentation*

Marathe CS, Feinle –Bisset C, Bound M, Standfield S, Jones KL, Horowitz M & Rayner CK.
The effect of intraduodenal glucose delivery on the oral disposition index in health and type 2 diabetes.

*American Diabetes Association Annual Scientific Sessions, Chicago*

2013 – *Poster presentation*

Marathe CS, Rayner CK, Jones KL, Horowitz M.
The size of the incretin effect is dependent on the small intestinal glucose load in health.

*American Diabetes Association Annual Scientific Sessions, Philadelphia*

2012 – *Oral presentation*

Marathe CS, Horowitz M, Rayner CK, Lange K, Jones KL.
Biphasic relationship between oral glucose tolerance and gastric emptying in healthy subjects.

*European Association for the Study of Diabetes, Lisbon, Portugal*

2011 – *Poster Presentation*

(Recipient of EASD Travel Grant Award)
LIST OF PUBLICATIONS ARISING FROM THIS THESIS

Marathe CS, Rayner CK, Jones KL, Horowitz M.

Novel insights into the effects of diabetes on gastric motility.

PMID: 26647088

Marathe CS, Horowitz M, Trahair LG, Wishart JM, Bound M, Lange K, Rayner CK, Jones KL.

Relationships of early and late glycemic responses with gastric emptying during an oral glucose tolerance.

PMID: 26171801

Marathe CS, Feinle-Bisset C, Pilichiewicz A, Lange K, Jones KL, Rayner CK, Kahn SE, Horowitz M.

The duodenal glucose load impacts the oral disposition index in healthy subjects.

PMID: 25981372

Marathe CS, Bound M, Lange K, Jones KL, Rayner CK, Horowitz M.

Ethnic disparities in insulin and glucose-dependent insulinotropic peptide (GIP) responses to intraduodenal glucose in health.

PMID: 25399343

Small intestinal glucose exposure determines the magnitude of the incretin effect in health and type 2 diabetes.


Marathe CS, Rayner CK, Jones KL, Horowitz M.

Relationships between gastric emptying, postprandial glycemia, and incretin hormones.


Marathe CS, Rayner CK, Jones KL, Horowitz M.

Glucagon-like peptides 1 and 2 in health and disease: a review.

Peptides. 2013 Jun; 44:75-86. doi: 10.1016/j.peptides.2013.01.014. PMID: 23523778

Marathe CS, Rayner CK, Jones KL, Horowitz M.

Effects of GLP-1 and incretin-based therapies on gastrointestinal motor function.

CHAPTER 1
CHAPTER 1. RELATIONSHIPS BETWEEN GASTRIC EMPTYING, POSTPRANDIAL GLYCAEMIA AND INCRETIN HORMONES

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1.1 INTRODUCTION

The importance of achieving tight glycaemic control, usually assessed by glycated haemoglobin (HbA1c), for both the prevention and to delay in the progression of diabetes-related microvascular complications, is established and the ADA/EASD joint committee has recommended an HbA1c <7% as the goal in type 2 patients (Inzucchi, Bergenstal et al. 2012). The relative contributions of pre- and postprandial glycaemia to HbA1c have been clarified during the last decade, following the seminal report by Monnier et al. indicating that in type 2 diabetes, postprandial glycaemic excursions account for about 70% of variability when HbA1c is <7.3%, while the contribution of ‘fasting’ glycaemia is greater in less well controlled patients (Monnier, Lapinski et al. 2003). Subsequent studies have confirmed the predominance of postprandial glycaemia in determining overall glycaemic control in ‘well controlled’ type 2 patients managed by oral hypoglycaemic agents, or basal insulin (Riddle, Umpierrez et al. 2011). The importance of postprandial glycaemia to overall glycaemic control is not surprising considering that (i) humans in modern societies spend only about 3 or 4 hours before breakfast in a truly fasting state, because in health, gastric emptying of meals occurs at an overall rate of 1-4 kcal/minute (Horowitz and Dent 1991), and (ii) postprandial hyperglycaemia occurs frequently in diabetes (Inzucchi, Bergenstal et al. 2012). The relevance of postprandial glycaemia is further increased by the recognition that it may
represent an independent risk factor for adverse cardiovascular outcomes in both diabetic and non-diabetic populations (Standl, Schnell et al. 2011).

The determinants of postprandial glycaemia include preprandial glycaemic levels, meal composition, gastric emptying, insulin secretion, small intestinal glucose absorption of, and hepatic and peripheral glucose metabolism. Furthermore, the relative contributions of each of these factors may vary over time during the postprandial state. Nevertheless, both the rate of gastric emptying and the secretion and action of the ‘incretin’ hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), exert a major influence (Chang, Rayner et al. 2010). The latter limit postprandial glycaemia through their insulinotropic and, in case of GLP-1, glucagonostatic actions. They account for the ‘incretin effect’ – a substantial augmentation of insulin secretion following oral or enteral glucose exposure, when compared with an isoglycaemic intravenous glucose infusion, which is diminished in type 2 diabetes (Drucker and Nauck 2006). GIP and GLP-1 are released from entero-endocrine cells located most densely in the proximal small intestine and distal small intestine/colon respectively, following nutrient exposure. Each macronutrient has the capacity to stimulate GLP-1 and GIP release, although the mechanisms underlying secretion differ, and fat and carbohydrate may be more potent stimuli than protein (Baggio and Drucker 2007). Carbohydrates, for example, stimulate incretin secretion through a number of interrelated mechanisms, which are likely to include the sodium-glucose co-transporter-1 (SGLT-1) and intestinal ‘sweet taste’ receptors (Baggio and Drucker 2007). Following their release, the incretin hormones are rapidly degraded to ‘inactive metabolites’ by the ubiquitous enzyme, dipeptidyl peptidase-4 (DPP-IV). While GIP may be the most important incretin hormone in health, its capacity to stimulate insulin is markedly diminished in type 2 diabetes (Nauck, Heimesaat et al. 1993, Baggio and Drucker 2007).
Even relatively minor variations in gastric emptying can have a major impact on the postprandial glycaemic profile in health and type 2 diabetes (Gonlachanvit, Hsu et al. 2003, O'Donovan, Doran et al. 2004, Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012). It is now recognized that complex, inter-dependent relationships exist between gastric emptying, the incretin axis, and postprandial glycaemia, with the rate of gastric emptying having a major impact on the magnitude of both the glycaemic excursion and incretin hormone secretion, and conversely, acute hyperglycaemia and GLP-1 exerting negative feedback on gastric emptying (Chang, Rayner et al. 2010). This review focuses on these inter-relationships, summarised in figure 1, and the consequent implications for dietary and pharmacological strategies to manage postprandial glycaemia in type 2 diabetes.

**Figure 1.** Summary of the inter-dependent relationships of gastric emptying, incretin hormones and postprandial glycaemia.
1.2 GASTRIC EMPTYING IN HEALTH AND DIABETES

It is not well recognized that in both health and diabetes, the rate of gastric emptying shows wide inter-individual, with much less intra-individual, variation (Horowitz and Dent 1991). Gastric emptying is determined by the integration of motor activity of the stomach and upper small intestine, controlled by electrical slow waves generated by the interstitial cells of Cajal (ICC) (Horowitz and Dent 1991, Kashyap and Farrugia 2010). The proximal stomach initially relaxes to accommodate a meal and the antrum then grinds solids to a small particle size (1-2 mm), before the chyme is pumped across the pylorus, predominantly in a pulsatile manner (Horowitz and Dent 1991). These processes are regulated primarily by inhibitory feedback arising from the interaction of nutrients with the small intestine, rather than by ‘intragastric’ mechanisms, and modulated by both stimulation of the vagus nerve and the secretion of gut hormones, including GLP-1, cholecystokinin (CCK) and peptide YY (PYY). The magnitude of small intestinal inhibitory feedback is dependent on the nutrient load and the length of intestine exposed and is associated with fundic relaxation, suppression of antral contractions, and stimulation of tonic and phasic contractions localised to the pylorus, to retard the subsequent rate of nutrient delivery to the intestine (Horowitz and Dent 1991).

While it has been suggested for many years that the gastric motor function is frequently disordered in diabetes – the term ‘gastroparesis diabeticorum’ was introduced by Kassander more than fifty years ago (Kassander 1958) – it has only much more recently been confirmed that abnormally delayed gastric emptying occurs frequently with both type 1 and type 2 diabetes (Chang, Rayner et al. 2010). The true prevalence, however, remains uncertain because of a lack of population-based studies and inconsistent criteria to define gastroparesis, including variations in the technique used to quantify gastric emptying (where scintigraphy remains the ‘gold standard’ method and stable isotope breath tests and ultrasonography represent acceptable alternatives), the desirable blood glucose levels during the emptying measurement, the magnitude of the delay in gastric emptying regarded as abnormal, and
whether the presence of gastrointestinal symptoms represents a pre-requisite for the diagnosis. In relation to the latter, it has been suggested that gastroparesis should not be diagnosed in the absence of upper gastrointestinal symptoms (Jung, Choung et al. 2009), but we believe this is inappropriate given that delayed gastric emptying can have other manifestations, particularly in relation to glycaemic control. Nevertheless, there is no doubt that the prevalence of delayed gastric emptying with symptoms is less than that of delayed emptying per se. While recognising these limitations, it appears that the gastric emptying of solids and/or nutrient liquids is delayed in 30-50% of patients with longstanding type 1 or type 2 diabetes, and is sometimes abnormally rapid (Bharucha, Camilleri et al. 2009, Chang, Rayner et al. 2010, Kashyap and Farrugia 2010). A high prevalence (approximately 47%) of delayed gastric emptying has recently been reported in the DCCT cohort (Bharucha, Batey-Schaefer et al. 2015). As would be predicted, motor dysfunction of different regions of the stomach is also frequently observed, but is heterogeneous (Kashyap and Farrugia 2010). This high prevalence of disordered emptying is not surprising given that it is consistent with the prevalence of other diabetic complications, including peripheral neuropathy. Because the relationship of gastrointestinal symptoms – such as fullness, nausea or vomiting – with disordered emptying is relatively weak (Horowitz, Maddox et al. 1991, Jones, Russo et al. 2001, Bharucha, Camilleri et al. 2009), and the magnitude of delay in gastric emptying is in many cases modest (Horowitz, Maddox et al. 1991), it is likely that many, if not the majority, of such patients do not come to clinical attention. Nevertheless, hospital admissions in symptomatic patients given a diagnosis of diabetic gastroparesis are reported to be increasing (Wang, Fisher et al. 2008).

Diabetic gastroparesis has long been attributed to the presence of irreversible autonomic (vagal) neuropathy, but the pathogenesis is now recognised to be heterogeneous. Important insights have resulted from the work of the Gastroparesis Clinical Research Consortium in the United States. Loss or dysfunction of ICC and defects in inhibitory transmission, particularly
involving neuronal nitric oxide synthase, appear to be of central importance, as well as abnormalities in immune cells (CD 45, CD 206) and an up-regulation of haeme oxygenase-1 in macrophages, which impacts on the enteric neurotransmitter carbon monoxide (Kashyap and Farrugia 2010, Grover, Farrugia et al. 2011). The prognosis in patients with delayed gastric emptying is not necessarily poor (Kong, Horowitz et al. 1999) - preliminary data indicate that there is little, if any, change in gastric emptying over periods of up to 25 years (Chang, Russo et al. 2012) and that mortality is not increased (Kong, Horowitz et al. 1999), although the latter may not apply to patients with severe gastroparesis.

1.3 IMPACT OF GASTRIC EMPTYING ON GLYCAEMIA AND INCRETIN HORMONES

The recognition that gastric emptying is a major determinant of postprandial glycaemia in health, as well as type 1 and type 2 diabetes, is relatively recent (Horowitz, Edelbroek et al. 1993, Ishii, Nakamura et al. 1994, Jones, Horowitz et al. 1996, Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012). In retrospect, such a relationship is not surprising given the substantial inter-individual variation in gastric emptying in health, which is even greater in patients with diabetes because of the frequent occurrence of delayed, and occasionally accelerated, gastric emptying. Gastric emptying accounts for about 35% of the variance in the glycaemic response (both peak and total area under the curve) to oral glucose and/or carbohydrate-containing meals in health (Horowitz, Edelbroek et al. 1993) and type 2 diabetes (Jones, Horowitz et al. 1996). Insulin-treated patients with gastroparesis initially require less insulin to maintain euglycaemia postprandially when compared to patients with normal emptying (Ishii, Nakamura et al. 1994). In type 2 patients not managed with insulin, slowing gastric emptying decreases postprandial glycaemic excursions, while acceleration of emptying has the opposite effect (Gonlachanvit, Hsu et al. 2003). In patients with cystic fibrosis and concomitant pancreatic exocrine insufficiency, gastric emptying of high
fat/carbohydrate meals is accelerated and incretin secretion impaired due to maldigestion of nutrients, leading to elevated postprandial glucose levels, even in those without overt diabetes. In this situation, pancreatic enzyme supplementation given with a meal slows gastric emptying and restores the GLP-1 response, thereby diminishing postprandial glycaemia substantially (Kuo, Stevens et al. 2011).

The relationship of glycaemia with small intestinal glucose delivery has recently been shown to be non-linear, based on the outcome of studies in which glucose was infused directly into the duodenum at rates spanning the normal range of gastric emptying (i.e. 1-4 kcal/min) in healthy young (Pilichiewicz, Chaikomin et al. 2007) and older subjects (Trahair, Horowitz et al. 2012), and type 2 patients managed by diet alone (Ma, Pilichiewicz et al. 2012). In these groups, intraduodenal infusion of glucose at 1 kcal/min was associated with only a modest rise in blood glucose, and while the glycaemic responses to loads of 2 kcal/min, 3 kcal/min and 4 kcal/min were substantially greater, there was little difference between them (Figure 2). The latter probably reflects the much greater plasma insulin response to the higher intraduodenal glucose loads (i.e. 3 and 4 kcal/min), which is in turn, attributable to a much greater GLP-1 response. Plasma GIP, unlike GLP-1, rises in an approximately linear fashion with increasing glucose loads (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012). In other studies of healthy subjects and type 2 patients, the effects of initially more rapid intraduodenal glucose delivery, when compared with delivery of the same amount of glucose at a constant rate, were explored (O'Donovan, Doran et al. 2004, Chaikomin, Doran et al. 2005). While the initial plasma insulin, GLP-1 and GIP responses were predictably greater with the former approach, there was no difference in the overall glycaemic response, implying that the initial increase in insulin was inadequate to compensate for the greater load of glucose absorbed. A fundamental implication of these studies is that the magnitude of the incretin effect in a given individual will be dependent on their rate of gastric emptying, i.e. the incretin effect should be greater in an individual, healthy
or with type 2 diabetes, whose stomach empties at 4 kcal/minute when compared with another in whom emptying occurs at 2 kcal/minute, although the former situation would also favour an initially greater postprandial glycaemic excursion. Hence, studies relating to incretin hormone secretion should probably take into account the rate of gastric emptying (which has hitherto frequently not been the case) and the incretin hormone responses to a meal, particularly that of GLP-1, appear to represent a dynamic compensatory mechanism to minimise postprandial glycaemia when emptying is relatively more rapid. In a healthy individual, the relative importance of GIP and GLP-1 in determining the incretin effect is likely to be dependent on the rate of gastric emptying. In type 2 diabetes, the magnitude of GLP-1 response is likely to be crucial given the reduced insulinotropic effect of GIP (Drucker and Nauck 2006). We would speculate that to minimise postprandial glycaemic excursions in type 2 patients, gastric emptying of carbohydrate should be slowed to about 1 kcal/minute in those whose emptying is more rapid than this. As has been suggested, the prompt amelioration of type 2 diabetes after Roux-en-Y gastric bypass surgery is likely to reflect, at least in part, an exaggerated incretin (particularly GLP-1) response (Naslund, Gryback et al. 1997) consequent to dramatically accelerated gastric emptying of nutrient-containing liquids and ‘semi-solids’ (Horowitz, Cook et al. 1982).

**Figure 2 (see following page).** Effect of intraduodenal glucose load on glycaemia, plasma insulin and incretin hormones. Blood glucose (a), plasma insulin (b), glucagon-like peptide-1 (GLP-1) (c) and glucose-dependent insulinotropic polypeptide (GIP) (d) concentrations in response to a 120-min intraduodenal glucose infusion at 1 kcal/min (G1), 2 kcal/min (G2), 4 kcal/min (G4) or (iv) saline control (S) in 10 healthy subjects and eight Type 2 patients. Data are presented as mean ± SEM. *P < 0.05 vs. control, #P < 0.05 vs. G1, §P < 0.05 vs. G2. Adapted from Ma et al. Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association. 2012;29(5):604-8 with permission.
As well as being a determinant of glycaemia, gastric emptying is itself modulated by acute changes in the blood glucose concentration (Rayner, Samsom et al. 2001, Chang, Rayner et al. 2010). While there is a lack of consensus in relation to the magnitude of the effect of acute hyperglycaemia, and the potential influence of chronic elevation of blood glucose, it is clear that marked acute hyperglycaemia (i.e. blood glucose level ~ 15mmol/L) delays gastric emptying substantially in both health and type 1 diabetes, when compared to euglycaemia (~5 mmol/L). Emptying is slowed even at ‘physiological’ degrees of hyperglycaemia (~8mmol/L) (Rayner, Samsom et al. 2001), and accelerated during insulin-induced hypoglycaemia (Russo, Stevens et al. 2005); the latter response is evident even in patients with autonomic neuropathy and gastroparesis, and likely represents an important counter-regulatory mechanism to facilitate carbohydrate absorption (Russo, Stevens et al. 2005). Acute hyperglycaemia attenuates the gastrokinetic effect of erythromycin (Jones, Berry et al. 1999) and this effect is likely also to apply to other prokinetic drugs. It remains to be determined whether the effect of drugs that slow gastric emptying is also modulated by acute changes in the blood glucose concentration, but this appears intuitively likely. The mechanisms by which acute hyperglycaemia modulates gastric emptying are poorly defined, but nitrergic pathways appear important (Kuo, Gentilcore et al. 2009). There is limited evidence that ‘chronic’ hyperglycaemia, as assessed by glycated haemoglobin, also impacts on gastric emptying, as suggested in the DCCT cohort (Bharucha, Batey-Schaefer et al. 2015).

1.4 EFFECTS OF ENDOGENOUS AND EXOGENOUS INCRETIN HORMONES ON GASTRIC EMPTYING

GIP and GLP-1 differ in their effects on gastric emptying. Studies employing the GLP-1 receptor antagonist, exendin 9-39, indicate that endogenous GLP-1 slows gastric emptying modestly (Deane, Nguyen et al. 2010) and affects intra-gastric meal distribution (Witte, Gryback et al. 2011), as a result of suppression of antro-duodenal motility and stimulation of pyloric contractions (Schirra, Nicolaus et al. 2006). That not all investigators have
demonstrated effects on emptying probably reflects methodological issues, including the composition of the test meal and the use of sub-optimal techniques to measure gastric emptying (Salehi, Vahl et al. 2008, Witte, Gryback et al. 2011). The indirect assessment of gastric emptying using the absorption kinetics of oral paracetamol is imprecise, but has been used widely.

