

**Gastric emptying and its relationship
with postprandial glycaemic control in
young people with cystic fibrosis and
type 1 diabetes.**

A thesis submitted by

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TABLE OF CONTENTS

THESIS AIMS	5
THESIS SUMMARY	6
DECLARATION.....	10
ACKNOWLEDGEMENTS	11
PUBLICATIONS ARISING FROM THE THESIS.....	14
CHAPTER 1. GASTRIC EMPTYING IN HEALTH AND THE DIAGNOSIS OF DISORDERED EMPTYING.....	15
1.1 Gastric emptying in health.....	15
1.2 Diagnosis of disordered gastric emptying	16
1.2.1 Scintigraphy.....	16
1.2.2 ¹³ C - breath test.	17
1.2.3 Ultrasonography	18
1.2.4 Dynamic MRI	18
1.2.5 Wireless motility capsule	18
1.2.6 Paracetamol absorption test.....	19
1.2.7 Barium Meal	19
1.3 Conclusion	20
2 CHAPTER 2: GASTRIC EMPTYING IN CYSTIC FIBROSIS	21
2.1 Summary.....	21
2.2 Introduction	22
2.3 CFRD Prevalence and Significance	22
2.4 CFRD Pathogenesis	24
2.5 CFRD Diagnosis	25
2.6 CFRD Current Management	27
2.7 Determinants of glycaemia in CF	30
2.7.1 Gastric emptying in health and CF	30
2.7.2 Gut hormone secretion	33
2.7.3 Insulin secretion and action	34
2.8 Management of CFRD and the role of gastric emptying and incretin hormones.....	35

2.8.1	Pancreatic Enzyme Supplementation	36
2.8.2	Potential Future Research	36
2.9	Conclusion	38
3	CHAPTER 3: GASTRIC EMPTYING IN DIABETES AND ITS RELATIONSHIP TO GASTROINTESTINAL SYMPTOMS AND AUTONOMIC FUNCTION.	47
3.1	Introduction	47
3.2	Gastric emptying in diabetes	48
3.3	Pathogenesis of abnormal gastric emptying	49
3.3.1	Autonomic (vagal) neuropathy	49
3.3.2	Cellular dysfunction	50
3.3.3	Impact of glycaemia.....	51
3.4	Natural history and prognosis.....	51
3.5	Gastrointestinal symptoms in type 1 diabetes and their relationship to gastric emptying.....	52
3.6	Management of disordered emptying.....	54
3.6.1	Dietary Modifications:	54
3.6.2	Pharmacological agents	55
3.6.3	Non-pharmacological therapies	58
4	CHAPTER 4: METHODS	59
4.1	Introduction	59
4.2	Subjects	59
4.3	Measurements	60
4.3.1	Gastric emptying.....	60
4.3.2	Autonomic nerve function	61
4.3.3	Gastrointestinal symptoms assessment	62
4.3.4	Biochemistry/ Hormones.....	63
4.4	Statistical analysis.....	64
4.5	Conclusion	64
5	CHAPTER 5: PANCREATIC ENZYME SUPPLEMENTATION IMPROVES THE INCRETIN HORMONE RESPONSE AND ATTENUATES POSTPRANDIAL GLYCAEMIA IN ADOLESCENTS WITH CYSTIC FIBROSIS: A RANDOMIZED CROSSOVER TRIAL.....	65
5.1	Summary.....	65

5.2	Introduction	66
5.3	Methods	68
5.3.1	Subjects.....	68
5.3.2	Protocol	68
5.3.3	Measurements.....	69
5.3.4	Statistical analysis	70
5.4	Results	71
5.5	Discussion.....	74
	Follow-Up	81
	Analysis	81
	Enrollment	81
	Crossover	81
1.1	AllAllocation	81
6	CHAPTER 6: RAPID GASTRIC EMPTYING IS A MAJOR DETERMINANT OF POSTPRANDIAL GLYCAEMIA IN ADOLESCENTS WITH TYPE 1 DIABETES.	87
6.1	Summary.....	87
6.2	Introduction	88
6.3	Methods	90
6.3.1	Subjects.....	90
6.3.2	Protocol	91
6.3.3	Measurements.....	91
6.3.4	Statistical analysis	93
6.4	Results	94
6.5	Discussion.....	95
	BIBLIOGRAPHY	111

THESIS AIMS

1. To quantify gastric emptying in adolescents with exocrine pancreatic insufficient cystic fibrosis and examine its relationship with postprandial glycaemia, incretin hormone response, and pancreatic enzyme replacement therapy.

2. To quantify gastric emptying in adolescents with type 1 diabetes and examine its relationship with postprandial glycaemia, gastrointestinal symptoms and autonomic function.

HYPOTHESES

In adolescents with exocrine pancreatic insufficient cystic fibrosis, both with and without cystic fibrosis related diabetes:

- i) Gastric emptying of a high fat/carbohydrate meal will be abnormally rapid.
- ii) Abnormal emptying will be associated with postprandial hyperglycaemia and deficient GIP, GLP-1 and insulin responses.
- iii) These abnormal responses will be ameliorated by pancreatic enzyme

In adolescents with type 1 diabetes

- (i) Gastric emptying is slower than in controls
- (ii) Cardiac autonomic dysfunction relates to gastric emptying
- (iii) Gastrointestinal symptoms are associated with abnormal gastric emptying.

THESIS SUMMARY

This thesis examines gastric emptying and its relationship to postprandial glycaemia in two groups of young people; those with cystic fibrosis (CF) and those with type 1 diabetes (T1D). In particular, the relationship of gastric emptying to postprandial glycaemia, and incretin hormone responses, and the effect of pancreatic enzyme supplementation therapy (PERT) is investigated in CF, while in T1D the relationship of gastric emptying to postprandial glycaemia, gastrointestinal symptoms and autonomic function is investigated.

Optimal glycaemic control, as measured by glycated haemoglobin (HbA1c), is critical in preventing long term micro- and macro-vascular complications of diabetes. Gastric emptying is a major determinant of overall glycaemic control, accounting for at least 30% of the variation in postprandial blood glucose concentrations in adults with diabetes (Monnier et al., 2003, Horowitz et al., 1993). Both the magnitude of postprandial glycaemic excursions and peak postprandial blood glucose relate to the rate of gastric emptying in health and in type 2 diabetes (Horowitz et al., 1993, Jones et al., 1995), so that rapid emptying and delivery of nutrients to the small intestine results in a sharp, early rise in blood glucose.

Gastric emptying is the result of co-ordinated gastric motor activity of the proximal and distal stomach, and is influenced by multiple variables including the neurohumoral axis, in particular the incretin hormones, and the autonomic nervous system. The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintrophic polypeptide (GIP), play a critical role in postprandial

glycaemic control, with up to 70% of the total insulin response to oral glucose attributed to their actions in health (Baggio and Drucker, 2007). They are secreted from intestinal enteroendocrine cells in response to nutrient exposure; GLP-1 has both glucose-dependent insulinotropic and glucagonostatic properties, and also plays an important role in the slowing of gastric emptying. GIP predominantly improves postprandial glycaemia through its insulinotropic effects, as it does not appear to influence the rate of gastric emptying, and may stimulate glucagon secretion (Nauck et al., 1997).

While diabetes management has focused on fasting or pre-prandial glycaemia, the contribution of postprandial glycaemia to HbA1c, particularly when the latter is only moderately elevated (HbA1c <7.5%) is being increasingly recognised (Monnier et al., 2003, Horowitz et al., 1993). Consequently, there is now growing interest in dietary and pharmaceutical therapies that modify the rate of gastric emptying for optimising postprandial glycaemic control in diabetes.

The mean life expectancy for patients with CF is improving, and with this has come the increasing clinical challenge of managing the associated long term co-morbidities, in particular CF related diabetes (CFRD)(Lanng et al., 1994). CFRD is associated with worsening nutritional state and pulmonary function, and increased mortality. Abnormalities of carbohydrate metabolism in CF represent a continuum from normal, through pre-diabetes, to overt diabetes, with CFRD characterised by postprandial, rather than fasting or pre-prandial, hyperglycaemia. Insulin is currently recommended to treat CFRD; however, it is a demanding treatment for patients who already require other complex and time consuming regimens, and is associated with

a risk of hypoglycaemia. Exocrine pancreatic insufficiency with associated fat malabsorption affects approximately 80% of CF patients, and fat digestion often remains abnormal despite PERT (Baker et al., 2005, Symonds et al., 2003). Lipolytic products in the small intestine induce both release of the incretin hormones, and other neurohumoral feedback that slows gastric emptying (Borovicka et al., 2000, Hedde et al., 1989). Fat malabsorption in adults is, therefore, associated with abnormally rapid gastric emptying of high fat meals and accelerated absorption of carbohydrates, resulting in postprandial hyperglycaemia (Carney et al., 1995, Kuo et al., 2010). Few studies have assessed gastric emptying and/or incretin responses in CF patients, with inconsistent observations (Pauwels et al., 2011, Cucchiara et al., 1996, Kuo et al., 2011, Collins et al., 1997). An initial pilot study in 5 adults indicated that PERT slows gastric emptying of a high fat/high carbohydrate meal, with enhanced incretin hormone secretion, and a reduction in postprandial glycaemia (Kuo et al., 2011).

The study reported in Chapter 5 aimed to assess whether PERT slowed gastric emptying, increased incretin secretion and improved postprandial glycaemia in adolescents with CF. This study showed that in adolescents with pancreatic insufficient CF, PERT markedly attenuates postprandial hyperglycaemia by slowing gastric emptying and augmenting incretin hormone secretion. It illustrates the importance of adequate and timely PERT, not just for fat absorption, but also in the management of postprandial hyperglycaemia, which has not been a focus of clinical management.

The prevalence of T1D in children under 15 years has doubled over the last 20 years in Australia , with 2 young people diagnosed each day (Guariguata et al., 2013). Evidence for altered gastric emptying in adults with diabetes is abundant, with delayed gastric emptying more commonly reported, often in association with autonomic dysfunction (Rayner et al., 2001, Jones et al., 2002). Gastrointestinal symptoms can suggest the presence of abnormal gastric emptying, however the correlation between the two is relatively poor (Samsom et al., 2003, Bharucha et al., 2009). Abnormal autonomic function is implicated in the pathogenesis of delayed gastric emptying in patients with long standing diabetes. Abnormalities of heart rate variability (HRV), a measure of cardiovascular autonomic function, can be detected in adolescents with T1D before the appearance of symptoms (Pfeifer et al., 1984, Wawryk et al., 1997). Gastric emptying and its relationship to postprandial glycaemia, gastrointestinal symptoms, and autonomic function, has not been assessed in young people with T1D.

The study in Chapter 6 aimed to assess gastric emptying in adolescents with T1D, and its relationship with postprandial glycaemia, gastrointestinal symptoms and autonomic function. This study showed that adolescents with T1D have rapid gastric emptying compared with healthy controls, and that this is associated with large postprandial glycaemic excursions. Fasting hyperglycaemia is, however, associated with slower gastric emptying and gastrointestinal symptoms in this group. This raises the question of the potential benefit of therapies that modify gastric emptying in the management of postprandial glycaemia in T1D.

DECLARATION

Name: Shiree Perano

Program: Master of Philosophy

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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PUBLICATIONS ARISING FROM THE THESIS

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Perano S J, Couper J J, Horowitz M, Martin, A J, Kritas S, Sullivan T, Rayner C K. Pancreatic enzyme supplementation improves the incretin hormone response and attenuates postprandial glycaemia in adolescents with cystic fibrosis: a randomised crossover trial. *JCEM* 2014; 99:2486-93 (THESIS CHAPTER 5)

Perano S, Rayner C, Horowitz M, Kritas S, Donaghue K, Mpundu-Kaambwa C, Giles L, Couper J. Gastric emptying is rapid in adolescents with type 1 diabetes and impacts on postprandial glycaemia. *J Clin Endocrinol Metab* 2015;100 (6):2248-53 (THESIS CHAPTER 6)

CHAPTER 1. GASTRIC EMPTYING IN HEALTH AND THE DIAGNOSIS OF DISORDERED EMPTYING.

1.1 Gastric emptying in health

In health, gastric emptying involves complex motor co-ordination of the proximal and distal stomach, the pylorus and upper small intestine. In the fasting state gastric motility is characterised by the 'migrating motor complex', which consists of 3 phases of gastric motility: phase I- motor quiescence (40minutes), phase II- irregular contractions (50minutes), and phase III- regular contractions at 3 per minute (5-10minutes) (Horowitz and Akkermans, 2004). The postprandial pattern commences after meal ingestion and is characterised by irregular antral contractions, an increase in tonic and phasic pyloric pressures, and proximal stomach relaxation (Horowitz and Dent, 1991). The antrum grinds food into small particles (<2mm) and pumps chyme into the duodenum against pyloric resistance. Interstitial cells of Cajal (ICC) are specialised pace-maker cells that control the maximum contraction frequency of the antrum and pylorus, by generating "slow waves" at approximately 3 waves per minute. Nutrient emptying from the stomach occurs at a rate of 1-4 kcal/min and is primarily controlled by the neurohumoral axis (including GLP-1, cholecystokinin (CKK) and peptide YY (PYY)) in response to nutrient absorption (Horowitz and Akkermans, 2004, Fried et al., 1991, Deane et al., 2010). Solids and liquids have different emptying profiles, with solids having an initial lag phase followed by linear emptying. The lag phase represents the time taken for the meal to move to the distal stomach and subsequently be ground into small particles. Liquids are emptied preferentially to solids in mixed meals, and have

minimal lag phase and an exponentially increasing rate of emptying, which becomes more linear as the calorie content of the liquid increases (Camilleri, 2006).

1.2 Diagnosis of disordered gastric emptying

In clinical practice, investigations to exclude delayed gastric emptying are warranted in symptomatic patients and in cases of unexplained postprandial hypoglycaemia, however the correlation between upper gastrointestinal symptoms and objective evidence of abnormal gastric emptying is weak. Gastroparesis may be diagnosed based on a combination of upper gastrointestinal symptoms and evidence of delayed gastric emptying, in the absence of mechanical obstruction, although markedly delayed gastric emptying can be asymptomatic (Kassander, 1958). When assessing the rate of gastric emptying it is important to control for reversible variables that alter emptying, such as medication, hyperglycaemia, and smoking. Methods for assessing gastric emptying are summarised below.

1.2.1 Scintigraphy

Scintigraphy represents a quantitative, reproducible, non-invasive test, which uses radiolabelled solid and/or liquid nutrient meals to assess gastric emptying. Scintigraphy is considered the gold standard for diagnosis of abnormal gastric emptying. There is however, still some debate around how best to standardise the technique. The Neurogastroenterology and Motility Society's consensus statement published in 2008 recommends a low fat egg white meal labelled with ^{99m}Tc -sulfur colloid, with gastric emptying measured over 4 hours (Camilleri et al., 2008). There is no evidence to demonstrate the superiority of a solid meal, when compared with a nutrient liquid, in assessing gastric emptying, although in some cases the emptying of one but not the other is abnormal. Gastric emptying is therefore ideally assessed

by a dual radiolabelled meal that allows the assessment of emptying of both solid and liquid components (Horowitz et al., 1986) . Scintigraphy can also provide information on retention in both the proximal and distal regions of the stomach. Its disadvantages include exposure to radiation, which is particularly relevant in the paediatric population, the high cost, and the fact that its use is restricted to specialised centres.

1.2.2 ^{13}C - breath test.

This test utilises ^{13}C - octanoate or ^{13}C - actate added to a test meal as a non-invasive measure of gastric emptying. The ^{13}C label is absorbed when the meal reaches the small intestine. It is then metabolised to $^{13}\text{CO}_2$ by the liver and excreted by the lungs during expiration. The concentration of $^{13}\text{CO}_2$ in breath samples provides an indirect measure of the rate of gastric emptying, with the assumption that gastric emptying is the rate limiting step. The ^{13}C - breath test correlates well with scintigraphy with a sensitivity of 75% and specificity of 86% (Ziegler et al., 1996), and has been validated in healthy adults (Choi et al., 1997) and adolescents (Hauser et al., 2006), as well as adults with diabetes (Ziegler et al., 1996). It involves no radiation exposure, making it an attractive option for paediatric assessment. The ^{13}C - breath test is a low cost technique and is accessible in many centres, as samples can be collected, transported, and analysed in a centralised laboratory at a later time. There is low intra-individual variation however abnormal hepatic or pulmonary function may affect the results. A correction factor has been developed for cystic fibrosis patients to account for the influence of abnormal pulmonary function (Amarri et al., 1998).

1.2.3 Ultrasonography

Ultrasound has the ability to assess gastric emptying, transpyloric flow of liquid gastric contents, and accommodation of the proximal stomach. It is comparable to scintigraphy in quantifying emptying of low and high nutrient liquids. Two-dimensional ultrasonography measures gastric emptying indirectly by changes in antral area over time (Hveem et al., 1996). The recent development of 3-dimensional imaging has allowed a more comprehensive assessment, including intragastric meal distribution, and has been validated in healthy subjects and in gastroparesis patients (Gentilcore et al., 2006b, Gilja et al., 1999). Advantages of ultrasonography are similar to those of the breath test, ie. low cost and lack of radiation exposure, however it does require specialist equipment and training, and accuracy is operator-dependent. Furthermore assessment is limited to liquid meals.

1.2.4 Dynamic MRI

Dynamic MRI is a non-invasive test that quantifies both gastric motility and emptying. It has the ability to differentiate phases of solid and liquid emptying, gastric secretions and intra-gastric air. Emptying measured by MRI correlates strongly with scintigraphy (Parkman et al., 2010); however the drawbacks are the high cost and time consuming interpretation of results.

1.2.5 Wireless motility capsule

The wireless motility capsule is a non-digestible capsule that measures luminal pH, temperature, and pressure during gastrointestinal transit. Gastric emptying time is inferred from an abrupt rise in pH when the capsule is emptied into the small intestine; this would typically occur after the emptying of digestible meal components. Results in healthy subjects and in gastroparesis correlate well with scintigraphy, with a sensitivity of 87% and specificity of 92% in discriminating

between normal and delayed gastric emptying (Rao et al., 2009). Green et al compared the wireless motility capsule test with 2-hour scintigraphy in 21 young people aged 8-17 year who had severe upper gastrointestinal symptoms. The capsule diagnosed gastroparesis with 100% sensitivity and 50% specificity when compared to scintigraphy (Green et al., 2013). Assessment of gastric emptying over 2 hours, not the standard 4 hours, may explain the low specificity. More cases of gastroparesis may have been detected with a 4 hour study. Impaired bicarbonate secretion, as seen in those with pancreatic insufficiency, would only be relevant in the duodenum, after the capsule has emptied from the stomach. A limitation of the capsule is that it a large indigestible solid and tends to empty with phase 3 of the migrating motor complex. It does not therefore, perfectly reflect gastric emptying of nutrient liquids or digestible solids however it does correlate moderately well.

This is a promising diagnostic technique; however it is expensive and further data, particularly in the paediatric population, are required.

1.2.6 Paracetamol absorption test

This is a simple, relatively non-invasive bedside test to assess the gastric emptying of liquids. It should be considered as a screening tool, at best, due to its variable accuracy (Willems et al., 2001). The principle of this test is similar to the ^{13}C - breath test, with paracetamol added to a meal. Paracetamol empties into the small intestine with the liquid component of the test meal, where it is absorbed and rapidly enters into the blood stream. Serial serum paracetamol concentrations therefore provide an indirect measure of the rate of gastric emptying.

1.2.7 Barium Meal

This test uses a non-nutrient contrast load, barium, to exclude mucosal lesions or mechanical obstruction. It has limited capacity to assess gastric emptying,

since it does not involve the capacity of the stomach to empty nutrients, and the retention of barium is difficult to quantify.

1.3 Conclusion

In health, gastric emptying is a complex and tightly regulated process. While there have been advances in diagnostic techniques, assessment of gastric emptying remains challenging. This is particularly so in a paediatric population, where the gold standard, scintigraphy, is not acceptable due to the radiation exposure that it entails. For the studies reported in this thesis, a ^{13}C breath test was used to assess gastric emptying, as it was deemed the most accurate, available, and acceptable test in this population.

