

**A Novel Nutritional Approach to the
Management of Type 2 Diabetes:
Effects of Nutritional Preloads on
Postprandial Blood Glucose and Gastric
Emptying in Type 2 Diabetes Mellitus**

**A thesis submitted by
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MBBS, FRACP**

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Doctor in Philosophy**

**Discipline of Medicine
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THESIS SUMMARY

This thesis focuses on the impact of dietary protein and fibre preloads on postprandial blood glucose and gastric emptying in type 2 diabetes mellitus.

Key themes relate to:

- 1) The longitudinal evaluation of gastric emptying in type 2 diabetes
- 2) The evaluation of gastric emptying as measured by means of a ^{13}C -octanoic acid breath test in older adults with type 2 diabetes
- 3) The evaluation of the acute effects of low dose 'preloads' of whey and guar, given alone or in combination before a meal, on postprandial glycaemia, GLP-1, insulin, and gastric emptying
- 4) The evaluation of the effects of 12 weeks' treatment with a whey/guar preload on gastric emptying, postprandial glycaemia, and glycated haemoglobin (HbA1c), in type 2 diabetes.

It is well established that the rate of gastric emptying plays a major role in determining the early postprandial glycaemic response, and that interventions that slow gastric emptying can reduce postprandial glycaemic excursions in type 2 diabetes. Gastric emptying is regulated by inhibitory feedback arising from the interaction of nutrients with the small intestine, mediated in part by the secretion of gut hormones. Gastric emptying is often disordered in people with type 2 diabetes; the emptying of solids and/or nutrient liquids is abnormally delayed in up to 30-50% with patients with longstanding type 2 diabetes, while gastric emptying may be accelerated in 'early' type 2 diabetes, although this has not been a consistent observation. Dietary strategies that slow gastric emptying, such as the use of 'preloads' (small quantities of macronutrient given in advance of the main meal), represent an appealing approach to reducing postprandial glycaemia. However, if modulating the rate of gastric emptying is to be

developed further as a therapeutic strategy for postprandial glycaemic control, additional information is required about the natural history of gastric emptying in these patients. In the study reported in Chapter 2, I explored how the rate of gastric emptying changed over an interval of some 14 years in a group of patients with type 2 diabetes.

The intra-individual variation of gastric emptying in health is modest, so that emptying is highly reproducible, although there is substantial inter-individual variation. Studies from tertiary referral centres involving patients with longstanding (typically 8-12 years) type 2 diabetes, relatively poor glycaemic control (HbA1c >8.5%), and a high prevalence of microvascular complications, indicate that 30-50% have abnormally delayed emptying of solids and/or nutrient liquids, whether studied by scintigraphy or stable isotope breath test, while a few have rapid emptying. Conversely, patients with 'early' type 2 diabetes (< 2 years) and/or an absence of autonomic neuropathy have been reported to have abnormally rapid emptying for solids and/or liquids, although this has not been observed in all series. Gastric emptying has a profound impact on control of glycaemia in type 2 diabetes, such that more rapid emptying of a glucose drink is associated with a higher blood glucose response. Scintigraphy is the gold standard technique used to quantify gastric emptying, but requires exposure of patients to ionising radiation and access to specialised equipment and personnel to undertake the assessment. An alternative method of measuring gastric emptying is by means of a stable isotope breath test, which can be used in an office-based setting, and has been validated against scintigraphy in both health and type 2 diabetes. There is also conflicting information regarding the effect of ageing on gastric emptying, with reports that emptying is either slowed, accelerated, or unchanged when compared to the young. When assessing the effects of type 2 diabetes on gastric emptying, it is important to select an age-matched healthy control group, particularly since type 2 diabetes cohorts tend to be older than the general population. In the study reported in Chapter 3, I evaluated the rate of gastric emptying using a

¹³C-octanoic acid breath test in community-based patients with type 2 diabetes, and compared this with age-matched healthy controls.

Nutritional strategies to reduce postprandial glycaemia are attractive, and represent the greatest opportunity for optimising glycaemic control at an affordable cost as the healthcare demands of society escalate. Both the rate of gastric emptying, and the actions of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), are major determinants of postprandial glycaemic excursions. In type 2 diabetes, the insulintropic effect of GIP is diminished, whereas GLP-1 retains its capacity to stimulate insulin secretion, and also slows gastric emptying and suppresses glucagon secretion and energy intake. Stimulation of GLP-1 secretion is appealing in the management of type 2 diabetes. Whey protein, a by-product of the cheese-making process, can reduce postprandial glycaemia when taken with, or before, a meal, through interrelated mechanisms including enhancement of insulin and gut hormone secretion, slowing of gastric emptying, and reductions in appetite and energy consumption. Guar gum is a viscous soluble fibre, and when given with a meal, can decrease postprandial glycaemic excursions by slowing gastric emptying and inhibiting small intestinal absorption of glucose, associated with reduced, rather than increased plasma insulin levels, as well as attenuation of plasma GLP-1. Accordingly, combining both guar gum and whey protein in a dietary supplement may be advantageous. However, the relative contribution of whey and guar to glucose-lowering and slowing of gastric emptying when used alone, and whether their actions are additive or synergistic when given together, are uncertain. In the study reported in Chapter 4, I evaluated the comparative acute effects of whey protein and guar gum preloads, either alone or in combination, on postprandial glycaemia, GLP-1, insulin, and gastric emptying in type 2 diabetes.

For the majority of people with type 2 diabetes who have relatively good overall glycaemic control ($\text{HbA1c} \leq 7.9\%$), postprandial glycaemia predominates over fasting blood glucose in contributing to HbA1c. Indeed, a 'target' HbA1c of $\leq 7\%$ is difficult to achieve without

minimizing postprandial glycaemic excursions. We have previously established that acute administration of high doses of whey protein preloads (50g) slows gastric emptying of the subsequent meal. An acute study in subjects with type 2 or pre-diabetes reported a preload incorporating low-dose whey (17g) together with guar (5g) slows gastric emptying and reduces postprandial glycaemia. However, it remains to be determined if a 'pre-load' strategy to reduce glycaemia are sustained with long-term use. Similarly, while a pre-load slows gastric emptying acutely, it is unknown whether this effect is sustained with daily pre-load administration. Moreover, whey supplements incur a substantial energy burden, so it is also important to determine if patients compensate by adjusting their overall intake with long-term use. In the study reported in Chapter 5, I evaluated the effects of a twice daily low dose whey/guar preload taken 15 minutes before breakfast and dinner over 12 weeks, in 79 patients with type 2 diabetes and relatively good glycaemic control treated with diet or metformin only, on HbA1c, gastric emptying, postprandial glycaemia and body weight and composition, in a single-blind, randomized, placebo-controlled trial.

DECLARATION

I, Linda Ernestine Watson (nee Mignone),

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

Date: 26/02/2018

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LE. Watson, L. K. Phillips, T. Wu, M. J. Bound, K.L. Jones, M. Horowitz, C. K. Rayner.

Discipline of Medicine, The University of Adelaide, Adelaide Australia

Australian Diabetes Society – ADEA Annual Meeting,

Perth, August 2017

A low-dose whey protein preload slows gastric emptying and improves post-prandial glycaemia in type 2 diabetes (Oral Presentation) L.E.Watson, T.Wu, M.Bound,

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A low-dose whey protein preload slows gastric emptying and improves post-prandial glycaemia in type 2 diabetes (Oral Presentation) L.E.Watson, T.Wu, M.Bound, H.Checklin, K.Jones, M.Horowitz, C.Rayner. Discipline of Medicine, The University of Adelaide, Adelaide Australia

Australian Diabetes Society – ADEA Annual Meeting,

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A low-dose whey protein preload slows gastric emptying and improves post-prandial glycaemia in type 2 diabetes (Clinical Oral Presentation) L.E.Mignone, T.Wu, M.Bound, H.Checklin, K.Jones, M.Horowitz, C.Rayner. Discipline of Medicine, The University of Adelaide, Adelaide Australia

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A low-dose whey protein preload slows gastric emptying and improves post-prandial glycaemia in type 2 diabetes (Poster Presentation) L.E.Mignone, T.Wu, M.Bound, H.Checklin, K.Jones, M.Horowitz, C.Rayner. Discipline of Medicine, The University of Adelaide, Adelaide Australia

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CHAPTER 1 – WHEY PROTEIN, GUAR GUM, POSTPRANDIAL GLYCAEMIA AND GASTRIC EMPTYING

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1.1 Introduction

It is well established that in both type 1 and type 2 diabetes the risk of microvascular, and to a lesser extent macrovascular complications, is closely related to ‘average’ glycaemic control as assessed by glycated haemoglobin (HbA1c). In people with type 2 diabetes who have relatively good glycaemic control, postprandial hyperglycaemia predominates over preprandial blood glucose in contributing to HbA1c (Monnier, Lapinski et al. 2003; Riddle, Umpierrez et al. 2011). Accordingly, focusing on postprandial glycaemia in patients with mild or moderate elevation of HbA1c is now appreciated as an important management strategy; indeed, achieving a ‘target’ HbA1c of $\leq 7.0\%$ is difficult without minimising postprandial glycaemic excursions (Inzucchi, Bergenstal et al. 2012; Monnier 2000). The potential use of dietary manipulations to reduce postprandial glycaemia is intuitively appealing, particularly given the escalation in health care costs with the rising incidence of type 2 diabetes.

Whey, a by-product of cheese making, is gaining recognition as an important functional food (Smithers 2008). Whey protein has been demonstrated to diminish postprandial glycaemia through various interrelated mechanisms including enhancement of insulin and incretin hormone secretion, slowing of gastric emptying, and reductions in appetite and energy consumption (Figure 1). These properties suggest the potential for whey in the management of type 2 diabetes. However, whey protein cannot be endorsed as a potential treatment until further studies show that it improves long-term glycaemic control without significant adverse outcomes.

This literature review will explore the different forms of whey protein and compare the effects of whey with other sources of protein in reducing postprandial glycaemia. It will address the mechanisms by which whey lowers glycaemia, the factors that need to be considered for optimal use of whey, and the effects of long term consumption of whey protein on glycaemic control, together with its potential adverse effects.

1.1.1 Comparison of whey and casein and forms of whey protein – isolate, concentrate and hydrolysate.

Milk proteins are an important amino acid source for young mammals; they facilitate uptake of nutrients and trace elements (Sharma 2011) and provide a source of bioactive peptides with a range of physiological functions (Nagpal, Behare et al. 2011; Pihlanto-Lappala 2011; Sharma 2011). Cow's milk contains about 3.5g of protein per 100ml, of which whey accounts for about 20% and casein 80% (Madureira, Tavares et al. 2010; McGregor & Poppitt 2013; Pihlanto 2011).

Whey consists of a heterogeneous group of proteins (van Meijl, Vrolix et al. 2008), including beta-lactoglobulin (35%), alpha-lactalbumin (12%), proteose peptone (12%), immunoglobulins (8%), and bovine serum albumin (5%) (de Wit 1998; Krissansen 2007; Madureira, Tavares et al. 2010). When chymosin is used in the cheese-making process, glycomacropeptide - which is high in branched chain amino acids - accounts for about 12% of total protein in whey (Marshall 2004). Up to 1% of the total protein content of whey comprises 'low abundance' proteins, including lactoferrin, and lactoperoxidase (Krissansen 2007). All these proteins have been reported to have nutritional and/or physiological functions (Smithers 2008).

Whey is seen as a more attractive protein for use as a dietary supplement compared to casein, due to differences in the amino acid composition and absorption kinetics between the two proteins (Bendtsen, Lorenzen et al. 2013). Whey protein has a higher proportion of branched chain amino acids than casein, (Hall, Millward et al. 2003) and is more soluble in the acidic

environment of the stomach, leading to more rapid digestion (Boirie, Dangin et al. 1997) – hence it is termed a ‘fast’ protein (Petersen, Ward et al. 2009), while casein is a ‘slow’ protein (Bendtsen, Lorenzen et al. 2013; Mahe, Roos et al. 1996). Using ¹³C-leucine-labelled whey and casein protein, Boirie et al demonstrated in healthy subjects that whey protein results in more rapid appearance, and higher peak plasma concentrations of amino acid, when compared with casein (Boirie, Dangin et al. 1997), while Stranstrup et al reported that levels of amino acids after a fat rich meal containing whey were substantially higher when compared to the same meal containing casein (Stranstrup, Schou et al. 2014). As a result of greater solubility, more rapid digestion, and resultant higher plasma concentrations of amino acids, whey appears to be the more favourable protein to provide nutritional and functional benefits.

Forms of whey protein – isolate, concentrate and hydrolysate

Whey protein is available in three forms: concentrate, isolate, and hydrolysate. Whey protein concentrate contains 35-80% protein, with fat, lactose and minerals making up the remainder; whey protein isolate contains 85-90% protein and very little fat or lactose (Marshall 2004; Smithers 2008; Walzem, Dillard et al. 2002); and whey protein hydrolysate consists of proteins that have undergone hydrolysis by proteolytic enzymes (Krissansen 2007). Whey hydrolysates and isolates are more costly than whey concentrates, which is an important consideration if whey protein is to be used for a prolonged period of time in the management of type 2 diabetes. It is therefore important to consider the evidence that one form of whey protein is more ‘functional’ than another.

Protein hydrolysates are usually more rapidly absorbed than the intact protein (Manninen 2004), but since intact whey is already a rapidly digested protein, any difference is likely to be minimal (Baro, Guadix et al. 1995; Boza, Martinez-Augustin et al. 1995). Some studies have suggested that whey hydrolysates may stimulate insulin and glucose-dependent insulinotropic polypeptide (GIP) secretion to a greater degree than the intact protein (Calbet & Holst 2004; Power, Hallihan et al. 2009). Mortensen et al investigated the effects of adding

45g of four different whey protein formulations (whey hydrolysate, whey isolate, alpha-lactalbumin enhanced whey, and caseino-glyco-macro-peptide (CGMP) enhanced whey) to a high fat/carbohydrate meal in subjects with type 2 diabetes (Mortensen, Holmer-Jensen et al. 2012), and reported that the first phase insulin response (as assessed by the incremental area under the curve (iAUC) up to 30 min) was enhanced after whey hydrolysate compared with the other three supplements, and that whey isolate and whey hydrolysate yielded a greater overall insulin response (iAUC at 480min) than the other two supplements, without any difference between them. Whey proteins which have been hydrolysed are, however, usually less palatable (Claessens, Calame et al. 2009), which detracts from their potential therapeutic use. There is no compelling evidence that one form of whey protein is significantly more potent than another, particularly in relation to reduction of postprandial glycaemia, so consideration of palatability and cost must also be taken into account.

