# Gastric and small intestinal motor function in health and disease – implications for glucose absorption, incretin hormone release, and postprandial blood glucose regulation

A thesis submitted by

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# Thesis summary

The human digestive tract is a complex system that, in addition to the digestion and absorption of nutrients, serves an important neuroendocrine role. The focus of this thesis is to examine how changes in the motor function of the gastroduodenal region influence glucose absorption, gut hormone secretion, and postprandial blood glucose regulation, in different human populations, including the healthy young and those with cystic fibrosis. The studies included utilise a mix of established and novel techniques to evaluate gastroduodenal motor function and glucose absorption, and provide insights into the function of the human gut.

Strict overall glycaemic control dramatically reduces the incidence and progression of micro-, and probably macrovascular, complications associated with type 1 and type 2 diabetes. Postprandial glycaemia is now recognised as an important determinant of overall glycaemia, as indicated by the glycated haemoglobin (HbA1c). The rate of glucose absorption after a meal has a major influence on postprandial glycaemia and has, therefore, been a focus of increasing research interest in recent years. Postprandial blood glucose concentrations are a poor indicator of glucose absorption due to peripheral glucose uptake and hepatic glucose release. The glucose analogue 3-O-methylglucose (3-OMG) is absorbed in the small intestine by the same mechanism as glucose, but is not metabolised, and its plasma concentrations are widely used as an index of glucose absorption. However, analysis of plasma 3-OMG concentrations requires chromatographic

methods which are labour-intensive and costly. By labeling 3-OMG with the $^{14}C$ radioisotope, plasma $^{14}C$-3-OMG activity can be measured by the rapid and inexpensive method of liquid scintillation counting. In Chapter 6, plasma $^{14}C$-3-OMG activity was shown to correlate closely to plasma concentrations of 3-OMG, after concomitant oral administration. $^{14}C$-3-OMG therefore represents a convenient alternative to 3-OMG, for measuring enteral glucose absorption.

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are secreted by the L and K cells in the intestines respectively, in response to nutrient-gut interactions. Their main function is the augmentation of glucose-induced insulin release from the pancreas, the so-called “incretin effect”. GLP-1 also possesses a potent inhibitory effect on gastric emptying, arguably the dominant mechanism through which GLP-1 lowers postprandial blood glucose. However, unlike GIP, the release of which is roughly proportional to the amount of glucose entering the small intestine, a caloric threshold of 1.8 kcal/min has been reported to exist for the release of GLP-1, below which the GLP-1 secretory mechanism is not stimulated. In the study described in Chapter 7, by performing a retrospective analysis of data collated from several studies performed previously, a transient, early release of GLP-1, in response to intraduodenal glucose delivery at the rate of 1 kcal/min, was demonstrated. While the functional significance of this observation remains uncertain, this early release of GLP-1 might serve the role of “priming” the gluco-regulatory system, in anticipation for the subsequent arrival of a larger nutrient

load. Furthermore, the mechanism for this early, transient release of GLP-1 remains to be further investigated, as the GLP-1 secreting intestinal L-cells are located most densely in the distal, rather than the proximal, small intestine.

It is established that differences in the rate of gastric emptying contribute to approximately one-third of the variation in the initial rise in postprandial glycaemia, but the contribution made by duodenal motor activity is much less well defined. An increase in duodenal motility, as measured by the number of pressure waves and propagated pressure wave sequences, has been shown to be associated with increased glucose absorption. More recently, using a combined manometry and impedance monitoring technique, it has been demonstrated that duodenal flow events may be a more important determinant of glucose absorption than pressure waves. Impedance monitoring is capable of measuring intraluminal movement of both fluid and air, and can be used in the proximal small intestine to measure the flow of intraluminal chyme. Compared to manometry, impedance monitoring correlates better with fluoroscopy, for measuring movement of small intestinal intraluminal content. In Chapter 8, it was demonstrated that despite stimulating duodenal pressure waves pharmacologically, using the prokinetic agent metoclopramide, there was no concomitant increase in the number of duodenal flow events, as measured by impedance monitoring, and no associated change in glucose absorption. These findings are consistent with those of a previous study using the anti-motility agent, hyoscine butylbromide, and both reinforce the importance of duodenal flow events in determining glucose

absorption, and highlight the value of combining impedance monitoring with manometry in assessing small intestinal motor function and nutrient absorption.

Delayed gastric emptying affects up to 50% of outpatients with long-standing type 1 and type 2 diabetes, often causing persistent upper gut symptoms that are difficult to manage. Acute hyperglycaemia, in a dose-dependent manner, exerts a number of reversible effects on upper gut motor function, including relaxation of the gastric fundus, suppression of antral motility, stimulation of pyloric contractions, and slowing of gastric emptying. In contrast to gastric motor function, data regarding the effects of hyperglycaemia on small intestinal motor function are scarce. Furthermore, there is little information regarding the effects of hyperglycaemia on incretin hormone release and intestinal glucose absorption. The study described in Chapter 9, using the combined manometry and impedance monitoring technique, demonstrated that acute hyperglycaemia in the physiological postprandial range (~9 mmol/L) had minimal impact on duodenal pressure waves and flow events, but reduced fasting plasma GLP-1 concentrations, and increased postprandial GIP secretion and small intestinal glucose absorption. The mechanism for these observations remains to be determined, but may involve changes in the small intestinal mucosa related to hyperglycaemia.