2 CHAPTER 2: GASTRIC EMPTYING IN CYSTIC FIBROSIS

2.1 Summary

As the average life expectancy of patients with cystic fibrosis (CF) improves, the long term co-morbidities assume increasing importance. CF related diabetes (CFRD) has adverse effects on both nutrition and pulmonary function, and is associated with increased mortality. Abnormalities of glucose metabolism in CF represent a continuum; however the predominant abnormality is postprandial, not pre-prandial, glycaemia. Insulin is currently recommended as the treatment of choice for CFRD, but its use is associated with a number of limitations, including hypoglycaemia. Both the rate of gastric emptying and the consequent release of the 'incretin' hormones, glucose-dependent insulintropic polypeptide (GIP) and glucagon-like-peptide-1 (GLP-1), from the gut are important determinants of overall glycaemic control, particularly postprandial glycaemia. Both are abnormal in conditions associated with exocrine pancreatic insufficiency. Incretin based therapies that have the capacity to slow gastric emptying and/or modulate the release of 'incretin' hormones, are now used widely in type 2 diabetes (T2D). This paper explores the determinants of glycaemic control in CF, with a particular focus on the roles of gastric emptying and 'incretin' hormones, providing a rationale for the use of therapies that delay gastric emptying, including incretin mimetics, to minimise postprandial glycaemia and improve nutritional status.

2.2 Introduction

The establishment of specialised CF centres, and substantial nutritional and pharmaceutical advances during the last 60 years, have improved mean life expectancy for CF by more than 38 years, with the consequent clinical challenge of prevention and management of long-term co-morbidities, of which CFRD has increasing prominence (Bethesda). The abnormalities of glucose metabolism in CF represent a continuum from normal, through pre-diabetes, to overt diabetes with the pathogenesis characterised by postprandial, rather than pre-prandial, hyperglycaemia. Insulin is currently the treatment of choice for CFRD although the acceptance and compliance with this therapy is challenging due to the already high burden of care in the CF population. Newer therapies are available that specifically modify postprandial glycaemia, such as those based on the incretin system, which are widely utilised in the management of T2D. These therapies may represent a logical treatment for CFRD, as monotherapy or in combination with basal insulin, as they specifically address postprandial hyperglycaemia.

2.3 CFRD Prevalence and Significance

The prevalence of CFRD increases with age, such that more than 50% of patients over 40 years are affected (Moran et al., 2009a). The mortality rate for CFRD has been estimated at 3.5 per 100 person years, from a peak 20 years ago of 6.2 per 100 person years, but remains substantially higher than in CF patients without diabetes (Moran et al., 2009a). This improvement is largely due to increased awareness and detection in the pre-diabetic state, which is imperative in light of persuasive evidence that the optimal management and long-term prognosis of CF, is affected greatly by the presence of carbohydrate intolerance. The long term

implications of CFRD for pulmonary health and body mass index (BMI) are considered of greater relevance than the microvascular and macrovascular complications classically associated with type 1 diabetes (T1D) and T2D, although with increased survival microvascular complications have now become apparent, as will be discussed. CFRD with fasting hyperglycaemia is associated with a decline in pulmonary function and nutrition, an increase in the incidence of *Pseudomonas aeruginosa* and *Burkholderia cepacia* infection, doubling of hospitalisation rates, and an increase in the prevalence of liver disease (Marshall et al., 2005). CFRD also adversely affects prognosis after lung transplantation (Belle-van Meerkerk et al., 2012) .

Whether these associations are a direct effect of CFRD, or reflect more severe disease is uncertain; however the degree of clinical decline, particularly in respiratory function, correlates directly with glycaemic control. Moreover, there is now persuasive evidence that the decline in nutritional and pulmonary status occurs 2- 6 years before the diagnosis of CFRD, when postprandial hyperglycaemia is less marked (Bizzarri et al., 2006, Moran et al., 2009b). This suggests that even 'early' carbohydrate intolerance, with relatively modest postprandial glycaemic excursions, is metabolically detrimental to pulmonary function. Upper airway glucose concentrations are higher in CF when compared with healthy subjects, and those with T1D or T2D, with the potential to facilitate bacterial growth and subsequent lung damage (Brennan et al., 2007). Direct toxic effects of hyperglycaemia on airway function remain to be established.

Microvascular complications occur in CFRD, albeit with a lower prevalence than in T1D or T2D, and are related both to the duration of diabetes and glycaemic control. Ten years from the diagnosis of CFRD, 50% of patients are reported to have

peripheral neuropathy, 16% retinopathy and 14% microalbuminuria (Schwarzenberg et al., 2007). Macrovascular complications in CFRD have not featured in the literature, possibly reflecting both the shorter life expectancy and beneficial effect conferred by the genetic mutation and fat malabsorption.

2.4 CFRD Pathogenesis

The pathogenesis of CFRD is multifactorial, with both the CF genotype and innate and adaptive immunity contributing to β cell destruction (Rana et al., 2010, Rana et al., 2011). CFRD is characterised by predominantly postprandial, rather than pre-prandial, hyperglycaemia. This contrasts with T1D, and the proportional elevation of postprandial glucose in CF, relative to fasting glucose, is greater than in T2D. The primary defect has been regarded as insulin deficiency, with a variable contribution from insulin resistance, dependent on clinical state, infection, inflammation and concurrent steroid medication. There is, however, a poor correlation between clinical diabetes and the degree of islet cell damage, suggesting that other factors, such as autoimmunity, may be involved. However, the prevalence of islet antibodies and T1D susceptibility alleles in CFRD appear to be comparable to the general population, although CFRD and T1D co-exist in a minority (Lanng et al., 1993a). It has been suggested that CFRD may be more closely related to T2D, with islet amyloid deposits, as seen in T2D, being evident in 69% at autopsy (Couce et al., 1996). It is not clear whether amyloid deposits play a direct role in the pathogenesis of β cell death or are simply a marker of increasing β cell stress (Huang et al., 2007a, Huang et al., 2007b). Susceptibility genes that increase the risk of T2D in the general population have been found in CFRD, strongly suggesting

these genes may increase the propensity to diabetes in CF, and a family history of T2D increases the risk of CFRD substantially (Lanng et al., 1993a).

The cystic fibrosis transmembrane conductance regulator (CFTR) protein may play a direct role in insulin secretion. Ivacaftor, a CFTR potentiator, is a new therapy which improves chloride transport through the dysfunctional CFTR protein in individuals with the G551D mutation. The implications for glycaemic control are uncertain; however in a pilot study of 5 CF patients Ivacaftor improved insulin secretion, albeit without affecting glycaemic control (Bellin et al., 2013).

2.5 CFRD Diagnosis

Carbohydrate intolerance in CF represents a continuum on which patients fluctuate, depending on clinical variables including infection, energy requirement, nutritional state, gastrointestinal absorption and steroid therapy. The diagnosis of CFRD is challenging, not just because of this intra-individual variability, but also because of the lack of a 'gold standard' diagnostic test. In practice, carbohydrate intolerance in CF is commonly classified into categories of normal glucose tolerance, indeterminate glucose tolerance, impaired glucose tolerance, CFRD without fasting hyperglycaemia, and CFRD with fasting hyperglycaemia (Table 1) based on an oral glucose tolerance test (OGTT, 1.75g/kg body weight, maximum 75g) using fasting and 120 minute glucose levels (Moran et al., 2009a).

Previously, the OGTT was regarded as the diagnostic gold standard with high sensitivity (Moran et al., 1999); however more recent evidence indicates that many patients experience large glycaemic excursions at 30, 60 and 90 minutes after oral glucose, which may be clinically significant, but have normal blood glucose levels at baseline and 2 hours (Hameed et al., 2010). While there is a relationship between

the 2 hour OGTT blood glucose value and glycaemic excursions following a mixed meal in healthy subjects, and those with impaired glucose tolerance or overt diabetes (Meier et al., 2009), the absolute blood glucose concentrations vary substantially between the two tests. The OGTT tends to elicit greater glycaemic excursions, while a mixed meal more accurately represents the glycaemic variations occurring in everyday life. In CF, this is particularly important, since exocrine pancreatic insufficiency may influence the response to a mixed meal, but not to oral glucose. Moreover, unless blood glucose is measured more frequently than at 2 hours (eg. every 30 min), the OGTT may miss an early glycaemic peak. The OGTT also has poor specificity, with up to 58% of patients with impaired glucose tolerance being shown to revert to normal glucose tolerance and only 14% progressing to CFRD over the following 5 years (Lanng et al., 1995). Measurement of insulin and C-peptide responses to an OGTT may aid in detecting abnormalities in carbohydrate metabolism and progression. A delayed and reduced insulin peak and first phase insulin response to oral and intravenous glucose tolerance tests are evident in CF subjects with impaired, compared with those with normal glucose tolerance (Tofe et al., 2005). This information may facilitate identification of those at high risk of progressing to CFRD however more studies are required. HbA1c cannot be used to screen for CFRD, as levels are often falsely normal in CF and do not reliably correlate with glycaemic control.

Current diagnostic guidelines for CFRD include one of the following criteria;

- 2 hour OGTT blood glucose >11.1mmol/L
- Fasting blood glucose >7mmol/L
- HbA1c \geq 6.5%

- Classic symptoms of diabetes (ie. polyuria, polydipsia) with a random blood glucose $>11.1\text{mmol/L}$

During periods of acute illness a diagnosis of CFRD can be made if the fasting blood glucose is $>7\text{mmol/L}$. or 2 hour postprandial blood glucose is $>11.1\text{mmol/L}$ for greater than 48hrs (Moran et al. 2010).

2.6 CFRD Current Management

The 2009 International Society for Pediatric and Adolescent Diabetes (ISPAD) clinical practice guidelines for the management of CFRD recommend a combination of basal (long-acting) and bolus (rapid-acting) insulin (O'Riordan et al., 2009). Pump insulin therapy offers the greatest flexibility and a reduction in the number of injections required (Sulli and Shashaj, 2003); however insulin regimens need to take into consideration the patients' individual needs and current treatment burden. Basal insulin doses start around 0.25u/kg/24h and are titrated to fasting blood glucose levels with suggested pre-meal insulin doses of $0.5\text{-}1\text{u}$ per 15g of carbohydrate and 'correction' doses added as required (O'Riordan et al., 2009).

While a basal-bolus insulin regimen is recommended, a 2013 Cochrane review was unable to identify sufficient evidence to inform on the optimal management of hyperglycaemia in CF (Onady and Stolfi, 2013). The relative benefits of postprandial, versus pre-prandial, glycaemic control have not been evaluated in CF; however in T2D it is now recognised that postprandial glycaemic excursions are a major determinant of 'overall' glycaemic control, particularly in the lower range of HbA1c.

The best management of CFRD without fasting hyperglycaemia and impaired or indeterminate glucose tolerance also remains unclear, and determining the

optimal therapy for pre-diabetes is regarded as an urgent priority by the CF Foundation, American Diabetes Association and Pediatric Endocrine Society (Moran et al., 2010). The outcomes of several studies indicate insulin therapy can ameliorate the decline in pulmonary function and BMI seen in the early stages of impaired glucose tolerance (Bizzarri et al., 2006, Moran et al., 2009b). In adults, an 8% increase in FEV1 and 42% decline in pulmonary exacerbations were evident during 12 months of basal insulin (Glargine) therapy (Moran et al., 2009b), with reversal of chronic weight loss (Bizzarri et al., 2006). These effects are attributable to insulin's anabolic role, increasing protein synthesis and BMI with enhanced respiratory muscle strength, rather than a direct effect of improved glycaemic control.

It is well recognised that it can be difficult to achieve satisfactory glycaemic control in CF subjects using insulin therapy. Not infrequently, the therapeutic emphasis has been on pre- rather than postprandial glycaemic control. In determining the optimal insulin regimen and maximising compliance, current therapies and social supports need to be taken into consideration. Because insulin therapy is invasive, requires regular injections, and is associated with a high risk of hypoglycaemia, acceptability of this therapy in the early stages of carbohydrate intolerance, or indeed even in overt CFRD, is likely to be limited. There are few established alternative therapeutic options, given traditional oral hypoglycaemic agents are not currently recommended in CF due to limited data and the risk of adverse effects (Onady and Stolfi, 2013). Metformin is associated with gastrointestinal side effects, has a theoretical risk of lactic acidosis, and is better suited to obese patients with insulin resistance. Sulphonylureas increase insulin secretion, but bind to the CF transmembrane conductance regulator, raising

concerns they may interfere with other CF therapies. Thiazolidinediones primarily target peripheral insulin resistance, which is not the dominant feature in CFRD.

Glycaemic control in this population is further challenged by states of increased insulin resistance such as acute pulmonary infection and pregnancy, and following lung transplantation. Medications, such as corticosteroids, given during an acute pulmonary exacerbation, combined with increased insulin resistance can result in a temporary requirement for exogenous insulin. In those with CFRD acute infection may increase insulin requirements 4 fold (O'Riordan et al., 2009). With resolution of the infection requirements typically return to previous levels.

During pregnancy, insulin resistance increases, putting women with CF at greater risk of developing gestational diabetes when compared to healthy females. Monitoring of blood glucose at routine appointments, an OGTT on confirmation of pregnancy (if one has not occurred the prior 6 months), and at the end of the 1st and 2nd trimester, is recommended (Moran et al., 2010).

Lung transplantation does not appear to increase the risk of diabetes in CF patients compared with non-CF transplant patients (Belle-van Meerkerk et al., 2012); however glycaemic control deteriorates in the majority of CFRD patients following transplantation (Valour et al., 2013).

Hypoglycaemia can occur in CFRD in the context of insulin therapy, although normal hypoglycaemic awareness is generally maintained. Education is paramount in reducing the risk. The non-diabetic CF population may have a greater tendency to fasting hypoglycaemia than the healthy population (Battezzati et al., 2011).

2.7 Determinants of glycaemia in CF

Key determinants of postprandial glycaemic control in CF include gastric emptying, gut hormone secretion (particularly the incretins, GIP and GLP-1, but also cholecystokinin (CCK) and peptide tyrosine tyrosine (PYY)), the insulin and glucagon response, pancreatic enzyme supplementation and the high fat/ high energy diet recommended for these patients. Gastric emptying and incretin hormone secretion are central to postprandial glycaemic control (Marathe et al., 2013); but have received little attention in CF.

2.7.1 Gastric emptying in health and CF

In health, individual gastric emptying rates vary substantially between 1-4 kcal/min (Trahair et al., 2012), with regulation predominantly arising through inhibitory feedback triggered by carbohydrate, protein and fat digestion in the small intestine. The presence of lipolytic products in the intestinal lumen contributes substantially to the neurohumoral feedback mechanisms that slow gastric emptying. The use of the lipase inhibitor, orlistat, in T2D is associated with accelerated gastric emptying and accentuation of postprandial hyperglycaemia (O'Donovan et al., 2004). The rate of gastric emptying is in turn a fundamental determinant of the release of gut peptides and the rate of carbohydrate absorption, both of which are central to postprandial glycaemic control. Gastric emptying accounts for around one third of the variation in peak glucose response after an oral glucose load in health or T2D (Horowitz et al., 1993). Relatively more rapid gastric emptying (3-4kcal/min) is associated with a greater initial rise in blood glucose, while slower emptying (<1.5kcal/min) provides a more controlled rise (Ma et al., 2012).

Multiple variables may influence the rate of gastric emptying, including meal composition and volume, posture, illness, glycaemia and medications. Variables of

particular relevance to CF are pancreatic enzyme supplementation, glycaemia and the high fat/ high energy diet prescribed for these patients. Exocrine pancreatic insufficiency affects around 80% of CF patients and requires pancreatic enzyme supplementation; however, the latter fails to normalise fat absorption in around 20% (Symonds et al., 2003). The relationship between gastric emptying of nutrients and supplemental enzymes is complex, and co-ordination of both to optimise nutrient absorption is therapeutically challenging, particularly when gastric emptying is disordered (either abnormally fast or slow) (Meyer and Lake, 1997, Symonds et al., 2003). It is recognised that there is substantial inter-individual variation in gastric emptying of food and enzymes respectively, with enzymes emptying on average 60 minutes before food when given together (Taylor et al., 1999). Meal composition and the size of spheres in enzyme formulations are important. Mixing of enzymes with the liquid component of a meal can result in rapid non-parallel emptying, given that liquids empty more rapidly than solids. There is inconsistent information in relation to the impact of the size of the enzyme sphere on the rate of emptying; however, smaller spheres ($\leq 1\text{mm}$) generally empty more rapidly, and emptying of spheres $\geq 1.6\text{mm}$ may be delayed up to 3 hours after a meal (Meyer and Lake, 1997). Pancreatic enzyme efficacy is further impaired in CF by a lower intestinal pH, due to impaired HCO_3 secretion, resulting in delayed dissolution of the enteric coating on enzyme formulations (Guarner et al., 1993).

Gastric emptying is itself influenced by acute changes in blood glucose concentrations, with hyperglycaemia delaying gastric emptying, which in turn slows the absorption of ingested carbohydrate and reduces the propensity for further hyperglycaemia (Barnett and Owyang, 1988). Conversely, in health and T1D, insulin-induced hypoglycaemia accelerates emptying, increasing the delivery of nutrients to

the small intestine, and again providing an adaptive response (Schvarcz et al., 1995).

The high fat diet prescribed in CF would be expected to favour slow gastric emptying, since fat is a potent inhibitor of emptying. However, ingestion of a diet high in fat in healthy subjects is associated with relatively more rapid emptying of fat, presumably reflecting a reduction in small intestinal inhibitory feedback (Cunningham et al., 1991). In patients with CF, the majority of whom have pancreatic exocrine insufficiency, the emptying of high fat meals is further complicated by fat maldigestion, an issue which is discussed in detail subsequently. Protein, such as whey, has been shown to slow gastric emptying through enhanced gut hormone secretion (Ma et al., 2009). Digestion of protein is also impaired in pancreatic insufficient CF, although to a lesser degree than fat, which may contribute to rapid gastric emptying in CF.

Despite the potential importance of abnormal gastric emptying in CF patients, only a limited number of studies have evaluated gastric emptying in this population (Kuo et al., 2011, Collins et al., 1997, Symonds et al., 2003, Roulet et al., 1980, Pauwels et al., 2011, Perano et al., 2014, March 26.), with inconsistent results, attributable to differences in subject characteristics, meal composition, use of pancreatic enzymes and the method used to measure gastric emptying. Studies have reported abnormally rapid (Kuo et al., 2011, Collins et al., 1997) or delayed (Pauwels et al., 2011) emptying, or no difference from controls (Symonds et al., 2003, Roulet et al., 1980, Perano et al., 2014, March 26.). A bimodal pattern of gastric emptying has also been proposed, with more rapid emptying 'early' in the disease reflecting gut adaptations to the high energy diet, and delayed emptying 'later', when malnutrition is more likely to be present (Collins et al., 1997). Rapid

gastric emptying has been shown in exocrine pancreatic insufficiency from other causes, such as chronic alcoholism (Long and Weiss, 1974).

There is no consistent evidence of abnormal gastric musculature or innervation altering gastric emptying in CF; therefore it may be reasonable to conclude that gastric emptying in CF with exocrine pancreatic insufficiency, in the absence of enzyme replacement, will be more rapid due to fat malabsorption, since fat is crucial in regulating gastric emptying. In a small study of adults with CF we demonstrated rapid emptying of a high fat/ carbohydrate meal, associated with marked postprandial hyperglycaemia. Pancreatic enzyme supplementation slowed gastric emptying and markedly decreased the postprandial glycaemic excursion (Fig.1-2) (Kuo et al., 2011). We recently reported the same phenomenon in a study of 14 paediatric CF patients (Fig.3) (Perano et al., 2014, March 26.).