1.2 Role of the incretin hormones, GIP, GLP-1 in protein induced insulin secretion

The phenomenon by which insulin secretion is increased when glucose is given by the enteral route, when compared to an isoglycaemic intravenous glucose infusion, is called the ‘incretin effect’, and is attributed to the secretion of ‘incretin’ hormones from the gut. The two known incretin hormones, glucagon-like-peptide-1 (GLP-1) and GIP, exert their insulinotropic actions through distinct G-protein-coupled receptors that are highly expressed on beta cells (Campbell & Drucker 2013). After oral glucose, about two thirds of the plasma insulin response can be attributed to the effects of GIP and GLP-1. The insulinotropic effects of both GIP and GLP-1 are glucose-dependent, requiring a substantial elevation of blood glucose (>8mmol/L) to be manifested (Holst & Gromada 2004). Incretin based therapies, such as GLP-1 receptor agonists, are attractive for this reason, as insulin release is only triggered in the presence of elevated glucose concentrations, with consequently minimal risk of hypoglycaemia.

Incretin hormones may play an important role in protein-stimulated insulin release in health and type 2 diabetes (Gannon, Nuttall et al. 1988). GIP and GLP-1, when infused intravenously to mimic physiological increments after a meal, have been reported to potentiate the insulin secretory response to IV administration of an amino acid mixture (Fieseler, Bridenbaugh et al. 1995). In a study of oral administration of protein and amino acids in health, a whey drink resulted in a greater GIP response than a drink containing the essential amino acids found in whey, with an associated augmentation of the insulin response (Nilsson, Holst et al. 2007). Additionally, the stimulation of insulin secretion from murine islets *in vitro* by whey was inhibited by GIP receptor antagonists (Salehi, Gunnerud et al. 2012). The effects of the GLP-1 antagonist, exendin 9-39, on whey-induced insulin secretion have not been evaluated. However, it is clear that the insulintropic effects of whey, at least in part, involve the incretin axis.

In humans, fats and carbohydrates are reported to be the most potent stimuli for GLP-1 and GIP secretion (Baggio & Drucker 2007), although the effects of protein on incretin secretion are less well studied than the other macronutrients (Wu, Rayner et al. 2010). Nevertheless, whey protein is reported to stimulate GLP-1 and GIP release (Frid, Nilsson et al. 2005; Hall, Millward et al. 2003; Nilsson, Holst et al. 2007; Nilsson, Stenberg et al. 2004; Salehi, Gunnerud et al. 2012; Simpson, McDonald et al. 1985). Bowen et al showed that plasma active GLP-1 concentrations were higher after intake of a whey protein beverage compared to a glucose or fructose drink (Bowen, Noakes et al. 2007), but the mechanisms mediating protein-induced incretin secretion remain largely unknown (Wu, Rayner et al. 2010).

Although the capacity for GIP to stimulate insulin is markedly diminished in type 2 diabetes, at least in part due to the effects of chronic hyperglycaemia (Marathe, Rayner et al. 2013), GLP-1 retains much of its activity. As whey protein can augment incretin hormone secretion and enhance protein-stimulated insulin release, it seems reasonable to view whey as a potential therapeutic agent in the treatment of type 2 diabetes.

1.3 Effects of whey on postprandial glycaemia

1.3.1 Role of gastric emptying

It is now well established that gastric emptying plays a major role in determining postprandial blood glucose concentrations, particularly the ‘early’ glycaemic response, and that slowing gastric emptying can diminish postprandial glycaemic excursions in health and diabetes (Horowitz, Edelbroek et al. 1993; Jones, Horowitz et al. 1996; Kojecky, Bernatek et al. 2008; Rayner, Samsom et al. 2001). In healthy humans, the addition of protein to oral glucose lowers postprandial blood glucose concentrations acutely, probably predominantly by slowing gastric emptying (Karamanlis, Chaikomin et al. 2007). Similarly, a ‘preload’ of whey has been shown to slow gastric emptying of a subsequent meal in both health (Hall, Millward et al. 2003), and in type 2 diabetes (Ma, Stevens et al. 2009).

The effects of whey on gastric emptying, postprandial glycaemia, and the secretion of incretin hormones, are interdependent. The incretins not only have major insulinotropic effects, but GLP-1 also slows gastric emptying, suppresses energy intake and has glucagonstatic effects to improve postprandial glycaemia (Marathe, Rayner et al. 2013). Reports that GLP-1 secretion is impaired in longstanding type 2 diabetes (Toft-Nielsen, Damholt et al. 2001; Vilsboll, Krarup et al. 2001) did not take potential differences in gastric emptying rates into account; furthermore, it has been shown that in patients with type 2 diabetes managed by diet or metformin only, the GLP-1 response to an intraduodenal glucose challenge is apparently normal (Jones, Horowitz et al. 1996). That GLP-1 secretion is intact in type 2 diabetes adds to the rationale for using a nutritional approach to enhance the secretion of endogenous GLP-1. Moreover, gastric emptying and appetite are inhibited by gut hormones other than the incretins, including cholecystokinin (CCK) and peptide YY (PYY) (Allen, Fitzpatrick et al. 1984; Nguyen, Fraser et al. 2007; Yamagishi & Debas 1978). Stimulation of these hormones by nutritional supplements could also be beneficial in reducing postprandial glycaemia.

Antropyloroduodenal motility

Interactions between nutrients and the small intestine can induce feedback on gut function to suppress antral motility and stimulate pyloric contractions, with resultant slowing of gastric emptying (Phillips, LK, Deane et al. 2015). In both healthy young and older humans, intraduodenal delivery of whey suppresses antral and duodenal waves and increases isolated pyloric pressure waves. Such changes in antropyloric motility in response to nutrient ingestion also appear to be independently related to subsequent energy intake in healthy young subjects (Seimon, Lange et al. 2010). Soenen et al examined the effects of intraduodenal whey protein infusion on appetite and subsequent *ad libitum* energy intake in relation to antropyloroduodenal motility. They reported that energy intake at a buffet meal was inversely related to the number of isolated pyloric pressure waves, and positively related to the number of antral pressure waves (Soenen, Giezenaar et al. 2014), supporting a relationship between antropyloroduodenal motor activity and feeding behaviour.

1.3.2 Potential impact of whey on dipeptidyl peptidase-IV, alpha- glucosidase, and glucagon

The incretin hormones are rapidly degraded to inactive metabolites by dipeptidyl peptidase-IV (DPP-IV). More than 50% of the GLP-1 newly secreted from intestinal L cells is degraded before reaching the systemic circulation (Hansen, Deacon et al. 1999), mainly by DPP IV present in the endothelium of the capillary bed in close proximity to the L cells (Baggio & Drucker 2007; Hansen, Deacon et al. 1999). Whey hydrolysates, produced using digestive enzymes such as pepsin and trypsin, have been found to inhibit the activity of DPP-IV *in vitro* (Lacroix & Li-Chan 2013, 2014; Nongonierma & FitzGerald 2013; Tulipano, Sibilia et al. 2011). For rodents *in vivo*, ingestion of whey protein can reduce DPP-IV activity in the proximal small bowel, thereby increasing intact incretin hormone concentrations (Gunnarsson, Winzell et al. 2006). Further *in vivo* studies, particularly in humans, are required to confirm this phenomenon, and establish its durability with long term ingestion of whey (Drucker 2006).

Alpha glucosidase is an enzyme that hydrolyzes starch and disaccharides to enable absorption of glucose at the small intestinal brush border. *In vitro* studies have shown that whey protein hydrolysate has a modest effect to inhibit alpha-glucosidase (Lacroix & Li-Chan 2013), which may be clinically relevant given that alpha-glucosidase inhibitors, such as acarbose, are used widely in the management of type 2 diabetes to improve postprandial glycaemia. Human studies are required to further evaluate this mechanism and the magnitude of the glucose lowering effect attributable to it.

Glucagon, secreted from the alpha cells of the pancreas, primarily acts on the liver to initiate glycogenolysis and gluconeogenesis, which then increases endogenous glucose production. Glucagon secretion is exaggerated in response to a meal in patients with type 2 diabetes (Young 2005), and ingested protein results in an increase in plasma glucagon levels (Ahmed, Nuttall et al. 1980). It might therefore be expected that protein ingestion would increase blood glucose concentrations, but this is not necessarily the case.

Calbet et al gave 6 healthy adults four test meals containing glucose, cow's milk solution, pea and whey peptide hydrolysates, and found that the glucagon response was linearly related to the increase in plasma amino acids. Despite this, plasma glucose levels after whey hydrolysates decreased by about 1.5 mmol/L from baseline to 180 minutes, most likely due to the effects of insulin, which is stimulated concurrently and is particularly effective at suppressing glycogenolysis (Calbet & MacLean 2002).

1.3.3 Amino acids as a stimulus for insulin secretion

It has been established for many years that ingested protein stimulates insulin secretion (Floyd, Fajans et al. 1966a; Karamanlis, Chaikomin et al. 2007), an effect observed in both healthy subjects and in those with type 2 diabetes. This effect is enhanced when protein is co-ingested with carbohydrates when compared with the ingestion of carbohydrate or protein alone, suggesting a synergy between oral protein and glucose (Manders, Hansen et al. 2014;

Manders, Wagenmakers et al. 2005; Nuttall, Mooradian et al. 1984; Pallotta & Kennedy 1968; Rabinowitz, Merimee et al. 1966). In a recent comparison of four protein sources, the greatest postprandial insulin response was associated with whey compared to casein, gluten or cod, and was attributed to the more rapid appearance of amino acids in plasma when derived from whey (Stanstrup, Schou et al. 2014).

Whey protein is a rich source of essential amino acids and branched chain amino acids known to have potent insulinotropic properties (Holmer-Jensen, Hartvigsen et al. 2012). The branched chain amino acids – leucine, valine, and isoleucine – are more insulinogenic than other amino acids (Nilsson, Stenberg et al. 2004; van Loon 2012). In the 1960s, Floyd et al showed that amino acids, given either intravenously or orally, had the capacity to stimulate insulin secretion and reduce blood glucose concentrations (Fajans, Knopf et al. 1963; Floyd, Fajans et al. 1966a, 1966b). The insulinotropic effect of whey, at least in part, reflects a direct effect of amino acids to stimulate beta cells (Blachier, Mourtada et al. 1989; Newsholme, Brennan et al. 2005; Salehi, Gunnerud et al. 2012; Sener, Blachier et al. 1989; van Loon, Saris et al. 2000); the underlying mechanisms are complex and involve mitochondrial metabolism (Newsholme, Brennan et al. 2005).

Amino acids can stimulate insulin secretion in type 2 diabetes as well as in health. Van Loon et al reported that patients with long standing type 2 diabetes who co-ingested an amino acid/protein mixture (wheat protein hydrolysate) with a carbohydrate meal almost trebled their insulin response, when compared to ingestion of carbohydrate alone. This preserved stimulation of insulin by amino acids in type 2 diabetes contrasts with the diminished insulin response to carbohydrates, when compared with healthy controls (van Loon, Kruijshoop et al. 2003). Similarly, addition of casein to carbohydrate has also been noted to potentiate insulin secretion in longstanding type 2 diabetes. That amino acids derived from ingested proteins remain a strong stimulus for insulin secretion, even in patients with long standing type 2 diabetes, supports their potential efficacy in the management of this condition (Manders, Hansen et al. 2014).

1.4 Whey and appetite regulation

1.4.1 CCK, GLP-1, PYY, ghrelin

Reduction in energy expenditure and appetite may be achieved through manipulation of dietary macronutrient composition (Bowen, Noakes et al. 2006). Protein has been shown to be more satiating than other macronutrients such as carbohydrate and fat (Bendtsen, Lorenzen et al. 2013; Clifton, PM & Keogh 2007), and has also been reported to increase satiety (Latner & Schwartz 1999; Poppitt, McCormack et al. 1998; Porrini, Crovetti et al. 1995). Whey protein, in particular, has been shown to enhance satiety and reduce food intake at the next meal in acute studies (Akhavan, Luhovyy et al. 2010; Zafar, Waslien et al. 2013), and this effect is thought to be mediated by gut hormones (Hall, Millward et al. 2003; Luhovyy, Akhavan et al. 2007), specifically by stimulation of CCK, PYY and GLP-1, and by suppression of the orexigenic hormone, ghrelin (Bendtsen, Lorenzen et al. 2013).

Bowen et al reported prolonged postprandial suppression of ghrelin, and elevation of GLP-1 and CCK, after consumption of whey, gluten and soy based preloads compared with glucose, and this was associated with reduction of energy intake at an *ad libitum* meal. CCK is typically associated with satiation; however, in this study there was a trend for an inverse relationship between CCK and subsequent energy intake, which suggests that CCK can also contribute to satiety (Bowen, Noakes et al. 2006). Similarly, in a study where hunger scores were reduced after whey ingestion compared to casein, the CCK and GLP-1 responses were higher following whey, which may have contributed to its greater satiating effect (Hall, Millward et al. 2003). Other studies have reported that PYY concentrations are higher after whey compared with other proteins, but with comparable CCK and ghrelin responses (Akhavan, Luhovyy et al. 2014).

1.4.2 Direct effects of amino acids on hunger

Elevation in plasma concentrations of amino acids after ingestion of whey may affect appetite (Mellinkoff, Frankland et al. 1956; Veldhorst, M, Smeets et al. 2008) by hitherto poorly defined mechanisms, including vagal feedback and direct suppression of hunger at the level of the hypothalamus (Fromentin, Darcel et al. 2012). The greater suppression of hunger by whey, when compared to soy or casein, is associated with increased concentrations of the amino acids leucine, lysine, tryptophan, isoleucine, and threonine (Veldhorst, MA, Nieuwenhuizen et al. 2009). Furthermore, tryptophan is synthesised into serotonin, which itself is known to influence food intake (Beulens, Bindels et al. 2004; Veldhorst, M, Smeets et al. 2008).

1.5 Whey protein and energy expenditure

Energy expenditure from thermogenesis, which increases oxygen consumption and body temperature, is thought to induce feelings of satiety (Westerterp-Plantenga, Rolland et al. 1999) . Of the macronutrients, dietary protein stimulates thermogenesis and satiety more than carbohydrate or fat (Veldhorst, M, Smeets et al. 2008). Acheson et al reported that whey protein elicits a greater thermic response than protein composed of either casein or soy, where protein accounted for 50% of the energy content of the meal (Acheson, Blondel-Lubrano et al. 2011). This may be because whey protein, as a ‘fast’ protein, is rapidly digested to result in greater postprandial protein synthesis (Boirie, Dangin et al. 1997). In particular, leucine, which is present in high concentrations in whey (Jakubowicz & Froy 2013), has been shown to stimulate muscle protein synthesis (Layman & Walker 2006) and may also increase postprandial energy expenditure (Jakubowicz & Froy 2013).