Acute, intravenous infusion of GLP-1 (in pharmacological doses) slows gastric emptying markedly in both healthy subjects and type 2 patients, in a dose-dependent manner (Nauck, Niedereichholz et al. 1997, Meier, Gallwitz et al. 2003, Little, Pilichiewicz et al. 2006), by mechanisms that include relaxation of the proximal stomach, reduction of antral and duodenal motility and an increase in pyloric tone (Schirra, Houck et al. 2000), and which involve vagal pathways (Wettergren, Wojdemann et al. 1998). With pharmacological doses of GLP-1, nausea appears to occur rarely, unlike the situation with GLP-1 agonists. By contrast, in healthy subjects, exogenous GIP in pharmacological doses does not slow gastric emptying (Meier, Goetze et al. 2004) and may accelerate it modestly (Edholm, Degerblad et al. 2010).

The magnitude of the retardation of gastric emptying induced by exogenous GLP-1 is influenced by the ‘baseline’ rate of emptying, i.e. slowing is more marked in those with more rapid gastric emptying. For example, in the critically ill, GLP-1 slows gastric emptying when the latter is normal, but not when it is delayed (Deane, Chapman et al. 2010). Importantly, the reduction in postprandial glycaemia induced by exogenous GLP-1 is also closely related to the magnitude of the slowing of gastric emptying, being greater when baseline emptying is relatively more rapid (Little, Pilichiewicz et al. 2006, Deane, Chapman et al. 2010) (Figure 3). Indeed, the effect of acute administration of GLP-1 to slow gastric emptying outweighs its insulinotropic effect, so that while GLP-1 stimulates insulin during hyperglycaemia in the fasted state, postprandial insulin concentrations are suppressed, rather than stimulated, in both health and type 2 diabetes (Nauck, Niedereichholz et al. 1997, Little, Pilichiewicz et al. 2006),
and when the GLP-1-induced slowing of gastric emptying is reversed by administering erythromycin, the insulinotropic action of GLP-1 is unmasked (Meier, Kemmeries et al. 2005). Accordingly, it is arguable whether GLP-1 should be regarded as a true ‘incretin’ hormone according to Creutzfeldt’s definition (Horowitz and Nauck 2006). No studies have evaluated the effect of exogenous GLP-1 on gastric emptying in patients with gastroparesis, although it is known that the relaxation of the proximal stomach induced by exogenous administration of GLP-1 is attenuated in diabetic patients with autonomic neuropathy (Delgado-Aros, Vella et al. 2003). Hence the magnitude of the slowing of gastric emptying induced by GLP-1 will almost certainly be reduced in gastroparesis. It is not known whether the GLP-1 induced slowing of gastric emptying can be ‘over-ridden’ by hypoglycaemia, which, as discussed, is associated with prompt acceleration of emptying (Russo, Stevens et al. 2005). A recent study reported that exogenous GLP-1 attenuated, but did not abolish, the accelerated gastric emptying induced by insulin clamp studies involving healthy volunteers (Plummer, Jones et al. 2014). This issue should be addressed, particularly when GLP-1 agonists are used in combination with sulfonylureas, or insulin when the propensity to hypoglycaemia will be increased. Furthermore, hyperglycaemia, induced by glucose clamp studies, potentiates the slowing of gastric emptying induced by exogenous GLP-1 (Plummer, Jones et al. 2015).

A recent report suggests that there may be rapid tachyphylaxis to the slowing of gastric emptying induced by exogenous GLP-1 (Nauck, Kemmeries et al. 2011). In that study, two liquid ‘meals’, separated by 4 hours, were given to healthy volunteers during intravenous infusion of GLP-1 or placebo. GLP-1 was shown to slow emptying of both meals markedly, but the magnitude of slowing of the second meal was less. It was suggested that this tachyphylaxis occurs at the level of the vagus nerve (Nauck, Kemmeries et al. 2011). Consistent with this is a recent study in which exogenous GLP-1 was administered in in healthy volunteers in three regimens, a 4.5 h infusion (‘acute’), two 4.5 h infusions separated by 20 h (‘intermittent’) and a continuous 24 h infusion (‘prolonged’), along with a mashed
potato meal and demonstrated that gastric emptying and postprandial glycaemia were both reduced with ‘acute’ and ‘intermittent’, but not with ‘prolonged’ regimen (Umapathysivam, Lee et al. 2014). These observations are of considerable interest and likely to be relevant to the observed effects of GLP-1 agonists on gastric emptying.

Figure 3. Relationship between the effect of GLP-1 (1.2 pmol/kg/min IV) on gastric emptying and the rate of gastric emptying on placebo in critically ill patients (n=25). Gastric emptying of a 100 ml nutrient liquid (Ensure) labelled with 13C octanoic acid was measured with a breath test and the gastric emptying coefficient (GEC) determined. A lower GEC is indicative of more rapid gastric emptying. R = -0.48, P < 0.001 (Reprinted with permission from Deane AM et al (Deane, Chapman et al. 2010)

1.5 MODULATION OF GASTRIC EMPTYING TO MINIMISE POSTPRANDIAL GLYCAEMIC EXCURSIONS IN TYPE 2 DIABETES

A number of strategies have been proposed to optimize postprandial glycaemic control based on modulation of gastric emptying, stimulated by insights relating to the impact of emptying on glycaemia and incretin hormone secretion. The focus of these strategies has been type 2 diabetes, underpinned by the rationale that a slower rate of nutrient delivery to the small intestine would be desirable to compensate for the delay in insulin release, and the resistance to its actions, characteristic of this disease. The approaches include modifying the
macronutrient content of meals, the use of fat and protein ‘preloads’, and pharmacological agents, particularly GLP-1-based therapies and the amylin analogue, pramlintide (Chang, Rayner et al. 2010).

In type 1 diabetes, gastric emptying needs to be predictable, rather than specifically normal, delayed or more rapid, to allow for accurate dosing of exogenous, short-acting insulin, although gastroparesis probably increases the propensity for hypoglycaemia in the postprandial period (Horowitz, Jones et al. 2006), providing a rationale for measurement of emptying in patients with otherwise unexplained hypoglycaemia. Studies using gastrokinetic drugs to improve glycaemic control in type 1 patients have substantial methodological limitations and yielded inconsistent observations.

A number of studies have evaluated the effects of modifying the macronutrient and fibre content of meals, based on their putative effects on gastric emptying and/or incretin secretion. For example, increasing dietary fibre (Chandalia, Garg et al. 2000), or adding guar gum (Lorusso, Mikhaylova et al.) slows emptying and reduces postprandial glucose in type 2 diabetes, probably as a result of retardation of both gastric emptying and intestinal glucose absorption. Incorporating fat into a carbohydrate-rich meal also slows gastric emptying and improves the postprandial glycaemic profile, albeit at the cost of increasing the energy load (Cunningham and Read 1989). An increase in the protein content of the diet from 15 to 30% for 5 weeks reduces postprandial glycaemia, as well as HbA1c, in type 2 patients (Gannon, Nuttall et al. 2003). In the latter study, the higher protein content was at the cost of carbohydrates, and total energy intake remained stable. Longer-term studies are indicated to determine whether these benefits are sustained.
As discussed, fat, protein and carbohydrate stimulate incretin secretion by various mechanisms and retard gastric emptying. These effects underlie the novel strategy of administering a small amount of macronutrient (a ‘preload’) 30-60 minutes before a meal, with the rationale of triggering neurohormonal feedback via stimulation of GLP-1 and GIP, as well as PYY and CCK from the small intestine, to slow gastric emptying, stimulate insulin, and suppress glucagon, in advance of the main meal and, potentially, to reduce subsequent energy intake (Wu, Rayner et al. 2010). Fat is a potent inhibitor of gastric emptying because of its high caloric density, but while acute administration of a fat preload predictably slows gastric emptying markedly, it results in only a modest reduction in peak postprandial glucose in type 2 patients (Gentilcore, Chaikomin et al. 2006). On the other hand, a 55g whey protein preload, when given acutely to type 2 patients, slows gastric emptying, stimulates GIP and GLP-1, and markedly reduces postprandial glycaemic excursions (Ma, Stevens et al. 2009) (Figure 4). The latter effect is likely to be attributable in part to the stimulation of insulin secretion by absorbed amino acids. In view of these promising observations, studies to evaluate the optimum dose and composition of protein preloads, and determine whether the beneficial effects on glycaemia are sustained with chronic use, are indicated.
Figure 4. Gastric emptying (A), concentrations of blood glucose (B), plasma insulin (C), plasma GLP-1 (D), plasma GIP (E) and plasma CCK (F) in response to a mashed potato meal in eight type 2 diabetic patients. In each study day, subjects ingested 350mL of beef-flavored soup 30 minutes before a radiolabeled mashed potato meal; either 55g of whey protein was added to the soup (whey preload) or no whey was given (no whey). Data are means±SEM. *, P<0.05: whey preload versus whey in meal; #, P<0.05: whey in meal versus no whey; §, P<0.05: whey preload versus no whey. Reprinted with permission from Ma J et al (Ma, Stevens et al. 2009)
A potential disadvantage of all macronutrient preloads is that they involve additional energy consumption. Two recent studies evaluating the effects of preloads entailing minimal additional energy are, accordingly, of interest (Ma, Chang et al. 2010, Wu, Zhao et al. 2012). Sucralose, a non-calorific artificial sweetener, stimulates GLP-1 in vitro, but apparently has no effect on incretin secretion in healthy humans when administered as a preload (Ma, Bellon et al. 2009). A non-metabolised SGLT-1 substrate, 3-O-methylglucose (3OMG), does stimulate GLP-1, slow gastric emptying and diminish the glycaemic response to a subsequent oral glucose load (Wu, Zhao et al. 2012). Further evaluation of the effects of preloads that entail minimal added energy intake is warranted. A recent study indicates that bile acids can stimulate endogenous GLP-1 in type 2 patients when administered directly to the distal large intestine (Adrian, Gariballa et al. 2012).

**Pharmacological agents: GLP-1 agonists, DPP-IV inhibitors and pramlintide**

While the beneficial effect of GLP-1 agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors on glycaemia in type 2 diabetes has been attributed to glucose-dependent insulinotropic and glucagonostatic properties, GLP-1 agonists also slow gastric emptying and that this is, at least in some cases, an important mechanism by which they lower postprandial glucose excursions. A number of GLP-1 receptor agonists such as exenatide, exenatide LAR, liraglutide, lixisenatide, albiglutide, dulaglutide have been approved for use in different parts of the world, while others such as semaglutide are in late phase development. Because of variable half-lives, there are major differences between these drugs in the frequency of dosing and the resulting plasma drug levels. It is also recognised that they vary in the magnitude of their effects on pre- versus postprandial glycaemia (Drucker and Nauck 2006), although differences in effects on HbA1c hitherto appear subtle (Inzucchi, Bergenstal et al. 2012). All of these drugs cause gastrointestinal adverse effects such as anorexia, nausea and diarrhoea, and are associated with modest weight loss. Most patients respond to GLP-1 agonists, but
there is substantial inter-individual variation in the response, and certainly not all achieve the target HbA1c of <7%. With some rare exceptions, no clear factors that have hitherto been established to indicate which patients will respond best to these drugs, except that the reduction in HbA1c is predictably greater when baseline HbA1c is higher. The relative effect of these drugs on gastric emptying as a determinant of their effect on glycaemia has received inappropriately little attention.

It is now clear that all GLP-1 agonists have the capacity to slow gastric emptying, in a variable, but often marked manner, when administered acutely, although emptying has often been quantified by the suboptimal paracetamol absorption test. Effects on gastric emptying have been most comprehensively examined for exenatide given twice daily, which slows emptying in a dose-dependent manner in type 2 patients, apparently irrespective of the presence of cardiovascular autonomic neuropathy (Kolterman, Buse et al. 2003, Linnebjerg, Park et al. 2008). Indeed, the slowing of emptying appears to be the predominant mechanism by which exenatide reduces postprandial glycaemia (Linnebjerg, Park et al. 2008) (Figure 5), and the magnitude of this effect is dependent on the ‘baseline’ rate of emptying (Linnebjerg, Park et al. 2008), as is the case with exogenous GLP-1 (Deane, Chapman et al. 2010). Hence, like GLP-1, GLP-1 agonists would be anticipated to have minimal, if any, effect on gastric emptying in gastroparesis. Moreover, the reduction in postprandial glycaemia and impact on postprandial insulin induced by GLP-1 agonists are also related to the degree to which emptying is slowed, at least in the case of exenatide twice daily and lixisenatide, i.e. when the slowing of gastric emptying is substantial, improvement in postprandial glycaemia is most marked, while postprandial insulin secretion is diminished, rather than stimulated (Linnebjerg, Park et al. 2008, Lorenz, Pfeiffer et al. 2012).
Figure 5. Relationship between the effect of exenatide sc bd for 5 days on postprandial glycaemia (plasma glucose AUC (0-3 hours)) and gastric emptying (50% emptying or t50) of a radiolabeled solid meal in type 2 patients (n = 17). Placebo, white circles; 5 μg exenatide, black triangles; and 10 μg exenatide, black squares. R = −0.49, P < 0.0001. A longer t50 is indicative of slower gastric emptying (Reprinted with permission from Linnebjerg H et al (Linnebjerg, Park et al. 2008)).

The different duration of action of GLP-1 agonists appears to determine their impact on gastric emptying with repeated dosing. Evidence from animal and human studies indicates that the slowing of gastric emptying induced by a long-acting formulation of exenatide (‘exenatide LAR’) (Drucker, Buse et al. 2008) and liraglutide (Jelsing, Vrang et al. 2012), and presumably other ‘long acting’ GLP-1 agonists, but not exenatide twice daily, or lixisenatide (which are ‘short acting’), diminishes with time, probably reflecting the tachyphylaxis phenomenon reported with GLP-1, initially by Nauck et al (Nauck, Kemmeries et al. 2011) and confirmed by UmapathySivam et al (Umapathysivam, Lee et al. 2014). For example, in mice, the initial marked slowing of paracetamol absorption induced by acute administration of liraglutide diminishes within 2 weeks of continuous dosing, whereas the initially comparable marked slowing of paracetamol absorption induced by exenatide is sustained (Jelsing, Vrang et al. 2012). In a human study comparing exenatide twice daily with exenatide LAR (administered once a week), the latter did not slow paracetamol absorption significantly at 14
weeks, while exenatide twice daily did so (Drucker, Buse et al. 2008). This is not to suggest that ‘long acting’ GLP-1 agonists, such as liraglutide, do not have any sustained effect to slow gastric emptying, rather that the magnitude of this effect diminishes with time. In type 2 patients, liraglutide slowed paracetamol absorption slightly after administration for 3 weeks (Flint, Kapitza et al. 2011), despite significant glucose-lowering which would favour more rapid gastric emptying (Rayner, Samsom et al. 2001). Moreover, the reduction in paracetamol absorption was related to the magnitude of the decrease in postprandial glycaemia, consistent with the concept that even modest slowing of gastric emptying can effect postprandial glycaemic excursions. As a ‘short-acting’ GLP-1 agonist, lixisenatide appears likely to have a sustained, major effect to slow gastric emptying (Lorenz, Pfeiffer et al. 2012), so that after 4 weeks’ administration, it lowers postprandial glucose much more than liraglutide, and suppresses, rather than stimulates, postprandial insulin (Kapitza, Coester et al. 2011). Hence, it appears that, in the longer term, ‘short acting’ GLP-1 agonists may act predominantly by lowering postprandial glycaemia (through a sustained, substantial inhibition of gastric emptying), while the ‘long acting’ GLP-1 agonists, predominantly lower pre-prandial hyperglycaemia through insulino tropic and glucagonostatic effects. Accordingly, the choice of GLP-1 agonists may, in future, be dictated by whether the dominant target is pre- or postprandial glycaemic control, and by the baseline rate of gastric emptying. A ‘short-acting’ drug would intuitively be most effective at lowering postprandial glycaemia in those with normal, or rapid emptying and relatively lower HbA1c, while those with already delayed emptying are less likely to require a focus on postprandial glucose, given that when duodenal carbohydrate delivery is ≤1 kcal/min, there appears to be little rise in blood glucose (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012). These hypotheses now warrant formal evaluation.

There is increasing interest in combining a GLP-1 agonist with exogenous basal insulin in type 2 diabetes (Buse, Bergenstal et al. 2011, Holst and Vilsboll 2013), based on the rationale
that the latter primarily targets preprandial glucose, but is associated with weight gain and an increased risk of hypoglycaemia (Inzucchi, Bergenstal et al. 2012) and the addition of a GLP-1 agonist that targets postprandial blood glucose, by slowing gastric emptying, while inducing weight loss, and without increasing the risk of hypoglycaemia, would, therefore, provide a complementary strategy to optimize glycaemic control. There is now clear evidence to support this strategy. For example, in a study by Buse et al (Buse, Bergenstal et al. 2011), exenatide twice daily improved glycaemic control (reduction in HbA1c of 0.7%) in type 2 patients managed with insulin glargine, associated with modest weight loss, and without an increased hypoglycaemia. While, there is hitherto no evidence of an increased risk of hypoglycaemia with the addition of a GLP-1 agonist to basal insulin, this issue should be viewed circumspectly. While the majority of the patients will have an intact glucagon response to hypoglycaemia, it would also be relevant to know whether hypoglycaemia ‘overrides’ the deceleration of gastric emptying induced by the GLP-1 agonist. More recently, a 26-week trial of a fixed ratio combination of insulin and GLP-1 (insulin degludec and liraglutide – ‘IDegLira’), administered as a once-daily injection, was found to have comparable improvement in glycaemia to its individual components without significant hypoglycaemia or weight gain (Gough, Bode et al. 2014). This approach appears promising but is, currently, expensive and long-term studies showing sustained improvement in glycaemia are awaited (Del Prato 2014).

The DPP-IV enzyme acts on both GLP-1 and GIP, and DPP-IV inhibitors can be given orally, unlike GLP-1 agonists. An important distinction from GLP-1 agonists is that DPP-IV inhibitors have minimal, if any, effect on gastric emptying. For example, 2 days’ dosing with 100 mg sitagliptin failed to affect gastric emptying (Stevens, Horowitz et al. 2012), and Vella et al. similarly found no change in gastric emptying following 10 days administration of vildagliptin (50mg) in type 2 patients (Vella, Bock et al. 2007). In contrast, Woerle et al reported a modest slowing of gastric emptying following a single dose of vildagliptin
(100mg) in type 2 patients (Woerle, Lindenberger et al. 2007), and in the study by Aulinger et al the ‘overall’ impression of action of DPP-IV inhibition in type 2 diabetes was of a modest slowing of gastric emptying (Aulinger, Bedorf et al. 2014), raising the possibility that tachyphylaxis may have been responsible for the negative outcome of repeated dosing. In healthy subjects, the magnitude of the initial rise in glucose after a carbohydrate meal is related to the rate of gastric emptying on sitagliptin, although sitagliptin itself had no significant effect on emptying (Stevens, Horowitz et al. 2012). This indicates that gastric emptying is, as would be predicted, also an important determinant of postprandial glycaemia in the presence of DPP-IV inhibition. This concept is supported by a recent study in which a protein preload enhanced the glucose-lowering efficacy of vildagliptin (Wu, Little et al. 2016). The lack of effect of DPP-IV inhibitors on gastric emptying is likely to contribute to their apparently lesser effect on postprandial glycaemia than GLP-1 agonists in clinical trials.

Amylin, a pancreatic hormone co-secreted with insulin by the beta cell, slows gastric emptying, in addition to suppressing glucagon. The synthetic amylin analogue, pramlintide, available in the USA for the management of diabetes, also slows gastric emptying, no doubt contributing to its beneficial effect on postprandial glycaemia (Samsom, Szarka et al. 2000).