Nitric oxide is a major inhibitory neurotransmitter in the gut, and an increase in its availability has effects on gastropyloric motility and gastric emptying that are

similar to those observed during acute hyperglycaemia. Therefore, nitric oxide may be a mediator of the effects of hyperglycaemia on upper gut motor function. Using the specific nitric oxide synthase inhibitor, NG-nitro-L-arginine-methylester (L-NAME), the study described in Chapter 10 demonstrated that the delay in gastric emptying induced by acute hyperglycaemia (~15 mmol/L) was indeed mediated by nitric oxide, and may involve the modulation of tonic pyloric activity. In addition, nitric oxide may be involved in the release of insulin.

Cystic fibrosis (CF) affects approximately 1 in 2,500 live births in western societies, and the life-expectancy of these patients has risen dramatically as a result of improved medical care. However, this is accompanied by a rapid rise in many long term co-morbidities such as diabetes, which affects ~75 % of all CF patients by the age of 30. Cystic fibrosis-related diabetes (CFRD) is distinct from type 1 and type 2 diabetes, and is characterised by postprandial, rather than fasting, hyperglycaemia. Persistent fat malabsorption occurs in up to 20 % of CF patients, despite pancreatic enzyme supplementation, and fat malabsorption is known to accelerate gastric emptying in both healthy subjects and type 2 diabetes patients. The breakdown of fat is also required to stimulate the release of incretin hormones from the gut. Therefore, fat digestion in CF may be an important factor in determining the rate of gastric emptying and incretin hormone secretion, and consequently, postprandial glycaemia. The study described in Chapter 11 demonstrated that without pancreatic enzyme supplementation, CF patients had more rapid gastric emptying, reduced incretin hormone secretion, and exaggerated

postprandial glycaemic excursions compared to healthy subjects, after a solid high fat, high carbohydrate meal, and that these abnormalities were either substantially improved or normalised by pancreatic enzyme supplementation. Furthermore, the failure of enzyme supplementation to normalise GIP secretion, as opposed to the complete restoration of the GLP-1 response, suggests that mixing of enzymes with food in the proximal small intestine, where GIP-secreting K cells are predominantly located, is suboptimal. Therefore, strategies to optimise mixing between food and enzymes in the proximal small intestine, or incretin-based approaches such as the administration of GIP analogues, represent potential novel approaches in the management of postprandial hyperglycaemia and diabetes in CF.

The recent rapid rise in the prevalence of type 2 diabetes, and the importance of good overall glycaemic control in reducing the long term complications of diabetes, has prompted intense research into new ways to optimise diabetes management. Gastroduodenal motor function has a major influence on glucose absorption, incretin hormone secretion, and postprandial glycaemia, and thus represents an ideal therapeutic target, illustrated by the recent development of GLP-1-based therapies (such as the GLP-1 analogue exenatide, and the DPP-IV inhibitor sitagliptin) for the treatment of type 2 diabetes. However, many areas are still incompletely understood. Further studies are warranted to investigate the relationships between gastroduodenal motor function, glucose absorption, and

incretin hormone secretion, and their impact on postprandial blood glucose regulation.

# Declaration

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# Publications arising from this thesis

The materials in this thesis formed the basis for the publications listed below:

Kuo P, Rayner CK, Jones KL, and Horowitz M. Pathophysiology and Management of Diabetic Gastropathy: A Guide for Endocrinologists. Drugs 2007;67(12):1671-87.

Kuo P, Rayner CK, and Horowitz M. Gastric emptying, diabetes, and aging. Clinics in Geriatric Medicine 2007;23(4):785-808.

Kuo P, Chaikomin R, Pilichiewicz A, O'Donovan D, Wishart JM, Meyer JH, Jones KL, Feinle-Bisset C, Horowitz M, and Rayner CK. Transient, early release of glucagon-like peptide-1 during low rates of intraduodenal glucose delivery. Regulatory Peptides 2008;7;146(1-3):1-3.

Kuo P, Gentilcore D, Nair N, Stevens JE, Hausken T, Gilja OH, Wishart JM, Lange K, Horowitz M, Jones KL, and Rayner CK. The nitric oxide (NO) synthase inhibitor, NG-nitro-L-arginine-methyl-ester (L-NAME), attenuates the delay in gastric emptying induced by hyperglycaemia in healthy humans. Neurogastroenterol Motil 2009;21(11):1175-e103.

Kuo P, Bellon M, Wishart JM, Smout AJ, Holloway RH, Fraser RJ, Horowitz M, Jones KL, Rayner CK. Effects of metoclopramide on duodenal motility and flow events, glucose absorption, and incretin hormone release, in response to intraduodenal glucose infusion. (Submitted for publication)

Kuo P, Stevens JE, Russo A, Maddox A, Wishart JM, Jones KL, Greville HW, Hetzel DJ, Chapman IM, Horowitz M, and Rayner CK. Gastric emptying, incretin hormone secretion, and postprandial glycaemia in cystic fibrosis – effects of pancreatic enzyme supplementation. (Submitted for publication)

Kuo P, Wishart JM, Bellon M, Smout AJ, Holloway RH, Fraser RJ, Horowitz M, Jones KL, Rayner CK. Effects of physiological hyperglycaemia on duodenal motility and flow events, glucose absorption, and incretin hormone secretion in healthy humans. (Submitted for publication)

Kuo P, Chaikomin R, Bellon M, Jones KL, Horowitz M, Rayner CK. Validation of $^{14}$C-3-O-methylglucose as a measure of intestinal glucose absorption in humans. (Submitted for publication)