1.6 Therapeutic implications of whey protein in type 2 diabetes

1.6.1 Is whey protein effective in reducing postprandial glycaemia in type 2 diabetes?

Although it is clear that whey has an insulinotropic effect, it is less clear as to whether the magnitude of insulin stimulation is sufficient to reduce postprandial glycaemia in patients with type 2 diabetes, who tend to be insulin-resistant, and often exhibit hyperinsulinaemia (Gunnerud, U, Holst et al. 2012; Gunnerud, UJ, Ostman et al. 2013; Nilsson, Stenberg et al. 2004; Wildova, Dlouhy et al. 2013). Insulin sensitivity, assessed using a euglycaemic-hyperinsulinaemic clamp, impacts on the capacity for acute administration of protein to reduce blood glucose concentrations in healthy subjects (Brand-Miller, Colagiuri et al. 2000), and this may explain why some studies of patients with type 2 diabetes reported no reduction in blood glucose despite stimulation of insulin after a protein meal (Simpson, McDonald et al. 1985; Tessari, Kiwanuka et al. 2007).

Frid et al evaluated the effect of adding whey protein to high glycaemic index meals taken at breakfast and lunch in patients with type 2 diabetes. Plasma insulin responses were higher after both breakfast (31%) and lunch (57%) with whey (27.6g) when compared to lean ham or lactose (Frid, Nilsson et al. 2005). There was a reduction in blood glucose excursions after lunch but not breakfast, which might be related to either the differing meal content, or to higher insulin resistance seen in the fasting state (Plat, Byrne et al. 1996) affecting responses after breakfast.

Conversely, other studies in type diabetes have reported up to 3 or 4 fold increases in insulin responses to meals containing protein and carbohydrate, when compared to carbohydrate alone, with concomitant reductions in postprandial glycaemia (Manders, Koopman et al. 2006; Manders, Wagenmakers et al. 2005). Nuttall et al evaluated nine male subjects with diet controlled type 2 diabetes and showed that the blood glucose response (AUC) to protein and glucose ingestion was one third lower than after glucose alone, and the mean insulin AUC was also considerably greater (Nuttall, Mooradian et al. 1984). While these studies used beef or casein, whey is also effective for both stimulating insulin secretion and reducing postprandial

glycaemia in individuals with type 2 diabetes and/or insulin resistance (Ma, Stevens et al. 2009; Mortensen, Hartvigsen et al. 2009).

1.6.2 Timing of whey protein, ‘preloads’ and gastric emptying

The concept of a ‘preload’ refers to administration of a small load of macronutrient at a fixed interval before a meal, so that the presence of nutrients in the small intestine induces the release of GLP-1 and GIP, and other gut peptides such as CCK and PYY, to slow gastric emptying and stimulate insulin secretion in advance of the main nutrient load. In health, whey protein preloads have been shown to slow gastric emptying, as assessed by the plasma concentrations of oral paracetamol given with the meal, and enhance post-prandial GLP-1 levels (Akhavan, Luhovyy et al. 2014). Similarly, whey given immediately before a meal, with or without additional amino acids, reduces the postprandial glycaemic response by over a third (iAUC 0-60min), associated with an increase in the early postprandial plasma insulin and GLP-1 responses (Gunnerud, UJ, Heinzle et al. 2012).

The capacity for a whey preload to stimulate incretin hormone secretion and slow gastric emptying has also been established in subjects with type 2 diabetes (Ma, Stevens et al. 2009). Ma et al reported in type 2 patients that a 55g whey protein preload, given 30 minutes before a meal, slows gastric emptying when compared to either a nutrient-free preload or ingestion of whey with the meal (Ma, Stevens et al. 2009). In this study, gastric emptying was quantified using scintigraphy, which represents the ‘gold standard’. Whey protein markedly reduced postprandial glucose excursions (iAUC after whey preload about half that of control), and stimulated insulin and CCK, as well as GIP and GLP-1. Both the GLP-1 response and the reduction in postprandial glycaemia were greater when whey was given as a preload, when compared to ingestion with the meal. Accordingly, this study not only established that whey can slow gastric emptying substantially in type 2 diabetes, but that the timing of supplementation is pivotal to the stimulation of incretins and other gut hormones. These acute

effects of whey preloads to improve postprandial glycaemia were recently confirmed in another study in type 2 patients (Jakubowicz, Froy et al. 2014). While whey has been shown to slow gastric emptying acutely, it remains to be seen whether this effect is sustained with long term administration.

1.6.3 Is the dose of whey protein important?

When assessing the magnitude of glycaemic responses after whey protein consumption, one should consider not only the timing of ingestion (e.g. whether giving as a preload), but also the dose, since the effects of whey on glycaemic responses, as well as appetite, appear to be dose-dependent (Akhavan, Luhovyy et al. 2010; Petersen, Ward et al. 2009). Preloads of whey concentrate in doses of 5g, 10g, 20g, and 40g, and control, were given to 22 healthy individuals, followed 30 minutes later by a standardised pizza meal; the 20g and 40g whey preloads suppressed appetite more than control, or 5 or 10g whey protein, as assessed by visual analogue questionnaires (Akhavan, Luhovyy et al. 2010). In addition, whey protein reduced postprandial glucose in a dose-dependent manner. Poppitt et al gave 50 overweight women drinks containing 5g, 10g or 20g whey, or control, 120 minutes after a standardized breakfast, and found that there was a tendency for hunger and fullness to be dose-related, although this did not reach statistical significance (Poppitt, Proctor et al. 2011).

In healthy volunteers, whey protein taken with a meal increases insulin and reduces postprandial glycaemia in a dose dependent manner (Gunnerud, UJ, Ostman et al. 2013). Gunnerud et al found that a drink containing 25 g glucose and either 4.5g, 9g or 18g whey protein, reduced postprandial glycaemia (iAUC) by 25%, 37% and 46% respectively, compared to a 25g glucose alone; the reductions with 9g and 18g whey were statistically significant. There was also dose-dependent increase in insulin (iAUC 0 – 120min), which reached statistical significance with the highest dose of whey (Gunnerud, UJ, Ostman et al. 2013).

While whey has convincing dose-dependent effects on glucose, insulin and appetite, the optimal dose for improving long-term glycaemic control in people with type 2 diabetes is yet to be determined.

1.6.4 Effects of long term consumption of whey protein on glycaemic control

High protein diets induce weight loss and preserve lean mass (Clifton, P 2012). However, there is a paucity of data relating to whether whey has the capacity to reduce glycated haemoglobin with ongoing treatment in patients with type 2 diabetes.

A 5 week study in 8 men with type 2 diabetes showed that a diet containing 30% versus 15% of total energy derived from protein, with a corresponding decrease in carbohydrate content, was associated with a greater (by about 0.5%) decrease in glycated haemoglobin (Gannon, Nuttall et al. 2003). In another study, 72 non-diabetic obese men were randomised to receive supplements of either whey protein isolate, casein, or glucose (each 54g/day), 30 minutes before breakfast and the evening meal for 12 weeks. Improvements in fasting insulin and homeostasis model assessment of insulin resistance score of almost 10% were observed with whey compared to control, but there was no difference in the fasting serum glucose (Pal, Ellis et al. 2010).

In considering the use of whey protein in the management of diabetes, it is also important to recognise the potential adverse effects of longer term supplementation. There have been concerns that high protein diets could potentially reduce bone density and impair renal function. However, a recent two year weight loss study in postmenopausal women found no clinically significant effect of a high protein diet on bone density (Jesudason, D, Nordin et al. 2013); nor was there any reduction in renal function in a one year weight loss study in patients with type 2 diabetes with microalbuminuria, assigned to a high protein diet (≥ 90 g protein/day) (Clifton, P 2012; Jesudason, DR, Pedersen et al. 2013).

The effects of additional energy intake associated with protein supplements should also be considered if using this strategy over the long term. Subjects tend to compensate for the

additional energy load by eating less at a subsequent *ad libitum* meal in acute and short term (5 day) studies (Bertenshaw, Lluch et al. 2009; Potier, Fromentin et al. 2009). This is supported by a 12 week study in which overweight men received 54g whey supplements per day, but showed no change in body composition (Pal, Ellis et al. 2010). Age may be an important determinant of this effect, however; Soenen et al observed that older men (aged 68 to 81 years), had less capacity to compensate for the additional energy intake associated with whey administration when compared to young men (Soenen, Giezenaar et al. 2014).

Whey's ability to slow gastric emptying is one of the main mechanisms by which postprandial glycaemia is reduced acutely after a meal. However, it is unknown whether the capacity for whey to slow gastric emptying is sustained with prolonged exposure, or whether there is an adaption to this macronutrient of the gut feedback mechanisms that control gastric emptying, as has been demonstrated for carbohydrates and fats (Cunningham, Daly et al. 1991). It would therefore be important to establish whether slowing of gastric emptying induced by whey is sustained with prolonged exposure; this appears to be the case over four weeks in a small pilot study (Ma, Jesudason et al. 2015)

1.7 Guar gum, postprandial glycaemia and gastric emptying

Guar gum is a viscous soluble fibre, and when given with a meal, can decrease postprandial glycaemic excursions by slowing gastric emptying (Russo, Stevens et al. 2003) and inhibiting small intestinal absorption of glucose (Jenkins, Goff et al. 1976; O'Donovan, D, Feinle-Bisset et al. 2005), associated with reduced, rather than increased plasma insulin levels, as well as attenuation of plasma GLP-1 and GIP. Dietary fibre also increases feelings of fullness (Evans & Miller 1975; Raben, Christensen et al. 1994). Guar gum forms a gel in the stomach, leading to prolonged gastric distention, an important signal in satiety (Plata-Salaman 1991). Pasman et al report reduced energy intake and lower hunger scores when a high dosage of guar gum fibre supplement was taken for one week under free living conditions in obese women

(Pasman, Saris et al. 1997). Other possible mechanisms of guar gum inducing prolonged feelings of satiety include the effects of increased viscosity of the meal (van Nieuwenhoven, Kovacs et al. 2001).

Guar supplementation has also been associated with reductions in waist circumference, HbA1c and serum trans-fatty acids in patients with type 2 diabetes (Dall'Alba, Silva et al. 2013). Groop et al evaluated the effects of 15g guar gum supplementation in patients with diet controlled type 2 diabetes for 48 weeks and demonstrated that guar gum improved long-term glycaemic control, postprandial glucose tolerance as well as lipid concentrations (Groop, Aro et al. 1993). Similarly de Carvalho et al report that a breakfast consumed with a high fibre guar gum supplement (9.1g) was associated with lower postprandial glucose after breakfast than a breakfast with normal amounts of fibre (2.4g) (de Carvalho, de Paula et al. 2017), although reports of guar gum supplements on glycaemia are conflicting (Bhardwaj, Dasgupta et al. 1994; Lim, Ee et al. 1990; Uusitupa, Siitonen et al. 1989; Vuorinen-Markkola, Sinisalo et al. 1992). In health, varying doses of guar gum (2.5, 7.5 or 12.5 g) added to a liquid meal reduced the rise in blood glucose compared with control, and a threshold was reached by the smallest dose (2.5g) to the effect on the glucose response (Torsdottir, Alpsten et al. 1989). Low doses of guar gum (4g) given twice daily has also been demonstrated to reduce HbA1c by 0.6% in non-insulin dependent diabetes (Torsdottir, Alpsten et al. 1989) suggesting lower doses may have therapeutic role in management of type 2 diabetes (Clifton, PM, Galbraith et al. 2014).

1.8 Conclusions

The acute effects of whey protein on postprandial glycaemic excursions appear promising, but the long term efficacy and optimal application in the management of type 2 diabetes remain to be determined.

Patients most likely to benefit from postprandial glucose lowering by whey protein are those with mild to moderate elevation of HbA1c, who have relatively well controlled fasting glucose, since this is the group of patients in whom postprandial glycaemia makes the greatest relative contribution to HbA1c. However, combining a dietary strategy with pharmacological agents in less well controlled patients should also be evaluated, such as the combination of insulin to control fasting glucose, together with whey protein to reduce postprandial glycaemia; such a concept has proven to be effective with the combination of basal insulin and short-acting GLP-1 receptor agonists (Buse, Bergenstal et al. 2011). Moreover, the combination of whey protein with a DPP-IV inhibitor should also be examined, given the potential to augment the stimulation of GLP-1.

The timing of protein ingestion is important when aiming to stimulate incretin secretion and suppress appetite in advance of the main meal Wu, Zhao et al. (2012), and this, together with the optimal dose of whey protein, requires further refinement.

Guar gum reduces postprandial glycaemia, due to slowing of GE and small intestinal absorption of glucose (Russo, Stevens et al. 2003), and low doses may improve glycaemic control in type 2 diabetes.

CHAPTER 2 – LONGITUDINAL EVALUATION OF GASTRIC EMPTYING IN TYPE 2 DIABETES

2.1 Summary

In type 1 diabetes, the natural history, even over 25 years, is for gastric emptying to remain stable, however little is known about the intra-individual stability of gastric emptying measurements over time in type 2 diabetes. We evaluated the natural history of gastric emptying in type 2 diabetes. 12 patients with type 2 diabetes (7 female; age 65.6 ± 1.2 years; duration of known diabetes 22.9 ± 1.5 years) were invited to return for repeat measurements of gastric emptying using the same dual-labeled solid and liquid meal, a mean of 14.0 ± 0.5 years after their initial study. Blood glucose levels, glycated haemoglobin, upper gastrointestinal symptoms and autonomic nerve function at baseline and follow up were also compared. Gastric emptying of solids was more rapid at follow up than at baseline (treatment effect $P < 0.05$), while emptying of liquids was comparable at baseline and follow up (treatment effect $P = 0.2$). Gastric emptying of the solid component was abnormally slow (based on T100min) in 6 subjects at baseline and 1 subject at follow up. Liquid emptying was abnormally slow in 6 subjects at baseline, and 5 subjects at follow up. Two patients were insulin treated at baseline, and 6 at follow up. HbA1c was higher at follow up ($P < 0.05$); however, fasting blood glucose ($P = 0.6$), postprandial blood glucose excursions ($P = 0.07$), autonomic nerve function ($P > 0.999$), and total upper gastrointestinal symptom score ($P = 0.1$) did not differ. In conclusion, patients with long-term type 2 diabetes, gastric emptying of solids and liquids does not usually become more delayed over time, and abnormally slow gastric emptying of solids may improve.