1.6 CONCLUSION

Gastric emptying, which exhibits a substantial inter-individual variation in health, is frequently abnormally delayed in patients with long-standing diabetes and a major determinant of postprandial glycaemia and the secretion of the incretin hormones, GIP and GLP-1. The relation of glycaemia and GLP-1 secretion with small intestinal glucose delivery appears to be non-linear in health and type 2 diabetes. Macronutrients, particularly protein ‘pre-loads’, show promise in the management of type 2 diabetes by stimulating incretin and insulin secretion and slowing gastric emptying. Acute, exogenous GLP-1 slows gastric
emptying, and thereby carbohydrate absorption, but there is tachyphylaxis to this effect. GLP-1 agonists also slow gastric emptying and, when administered acutely, this may represent their dominant mechanism of glucose-lowering. With both exogenous GLP-1 and GLP-1 agonists, the magnitude of slowing of gastric emptying, and consequent reduction in postprandial glucose, are greater when baseline gastric emptying is relatively more rapid. The slowing of gastric emptying induced by ‘long-acting’ GLP-1 agonists, such as exenatide LAR and liraglutide, appears to diminish with time, in contrast to ‘short-acting’ agonists such as exenatide twice daily and lixisenatide. Hence, in an individual type 2 patient, the impact of a GLP-1 agonist on postprandial glycaemia is likely to be dependent on both the baseline rate of emptying and the choice of GLP-1 agonist. If postprandial glycaemia is to be targeted preferentially, ‘short-acting’ analogues are likely to be optimally combined with basal insulin.
CHAPTER 2
CHAPTER 2. RELATIONSHIPS OF EARLY AND LATE GLYCAEMIC RESPONSES WITH GASTRIC EMPTYING DURING AN ORAL GLUCOSE TOLERANCE TEST

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2.1 SUMMARY

The ‘early’ glycaemic response during a 75g OGTT is related directly to the rate of gastric emptying. There is little information about the impact of gastric emptying on the blood glucose at either 60 min (a predictor of type 2 diabetes) or 120 min (used diagnostically). We evaluated the relationships between glycaemic responses at 30, 60 and 120 min and gastric emptying following a 75g OGTT in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D). 82 subjects without diabetes (57 NGT, 25 IGT) and 16 with T2D consumed a 75g glucose drink labelled with 99m Tc-sulfur colloid. Gastric emptying (by scintigraphy) and glycaemia were measured from t=0-120 min and relationships between blood glucose (absolute, change from baseline and AUC) and GE at 30, 60 and 120 min determined. There were no differences in gastric emptying. There were relationships between the blood glucose at 30 min and gastric emptying (NGT r=0.40, P<0.01; IGT r=0.49, P=0.02; T2D r=0.62, P=0.01). There was also a relationship between the blood glucose at 60 min and gastric emptying in IGT (r=0.52, P=0.02) and T2D (r=0.77, P<0.01), but not NGT (r=0.16, P=0.24). In NGT, there was an inverse relationship between blood glucose at 120 min and gastric emptying (r=−0.30, P=0.02), but not in IGT (r=0.05, P=0.82) or T2D (r=0.37, P=0.16). In conclusion, gastric emptying is a determinant of the
glycaemic response to an OGTT in NGT, IGT and T2D but these relationships differ and are
time-dependent.

2.2 INTRODUCTION

The 75 g oral glucose tolerance test (OGTT), the ‘gold standard’ for the diagnosis of impaired
 glucose tolerance and diabetes, is known to exhibit considerable variability (Mooy, Grootenhuis et al. 1996), at least in part due to variations in gastric emptying (GE), which
determines the exposure of the small intestine to glucose (Collins, Horowitz et al. 1983). In
health, GE of glucose is reproducible and tightly regulated, usually in the range of 1-4 kcal/min (Brener, Hendrix et al. 1983). This substantial inter-individual variation is even
greater in type 2 diabetes because of the high prevalence of delayed and, sometimes
accelerated, gastric emptying (Horowitz, Harding et al. 1989, Bharucha, Camilleri et al. 2009,
Phillips, Deane et al. 2015), and it has been suggested that relatively more rapid GE may
predispose to the development of type 2 diabetes (Phillips 2006). These observations have
stimulated the development of dietary (Ma, Stevens et al. 2009, Wu, Zhao et al. 2012, Wu,
Bound et al. 2013) and pharmacological (Linnebjerg, Park et al. 2008, Lorenz, Pfeiffer et al.
2013) treatments designed to improve postprandial glycaemic control in type 2 patients by
slowing gastric emptying.

Studies involving small cohorts indicate that gastric emptying is a major determinant of the
magnitude of the initial rise (i.e. ~30 min) in blood glucose after a 75g oral glucose load, or a
carbohydrate-containing meal, accounting for about 35% of the variance in the initial
glycaemic response, in both health and type 2 diabetes (Mourot, Thouvenot et al. 1988,
Horowitz, Edelbroek et al. 1993, Schwartz, McMahan et al. 1995, Jones, Horowitz et al. 1996,
Gonlachanvit, Hsu et al. 2003), i.e. when GE is relatively faster, the initial rise in blood
 glucose is greater. The outcome of recent studies employing direct infusion of glucose into
the duodenum at rates within the ‘physiological’ range of gastric emptying, suggest that the relationship of the initial glycaemic response with GE is non-linear in healthy young (Pilichiewicz, Chaikomin et al. 2007, Marathe, Rayner et al. 2014) and old (Trahair, Horowitz et al. 2012), as well as type 2 patients (Ma, Pilichiewicz et al. 2012, Marathe, Rayner et al. 2014). In an OGTT, there is evidence that 60 min blood glucose predicts progression to type 2 diabetes better than the traditional 120 min, correlating with insulin secretion and resistance (Abdul-Ghani, Williams et al. 2007, Abdul-Ghani, Abdul-Ghani et al. 2008). Accordingly, a 60 min plasma glucose of 8.6 mmol/l or greater is a strong predictor of future type 2 diabetes (Abdul-Ghani, Abdul-Ghani et al. 2008, Abdul-Ghani, Lyssenko et al. 2009), irrespective of glucose tolerance (i.e. fasting and 120 min glucose) status. There has also been interest in the ‘shape’ of the plasma glucose curve during an OGTT to predict type 2 diabetes (Tschritter, Fritsche et al. 2003, Abdul-Ghani, Lyssenko et al. 2010), with evidence that the initial rise in plasma glucose is determined primarily by hepatic insulin resistance, while rate of decline in plasma glucose thereafter is strongly influenced by the rate of glucose uptake in peripheral tissues, particularly skeletal muscle (Abdul-Ghani, Matsuda et al. 2007). We recently reported, in a cohort of 87 older subjects (31 with NGT and 46 with IGT), that the magnitude of the rise in blood glucose at 60 min in an OGTT is more closely related to GE in subjects with IGT than in health (Trahair, Horowitz et al. 2014), consistent with the concept that gastric emptying may affect the risk of type 2 diabetes (Phillips 2006). There is also little information about the impact of GE on the blood glucose at 120 min in an OGTT, the time point used diagnostically (Alberti and Zimmet 1998) or the ‘overall’ glycaemic response from 0-120 min, and how this relationship may differ in impaired glucose tolerance and type 2 diabetes compared to health. In our recent study, the blood glucose at 120 min was inversely related to gastric emptying in NGT, probably reflecting a strong early insulin response, and intact insulin sensitivity but this relationship tended to be direct in IGT (Trahair, Horowitz et al. 2014). In this study, we did not assess type 2 patients and, most importantly, gastric emptying was quantified using a stable isotope breath test where the 50% gastric emptying (T50) time
should be regarded as ‘notional’, rather than the ‘gold standard’ of scintigraphy, which allows GE to be quantified precisely (Phillips, Deane et al. 2015).

We have now investigated the impact of GE, measured by scintigraphy, on the glycaemic responses at 30, 60 and 120 min during an OGTT in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D).

2.3 RESEARCH DESIGN & METHODS

Subjects

Studies were performed in 82 Caucasian volunteers without diabetes and 16 patients with recently diagnosed type 2 diabetes managed by diet alone (T2D), recruited through advertisement. Subjects with a history of gastrointestinal disease, significant medical illness, other than diabetes, or taking medication known to affect gastrointestinal motility, were excluded. The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, which conformed to the principles of the Declaration of Helsinki, all subjects provided written, informed consent before participating.

Protocol

Each subject was asked to fast overnight (14 hours for solids and 12 hours for liquids) and attend the Department of Nuclear Medicine, PET and Bone Densitometry (Royal Adelaide Hospital) at 0800h. While sitting in front of a gamma camera, each subject drank 350 mL water containing 75 g glucose labelled with 20MBq $^{99m}$Tc-sulphur colloid over 3 minutes. Time zero (t=0) was taken as the end of drink consumption (Horowitz, Edelbroek et al. 1993, Jones, Horowitz et al. 1996).
Measurements of gastric emptying, blood glucose and autonomic neuropathy

Gastric emptying data were acquired in 30s frames for the first 30 minutes followed by 3 minute frames for the subsequent 120 minutes. After ≈ 150 minutes, each subject drank 100 ml of water labelled with 5MBq of $^{99m}$Tc-sulfur colloid to enable a lateral image of the stomach to be recorded, which was used to derive correction factors for tissue attenuation (Collins, Horowitz et al. 1983). Data were also corrected for subject movement and radionuclide decay (Horowitz, Edelbroek et al. 1993, Jones, Horowitz et al. 1996). From the gastric emptying curves the intra-gastric retentions at t=30, 60 and 120 min were measured, as well as the time for 50% emptying ($T_{50}$) (Horowitz, Edelbroek et al. 1993). The rate of gastric emptying, expressed as kcal/min, was derived from the $T_{50}$.

Blood glucose (mmol/L) was determined with a portable glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA) at t = –5 min and then at 15, 30, 60, 120, 180 and 240 min. Based on the glycaemic response, the non-diabetic subjects were classified, according to the WHO criteria (Alberti and Zimmet 1998), as having either NGT (fasting blood glucose <6.1 mmol/L, and 2 hour <7.8 mmol/L) or IGT (2 hour blood glucose <11.1 mmol/L, but >7.8 mmol/L). In type 2 patients, standardized cardiovascular reflexes were examined after the completion of the gastric emptying measurement in order to assess autonomic nerve function (Ewing and Clarke 1982, Jones, Horowitz et al. 1996).

Statistical analysis

Blood glucose concentrations, expressed as both absolute values and change from baseline, were evaluated from t=0-120 min. Blood glucose values at 30 or 60 min (absolute or change from baseline) were taken to reflect ‘early’ responses, while the glycaemic response at 120 min (absolute or change from baseline) was regarded as the ‘late’ response. The iAUC for
blood glucose between t=0-120 min was determined using the trapezoidal rule. Pearson’s correlation was used to assess linear relationships between variables. Linear regression was used to assess the relationship between initial glycaemic responses and GE, measured with the T50 values. The linear model was adjusted for age, BMI and gender to assess potential improvement of the model fit. Non-linear relationships were assessed with exponential, S-curve and quadratic models. Statistical significance was accepted at P<0.05 and data are presented as mean values ± standard error (SE). The software used for the statistical analysis was SPSS (IBM Corp., Armonk, NY, USA) and for drawing graphs GraphPad Prism for Macintosh (GraphPad, San Diego USA).

2.4 RESULTS

All subjects tolerated the study well and there were no adverse events. The demographic variables are summarized in Table 1. Of the 82 volunteers without diabetes, 57 had normal glucose tolerance (NGT) and 25 had impaired glucose tolerance. In the T2D cohort, HbA1c was 7.5±0.6%, and duration of known diabetes <12 months; four had definite autonomic neuropathy. The cohorts with IGT and T2D were older (P<0.01) and both BMI (P<0.01) and fasting glucose (P<0.01) were, as expected, higher in the IGT and T2D groups than those with NGT. In the NGT group, the blood glucose approached fasting levels by 120 min (P=NS), but remained elevated in the IGT (P<0.05) and T2D (P<0.05) groups. In all subjects, GE approximated an overall linear pattern. There was no difference in the rate (kcal/min) of gastric emptying between the groups (1.48±0.04 for NGT vs. 1.40±0.08 for IGT vs. 1.60±0.1 for T2D, kcal/min, P=0.11). Gastric emptying had not been completed by 120 min in any subject across all three groups (Figure 1).
Table 1. Demographic variables of the NGT, IGT and T2D groups. Continuous data are presented as mean ± SEM. NGT – Normal glucose tolerance, IGT – Impaired glucose tolerance, T2DM – type 2 diabetes. P-values were calculated using ANOVA

<table>
<thead>
<tr>
<th></th>
<th>NGT (n=57)</th>
<th>IGT (n=25)</th>
<th>T2DM (n=16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>39 (68.4%)</td>
<td>12 (48.0%)</td>
<td>11 (68.8%)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>34.9±2.2</td>
<td>57.1±3.9</td>
<td>57.1±3.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.3±0.6</td>
<td>25.6±0.7</td>
<td>28.7±1.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Figure 1. Blood glucose concentrations (mmol/L) (A) and gastric emptying (B) during an oral glucose tolerance test (OGTT) in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (T2D).

Relationships of ‘early’ (30 and 60 min) glycaemic responses with gastric emptying

NGT (figures 2A and 2B)

Blood glucose at 30 min was related directly to the rate of gastric emptying (r=0.40, P<0.01), as was the change in blood glucose at 30 min (r=0.43, P<0.01). In contrast, at 60 min there...
was no significant relationship of either absolute blood glucose ($r=0.16$, $P=0.24$), or the change from baseline at 60 min ($r=0.13$, $P=0.32$), with gastric emptying. Analysis of the relationship of change in blood glucose at 30 min with gastric emptying demonstrated that a non-linear relationship, such as a S-curve ($r=0.40$, $P<0.05$), was as compatible as a linear relationship.

**IGT (figures 2E and 2F)**

Blood glucose at 30 min was related directly to the rate of gastric emptying ($r=0.49$, $P=0.02$), as was the relationship of the change in blood glucose at 30 min ($r=0.54$, $P=0.01$). There was a significant relationship of both absolute blood glucose at 60 min ($r=0.51$, $P=0.02$) and change from baseline ($r=0.52$, $P=0.01$) with gastric emptying.

**T2D (figures 2I and 2J)**

Blood glucose at 30 min was related directly to gastric emptying ($r=0.62$, $P=0.01$), but not the change in blood glucose at 30 min ($r=0.23$, $P=0.39$). There was a significant relationship between the absolute blood glucose at 60 min ($r=0.77$, $P<0.01$), but not change in blood glucose at 60 min from baseline ($r=0.21$, $P=0.44$), with the rate of gastric emptying.

**Relationships of ‘late’ (120 min) and ‘overall’ glycaemic responses with gastric emptying**

**NGT (figures 2C and 2D)**

Both the absolute blood glucose at 120 min ($r= -0.30$, $P=0.02$) and change from baseline ($r= -0.31$, $P=0.02$) were inversely related to the rate of gastric emptying. There was no relationship between the overall glycaemic response ($i\text{AUC}_{0-120}$) and gastric emptying ($r=0.18$, $P=0.16$).
IGT (figures 2G and 2H)

There was no relationship between either the absolute blood glucose at 120 min ($r=0.05$, $P=0.82$), or change from baseline ($r=0.07$, $P=0.74$) with gastric emptying. There was a direct relationship between the overall glycaemic response (iAUC$_{0-120}$) and gastric emptying ($r=0.47$, $P=0.03$).

T2D (figures 2K and 2L)

There was no relationship between either the absolute blood glucose at 120 min ($r=0.37$, $P=0.16$), or change from baseline ($r=0.02$, $P=0.95$), and gastric emptying. There was no relationship between the overall glycaemic response (iAUC$_{0-120}$) and gastric emptying ($r=0.24$, $P=0.37$).

**Relationship of glycaemia with gastric emptying adjusted for age, gender and BMI**

Adjusting the linear model for age, gender and BMI did not improve the model fit significantly in any of the groups.

**Figure 2 (see following page).** Relationships between blood glucose at 30, 60 and 120 min and incremental area under the curve for 120 min (iAUC$_{0-120}$) with gastric emptying expressed as kcal/min based on $T_{50}$ in subjects with NGT (A-D), IGT (E-H) and T2D (I-J).
2.6 DISCUSSION

This study has evaluated the relationship of the ‘early’ and ‘late’ glycaemic responses in a 75g OGTT with gastric emptying in NGT, IGT and T2D, quantifying the latter with the ‘gold standard’ technique of scintigraphy. Key observations are that: i) the ‘early’ blood glucose concentration (30 min in all three groups and 60 min in IGT and T2D) was related directly to gastric emptying i.e. when gastric emptying was relatively more rapid, glucose was greater, and in the NGT group, a non-linear relationship between the rise in blood glucose at 30 min and gastric emptying was as compatible as a linear relationship; ii) the 120 min blood glucose was inversely related to gastric emptying in subjects with NGT, but not in IGT or T2D; iii) the iAUC$_{0-120}$ blood glucose correlated positively with gastric emptying only in those with IGT; and iv) there were no differences in gastric emptying between the three groups. Accordingly, gastric emptying of a 75g glucose load is a determinant of glycaemia in health, IGT and T2D, but the relationships differ among the groups and are time-dependent, probably reflecting differences in insulin secretion and sensitivity.

Evaluation of the relationship of gastric emptying with postprandial glycaemia is complicated by their inter-dependency. Acute hyperglycaemia retards gastric emptying (Fraser, Horowitz et al. 1990), even at blood glucose concentrations within the normal postprandial range (Schvarcz, Palmer et al. 1997), while insulin-induced hypoglycaemia accelerates it (Russo, Stevens et al. 2005). Studies employing direct intraduodenal glucose infusions to ‘control’ for gastric emptying have suggested a non-linear relationship between the initial glycaemic response and the rate of small intestinal glucose exposure in healthy subjects (Pilichiewicz, Chaikomin et al. 2007, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014) and type 2 diabetes (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014). Accordingly, while there was a modest glucose rise during a 1 kcal/min intraduodenal glucose infusion, and a predictably greater glycaemic
response to rates of 2, 3 and 4 kcal/min, there was little difference between the latter rates. This was accounted for by a substantially greater insulin response to the higher rates of intraduodenal glucose infusions, probably triggered by increased GLP-1 secretion (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014). In the current study, the relationship of blood glucose at 30 min with gastric emptying fitted a non-linear, as well as a linear function. That a non-linear function was no better may reflect the fact that in the majority of the subjects, the rate of gastric emptying was less than 2 kcal/min and did not exceed 3 kcal/min in any, and it is only at higher rates of emptying where a plateau in the blood glucose response would be anticipated (Pilichiewicz, Chaikomin et al. 2007, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014).