2.7.2 Gut hormone secretion

The “incretin” hormones, GLP-1 and GIP, are secreted from intestinal L and K cells respectively in response to nutrient digestion and exposure (Baggio and Drucker, 2007) and are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DDP-4) to form ‘inactive’ metabolites. Up to 70% of the total insulin response to oral glucose can be attributed to the actions of GLP-1 and GIP, making these peptides critical to postprandial glycaemic control (Baggio and Drucker, 2007). GLP-1 improves postprandial glycaemia through slowing gastric emptying and its glucose-dependent insulinotropic and glucagonostatic properties. GIP is insulinotropic, and can stimulate, rather than suppress, glucagon secretion, but appears not to affect gastric emptying (Nauck et al., 1997). Both the nutrient load and the rate at which nutrients empty to the small intestine are fundamental determinants of GLP-1 and GIP secretion. With increasing rates of small intestinal glucose exposure, there is a

linear increase in GIP secretion. By contrast, GLP-1 secretion is minimal at low rates (<2kcal/min) of glucose exposure, but increases substantially at higher rates (3-4kcal/min) in both health and T2D (Ma et al., 2012).

CCK and PYY may also influence postprandial glycaemic control through inhibition of gastric emptying, with their release also being dependent on nutrient digestion. CCK is released from I-cells in the duodenum and PYY predominantly by the L cells in the distal small intestine and colon, both in response to fatty acids, amino acids and glucose.

Based on the above, it may be anticipated in CF, associated with untreated pancreatic insufficiency, that meal induced secretion of GLP-1, GIP, CCK and PYY would be impaired. No studies have evaluated CCK or PYY in CF, and evidence relating to GLP-1 and GIP secretion is limited and conflicting, probably reflecting methodological inconsistencies (Kuo et al., 2011, Lanng et al., 1993b). Our recent work, in both adult and paediatric CF patients, has provided persuasive evidence of a marked reduction in GLP-1 and GIP secretion, in response to a high fat/high carbohydrate meal, when compared to control responses, (Kuo et al., 2011, Perano et al., 2014, March 26.). Pancreatic enzyme supplementation restored GLP-1 and GIP secretion, though the latter was still not normalised in the adult group (Fig.2), perhaps suggesting suboptimal mixing of enzymes and nutrients in the most proximal small intestine, from which most of GIP is derived.

2.7.3 Insulin secretion and action

That CFRD represents a state of insulin deficiency is evidenced by the loss of 1st phase insulin secretion, characterised by a delay of at least 60 minutes in the insulin response to a meal when compared to health, together with a reduction in peak insulin levels (Lanng et al., 1993b, Moran et al., 1991). First phase insulin

secretion is critical to the regulation of postprandial glycaemia, and in CF, impairment is evident in early stages of abnormal carbohydrate metabolism including some with a normal OGTT (Moran et al., 1991). Insulin resistance as assessed by homeostatic model assessment (HOMA-IR), is also evident in CFRD, but probably plays a lesser role in postprandial glycaemic control, and assumes greater importance during infection, or treatment with corticosteroids. Dysregulation of glucagon secretion may occur in CF, as a result of damage to pancreatic α cells (Moran et al., 1991, Lanng et al., 1993b). A diminished glucagon response to insulin-induced hypoglycaemia is evident in pancreatic insufficient CF subjects (Moran et al., 1991); conversely, an impaired capacity to suppress glucagon in response to oral glucose is increasingly evident as carbohydrate tolerance worsens (Lanng et al., 1993b). Increases in insulin clearance, hepatic gluconeogenesis and glucose absorption may also contribute to glycaemic dysregulation in CF.

2.8 Management of CFRD and the role of gastric emptying and incretin hormones.

Many of the current recommendations for the management of CFRD are based on expert consensus, due to the lack of higher levels of evidence and the limited therapeutic options available (Littlewood et al., 2004). The pathogenesis of abnormal carbohydrate metabolism in CFRD, particularly in the early stages, is dominated by postprandial, not pre-prandial hyperglycaemia, so the former should logically be the dominant therapeutic focus. Therapies that target postprandial hyperglycaemia may act by modifying gastric emptying and/or incretin hormone secretion; in particular, pancreatic enzyme supplementation, the use of macronutrient 'pre-loads', and incretin-based therapies, particularly GLP-1 agonists, may be of relevance in CF. There may also be additional benefit in combining these

therapies with basal insulin to target postprandial and pre-prandial hyperglycaemia respectively. The combination of basal insulin with a GLP-1 agonist represents an approach attracting increasing attention in the management of T2D (Inzucchi et al., 2013).

2.8.1 Pancreatic Enzyme Supplementation

Optimising pancreatic enzyme supplementation, through adequate dosing and timing of administration, is an important first step in achieving postprandial glycaemic control, with improved fat digestion stimulating gut hormone secretion and attenuating any tendency for rapid gastric emptying (Kuo et al., 2011). In the early stages of carbohydrate intolerance, this alone may be an effective management strategy. Pancreatic enzymes given prior to a meal at a dose appropriate for the fat content (500-4000IU lipase/gram of dietary fibre, mean 1800IU lipase/gram of fat/meal, maximum 10,000u lipase/kg/day (Anthony et al., 1999)) would be a reasonable starting point, with efficacy assessed by postprandial blood glucose concentrations, particularly at 30 and 60 minutes. Dosing based on body weight alone is simpler; however dosing based on the meal fat content is more likely to replicate the body's normal response. Combination therapy with a proton pump inhibitor, reducing duodenal acidity, may further optimise pancreatic enzyme efficacy. Additional information is required particularly regarding further therapeutic benefits of increased doses, and optimal timing in relation to the meal.

2.8.2 Potential Future Research

Macronutrient 'pre-load'

The novel 'pre-load' concept is currently being explored in T2D for the management of postprandial hyperglycaemia. A macronutrient pre-load is consumed 30-60 minutes prior to a meal to prime neurohumoral feedback, resulting in pre-emptive

slowing of gastric emptying and increased incretin hormone secretion. A fat pre-load, known to be potent in slowing gastric emptying, appears to have only a limited effect on overall postprandial glycaemia in T2D. In contrast, acute administration of a whey protein preload slows gastric emptying, stimulates incretin hormones, and markedly increases insulin secretion, probably predominantly via amino acids, thereby markedly reducing postprandial glycaemia (Ma et al., 2009, Gentilcore et al., 2006a). The increase in calorie consumption with a 'pre-load', a potential disadvantage in T2D, would not be an issue in CF; however the current CF dietary prescription of 3 meals and 3 snacks per day make a 'pre-load' less feasible. Further studies assessing the optimal quantity, timing and long term benefits, in particular the effect of a protein pre-load before the main meals in CF is required.

Incretin-based therapies

Incretin-based therapies, particularly GLP-1 agonists, are effective in the management of T2D (Inzucchi et al., 2013). In Australia GLP-1 agonists currently licensed for use in adults are exenatide, liraglutide, lixisenatide and exenatide LAR (long acting release), with a number of others in late phase development. The half-life of subcutaneously administered GLP-1 agonists appears to dictate whether efficacy is primarily preprandial ('non-prandial') or postprandial ('prandial'). 'Prandial' agonists (exenatide, lixisenatide) have a short duration of action, exerting a greater effect on postprandial glycaemia, predominantly through potent slowing of gastric emptying and postprandial insulin suppression (Kapitza et al., 2011;). 'Non-prandial' agonists (liraglutide and exenatide LAR), are longer acting and better suited for preprandial glycaemic control, since their predominant mode of action is to stimulate insulin and suppress glucagon secretion (Umapathysivan et al., 2014). The mechanisms underlying glucose-lowering induced by 'prandial' GLP-1 agonists

suggest potential benefits even in the early stages of the continuum of abnormal carbohydrate metabolism in CF; however this has yet to be investigated. The combination of a GLP-1 agonist for control of postprandial glycaemia, in conjunction with basal insulin for pre-prandial glycaemic control, is being used to good effect in T2D (Holst JJ and Vilsboll, 2013). This therapeutic combination may provide more effective pre- and postprandial glycaemic control in CFRD with fasting hyperglycaemia than insulin therapy alone, and should be explored further.

Adverse effects associated with GLP-1 agonists include gastrointestinal symptoms and modest weight loss, both of which would of concern in the CF population. In T2D however, only a minority of patients cannot tolerate therapy due to nausea, particularly if the dosage is increased gradually (Fakhoury et al., 2010). It is not known whether GLP1 agonists affect body weight in those with a normal or low BMI. Furthermore, rates of obesity (BMI>30kg/m² in adults), previously not considered an issue in CF, are now increasing in CF centres in Europe, the USA and Australia (Hanna and Weiner, 2012, Viviani et al.). Recent concerns relating to the potential association of incretin-based therapies with pancreatitis, pancreatic cancer, and medullary cell carcinoma have diminished (Egan et al., 2014) but need to be recognised, particularly in this patient group with pancreatic exocrine insufficiency.

2.9 Conclusion

The pivotal roles of pancreatic enzyme therapy, gastric emptying and incretin hormones in the pathophysiology of CFRD, characterised by postprandial hyperglycaemia, have largely been ignored. New therapies that act on gastric emptying and the incretin axis should be of considerable interest in the management of postprandial glycaemia in CF. With a high priority being given to finding

appropriate and acceptable therapies for pre-diabetes and CFRD, the optimisation of pancreatic enzyme supplementation, which is fundamental in the management of carbohydrate intolerance in CF, should not be overlooked. The benefit of a macro-nutrient 'pre-load', and efficacy, tolerability and long term safety of incretin-based therapies, should be further explored in this population.

Categories	Fasting plasma glucose (mmol/L)	2hr plasma glucose (mmol/L)
Normal glucose tolerance	<7.0	<7.8
Indeterminate glucose tolerance	<7.0	<7.8 with levels during 2 hours \geq 11.1
Impaired glucose tolerance	<7.0	7.8-11.1
CFRD without fasting hyperglycaemia	<7.0	\geq 11.1
CFRD with fasting hyperglycaemia	\geq 7.0	OGTT not necessary

Table 1. Categories of carbohydrate intolerance in CF by OGTT.

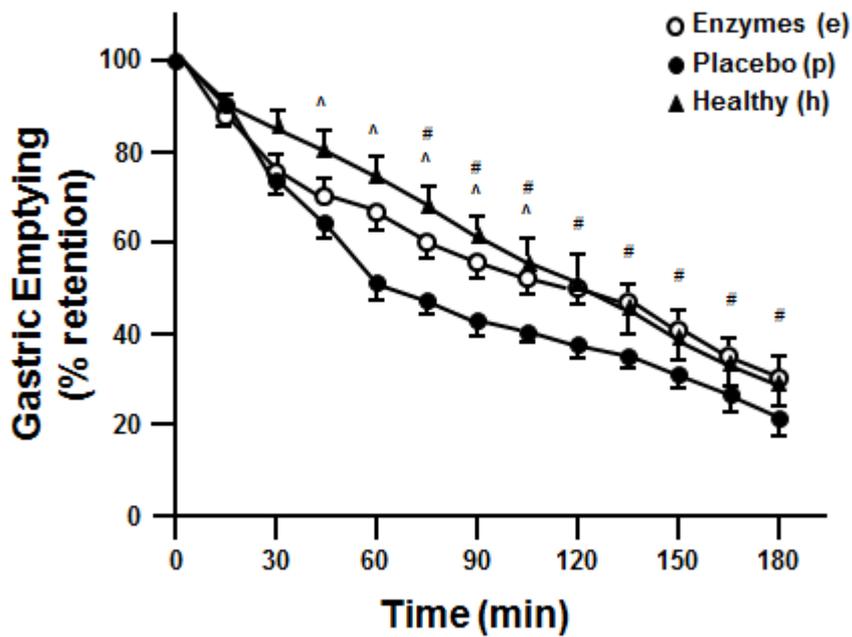


Fig 1. Gastric emptying of a meal in healthy subjects (n= 6) and CF patients (with and without pancreatic enzyme replacement therapy (PERT), n=5). Results represent means \pm SE. Emptying was faster in CF with placebo than in healthy subjects ($P < 0.001$, group-by-time interaction; Δ , points of significant difference). PERT normalised gastric emptying compared with placebo in CF patients ($P < 0.001$, treatment-by-time interaction; #, points of significant difference). Reprinted from Kuo et al (Kuo et al., 2011), with permission.

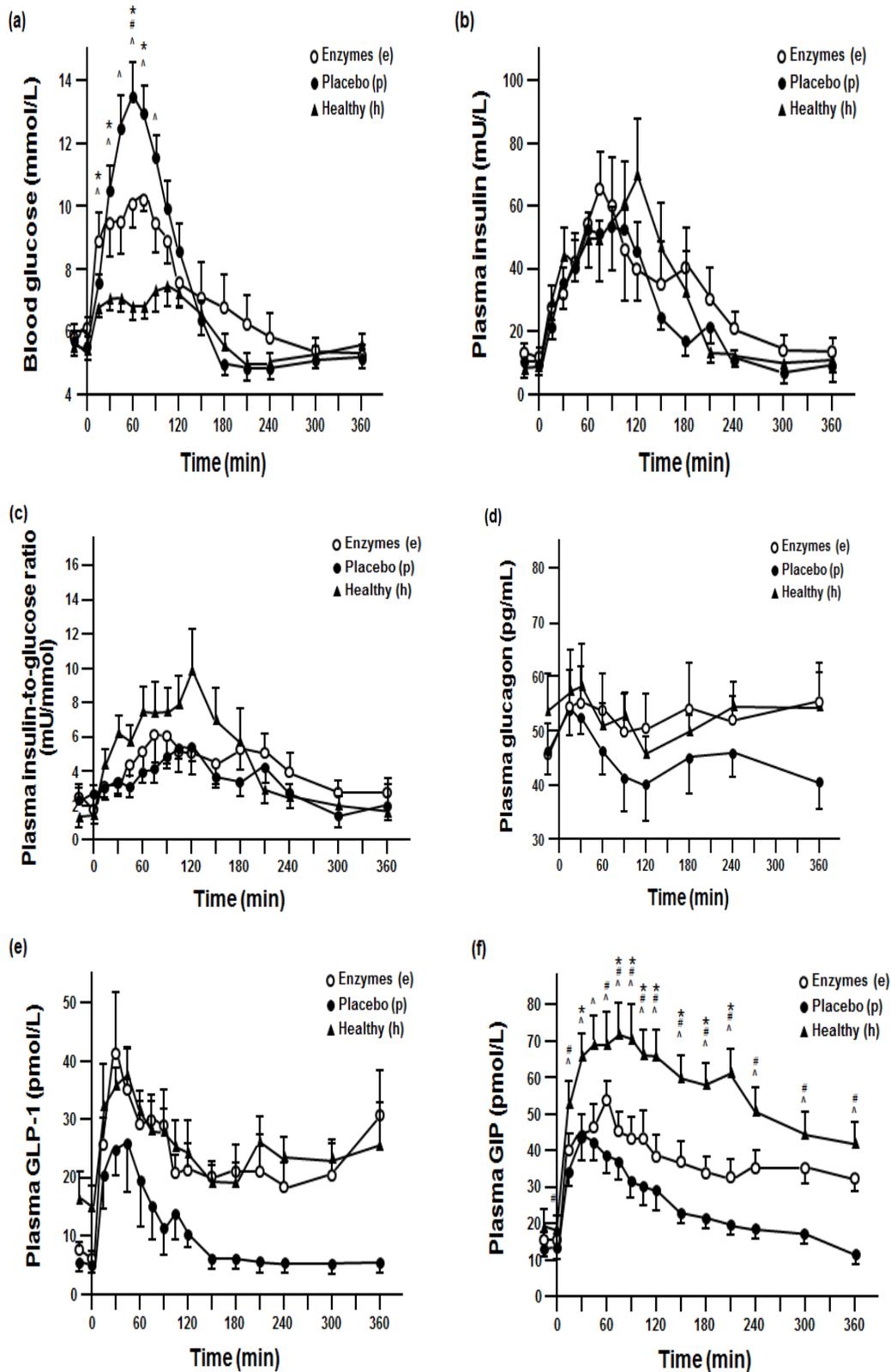


Figure 2.

Fig 2. A. Plasma blood glucose, B. insulin, C. insulin-to glucose ratio, D. glucagon, E. GLP-1, and F. GIP concentrations in CF patients with and without pancreatic enzyme replacement therapy (PERT) and in healthy subjects after a mashed potato meal. Results represent mean \pm SE. A, Blood glucose was higher in CF with placebo than in healthy subjects ($P < 0.001$, group-by-time interaction; \wedge , points of significant difference). In CF patients PERT lowered blood glucose ($P < 0.001$, treatment-by-time interaction; $\#$, points of significant difference). B, Insulin concentrations did not differ between the groups. C, Insulin-to-glucose ratio was lower in CF patients than healthy subjects ($P < 0.05$, group effect), and this did not improve with PERT. D, Glucagon concentrations did not differ between CF patients and healthy subjects but tended in CF patients to be higher after PERT ($P = 0.08$, treatment effect). E, GLP-1 was lower in CF patients than healthy subjects ($P < 0.01$, group effect), and this deficiency was completely reversed with PERT. F, GIP concentrations were lower in CF patients than in healthy subjects ($P < 0.001$, group-by-time interaction; \wedge , points of significant difference). PERT increased plasma GIP secretion in CF patients ($P < 0.001$, treatment-by-time interaction; $\#$ points of significant difference), but GIP remained lower than in healthy subjects ($P < 0.01$, group-by-time interaction; $*$, points of significant difference). Reprinted from Kuo et al (Kuo et al., 2011), with permission.

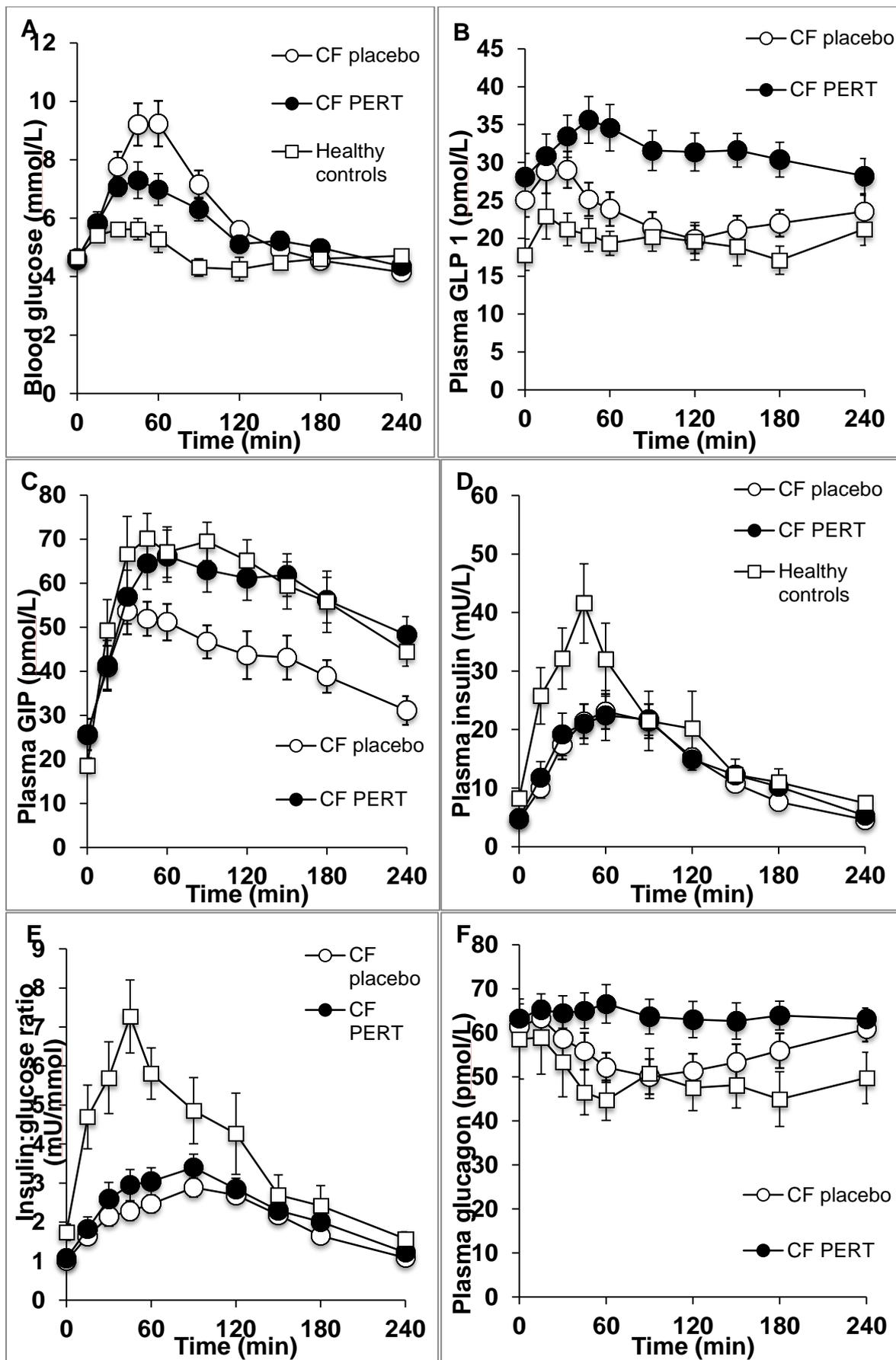


Figure 3.