2.2 Introduction

Gastric emptying is often disordered in people with type 2 diabetes (Phillips, LK, Deane et al. 2015), with abnormally delayed emptying of solids and/or nutrient liquids observed in 30 - 50% with longstanding type 2 diabetes (Chang, Rayner et al. 2010; Horowitz, Harding et al. 1989; Marathe, Rayner et al. 2013). While gastric emptying may be accelerated in early type 2 diabetes (Phillips, WT, Schwartz et al. 1992; Schwartz, Green et al. 1996), this has not been a consistent observation (Jones, Horowitz et al. 1996). Gastric emptying is a major determinant of postprandial glycaemia (Horowitz, Edelbroek et al. 1993), and pharmacological or nutritional therapies that slow gastric emptying, including ‘short-acting’ GLP-1 receptor agonists, pramlintide, acarbose, or nutrient preloads (Ma, Jesudason et al. 2015), are effective in lowering postprandial glycaemic excursions. In healthy subjects, there is substantial inter-individual variation in the rate of gastric emptying (Collins, Horowitz et al. 1983), but the intra-individual variation is modest, so that emptying is highly reproducible (Camilleri, Iturrino et al. 2012; Cremonini, Mullan et al. 2002; Nair, Brennan et al. 2009). In both type 1 and type 2 diabetes, the inter-individual variation of gastric emptying is predictably greater than in health because of the higher prevalence of abnormal gastric emptying (Horowitz, Harding et al. 1989). In type 1 diabetes, the natural history, even over 25 years, is for gastric emptying to remain stable (Chang, Russo et al. 2012). However, little is known about the intra-individual stability of gastric emptying measurements over time in type 2 diabetes. If modulating the rate of gastric emptying is to be developed further as a therapeutic strategy for postprandial glycaemic control, additional information is required about the natural history of gastric emptying in these patients.

2.3 Subjects and methods

2.3.1 Subjects

We examined the records of 167 patients with diabetes mellitus who had scintigraphic measurement of gastric emptying for research purposes in our laboratory between 2000 and 2005 (Russo, Stevens et al. 2004; Stevens, Russo et al. 2008). After excluding 45 patients known to have died (Births Deaths and Marriages Register), 42 who had type 1 diabetes, and 27 whose current address could not be determined, letters of invitation to participate in the study were sent by mail. Twenty one patients declined to participate, and 4 were excluded due to advanced age, iron deficiency, opiate use, and RYGB surgery.

Twelve patients with type 2 diabetes (7 female, 5 male; mean age 65.6 ± 1.2 years; body mass index (BMI) 31.4 ± 1.0 kg/m²; duration of known diabetes 22.9 ± 1.5 years) were, therefore, available to undergo repeat measurement of gastric emptying, which was undertaken a mean of 14.0 ± 0.5 years after their initial study.

One patient who was taking weekly exenatide QW withheld his medication and was studied 2 weeks after the last dose; no other subject was taking medication known to affect gastrointestinal motility. Written informed consent was obtained from each participant, and the protocol was approved by the Royal Adelaide Hospital Research Ethics Committee.

At the initial study ('baseline'), the mean age was 51.6 ± 1.2 years, body mass index was 30.5 ± 0.9 kg/m², and duration of known diabetes was 8.9 ± 1.5 years. At follow up, the mean age was 65.6 ± 1.2 years, BMI 31.2 ± 1.0 kg/m², and duration of known diabetes 22.9 ± 1.5 years. Insulin was used in the management of diabetes in 2 patients at baseline and 6 patients at follow up (Table 1).

2.3.2 Study protocol

Measurement of Gastric emptying

Gastric emptying was evaluated using a dual isotope scintigraphic method identical to that used at baseline, providing concurrent measurement of solid and nutrient liquid emptying (Collins, Horowitz et al. 1983; Jones, Horowitz et al. 1995). Each patient presented to the department after an overnight fast, and were given their usual insulin dose or anti-hyperglycaemic medication with the meal. The solid component comprised 100g minced beef labeled with 20MBq ^{99m}Tc -sulfur colloid chicken liver, and the liquid component was 150mL 10% glucose labeled with 7 MBq ^{67}Ga -ethylene-diamine-tetraacetic acid (EDTA) (Horowitz, Maddox et al. 1991; Jones, Russo et al. 2002). Each study was performed with the patient seated with their back against a gamma camera. Data were acquired for 120 minutes, with 1-minute frames for the first hour, and at 3-minute intervals thereafter. Patients were instructed to consume the meal within 5 minutes, and time zero ($t=0$) was defined as the time of meal completion. The retention (%) of solid and liquid in the stomach at 30, 60, 90, and 120 minutes was quantified, along with the percentage of solid retained at 100 minutes (T100min), and the time taken for 50% of the liquid to empty (T50%). Gastric emptying results were classified as normal or abnormal using a control range (mean \pm 2SD) established in healthy volunteers (solid emptying T100min (12-61%), and liquid emptying T50% (8-31min)) (Horowitz, Maddox et al. 1991; Jones, Russo et al. 2002).

Assessment of Gastrointestinal Symptoms

Symptoms of hunger, desire to eat, and fullness were assessed before and after the meal using validated 100mm visual analog scales (Parker, Sturm et al. 2004; Sepple & Read 1989). Upper gastrointestinal symptoms were assessed by a validated questionnaire (Horowitz, Maddox et al. 1991). Gastric (anorexia, nausea, early satiation, abdominal bloating/fullness, vomiting, abdominal pain) and oesophageal symptoms (dysphagia, heartburn, acid regurgitation) were graded as 0 (none), 1 (mild; the symptom could be ignored), 2 (moderate; the symptom could not be ignored, but did not influence daily activities), or 3 (severe; the symptom influenced

daily activities). A total symptom score was calculated as the score for both gastric and oesophageal symptoms, with a potential maximum score of 27 (Horowitz, Maddox et al. 1991).

Assessment of Autonomic Neuropathy

Autonomic nerve function was evaluated using standardised cardiovascular reflex tests (variation in heart rate during deep breathing, heart rate response to standing, and fall in systolic blood pressure in response to standing). Each test result was scored as 0 = normal, 1 = borderline, 2 = abnormal. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing & Clarke 1982).

Assessment of Glycaemia

At baseline and follow up, glycated haemoglobin (HbA1c) was measured from a fasting blood sample (SA Pathology, Adelaide, Australia), and blood glucose concentrations were measured using a glucometer (Optium Xceed, Abbott Laboratories, Bedford, MA, USA) immediately before ingestion of the meal (-5min) and then at 30, 60, 90, 120 minutes after meal ingestion.

2.3.3 Statistical analysis

Data were evaluated using repeated measures analysis of variance (ANOVA), and are shown as mean values \pm SEM. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed if ANOVAs revealed significant interactions. Incremental areas under the curves (iAUC) for blood glucose concentrations were calculated using the trapezoidal rule (Wolever 2004). Comparisons between baseline and follow up were

evaluated using paired t-tests. Analyses were performed using Prism 7.0 (La Jolla, CA, USA). $P < 0.05$ was considered statistically significant.

2.4 Results

2.4.1 Gastric emptying

As a group, gastric emptying of solids was more rapid at follow up than at baseline (treatment effect $P < 0.05$, and treatment*time interaction $P < 0.05$), with significant differences at $t = 90$ and 120 minutes ($P < 0.0001$ for both), while emptying of liquids was comparable at baseline and follow up (treatment effect $P = 0.2$). Gastric emptying of the solid component was abnormally slow (based on T100min) in 6 subjects at baseline and 1 subject at follow up. Liquid emptying was abnormally slow in 6 subjects at baseline, and 5 subjects at follow up; 4 of these subjects had delayed liquid emptying at both baseline and follow up (Figure 1). Both solid and liquid gastric emptying were delayed in 4 subjects at baseline and none at follow up. No subject had abnormally rapid gastric emptying of either solids or liquids at baseline or follow up.

2.4.2 Gastrointestinal symptoms

Fasting scores for hunger (30.8 ± 8.5 mm at baseline vs 39.7 ± 9.1 mm at follow up, $P = 0.07$), desire to eat (34.8 ± 9.1 mm vs 43.8 ± 10.2 mm, $P = 0.2$), and fullness (9.2 ± 4.1 mm vs 13.5 ± 7.9 mm, $P = 0.6$) did not differ between baseline and follow up. Postprandial scores for hunger (0 -120min) were lower at follow up than at baseline (treatment*time interaction $P < 0.05$, however post-hoc comparisons were non-significant); ratings of desire to eat ($P = 0.2$) and fullness ($P = 0.5$) were similar (Figure 2).

Eleven subjects had upper gastrointestinal symptoms at baseline and 11 at follow up. The mean score for oesophageal symptoms was greater at follow up than at baseline (baseline 0.8 ± 0.3 vs follow up 1.9 ± 0.3 , $P=0.04$), but there were no differences in either gastric ($P=0.4$) or total upper gastrointestinal symptom scores ($P=0.1$) between baseline or follow up.

2.4.3 HbA1c and blood glucose concentrations

The mean HbA1c was higher at follow up ($9.0 \pm 0.3\%$ (73.5 ± 3.5 mmol/mol)) when compared to baseline ($7.4 \pm 0.4\%$ (56.8 ± 4.5 mmol/mol), $P<0.05$). Fasting blood glucose concentrations were comparable at baseline (9.7 ± 0.8 mmol/L) and follow up (10.4 ± 0.8 mmol/L) ($P=0.6$). Blood glucose concentrations increased postprandially, and tended to be higher at follow up than at baseline ($P=0.07$ for treatment*time interaction; $P=0.1$ for comparison of iAUC (baseline 223.8 ± 34.73 mmol/L.min vs follow up 300 ± 54.17 mmol/L.min) (Figure 3).

2.4.4 Autonomic neuropathy

Seven subjects had evidence of cardiovascular autonomic neuropathy at baseline, and 6 at follow up; five of these had abnormal tests at both baseline and follow up. The mean score for autonomic nerve function score did not change between baseline and follow up ($P>0.999$).

2.5 Discussion

This study has evaluated the natural history of gastric emptying in patients with type 2 diabetes. During a mean follow-up of 14 years, gastric emptying of solids tended to improve in those who had abnormally slow emptying at baseline. Emptying of nutrient liquids was stable over time, such that if it was abnormally slow at baseline, it was also slow at follow up. Moreover, for both solids and liquids, emptying that was initially in the normal range generally did not become slower over time.

We previously evaluated the natural history of gastric emptying using scintigraphy in patients with predominantly type 1 diabetes, and observed no overall change over time in the rate of emptying of either solids or liquids. Jones et al studied twenty patients with diabetes – 16 type 1 and 4 type 2 – and reported no differences in either solid or liquid gastric emptying over a period of 12 years (Chang, Russo et al. 2012; Jones, Russo et al. 2002). Similarly, Chang et al evaluated the same group 25 years after the initial study and found that gastric emptying remained stable over that period (Chang, Russo et al. 2012). Our findings in patients with type 2 diabetes suggest similar stability in those with normal solid emptying, and in those with either slow or normal liquid emptying on the initial study. However, our finding that initially slow solid emptying can become more rapid over time in type 2 diabetes contrasts with these longitudinal studies of predominantly type 1 patients (Chang, Russo et al. 2012; Jones, Russo et al.). The reasons for this difference are unclear.

Prevailing blood glucose concentrations influence gastric emptying, such that acute hyperglycaemia – even at physiological postprandial levels – is associated with slowing of gastric emptying compared with euglycaemia, in both health and type 1 diabetes (Fraser, Horowitz et al. 1990; Phillips, LK, Deane et al. 2015; Schvarcz, Palmer et al. 1997). In our study, the delay in solid gastric emptying at baseline compared to follow up is not attributable to differences in blood glucose concentrations, which were similar during the fasting state on both occasions and tended to be higher postprandially at follow up. Other than the reversible influence of acute hyperglycaemia, the pathophysiology of delayed gastric emptying is complex and heterogeneous. Irreversible autonomic neuropathy potentially contributes, but its relationship with the rate of gastric emptying is, at best, weak (Chang, Russo et al. 2012; Jones, Russo et al. 2001), particularly in type 2 diabetes (Cotroneo, Grattagliano et al. 1991; Horowitz, Harding et al. 1989). In our patients, autonomic neuropathy was present in 3/6 patients who had delayed gastric emptying at baseline, and we did not observe a relationship between autonomic function scores and solid gastric emptying.

In response to the same meal, postprandial hunger scores overall were lower at follow up compared with baseline. This finding was not unexpected, as aging is known to be associated with some degree of physiological anorexia (Cook, Andrews et al. 1997). Oesophageal symptoms such as dysphagia, heartburn, and acid regurgitation worsened over time, however there were no significant changes in total upper gastrointestinal symptoms between baseline and follow up.

It has been postulated that accelerated gastric emptying may precede the development of type 2 diabetes, and/or contribute to worsening of glycaemic control (Phillips, WT, Schwartz et al. 1991, 1992; Schwartz, Green et al. 1996). Rapid gastric emptying has also been demonstrated in adolescents with type 1 diabetes, and is predictably associated with a greater rise in postprandial blood glucose (Perano, Rayner et al. 2015). Similarly, studies evaluating patients with longstanding type 2 diabetes have reported abnormally rapid gastric emptying in approximately 20% (Bharucha, Camilleri et al. 2009). No patient in our study had abnormally rapid emptying of solid or liquid at either baseline or follow up. It is well established that the relationship between liquid and solid gastric emptying in type 2 diabetes is weak, such that those with severe gastroparesis of solids may have normal gastric emptying of liquids, while up to 24% of those with delayed emptying of a nutrient liquid have normal solid emptying (Abell, Camilleri et al. 2008; Horowitz, Maddox et al. 1991). In our study, all 6 patients with delayed liquid gastric emptying at follow up demonstrated normal emptying of solids, while emptying of the liquid component – which contained almost all of the carbohydrate content – remained stable over time. It is likely that the deterioration in glycaemic control evident in our patients at follow up, as reflected in the HbA1c, was attributable to progressive beta cell failure, an established feature of type 2 diabetes (Fonseca 2009). That we did not observe any significant difference in postprandial incremental glucose concentrations over time in our patients, is consistent with the lack of change in liquid emptying.

Potential mechanisms by which initially slow gastric emptying could become more rapid over time may include the development of impaired proximal gastric accommodation to a meal, or a loss of factors that inhibit emptying (Nowak, Johnson et al. 1995; Weytjens, Keymeulen et al. 1998). An example of the latter is amylin, a pancreatic hormone co-secreted with insulin, which often reaches low or undetectable plasma concentrations in patients with type 1 or late-stage type 2 diabetes (Samsom, Szarka et al. 2000; Thompson, Pearson et al. 1998). Accordingly, relative amylin deficiency could potentially contribute to an increase in the rate of gastric emptying over time in longstanding type 2 diabetes (Hieronymus & Griffin 2015); however, we did not measure plasma amylin concentrations in our patients.