In our recent study (Trahair, Horowitz et al. 2014), the blood glucose at 60 min during an OGTT was more closely related to gastric emptying in subjects with IGT than in those with NGT. The current study confirms and extends these observations. That there were positive relationships between the blood glucose at 60 min with gastric emptying in IGT and T2D, but not in those with NGT, is likely to reflect the delay in insulin release and impaired insulin sensitivity associated with IGT (Nathan, Davidson et al. 2007) and T2D (Kahn, Cooper et al. 2014) leading to a later peak in blood glucose. The 60 min blood glucose value during an OGTT may have greater diagnostic value than 120 min value, particularly in predicting progression to T2D (Abdul-Ghani, Williams et al. 2007, Abdul-Ghani, Abdul-Ghani et al. 2008) and the demonstrated dependence on gastric emptying is, accordingly, consistent with the concept that rapid gastric emptying predisposes to T2D (Schwartz, McMahan et al. 1995, Phillips 2006). The relationship of glycaemia at 120 min with gastric emptying differed among the three groups. As in previous studies (Horowitz, Edelbroek et al. 1993, Trahair, Horowitz et al. 2014) in NGT, when gastric emptying was relatively more rapid, the blood glucose at 120 min was proportionately less, but this was not the case in IGT or T2D, the
relationship tended to be direct, rather than inverse. Again, these differences are likely to be attributable to the well-characterized impairments in insulin secretion and sensitivity in the latter two groups (Nathan, Davidson et al. 2007, Kahn, Cooper et al. 2014). This is supported by our observation that in subjects with IGT, the blood glucose at 180 min after a 75g glucose load is inversely related to GE as measured with a breath test (Trahair, Horowitz et al. 2014) and the concept that, in non-diabetic subjects, the 120 min glucose during an OGTT is a marker of insulin sensitivity in skeletal muscle (Winner, Norton et al. 2014).

There is little information about the impact of gastric emptying on the ‘overall’ (0-120 min) glycaemic response to an OGTT, or a meal. In the current study, gastric emptying had not been completed by 120 min in any subject, a phenomenon that is not well recognised, but predictable given that complete gastric emptying within 120 min would have required an emptying rate of $\geq 2.5$ kcal/min, which occurs in only a minority of healthy subjects. That blood glucose levels had normalised by 120 min in the NGT subjects, despite ongoing emptying, reflects the effective glucose counter-regulation in this group; in contrast, in both IGT and T2D groups, the blood glucose remained markedly elevated over baseline at 120 min. Hence, the $iAUC_{0-120}$ was indicative of the ‘total’ AUC only in the NGT group. It is intuitively likely that gastric emptying will be of greater importance to the overall glycaemic response as beta cell function diminishes, and the observed direct relationship in the IGT group is consistent with this. The above insights are of relevance to the use of dietary strategies (e.g. protein preloads (Ma, Stevens et al. 2009, Wu, Zhao et al. 2012, Wu, Bound et al. 2013)) and pharmacological approaches (e.g. short-acting GLP-1 agonists (Linnebjerg, Park et al. 2008, Lorenz, Pfeiffer et al. 2013)) to reduce postprandial glycaemia by slowing gastric emptying, as well as the diagnosis of impaired glucose tolerance / diabetes in pregnancy, given that the latter may be associated with a delay in gastric emptying (Bonde, Vilsboll et al. 2013).
There were no differences in gastric emptying between the groups. Longstanding T2D is associated with a high prevalence of delayed (and sometimes accelerated) gastric emptying; although in many cases the magnitude of the delay is modest (Jones, Russo et al. 2001, Bharucha, Camilleri et al. 2009, Marathe, Rayner et al. 2013, Phillips, Deane et al. 2015). Given that acute hyperglycaemia slows gastric emptying, including elevations that are within the normal postprandial range (Fraser, Horowitz et al. 1990, Schvarcz, Palmer et al. 1997), and blood glucose levels were higher in the IGT and T2D, it would not be surprising if both IGT and ‘early’ T2D were associated with a modest, ‘overall’, acceleration of gastric emptying during euglycaemia, as has been suggested (Phillips 2006). Age and BMI were greater in the IGT and T2D groups. Healthy aging, however, appears to have little, if any, effect on gastric emptying (Horowitz, Maddern et al. 1984). While observations relating to the effect of obesity on gastric emptying are inconsistent, the reported differences have, in general, been modest (Seimon, Brennan et al. 2013, Acosta, Camilleri et al. 2015). Moreover, the observed relationships were not affected by ‘adjustment’ for differences in age and BMI.

In interpreting our observations, specific limitations should be recognised. The number of IGT and T2D patients, in particular, was relatively small and all had ‘early’ T2D and thus it is possible that the study was not sufficiently powered to detect all relationships between glycaemia and gastric emptying. It would be of interest to study subjects with longstanding T2D associated with microvascular complications as well as those with poor glycaemic control. In the T2D subjects, there was a discrepancy of absolute and incremental blood glucose with the rate of gastric emptying, which is likely to reflect greater variability of the increment compared to the absolute value, and warrants further definition. Blood glucose was determined by portable glucometer, but the differences in glucose between the groups were marked. We did not evaluate insulin, glucagon or incretin hormones (GIP and GLP-1), as these were available in only a subset of the total cohort. Importantly, the demonstration of relationships does not establish causality and we did not evaluate glucose kinetics (i.e. oral
absorption, endogenous production or rate of utilization), which would be of considerable interest.

In conclusion, we have confirmed that in health, there is an inverse relationship between blood glucose at 120 min in an OGTT and gastric emptying and that the relationship of the blood glucose at 30 min with gastric emptying is direct, and possibly non-linear. In contrast to NGT, there is a strong direct relationship between the blood glucose at 60 min and gastric emptying in both IGT and T2D and, in the latter group; the relationship at 120 min also tends to be direct. Hence, the impact of gastric emptying on glycaemia during an OGTT is highly dependent on glucose tolerance.
CHAPTER 3
CHAPTER 3. RELATIONSHIPS OF THE EARLY INSULIN SECRETORY RESPONSE AND ORAL DISPOSITION INDEX WITH GASTRIC EMPTYING IN SUBJECTS WITH NORMAL GLUCOSE TOLERANCE

Marathe CS et al – Submitted for publication

3.1 SUMMARY

The oral disposition index, the product of the early insulin secretory response during an oral glucose tolerance test and insulin sensitivity, is used widely for both the prediction of, type 2 diabetes and evaluation of the response to interventions, in type 2 diabetes. Gastric emptying, which determines small intestinal exposure of nutrients, modulates postprandial glycaemia. The aim of this study was to determine whether the insulin secretory response and the disposition index (DI) related to gastric emptying in subjects with normal glucose tolerance. Thirty-nine subjects consumed a 350mL drink containing 75g glucose labelled with $^{99m}$Tc-sulfur colloid. Gastric emptying (by scintigraphy), blood glucose (G) and plasma insulin (I) were measured between t=0 to 120 min. The rate of gastric emptying was derived from the time taken for 50% emptying (T50) and expressed as kcal/min. The early insulin secretory response was estimated by the ratio of the change in insulin ($\Delta I_{0-30}$) to that of glucose at 30 min ($\Delta G_{0-30}$) represented as $\Delta I_{0-30}/\Delta G_{0-30}$. Insulin sensitivity was estimated as 1/fasting insulin and the DI was then calculated as $\Delta I_{0-30}/\Delta G_{0-30} \times 1/$fasting insulin. There was a direct relationship between $\Delta G_{0-30}$ and gastric emptying ($r=0.47$, P=0.003). While there was no association of either $\Delta I_{0-30}$ ($r=0.16$, P=0.34) or fasting insulin ($r=0.21$, P=0.20), there were inverse relationships between the early insulin secretory response ($r=-0.45$, P=0.004) and the DI ($r=-0.33$, P=0.041), with gastric emptying. We conclude that gastric emptying is
associated with both insulin secretion and the disposition index in subjects with normal glucose tolerance, such that when gastric emptying is relatively more rapid, both the early insulin secretory response and the disposition index are less.

3.2 INTRODUCTION

There is a hyperbolic relationship between the insulin secretory response and insulin sensitivity during an OGTT, such that the composite of the two – the oral disposition index (DI) – is a constant (Utzschneider, Prigeon et al. 2009). Thus, normal glucose tolerance (NGT) is maintained as long as the islet \( \beta \) cells have the capacity to up-regulate insulin secretion and compensate for diminished insulin sensitivity. The failure of \( \beta \) cells to meet insulin secretory demand is fundamental to the pathogenesis of type 2 diabetes, so that with the progression from NGT to impaired glucose tolerance (IGT) to type 2 diabetes there is a progressive shift in this curve downwards and to the left and a reduction in the disposition index (Utzschneider, Prigeon et al. 2009). A low DI at baseline was shown, in a community study of >600 people, to be predictive of progression to type 2 diabetes (Utzschneider, Prigeon et al. 2009) and, in type 2 diabetes, a lower DI is associated with the failure of a pharmacological intervention to slow progression of the disease process (Kahn, Lachin et al. 2011).

Along with insulin secretion and sensitivity, gastric emptying is now recognised as a major determinant of oral glucose tolerance in health (Horowitz, Edelbroek et al. 1993) and type 2 diabetes (Jones, Horowitz et al. 1996); when gastric emptying is more rapid, the initial glycaemic response is greater (Marathe, Rayner et al. 2013). The emptying of liquid nutrients, such as glucose during an OGTT, approximates an overall linear pattern, but exhibits a wide inter-individual variation of between 1-4 kcal/min in healthy subjects, a range which is even wider in type 2 diabetes because of the prevalence of both delayed and accelerated gastric
emptying (Brener, Hendrix et al. 1983, Lin, Doty et al. 1989, Jones, Russo et al. 2001). Moreover, even relatively minor variations in gastric emptying can have a major effect on the postprandial glycaemic excursion (Thompson, Wingate et al. 1982, Horowitz, Edelbroek et al. 1993, Jones, Horowitz et al. 1996). The exposure of the small intestine to nutrients, including glucose, as a result of gastric emptying triggers the release of the incretin hormones (GIP and GLP-1) (Nauck 2011), which have a major impact on postprandial insulin secretion, accounting for 50-70% of insulin release in health during an OGTT (Nauck 2011). The secretion of both GIP and GLP-1 is influenced by the rate of entry of glucose into the duodenum (Pilichiewicz, Chaikomin et al. 2007, Marathe, Rayner et al. 2014).

Based on the above evidence, we hypothesized that gastric emptying impacts on the insulin secretory response and the oral disposition index, as well as glycaemia, and in this study, determined these relationships in a cohort with normal glucose tolerance.

3.3 RESEARCH DESIGN & METHODS

Subjects

39 healthy Caucasian volunteers (27 male and 12 female participants, mean age 30.1±1.9 yrs, mean BMI 24±0.6 kg/m2) were recruited through advertisement and are a subset of the subjects enrolled for the study in Chapter 2. Subjects with a history of gastrointestinal disease, other significant medical illness, or taking medication known to affect gastrointestinal motility, were excluded. The Royal Adelaide Hospital Human Research Ethics Committee approved the study protocol, which conformed to the principles of the Declaration of Helsinki, and all subjects provided written, informed consent before their participation.
Protocol

Each subject fasted overnight (14 hours for solids and 12 hours for liquids) and attended the Department of Nuclear Medicine, PET and Bone Densitometry (Royal Adelaide Hospital) at 0800h. While sitting in front of a gamma camera, each subject drank 350mL water containing 75g glucose labelled with 20MBq $^{99m}$Tc-sulphur colloid over 3 minutes. Time zero (t=0) was taken as the end of drink consumption (Horowitz, Edelbroek et al. 1993, Jones, Horowitz et al. 1996).

**Measurements of gastric emptying, blood glucose and plasma insulin**

Gastric emptying data were acquired in 30s frames for the first 30 minutes, followed by 3 minute frames for the subsequent 120 minutes. After ≈ 150 minutes, each subject drank 100mL of water labeled with 5MBq of $^{99m}$Tc-sulfur colloid to enable a lateral image of the stomach to be recorded, which was used to derive correction factors for tissue attenuation (Collins, Horowitz et al. 1983). Data were also corrected for subject movement and radionuclide decay (Horowitz, Edelbroek et al. 1993, Jones, Horowitz et al. 1996). From the gastric emptying curves the intra-gastric retention at t=30, 60 and 120 min were determined, as well as the time for 50% emptying (T50) (Horowitz, Edelbroek et al. 1993). The rate of gastric emptying, expressed as kcal/min, was derived from the T50.

Venous blood was obtained at 0, 30, 60 and 120 min. Plasma was separated by centrifugation and stored at −70°C for subsequent assays. Blood glucose concentrations were determined using a glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA) and plasma insulin concentrations by ELISA (10-1113 Mercodia, Uppsala, Sweden), with assay sensitivity of 1.0mU/L and coefficient of variation 2.5% within assays and 7.4% between assays (Trahair, Horowitz et al. 2012). All subjects had normal glucose tolerance according
to WHO criteria (blood glucose concentrations <6.1mmol/L fasting, and <7.8mmol/L at 2 hours) (Alberti and Zimmet 1998).

**Calculation of early insulin response, insulin sensitivity and the oral disposition index**

The insulin secretory response was estimated as the ratio of change in insulin to that of glucose at 30 min, represented as $\Delta I_{0.30}/\Delta G_{0.30}$ (Retnakaran, Qi et al. 2009). Insulin sensitivity was estimated as $1/$fasting insulin (Utzschneider, Prigeon et al. 2009). The disposition index was then calculated as $\Delta I_{0.30}/\Delta G_{0.30} \times 1/$fasting insulin (Utzschneider, Prigeon et al. 2009).

**Statistical analysis**

Blood glucose and plasma insulin concentrations at t=0, 30, 60 and 120 min are shown as changes from baseline values (t=0 min). Pearson’s correlation was used to assess linear relationships between variables. Linear regression was used to assess the relationships of the change in glucose at 30 min, $1/$fasting insulin, change in insulin at 30 min, early insulin secretory response (i.e. change in insulin relative to the change in glucose at 30 min) and the oral disposition index with gastric emptying. Data are presented as mean values ± standard error (SE), with statistical significance accepted at P<0.05.

**3.4 RESULTS**

All subjects tolerated the study well and there were no adverse events. In all cases, gastric emptying approximated an overall linear pattern and the mean rate was $1.5\pm0.06$ kcal/min (mean±SEM). Blood glucose levels (mmol/L) at baseline, 30, 60 and 120 min were $4.9\pm0.1$, $8.4\pm0.2$, $7.9\pm0.3$ and $5.7\pm0.2$ respectively (mean±SEM).
Relationships of changes in glucose and insulin at 30 min, and insulin sensitivity, with gastric emptying (Fig 1 A, B and C)

While the change in blood glucose at 30 min (\(\Delta G_{0.30}\)) was related directly to gastric emptying (\(r=0.47, P=0.003\)), there was no significant relationship of the latter with the change in plasma insulin at 30 min (\(\Delta I_{0.30}\)) (\(r=0.16, P=0.34\)). There was also no relationship between insulin sensitivity (1/fasting insulin) and gastric emptying (\(r=0.21, P=0.20\)).

Relationships of early insulin secretory response and the oral disposition index with gastric emptying (Fig 2 A and B)

Both the early insulin secretory response (\(\Delta I_{0.30}/\Delta G_{0.30}\)) (\(r=-0.45, P=0.004\)) and the oral disposition index (\(\Delta I_{0.30}/\Delta G_{0.30} \times 1/\text{fasting insulin}\)) (\(r=-0.33, P=0.041\)) were related inversely to the rate of gastric emptying.

3.5 DISCUSSION

We have evaluated, in subjects with normal glucose tolerance, the relationships of glycaemia, early insulin secretory response and oral disposition index during a 75g OGTT with gastric emptying, quantifying the latter with the ‘gold standard’ technique of scintigraphy. We confirmed that the early rise in blood glucose was related directly to the rate of gastric emptying. Novel, and important, observations are that both the early insulin secretory response and the oral disposition index, are inversely associated with gastric emptying.

We estimated the early insulin secretory response as the rise (change) in insulin relative to the change in glucose over 30 min following ingestion of oral glucose as in previous studies (Utzschneider, Prigeon et al. 2009). In health, the stimulation of insulin secretion by an
oral/enteral glucose load primarily reflects the result of direct action of glucose on the β cell and the release of the incretin hormones, GIP and GLP-1, which are both insulinotropic when blood glucose levels are elevated above ≈ 8 mmol/L, but not during euglycaemia (Dupre, Ross et al. 1973, Schmidt, Siegel et al. 1985). As noted previously (Horowitz, Edelbroek et al. 1993), there was no relationship between the increase in plasma insulin at 30 min and gastric emptying, which in part, is likely to reflect the latency of the plasma insulin response to an increase in blood glucose. The magnitude of the elevation in blood glucose was above the threshold required for GIP and GLP-1 stimulated insulin secretion in ≈ 50% of the cohort, and changes in incretin secretion may, accordingly, also be relevant. Studies by ourselves and others (Pilichiewicz, Chaikomin et al. 2007, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014) have established that patterns of GIP and GLP-1 release have different relationships with the rate of glucose entry to the duodenum. In health, when gastric emptying is <2 kcal/min (in our cohort, the mean gastric emptying was ≈ 1.5 kcal/min and no subject had an emptying rate ≥3 kcal/min), GIP, rather than GLP-1, is likely to be the dominant incretin; this cannot be the case in type 2 patients, because the insulinotropic effect of GIP, but not GLP-1, is markedly attenuated (Nauck, Heimesaat et al. 1993). Both GLP-1 and insulin secretion are markedly increased when duodenal glucose delivery exceeds 3 kcal/min. We would suggest that in healthy subjects, the relationship between incretin-mediated insulin secretion and gastric emptying after oral glucose will be non-linear, so that when the rate of gastric emptying increases from ≈1 kcal/min to 3 kcal/min, there is a relative reduction in the early insulin secretory response, but when gastric emptying is ≥ 3 kcal/min, the increase in insulin levels will be much greater (related to substantially greater GLP-1 secretion) and potentially adequate to compensate for the increase in blood glucose, so that the early insulin response (∆I_{0-30}/∆G_{0-30}) is constant. Further studies are indicated to address this hypothesis.

The observed inverse relationship between the oral disposition index and gastric emptying (Fig 2B) is consistent with the relationship between the early insulin secretory response and
gastric emptying, particularly given the absence of a relationship between insulin sensitivity and gastric emptying. Accordingly, in healthy when the early insulin secretory response is reduced, the oral disposition index is lower.