Fig 3. A. Plasma blood glucose, B. GLP-1, C. GIP, D. insulin, E. insulin-to- glucose ratio, F. and glucagon concentrations in CF patients with placebo and PERT and in healthy controls after a high fat pancake meal. Results represent mean \pm SE. A, Blood glucose was higher in CF with placebo than controls ($P < 0.0001$). PERT partially normalised blood glucose levels ($P = 0.0002$). B, iAUC GLP-1 was lower in CF than controls ($P = 0.04$), and normalised with PERT. C, iAUC GIP was lower in CF than controls ($P = 0.003$), and normalised with PERT. D, Insulin concentrations were lower in CF than controls ($P = 0.02$) and did not normalise with PERT ($P = 0.4$). E, Insulin-to-glucose ratio was lower in CF than controls and did not normalise with PERT ($P = 0.3$). F, Glucagon concentrations were similar between CF and controls ($P = 0.3$) and increased with PERT ($P < 0.0001$). Reprinted from Perano et al (Perano et al., 2014, March 26.), with permission.

Dr Shiree Perano – Preparation of manuscript

A/Prof Chris Rayner — Correction of manuscript.

Professor Jennifer Couper – Correction of manuscript.

Dr James Martin - Correction of manuscript.

Professor Michael Horowitz- Correction of manuscript.

I approve the final draft submitted and give permission for this paper to be included in the thesis.

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3 CHAPTER 3: GASTRIC EMPTYING IN DIABETES AND ITS RELATIONSHIP TO GASTROINTESTINAL SYMPTOMS AND AUTONOMIC FUNCTION.

3.1 Introduction

The importance of gastric emptying in diabetes is increasingly being recognised, given that it is a major determinant of overall glycaemic control, accounting for up to 30% of the variation in postprandial blood glucose in health and diabetes (Horowitz et al., 1993). Postprandial glycaemic control, as compared with fasting or pre-prandial blood glucose, exerts a greater effect on overall HbA1c in T2D in patients with an HbA1c that is only modestly elevated, particularly <8 % (Monnier et al., 2003), and is also an important risk factor in the development of macrovascular disease (Nathan, 2014).

Disordered gastric emptying was first described in diabetic patients by Boas in 1925 and the term “diabetic gastroparesis” was coined by Kassarjian in 1945 (Kassarjian, 1958, Boas, 1925). Diabetic gastroparesis was initially thought to be a condition affecting primarily T1D, as a result of irreversible vagal nerve damage. Gastrointestinal symptoms were regarded as a reliable predictor of gastroparesis, and the prognosis was deemed to be poor. Over the last 25 years there have been major advances in the understanding of the pathogenesis and clinical significance of disordered gastric emptying, and improved diagnostic techniques are available. It is now apparent that the relationship of gastric emptying to gastrointestinal symptoms, and autonomic function, is more complex than initially thought. This chapter explores the current evidence on gastric emptying in health and in diabetes, the role of autonomic function, and its relationship to gastrointestinal symptoms, with particular reference to the current paediatric evidence base.

3.2 Gastric emptying in diabetes

Abnormal gastric emptying is a recognised, but often overlooked, complication of diabetes. Gastric motor dysfunction in this condition is heterogeneous and cannot be attributed to a single abnormality (Kashyap and Farrugia, 2010). Discordant gastric emptying of solids and liquids can occur, eg. with delayed emptying of solids associated with normal nutrient liquid emptying (Jones et al., 1996).

The prevalence of disordered gastric emptying within the diabetic population is uncertain due to differences in study methodology, measurement techniques and diagnostic criteria. The apparent increase in prevalence over time is likely due to an underestimation by earlier studies, with improved diagnostic techniques and increased awareness now allowing more accurate assessment. What clinicians may regard as “diabetic gastroparesis”, with frequent episodes of vomiting and hospitalisation, can be recognised as the severe end of a spectrum, and represents a relatively uncommon, late complication. This contrasts with delayed gastric emptying which is prevalent in up to 50% of patients with diabetes (Samsom et al., 2003, Horowitz et al., 1986), and is often asymptomatic. Accelerated gastric emptying is reported in a minority of patients with diabetes, predominantly in ‘early’ T2D cohorts and in some patients with long standing T1D (Phillips et al., 1991, Bharucha et al., 2009). In animal models of diabetes rapid emptying occurs 1-2 weeks after the onset of hyperglycaemia (Choi et al., 2007), although this effect has not been reported in humans. The prevalence of abnormal gastric emptying in the paediatric T1D population is unknown. A limited number of studies that have assessed gastric emptying report either no difference or delayed emptying (Cucchiara et al., 1998, Heptulla et al., 2008, Ersoy et al., 2013). As with the adult

literature, this inconsistency is most likely due to differences in study methodology and diagnostic techniques.

3.3 Pathogenesis of abnormal gastric emptying

Gastric emptying involves the complex co-ordination of the extrinsic nervous system, enteric nervous system, interstitial cells of Cajal (ICC), smooth muscle, and immune cells. The pathogenesis of disordered gastric emptying is likely to be the result of an abnormality of more than one of these elements.

3.3.1 Autonomic (vagal) neuropathy

Autonomic dysfunction affecting the vagal nerve was the first abnormality implicated in the pathogenesis of gastroparesis (Rundles, 1945). The connection was made between irreversible vagal nerve damage and abnormal gastric emptying based on the similarity of gastrointestinal symptoms in patients with surgical vagotomies to those with long standing diabetes. This association was further supported by the detection of a diminished vagal nerve response to sham feeding in patients with diabetic gastroparesis (Gaddipati et al., 2006). Animal models of diabetes indicate damage to smaller unmyelinated and myelinated vagal nerve fibres, although this has not carried over to human studies (Yagihashi and Sima, 1986). There is no test to assess vagal nerve function in the gut directly, and cardiovascular autonomic function tests are often used as a surrogate marker (Ewing and Clarke, 1982). Despite initial studies indicating an increased prevalence of disordered gastric emptying in diabetic patients with cardiovascular autonomic neuropathy (Jones et al., 2002, Horowitz et al., 1989), the relationship appears to be relatively weak (Horowitz et al., 1991, Buysschaert et al., 1987).

3.3.2 Cellular dysfunction

Reduced levels of nitric oxide- an important enteric neurotransmitter- secondary to a reduction in neuronal nitric oxide synthase (nNos) activity is evident in animal models and in humans with gastroparesis (Takahashi et al., 1997, Chandrasekharan and Srinivasan, 2007). This inhibition may be the result of advanced glycation end products binding to the neurons (Watkins et al., 2000). Despite these reports, therapies that increase NO levels (eg. nitroglycerine or sildenafil) do not normalise delayed gastric emptying in humans (Sun et al., 1998, Dishy et al., 2004).

The ICCs have numerous functions in the gastrointestinal tract (Farrugia, 2008). Loss or dysfunction of ICC is one of the most consistent findings in diabetic gastroparesis, and may be central in the pathogenesis (Wang et al., 2008, Forster et al., 2005). Previously, human studies have been limited due to difficulties in specimen collection. The National Institutes of Health in the USA has since established the Gastroparesis Clinical Research Consortium (GpCRC) to follow the largest cohort of patients with gastroparesis. Data from this cohort indicate that half the patients have substantial (more than 25%) reduction in ICC numbers (Grover et al., 2011). The loss of ICC is the result of an imbalance between their ability to regenerate and repair, depletion of stem cell factors including haem oxygenase-1, and damage due to the increased oxidative state of diabetes. Haem oxygenase-1, important in the ICC's defence against oxidative stress, is diminished in non-obese diabetic (NOD) mice (Choi et al., 2010), with preliminary studies indicating that therapies which influence the haem oxygenase/ carbon-monoxide pathway may be of benefit in the management of gastroparesis (Kashyup et al., 2010)

3.3.3 Impact of glycaemia

The relationship between glycaemic control and gastric emptying is complex and reciprocal. Acute changes in blood glucose have a substantial effect on the rate of gastric emptying, both in health and diabetes. Hyperglycaemia (>15mmol/L) slows gastric emptying of both solids and liquids (Fraser et al., 1990), with this effect even being observed at blood glucose levels as low as 8mmol/L (Schvarcz et al., 1997). Insulin induced hypoglycaemia, on the other hand, accelerates gastric emptying in health and uncomplicated T1D (Schvarcz et al., 1995, Schvarcz et al., 1993). These effects of glycaemic variation on gastric emptying potentially represent a protective mechanism to maintain euglycaemia; however the ability to delay gastric emptying in the face of hyperglycaemia appear to be impaired in T1D compared with healthy subjects (Woerle et al., 2008).

The impact of long term glycaemic control on gastric motor function is less clear. Several longitudinal studies found no change in gastric emptying, despite improvements in glycaemic control over this time. This may in part be due to deterioration in motor function over time, which counteracts the improvement in glycaemia (Chang et al., 2012) . Delayed gastric emptying has been associated with higher HbA1c and longer duration of diabetes (Cucchiara et al., 1998, Ersoy et al., 2013).

3.4 Natural history and prognosis

There are few longitudinal studies documenting the natural history of disordered gastric emptying. Gastric emptying remained stable over a 25 year period in 13 patients with diabetes (Chang et al., 2010). These findings may however, be related to improved glycaemic control offsetting a deterioration in autonomic function.

The natural history of abnormal gastric emptying in paediatric T1D is unknown. In paediatric patients with gastroparesis from all causes, of which T1D comprised only 4%, there was an improvement in 60% of patients over a 2 year period (Waseem et al., 2012); however, the majority of this cohort had idiopathic gastroparesis in which the underlying pathology is likely to differ from diabetes.

Gastroparesis was once thought to carry a poor prognosis, associated with increased hospitalisation, upper gastrointestinal symptoms and mortality (Hyett et al., 2009). On the contrary, Kong et al, followed a cohort of diabetic patients for 9 years and found no association between gastroparesis and increased mortality (Kong et al., 1999). A recent review of 86 diabetic patients over a 25 year period also concluded that delayed gastric emptying was not associated with a poor prognosis or higher mortality rate (Chang et al., 2012). It is likely that the prognosis remains poor in those with symptomatic gastroparesis, but in those with asymptomatic pathology there appears to be no impact on long term prognosis.

3.5 Gastrointestinal symptoms in type 1 diabetes and their relationship to gastric emptying.

Estimates of the prevalence and the determinants of gastrointestinal symptoms in diabetes are conflicting and limited, once again with the majority of the literature derived from the adult population, and including both T1D and T2D. Accurate symptom assessment was further limited by the lack of a diabetes-specific instrument, until a validated Diabetes Bowel Symptom Questionnaire (DBSQ) was developed in 2003 (Quan et al., 2003). The prevalence of gastrointestinal symptoms has been reported to be higher in adults with diabetes than in a non-diabetic population, with symptoms occurring more frequently in females and those with poor

glycaemic control, as measured by HbA1c (Bytzer et al., 2001, Ricci et al., 2000). Symptoms potentially attributable to gastroparesis have been reported in 5-10% of patients with diabetes in the community, with higher rates observed in tertiary referral centres (Parkman et al., 2010).

Gastrointestinal symptoms are often over looked in young people with T1D, with the prevalence and relationship to gastric emptying and glycaemia poorly defined (Burghen et al., 1992, Vazeou et al., 2004, Vogiatzi et al., 1996). Vazeou et al assessed recurrent abdominal pain, dyspepsia and constipation in 118 adolescents with T1D by questionnaire, and found no difference in symptom frequency when compared to controls. No correlation was found between gastrointestinal symptoms and HbA1C, duration diabetes, age, sex, BMI, or insulin dose (Vazeou et al., 2004).

The relationship of gastrointestinal symptoms to gastric emptying is tenuous (Horowitz and Dent, 1991, Punkkinen et al., 2008) and the use of symptoms as a clinical tool to predict disordered gastric emptying is unreliable (Samsom et al., 2003, Bharucha et al., 2009). It would be a natural assumption that gastrointestinal symptoms were attributable to disordered gastric emptying, however it is now evident that symptom aetiology is complex, with a multitude of variables, including age, gender, BMI, visceral hypersensitivity and psychological/ psychiatric conditions, potentially contributing (Rayner et al., 2000, Talley et al., 2001). Glycaemia also plays a role in symptom perception; for example, the perception of gastrointestinal symptoms appears heightened during hyperglycaemia, when compared to euglycaemia (Hebbard et al., 1996, Rayner et al., 2000). The perception of fullness,

in particular, has been correlated with the postprandial glycaemia (Jones et al., 1997).

3.6 Management of disordered emptying

Management is challenging due to an incomplete understanding of the pathophysiology, overlap of symptoms with other disorders, and paucity of effective therapeutic options. There is a lack of data in the paediatric population; consequently clinical practice is guided by adult evidence, often based on mixed cohorts of T1D and T2D. Furthermore, few studies have compared therapies directly. Symptom control, optimising nutrition, and improving glycaemic control are the cornerstones of the management of disordered gastric emptying, whether accelerated or delayed. In patients treated with insulin, achieving predictable delivery of nutrients to the small intestine to match the onset of action of exogenous insulin, is an increasing goal, not only to reduce long term diabetes complications but also to minimise the inhibitory effects of hyperglycaemia on gastric emptying.

3.6.1 Dietary Modifications:

Patients with rapid emptying may benefit from increased dietary fibre or increased fat content of the meal to slow gastric emptying (Chandalia et al., 2000, Gentilcore et al., 2006a). Protein pre-loads, in the form of whey protein, taken prior to a meal to prime the neurohumoral feedback mechanism, are also an effective strategy to delay gastric emptying (Ma et al., 2009). Protein pre-loads have the added glycaemic advantage, in T2D, of increasing insulin secretion (Ma et al., 2009). Dietary modification to accelerate gastric emptying, such as increasing the liquid content of meals, reducing fat and fibre intake and eating 4-6 small frequent meals

over the day, may be of benefit in cases of delayed gastric emptying (Olausson et al., 2008), although the evidence base for dietary manipulation in this setting is limited.

3.6.2 Pharmacological agents

Therapeutic agents used to influence gastric emptying in adults with diabetes include prokinetics to accelerate emptying (most commonly erythromycin, metoclopramide, and domperidone), and agents, such as GLP-1 receptor agonists (eg. exenatide and liraglutide) and amylin analogues (pramlintide), that slow gastric emptying.

Prokinetics accelerate gastric emptying through increased antral contractility and improved antropyloroduodenal coordination (Khoo et al., 2009), although their efficacy is reduced in the presence of acute hyperglycaemia. Metoclopramide and domperidone have additional antiemetic properties. Of the three prokinetics, erythromycin may be superior in terms of accelerating gastric emptying and reducing symptoms (Sturm et al., 1999). In several studies, it was the most commonly prescribed therapy for paediatric gastroparesis of all causes, despite being associated with the highest rate of side effects and the potential development of tachyphylaxis with long term use (Waseem et al., 2012, Ambartsumyan and Rodriguez, 2014). Metoclopramide was the second most commonly used prokinetic and has the clinical advantage of subcutaneous administration; however its use is now limited by an FDA 'black box' warning due to the risk of irreversible tardive dyskinesia. Domperidone was reported as the most effective prokinetic, however it was the least frequently used, and is not widely available in some markets (eg. USA) (Ambartsumyan and Rodriguez, 2014). A minority of patients have been

managed with tegaserod, cisapride, and azithromycin. Cisapride and tegaserod have both been withdrawn from the market due to potential life threatening cardiac side effects.

Other potential therapies under development that accelerate gastric emptying are motilin receptor agonists which avoid tachyphylaxis (Mitemcinal (GM611) and GSK962040), and ghrelin agonists (Sanger and Lee, 2008, McCallum et al., 2007). Ghrelin is an endogenous ligand for receptors expressed on vagal afferent neurons and enteric neurons in the stomach Phase II trials of ghrelin agonists (GM-131, T2P-101 and T2P-102) for the treatment of diabetic gastroparesis are currently being conducted.

GLP-1 receptor agonists slow gastric emptying, with the magnitude of delay directly related to the baseline rate of emptying, such that those with initially more rapid gastric emptying are most likely to benefit (Linnebjerg et al., 2008). The differing half-life of each agonist determines the dosing regimen and also appears to dictate whether efficacy is primarily pre- or postprandial. Short acting agonists, exenatide BD and lixisenatide, exert a greater effect on postprandial glycaemia in T2D predominantly through potent slowing of gastric emptying, with a corresponding degree of postprandial insulin suppression (Kapitza et al., 2011;). Longer acting agents, such as liraglutide and exenatide LAR, may be more appropriate choices for pre-prandial glycaemic control, since their predominant mode of action is to stimulate insulin and suppress glucagon secretion; this appears to be because there is tachyphylaxis to the slowing of gastric emptying with sustained activation of GLP-1 receptors (Nauck et al., 2011, Umapathysivan et al., 2014).

GLP-1 agonists are utilised frequently in the management of T2D to improve postprandial glycaemic control through their effect on gastric emptying. A small study of exenatide, a short acting GLP-1 receptor agonist, in adolescents with T1D showed a reduction in postprandial glycaemia with an associated delay in gastric emptying (Raman et al., 2010). Long term efficacy of these agents is still being established, although prolonged use of long acting GLP-1 agonists (such as exenatide LAR) is limited by the development of tachyphylaxis (Nauck et al., 2011). While these agents show promise, for their effects both on glycaemia and gastric emptying, further studies need to be conducted to assess long term effects, and efficacy in both T1D, and in paediatric patients.

Pramlintide, an amylin analogue, slows gastric emptying in T1D and T2D. It has the additional glycaemic benefits of glucagon suppression and increased satiety (Vella et al., 2002). The mechanism of action, through vagal nerve inhibition, suggests that its effect would be reduced in those with vagal nerve dysfunction.

Therapies to provide symptom relief include antihistamines, antiemetics, and tricyclic antidepressants (TCA). The addition of antiemetics, prochlorperazine and ondansetron, may reduce nausea and/or vomiting. Prochlorperazine is associated with extrapyramidal side effects, and there is limited evidence for the use of ondansetron in diabetes. TCAs, selective serotonin reuptake inhibitors, gabapentin, and opiates may be used for pain management, however the evidence is limited.

3.6.3 Non-pharmacological therapies

Non-pharmacological therapies such as intrapyloric botulinum toxin injection and gastric electrical stimulation have been assessed in adult and paediatric populations. The former did not improve gastric emptying or symptoms more than sham control in 2 adult trials, while the latter has not yet been adequately evaluated in sham-controlled fashion.

4 CHAPTER 4: METHODS

4.1 Introduction

The studies in this thesis utilise the following techniques: measurement of gastric emptying by ^{13}C Na-octanoate breath test, evaluation of cardiovascular autonomic function by measures of heart rate variability (HRV), quantification of chronic gastrointestinal symptoms by standardised questionnaire, quantification of acute symptoms by validated visual analogue scale and assays for the measurement of glucose and gut hormones.

4.2 Subjects

In the study described in Chapter 5, subjects were male and female patients aged 10– 18 years, with confirmed CF and documented exocrine pancreatic insufficiency (faecal elastase < 200ug elastase/g stool), requiring regular pancreatic enzyme replacement therapy (PERT). They were recruited consecutively over a 3 month period from either the CF clinic, or at point of discharge from the Women's and Children's Hospital by the primary investigator. Healthy age- and sex-matched controls, who were school friends of the CF subjects, were also recruited. For the study reported in Chapter 6 subjects were male and female patients aged 10 – 18 years with confirmed T1D of greater than 12 months duration. They were recruited consecutively over a 5 month period from the T1D clinics at the Women's and Children's Hospital. Age-and sex- matched healthy control subjects recruited were friends and siblings of the T1D patients. Additional control subjects for gastric emptying were included from previous work at the Women's and Children's Hospital. HRV control subjects' data was also provided by co-investigators from the Children's Hospital Westmead, Sydney, from their large control database.