There are limitations to our study. The number of patients was small, and larger cohorts should be studied to confirm our findings. We did not assess patients under identical fasting glucose conditions, but this seems unlikely to account for the changes in solid meal emptying over time. Patients assessed at baseline had a mean duration of diabetes of 9 years, so our data do not provide information as to how gastric emptying might change in the early phase of diabetes. We acknowledge that selection of study participants was potentially biased, since they had previously participated in research studies concerning experimental therapies for gastroparesis within our department (Russo, Stevens et al. 2004; Stevens, Russo et al. 2008).

The main clinical implication of our findings relates to the relative stability of gastric emptying in type 2 diabetes, particularly if gastric emptying is within the normal range on initial evaluation. These patients would be candidates for pharmacological or dietary therapies that slow gastric emptying to improve postprandial glycaemic excursions. Patients with initially slow emptying of solids, on the other hand, do not inexorably progress and indeed may improve over time, so that they may merit re-evaluation if such therapies were to be considered later in the course of their management.

Patient	Sex	Age at follow up (Yrs)	Follow up period (Yrs)	Duration of Diabetes at follow up (Yrs)	BMI (kg/m ²)		Fasting Glucose (mmol/L)		HbA1c %		Solid T100min (%) (RR 12-61min)		Liquid T50% (min) (RR 8 - 31min)		Treatment	
					B	F	B	F	B	F	B	F	B	F	B	F
1*	F	63	15	21	30.9	35.2	9.3	10.2	9.3	8.1	62 †	31	32 †	39 †	Insulin + oral agents	Insulin + oral agents
2	M	62	14	32	35.6	33.0	9.0	7.1	6.4	10.2	54	35	33 †	43 †	Oral agents	Oral agents
3	F	72	15	20	24.8	32.0	9.7	6.0	7.9	8.2	36	35	17	30	Oral agents	Insulin + oral agents
4	M	64	15	28	35.4	37.5	14.3	13.0	9.1	8.6	27	28	11	26	Insulin + oral agents	oral agents
5	F	66	14	22	30.3	29.2	10.0	11.9	7.9	8.7	59	72 †	34 †	28	Oral agents	Insulin + oral agents
6*	F	59	14	15	30.2	28.8	7.7	12.5	5.4	8.7	78 †	26	28	9	Diet controlled	Oral agents
7*	M	65	16	22	31.0	33.6	5.8	14.9	4.9	10.9	62 †	57	60 †	39 †	Oral agents	Oral agents
8*	F	73	15	28	29.9	28.5	11.5	6.6	8.0	8.8	88 †	50	58 †	21	Oral agents	Oral agents
9	F	62	11	27	27.4	25.0	14.0	12.7	8.3	8.3	48	41	26	36 †	Oral agents	Oral agents
10	F	66	10	16	28.0	27.8	7.9	8.7	7.5	8.8	55	30	27	27	Oral agents	Insulin + oral agents
11*	M	63	15	23	29.5	31.0	10.8	9.9	8.6	8.4	98 †	51	22	27	Oral agents	Insulin + oral agents
12*	M	70	15	20	32.6	34.8	6.7	10.7	5.6	10.7	83 †	59	79 †	53 †	Oral agents	Insulin + oral agents

Table 1. Characteristics, gastric emptying and treatment of patients with type 2 diabetes (* indicates patients

with delay in solid gastric emptying at baseline but normal solid emptying at follow up: † indicates patients with

abnormal gastric emptying for either solid or liquid) B=baseline, F=follow up.

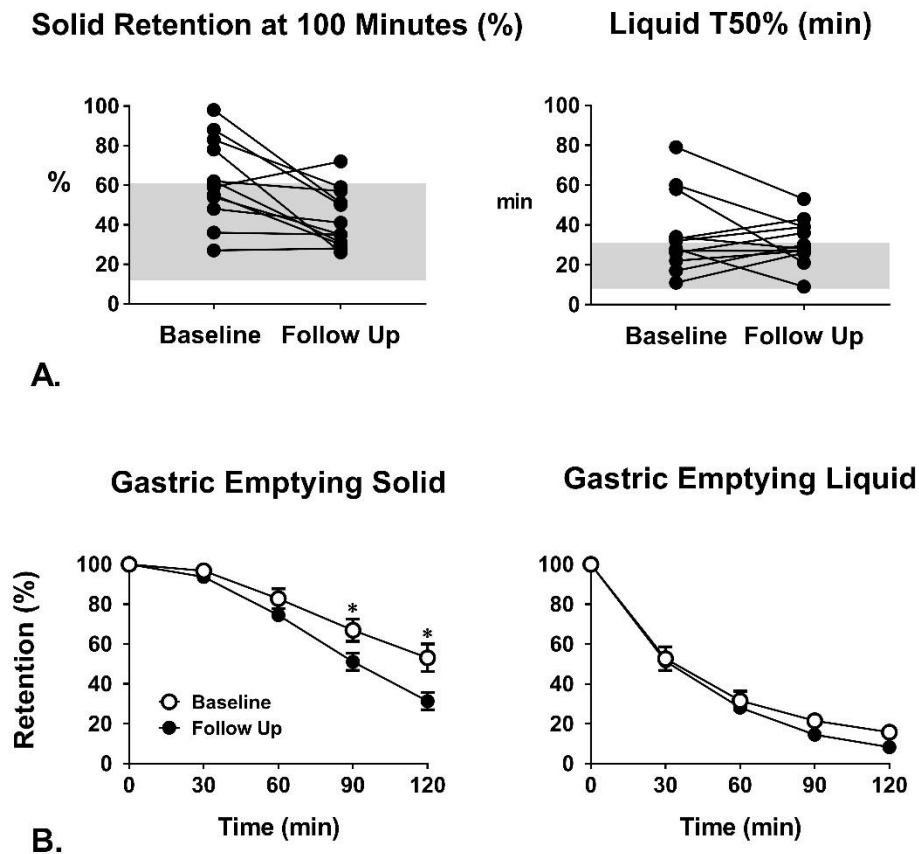


FIGURE 1: A: Gastric emptying of solid (retention at 100 minutes) and liquid (50% emptying time {T50}) meal components measured at baseline and follow up in 12 patients with type 2 diabetes. Normal ranges are indicated by the shaded areas. B: Gastric emptying (mean±SEM) of solid and liquid meal components measured at baseline (white circles) and follow up (black circles) in 12 patients with type 2 diabetes. * indicates $P < 0.0001$ for baseline versus follow up by repeated measures ANOVA.

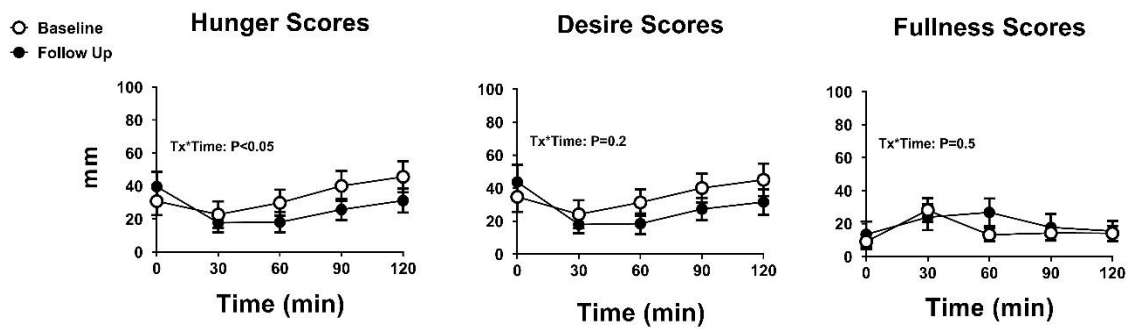


FIGURE 2: Visual analog scale ratings for hunger, desire to eat, and fullness at baseline (white circles) at follow up (black circles). Repeated-measures ANOVA was used to determine significance between baseline and follow up for hunger ($P < 0.05$), desire to eat ($P = 0.2$), and fullness ($P = 0.5$).

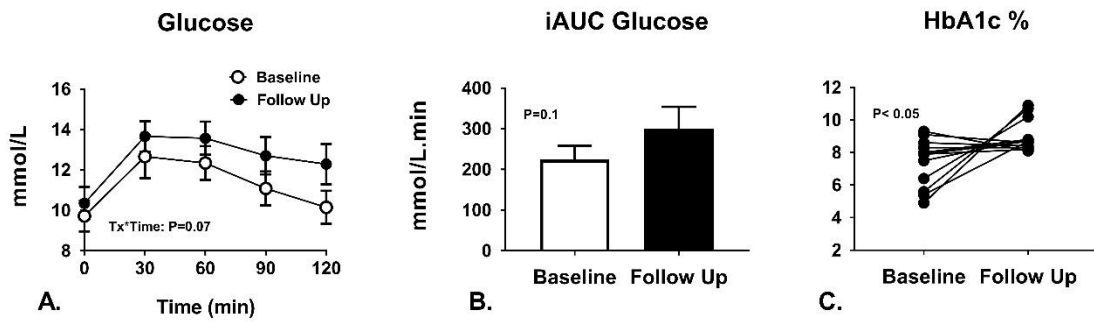


FIGURE 3: A: Postprandial blood glucose concentrations (Treatment*Time $P=0.07$, repeated measures ANOVA) B: iAUC for postprandial blood glucose concentrations (mean values \pm SEM, paired t -test baseline and follow up, $P=0.1$), and C: HbA1c (values for individual patients at baseline and follow up, paired t test baseline vs follow up, $P<0.05$)

CHAPTER 3 – GASTRIC EMPTYING IN PATIENTS WITH WELL CONTROLLED TYPE 2 DIABETES COMPARED TO HEALTHY CONTROLS

3.1 Summary

The intra-individual variation of gastric emptying in health is modest, so that emptying is highly reproducible, although there is substantial inter-individual variation. Studies from tertiary referral centres involving patients with longstanding (typically 8-12 years) type 2 diabetes, relatively poor glycaemic control (HbA1c >8.5%), and a high prevalence of microvascular complications, indicate that 30-50% have abnormally delayed emptying of solids and/or nutrient liquids, whether studied by scintigraphy or stable isotope breath test, while a few have rapid emptying. Conversely, patients with 'early' type 2 diabetes (< 2 years) and/or an absence of autonomic neuropathy have been reported to have abnormally rapid emptying for solids and/or liquids, although this has not been observed in all series. We evaluated the rate of gastric emptying in community-based patients with type 2 diabetes and good glycaemic control, compared to age- and body mass index (BMI)-matched healthy controls. 71 patients with type 2 diabetes treated with diet alone or metformin (40 male; age 64.8 ± 0.8 years; BMI 29.8 ± 0.6 kg/m²; HbA1c $6.7 \pm 0.1\%$ (49.8 ± 0.6 mmol/mol) and 19 controls (13 male; age 67.4 ± 1.9 years; BMI 29.0 ± 0.9 kg/m²; HbA1c $5.5 \pm 0.1\%$ (36.7 ± 0.8 mmol/mol)) had gastric emptying of a mashed potato meal evaluated using a ¹³C-octanoic acid breath test, with concurrent measurement of blood glucose concentrations. Gastric emptying was more rapid in type 2 diabetes patients than controls (median half-emptying time (T50) 158 (range 108-265) min vs 198 (106-435) min, $P < 0.05$). There was a relationship between the blood glucose increment 60min after the meal and T50 in the type 2 diabetes group ($r = -0.48$, $P < 0.0001$), such that when gastric emptying was slower, the rise in

postprandial glucose was smaller. Similarly, there were relationships between area under the blood glucose curve (AUC) and T50 between 0 – 60min ($r = -0.35$, $P= 0.003$) and 0 – 120min ($r = -0.35$, $P= 0.0026$).

In conclusion, as a group, well controlled type 2 diabetes patients have relatively rapid gastric emptying, which is a major determinant of postprandial glycaemia. This provides a strong rationale for the use of interventions that slow gastric emptying in order to improve postprandial glycaemic control in these patients.

3.2 Introduction

It is now appreciated that the rate of gastric emptying, which exhibits substantial inter-individual, but low intra-individual variation in both health (Camilleri, Iturrino et al. 2012; Cremonini, Mullan et al. 2002) and diabetes (Horowitz, Harding et al. 1989), is a major determinant of the ‘early’ postprandial glycaemic response after a glucose drink (Horowitz, Edelbroek et al. 1993; Jones, Horowitz et al. 1995) or a solid meal containing carbohydrate (Linnebjerg, Park et al. 2008). Interventions that slow gastric emptying, including ‘short-acting’ GLP-1 receptor agonists, pramlintide, acarbose, and nutrient preloads (Ma, Jesudason et al. 2015), have the capacity to attenuate postprandial glycaemic excursions in type 2 diabetes (Gonlachanvit, Hsu et al. 2003). Moreover, reduction in postprandial glycaemia with GLP-1 agonists has been shown to be related to the magnitude of the slowing of gastric emptying, such that when ‘baseline’ gastric emptying is relatively more rapid, the reduction of postprandial glycaemia is greater (Deane, Chapman et al. 2010; Little, Pilichiewicz et al. 2006). Accordingly, the baseline rate of gastric emptying is likely to be important in determining the response to therapy.

Gastric emptying is often disordered in people with type 2 diabetes, although there have been marked differences between studies which may relate, at least in part, to the characteristics of

the cohort selected. Studies from tertiary referral centres involving patients with longstanding (typically 8-12 years) type 2 diabetes, relatively poor glycaemic control (HbA1c >8.5%), and a high prevalence of microvascular complications, indicate that 30-50% have abnormally delayed emptying of solids and/or nutrient liquids, whether studied by scintigraphy (Bharucha, Kudva et al. 2015; Horowitz, Harding et al. 1989) or stable isotope breath test (Matsumoto, Yoshimura et al. 2007), while a few have rapid emptying. Conversely, patients with “early” type 2 diabetes (< 2 years) (Phillips, WT, Schwartz et al. 1992; Schwartz, Green et al. 1996) and/or an absence of autonomic neuropathy (Bertin, Schneider et al. 2001; Frank, Saslow et al. 1995) have been reported to have abnormally rapid emptying for solids and/or liquids, although this has not been observed in all series (Jones, Horowitz et al. 1996). A recent study of patients with longstanding type 2 diabetes but relatively good glycaemic control (HbA1c ~7%) indicated that the rate of gastric emptying (measured by ¹³C octanoic acid breath test) did not differ from healthy controls (Boronikolos, Menge et al. 2015); it is well established that acute hyperglycaemia – even at physiological postprandial levels – is associated with slowing of gastric emptying compared with euglycaemia (Schvarcz, Palmer et al. 1997), although the influence of chronic glycaemic control, as assessed by HbA1c, is less clear (Bharucha, Kudva et al. 2015; Boronikolos, Menge et al. 2015). Other factors that potentially influence the rate of gastric emptying include obesity, which has been associated with accelerated emptying (Cardoso-Junior, Coelho et al. 2007; Gryback, Naslund et al. 1996), and advancing age, in which emptying is modestly slowed (Horowitz, Maddern et al. 1984; Moore, Tweedy et al. 1983).