The limitations of our study should be recognized: the sample size was relatively small, and we measured glucose concentrations in blood rather than plasma (in general, the latter values are slightly higher). Moreover, the plasma insulin response is influenced by hepatic insulin extraction (Kautzky-Willer, Thomaseth et al. 1996, Rudovich, Rochlitz et al. 2004), which we did not quantify; and in this regard measurement of C-peptide would have been optimal. While we studied subjects with NGT, we recently reported that the relationships of gastric emptying with the early glycaemic response (30 and 60 min) during an oral glucose tolerance test are stronger in subjects with IGT and T2DM compared with NGT (Marathe, Horowitz et al. 2015). Moreover, given the marked impairment in β-cell function in these states and the loss of an insulinotropic action of GIP in type 2 diabetes (Nauck, Heimesaat et al. 1993), the relationships of the early insulin secretory response and disposition index with gastric emptying may well be stronger than we observed in health, but this needs to be confirmed. An implication of our observations is that relatively more rapid gastric emptying would predispose to type 2 diabetes, as has been suggested (Phillips, Schwartz et al. 1991, Marathe, Horowitz et al. 2015), not only because the initial glucose excursion is greater, but also because the early insulin secretory capacity is relatively diminished, so that compensation is inadequate. Interestingly, ‘early’ type 2 diabetes has been associated with more rapid gastric emptying (Phillips, Schwartz et al. 1991, Phillips, Schwartz et al. 1992), and the latter has also been observed in certain ethnic groups that are known to be predisposed to type 2 diabetes (Phillips 2006). If this is the case, slowing of gastric emptying, either by dietary (Gentilcore, Chaikomin et al. 2006, Ma, Stevens et al. 2009) or pharmacological strategies (e.g. ‘short-acting’ GLP-1 agonists (Linnebjerg, Park et al. 2008, Lorenz, Pfeiffer et al. 2013)), may potentially slow the progression of type 2 diabetes.
In conclusion, in individuals with normal glucose tolerance, both the early insulin secretory response and oral disposition index are inversely associated with the rate of gastric emptying. Whether similar relationships are observed in individuals with diminished glucose tolerance and type 2 diabetes remains to be determined.
**Figure 1.** Relationships between A) change in blood glucose from t=0 to t=30 min, B) change in plasma insulin from t=0 to t=30 min and C) 1/fasting insulin with gastric emptying expressed as kcal/min based on T_{50} in subjects with normal glucose tolerance. T=0 min was the sample taken just prior to, and t=30 min the sample taken 30 min after, consuming the glucose drink.
Figure 2. Relationships between A) early insulin secretory response, and B) oral disposition index with gastric emptying expressed as kcal/min based on T_{50} in subjects with normal glucose tolerance.
CHAPTER 4
CHAPTER 4. THE IMPACT OF DUODENAL GLUCOSE LOAD ON THE MAGNITUDE OF THE INCRETIN EFFECT IN HEALTH AND TYPE 2 DIABETES

Adapted from Marathe CS et al Diabetes, August 2014, Vol 63 (8), pages 2668-75

4.1 SUMMARY

The potential influence of gastric emptying on the ‘incretin effect’, mediated by glucose-dependent insulino tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), is unknown. The objectives of this study were to determine the effects of intraduodenal glucose infusions at 2 (ID2) and 4 (ID4) kcal/min (equating to two rates of gastric emptying within the physiological range) on the size of the incretin effect, gastrointestinal glucose disposal and plasma GIP, GLP-1 and glucagon, in health and type 2 diabetes. 10 healthy men and 11 men with type 2 diabetes, managed by diet or metformin only were studied. In both groups, GIP, GLP-1 and the magnitude of incretin effect were greater with ID4 than ID2, as was gastrointestinal glucose disposal. In both groups, plasma glucagon was suppressed by ID2, but not ID4. Based on these data, the author concludes that the rate of small intestinal glucose exposure (i.e. glucose load) is a major determinant of the comparative secretion of GIP and GLP-1, as well as the magnitude of both the incretin effect and gastrointestinal glucose disposal in health and type 2 diabetes.

4.2 INTRODUCTION

It was established in 1964 that the insulin response to oral, or enteral, glucose is much greater than that to an isoglycaemic intravenous glucose infusion (Elrick, Stimmeler et al. 1964,
McIntyre, Holdsworth et al. 1964). This ‘incretin effect’, mediated by glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino tropic polypeptide (GIP) (Baggio and Drucker 2007), is calculated by comparing the plasma insulin, or C-peptide, responses to isoglycaemic oral and intravenous glucose loads. In health, the ‘incretin effect’ ranges between 40% and 70% (Perley and Kipnis 1967), independent of glycaemia (Salehi, Aulinger et al. 2012), and has been reported to be diminished in patients with type 2 diabetes (Nauck, Stockmann et al. 1986), who exhibit marked attenuation of the insulino tropic effect of GIP, at least in part as a result of hyperglycaemia (Nauck, Heimesaat et al. 1993, Hojberg, Vilsboll et al. 2009). These latter observations have stimulated the development of ‘GLP-1 based’ drugs for the management of type 2 diabetes (Drucker and Nauck 2006). Paradoxically, GIP may be the more important incretin in health (Nauck, Heimesaat et al. 1993).

Gastric emptying determines the rate of nutrient delivery to the small intestine, and modulates postprandial glycaemic excursions in health and diabetes (Horowitz, Edelbroek et al. 1993, Jones, Horowitz et al. 1996, Marathe, Rayner et al. 2013). There is substantial inter-individual variation in the overall rate of gastric emptying in health (1-4 kcal/min) (Collins, Horowitz et al. 1983), which is even greater in type 2 diabetes because of the high prevalence of delayed, and sometimes accelerated, gastric emptying (Horowitz, Harding et al. 1989, Bharucha, Camilleri et al. 2009, Kashyap and Farrugia 2010). In contrast, the intra-individual variation in gastric emptying is low, such that rates of emptying are reproducible over time, even in longstanding diabetes (Collins, Horowitz et al. 1983, Jones, Russo et al. 2002, Chang, Russo et al. 2012). We have shown that the relationships of glycaemic and GLP-1 responses with the rate of small intestinal glucose delivery are non-linear in healthy young and older subjects and type 2 patients (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012), in contrast to GIP responses. Hence, both the absolute, and comparative, secretion of GIP and GLP-1 in response to enteral glucose appear to be critically dependent on the rate of gastric emptying. As well as the substantial inter-individual variation
in its magnitude in health (Perley and Kipnis 1967, Meier 2009), it has been suggested that the incretin effect increases with increasing glucose loads in both health and type 2 diabetes (Nauck, Homberger et al. 1986, Bagger, Knop et al. 2011). However, a fundamental limitation in interpreting the outcome of such studies has been their failure to assess, or account for, gastric emptying.

Measurement of gastrointestinal glucose disposal (GIGD) provides an assessment of the role of the gastrointestinal tract in glucose homeostasis and takes into account not only the actions of the incretin hormones, but other factors, including glucagon suppression and hepatic glucose uptake (Holst, Knop et al. 2011). GIGD increases with the oral glucose load in health and may be reduced in type 2 diabetes (Bagger, Knop et al. 2011). However, the few studies evaluating GIGD have also failed to take into account the potential impact of gastric emptying. In health, glucagon is suppressed following ingestion of carbohydrate-containing meals (Unger 1985), while type 2 diabetes is characterised by elevated fasting glucagon concentrations and impaired glucagon suppression following oral nutrients (Baron, Schaeffer et al. 1987, Shah, Vella et al. 2000). Somewhat surprisingly, the potential impact of the rate of small intestinal glucose exposure on glucagon suppression has not been evaluated.

We have now addressed the fundamental questions of whether: 1) the incretin effect and gastrointestinal glucose disposal increase and 2) the capacity to suppress glucagon is affected, with increasing rates of duodenal glucose delivery in health and type 2 diabetes.

4.3 RESEARCH DESIGN & METHODS

Subjects

Ten healthy Caucasian men (age 47±3 years, BMI 29.3±1 kg/m²) and eleven Caucasian men with type 2 diabetes [age 64±2 years, BMI 31±1.3 kg/m², HbA1c 6.9±0.2 % (52±2.2
mmol/mol) and duration of known diabetes 4.9±1.3 years), managed by diet or metformin only, were studied. Subjects with a history of gastrointestinal disease, or significant medical illness, apart from diabetes, or taking medication known to affect gastrointestinal motility, were excluded. Patients with type 2 diabetes were examined for the presence of microvascular complications. The study protocol, which conformed to the principles of the Declaration of Helsinki, was approved by the Royal Adelaide Hospital Research Ethics Committee, and all subjects provided written, informed consent before participating.

**Study Protocol**

Each subject attended the laboratory at 0800h following an overnight fast (14 hours for solids and 12 hours for liquids), on four separate days. On the first two study days, subjects were randomised to receive an ID glucose infusion at either 2 kcal/min or 4 kcal/min. On the last two study days, they were given IV glucose at a variable rate designed to match the blood glucose profile of the first two visits.

**Protocol: Intraduodenal infusion days**

A manometric assembly (Dentsleeve, Ontario, Canada), incorporating an infusion channel that opened 12cm distal to the pylorus, was inserted through the nose and positioned in the duodenum, as determined by measurement of the transmucosal potential difference (TMPD) in antral and duodenal side holes (Heddle, Fone et al. 1988). When the catheter was positioned correctly, ID infusion of 25% glucose at 2 or 4 kcal/min commenced (t=0 min) and continued for 120 min, when the ID catheter was removed. Observations continued until (t=240 min).
Protocol: Isoglycaemic intravenous glucose infusion days

An IV cannula was placed in an antecubital vein to administer 25% glucose to mimic the glycaemic excursions on the preceding ID study visit. The infusion was adjusted according to measurements of blood glucose taken every 5 minutes (Nauck, Stockmann et al. 1986).

Measurements

_Blood glucose, plasma insulin, C-peptide, GLP-1, GIP and glucagon_

An IV cannula was placed in an antecubital vein (contralateral to that used for IV infusions) for blood sampling, and the forearm was kept warm with a heat pack. Blood glucose concentrations were determined immediately, using a portable glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA).

For measurements of plasma insulin, C-peptide, GLP-1, GIP and glucagon, blood samples were collected at t= -5 min and then at 15, 30, 45, 60, 75, 90, 105, 120, 180 and 240 min into ice-chilled EDTA tubes. Plasma was separated by centrifugation (3,200rpm, 15 min, 4°C) within 30 min of collection and stored at −70°C until assayed. Plasma insulin was measured by enzyme linked immunosorbent assay (ELISA) (10-1113, Mercodia, Uppsala, Sweden), with sensitivity 1.0mU/L and coefficient of variation (CV) 2.5% within assays and 7.4% between assays. C-peptide was measured by ELISA (10-1136-01, Mercodia, Uppsala, Sweden). Sensitivity was 15pmol/L and the intra and inter-assay CVs were 11.9% and 1.5%, respectively. Plasma total GLP-1 and total GIP were measured on the intraduodenal infusion days at all time points, and at t=0 min and t=120 min on the intravenous infusion days. Plasma total GLP-1 was measured by radioimmunoassay (RIA) (GLPIT-36HK, Millipore, Billerica, MA) with sensitivity 3pmol/L and intra and inter-assay CVs 7.6% and 6.0%, respectively. Plasma GIP was measured by RIA (Perkin Elmer, Boston, MA) with sensitivity
2pmol/L and intra and inter-assay CVs 5.1% and 8.7%, respectively. Plasma glucagon was measured by RIA (GL-32K, Millipore, Billerica, MA) with sensitivity 20pg/ml and intra and inter-assay CVs 11.3% and 6.0%, respectively. Standardised international unit conversion was undertaken as per www.soc-bdr.org.

**Incretin effect & gastrointestinal glucose disposal (GIGD)**

The incretin effect was calculated from the incremental area under the curve (iAUC) using plasma C-peptide concentrations during the duration of ID or IV infusion (0-120 min) as \[
\left(\frac{\text{iAUC}_{\text{ID}(0-120)} - \text{iAUC}_{\text{IV}(0-120)}}{\text{iAUC}_{\text{ID}(0-120)}}\right) \times 100
\]
and expressed as a percentage (Nauck, Stockmann et al. 1986). GIGD, the amount of glucose required by intravenous infusion to ‘copy’ the glucose excursions after the oral load, was calculated as follows: if 25g intravenous glucose is required to copy a 75g oral glucose load, the GIGD amounts to 100 \times (75 – 25)/75 = 66% (Hare, Vilsboll et al. 2010).

**Statistical Analysis**

Blood glucose and plasma insulin, C-peptide, GLP-1, GIP, and glucagon were expressed as absolute values. Blood glucose and plasma hormone data were evaluated from t=0 min to t=240 min. Peak hormone concentrations, and the times at which these occurred, were calculated. The iAUC for glucose and hormone concentrations from t=0 min to t=120 min were determined using the trapezoidal rule. For intragroup data, one-way ANOVA tests were used to analyze baseline values and paired t-tests for peak values and iAUC. Unpaired t-tests and repeated measures ANOVA were used to analyze intergroup data. Bonferroni adjustment post-hoc tests were used when a significant interaction effect was seen in the intergroup data. Levene’s F-tests were used to test the difference in variance of incretin effects between the two groups. Statistical significance was accepted at P<0.05, and data are presented as mean values ± standard error (SE).
4.4 RESULTS

All subjects tolerated the study well. The control subjects were younger than the type 2 patients (47±3 vs. age 64±2 years, P<0.05), but BMI (29.3±1 vs. 31±1.3 kg/m², P=NS) was comparable. None of the type 2 patients had evidence of macro- or microvascular complications.

A. CONTROL SUBJECTS

Blood glucose, plasma insulin, C-peptide and glucagon (Figure 1) (Table 1)

Baseline (t=0 min) blood glucose, and plasma insulin, C-peptide and glucagon concentrations were similar between the four treatments.

There was no significant difference in the glycaemic response to ID2 and ID4. Following cessation of the ID glucose infusion, the blood glucose concentrations fell, particularly after ID4 when some subjects exhibited glucose concentrations in the hypoglycaemic range (<3mmol/L).

For both ID2 and ID4, the plasma insulin and C-peptide responses were much greater compared with the isoglycaemic intravenous glucose loads, as assessed by both peak insulin and iAUC (0-120 min) (P<0.05). The plasma insulin and C-peptide responses were much greater to ID4 than ID2 (P<0.05).

Following ID2, glucagon was suppressed at t=120 min when compared to baseline (43.3±5.7 vs. 68.7±11.8ng/L, P<0.05). However, following ID4, glucagon suppression was evident only initially, at ≈t=60 min compared to baseline (50.4±8.0 vs. 65.6±9.8ng/L, P<0.05) and concentrations then increased until t=120 min, so that at t=120 min there was no difference
from baseline (67.7±14.5ng/L, P=NS). Glucagon was suppressed at t=120 min compared to baseline in response to both IV2 and IV4 (P<0.05), without any difference between them.

Table 1. Results for glucose, insulin, C-peptide, and glucagon in BMI-matched controls and type 2 patients

<table>
<thead>
<tr>
<th></th>
<th>BMI-matched controls (n = 10)</th>
<th>Type 2 diabetes (n = 11)</th>
<th>ANOVA P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
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<tr>
<td>Baseline</td>
<td></td>
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</tr>
<tr>
<td>ID2</td>
<td>5.7 ± 0.2</td>
<td>6.2 ± 0.5</td>
<td>0.199</td>
</tr>
<tr>
<td>ID4</td>
<td>5.7 ± 1.1</td>
<td>7.9 ± 0.4</td>
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</tr>
<tr>
<td>IV2</td>
<td>5.9 ± 0.2</td>
<td>8.3 ± 0.4</td>
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</tr>
<tr>
<td>IV4</td>
<td>5.9 ± 0.2</td>
<td>8.3 ± 0.5</td>
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</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ID2</td>
<td>10.4 ± 0.7</td>
<td>15.1 ± 0.8</td>
<td>0.003</td>
</tr>
<tr>
<td>ID4</td>
<td>11.7 ± 0.7</td>
<td>15.3 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV2</td>
<td>10.7 ± 0.4</td>
<td>16.2 ± 0.8</td>
<td>0.144</td>
</tr>
<tr>
<td>IV4</td>
<td>12.0 ± 0.8</td>
<td>18.3 ± 1.2</td>
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<tr>
<td>AUC (0-120)</td>
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<tr>
<td>ID2</td>
<td>377 ± 34.7</td>
<td>478 ± 31.3</td>
<td>&lt;0.001</td>
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<tr>
<td>ID4</td>
<td>461 ± 63.5</td>
<td>751 ± 68.3</td>
<td>0.007*</td>
</tr>
<tr>
<td>IV2</td>
<td>385 ± 36.7</td>
<td>499 ± 51.2</td>
<td>0.009</td>
</tr>
<tr>
<td>IV4</td>
<td>462 ± 63.1</td>
<td>700 ± 56.4</td>
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<td><strong>Insulin</strong></td>
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<tr>
<td>Baseline</td>
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</tr>
<tr>
<td>ID2</td>
<td>4.1 ± 1.1</td>
<td>7.1 ± 1.2</td>
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<tr>
<td>ID4</td>
<td>5.5 ± 2.2</td>
<td>7.4 ± 1.3</td>
<td>0.157</td>
</tr>
<tr>
<td>IV2</td>
<td>5.9 ± 1.7</td>
<td>9.5 ± 1.8</td>
<td>0.801</td>
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<tr>
<td>IV4</td>
<td>5.6 ± 1.4</td>
<td>8.5 ± 1.1</td>
<td></td>
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<tr>
<td>Peak</td>
<td></td>
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</tr>
<tr>
<td>ID2</td>
<td>53.7 ± 13.5</td>
<td>49.7 ± 15.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ID4</td>
<td>137 ± 30.8</td>
<td>100 ± 24.4</td>
<td>0.793</td>
</tr>
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<td>IV2</td>
<td>28.8 ± 7.1</td>
<td>29.4 ± 4.9</td>
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<tr>
<td>IV4</td>
<td>40.8 ± 17.1</td>
<td>30.2 ± 8.9</td>
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</tr>
<tr>
<td>AUC (0-120)</td>
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</tr>
<tr>
<td>ID2</td>
<td>16,101 ± 5,535</td>
<td>13,081 ± 4,156</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ID4</td>
<td>53,269 ± 12,746</td>
<td>44,838 ± 11,384</td>
<td>0.484</td>
</tr>
<tr>
<td>IV2</td>
<td>7,805 ± 2,261</td>
<td>5,069 ± 1,261</td>
<td>0.900</td>
</tr>
<tr>
<td>IV4</td>
<td>12,800 ± 5,939</td>
<td>7,304 ± 1,810</td>
<td></td>
</tr>
<tr>
<td><strong>C-peptide</strong></td>
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<tr>
<td>Baseline</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ID2</td>
<td>369 ± 74.5</td>
<td>676 ± 96.3</td>
<td>0.109</td>
</tr>
<tr>
<td>ID4</td>
<td>379 ± 92.8</td>
<td>644 ± 70.7</td>
<td>0.018</td>
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<tr>
<td>IV2</td>
<td>430 ± 91.8</td>
<td>711 ± 101</td>
<td>0.765</td>
</tr>
<tr>
<td>IV4</td>
<td>400 ± 84</td>
<td>734 ± 79</td>
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<tr>
<td>Peak</td>
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<tr>
<td>ID2</td>
<td>2,137 ± 253</td>
<td>1,880 ± 294</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ID4</td>
<td>3,553 ± 572</td>
<td>3,754 ± 377</td>
<td>0.845</td>
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<tr>
<td>IV2</td>
<td>1,320 ± 227</td>
<td>1,533 ± 183</td>
<td>0.170</td>
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<td>IV4</td>
<td>1,510 ± 339</td>
<td>1,999 ± 203</td>
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<tr>
<td>AUC (0-120)</td>
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<tr>
<td>ID2</td>
<td>114,500 ± 16,647</td>
<td>65,960 ± 12,942</td>
<td>&lt;0.001</td>
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<tr>
<td>ID4</td>
<td>199,946 ± 35,116</td>
<td>178,167 ± 26,861</td>
<td>0.132</td>
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<tr>
<td>IV2</td>
<td>58,566 ± 7,557</td>
<td>39,504 ± 9,270</td>
<td>0.620</td>
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<tr>
<td>IV4</td>
<td>77,606 ± 16,472</td>
<td>44,736 ± 8,410</td>
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<td><strong>Glucagon</strong></td>
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<tr>
<td>Baseline</td>
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<td></td>
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<tr>
<td>ID2</td>
<td>57.4 ± 4</td>
<td>82.5 ± 5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ID4</td>
<td>56.5 ± 4</td>
<td>82.2 ± 3.7</td>
<td>0.203</td>
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<td>IV2</td>
<td>56 ± 3.6</td>
<td>91.4 ± 5.2</td>
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<td>IV4</td>
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<td>Δ (0-60 min)</td>
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<tr>
<td>ID2</td>
<td>−22.6 ± 5</td>
<td>−10.8 ± 4</td>
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<tr>
<td>ID4</td>
<td>−7 ± 6</td>
<td>12.8 ± 8</td>
<td>0.007</td>
</tr>
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</table>
A. BMI-Matched Controls

**Figure 1.** Blood glucose, plasma insulin, C-peptide, and glucagon concentrations at baseline and in response to a 120-min intraduodenal glucose infusion at 2 and 4 kcal/min and corresponding isoglycemic intravenous glucose infusions in BMI-matched controls. Data are mean ± SEM. Filled circles with bold line, intraduodenal glucose infusion; empty circles with dotted line, isoglycemic intravenous infusion. ID, intraduodenal; IV, intravenous; T2DM, type 2 diabetes.
Table 2. Results for GLP-1, GIP, Incretin effect and GIGD in BMI-matched controls and type 2 diabetes.