4.3 Measurements

4.3.1 Gastric emptying

Scintigraphy is the 'gold standard' diagnostic test for measuring gastric emptying; however it is not an acceptable test in the paediatric population due to the radiation exposure it entails. The ^{13}C Na-octanoate breath test correlates well with scintigraphy, as detailed in Chapter 1, and is an acceptable alternative to measure gastric emptying in children (Hauser et al., 2006).

In the study described in Chapter 6, a standardised solid pancake meal, consisting of Green's™ Pancake and Pikelet Mix (70g mixed with 50mL water) labelled with 100mg ^{13}C Na-octanoate and cooked with 10g butter (total of 8.8g fat, 30.3g carbohydrate, 142.7kcal), was consumed within 5 min. In the study described in Chapter 5, the standard pancake meal was modified with the addition of extra fat (35mL polyunsaturated oil), in order to replicate the high fat diet prescribed to CF patients, providing a total of 40.7 g fat, 30.3 g carbohydrate, and 426.7 kcal. In each study, the meal was consumed between 7.30-8.30am after an overnight fast. Breath samples were collected over 4 hours, with time zero ($t=0$) defined as the time of meal completion. During this time, the subjects remained seated and were allowed sips of water, up to 100mls, on request. No further food was consumed during the data collection period.

Breath samples were analysed for $^{13}\text{CO}_2$ using an isotope ratio mass spectrometer (Europa Scientific ABCA 20/20, Crewe, England). The $^{13}\text{CO}_2$ excretion rate was used to calculate the gastric half emptying time ($t_{1/2}$) using a non-linear regression model, as described previously (Omari et al., 2005). Abnormal hepatic or pulmonary metabolism may affect the rate of $^{13}\text{CO}_2$ excretion. To minimise this in CF subjects, a crossover study design was chosen for the study reported in Chapter 5,

with the CF subjects acting as their own controls. A washout period for the ^{13}C label of 48 hours was allowed between study days

4.3.2 Autonomic nerve function

While vagal nerve function cannot be measured directly, cardiovascular autonomic function is considered a reliable surrogate measure. Traditionally the Ewing battery of tests was performed to assess cardiovascular autonomic function (Ewing et al., 1985); however power spectral analysis of heart rate variability (HRV) is a more sensitive measure (Molgaard et al., 1992, Malpas and Maling, 1990, Weston et al., 1996). In Chapter 6, cardiovascular autonomic function was assessed by a 10 minute continuous ECG recording taken using the LabChart Pro system (AD Instruments, Amsterdam, Holland). Recordings were performed in a quiet room with subjects lying supine at a standardised time point ($t=210\text{mins}$) during the study. All subjects were fasted, and no caffeine had been consumed within 12 hours. The whole recording was analysed after exclusion of ectopic beats ($<500\text{ ms}$, $>1100\text{ms}$). The trace was visually scanned for R-waves amongst artefact and ectopic beats by the principal investigator, and R-waves manually marked for later analysis. The variation between R-wave intervals, at rest, represents the beat-by-beat variations in cardiac autonomic function (NN interval). Derived time domain measures included the following: standard deviation of mean NN intervals (SDNN) (where NN is determined from adjacent QRS complexes) and root mean squared difference of successive NN intervals (RMSSD), which are estimates of overall HRV. Frequency domain measures included the following: low frequency (LF), defined as $>0.04\text{ Hz}$ and $<0.15\text{ Hz}$, and high frequency (HF) components, defined as $>0.15\text{ Hz}$ and $<0.4\text{ Hz}$, and the LF:HF ratio, considered an estimate of the relative sympathetic and parasympathetic balance. Overall HRV is expressed as the triangular index (total

number of NN intervals/ number of NN intervals in the modal bin, where modal bin is the most commonly occurring NN interval determined by histogram plot) .

4.3.3 Gastrointestinal symptoms assessment

Chronic gastrointestinal symptoms were assessed by the modified Diabetes Bowel Symptom Questionnaire (Quan et al., 2003). This consists of questions assessing seven symptom groups for the preceding 3 months. The symptoms groups are: abdominal pain, nausea and vomiting, bloating, gastro-oesophageal reflux, dysphagia, diarrhoea, and constipation. To apply the questionnaire to a paediatric population, symptom questions not relevant to children were removed. The questionnaire was administered during the gastric emptying study by the principal investigator. Symptom frequency was recorded as 1 (not at all), 2 (sometimes; less than $\frac{1}{4}$ of the time), 3 (often; more than $\frac{1}{4}$ of the time), 4 (very often; more than $\frac{1}{2}$ the time), or 5 (almost always; more than $\frac{3}{4}$ of the time). Severity was graded as 1 (none), 2 (very mild; the symptom could be ignored), 3 (mild; the symptom could not be ignored, but did not affect daily activities), 4 (moderate; symptom cannot be ignored and affects daily activities), 5 (severe; affects lifestyle), or 6 (very severe; affects lifestyle a great deal). Symptoms were considered significant if they occurred $> \frac{1}{4}$ of the time (score ≥ 3), and severity was considered significant if lifestyle was affected (score ≥ 4).

The occurrence of symptoms of nausea, bloating, fullness, and anxiety during the gastric emptying study was assessed by a visual analogue questionnaire (Shields et al., 2003). Each question was accompanied by a 100mm horizontal line, with 0mm corresponding to no symptom sensation and 100mm to maximum possible sensation. Subjects recorded their response as a vertical mark along the line. Scores were quantified by measuring the distance from start point (left end of the line) to the

mark, thereby generating a score out of 100. Visual analogue questionnaires were completed at time points-25, 30, 60, 120, 180 and 240 minutes during the gastric emptying study.

4.3.4 Biochemistry/ Hormones

Blood samples for hormone analysis was placed into ice-chilled tubes containing EDTA. No inhibitors were added. Plasma was separated soon after collection by centrifugation at 3200rpm for 15 minutes at 4°C, and stored at -70°C for subsequent analysis.

Blood glucose

Blood glucose was measured immediately by portable glucometer (MediSense Optium Xceed, MediSense Inc) to ensure subject safety. The laboratory glucose oxidase method was used for subsequent analysis.

Plasma GLP-1

Plasma total GLP-1 was measured by RIA (GLPIT-36HK, Millipore, Billerica, MA), with sensitivity of 3 pmol/L, and intra- and inter-assay coefficients of variation (CVs) of 6.5% and 6.5% respectively (Kuo et al., 2010).

Plasma GIP

Plasma total GIP was measured by RIA, with sensitivity of 2 pmol/L and intra- and interassay CVs both 15% (Kuo et al., 2010) .

Serum insulin

Serum insulin was measured by ELISA (10-1113, Mercodia, Uppsala, Sweden), with sensitivity of 1.0 mU/L and intra- and inter-assay CVs of 2.9% and 8.0% (Kuo et al., 2010).

Serum glucagon

Serum glucagon was measured by RIA (GL-32K, Millipore, Billerica, MA), with sensitivity of 20 pg/mL, and intra- and inter-assay CVs of 5.1 % and 12.9% (Kuo et al., 2010)

4.4 Statistical analysis

Results are given as mean (standard deviation) where the data were normally distributed, and median (interquartile range) where this was not the case, unless otherwise stated. Specific statistical methods used for each study are described in the respective chapters.

4.5 Conclusion

All methods used in this thesis are well described, validated, and considered appropriate and acceptable for use in a paediatric population. The methods were all well tolerated by subjects and represented the best techniques to address the hypotheses in each study, taking into consideration the needs of the subject cohort.

5 CHAPTER 5: PANCREATIC ENZYME SUPPLEMENTATION IMPROVES THE INCRETIN HORMONE RESPONSE AND ATTENUATES POSTPRANDIAL GLYCAEMIA IN ADOLESCENTS WITH CYSTIC FIBROSIS: A RANDOMIZED CROSSOVER TRIAL.

5.1 Summary

Cystic fibrosis related diabetes (CFRD) is characterised by postprandial, rather than fasting, hyperglycaemia. Gastric emptying and the release of the incretin hormones [glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP)] are central to postprandial glycaemic control. Lipolysis is required for fat to slow gastric emptying and stimulate incretin release.

We aimed to determine the effect of pancreatic enzyme replacement therapy (PERT) on postprandial glycaemia in adolescents with cystic fibrosis (CF).

In a double blinded randomised crossover trial 14 adolescents (13.1 ± 2.7 years) with pancreatic insufficient CF consumed a high fat pancake, with either PERT (50,000IU lipase) or placebo. Gastric emptying was measured by breath test and blood sampled frequently for plasma blood glucose, insulin, glucagon, GLP-1 and GIP. Data were compared also with 7 healthy subjects.

CF subjects had postprandial hyperglycaemia compared to controls (area under the curve, $P < 0.0001$). PERT reduced postprandial hyperglycaemia ($P = 0.0002$), slowed gastric emptying ($P = 0.003$), and normalised GLP-1 and GIP secretion ($P < 0.001$ for each) when compared to placebo, without affecting insulin. Slowing of gastric emptying by PERT was closely related to the reduction in peak blood glucose ($r = -0.69$, $P = 0.007$).

In young people with pancreatic insufficient CF, PERT markedly attenuates postprandial hyperglycaemia by slowing gastric emptying and augmenting incretin hormone secretion.

5.2 Introduction

The mean life expectancy for those with cystic fibrosis (CF) has improved by over 38 years in the last 6 decades; however, with this has come the increasing clinical challenge of managing the associated long term co-morbidities (Lanng et al., 1994). An increasingly prevalent co-morbidity is CFRD, with more than 50% over 40 years of age affected (Van den Berg et al., 2009, Moran et al., 2009a, Lanng et al., 1994). CFRD is associated with worsening nutritional state and pulmonary function, and increased mortality. Abnormalities of carbohydrate metabolism in CF represent a continuum from normal, through pre-diabetes, to overt diabetes. Insulin is currently recommended to treat CFRD; however, it is a demanding treatment for patients who already require other complex and time consuming regimens, and is associated with a risk of hypoglycaemia. The optimal management of pre-diabetes in CF is not resolved.

Gastric emptying and the incretin hormones, GLP-1 and GIP, are central to postprandial glycaemic control (Marathe et al., 2013). Relatively more rapid gastric emptying results in a prompt postprandial rise in blood glucose, while relatively slower emptying provides a more controlled rise (Ma et al., 2012). The incretin hormones, GLP-1 and GIP, are secreted from intestinal entero-endocrine cells primarily in response to the products of nutrient digestion, including fatty acids (Baggio and Drucker, 2007, Martin et al., 2011). They are important determinants of postprandial glycaemia through their actions to stimulate insulin under conditions of

elevated blood glucose; GLP-1 suppresses glucagon and slows gastric emptying while GIP may stimulate glucagon (Nauck et al., 1997, Baggio and Drucker, 2007).

Exocrine pancreatic insufficiency with associated fat maldigestion affects approximately 80% of CF patients and fat digestion often remains abnormal despite PERT (Baker et al., 2005, Symonds et al., 2003). In addition to stimulating release of the incretin hormones, lipolytic products in the small intestine induce neurohumoral feedback to slow gastric emptying that involves, amongst other mechanisms, secretion of peptide YY and cholecystokinin (Hedde et al., 1989, Borovicka et al., 2000, Feinle et al., 2003). Fat maldigestion is, therefore, associated with abnormally rapid gastric emptying of high fat meals (Carney et al., 1995), and accelerated absorption of carbohydrates, resulting in postprandial hyperglycaemia (Kuo et al., 2011). The use of the lipase inhibitor, orlistat, in type 2 diabetes is associated with accelerated gastric emptying and accentuated postprandial hyperglycaemia (O'Donovan et al., 2004).

Few studies have assessed gastric emptying and/or incretin responses in CF patients, with inconsistent observations (Roulet et al., 1980, Cucchiara et al., 1996, Pauwels et al., 2011, Collins et al., 1997, Kuo et al., 2011, Symonds et al., 2003). Our initial pilot study in 5 adults indicated that PERT can slow gastric emptying of a high fat/high carbohydrate meal, with enhanced incretin hormone secretion, and a reduction in postprandial glycaemia (Kuo et al., 2011). This study therefore aimed to assess whether PERT slows gastric emptying, increases incretin secretion and improves postprandial glycaemia in adolescents with CF.

5.3 Methods

5.3.1 Subjects

Fifteen subjects aged 10 – 18 years with confirmed CF, and documented exocrine pancreatic insufficiency requiring regular PERT, were recruited consecutively over a 3 month period from either the CF clinic, or at point of discharge from the Women's and Children's Hospital by the primary investigator (fig 1). Patients with severe pulmonary disease ($FEV_1 < 30\%$ predicted), intercurrent infection or acute pulmonary exacerbation, significant liver disease (Child-Pugh score > 6), previous gastrointestinal surgery (apart from uncomplicated appendectomy), or concurrent medications that could affect gastrointestinal motility (eg erythromycin) or glycaemia (eg. prednisone), were excluded. Age- and sex-matched healthy controls, who were school friends of the CF subjects, were also recruited. The protocol was approved by the Human Research Ethics Committee of the Women's and Children's Health Network. Written informed consent was obtained from each participant and a parent.

5.3.2 Protocol

CF subjects were studied on 2 days each, receiving either placebo or PERT in randomised double blind order. Placebo (microcellulose) or PERT (Creon 50,000IU (Abbott Products Pty Ltd, Pymble, NSW, Australia) were packaged into generic capsules, randomised, and distributed by the hospital pharmacy clinical trials unit. Randomisation was by computer generated sequence, as for our previous trial (Kuo et al., 2011) with the primary investigator collecting medication packs, labelled day 1 and day 2, before the study day. All other investigators, subjects and their treating clinicians were blinded to the randomisation order until investigation of all subjects was complete. Each study commenced at 8am with the subject having fasted from

9pm the previous evening. Study days were separated by at least 48 hours to ensure that no residual ^{13}C Na-octanoate was present at the time of the second study. An IV cannula was inserted at the start of the study for blood sampling, or a peripherally inserted central catheter (PICC) was used, if already in situ. Placebo or PERT capsules (Creon 50,000IU providing 10,000IU of lipase per 8g fat) were taken at the start of the meal ($t = -10$ min), which was then consumed within 10 min.

The meal consisted of Green's™ Pancake and Pikelet Mix (70g mixed with 50mL water and 35mls of polyunsaturated oil) labelled with 100mg ^{13}C Na-octanoate and cooked with 10g butter (total of 40.7 g fat, 30.3 g carbohydrate, 426.7 kcal). This meal is representative of the high fat diet prescribed for CF subjects. Control subjects were studied on one day each, with the same meal but no PERT or placebo capsules. Breath samples for measurement of gastric emptying and blood samples for glucose, insulin, glucagon, GLP-1, and GIP were collected at $t = -15, -5, 15, 30, 45, 60, 90, 120, 150, 180, 240$, min on each study day (Table 1).

5.3.3 Measurements

Gastric emptying

Breath samples were analysed for $^{13}\text{CO}_2$ using an isotope ratio mass spectrometer (Europa Scientific ABCA 20/20, Crewe, England). The $^{13}\text{CO}_2$ excretion rate was used to calculate the gastric half-emptying time ($t_{1/2}$) using a non-linear regression model, as described (Omari et al., 2005).

Blood glucose, plasma insulin, plasma glucagon, and plasma GLP-1 and GIP

Blood glucose was measured by the glucose oxidase method and the remainder of the sample was placed in ice-chilled tubes containing EDTA (no inhibitors were added). Plasma was separated by centrifugation and stored at -70 C

for subsequent analysis of plasma insulin, glucagon, GLP-1, and GIP by previously described assays (Kuo et al., 2010). Total GLP-1 was measured by RIA (GLPIT-36HK, Millipore, Billerica, MA), with sensitivity of 3 pmol/L, and intra- and inter-assay coefficients of variation (CVs) of 6.5% and 6.5% respectively. Total GIP was measured by RIA, with sensitivity of 2 pmol/L and intra- and interassay CVs both 15%. Insulin was measured by ELISA (10-1113, Mercodia, Uppsala, Sweden), with sensitivity of 1.0 mU/L and intra- and inter-assay CVs of 2.9% and 8.0%. Glucagon was measured by RIA (GL-32K, Millipore, Billerica, MA), with sensitivity of 20 pg/mL, and intra- and inter-assay CVs of 5.1 % and 12.9 %

5.3.4 Statistical analysis

The primary outcome was postprandial glycaemia measured as peak blood glucose and area under the curve (AUC) for blood glucose; secondary outcomes were gastric emptying and incretin hormone (GLP-1 and GIP) response. To have 80% power ($p < 0.05$ two-tailed) to detect a difference in mean peak postprandial blood glucose of 2mmol/L between PERT and placebo conditions in CF patients, assuming a SD of 2.01 mmol/L (Kuo et al., 2011) and a correlation of 0.5 between conditions, a sample size of 11 CF subjects was required. Fifteen subjects were recruited to allow for possible withdrawal from the study. Statistical analyses were performed using SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA). Outcomes for postprandial glycaemia, gastric half-emptying time, incretin hormones, insulin and glucagon were compared within CF subjects given placebo and PERT, and against the healthy control group, using linear generalised estimating equations (GEE). A GEE approach was chosen to account for dependence in the data resulting from repeated measures in the CF patients. To satisfy the assumptions of the linear GEE model, gastric half emptying data were log transformed, giving geometric means and

confidence intervals. AUC for blood glucose and plasma hormone concentration curves were calculated using the trapezoidal rule; for comparison of GLP-1, the incremental rather than absolute AUC was used, since baseline values differed between CF patients and healthy controls. Pearson correlation coefficients were used to assess relationships between the various measures. Incremental AUCs for GLP-1 (with negative values removed) did not follow a normal distribution therefore non-parametric tests were used: Wilcoxon signed rank sum tests for paired comparisons between the crossover groups and Wilcoxon-Mann-Whitney tests for unpaired comparisons with control subjects.

5.4 Results

Fifteen CF subjects and 7 healthy controls were recruited. The studies were well tolerated. One CF subject withdrew from the study before completing the second visit, and was excluded. Control subjects were matched for age and body mass index, but were taller than the CF subjects (table 2). Of the CF group, 9 subjects were $\Delta F508$ heterozygous, 3 were $\Delta F508$ homozygous, and 2 had other mutations. Nine CF subjects were studied as inpatients, after their acute illness had resolved, just before discharge. 12/14 CF subjects had recorded a normal oral glucose tolerance test result as part of annual screening within the preceding 12 months.

Blood glucose

Fasting blood glucose concentrations did not differ between CF subjects and controls ($P = 0.87$). After the meal, the peak blood glucose concentration was higher in the CF placebo group compared to controls (mean $9.8 \pm$ standard error [SE] 0.7

versus 6.1 ± 0.2 mmol/L, $P < 0.0001$) and was lowered, but did not normalise with PERT (8.0 ± 0.5 mmol/L, $P = 0.0005$). The blood glucose AUC 240 min was higher in the CF placebo group than controls (AUC mean $1444 \pm SE 41$ versus 1145 ± 35 mmol/L.min, $P < 0.0001$), and was lowered, but did not normalise, with PERT (1349 ± 41 mmol/L.min, $P = 0.0002$) (figure 2A). The two subjects who had been diagnosed with CFRD had normal fasting blood glucose concentrations, while their postprandial glucose AUC responses, with or without PERT, were elevated but not outside the range seen in the other CF subjects.

Gastric emptying

The gastric half-emptying time did not differ between the CF placebo group and controls [geometric mean 127 (95% CI 90-180)] and 137 (95% CI 108-172)], although one third of the CF subjects had gastric emptying rates that were faster than the control range. PERT increased gastric half emptying time compared to placebo [202 (95% CI 147-277) min with PERT, $P = 0.003$] (figure 3A, B).

The reduction in post-prandial blood glucose levels and slowed gastric emptying did not correlate with changes in GIP or GLP-1 concentrations.