Accordingly, in assessing the effects of type 2 diabetes on gastric emptying, we sought to evaluate a cohort of community-based patients with good glycaemic control (HbA1c ≤ 7.9%), both to reduce heterogeneity, and in view of the fact that this group is most likely to benefit from interventions that specifically target postprandial glycaemia (Monnier, Lapinski et al. 2003). We compared these patients to a group of healthy controls matched for age and BMI,

given the potential influence of each of these variables on gastric emptying. While the gold standard technique to quantify gastric emptying is scintigraphy, this involves exposure to ionising radiation and requires specialised equipment and personnel to undertake the assessment. We therefore evaluated gastric emptying using a stable isotope breath test, which can be used in an office-based setting, and has been validated against scintigraphy in both health (correlation between half-emptying time determined by breath test and scintigraphic half-emptying time $r = 0.89$, $P < 0.0001$) (Ghoos, Y. F., Maes, B. D. et al. 1993) and type 2 diabetes (compared to the scintigraphic technique, sensitivity of the breath test to detect abnormally slow gastric emptying was 75% and specificity 86%) (Ziegler, Schadewaldt et al. 1996). Our hypothesis was that gastric emptying would be more rapid in community-based patients with type 2 diabetes and good glycaemic control than in age- and BMI-matched healthy controls.

3.3 Research design and methods

3.3.1 Subjects

Patients with type 2 diabetes were recruited for a study evaluating a nutritional therapy for type 2 diabetes (Clinical Trial Registry number and website: ACTRN12614001131640, The Australian New Zealand Clinical Trials Registry, <http://www.anzctr.org.au/>; (Mignone LE 2016)). Healthy controls were recruited by advertisement and screened for eligibility, including age and BMI within the range observed in the patient group. The type 2 diabetes patients had been diagnosed by ADA criteria, were managed by diet and/or metformin, and had HbA1c $\geq 6.0\%$ and $\leq 7.9\%$ at the time of screening. Healthy controls were excluded if they had evidence of type 2 diabetes by fasting blood glucose or HbA1c. Both type 2 diabetes patients and healthy controls were excluded if they reported significant gastrointestinal symptoms, a history of gastrointestinal disease including known gastroparesis, bariatric surgery or requirement for medications known to affect gastrointestinal function or appetite,

and they were screened visit to exclude those with kidney or liver disease. All participants completed a standardised questionnaire to assess upper gastrointestinal symptoms (Quan, Talley et al. 2003). ‘Gastric’ (anorexia, nausea, early satiation, abdominal bloating/fullness, vomiting, abdominal pain) and ‘oesophageal’ symptoms (dysphagia, heartburn, acid regurgitation) were graded as 0 (none), 1 (mild; the symptom could be ignored), 2 (moderate; the symptom could not be ignored, but did not influence daily activities), or 3 (severe; the symptom influenced daily activities) (Horowitz, Maddox et al. 1991). The healthy volunteer sample size of 19 was calculated to have 80% power at $\alpha = 0.05$ to detect a 20% difference in the gastric emptying T50 (18.4 minutes) (Delbende, Perri et al. 2000); a difference of this magnitude would be expected to be associated with a clinically significant change in postprandial glycaemia (Wu, Little et al. 2016). The Royal Adelaide Hospital Human Research and Ethics Committee approved the study, and all participants provided written informed consent.

3.3.2 Protocol

Gastric emptying was evaluated at a baseline visit in the type 2 diabetes patients, prior to the nutritional intervention being initiated, while the healthy control subjects were each studied on a single visit. All participants were asked to refrain from strenuous physical activity for 24 h before the study, and were provided with a standardized evening meal (energy content 592kcal) consisting of beef lasagne (McCain Foods, Australia) to be consumed with bread, a non-alcoholic beverage, and one piece of fruit at 1900h. Participants were then instructed to abstain from all food and nutrient beverages, but were allowed water until midnight, before attending the laboratory at 0800h.

On arrival, an intravenous cannula was inserted into a forearm vein for repeated venous blood sampling. The semi-solid test meal, consisting of 65g powdered potato (Deb; Unilever Australia) and 20g glucose, reconstituted with 200mL water and one egg yolk containing

100uL ¹³C-octanoic acid (368.5kcal: 61.4g carbohydrate, 7.4g protein and 8.9g fat), was then consumed within five minutes (t = 0 - 5 min). Breath samples were collected immediately before, and every 5 minutes after meal ingestion in the first hour, and every 15 minutes for a further 3 hours. Venous blood samples were taken immediately before meal at t = 0 and then at 15, 30, 60, 90, 120, 180 and 240 min. At the same intervals used for blood sampling, appetite and gastrointestinal sensations were assessed using validated 100 mm visual analog scales (Sepple & Read 1989).

3.3.3 Gastric emptying

¹³CO₂ in each breath sample was measured by a non-dispersive infrared spectrometer (FANci2, Fischer ANalysen Instrumente, Germany). The gastric half-emptying time (T50) was calculated using the formula described by Ghooos et al (Ghooos, Y. F., Maes, B. D. et al. 1993), which has been validated against scintigraphy (Chew, Bartholomeusz et al. 2003).

3.3.4 Blood glucose concentrations and cardiovascular autonomic function

Blood glucose concentrations were assessed using a glucometer (Optium Xceed, Abbott Laboratories, USA). After completion of the gastric emptying measurement, autonomic nerve function was evaluated using standardised cardiovascular reflex tests (variation in heart rate during deep breathing, heart rate response to standing, and fall in systolic blood pressure in response to standing). Each test result was scored as 0 = normal, 1 = borderline, 2 = abnormal. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing & Clarke 1982).

3.3.5 Statistical analysis

Comparisons between type 2 diabetes and healthy controls were made using unpaired t-tests, other than for gastric emptying (T50) data, which were not normally distributed and were

therefore compared using a Mann Whitney U test. Areas under the curve (AUCs) for blood glucose concentrations were calculated using the trapezoidal rule. Data for glucose and appetite sensations were evaluated by 2-way repeated measures analysis of variance (ANOVA) using treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed if ANOVAs revealed significant interactions. The distributions of sex, gastrointestinal symptoms, and autonomic neuropathy in each group were compared using Fisher's exact test. Relationships between change in blood glucose from baseline, HbA1c and gastric half-emptying time (T50) in the type 2 diabetes group were evaluated using the Pearson correlation coefficient. Analyses were performed using Prism 7.0 (La Jolla, CA, USA). $P < 0.05$ was considered statistically significant. Data are shown as means \pm SEM, and as median and range for T50.

3.4 Results

3.4.1 Baseline characteristics

71 patients with type 2 diabetes (40 male; age 64.8 ± 0.8 years; BMI 29.8 ± 0.6 kg/m²; HbA1c $6.7 \pm 0.1\%$ (49.8 ± 0.6 mmol/mol); mean duration of type 2 diabetes 5.1 ± 0.5 years; 37 treated with diet alone and 34 with metformin) and 19 healthy aged and BMI matched controls (13 male; age 67.4 ± 1.9 years; BMI 29.0 ± 0.9 kg/m²; HbA1c $5.5 \pm 0.1\%$ (36.7 ± 0.8 mmol/mol)) were studied. The prevalence of autonomic neuropathy was low and similar in both groups (4 in the 2 diabetes group, 1 in the control group).

3.4.2 Blood glucose concentrations

Fasting and postprandial blood glucose concentrations were higher in patients with type 2 diabetes than healthy controls ($P < 0.0001$ treatment effect; $P < 0.0001$, treatment x time

interaction), with significant differences at all time points ($t = 0$ to $t = 240$ min, $P < 0.05$) (Figure 1).

3.4.3 Upper gastrointestinal symptoms and appetite perceptions

The prevalence of upper gastrointestinal symptoms was similar in both groups; 47 patients in the type 2 diabetes group reported upper gastrointestinal symptoms (66%), and 12 in the control group (63%). The median total score for upper gastrointestinal symptoms in type 2 diabetes was 1 (0-8) and the main reported symptoms were acid regurgitation (32%), heartburn (27%) and fullness (27%). The median total score in the aged matched healthy controls was also 1 (0-4).

Fasting scores for hunger, desire to eat, projected food consumption, and fullness did not differ between type 2 diabetes patients and healthy controls, nor were there any differences between the groups for any of these sensations after the meal.

3.4.4 Gastric emptying

Gastric emptying was more rapid in the type 2 diabetes group (t_{50} 158 (108-265) min) than the control group (198 (106-435) min, $p < 0.05$) in the patients with type 2 diabetes, there was no difference in gastric emptying between those with and without upper gastrointestinal symptoms (164(115-265) min vs 156(108-255) min, $P = 0.1$) (Figure 2).

3.4.5 Relationship between blood glucose increments, HbA1c, and T50

There was a relationship between the blood glucose increment 60min after the meal and T50 in the type 2 diabetes group ($r = -0.48$, $P < 0.0001$), such that when gastric emptying was slower, the rise in postprandial glucose was smaller (Figure 3). Similarly, there were relationships between AUC 0 – 60min and T50 ($r = -0.35$, $P = 0.003$), and AUC 0 – 120min

and T50 ($r = -0.35$, $P = 0.0026$), but no relationship between AUC 0 – 240min and T50 ($P = 0.1$). No relationship was observed between gastric emptying and HbA1c ($P = 0.1$).

3.5 Discussion

We observed that gastric emptying is more rapid in relatively well controlled patients with type 2 diabetes compared with age- and BMI-matched healthy controls. Our observation that the magnitude of the blood glucose increment at 60 min is related to gastric emptying is consistent with the concept that the rate of emptying is an important determinant of postprandial glycaemia (Horowitz, Edelbroek et al. 1993; Jones, Horowitz et al. 1995). We have also confirmed that the area under the blood glucose curve over the first 1 – 2 hours after a meal is related to gastric emptying in type 2 diabetes.

Our findings are in contrast to some studies of patients with type 2 diabetes which indicated a high prevalence of delayed gastric emptying (Bharucha, Kudva et al. 2015; Horowitz, Harding et al. 1989; Matsumoto, Yoshimura et al. 2007) . It is important to note, however, that our patients differed from those studied previously, in that they were well controlled (mean HbA1c ~ 6.7%), had a relatively short duration of diabetes, and were managed on diet and metformin only. Our data are more consistent with reports that gastric emptying may be accelerated in ‘early’ type 2 diabetes, whether studied using a liquid (Phillips, WT, Schwartz et al. 1992) or solid (Schwartz, Green et al. 1996) high-carbohydrate meal using a scintigraphic technique. We evaluated gastric emptying using a semi-solid meal and a ^{13}C -octanoic acid breath test; the latter has previously been shown to correlate well with scintigraphy (Ghoos, Y. F., Maes, B. D. et al. 1993), although the T50 should be regarded as notional, rather than precise. Despite this, the consistency between our observations and those reported previously raises the question as to whether accelerated gastric emptying may, in fact, precede the development of type 2 diabetes (Phillips, WT, Schwartz et al. 1992; 1996). The lack of any patients with abnormally slow gastric emptying in our cohort is in agreement with the study of Boronikolos

et al (Boronikolos, Menge et al. 2015), where a ^{13}C -octanoic acid breath test was also used, and the patients were similarly well-controlled (HbA1c ~7%), albeit with a longer duration of diabetes (10 years).

Gastric emptying has a major impact in determining postprandial blood glucose concentrations, particularly the 'early' glycaemic response (1993; 1996). In patients with type 2 diabetes who are not treated with insulin, postprandial blood glucose excursions are increased by interventions that accelerate gastric emptying, and diminished by those that delay it (Gonlachanvit, Hsu et al. 2003), even when the slowing of emptying is quite modest (O'Donovan, DG, Doran et al. 2004; Pilichiewicz, Chaikomin et al. 2007). Moreover, therapies that slow gastric emptying are effective in patients who have normal or rapid emptying at baseline, but have minimal impact in those whose emptying is already delayed (Linnebjerg, Park et al. 2008). Our findings are of high clinical relevance, because the subset of patients with relatively good glycaemic control (HbA1c < 7.9%) is most likely to benefit from interventions that target postprandial glycaemia (Monnier, Lapinski et al. 2003; Riddle, Umpierrez et al. 2011), and we have shown that this group ought to respond well to interventions that slow gastric emptying. Evaluation of gastric emptying at baseline, such as using a point-of-care ^{13}C -octanoic breath test, may help individualise therapy in type 2 diabetes, although considering our data, one could argue that this is not essential in the well-controlled subgroup.

There is a poor correlation between upper gastrointestinal symptoms and gastric emptying in diabetes (Jones, Horowitz et al. 1995), which was confirmed in our study, as there was no difference in gastric emptying between those with or without upper gastrointestinal symptoms in the type 2 diabetes group. The prevalence of upper gastrointestinal symptoms was, however, similar in both the type 2 diabetes and control group. This may be explained by selection bias, given that we excluded patients who had significant symptoms. Our type 2 diabetes patients

also had relatively good glycaemic control, although the impact of glycaemic control on gastrointestinal symptoms remains to be determined.

A strength of our study is that both the type 2 diabetes and control groups were well matched for age, BMI, and prevalence of autonomic neuropathy. While the lack of inclusion of patients with poorly controlled diabetes or a high prevalence of complications might be seen as a limitation, it was not our intention to study patients in these categories, as they are less likely to benefit from interventions that slow gastric emptying.

In conclusion, patients with type 2 diabetes of relatively short duration and with good glycaemic control have more rapid gastric emptying than age-matched healthy controls, supporting the potential utility of interventions that slow gastric emptying to improve postprandial glycaemia in this group.