Incretin effect and gastrointestinal glucose disposal (GIGD) (Table 2)

The magnitude of the incretin effect, calculated using C-peptide, was greater for ID4 than ID2, (61.8±3.4% vs. 44.7±5.8%, P<0.05). GIGD was also greater for ID4 than ID2 (63.0% vs. 42.8%, P<0.05).

Plasma GLP-1 and GIP (Figure 2A) (Table 2)

Baseline plasma GLP-1 and GIP concentrations (t=0 min) before ID2 and ID4 were similar.

Following ID2, GLP-1 increased transiently (≈t=15 min), and then returned to baseline during the remainder of the infusion. In contrast, following ID4, GLP-1 increased progressively and remained elevated throughout the infusion, and the GLP-1 response was much greater to ID4 than ID2 (P<0.05).

There was a prompt rise in plasma GIP during both duodenal infusions. The GIP response was much greater to ID4 than ID2 (P<0.05).
Figure 2. Plasma GIP and GLP-1 concentrations at baseline and in response to a 120-min intraduodenal glucose infusion at 2 and 4 kcal/min in (A) BMI-matched controls and (B) type 2 patients. Data are mean ± SEM. Filled circles with bold line, intraduodenal glucose infusion at 2 kcal/min; empty circles with dotted line, intraduodenal glucose infusion at 4 kcal/min.
B. TYPE 2 PATIENTS AND COMPARISON WITH CONTROL SUBJECTS

Blood glucose, plasma insulin, C-peptide and glucagon (Figure 3) (Table 1)

Baseline (t=0 min) blood glucose, and plasma insulin, C-peptide and glucagon concentrations were similar between the four treatments in type 2 patients. Baseline blood glucose was greater in type 2 patients than controls (P<0.05). Fasting C-peptide (P<0.05), but not insulin (P=NS), was greater in healthy controls. Baseline plasma glucagon concentrations were greater in type 2 patients than the controls (P<0.05).

The overall glycaemic response in type 2 patients was greater to ID4 than ID2 as evaluated by the peak blood glucose and iAUC (0-120 min) (P<0.05). Following cessation of ID glucose infusion, the blood glucose concentrations fell to near baseline concentrations at t=240 min on all four study days. The overall glycaemic response to ID glucose, as evaluated by the peak blood glucose and iAUC (0-120 min), was greater than in the control group (P<0.05).

For both ID2 and ID4, the plasma insulin and C-peptide responses in type 2 patients were greater than the corresponding isoglycaemic intravenous glucose loads, as assessed by peak values and iAUC (0-120 min) (P<0.05). The insulin and C-peptide responses were much greater to ID4 than ID2 (P<0.05). The overall insulin or C-peptide responses, as assessed by peak values and iAUC (0-120 min), were similar in type 2 patients to controls (P = NS).
Figure 3. Blood glucose, plasma insulin, C-peptide, and glucagon concentrations at baseline and in response to a 120-min intraduodenal glucose infusion at 2 and 4 kcal/min and corresponding isoglycemic intravenous glucose infusions in type 2 patients. Data are mean ± SEM. Filled circles with bold line, intraduodenal glucose infusion; empty circles with dotted line, isoglycemic intravenous infusion. ID, intraduodenal; IV, intravenous; T2DM, type 2 diabetes.
Following ID2, plasma glucagon in type 2 patients decreased from baseline to $t=120$ min ($82.5\pm5.2$ vs. $57.7\pm4.3$ng/L, $P<0.05$). In contrast, in response to ID4, there was lack of suppression in plasma glucagon from baseline to $t=120$ min ($82.6\pm3.7$ vs. $123.1\pm24.1$ng/L, $P=\text{NS}$), which was first evident at $\approx t=90$ min. The magnitude of the change in glucagon concentrations from baseline to $t=60$ min was greater after ID2 than ID4 ($P<0.05$). Glucagon was suppressed from baseline to $t=60$ min in response to both IV2 and IV4, without any difference between them ($P=\text{NS}$).

While glucagon was suppressed by ID2 in both health and type 2 patients, there was impaired suppression in response to ID4 in the controls and no suppression of glucagon in type 2 patients.

**Incretin effect and gastrointestinal glucose disposal (GIGD) (Figure 4) (Table 2)**

The magnitude of the incretin effect in type 2 patients, calculated using plasma C-peptide, was substantially greater with ID4 than ID2, ($76\pm3\%$ vs. $34.3\pm11.6\%$, $P<0.05$) and comparable with controls at both ID2 and ID4 ($P=\text{NS}$ for both). The variance in the incretin effect, however, was greater in the type 2 group compared with controls at ID2 ($F=6.3$, $P<0.05$) but not ID4 ($F=0.4$, $P=\text{NS}$) (figure 4). GIGD was greater with ID4 than ID2 ($53.5\%$ vs. $33.1\%$, $P<0.05$) and comparable with controls at both ID2 and ID4 ($P=\text{NS}$ for both).
Figure 4. Variance in incretin effect in BMI-matched controls and type 2 patients. Data are mean ± SEM. T2DM, type 2 patients.

Plasma GLP-1 and GIP (Figure 2B) (Table 2)

Baseline plasma GLP-1 concentrations (t=0 min) in type 2 patients before ID2 and ID4 were similar, but were greater than in controls (P<0.05). Baseline plasma GIP concentrations (t=0 min) before ID2 and ID4 were similar in both groups (P =NS).

Following ID2, there was little, if any, change in GLP-1 in type 2 patients. In contrast, following ID4, GLP-1 increased progressively until ≈t=60 min and then stabilised. The iAUC for GLP-1 was much greater to ID4 than ID2 (P<0.05). The iAUC for the GLP-1 response was marginally less than in controls during both ID2 and ID4 (P<0.05).

There was a prompt rise in plasma GIP during both duodenal infusions in type 2 patients. The iAUC for GIP was much greater for ID4 than ID2 (P<0.05), but was comparable to controls at both ID2 and ID4 (P=NS).
4.5 DISCUSSION

We evaluated the effects of intraduodenal infusion of glucose at two rates within the physiological range of gastric emptying on glycaemia, insulinaemia, glucagonaemia, GLP-1 and GIP, and compared these responses to the effects of isoglycaemic intravenous glucose loads, in healthy subjects and type 2 patients. The major observations were: 1) there was no difference in the overall glycaemic response to ID2 and ID4 in controls, and in type 2 patients, the response to ID4 was only modestly greater; 2) there was minimal stimulation of GLP-1 secretion in response to ID2 but a sustained elevation in response to ID4, while GIP was stimulated substantially by ID2, and more by ID4, in both groups; 3) in both groups, the rises in plasma insulin and C-peptide and the magnitude of both the incretin effect and GIGD, were much greater in response to ID4 than ID2; 4) while there was sustained suppression of glucagon in response to ID2 and both isoglycaemic intravenous infusions, this was not the case with ID4 in either group.

While it is well established that the incretin effect represents a major contribution to the postprandial insulin response in health, the reasons for its low intra-individual, and substantial inter-individual variation, have hitherto not been determined (Perley and Kipnis 1967, Meier 2009). A recent study indicated that the magnitude of the incretin effect is independent of the circulating glucose load (Salehi, Aulinger et al. 2012), and it was suggested by these authors that the control of the incretin effect ‘occurs primarily at the level of the gastrointestinal tract’. That the rate of gastric emptying is an important determinant of postprandial glycaemia in health, as well as in type 1 and type 2 diabetes, is well established (Horowitz, Harding et al. 1986, Horowitz, Edelbroek et al. 1993, Rayner, Samsom et al. 2001). However, the non-linearity of the relationship of glycaemia with gastric emptying has only recently been recognized (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012). Our previous studies have shown that in health, the glycaemic
responses to intraduodenal glucose infusion at 2 kcal/min, 3 kcal/min or 4 kcal/min are comparable, attributable to substantially greater insulin and GLP-1 responses to the higher loads (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012). The outcome of the current study indicates that the incretin effect is dependent on the rate of gastric emptying in a given individual, with the inference that modulating the rate of emptying would impact on its magnitude. This observation has important implications for future studies evaluating the incretin effect, i.e. gastric emptying should be measured in most cases, and ideally, by a precise technique such as scintigraphy (Abell, Camilleri et al. 2008), given that modest changes in gastric emptying may have a major impact on glycaemia.

The incretin effect is known to be reduced in longstanding type 2 diabetes, in whom gastric emptying is frequently delayed, and sometimes more rapid (Bharucha, Camilleri et al. 2009, Chang, Rayner et al. 2010, Kashyap and Farrugia 2010, Bharucha, Batey-Schaefer et al. 2015), and this has been attributed to a reduced insulinotropic capacity of GIP (Hojberg, Vilsboll et al. 2009), rather than an absolute reduction in postprandial GIP or GLP-1 secretion (Nauck 2011). However, the studies that led to this conclusion did not quantify gastric emptying. In the current study, we have not only confirmed the increased secretion of both incretin hormones at 4 kcal/min compared to 2 kcal/min in both health and type 2 diabetes (Ma, Pilichiewicz et al. 2012), but have also shown that the incretin effect is substantially greater with higher rates of intraduodenal glucose infusion in both groups. Furthermore, GIGD is also dependent on the rate of duodenal glucose exposure in both health and type 2 diabetes, attesting to the importance of the rate of gastric emptying for glucose disposal, which has hitherto not been established.

It has been suggested that the incretin hormones, GIP and GLP-1, make a comparable contribution to the incretin effect in health (Toft-Nielsen, Madsbad et al. 1998, Vilsboll, Krarup et al. 2003), but our observations suggest that it is more likely that their relative
importance is dependent on the rate of small intestinal nutrient delivery. While GIP secretion increases approximately linearly with higher rates of duodenal glucose entry, the stimulation of GLP-1 is minimal at duodenal glucose loads $\leq 2$ kcal/min (Pilichiewicz, Chaikomin et al. 2007, Trahair, Horowitz et al. 2012). This implies that GIP is the major contributor to the incretin effect when gastric emptying of carbohydrate is less than 2 kcal/min, and that the importance of GLP-1 is greater at intraduodenal loads $\geq 3$ kcal/min (Trahair, Horowitz et al. 2012). The reason(s) underlying this phenomenon remain uncertain, but the distribution of enteroendocrine K and L cells (secreting GIP and GLP-1 respectively) within the gastrointestinal tract may be relevant - K cells are present most densely in the proximal small intestine, while L cells predominate in the distal ileum and colon (Baggio and Drucker 2007), and it is likely that at higher duodenal glucose loads, the maximal rate of proximal small intestinal glucose absorption is exceeded, so that a greater length, and more distal regions, of the gut are exposed. Furthermore, postprandial GLP-1 secretion is known to be exaggerated after gastric bypass surgery for obesity (Naslund, Gryback et al. 1997), which is associated with markedly accelerated gastric emptying of nutrient-containing liquids (Horowitz, Cook et al. 1982).

In response to ID2, glucagon was suppressed in both groups, however in response to ID4, glucagon was suppressed initially followed by a paradoxical rise in health, while there was no suppression at all in type 2 patients. The underlying reason(s) for the latter are again uncertain, but likely to reflect gastrointestinal mechanisms, particularly as isoglycaemic intravenous glucose was predictably associated with sustained suppression of glucagon in both groups. The incretin hormones and GLP-2, which is co-secreted from L cells with GLP-1, differ in their effects on glucagon (Lund, Vilsboll et al. 2011). While GLP-1 suppresses glucagon (Creutzfeldt, Kleine et al. 1996, Hare, Vilsboll et al. 2010), GIP and GLP-2 may stimulate its release (Meier, Goetze et al. 2004, Lund, Vilsboll et al. 2011). It is not known whether the ‘ratio’ of GLP-1 and GLP-2 release from L cells remains constant in response to varying
duodenal loads, and we did not measure GLP-2. The gastrointestinal tract could itself be an extra-pancreatic site of glucagon secretion (Knop 2009), consistent with observations in patients with gastric bypass surgery that oral ingestion of glucose enhances while IV glucose suppresses glucagon levels (Salehi, Prigeon et al. 2011).

There was no difference in the magnitude of the incretin effect, GIGD, or plasma insulin and C-peptide responses to ID or IV glucose between the healthy subjects and type 2 patients. However, there was a greater variance in the size of the incretin response to ID2 in the type 2 patients, such that three patients exhibited no incretin effect at this rate of duodenal glucose infusion. While the number of subjects was too small to explore potential associations with duration of diabetes or glycaemic control, one could speculate that these subjects may have been less responsive to the insulinotropic effects of GIP than the others. It would be of interest to evaluate patients with longstanding, complicated type 2 diabetes and determine whether chronic glycaemic control impacts on the incretin effect; the loss of this effect in such patients is likely to represent an epi-phenomenon of pancreatic beta cell failure (Meier and Nauck 2010).

While there were no major differences in basal or glucose-stimulated GIP, we found that baseline GLP-1 levels were marginally greater in type 2 patients than healthy controls, although the overall GLP-1 response was slightly less. Although these changes were modest, they differ from our recent report (Young, Chia et al. 2013), which found no difference between similar groups. Glucose and glucagon levels were predictably greater in type 2 patients.

In interpreting our observations, potential limitations should be recognised. We studied type 2 patients who had uncomplicated disease of short-duration and relatively good glycaemic control. We have assumed that the rate of duodenal glucose delivery is indicative of the
effects of a comparable gastric emptying rate following oral ingestion of glucose. While this was the only way by which ‘gastric emptying’ could be standardised, we did not measure gastric emptying in our subjects. We included only males in order to exclude potentially confounding effects of the menstrual cycle on gut motility and hormone secretion (Brennan, Feltrin et al. 2009), although we see no reason to expect a different outcome in women. While the mean BMI in our groups was comparable, there is some evidence that obesity may be associated with disordered incretin hormone secretion (Muscelli, Mari et al. 2008). It would also be of interest to determine whether similar effects would be observed with a combination of macronutrients, in particular fat, which is a potent stimulus for incretin hormone secretion and associated with an ‘incretin effect’ (Lindgren, Carr et al. 2011).

In conclusion, in health and well controlled type 2 diabetes, the magnitude of both the incretin effect and gastrointestinal glucose disposal are dependent on the rate of small intestinal glucose exposure, being substantially greater when glucose is infused intraduodenally at 4 kcal/min compared to 2 kcal/min. These observations imply that the rate of gastric emptying is a major determinant of the magnitude of the incretin effect in both health and type 2 diabetes. Furthermore, the relative secretion of GIP and GLP-1 is dependent on the rate of small intestinal glucose exposure and in health, it is likely that GIP is the dominant incretin at lower duodenal glucose loads. In type 2 diabetes, the incretin effect is highly dependent on GLP-1 secretion, given the diminished insulinotropic capacity of GIP. Hence, the rate of gastric emptying may be an even greater determinant of the incretin effect and potentially, glucose-lowering induced by DPP-IV inhibitors, in this group.
CHAPTER 5
CHAPTER 5. THE IMPACT OF DUODENAL GLUCOSE LOAD ON THE ORAL DISPOSITION INDEX IN HEALTH

Adapted from Marathe CS et al, Diabetic Medicine, 2015 Nov, Vol 32 (11); Pages 1500-3

5.1 SUMMARY

The oral disposition index (ratio of insulin response to insulin sensitivity) in healthy subjects is predictive of the development of type 2 diabetes. We sought to determine whether the rate of intraduodenal glucose delivery affects the disposition index in non-diabetic humans. 19 Caucasian males received glucose infusions via an intraduodenal (ID) catheter at either 2 kcal/min (ID2) or 4 kcal/min (ID4) for 120 min, on two separate days with measurements of blood glucose (G) and plasma insulin (I) at frequent intervals. Insulin release was estimated by the ratio of the change in insulin to that of change in glucose at 30 min represented as $\Delta I_{0-30}/\Delta G_{0-30}$. Insulin sensitivity was estimated as $1/\text{fasting insulin}$. The disposition index was then calculated as $\Delta I_{0-30}/\Delta G_{0-30} \times 1/\text{fasting insulin}$. The overall glycaemic response was comparable on both days, but the insulin response was much greater at ID4 ($P<0.05$). The disposition index at 30 min was greater ($P<0.05$) in response to ID4 than ID 2. In conclusion, the rate of duodenal glucose delivery has a major impact on insulin release and, thereby, the disposition index. These data indicate that the role of gastric emptying, which determines duodenal glucose delivery, warrants investigation as a potential predictor of the risk of future type 2 diabetes.

5.2 INTRODUCTION

Progressive beta cell failure is a pathogenetic feature of type 2 diabetes (Weyer, Bogardus et al. 1999, Kahn, Cooper et al. 2014), and well advanced by the time of diagnosis (Fonseca...
However, the development of postprandial hyperglycaemia frequently precedes fasting hyperglycaemia (Monnier, Colette et al. 2007). It is also evident that the deterioration in beta cell function occurs in a step-wise manner and both onset and rate are major determinants of the development of hyperglycaemia (Kahn, Cooper et al. 2014). The oral disposition index or DI (defined as the ratio of insulin release to insulin sensitivity) has emerged as a measure of beta cell function that enhances the prediction of future type 2 diabetes risk ‘over and beyond’ the oral glucose tolerance test, so that in individuals with a higher DI, the risk is substantially less (Utzschneider, Prigeon et al. 2009).

Gastric emptying i.e. the delivery of nutrients from stomach to duodenum, is a major determinant of both postprandial glycaemia and insulinaemia in health and type 2 diabetes (Marathe, Rayner et al. 2013). It is not well recognized that gastric emptying exhibits substantial inter-individual variation in health (1-4 kcal/min) (Collins, Horowitz et al. 1983) which is increased in type 2 diabetes because of the high prevalence of delayed, and sometimes accelerated, emptying (Marathe, Rayner et al. 2013). Moreover, gastric emptying has also been reported to vary between various ethnic groups (Phillips 2006), although not all major ethnicities have been evaluated. Studies employing intraduodenal (ID) glucose infusions at rates within the physiological range of gastric emptying have been used to characterise the impact of gastric emptying on postprandial glycaemia. These have established that the relationship between glycaemia and the rate of delivery of glucose to the small intestine is non-linear, such that in health (and to some extent, in well controlled type 2 diabetes) there is minimal increment in overall glycaemic response on increasing the infusion rate from 2 to 4 kcal/min, while insulin increase is much greater at 4 kcal/min (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012). The latter may be attributable to increased secretion of GIP, and, particularly, GLP-1, leading to an increased incretin effect in health, as well as in well controlled type 2 diabetes (Marathe, Rayner et al. 2014). Somewhat
surprisingly, the impact of the rate of duodenal glucose delivery on the oral disposition index has not been assessed.