Incretin hormones

Fasting GLP-1 concentrations were higher in the CF group than in controls ($P = 0.002$), while fasting GIP concentrations were similar ($P = 0.09$) (figure 2B,C). The postprandial incremental AUC for GLP-1 in the CF placebo group was less than in healthy controls (median 162 (IQR 147- 444) and 606 (461- 924) pmol/L.min, $P = 0.04$). PERT was associated with an increase in incremental AUC for GLP-1 (918 (767-1358) pmol/L.min, $P = 0.0009$), to levels comparable to those in healthy

controls ($P = 0.18$). In the CF placebo group the postprandial AUC for GIP was less than in controls (mean $10226 \pm SE 880$ and 14106 ± 973 pmol/L.min, $P = 0.003$).

PERT was associated with an increase in AUC for GIP (13676 ± 1080 pmol/L.min, $P < 0.001$), again to levels similar to controls ($P = 0.77$).

Insulin and glucagon:

Fasting insulin concentrations were lower in CF subjects than controls ($P = 0.001$) (Figure 2D). Postprandially, the AUC for insulin was less in the CF placebo group than controls (mean $3191 \pm SE 262$ versus 4607 ± 558 mU/L.min, $P = 0.02$), and did not increase with PERT (3402 ± 321 mU/L.min, $P = 0.41$), nor did the insulin to glucose ratio improve with PERT ($P = 0.26$) (figure 2E).

Fasting glucagon concentrations did not differ between CF subjects and controls ($P = 0.19$). The postprandial AUC 240min for glucagon was comparable in the CF placebo and control groups (mean $13284 \pm SE 781$ and 11730 ± 1215 pg/ml.min, $P = 0.28$), and increased with PERT (15343 ± 780 pg/ml.min, $P < 0.0001$) (figure 1F).

Relationship between postprandial glycaemia and gastric emptying

In the CF subjects, there was a strong inverse relationship between the change in gastric emptying and the change in peak postprandial blood glucose concentration between the placebo and enzyme days ($r -0.69$, $P=0.007$), ie. the greater the slowing of gastric emptying with PERT, the greater the reduction in peak blood glucose.

All results were the same if either the 2 subjects with CFRD, or the outlier with a t 1/2 at 695 min for gastric emptying, were excluded.

5.5 Discussion

This study has established that young people with CF have markedly greater glycaemic excursions after a high fat/high carbohydrate meal than healthy controls, even in the context of a normal fasting blood glucose and a normal oral glucose tolerance test. Provision of PERT lowered postprandial glycaemia substantially, albeit not to normal levels, and this was related to the slowing of gastric emptying with PERT. These findings are consistent with our previous observations in 5 adults with CF (Kuo et al., 2011), and extend them to include adolescents with cystic fibrosis. They provide evidence of a continuum of abnormal postprandial carbohydrate metabolism in the majority of CF subjects, which calls into question the clinical validity of the oral glucose tolerance test with baseline and 120 minute sampling as a screening tool in this population (Hameed et al., 2010).

In every day practice there may be clinical benefit in additional blood glucose sampling at 30, 60 and 90 minutes during an OGTT, to detect those with early carbohydrate intolerance that would be missed with the traditional baseline and 120 minute samples. Although the long term benefit of tight glycaemic control in these early stages, and the threshold for intervention and optimum type of treatment are still being established, early detection of these patients would allow closer glycaemic monitoring, particular during times of illness, and also the optimisation of regular and before-meal pancreatic enzyme supplementation.

Our CF subjects had abnormally low postprandial stimulation of the incretin hormones, GLP-1 and GIP, which was normalised with PERT. In CF, active and /or total postprandial GLP-1 and GIP have been reported to be increased (Ross et al.,

1981), reduced (Hillman et al., 2012, Adrian et al., 1980, Allen et al., 1983), or unchanged (Lanng et al., 1993b, Anzeneder et al., 2011). The stimulation of incretin secretion with PERT has been reported in adult patients with chronic exocrine pancreatic insufficiency due to other causes (Knop et al., 2007), and there are now several studies in CF demonstrating normalisation of total GLP-1 with PERT, with partial improvement in the GIP response, including that of Ross *et al* in children (Ross and Shaffer, 1981) and our own report in adults with CF (Kuo et al., 2011). This is the first study to our knowledge that demonstrates normalisation of the GIP response with PERT in CF subjects. Unlike our previous study, we found that fasting total GLP-1, but not GIP, concentrations were higher in young people with CF than in age- and BMI- matched controls. Studies in adults and children have described normal or reduced fasting total or active incretin concentrations (Kuo et al., 2011, Ross et al., 1981, Lanng et al., 1993b). The increased basal levels of GLP-1 observed in the current study may be explained by CF subjects consuming a high fat snack at supper time just prior to the overnight fast; patterns of nutrient ingestion the evening prior to blood sampling have been shown to affect basal GLP-1 concentrations (Chandarana et al., 2009). The tendency of CF subjects to malabsorb fat might also have resulted in persisting nutrient stimulation after the last meal, particularly in the distal gut where the GLP-1 secreting cells are primarily located, contributing to elevated 'fasting' GLP-1 hormone concentrations

Fasting insulin concentrations were lower in our CF patients than in controls, as were postprandial insulin concentrations both with and without PERT. Indeed, the provision of PERT, despite stimulating greater incretin hormone secretion, did not result in an increase in insulin concentrations after the meal, even when corrected for

prevailing blood glucose concentrations. These observations are in keeping with the concept of CF as a relatively insulinopaenic state, which is evident even before the diagnosis of CFRD (Lanng et al., 1993b, Mohan et al., 2009), and are consistent with the findings of our adult study (Kuo et al., 2011). It cannot be concluded, however, that the incretin hormones are devoid of insulinotropic effects in CF, as postprandial blood glucose after PERT was only modestly elevated, so it is possible that the mean blood glucose remained under the threshold required for stimulation of insulin by GLP-1 or GIP.

Postprandial glycaemia in our CF patients was not explained by abnormally high concentrations of glucagon on the placebo day, when compared to controls. However, we did observe an increase in the glucagon response with PERT, which was consistent with our previous study and could be attributed either to the increase in GIP concentrations, or to lower blood glucose levels with PERT inducing less suppression of glucagon.

Gastric emptying of the high fat/high carbohydrate meal in the CF group, as evaluated by stable isotope breath test, was not abnormally rapid when compared to controls; although 30% of the CF group did have rapid gastric emptying relative to controls. The products of fat digestion in the intestinal lumen induce potent inhibition of gastric emptying, so that emptying is typically accelerated in the setting of impaired fat digestion or absorption (O'Donovan et al., 2004, Heddle et al., 1989, Borovicka et al., 2000). There are limited data concerning gastric emptying in CF, with inconsistent results between studies, probably attributable to differences in subject characteristics, meal composition, previous dietary habits and nutritional

state, and the method used to measure gastric emptying. Gastric emptying has been reported as rapid (Kuo et al., 2011, Collins et al., 1997), delayed (Cucchiara et al., 1996, Pauwels et al., 2011), or comparable to controls (Roulet et al., 1980, Symonds et al., 2003). A previous study that used the ^{13}C Na-octanoate breath test also reported no difference in the rate of gastric emptying between young people with CF, studied without PERT, and controls (Symonds et al., 2003). It should be noted that our previous study in adults with CF, where gastric emptying was abnormally rapid, used scintigraphy to measure emptying, which is regarded as the 'gold standard'. We used a ^{13}C Na-octanoate breath test in the current study, particularly since it involves no exposure to ionising radiation, and has been well validated against scintigraphy in healthy subjects (Bromer et al., 2002, Perri et al., 2005). It is, however, an indirect measure that is dependent on substrate digestion, absorption, metabolism and excretion of carbon dioxide. The higher metabolic and respiratory rates associated with CF result in increased CO_2 production compared to healthy individuals (Buchdahl et al., 1988), but correcting for this did not change our findings. It remains possible, however, that variations in other aspects of digestion, absorption, metabolism and excretion of the carbon label in CF could have confounded our comparisons with controls.

Alternatively, it is possible that differences in the duration of fasting, perhaps reflected in elevated baseline GLP-1 hormone concentrations in the CF subjects, may have counterbalanced any tendency for accelerated emptying on the placebo day, when compared to healthy controls. Other potential factors that could tend to slow gastric emptying in the CF group include medications and intercurrent illness. However, CF subjects taking medications known to affect gastric emptying were

excluded, and we endeavoured to study patients only after resolution of disease flares.

Our observations highlight the fact that postprandial hyperglycaemia is the predominant abnormality of carbohydrate metabolism in CF, including those with CFRD, and that it is manifest early in the course of the disease. Therapies that specifically target postprandial glucose excursions are, therefore, likely to be of central importance in improving clinical outcomes and life expectancy. Our study has demonstrated that postprandial glycaemia can be lowered substantially by modulating the rate of gastric emptying and/or the incretin axis. It is clearly important that PERT be optimised, in regard to dose and timing (including giving it before eating), but also to ensure adequate mixing with ingested nutrients. Fat digestion is not necessarily normalised by current PERT regimens and our observations support the importance of further attempts to optimise PERT.

Further improvements in postprandial glycaemia might be gained through other agents that slow gastric emptying, such as 'prandial' administration of GLP-1 agonists, or by augmenting the action of the incretin hormones, such as with DPP-IV inhibitors. 'Prandial' GLP-1 agonists are being used successfully in type 2 diabetes, with or without basal insulin, to target postprandial hyperglycaemia (Fakhoury et al., 2010, Buse et al., 2011) and have the advantage of requiring less frequent administration and glucose monitoring than insulin therapy, and with a lower risk of hypoglycaemia (Pratley et al., 2011). These represent potentially significant advantages in a group that already has a high treatment burden.

Table 1 Study protocol for adolescents with cystic fibrosis (day 1 and day 2) and controls

Time (minutes)	-15	-10	-5	0	+15	+30	+45	+60	+75	+90	+105	+120	+150	+180	+210	+240
Blood glucose/ glucagon/ insulin/ GLP-1/GIP sample	Grey		Grey		Grey	Grey	Grey	Grey		Grey		Grey	Grey	Grey		Grey
Gastric emptying sample			Grey		Grey											
Pancreatic enzymes or placebo		Grey														
Pancake meal				Grey												

Grey square indicates time point variable occurred

Table 2. Subject and control characteristics

	CF (n=14)	Controls (n=7)	p value
Male	6	2	
Age (years)	13.1 (2.7)*	14.6 (3.1)	0.3
Puberty (Tanner ≥ 2)	7	5	
Height Z score	-0.4 (1.1)	1.1 (0.8)	0.005
Weight Z score	-0.2 (1.1)	0.6 (0.5)	0.1
BMI z score	0.12 (0.8)	0.2 (0.4)	0.8
Normal annual OGTT	12		
IGT	0		
CFRD	2		
FEV 1 (%)	92.4 (11.8)		
Pseudomonas positive last 12 months	5		

*mean (SD). p values calculated by ANOVA or Kruskal-Wallis test.

BMI, body mass index. OGTT, oral glucose tolerance test. IGT, impaired glucose tolerance. FEV, forced expiratory volume.

Figure 1 : Consort Flow Diagram

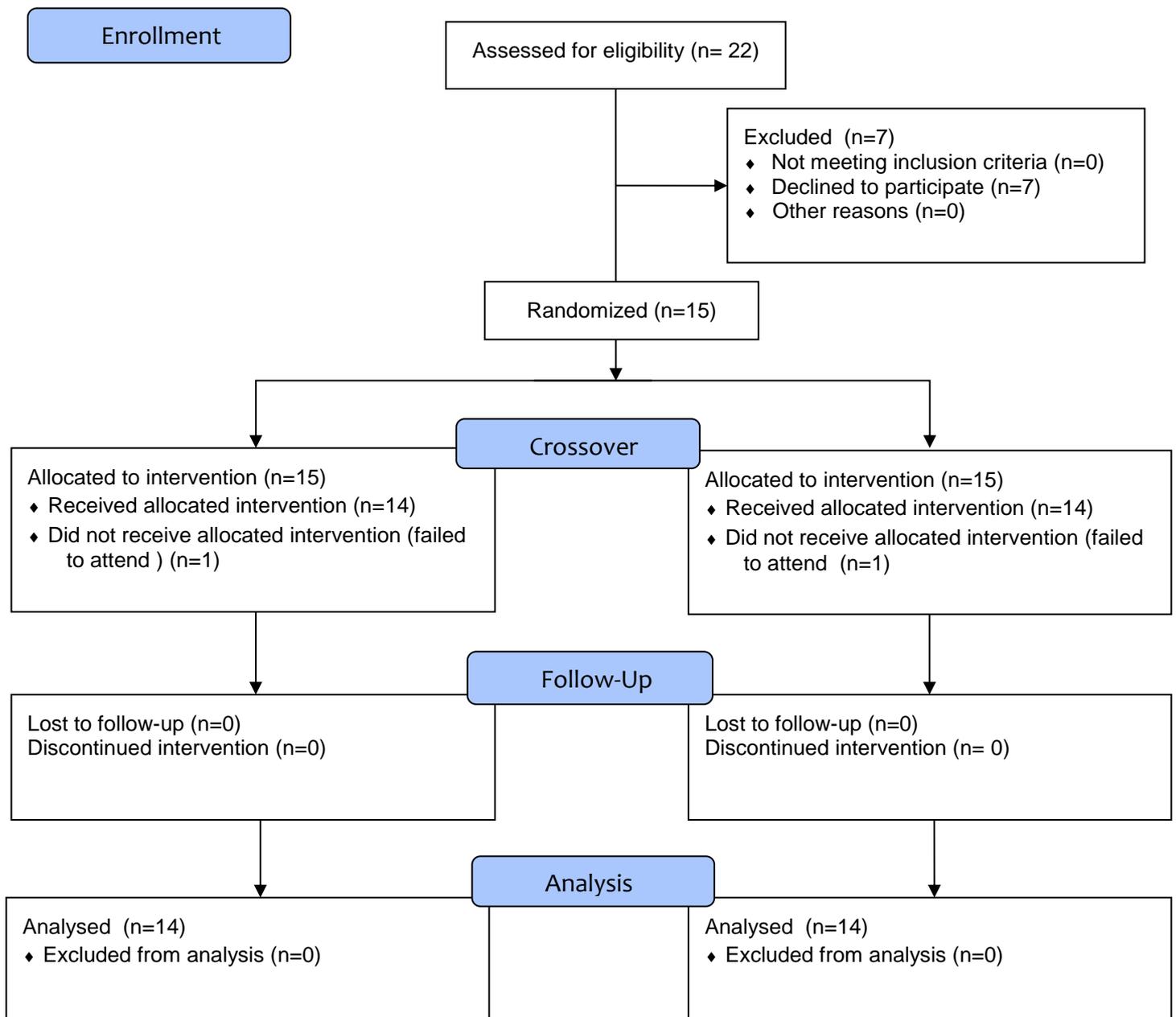


Figure 2.

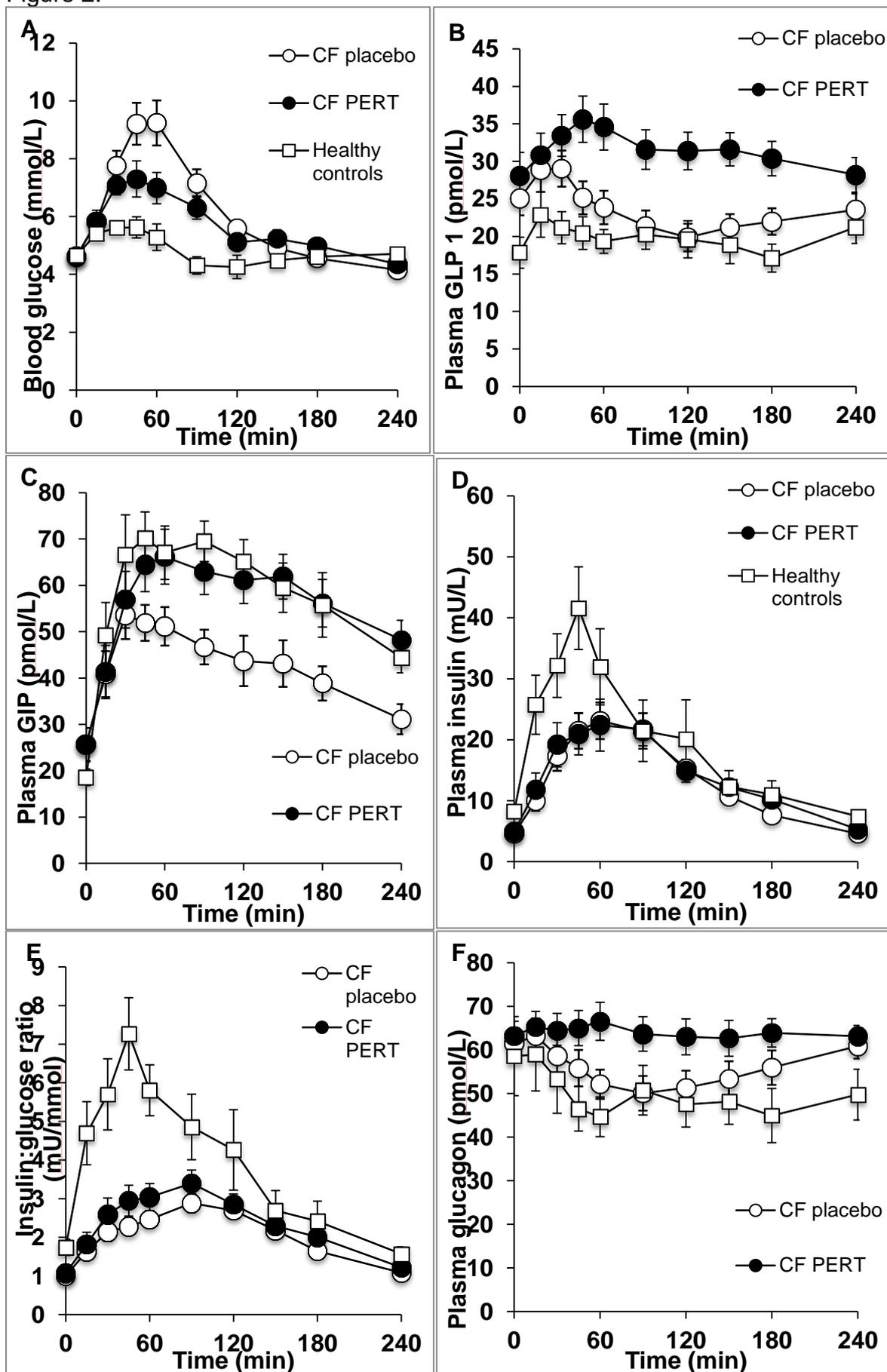


Figure 2. Plasma blood glucose (A), GLP-1 (B), GIP (C), insulin (D), insulin-to-glucose ratio (E), and glucagon (F) concentrations in CF patients with placebo and PERT and in healthy controls after a high fat pancake meal. Results represent mean \pm SE. A, Blood glucose was higher in CF with placebo than in controls ($P < 0.0001$). PERT reduced blood glucose but it remained higher than controls ($P = 0.0002$). B, iAUC GLP-1 was lower in CF than in controls ($P = 0.04$), and this was reversed by PERT. C, iAUC GIP was lower in CF than in controls ($P = 0.003$), and this was reversed by PERT. D, Insulin concentrations were lower in the CF than in controls ($P = 0.02$) and this did not improve with PERT ($P = 0.4$). E, Insulin-to-glucose ratio was lower in CF than in controls and this did not improve with PERT ($P = 0.3$). F, Glucagon concentrations were the same between CF and controls ($P = 0.3$). PERT increased glucagon in the CF ($P < 0.0001$)