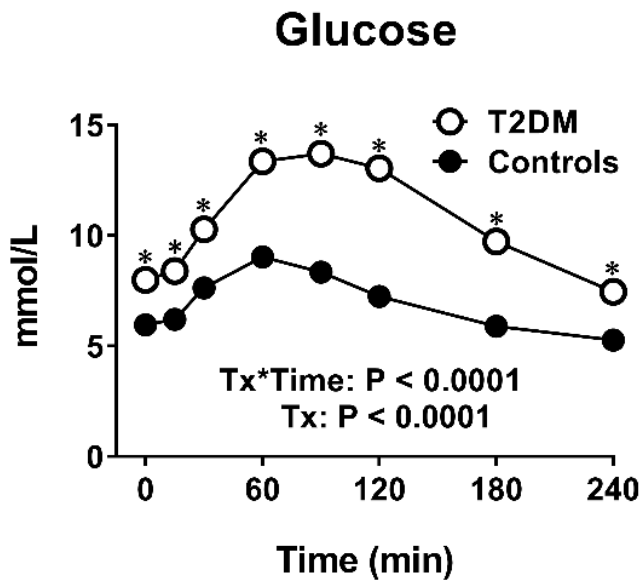


Figure 1: Blood glucose concentrations in response to a standardized carbohydrate meal in type 2 diabetes patients and aged matched healthy controls. * $P < 0.05$ (2-way repeated measures ANOVA). Data are mean values \pm SEM.

Gastric emptying

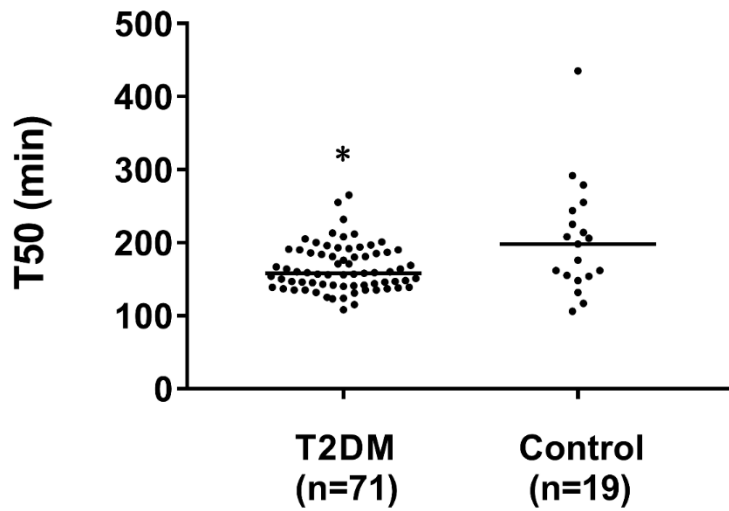


Figure 2: Individual results and medians (horizontal lines) for gastric half-emptying time (T50) in type 2 diabetes patients and control subjects as assessed by ^{13}C -octanoic acid breath test. * $P < 0.05$ (Mann Whitney U test).

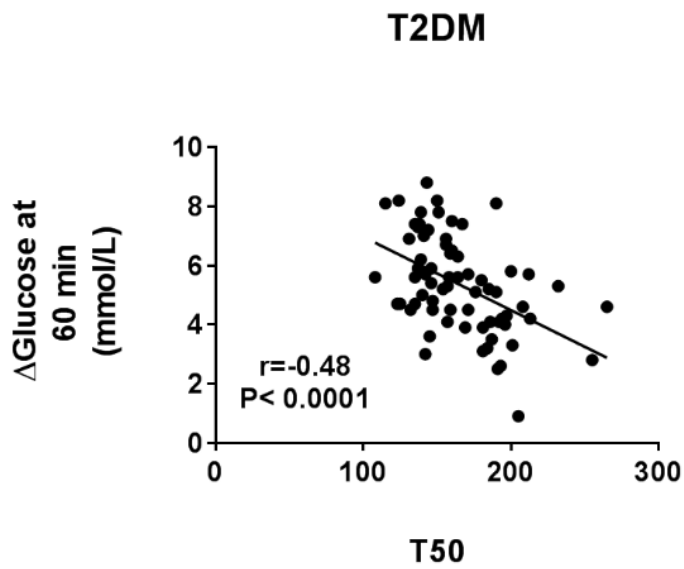


Figure 3: Relationship between the blood glucose increment 60 min after the meal and gastric half-emptying time (T50) in the type 2 diabetes patients, as evaluated using the Pearson correlation coefficient.

CHAPTER 4 - DIFFERENTIATING THE EFFECTS OF WHEY PROTEIN AND GUAR GUM PRELOADS ON POSTPRANDIAL GLYCAEMIA IN TYPE 2 DIABETES.

4.1 Summary

Whey protein and guar gum have both been reported to reduce postprandial glycaemia in health and type 2 diabetes, associated with stimulation of glucagon-like peptide-1 (GLP-1) and/or slowing of gastric emptying. We evaluated the acute effects of low dose ‘preloads’ of whey and guar, given alone or in combination before a meal, on postprandial glycaemia, GLP-1, insulin, and gastric emptying in type 2 diabetes. 21 patients with type 2 diabetes, managed by diet or metformin alone, were each studied on 4 days. They received a preload ‘shake’ 15min before a mashed potato meal (368.5kcal) labeled with ¹³C-octanoic-acid. The preloads comprised either (i) 17g whey (W), (ii) 5g guar (G), (iii) 17g whey + 5g guar (WG) each sweetened with 60mg sucralose, and (iv) 60mg sucralose alone (control; C), all dissolved in 150 mL water. Venous blood was sampled frequently for measurements of glucose, insulin, and GLP-1 concentrations. Gastric half-emptying time (T50) was calculated from breath ¹³CO₂ excretion over 240 min. Gastric emptying was slower with W (T50: 179.6 ± 6.1 min, P<0.05) and WG (T50: 197.6 ± 9.7 min, P<0.0001) when compared to C (T50: 162.9 ± 6.2 min), but did not differ between G (T50: 171.3 ± 7.0) and C (P>0.99). Postprandial blood glucose concentrations were lower with W and WG compared to C (each P <0.0001, treatment x time interaction), and lower after G than C only at 30min. GLP-1, insulin, and glucagon concentrations were higher after W than WG, G, or C (P <0.05, treatment x time interaction), without differences between the latter three. In conclusion, both whey and whey/guar preloads reduced postprandial glycaemia, associated with slowing of gastric emptying and stimulation of insulin and GLP-1 secretion. Low dose guar was less effective as a preload for glucose-lowering and did not slow gastric emptying.

4.2 Introduction

There is increasing recognition of the importance of lowering postprandial blood glucose, as opposed to fasting or pre-prandial glycaemia, to achieve target HbA1c and reduce glycaemic variability and cardiovascular risk in type 2 diabetes (Investigators 2016; Monnier 2000; Standl, Schnell et al. 2011). Nutritional strategies to reduce postprandial glycaemia are attractive, and represent the greatest opportunity for optimising glycaemic control at an affordable cost as the healthcare demands of society escalate.

Both the rate of gastric emptying, and the actions of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), are major determinants of postprandial glycaemic excursions (Chang, Rayner et al. 2010; Rayner, Samsom et al. 2001). In both health and type 2 diabetes, postprandial glycaemia is attenuated by interventions that slow gastric emptying, and exacerbated by those that accelerate it (Gonlachanvit, Hsu et al. 2003; Horowitz, Edelbroek et al. 1993; Rayner, Samsom et al. 2001). In health, GLP-1 and GIP both account for the augmentation in insulin secretion after oral compared to isoglycaemic intravenous glucose administration (the ‘incretin’ effect). In type 2 diabetes, the insulintropic effect of GIP is diminished, whereas GLP-1 retains its capacity to stimulate insulin secretion, and also slows gastric emptying (Deane, Nguyen et al. 2010; Nauck, Heimesaat et al. 1993) and suppresses glucagon secretion and energy intake (Wu, Rayner et al. 2016). Accordingly, stimulation of GLP-1 secretion is appealing in the management of type 2 diabetes.

Our group has developed the concept of giving macronutrient ‘preloads’ such as poorly absorbed carbohydrate (Wu, Zhao et al. 2012), or protein (Ma, Stevens et al. 2009)(Ma, Stevens et al. 2009)(Ma, Stevens et al. 2009)(Ma, Stevens et al. 2009)(Ma, Stevens et al. 2009)(Ma, Stevens et al. 2009)(Ma, Stevens et al. 2009) at a fixed interval before a meal, so that the presence of nutrients in the small intestine stimulates the

release of gut hormones, including GLP-1, augments insulin secretion (Fieseler, Bridenbaugh et al. 1995; Ma, Stevens et al. 2009), and slows gastric emptying of the meal. We have reported that 55g whey protein given 30 min before a high carbohydrate meal markedly reduced postprandial blood glucose (by ~3mmol/L) via these mechanisms in type 2 diabetes (Ma, Stevens et al. 2009). However, such a dose of whey entails a large energy load (210kcal), and would be relatively expensive if used regularly.

Guar gum is a viscous soluble fibre, and when given with a meal, can decrease postprandial glycaemic excursions by slowing gastric emptying (Russo, Stevens et al. 2003) and inhibiting small intestinal absorption of glucose (Jenkins, Goff et al. 1976; O'Donovan, D, Feinle-Bisset et al. 2005), associated with reduced, rather than increased plasma insulin levels, as well as attenuation of plasma GLP-1 and GIP. Guar supplementation has also been associated with reductions in waist circumference, HbA1c and serum trans-fatty acids in patients with type 2 diabetes (Dall'Alba, Silva et al. 2013). Accordingly, combining both guar gum and whey protein in a dietary supplement may be advantageous. A low dose of whey (17g), when combined with 5g guar and taken 15 minutes before a high carbohydrate test meal, was recently reported to reduce postprandial blood glucose excursions in type 2 diabetes (Clifton, PM, Galbraith et al. 2014), and has an energy burden of only 90kcal, making it preferable to higher dose preloads for regular consumption. However, the relative contribution of whey and guar to glucose-lowering and slowing of gastric emptying when used alone, and whether their actions are additive or synergistic when given together, are uncertain.

The aims of this study were to evaluate, in type 2 diabetes, the comparative acute effects of whey protein and guar gum preloads, either alone or in combination, on postprandial glycaemia, GLP-1, insulin, and gastric emptying.

4.3 Research design and methods

4.3.1 Subjects

Twenty one patients with type 2 diabetes (16 males, 5 females), managed by diet (n = 9) or a stable dose of metformin (n = 12) only, were studied after providing written, informed consent. Their age (mean \pm standard error) was 66 ± 2 years, BMI 30.8 ± 1.0 kg/m², HbA1c $6.4 \pm 0.1\%$ (46.4 ± 1.5 mmol/mol), and duration of known diabetes 6.3 ± 1.9 years. None had significant comorbidities of diabetes, were smokers, or were taking any medication known to affect gastrointestinal function. The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and conducted in accordance with the principles of the Declaration of Helsinki as revised in 2000. The trial was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615001272583).

4.3.2 Protocol

Each patient was studied on 4 occasions, separated by at least 4 days, in a single-blind, randomized, cross-over design. Patients were provided with a standardized evening meal consisting of beef lasagne (McCain Foods, Australia) to be consumed with bread, a non-alcoholic beverage, and one piece of fruit at 1900h on the evening before each study. Subjects were then instructed to abstain from all food and nutrient beverages, but were allowed to drink water until midnight, before attending the laboratory at 0800h. On arrival, an intravenous cannula was inserted into a forearm vein for repeated blood sampling.

Subjects were given a preload 'shake' containing either (i) 17g whey protein (W), (ii) 5g high molecular weight guar gum (G) (both provided by Omniblend Innovation, Australia), (iii) 17g whey protein + 5g guar gum (WG) each sweetened with 60mg sucralose, or (iv) 60mg sucralose alone (control; C). Each preload was dissolved in 150mL water, and consumed at t = -15 min within 2 min, followed by a standardised semi-solid test meal (t = 0-5 min). The

meal consisted of 65g powdered potato (Deb; Unilever Australia) and 20g glucose, reconstituted with 200mL water and one egg yolk containing 100uL ^{13}C -octanoate (368.5kcal: 61.4g carbohydrate, 7.4g protein and 8.9g fat). Breath samples were collected immediately before, and every 5 minutes after, meal ingestion for the first hour and every 15 minutes for a further 3 hours for measurement of gastric emptying. Venous blood samples (~15 mL) were taken immediately before administration of the preload ($t = -20$ min), and at $t = 0, 15, 30, 60, 90, 120, 180$ and 240 min for measurement of blood glucose, and plasma insulin, total GLP-1 and glucagon. Blood samples were placed in ice-chilled EDTA tubes and were centrifuged at 3200 rpm for 15 min. Plasma was separated and stored at -80°C for subsequent analysis. We did not record gastrointestinal symptoms in this study.

4.3.3 Measurements

4.3.3.1 Blood glucose, GLP-1, insulin and glucagon assays

Blood glucose concentrations were measured using a glucometer (Optium Xceed, Abbott Laboratories, USA). Plasma total GLP-1 was measured by radioimmunoassay (GLPIT-36HK; Millipore, USA), with sensitivity of 3pmol/L, and intra- and inter-assay CVs of 4.8% and 9.7% respectively. Plasma insulin was measured by ELISA immunoassay (10-1113; Mercodia, Sweden), with sensitivity of 1.0mU/L and intra- and inter-assay CVs of 2.7% and 5.8%. Plasma glucagon was measured by radioimmunoassay (GL-32K; Millipore, USA), with sensitivity of 20pg/mL, and inter- and intra-assay CVs of 13.2% and 3.6%.

4.3.3.2 Gastric emptying

Gastric emptying of the potato meal, labeled with ^{13}C -octanoic acid, was evaluated by excretion of $^{13}\text{CO}_2$ in the breath, measured by non-dispersive infrared spectrometer (FANci2, Fischer ANalysen Instrumente, Germany). The gastric half-emptying time (T50) was

calculated using the formula described by Ghooos et al (Ghooos, Y. F., Maes, B. D. et al. 1993), which has been validated against scintigraphy (Chew, Bartholomeusz et al. 2003).

4.3.4 Statistical analysis

Data relating to glucose, GLP-1, insulin and glucagon concentrations were evaluated by 2-way repeated measures analysis of variance (ANOVA) using treatment and time as factors and are shown as mean values \pm SEM. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed if ANOVAs revealed significant interactions. Incremental areas under the curves (iAUC) for blood glucose, GLP-1, insulin and glucagon concentrations were calculated using the trapezoidal rule (Wolever 2004). The iAUCs for glucose, GLP-1, insulin, and glucagon, and the gastric emptying T50, were compared using one-factor ANOVA. Based on our previous studies (Ma, Stevens et al. 2009; Thazhath, Wu et al. 2014), it was determined that inclusion of 21 participants would provide 80% power to detect a 0.6 mmol/l difference between treatments in mean blood glucose concentrations after the test meal, with Bonferroni adjusted $P < 0.05$, to allow for multiple post-hoc comparisons. Relationships between the change in blood glucose from baseline and the T50 were evaluated using the Pearson correlation coefficient. Analyses were performed using GraphPad Prism 7.0 (GraphPad Software, USA). $P < 0.05$ was considered statistically significant.