This study has evaluated the effect of two different rates of duodenal glucose delivery, 2 and 4 kcal/min (both within the physiological rate of gastric emptying) on the oral disposition index in health. The underlying hypothesis is that the oral disposition index would be greater at the higher duodenal delivery rates.

5.3 RESEARCH DESIGN & METHODS

Subjects

Data were derived from 19 non-diabetic overweight men (age 38.5±3 years, BMI 27±0.5 kg/m²) from our previously published studies (Pilichiewicz, Chaikomin et al. 2007, Marathe, Rayner et al. 2014). Subjects with known gastrointestinal disease, medical illness, or taking medication known to affect gastrointestinal motility, were excluded. Each study protocol conformed to the principles of the Declaration of Helsinki, and was approved by the Royal Adelaide Hospital Research Ethics Committee.

Study Protocol

Each subject attended the laboratory at 0800h following an overnight fast on two separate days and received, in randomised order, an ID glucose infusion at 2 kcal/min or 4 kcal/min. A manometric catheter (Dentsleeve, Ontario, Canada), incorporating an infusion channel opening 12cm beyond the pylorus, was inserted through the nose and positioned in the duodenum by monitoring the antro-duodenal transmucosal potential difference (Heddle, Fone et al. 1988). ID glucose (25g/100ml) was infused at 2 or 4 kcal/min from t=0 to 120 min. At
the end of 120 min, the ID catheter was removed. The subject was provided with a light lunch before leaving the laboratory.

**Measurements**

**Blood glucose and plasma insulin**

A cannula was placed in the antecubital vein and blood was sampled at \( t=0 \), 15, 30, 45, 60, 90, 105 and 120 minutes into ice-chilled EDTA tubes. Plasma was separated by centrifugation and stored at \(-70^\circ C\) for subsequent assays. Blood glucose concentrations were determined using a glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA) and plasma insulin concentrations by ELISA (10-1113 Mercodia, Uppsala, Sweden), the insulin assay having a sensitivity of 1.0mU/L and coefficient of variation 2.5% within assays and 7.4% between assays (Trahair, Horowitz et al. 2012).

**Calculation of glycaemic and insulinaemic response, insulin secretion, sensitivity and the oral disposition index**

The overall glycaemic (Glu) and insulinaemic (Ins) responses were estimated as the incremental area under the curve from \( t=0 \) to \( t=120 \) min (calculated using the trapezoidal rule) and represented as \( \text{Glu iAUC}_{0-120} \) or \( \text{Ins iAUC}_{0-120} \) respectively. Insulin secretion was estimated as the change in insulin to that of change in glucose at 30 min represented as \( \Delta I_{0-30}/\Delta G_{0-30} \) (Retnakaran, Qi et al. 2009). Insulin resistance was estimated as 1/fasting insulin (Utzschneider, Prigeon et al. 2009). The disposition index was then calculated as \( \Delta I_{0-30}/\Delta G_{0-30} \times 1/\text{fasting insulin} \) (Utzschneider, Prigeon et al. 2009).
Statistical Analysis

Paired Student’s t-tests were used to analyse the data. Significance was accepted at P<0.05, and data are presented as mean ± standard error (SE). Statistical analyses were performed using SPSS software (Version 22, Armonk, NY: IBM Corp USA).

5.4 RESULTS

Blood glucose, plasma insulin, insulin response, insulin sensitivity and disposition index (Table 1)

Fasting concentrations of blood glucose (P=NS) and plasma insulin (P=NS) were comparable on both days. The overall glycaemic response, as estimated by Glu iAUC$_{0-120}$, was comparable on both days (P=NS), but the overall insulin response, as estimated by Ins iAUC$_{0-120}$, was much greater with ID4 (P<0.001).

The insulin response, estimated as $\Delta I_{0-30}/\Delta G_{0-30}$, was substantially greater with ID4 than ID2 (P=0.02). Insulin sensitivity, estimated as the inverse of fasting insulin (0.36±0.04 for ID2 vs. 0.35±0.08 for ID4, P=NS), was similar on both days. The disposition index, estimated as $\Delta I_{0-30}/\Delta G_{0-30} \times 1/\text{fasting insulin}$, was, therefore, much greater in response to ID4 than ID2 (P<0.001) (table 1).
Table 1. Fasting blood glucose and plasma insulin levels, total glycaemic and insulinaemic responses, insulin secretory response and oral disposition index in people without diabetes (n = 19) who received intraduodenal glucose at 2 (ID2) or 4 (ID4) kcal/min for 120 min.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 19)</th>
<th>Paired t-test P</th>
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<tbody>
<tr>
<td><strong>Glucose – Baseline</strong></td>
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</tr>
<tr>
<td>ID2</td>
<td>5.6 ± 0.1</td>
<td>0.56</td>
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<tr>
<td>ID4</td>
<td>5.5 ± 0.1</td>
<td></td>
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<tr>
<td><strong>Insulin – Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID2</td>
<td>3.2 ± 0.3</td>
<td>0.67</td>
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<tr>
<td>ID4</td>
<td>3 ± 0.3</td>
<td></td>
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<tr>
<td><strong>Glu iAUC0-120</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID2</td>
<td>250 ± 25</td>
<td>0.14</td>
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<tr>
<td>ID4</td>
<td>284 ± 32</td>
<td></td>
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<tr>
<td><strong>Ins iAUC0-120</strong></td>
<td></td>
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<tr>
<td>ID2</td>
<td>2159 ± 303</td>
<td>&lt; 0.001</td>
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<tr>
<td>ID4</td>
<td>6641 ± 887</td>
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<tr>
<td><strong>ΔI0-30/ΔG0-30</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID2</td>
<td>6.4 ± 1</td>
<td>0.02</td>
</tr>
<tr>
<td>ID4</td>
<td>10.6 ± 1.6</td>
<td></td>
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<tr>
<td><strong>ΔI0-30/ΔG0-30 × 1/fasting insulin</strong></td>
<td></td>
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<tr>
<td>ID2</td>
<td>2.4 ± 0.5</td>
<td>0.03</td>
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<tr>
<td>ID4</td>
<td>3.9 ± 0.6</td>
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<tr>
<td><strong>ΔI0-60/ΔG0-60</strong></td>
<td></td>
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</tr>
<tr>
<td>ID2</td>
<td>9.2 ± 1.5</td>
<td>&lt; 0.01</td>
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<tr>
<td>ID4</td>
<td>29.2 ± 6.3</td>
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<tr>
<td><strong>ΔI0-60/ΔG0-60 × 1/fasting insulin</strong></td>
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<tr>
<td>ID2</td>
<td>3.5 ± 0.7</td>
<td>0.02</td>
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<tr>
<td>ID4</td>
<td>12.5 ± 4</td>
<td></td>
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</table>
Figure 1. Blood glucose (A) and plasma insulin (B) concentrations at baseline and in response to a 120-min intraduodenal glucose infusion at 2 kcal/min and 4 kcal/min. Data are mean ± SEM. Filled circles with bold line = intraduodenal glucose infusion at 2 kcal/min, filled circles with dotted line = intraduodenal glucose infusion at 4 kcal/min.
The key findings of this study are: 1) the estimated insulin response is greater at ID4 than ID2 and 2) the oral disposition index is greater at ID4 than ID2 in healthy subjects.

The oral disposition index has now been studied in a number of ethnic populations (Abdul-Ghani, Williams et al. 2007, Abdul-Ghani, Lyssenko et al. 2009, Utzschneider, Prigeon et al. 2009) and emerged as a valuable tool to identify individuals at high risk of future type 2 diabetes (DeFronzo and Abdul-Ghani 2009). Therefore, it is important to determine what physiological variables contribute to the disposition index. Studies that have employed intraduodenal glucose infusions at a constant rate have established that, while the overall glucose response (as measured by iAUC) is greater in response to 2 kcal/min than 1 kcal/min, the response to 2 kcal/min is comparable to 3 kcal/min and 4 kcal/min in healthy humans (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014). Substantially greater insulin responses to the higher rates of glucose delivery account for this phenomenon, and appear to be driven by enhanced secretion of the incretins, GIP and GLP-1 (Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014). While GIP increases linearly with increasing rates of duodenal glucose exposure, the GLP-1 response is non-linear, with a minimal, transient, increase in GLP-1 at rates $\leq 2$ kcal/min, but a substantial, and sustained, response to infusions $\geq 3$ kcal/min. This is consistent with the concept that the relative contribution of GIP and GLP-1 to glucose homeostasis is dependent on the rate of entry of glucose into the small intestine (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Marathe, Rayner et al. 2014, Wu, Ma et al. 2014), and has implications for the disposition index, which is derived from the insulin and glucose excursions. This explains why the disposition index was greater in response to 4 kcal/min than 2 kcal/min. As mentioned earlier, not only does the rate of gastric emptying vary widely in health and type 2 diabetes (Collins, Horowitz et al. 1983, Jones, Horowitz et al. 1996), it
also varies within various ethnic groups (Phillips 2006). The insulin secretory response (estimated as $\Delta l_{0.3d}/\Delta G_{0.3d}$) following oral glucose also varies with ethnicities – for example, a number of studies have shown the insulin secretory response to be lower in East Asian population compared with Caucasians. As gastric emptying is an important determinant of both the early glucose and insulin responses (Horowitz, Edelbroek et al. 1993), it should be considered when comparing populations at risk for type 2 diabetes.

This study should be regarded as ‘proof-of-principle’ - potential limitations relate to the route, and rate, of glucose delivery i.e. the glucose was delivered directly into the duodenum and the rate of duodenal delivery is assumed to be indicative of the effects of a comparable rate of gastric emptying after oral glucose. While only male subjects were evaluated to obviate the confounding effects of the menstrual cycle on gastrointestinal motility (Brennan, Feltrin et al. 2009), it would be surprising if the observations were not applicable to females. It would of interest to study the effect of gastric emptying on the disposition index following a standardized glucose tolerance test. The observations also suggest that community-based prospective studies to assess the impact of gastric emptying on the risk of type 2 diabetes would be desirable.
CHAPTER 6
CHAPTER 6. ETHNIC DISPARITIES IN INSULIN & GIP SECRETION IN RESPONSE TO INTRADUODENAL GLUCOSE IN HEALTH

Adapted from Marathe CS et al, Acta Diabetologica, 2015 Aug, Vol 52(4), Pages 817-9

6.1 SUMMARY

Insulin secretion following an oral glucose load varies widely within ethnic groups and appears to be less in East Asians than Caucasians (for uncertain reasons). This study has evaluated the comparative effects of an intraduodenal glucose infusion on glycaemia, insulinaemia and incretin hormones (GIP & GLP-1) in healthy Han Chinese & Caucasian subjects. 11 healthy Han Chinese & 8 Caucasian males received an intraduodenal glucose infusion at the rate of 4 kcal/min (ID4) for 120 min with concurrent measurements of blood glucose (G), plasma insulin (I), glucagon-like peptide 1 (GLP-1) and glucose-dependent insulino tropic polypeptide (GIP). Fasting, peak and $\text{AUC}_{(0-120)}$ glucose and fasting GIP and GLP-1 were comparable in both groups. Fasting insulin (P<0.01), insulin secretion (measured as $\Delta I_{0-30}/\Delta G_{0-30}$) and GIP (peak, P<0.01 and $\text{AUC}_{(0-120)}$, P=0.01) secretion were less in Han Chinese than Caucasian subjects, while there was no differences in GLP-1 (peak or $\text{AUC}_{(0-120)}$) secretion or the oral disposition index (measured as $\Delta I_{0-30}/\Delta G_{0-30} \times 1/$fasting insulin). These results indicate that the stimulation of insulin by duodenal glucose is lower in healthy Han Chinese than Caucasians, which may relate to a reduction in GIP secretion.

6.2 INTRODUCTION

The increasing prevalence of type 2 diabetes has increased the recognition of pathophysiological differences between ethnic groups (Kodama, Tojjar et al. 2013). In East
Asians with type 2 diabetes, BMI is usually less and the ‘visceral to subcutaneous fat ratio’ for a given BMI higher when compared to Caucasians (Huxley, James et al. 2008), consistent with the concept that impaired insulin secretion represents the primary defect in the pathogenesis of type 2 diabetes in East Asians, rather than insulin resistance (Matsumoto, Miyake et al. 1997, Fukushima, Suzuki et al. 2004, Rattarasarn, Soonthornpan et al. 2006). East Asians also appear to secrete less insulin than Caucasians following an oral glucose challenge for uncertain reasons. The oral disposition index, calculated as the product of insulin secretion and insulin sensitivity, is used to predict future risk of type 2 diabetes (Utzschneider, Prigeon et al. 2009) and the comparative difference between the two ethnic groups has not been reported. In Caucasians, the ‘incretin effect’ (the amplified insulin response following oral or enteral compared with isoglycaemic intravenous glucose), mediated by GIP and GLP-1, is attenuated in type 2 diabetes (Nauck, Stockmann et al. 1986), but a recent study (Oh, Kim et al. 2014) suggests that the ‘incretin effect’ may be preserved in East Asians with ‘early’ type 2 diabetes.

The rate of gastric emptying exhibits substantial inter-individual variation and is a major determinant of both postprandial glycaemia and insulinaemia and the secretion of incretin hormones (Marathe, Rayner et al. 2013). There is evidence to suggest that the rate of gastric emptying is dependent on ethnicity (Phillips 2006). Intraduodenal glucose infusions have been employed to control for gastric emptying (range 1-4 kcal/min in health)(Brener, Hendrix et al. 1983, Marathe, Rayner et al. 2013). These studies have established that incretin secretion following intraduodenal glucose is rate dependent and that while GIP increases linearly with the duodenal glucose load, there is minimal GLP-1 secretion at rates less than 2 kcal/min (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012, Trahair, Horowitz et al. 2012, Marathe, Rayner et al. 2014). However, at a duodenal glucose infusion rate of 4 kcal/min, which is at the upper end of the physiological rate of gastric emptying (Brener, Hendrix et al. 1983), both GIP and GLP-1 show sustained and substantial elevations
Information about the incretin hormone secretion in East Asians compared with Caucasians is limited. A recent review (Seino, Fukushima et al. 2010) suggests disparities in incretin hormone secretion (GIP and GLP-1) between East Asian and Caucasian subjects, although this may be based on data derived from different studies (Yabe, Kuroe et al. 2010) employing different assays, precluding meaningful comparison. The current study has evaluated insulin and incretin responses to intraduodenal glucose infusion in healthy Han Chinese and Caucasian volunteers. Our hypothesis was that insulin secretion would be less in the Han Chinese group and associated with differences in secretion of either GIP and/or GLP-1.

6.3 RESEARCH DESIGN & METHODS

Subjects

We studied eleven Han Chinese and eight Caucasian non-diabetic men; the latter were part of healthy control group included in a previous study (Marathe, Rayner et al. 2014). The Han Chinese subjects were all born on mainland China and had migrated to Australia less than 5 years previously. Subjects with gastrointestinal disease, or taking medication known to affect gastrointestinal motility, were excluded. The study protocol, which conformed to the principles of the Declaration of Helsinki, was approved by the Royal Adelaide Hospital Research Ethics Committee.

Study Protocol

Each study subject attended the laboratory at 0800h following an overnight fast and received an ID glucose infusion at 4 kcal/min. A manometric catheter (Dentsleeve, Ontario, Canada),
incorporating an infusion channel opening 12cm beyond the pylorus, was inserted through the
nose and positioned in the duodenum by monitoring antral and duodenal transmucosal
potential difference (Heddle, Fone et al. 1988). An IV cannula was placed in the antecubital
vein for blood sampling. ID glucose (25g/100ml) was infused at 4 kcal/min from t=0 to 120
min. Following this, the catheter was removed and each subject given a light lunch.

Measurements

Blood glucose, plasma insulin, GIP and GLP-1

Blood glucose was determined using a glucometer (Medisense Precision QID; Abbott
Laboratories, Bedford, MA). Blood samples were collected at frequent intervals (t=0, 15, 30,
45, 60, 90, 105 and 120 min) into ice-chilled EDTA tubes for insulin, GIP and GLP-1
measurements. Plasma was separated by centrifugation and stored at −70°C until assayed.
Plasma insulin was measured by ELISA (10-1113, Mercodia, Uppsala, Sweden) (Trahair,
and total GLP-1 (GLPIT-36HK, Millipore, Billerica, MA) were measured by
radioimmunoassay (RIA).

Calculation of insulin secretion, sensitivity & the disposition index

The total area under the curve (AUC) responses for insulin and glucose were calculated using
the trapezoidal method from t=0 to t=30 and or from t=0 to t=120 and represented as AUCI_{0-30}/AUCG_{0-30} or AUCI_{0-120}/AUCG_{0-120}, respectively. Insulin secretion was estimated as the
change in insulin divided by the change in glucose at 30 min (ΔI_{0-30}/ΔG_{0-30}). Insulin sensitivity
was estimated as 1/fasting insulin. The disposition index (DI) was calculated as ΔI_{0-30}/ΔG_{0-30}\times
1/fasting insulin.
Statistical Analysis

The sample size was determined by a professional biostatistician based on earlier published work (Pilichiewicz, Chaikomin et al. 2007, Ma, Pilichiewicz et al. 2012). Fasting, peak and total AUC for glucose and hormone concentrations from \( t=0 \) min to \( t=120 \) min, were determined. Unpaired t-tests were used to analyze the data. Significance was accepted at \( P<0.05 \), and data are presented as mean±standard error (SE).

6.4 RESULTS

All Han Chinese subjects tolerated the study well. The Han Chinese (HC) were younger than Caucasian (C) subjects (24.8±1.3 vs 45.3±3.8 years, \( P<0.01 \)); there was no difference in BMI, although mean values were less in the Han Chinese (25.1±1.7 vs. 28.3±0.7 kg/m\(^2\), \( P=\text{NS} \)).

Blood glucose, plasma insulin, insulin secretion, sensitivity & disposition index

There was no difference in fasting glucose (5.4±0.1 for HC vs 5.7±0.2 for C, mmol/L, \( P=0.10 \)), peak glucose (10.1±0.5 for HC vs 11.6±0.9 for C, mmol/L, \( P=0.20 \)) and overall glycaemic response (as measured by total AUC\(_{0-120}\)) (1012±58 for HC vs 1110±72 for C, mmol/L.min, \( P=0.30 \)) between the two groups. In contrast, fasting insulin (4.9±0.8 for HC vs 19.2±3.9 for C, mU/L, \( P<0.01 \)), peak insulin (192±25 for HC vs 643±181 for C, mU/L, \( P=0.01 \)) and the overall insulinaemic response (as measured by total AUC\(_{0-120}\)) (13234±2134 for HC vs 43133±12197 for C, mU/L.min, \( P=0.01 \)) was lower in the Han Chinese subjects.

Fasting (4.9±0.8 vs 19.2±3.9, mU/L, \( P<0.01 \)) and AUC\(_{0-120}\) (13234±2134 vs 43133±12197, mU/L.min, \( P=0.01 \)) insulin and insulin secretion (15.5±5.2 vs 63.2±22, \( P=0.02 \)) were lower in Han Chinese. The DI was not different (2.9±0.4 vs 3.5±1.3, \( P=0.63 \)).
Figure 1. Blood glucose and plasma insulin concentrations at baseline and in response to a 120-min intraduodenal glucose infusion at 4 kcal/min in Han Chinese (Filled circles with bold line) and Caucasian (Empty circles with dotted line) subjects. Data are mean ± SEM.