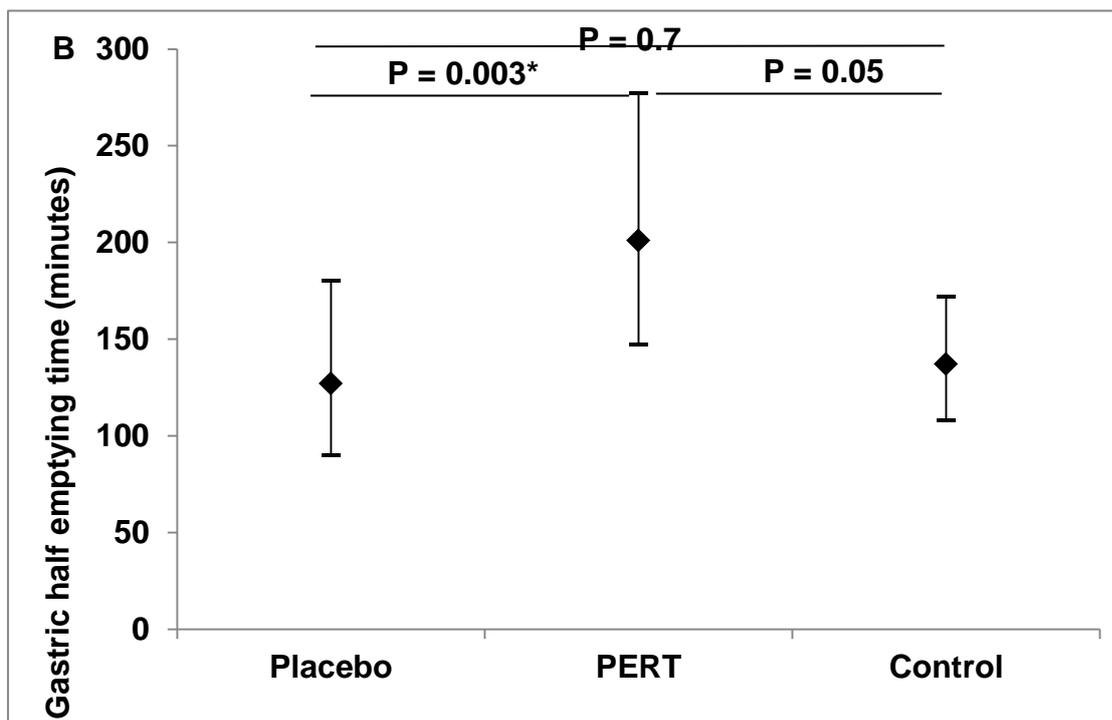
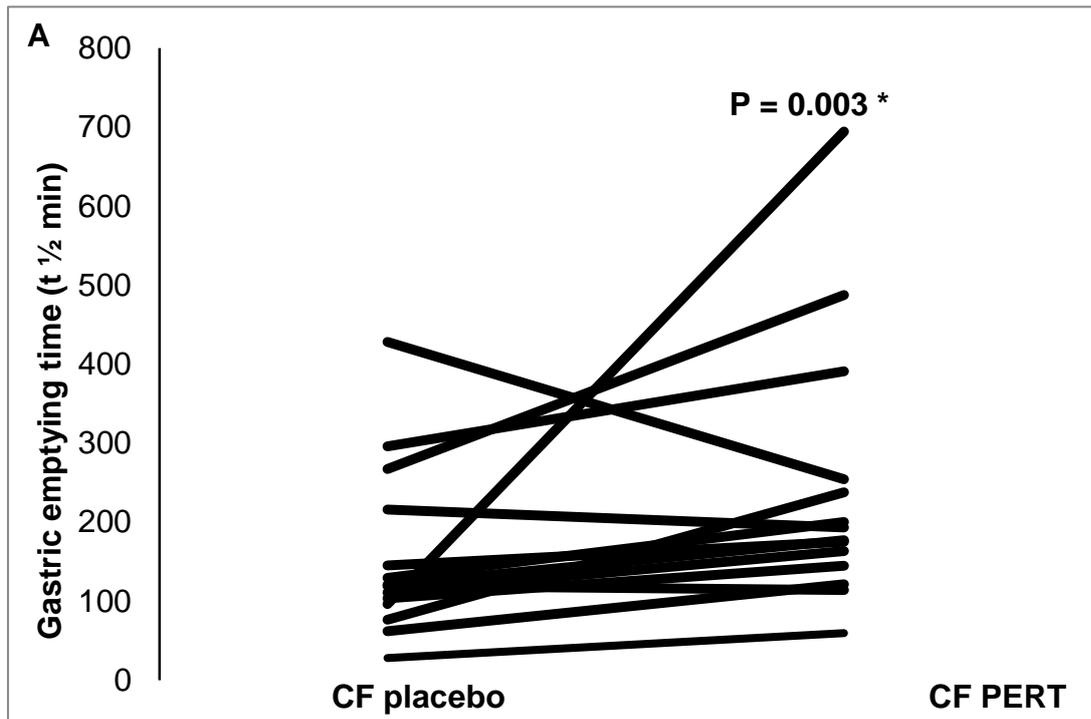


Figure 3. (A) Gastric half emptying time ($t_{1/2}$) for CF placebo and PERT groups (B) Gastric half emptying time for CF placebo and PERT and control groups. Results represent geometric mean with 95% confidence interval, * $P < 0.05$.

Dr Shiree Perano – Conducted study, including preparation of protocol, analysis of data and preparation of manuscript

Professor Jennifer Couper – Supervision, including preparation of protocol, analysis of data and correction of manuscript.

Professor Michael Horowitz – Supervision, including preparation of protocol, analysis of data and correction of manuscript.

Dr James Martin - Assisted in preparation of protocol and correction of manuscript.

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Thomas Sullivan - Assisted in statistical analysis and correction of manuscript.

A/Prof Chris Rayner – Supervision, including preparation of protocol, analysis of data, correction of manuscript, and assumes overall responsibility for the manuscript

I approve the final draft submitted and give permission for this paper to be included in the thesis.

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6 CHAPTER 6: RAPID GASTRIC EMPTYING IS A MAJOR DETERMINANT OF POSTPRANDIAL GLYCAEMIA IN ADOLESCENTS WITH TYPE 1 DIABETES.

6.1 Summary

Gastric emptying is a critical determinant of postprandial glycaemic control. This case control study aimed to quantify gastric emptying in adolescents with type 1 diabetes, compared with healthy controls, and examine its relationship with postprandial glycaemia, gastrointestinal symptoms and autonomic function.

We studied 30 adolescents (aged 15 ± 2.5 years, BMI z-score 0.6 ± 0.7) with type 1 diabetes and age and sex matched controls (gastric emptying $n = 20$, heart rate variability $n = 135$). Gastric half-emptying time of a solid meal was measured by ^{13}C -octanoate breath test. Cardio-autonomic function was measured by heart rate variability. Chronic and postprandial gastrointestinal symptoms were evaluated by questionnaire and visual analogue scales. Blood glucose concentrations were monitored frequently during the study.

Gastric emptying was more rapid in subjects with type 1 diabetes than controls (median half-emptying time 78 (IQR 61-99) versus 109 (71-124) minutes, $P = 0.02$). The postprandial rise in blood glucose at 60 minutes was strongly related to gastric half-emptying time ($R = -0.65$, $P = 0.0001$). Gastric emptying was slower in subjects with fasting hyperglycemia, but was not related to heart rate variability. Nausea, bloating and anxiety were related to fasting glycaemia.

Rapid gastric emptying is a major determinant of postprandial glycaemia in adolescents with type 1 diabetes. Therapies that modify the rate of gastric emptying may be of benefit in the management of postprandial glycaemia in this group.

6.2 Introduction

Optimal glycaemic control is critical in preventing the long term microvascular, and probably, macrovascular complications of diabetes (Nathan, 2014). Management has traditionally focused on fasting and pre-prandial glycaemia, however the fundamental contribution of postprandial glycaemia to HbA1c, and therefore the long term risk of complications, is now well recognised (Monnier et al., 2003, Woerle et al., 2007). Postprandial glycaemic control is influenced by the rate of gastric emptying, which accounts for up to 30% of postprandial blood glucose variability in health and diabetes (Horowitz et al., 1993, Jones et al., 2001). Both the magnitude of postprandial glycaemic excursions and peak postprandial blood glucose relate to the rate of gastric emptying (Jones et al., 2001, Horowitz et al., 1993), with more rapid emptying being associated with higher blood glucose after meals. Conversely, acute variations in blood glucose affect the rate of gastric emptying, with acute hyperglycaemia slowing gastric emptying and insulin induced hypoglycaemia accelerating it (Schvarcz et al., 1997); this may represent a protective mechanism to maintain euglycaemia.

In adults with long standing diabetes gastric emptying has been studied extensively. It is clear that delayed gastric emptying occurs frequently, affecting 30-50% of patients, although the magnitude of the delay is often modest (Jones et al., 2001, Samsom et al., 2003) and delayed emptying is weakly associated with markers of cardiac autonomic dysfunction (Rayner et al., 2001, Jones et al., 2002). Abnormally rapid emptying has been reported in patients with 'early' type 2 diabetes and some patients with longstanding type 1 diabetes (Bharucha et al., 2009, Phillips et al., 1991). In contrast the few studies that have assessed gastric emptying in

adolescents with type 1 diabetes have shown inconsistent results with either delayed emptying (Cucchiara et al., 1998, Heptulla et al., 2008), or no difference in emptying (Ersoy et al., 2013) described when compared with healthy controls.

Although gastrointestinal symptoms, particularly nausea, bloating and early satiety, are frequently reported in the adult diabetic population, they correlate poorly with the rate of gastric emptying and do not reliably predict abnormal gastric emptying (Jones et al., 2001, Samsom et al., 2003, Bharucha et al., 2009). Symptom assessment has usually focused on chronic symptoms, rather than evaluating meal-related symptoms concurrently with the assessment of gastric emptying. Glycaemia also potentially plays a role in symptom perception; patients with blood glucose levels $>11\text{mmol/L}$ have an increased perception of gastrointestinal symptoms, when compared with euglycaemia (Rayner et al., 2001). Gastrointestinal symptoms are often overlooked in children with type 1 diabetes, with their prevalence and relationship to gastric emptying and glycaemia poorly defined (Vogiatzi et al., 1996, Vazeou et al., 2004).

Autonomic dysfunction is an important complication of diabetes, known to be associated with increased mortality and morbidity, and is an important predictor of cardiovascular risk (Vinik et al., 2003, Burghen et al., 1992). Abnormal autonomic function is implicated in the pathogenesis of delayed gastric emptying in adults with long standing diabetes (Samsom et al., 2003, Jones et al., 2001). Abnormalities of heart rate variability (HRV), a measure of cardiovascular autonomic function, are evident in adolescents with type 1 diabetes (Pfeifer et al., 1984, Wawryk et al., 1997), but their relationship with gastric emptying has not been assessed.

We measured gastric emptying, postprandial blood glucose, gastrointestinal symptoms, and autonomic function in adolescents with type 1 diabetes. We hypothesised that, as in adults, gastric emptying would be slower than in healthy controls and be related to postprandial glycaemia, gastrointestinal symptoms and autonomic dysfunction.

6.3 Methods

6.3.1 Subjects

Thirty patients aged 10 – 18 years with confirmed type 1 diabetes of greater than 12 months duration were recruited consecutively over a 5 month period from the type 1 diabetes clinics at the Women's and Children's Hospital, which cares for >85% of such patients in South Australia. Patients with a history of gastrointestinal surgery, coeliac disease, or medications that affect gastrointestinal motility were excluded. Of 68 patients assessed, 20 did not meet the inclusion criteria, 17 declined, and one withdrew. 20 friends or siblings of the patients were recruited as healthy controls. Additional control data for HRV was obtained from community high school students (n=115).

Characteristics of the study groups were comparable (Table 1) and in the week prior to the study none of the type 1 patients had experienced ketosis (blood ketones >1.0 mmol/L). One patient was on thyroxine replacement and had been biochemically euthyroid for >6 months, while another had early background retinopathy. None of the remaining type 1 patients had any evidence of complications of diabetes. Twelve type 1 patients were taking metformin or placebo, or an angiotensin converting enzyme inhibitor with a statin or placebo, respectively. Medication was withheld and given at the completion of study measurements.

The protocol was approved by the Human Research Ethics Committee of the Women's and Children's Health Network. Written informed consent was obtained from each participant and a parent.

6.3.2 Protocol

Studies commenced at 8am with the subject having fasted from 9pm the previous evening. An IV cannula was inserted at the start of the study for blood sampling. Patients administered their usual rapid acting insulin according to their prescribed units of insulin/ grams of carbohydrate and insulin sensitivity factor, prior to the standardised test meal ($t = -15$ min), and then consumed the meal, consisting of 70g pancake mix (Green's™, NSW, Australia), 50mL water, and 100mg ^{13}C Na-octanoate, cooked with 10g butter (8.8g fat, 30.3g carbohydrate, 142.7kcal) within 5 minutes. Control subjects followed the same study protocol, including measurement of blood glucose concentrations. Breath samples for measurement of gastric emptying and blood samples for blood glucose concentrations were collected at $t = -25, -15, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240$ minutes (Table 1).

6.3.3 Measurements

Gastrointestinal symptoms

A Visual Analogue Questionnaire, validated for use in children over the age of 7 years (Shields et al., 2003), was administered during the gastric emptying study at $t = -25, 30, 60, 120, 180$ and 240mins to assess anxiety, meal-related nausea and bloating, and postprandial fullness. Chronic symptoms during the preceding 3 months were assessed by the modified Diabetes Bowel Symptom Questionnaire (Quan et al., 2003), which evaluated symptoms of abdominal pain, nausea and vomiting, bloating, gastro-oesophageal reflux, dysphagia, diarrhoea and

constipation, and was administered at the start of the study. Symptoms were considered significant if they occurred >25% of the time, and severe if they affected lifestyle. All questionnaires were administered and scored by one investigator.

Gastric emptying

Breath samples were analysed for $^{13}\text{CO}_2$ using an isotope ratio mass spectrometer (Europa Scientific ABCA 20/20, Crewe, England) and $^{13}\text{CO}_2$ excretion analysed using a non-linear regression model to calculate the gastric half-emptying time ($t_{1/2}$), time to maximum excretion (t_{max}), and gastric lag (time to first 5% of total $^{13}\text{CO}_2$ excreted)(Omari et al., 2005).

Blood glucose

Blood glucose was measured by the glucose oxidase method by YSI glucose analyser (YSI incorporated, Ohio, USA).

Autonomic function

A 10 minute continuous ECG recording was taken at $t = 180$ min (LabChart Pro, AD Instruments, Amsterdam, Holland). Recordings were performed in a quiet room with the subject supine at $t = 210$ mins. No caffeine had been consumed for 12 hours. The whole 10 minute recording was analysed, excluding ectopic beats (<500 ms, >1100ms). R-waves were marked after visual scanning by one investigator (SP). Derived time domain measures included the following: standard deviation of mean NN intervals (SDNN) (where NN is determined from adjacent QRS complexes) and root mean squared difference of successive NN intervals (RMSSD), which are estimates of overall HRV. Frequency domain measures included the following: low

frequency (LF), defined as >0.04 Hz and <0.15 Hz, and high frequency (HF) components, defined as >0.15 Hz and <0.4 Hz and the LF:HF ratio, considered an estimate of the relative sympathetic and parasympathetic balance (Cho et al., 2013). Overall HRV was expressed as the triangular index (total number of NN intervals/ number of NN intervals in the modal bin, where modal bin is the most commonly occurring NN interval determined by histogram plot) .

6.3.4 Statistical analysis

We calculated that 27 patients and 27 controls would provide 80% power ($p < 0.05$) to detect a 24 minute difference (SEM 15.4) in mean gastric half-emptying time. Statistical analyses were performed with STATA 13 software (StataCorp, Texas, USA).

Mean and standard errors (normally distributed variables) and median and interquartile ranges (for non-normally distributed variables) are reported. Independent samples t-tests were used for comparisons of normally distributed continuous variables and Mann-Whitney U-tests for comparisons of variables with skewed distributions. Chi-square tests of association were used to compare cases and controls for categorical variables. Pearson correlation coefficients were used to assess relationships between the different measures. Multiple linear regression was used to analyse relationships between gastric half-emptying time, gastrointestinal symptoms and postprandial change in blood glucose. P-values <0.05 were taken as significant.

6.4 Results

Other than diabetes the groups were well matched (Table 2). All subjects tolerated the protocol with no episodes of hypoglycaemia requiring treatment.

Gastric emptying

Gastric emptying, while within the control range, was more rapid in the type 1 diabetes patients (median half-emptying time 78; IQR 61- 99) than controls (109; IQR 71-124 minutes, $P = 0.02$) (Fig 1), and the difference remained significant when controls with chronic gastrointestinal symptoms were excluded. Gastric half-emptying time in those with type 1 diabetes did not relate to HRV and was independent of age, gender, BMI z-score, HbA1c, duration of diabetes, and total daily insulin dose.

Blood glucose concentrations

Fasting and peak postprandial blood glucose concentrations were higher in type 1 patients than controls (both $P < 0.05$) (Table 1). In type 1 subjects fasting blood glucose did not correlate with HbA1c ($R = -0.07$, $P = 0.72$). Postprandial glycaemic change was independent of whether insulin was administered by subcutaneous injection or by insulin pump.

Gastric emptying and glycaemia

The postprandial blood glucose increment ($t = 0-60\text{min}$) in type 1 patients was inversely related to the gastric half-emptying time ($R = -0.65$, $P = 0.0001$) (Fig. 2), while peak blood glucose and total area under the curve were not. Patients with fasting blood glucose levels above the median (14mmol/L) had slower gastric emptying than the remainder (half-emptying time 90; IQR 57-85 versus 73 IQR 72-

107 minutes, $P = 0.04$). There was no relationship between gastric emptying and postprandial glycaemia in controls.

Gastrointestinal symptoms and autonomic function

There was no difference between type 1 patients and controls in the prevalence of: (i) nausea, bloating, or anxiety before or after the meal, (ii) postprandial fullness, or (iii) chronic gastrointestinal symptoms.

There was no difference in heart rate or HRV between those with type 1 diabetes and controls.

Blood glucose and gastrointestinal symptoms

In type 1 diabetes, fasting nausea and bloating, and anxiety, were related to fasting blood glucose (regression co-efficient= 4.29, $P = 0.03$ and regression co-efficient= 4.30, $P = 0.03$ respectively), but no symptom was related to gastric emptying, after adjusting for blood glucose concentrations.

6.5 Discussion

We have shown, in contrast to our hypothesis based primarily on observations in adults, that adolescents with type 1 diabetes, as a group, have more rapid gastric emptying than healthy controls. Moreover, gastric emptying in these patients is clearly associated with greater postprandial glycaemic increments. Conversely, fasting hyperglycaemia is associated with slower gastric emptying, and an increase in upper gastrointestinal symptoms, as has been reported in adults (Woerle et al., 2007, Jones et al., 2001).

This study supports the concept that abnormalities of gastric emptying occur frequently in adolescents with type 1 diabetes, but to our knowledge it is the first to report rapid gastric emptying in this population. The limited number of previous studies in adolescents described either delayed (Heptulla et al., 2008, Cucchiara et al., 1998) or normal emptying (Ersoy et al., 2013). These differences may be attributed to variations in test meals, and diagnostic methods, or failure to account for the effects of glycaemia on gastric emptying. Cucchiara et al described delayed gastric emptying of a mixed meal by ultrasonography in 40 young people with type 1 diabetes; however the rate of gastric emptying may have been influenced by the study meal, which varied in energy content depending on the patient's age (Cucchiara et al., 1998). Ultrasonography is comparable to scintigraphy in the assessment of liquid emptying, however it is less reliable for a solid meal, and accuracy is also operator dependent. Heptulla measured gastric emptying by breath test and Ersoy's group used scintigraphy, which is considered the 'gold standard' technique (Heptulla et al., 2008, Ersoy et al., 2013). The ^{13}C breath test has been validated against scintigraphy, in healthy adults (Choi et al., 1997) and adolescents (Hauser et al., 2006), as well as adults with diabetes (Ziegler et al., 1996). It is considered acceptable as an alternate measure of gastric emptying in the paediatric setting, as it is a non-invasive test with no associated radiation exposure.

Heptulla et al ensured euglycaemia prior to the consumption of a liquid meal, followed by blood glucose monitoring postprandially, but their study was limited by a small sample size (n=7) and included only patients on insulin pump therapy; those on subcutaneous injection therapy were excluded (Heptulla et al., 2008). Ersoy et al had a sample size similar to our study (n=33) and ensured patients were not

hyperglycaemic when commencing the study, however glycaemia was not monitored after the meal (Ersoy et al., 2013).