Plasma GLP-1 and GIP

Fasting GIP levels (16.2±1.3 for HC vs 22±2.9 for C, pmol/L, P=0.06) tended to be less in Han Chinese and this was the case for peak GIP (57.9±3.5 for HC vs 88.3±9.2 for C, pmol/L, P<0.01) and overall GIP response (as measured by total AUC) (5836±337 for HC vs 7975±739 for C, pmol/L.min, P=0.01)

There was no difference in fasting (25±3.3 for HC vs 19.8±2.4 for C, pmol/L, P=0.24), peak GLP-1 (56.4±5.2 for HC vs 63.1±10 for C, pmol/L, P=0.54) or overall GLP-1 levels (as measured by total AUC) (4980±279 for HC vs 4990±701 for C, pmol/L.min, P=0.99) between the two groups.
**6.5 DISCUSSION**

The outcomes of this study indicate that in healthy Han Chinese subjects, insulin secretion is less and both insulin sensitivity and disposition index greater, than healthy Caucasian subjects in response to intraduodenal glucose infusion at 4 kcal/min, associated with a reduction in GIP, but comparable, GLP-1 secretion.

Type 2 diabetes is characterised by progressive defects in insulin secretion and increasing insulin resistance (Defronzo 2009). The latter is linked closely to BMI and is a characteristic feature of many ethnicities, particularly African American, Hispanic, South Asian and Caucasian groups (Kodama, Tojjar et al. 2013). In contrast, East Asians with type 2 diabetes, are not infrequently less obese than Caucasians with type 2 diabetes and it has been suggested that impairment in insulin secretion, rather than increased insulin resistance, is the principal glucoregulatory defect in this group (Fujimoto 1996). Our results are consistent with this concept - although basal insulin was less in the Han Chinese group, the glucose-stimulated insulin was much less. The reasons for this reduction in insulin secretory response remain uncertain, but reduced beta cell mass, beta cell failure and diminished incretin hormone
secretion have been postulated to be relevant (Kodama, Tojjar et al. 2013). Interestingly, the disposition index was comparable to the Caucasian subjects, reflecting an increase in insulin sensitivity in the Han Chinese subjects. A small number of studies (Han, Kim et al. 2010, Kozawa, Okita et al. 2010) (Yabe, Kuroe et al. 2010) have compared GIP and GLP-1 responses to oral glucose or mixed meals within East Asian populations and concluded that they are comparable. However, none of these studies included Caucasian subjects as part of the study design. The only direct comparison, as mentioned earlier, was in a recent review (Seino, Fukushima et al. 2010) in which the healthy Japanese subjects were reported to have higher total GIP and lower total GLP-1 secretion than Caucasians. Unfortunately, additional information regarding the subjects and their characteristics are not provided and there is no evidence that gastric emptying was measured; an important factor given potential ethnic differences in gastric emptying. Our findings are not in agreement with the study above and suggest that GIP responses are comparatively lower and given the comparable glycaemic responses, could, in part, contribute to the reduced insulin response seen in Han Chinese subjects.

The relative contribution of GIP and GLP-1 towards the ‘incretin effect’ is uncertain, but GIP is generally believed to the dominant incretin in health (Meier 2009). GIP loses its insulinotropic activity in type 2 diabetes, while GLP-1 retains it (albeit at supraphysiologic levels) (Nauck, Baller et al. 2004, Baggio and Drucker 2007). Recent studies suggest that incretin-based therapies (GLP-1 analogues and DPP-IV inhibitors) may be more effective in Asians with type 2 diabetes when compared with non-Asian (Kim, Hahn et al. 2013). Our finding that GLP-1 is not comparatively deficient in healthy Han Chinese men is potentially important, as it is the major therapeutic target in type 2 diabetes.

Limitations of our study are that the size of the cohorts was small and exclusively male, responses to intraduodenal, rather than oral, glucose were evaluated and there was a
difference in age between the groups, although GIP (and GLP-1) responses are apparently unaffected by age (Trahair, Horowitz et al. 2012). Mean BMI was higher in the Caucasians, albeit non-significantly, which may represent a confounder, although it appears that body weight does not affect the GIP response to nutrients (Wu, Ma et al. 2014). The observations should, therefore, be regarded as preliminary and ‘proof-of-principle’. They should stimulate further studies comparing the incretin secretory responses between various ethnicities; particularly given the implications for type 2 diabetes management and developing more effective therapeutic strategies.
CHAPTER 7. THE EFFECT OF DUODENAL GLUCOSE LOAD ON BLOOD PRESSURE IN TYPE 2 DIABETES

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7.1 SUMMARY

Postprandial hypotension occurs frequently in diabetes. We show in 9 type 2 patients, that the fall in systolic blood pressure is greater in response to intraduodenal glucose infused at 4 kcal/min than 2 kcal/min, implying that strategies to slow gastric emptying may be effective in the management of postprandial hypotension.

7.2 INTRODUCTION

As well as influencing postprandial glycaemia (Marathe, Horowitz et al. 2015), gastric emptying (GE) affects the postprandial hypotensive response in ‘healthy’ older subjects and type 2 patients, such that when GE is relatively more rapid, the fall in blood pressure is greater (Jones, Tonkin et al. 1998). In healthy older subjects, when gastric distension – which may influence blood pressure – is ‘bypassed’ by infusing glucose directly into the duodenum, the fall in systolic blood pressure is greater in response to 2 and 3 kcal/min than 1 kcal/min (O'Donovan, Feinle et al. 2002, Trahair, Vanis et al. 2012).

These observations are relevant to the pathogenesis and management of postprandial hypotension, defined as a fall in systolic blood pressure ≥20 mm Hg after a meal (Jansen and Lipsitz 1995), for which management is suboptimal (Jansen and Lipsitz 1995, Jansen, Kelly-Gagnon et al. 1996). Postprandial hypotension occurs frequently in diabetes (Jansen and
Lipsitz 1995). It is not known whether the rate of duodenal glucose delivery influences blood pressure in type 2 patients. This study has evaluated the effects of variations in intraduodenal glucose load within the normal range of GE (1-4 kcal/min) (Brener, Hendrix et al. 1983) on blood pressure in type 2 patients.

7.3 RESEARCH DESIGN & METHODS

Nine males with type 2 diabetes (age 62±2.4 yrs, HbA1c 6.7±2.4% (50mmol/mol), BMI 31±1.3 kg/m², managed by diet and/or metformin) were studied. None had evidence of microvascular complications or was known to have postprandial hypotension. Metformin, and any medications that could affect blood pressure, were withheld for ≥24 hours prior to each study day. The protocol was approved by the Royal Adelaide Hospital Research Ethics Committee and each subject provided written, informed consent. Data relating to glycaemia and incretin hormones have been reported previously (Marathe, Rayner et al. 2014) and described in detail in Chapter 4.

Each participant underwent paired studies on separate days. On each day, after an overnight fast, a manometric catheter (Dentsleeve, Ontario, Canada) was introduced intranasally and the tip allowed to pass into the duodenum by peristalsis (O'Donovan, Feinle et al. 2002). An intraduodenal glucose infusion (25g/100mL) at 2 kcal/min (2ID) or 4 kcal/min (4ID) was then commenced, and continued for 120 min. A cuff was placed on the right upper arm to measure systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) at baseline and then every 5 min for 120 min by an automated device (DINAMAP ProCare 100; GE Medical Systems, Milwaukee, MI, USA), and an intravenous cannula was placed in the contralateral antecubital vein for blood sampling. Subjects lay comfortably on a bed during the procedure. Blood glucose was determined using a glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA, USA) at baseline and then every 15 min for 120 min. Changes in
BP and HR from baseline were assessed between 0-60 min, given that the maximum fall in BP and the rise in HR following a meal, or in response to duodenal glucose infusion occurs during the first hour (Jansen and Lipsitz 1995, O'Donovan, Feinle et al. 2002). Areas above, or under these curves (AAC or AUC) between 0-60 min were calculated for SBP, DBP and HR and analysed using paired Student’s t tests. Within-treatment variation was analysed with one-way ANOVA. Differences in BP and HR at 30 min are presented, but not analysed statistically. Results are presented as mean±SEM and P<0.05 was considered significant.

7.4 RESULTS

All subjects tolerated the study well.

There was no difference in baseline SBP (127±6mmHg for 4ID and 128±5mmHg for 2ID). SBP fell at 30 min with 4ID (P<0.05), but not 2ID and the AAC\(_{0-60}\) was greater with 4ID (-347±134 versus -1.5±118, P<0.05) (Fig 1A). The mean difference in SBP between 2ID and 4ID at 30 min was 8.2±3.8mmHg. No subject experienced a fall in systolic BP >20mmHg.

There was no difference in baseline DBP (73±3mmHg for 4ID and 74±2mmHg for 2ID). DBP fell at 30 min with 4ID (P<0.01), but not 2ID, although there was no statistical difference in the AAC\(_{0-60}\) between the two treatments (-225±79 for 4ID and -64±98 for 2ID) (Fig 1B). The mean difference in DBP between 2ID and 4ID at 30 min was 2.7±1.7 mm Hg.

There was no difference in baseline HR (60±3 bpm for 4ID and 61±4 bpm for 2ID). There was a rise in HR at 30 min in response to 4ID (P<0.01) but not 2ID and the AUC\(_{0-60}\) was greater at 30 min with 4ID (336±72 versus 180±89, P<0.05) (Fig 1C). The mean difference in HR between 2ID and 4ID at 30 min was 2.8±1.4 bpm.
As reported (Marathe, Rayner et al. 2014) in Chapter 4, there was no difference in baseline blood glucose (8.0±0.5 mmol/L for 4ID and 8.0±0.5 mmol/L for 2ID, P=0.72) and the rise in blood glucose was slightly greater in response to 4ID (P <0.05).

**Figure 1.** Systolic blood pressure (mm Hg) (A), diastolic blood pressure (mm Hg) (B) and heart rate (bpm) (C) responses during intraduodenal glucose infusions at 2 kcal/min (solid circles, solid lines) and 4 kcal/min (empty triangles, dashed lines) in 9 patients with type 2 diabetes.

### 7.5 DISCUSSION

This study establishes that in patients with uncomplicated type 2 diabetes, the fall in systolic blood pressure and rise in heart rate are greater in response to glucose infused intraduodenally at 4 kcal/min than 2 kcal/min. These observations are consistent with those in healthy ‘older’ subjects, in whom a fall in systolic blood pressure occurs in response to intraduodenal infusion at 3 kcal/min (∼8mmHg), i.e. comparable to that observed in the current study, but not 1 kcal/min, and in whom heart rate increases more in response to 4 kcal/min than 2 kcal/min (O'Donovan, Feinle et al. 2002, Trahair, Vanis et al. 2012). The observed difference in the fall in systolic BP between the two infusions was substantial and, accordingly, probably clinically relevant if sustained, which is likely to be the case (Jones, Tonkin et al. 1998).
A number of factors, including splanchnic blood pooling, autonomic / baroreceptor imbalance, and the secretion of gut hormones, including GLP-1, may account for the fall in blood pressure (Jansen and Lipsitz 1995). There is considerable interest in the cardiovascular effects of GLP-1 (Ussher and Drucker 2014). While trials of GLP-1 agonists in type 2 diabetes have shown a modest reduction in blood pressure (≈2-6mmHg) and an increase in heart rate (≈3 beats/min) (Klonoff, Buse et al. 2008, Davies, Kela et al. 2011, Stonehouse, Walsh et al. 2011, Fonseca, Devries et al. 2014), the primary outcome in these studies was glycaemic control, and there was no distinction between fasting and prandial BP measurements. Our studies, including a recent report (Trahair, Horowitz et al. 2015), suggest that slowing gastric emptying by dietary or pharmacological strategies, including short-acting GLP-1 agonists, may prove useful in the management of postprandial hypotension.

Limitations of our study should be recognized; we did not include a ‘healthy’ control group – however, the mean age was 62 years, which is not usually associated with a substantial fall in blood pressure in health (Trahair, Vanis et al. 2012). We deliberately selected those with well-controlled, uncomplicated, diabetes. It is likely that the fall in blood pressure would be greater in long-standing type 2 diabetes complicated by autonomic neuropathy.

In conclusion, the fall in systolic blood pressure is dependent on the rate of glucose entry into the duodenum in uncomplicated type 2 diabetes.
CHAPTER 8. CONCLUSIONS

These studies reported in this thesis provide important insights into the role of the gut (and specifically gastric emptying) in blood glucose homeostasis and development of type 2 diabetes.

The significant relationship of the initial rise in blood glucose (i.e. at 30 min) during an OGTT with gastric emptying is well known such that when gastric emptying is relatively more rapid, the initial rise in glucose is greater. However, during an OGTT, the 120 min blood glucose is used for diagnosis/classification into glucose tolerance categories and 60 min glucose is gaining increasing traction as a predictor of type 2 diabetes; and their relationships with gastric emptying are uncertain. I explored these relationships (Chapter 2) in participants with normal and impaired glucose tolerance and type 2 diabetes. Accordingly, participants underwent concurrent assessments of OGTT and gastric emptying (measured with ‘gold standard’ technique of scintigraphy). It was demonstrated that the relations of blood glucose with gastric emptying differ with time, so that while rate of gastric emptying is a major determinant of 30 min glucose, the latter relationships are very much dependent on insulin sensitivity, presumably prominently at the level of skeletal muscle. These observations underlie the importance of slowing gastric emptying to diminish the initial rise in blood glucose. Strategies to lower gastric emptying (such as pre-loads and short-acting GLP-1 agonists) are already available as well as in development and likely to be used more widely.

A defect in early insulin secretion is fundamental to the development of type 2 diabetes. In addition to the postprandial glucose response, gastric emptying has also been linked to the secretion of incretin hormones, GIP and GLP-1 with evidence that while GIP is determined by the rate of gastric emptying, GLP-1 determines it. The incretin hormones are of particular importance to normal glucose tolerance where they may contribute up to 70% of postprandial insulin release. I, therefore, explored the relationships of the early insulin secretory response
and its composite with fasting insulin, the disposition index (which has emerged as an useful predictor of type 2 diabetes) with gastric emptying (Chapter 3) and found that relatively more rapid gastric emptying was associated with a reduction in the insulin secretory response as well as the disposition index. It should be recognised, however, that gastric emptying was not >3 kcal/min in any of the participants in this study. Studies with intraduodenal glucose are indicative of a non-linear pattern of postprandial insulin response when the glucose is infused at variable rates (i.e. between 1-4 kcal/min). At rates <2 kcal/min, there is minimal insulin response, but a much more pronounced response at 3 or 4 kcal/min. Interestingly, while GIP increases proportionately with an increasing rate of intraduodenal glucose infusion, between 1-4 kcal/min, there is minimal, and transient, GLP-1 response at a rate <2 kcal/min, but pronounced and sustained at 3 and 4 kcal/min. In the study described in Chapter 4, I demonstrated that the magnitude of incretin effect (i.e. the augmented insulin secretory response with oral compared with intravenous glucose) is affected by the rate of duodenal glucose infusion, with the incretin effect being substantially greater at 4 compared with 2 kcal/min in both health and well-controlled type 2 diabetes. This observation suggests that the contribution of incretin hormones to the incretin effect is not always equal and is likely to be dependent on the rate of gastric emptying – i.e. GIP the dominant incretin at rates <2 kcal/min with GLP-1 contributing only at rates >3 kcal/min, however, this concept warrants further evaluation. Gastrointestinal glucose disposal (GIGD) quantifies the contribution of the gut to the disposal of glucose and was also greater at 4 kcal/min. An unexpected finding was the impaired suppression of glucagon by the 4 kcal/min, as compared to 2 kcal/min, infusion in both health and type 2 diabetes. There are a number of potential explanations including the differential actions of the incretins, as well as GLP-2, on glucagon release and the possibility that the gut is an extra-pancreatic source of glucagon. Further investigation is required to clarify the issues. The estimated insulin secretory response and the disposition index at 4 kcal/min was also greater at 4 kcal/min than 2 kcal/min in health and, based on the outcome
of the study reported in Chapter 5 – is likely to reflect pronounced GLP-1 response at 4 kcal/min.

It is increasingly evident that differences exist in the key pathogenetic abnormalities favouring development of type 2 diabetes among the major ethnicities. For example, in Caucasians, insulin resistance is clearly a major issue while in East Asians insulin secretory defect may dominate. However, there is little clarity relating to the potential role of incretins in the variable insulin secretory response although one study (which had major methodological inadequacies) suggested that the GLP-1 response may be attenuated in Japanese subjects. In Chapter 6, I report on a study that compared incretin secretory patterns between small cohorts of Caucasians and Han Chinese men in response to 4 kcal/min duodenal glucose load. It was found, despite similar glycaemic responses, that both the insulin secretory and GIP responses were diminished in Han Chinese men, although GLP-1 responses were comparable. These preliminary findings require further testing to determine clearly whether incretin secretory patterns are indeed different among major ethnic groups in response to oral glucose and if there are differences in the magnitude of incretin effect. This would necessitate concurrent measurement of gastric emptying; studies in an aboriginal population would have particular relevance in Australia given the propensity of this population to the development of type 2 diabetes.

In Chapter 7, I report on a study evaluating the blood pressure responses following duodenal glucose at 2 and 4 kcal/min in men with type 2 diabetes. A fall in blood pressure post meal is termed ‘postprandial hypotension’ (PPH) when the fall in systolic BP is greater than 20mmHg within 2 hours of the meal and is sustained for 30 min. PPH occurs frequently in elderly and those with autonomic dysfunction, including type 1 and 2 diabetes. PPH is a potentially serious, but neglected, complication of type 2 diabetes. The study showed that the fall in BP was substantially greater in response to 4 kcal/min compared with 2 kcal/min in patients with
type 2 diabetes who did not have evidence of autonomic neuropathy. While underlying mechanisms remain unclear, a number of factors, including splanchnic blood pooling, autonomic / baroreceptor imbalance, and the secretion of gut hormones, including GLP-1, may contribute. In view of the observations in this study, type 2 patients with autonomic neuropathy should be evaluated, as well as the response to oral, rather than duodenal, glucose. Based on the outcome of another study (Trahair, Horowitz et al. 2015), short-acting GLP-1 agonists, which slow gastric emptying, may play a role in the management of PPH in type 2 diabetes.

SIGNIFICANCE OF THIS PhD PROJECT

Type 2 diabetes is characterised by a reduced insulin secretory response to glucose challenge resulting in a lower oral disposition index and a reduced incretin effect. My experiments have shown that following an oral challenge, rate of gastric emptying is a key determinant of not only the initial glycaemic response in health and type 2 diabetes but also the early insulin secretory response as well as the oral disposition index in the healthy cohort. This suggests that modulating gastric emptying (through pharmacological or dietary mechanisms) may impact on progression of type 2 diabetes and warrants further research.

My studies have shown that the secretory pattern of the incretin hormones, and more importantly, the magnitude of the incretin effect, is dependent on the rate of gastric emptying in both health and type 2 diabetes when tested using a intraduodenal glucose infusion. Some ethnic communities are disproportionately affected by the type 2 diabetes epidemic and disparities in insulin secretion and sensitivity are gradually being recognised. My preliminary study suggests that at a given rate of gastric emptying, the secretion of incretin hormones may differ amongst ethnic groups. I believe the set of experiments that make up this PhD thesis will be of considerable scientific interest and contribute to the field of gastrointestinal endocrinology.
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