We found that HRV and heart rate did not differ between patients and controls. In a recent study of 125 adolescent girls with type 1 diabetes, HRV was reduced, and resting heart rate was higher, when compared to controls (Cho et al., 2013). This difference may be explained by the higher BMI in the latter study cohort, as obesity is associated with sympathetic overdrive (Dangardt et al., 2011).

Both the rapid gastric emptying that we observed in our type 1 patients and its lack of relationship to autonomic dysfunction are in contrast to previous studies of adults with type 1 diabetes. The difference may be related to the shorter duration of the disease. It is also possible that the prevalence of gastroparesis in adults is now declining due to improved glycaemic control and a decrease in the risk of autonomic neuropathy. Longitudinal studies exploring the natural history of gastric emptying in diabetes are therefore indicated (Gubitosi-Klug, 2014).

The relationship between gastric emptying and glycaemia is complex and reciprocal. We observed that more rapid gastric emptying was associated with a greater increase in blood glucose during the first hour after the meal. This occurred despite rapid acting insulin being given 15 minutes before meal ingestion, as is generally recommended, although in our experience, adolescents often administer their insulin immediately before eating, which may further exacerbate early postprandial hyperglycaemia. Studies in health and in type 2 diabetes (Horowitz et al., 1993, Jones et al., 1995) corroborate this finding, with differences in the rate of gastric emptying accounting for up to 30% of postprandial glycaemic variation (Horowitz et al., 1993). Moreover, in type 1 patients with gastroparesis postprandial

insulin requirements are reduced (Ishii et al., 1994). As the rate of gastric emptying is a key determinant of the absorption of nutrients in the small intestine, it appears likely that the rapid emptying that we observed resulted in the large postprandial glycemic change, and not vice versa. Conversely, patients with a greater degree of fasting hyperglycaemia had slower gastric emptying, which is consistent with the adult literature (Schvarcz et al., 1997, Fraser et al., 1990), and suggests that gastric emptying would have been even faster if the subjects had been studied during euglycaemia. There is some evidence suggesting that adult type 1 patients may be less sensitive to the effect of elevated blood glucose concentrations on gastric emptying than type 2 or healthy subjects, (Woerle et al., 2008), but there are no previous data in adolescents. Rapid gastric emptying in this population may be the result of an abnormality in one or more of the elements involved in the regulation of gastric motility, including the intrinsic and extrinsic nervous system, and enteric hormones.

There was no relationship between the rate of gastric emptying and gastrointestinal symptoms. This is consistent with the literature, where any such relationship has been found to be weak at best, and the use of symptoms to predict disordered gastric emptying is therefore unreliable (Jones et al., 2001, Samsom et al., 2003, Bharucha et al., 2009). The majority of studies where symptoms have been assessed have, however, described chronic symptoms, and a strength of this study is the concurrent assessment of gastric emptying and gastrointestinal symptoms, even though this does not determine causality. Gastrointestinal symptoms may in part be attributable to disordered gastric emptying; however, it is more likely that symptom aetiology involves multiple variables, such as age, gender,

BMI, glycaemia, visceral hypersensitivity and psychological state (Talley et al., 2001, Rayner et al., 2001).

Fasting hyperglycaemia in our subjects was related to the presence of nausea and bloating, and anxiety. Glycaemia has been shown to play a role in symptom perception, with blood glucose levels >11 mmol/L associated with an increased perception of gastrointestinal symptoms, when compared with euglycaemia (Rayner et al., 2001). Fasting hyperglycaemia may have been secondary to anxiety and induced by the stress response to study conditions.

Strategies to reduce postprandial glycaemia include earlier administration of short-acting insulin and/or an increased dose; however the former is often difficult for adolescents to implement, and the latter increases the risk of hypoglycaemia. Modifying the rate of gastric emptying in order to optimise timing of the onset of insulin action and blunt the postprandial rise in glycaemia is an alternative therapeutic strategy. Dietary modification, such as the addition of fibre to a meal, slows gastric emptying and improves postprandial glycaemia in health and in type 2 diabetes (Chandalia et al., 2000, Benini et al., 1995, Hebbard et al., 1996). Alternatively, the addition of fat to a meal can also slow gastric emptying, with a modest improvement in postprandial glycaemia in adults with type 2 diabetes (Gentilcore et al., 2006a), and greater improvement in adolescents with type 1 diabetes (Lodefalk et al., 2008). A high fat diet, however, would not be advantageous in a population already at increased risk of vascular disease. Macronutrient preloads, such as whey protein, given prior to a meal, seem to be superior to fat in improving postprandial glycaemic control; the mechanism involves both slowing of gastric

emptying and stimulation of insulin secretion (Ma et al., 2009). Although the latter is unlikely to be of benefit in type 1 patients, the effects on gastric emptying would still be expected to be beneficial for glycaemia. Pharmacological agents that slow gastric emptying and improve postprandial glycaemic control include short acting ('prandial') glucagon-like peptide-1 (GLP-1) receptor agonists, such as exenatide, and the amylin analogue, pramlintide (Raman et al., 2010, Rodriguez et al., 2007). 'Prandial' GLP-1 receptor agonists are used in type 2 diabetes to improve postprandial glycaemic control through their ability to slow gastric emptying, stimulate insulin secretion and suppress glucagon production. The beneficial effect of exenatide on postprandial glycaemic control, primarily through the slowing of gastric emptying and glucagon suppression, has been shown in adults and children with type 1 diabetes (Raman et al., 2010, Dupre et al., 2004). Pramlintide delays and reduces peak postprandial blood glucose, in adults and children with type 1 diabetes (Rodriguez et al., 2007, Ratner et al., 2004), both by slowing gastric emptying and suppressing glucagon. Neither is associated with an increase in the frequency of hypoglycaemia

In summary, this study demonstrates that gastric emptying tends to be rapid in adolescents with type 1 diabetes, in contrast to the adult studies, and that it is a determinant of postprandial glycaemic excursions. Therapies that modify the rate of gastric emptying may be beneficial for optimising postprandial glycaemia in adolescents with type 1 diabetes, and should be investigated in subsequent studies.

Table 1. Study protocol for adolescents with type 1 diabetes and controls

Time (minutes)	-15	-5	0	+15	+30	+45	+60	+75	+90	+105	+120	+150	+180	+210	+240
Blood glucose sample	Grey	Grey	White	Grey											
Gastric emptying sample	White	Grey	White	Grey											
Acute symptom questionnaire	Grey	White	White	White	Grey	White	Grey	White	White	White	Grey	White	Grey	White	Grey
Chronic symptom questionnaire	White	Grey	White	White											
HRV trace	White	Grey	White												
Rapid acting insulin	Grey	White													
Pancake meal	White	White	Grey	White											

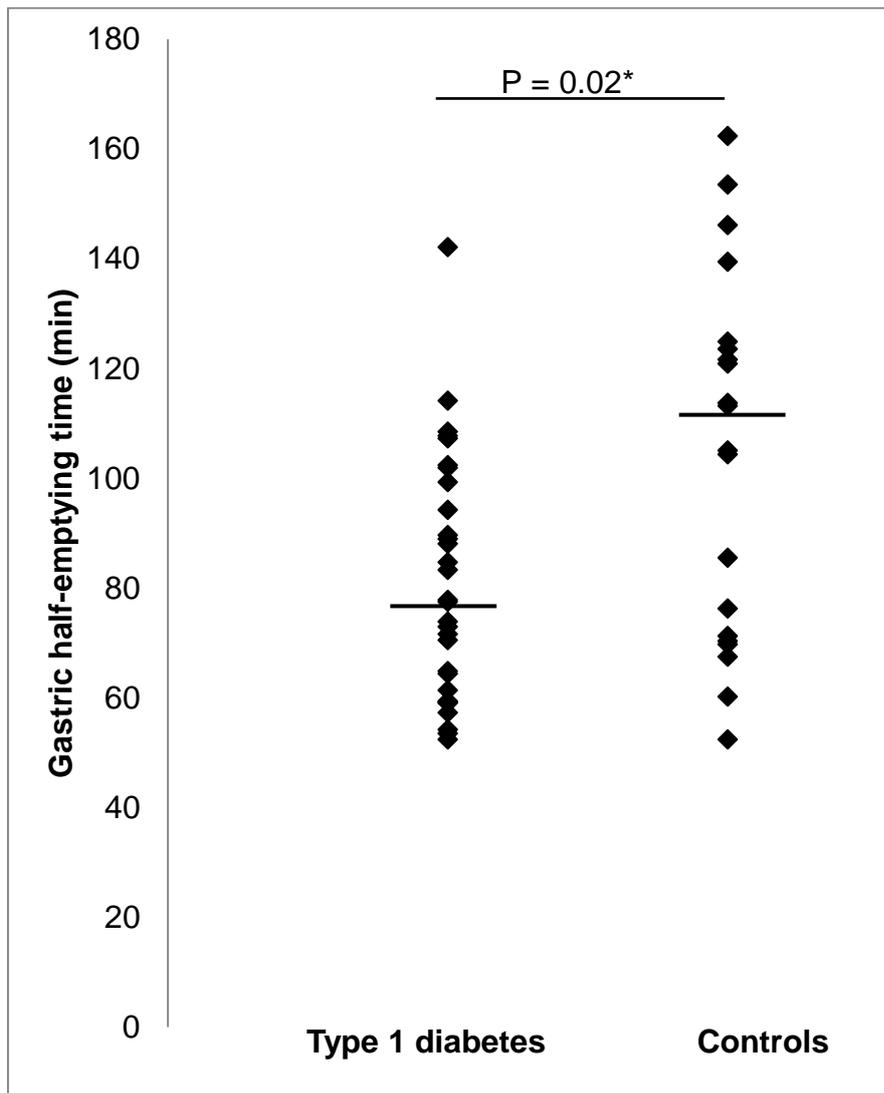
Grey square indicates time point variable occurred

Table 2: Characteristics of type 1 diabetes patients and controls

	Type 1 diabetes (n= 30)	Controls for gastric emptying (n=20)	P value ^a	Controls for HRV (n= 135)	P value ^b
Male n (%)	14 (47)	10 (50)	0.33	78 (53)	0.26
Age (yr)	15.1 ± 2.5	14.0 ± 3.5	0.22	15.5± 2.6	0.48
Height Z score	0.5 ±1.2	0.7 ± 2.5	0.72	0.29 ±1.4	0.50
Weight Z score	0.7 ± 0.9	0.5 ± 0.9	0.66	0.25 ± 1.0	0.04
Body mass index Z score	0.6 ± 0.7	0.6 ± 0.9	0.99	0.20 ± 1.0	0.04
Pubertal or postpubertal n (%)	21 (70)	11 (55)	0.82		
Insulin pump n (%)	17 (57)				
Diabetes duration (yr)	8.0 ± 4.6				
HbA1c(12 month mean)	8.7 ± 1.0				
Insulin total daily dose (units/ day)	45.9 ± 15.2				
Fasting blood glucose (mg/dl)	231 ± 92	83 ± 7	<0.001		
Change in blood glucose _{0-60mins} (mg/dl)	56 ± 70	13 ± 13	<0.001		
Peak blood glucose (mg/dl)	310 ± 67	117 ± 18	<0.001		
Fasting nausea/bloating n (%)	10 (33)	6 (40)	0.66		
Postprandial nausea/bloating n (%)	14 (47)	8 (53)	0.67		
Fullness t=30mins n(%)	17 (57)	8 (53)	0.83		
Anxiety n (%)	11 (37)	7 (47)	0.52		
≥2 chronic symptoms n (%)	5 (42)	4 (50)	0.40		
Severe chronic symptoms n (%)	8 (67)	6 (75)	0.67		
Heart rate (bpm) ^c	67 (60-74)			67 (60-76)	0.07
SDNN ^c	71 (52-87)			78 (55-104)	0.27
RMSSD ^c	68 (43-105)			76 (46-116)	0.67
LF power (ms ²) ^c	1042 (624-1982)			1507 (723-2623)	0.14
HF power (ms ²) ^c	1747 (510-4012)			1867 (912-4056)	0.81
LF : HF ^c	0.7 (0.4-1.1)			1.2 (0.8-1.2)	0.12
Triangular index ^c	16 (12-19)			17 (14-22)	0.23

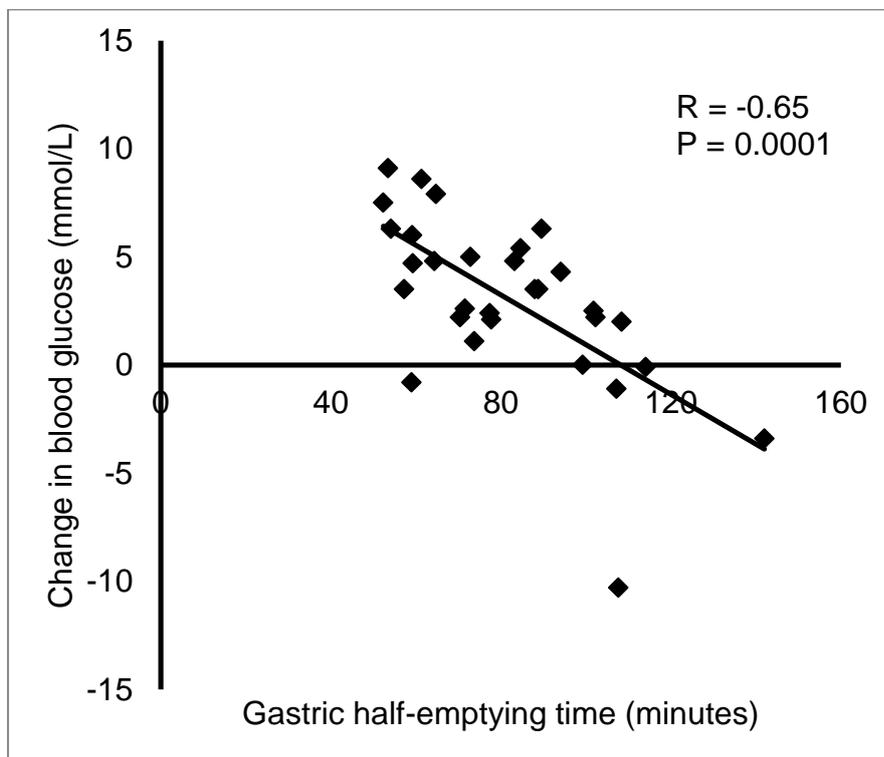
Table 1. Data as mean \pm SD. ^a Gastric emptying controls compared to type 1 diabetes patients. ^b Heart rate variability (HRV) controls compared to type 1 diabetes patients. ^c Data as median (IQR). SDNN, standard deviation of mean NN interval; RMSSD, root mean squared difference of successive NN intervals; LF, low-frequency; HF, high-frequency.

.

Figure 1. Gastric half-emptying time

Gastric half-emptying time (line indicates median) in type 1 diabetes (n=30) and controls (n=20).

Figure 2. Gastric emptying and change in postprandial blood glucose.



Relationship of change in postprandial glucose at 60min postprandial, and the gastric half-emptying time in patients with type 1 diabetes.

Dr Shiree Perano – Conducted study, including preparation of protocol, analysis of data and preparation of manuscript

A/Prof Chris Rayner – Supervision, including preparation of protocol, analysis of data and correction of manuscript.

Professor Michael Horowitz – Supervision, including preparation of protocol, analysis of data and correction of manuscript.

Stamatiki Kritas – Assisted in preparation of protocol and analysis of gastric emptying data and correction of manuscript.

Professor Kim Donaghue – Provided additional HRV control data, interpretation of HRV data and correction of manuscript.

Christine Mpundu-Kaambwa - Assisted in statistical analysis and correction of manuscript.

Lynne Giles - Assisted in statistical analysis and correction of manuscript.

Professor Jennifer Couper – Supervision, including preparation of protocol, analysis of data and correction of manuscript and assumes overall responsibility for the manuscript

I approve the final draft submitted and give permission for this paper to be included in the thesis.

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CHAPTER 7. CONCLUSION

This thesis provides new insight into gastric emptying and its relationship with postprandial glycaemia in young people with CF or T1D. These are two paediatric populations in which there have previously been few studies. In young people with T1D, gastric emptying was rapid when compared to healthy controls, and in both young people with CF and in T1D gastric emptying was strongly related to postprandial glycaemic excursions. In young people with CF PERT markedly attenuated postprandial hyperglycaemia by slowing gastric emptying and augmenting incretin hormone secretion.

In young people with CF, the effect of PERT in reducing postprandial hyperglycaemia highlights the importance of PERT, not only for improving fat malabsorption, but also in postprandial glycaemic control, a message that is not currently conveyed to patients and parents. Optimising PERT, through appropriate and timely dosing, is part of routine CFRD management, but can now be seen to be particularly important in the early stages of abnormal carbohydrate metabolism. Further studies are needed to explore the potential benefit of higher PERT doses, different PERT formulations, and different dosing schedules, before and during the meal, and the effect on postprandial glycaemia.

While PERT appears to 'normalise' incretin hormone concentrations, there may be additional benefit gained from pharmaceutical therapies, such as 'prandial' GLP-1 agonists, or DPP-IV inhibitors, either through their insulinotropic and glucagon suppressing actions, or their slowing of gastric emptying. In CFRD with fasting hyperglycaemia, the therapeutic combination of basal insulin therapy and a

'prandial' GLP-1 agonist may provide more effective pre- and postprandial glycaemic control than basal-bolus insulin therapy.

Studies of gastric emptying in adults with diabetes more commonly show delayed rather than rapid emptying, affecting approximately a third of patients. The work in this thesis, in contrast to adults, demonstrates rapid gastric emptying in young people with T1D. The rate of gastric emptying was also a major determinant of postprandial glycaemia, with rapid emptying associated with large postprandial glycaemic excursions. Conversely, fasting glycaemia influenced the rate of gastric emptying, with fasting hyperglycaemia associated with a relative slowing of emptying. This last finding is consistent with adult studies and illustrates the reciprocal relationship between gastric emptying and glycaemia. Optimising postprandial glycaemic control requires the consideration of both of these variables. Pharmacological therapies that slow gastric emptying in young people with T1D may be beneficial in the management of postprandial glycaemia, specifically prandial GLP-1 agonists, or an amylin agonist (eg. pramlintide). Optimal timing of rapid acting insulin, with adequate interval prior food consumption, and the use of a dual wave insulin bolus from an insulin pump (continuous subcutaneous insulin infusion), to better match insulin action to the rise in blood glucose, may also be of value.

The prevalence of gastrointestinal symptoms was similar in both T1D and controls, and did not relate to the rate of gastric emptying. While it remains important to recognise and investigate gastrointestinal symptoms in clinical practice, this study is further evidence that symptoms are not a reliable predictor of abnormal gastric emptying. Our findings also do not support a relationship between autonomic

dysfunction, as measured by HRV, and abnormal gastric emptying. Any relationship between autonomic function and gastric emptying is likely to be complex, and at best, account for only part of the variability.

Clinical research in a paediatric population comes with its own unique challenges and limitations. These studies are relatively unique in their use of the ^{13}C breath test as the diagnostic technique and further support its usefulness in this age group. Not only does the ^{13}C breath test involve no radiation exposure, it is easy to administer, and the breath samples are stable and can be stored and transported for analysis. These advantages facilitate large multicentre studies, in both young people with CF and T1D, to ratify and extend these studies. Longitudinal studies would provide valuable information on the unknown natural history of gastric emptying in this age group. This is particularly relevant in T1D, where the natural history of gastric emptying and its relationship with glucose variability is recognised as a knowledge gap in all age groups (Nathan, 2014). Further studies assessing therapies that modify gastric emptying, specifically ‘prandial’ GLP-1 agonists and amylin analogues, for the management of postprandial glycaemia in young people with CF and T1D are also warranted.

In summary, this thesis provides valuable insight into gastric emptying in young people with CF and with T1D, and its influence on postprandial glycaemic control. It highlights the importance of optimising and modifying current therapy; in CF through the timing and dosage of PERT, and in T1D through the timing and delivery of prandial insulin. It also indicates the potential for newer pharmacological agents which modulate gastric emptying to improve postprandial, and thereby overall, glycaemic control.

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