4.4 Results

4.4.1 Blood glucose concentrations

Fasting blood glucose did not differ between the four study days (W 7.4 ± 0.2 mmol/L, G 7.4 ± 0.2 mmol/L, WG 7.6 ± 0.2 mmol/L, C 7.4 ± 0.2 mmol/L). None of the preloads affected blood glucose in advance of the main meal. After the meal, blood glucose concentrations increased on each day before returning to baseline (Figure 1A). There was a significant treatment effect ($P < 0.05$) and treatment \times time interaction ($P < 0.0001$) on postprandial

glycaemia, such that blood glucose concentrations were lower after W and WG at $t = 30, 60, 90$ min, and lower after G at $t = 30$ min only, when compared to C. Blood glucose concentrations were lower after W than WG at $t = 120$ min, after W than G at $t = 60, 90$ and 120 min, and after WG than G at $t = 60$ and 90 min ($P < 0.05$ for each). Blood glucose concentrations were higher after W, G, and WG at $t = 180$ min when compared to C, without any difference at $t = 240$ min. Similarly, there was a significant treatment effect on iAUC for blood glucose concentrations ($P < 0.05$), such that iAUC was lower for W than G, and for WG than G ($P < 0.05$ for each) (Table 1). There was no difference in iAUC for G than C ($P > 0.99$).

4.4.2 Plasma insulin concentrations

Fasting plasma insulin concentrations did not differ between the four study days. Plasma insulin increased modestly in advance of the main meal after W, but not WG or G ($P < 0.05$, treatment x time interaction at $t = 0$ min). After the meal, plasma insulin concentrations increased on each day before returning to baseline (Figure 1B). There was a significant treatment effect ($P < 0.0001$) and treatment x time interaction ($P < 0.0001$) such that postprandial insulin concentrations were higher after W than C at $t = 15, 30$ and 60 min, after W than WG at $t = 15$ and 30 min, and after W than G at $t = 15, 30, 60$ and 90 min. Postprandial insulin concentrations were lower after G than C at $t = 30$ and 90 min ($P < 0.05$ for each). There was also a significant treatment effect on the overall iAUC for plasma insulin ($P < 0.05$), such that insulin concentrations were higher after W than G ($P < 0.05$) (Table 1).

4.4.3 Plasma GLP-1 concentrations

Fasting plasma GLP-1 concentrations did not differ between the four study days. Plasma GLP-1 increased in advance of the meal only after W ($P < 0.05$, treatment x time effect, at $t = 0$ min). After the meal, GLP-1 concentrations increased on each day before returning to baseline

(Figure 1C). There was a significant treatment effect ($P < 0.0001$) and treatment x time interaction ($P < 0.05$), such that postprandial GLP-1 concentrations were higher after W than C from $t = 15-180$ min, after W than WG from $t = 15-120$ min, after W than G from $t = 15-240$ min, and after WG than G at $t = 90$ and 120 min. Postprandial GLP-1 concentrations were lower after G than C at $t = 15$ and 60 min ($P < 0.05$ for each). There was a significant treatment effect on the iAUC for plasma GLP-1 ($P < 0.0001$), such that GLP-1 concentrations were higher after W than C, WG and G ($P < 0.05$ for each) (Table 1).

4.4.4 Plasma glucagon

Fasting plasma glucagon concentrations did not differ between the four study days. Plasma glucagon increased in advance of the main meal after W ($P < 0.0001$, treatment x time interaction, at $t = 0$ min) and WG ($P < 0.05$, treatment x time interaction, at $t = 0$ min) when compared to C, with a greater increase after W than WG ($P < 0.05$, treatment x time effect, at $t = 0$ min). After the meal, glucagon concentrations increased on each day before returning to baseline (Figure 1D). There was a significant treatment ($P < 0.0001$) and treatment x time interaction ($P < 0.0001$), such that glucagon concentrations were higher after W than C from $t = 15-120$ min, after W than WG at $t = 15, 30$ and 60 min, after W than G from $t = 15-120$ min, after WG than C from $t = 15-120$ min, and after WG than G from $t = 15-120$ min. There was also a significant treatment effect on the overall iAUC for plasma glucagon ($P < 0.0001$) such that glucagon concentrations were higher after W than C, after W than WG, after W than G, after WG than C, and after WG than G ($P < 0.05$ for each) (Table 1).

4.4.5 Gastric emptying

There was a treatment effect for gastric emptying (Figure 2), such that the T50 was greater after WG (197.6 ± 9.7 min) than either C (162.9 ± 6.2 min, $P < 0.0001$) or G (171.3 ± 7.0 ,

$P < 0.05$). The T50 was also greater after W (179.6 ± 6.1 min) than C ($P < 0.05$). While the mean T50 was numerically greater after WG than W, this difference did not achieve statistical significance ($P = 0.10$). Gastric emptying was similar between G and C ($P > 0.99$). In a pooled analysis of all subjects on all four study days, there was an inverse relationship between the change in blood glucose between $t = 0$ to 60 min and T50 ($r = -0.59$, $P < 0.0001$) such that when gastric emptying was slower (i.e. T50 greater), the rise in postprandial glucose was less (Figure 3).

4.5 Discussion

In patients with type 2 diabetes managed by diet or metformin alone who have relatively good glycaemic control, we observed that (i) a low dose whey preload reduced the postprandial glycaemic response to a high carbohydrate meal, associated with slowing of gastric emptying and stimulation of GLP-1, insulin and glucagon before and after the main meal; (ii) a low-dose guar preload reduced only the early postprandial glycaemic response, did not slow gastric emptying, and attenuated postprandial GLP-1 and insulin secretion; and (iii) the combined whey/guar preload exhibited similar effects on gastric emptying and postprandial glycaemia to those of whey alone, but was associated with relatively attenuated GLP-1, insulin and glucagon secretion.

Whey reduces postprandial glycaemia by stimulating the release of GLP-1 and augmenting insulin secretion (Fieseler, Bridenbaugh et al. 1995; Ma, Stevens et al. 2009) as well as slowing gastric emptying (Ma, Stevens et al. 2009), while guar reduces postprandial glycaemia through slowing gastric emptying (Russo, Stevens et al. 2003) and inhibiting small intestinal absorption of glucose (Jenkins, Goff et al. 1976; O'Donovan, D, Feinle-Bisset et al. 2005). In our study, both the whey and whey/guar preloads were effective in reducing postprandial glycaemia and slowing gastric emptying, supporting the use of either as a glucose-lowering therapy. Guar reduced the early rise in blood glucose at the 30 min time point only, and had a

neutral effect on gastric emptying whether given alone or when added to whey. This is in contrast to our previous report that showed slowing of gastric emptying when guar was added to a glucose drink (Russo, Stevens et al. 2003), although the dose of guar used in that study was higher (9g). The reduction in postprandial glycaemia with guar may, therefore, be related to inhibition of intestinal absorption of glucose as opposed to slowing of gastric emptying. When pooling data from all the preloads, however, we observed a strong correlation between the postprandial rise in blood glucose and the rate of gastric emptying, consistent with the concept that slowing of gastric emptying is a key mechanism for reducing postprandial glycaemia (Rayner, Samsom et al. 2001).

Whey protein is a rich source of essential and branched chain amino acids, which are known to have potent insulinotropic (Holmer-Jensen, Hartvigsen et al. 2012) and glucagonotropic (Calbet & MacLean 2002) properties. We did not measure plasma amino acid concentrations, but the observation that whey stimulated greater insulin and glucagon release than the other preloads supports the concept that amino acids are important in stimulating the secretion of these hormones.

Guar is reported to attenuate the rise in plasma GLP-1 to intraduodenal glucose, and whey protein to stimulate GLP-1 (Ma, Stevens et al. 2009). As expected, the whey preload in our study stimulated GLP-1 release prior to the meal, which was not observed with either the guar or control preloads. The addition of guar to the whey preload reduced this effect substantially, likely due to guar reducing the rate of digestion of whey and the exposure of the products of digestion to the distal small intestinal mucosa (O'Donovan, D, Feinle-Bisset et al. 2005), where the entero-endocrine cells that release GLP-1 are located. This suggests that the timing of preload ingestion in relation to the subsequent meal may be more critical for the whey preload than for the whey/guar combination, since the effects of the former would be optimised when the peak GLP-1 response coincides with the time of meal ingestion. Conversely, the whey/guar combination may prove to be effective even if given with, rather than in advance of, the meal.

Moreover, since glucose-lowering by the whey/guar preload does not appear to be dependent on the stimulation of insulin secretion, it may represent an advantage when given to patients with type 2 diabetes who have substantial beta cell dysfunction.

An advantage of our study is that we examined the effects of these preloads in patients with good overall glycaemic control ($\text{HbA1c} \leq 7.9\%$), a group in which overall HbA1c is particularly dependent on managing postprandial glycaemia (Monnier, Lapinski et al. 2003; Riddle, Umpierrez et al. 2011). There are, however, several limitations which should be appreciated. We did not evaluate the effects of varying the interval between preload administration and meal ingestion, and as discussed above, this may have differential effects depending on the nature of the preload. Guar, in particular, may reduce postprandial glycaemia if taken together with the meal, rather than as a preload (Jenkins, Wolever et al. 1980). Furthermore, we did not compare different doses of whey or guar; varying the dose of each may have impacted on the glucose lowering effect, although we wished to focus on low-dose preloads in the interests of limiting both their energy burden and cost.

In summary, in relatively well-controlled type 2 patients, both low-dose whey and whey/guar preloads reduced the postprandial glycaemic excursion in response to a high carbohydrate meal, associated with slowing of gastric emptying. The addition of guar to whey did not augment glucose-lowering compared to whey alone, but attenuated the secretion of GLP-1, insulin and glucagon. These observations indicate that further investigation on the efficacy of the whey/guar preload is warranted for postprandial glycaemic control in patients with more advanced type 2 diabetes or higher HbA1c.

Table 1 – Effects of preloads whey (W), guar (G), whey/guar (WG), or control (C) on iAUC, glucose, plasma insulin, plasma GLP-1, and plasma glucagon in response to a high carbohydrate meal in 21 patients with type 2 diabetes.

	C	W	G	WG	P value
Glucose iAUC _{-15 - 240min} (mmol/L·min)	597 ± 53	505 ± 58 ϵ	617 ± 58	506 ± 55 δ	<0.05
Insulin iAUC _{-15 - 240min} (mU/L·min)	5340 ± 629	7041 ± 1139 ϵ	4661 ± 530	5955 ± 833	<0.05
GLP-1 iAUC _{-15 - 240min} (pmol/L·min)	1180 ± 214	2197 ± 342* β ϵ	853 ± 183	1256 ± 181	<0.0001
Glucagon iAUC _{-15 - 240min} (pg/mL·min)	674 ± 107	4663 ± 383* β ϵ	851 ± 192	3140 ± 335 δ #	<0.0001
Data are mean ± SEM. One-factor ANOVA was used to determine statistical difference. Post hoc comparisons were adjusted by Bonferroni-Holm correction. *P<0.05 W vs. C, β P<0.05 W vs WG, ϵ P<0.05 W vs G, δ P<0.05 WG vs G #P<0.05 WG vs C					

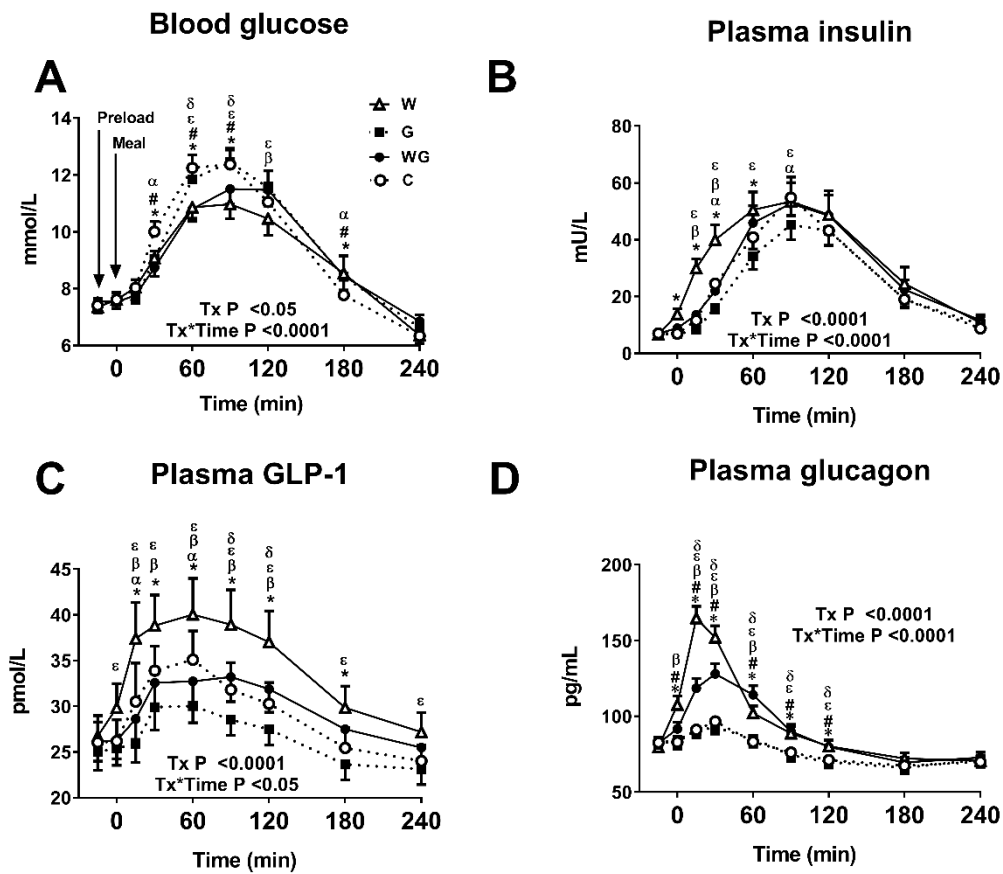


Figure 1: Effects of preloads whey (W), guar (G), whey/guar (WG), or control (C) on blood glucose (A), plasma insulin (B), plasma GLP-1 (C), and plasma glucagon (D) in response to a high carbohydrate meal in 21 patients with type 2 diabetes. Repeated-measures ANOVA was used to determine statistical difference. Post hoc comparisons were adjusted by Bonferroni-Holm correction. $P < 0.05$ for each treatment \times time interaction; * $P < 0.05$ W vs. C, # $P < 0.05$ WG vs C, $\alpha P < 0.05$ G vs C, $\beta P < 0.05$ W vs WG, $\epsilon P < 0.05$ W vs G, $\delta P < 0.05$ WG vs G. Data are mean values \pm SEM.

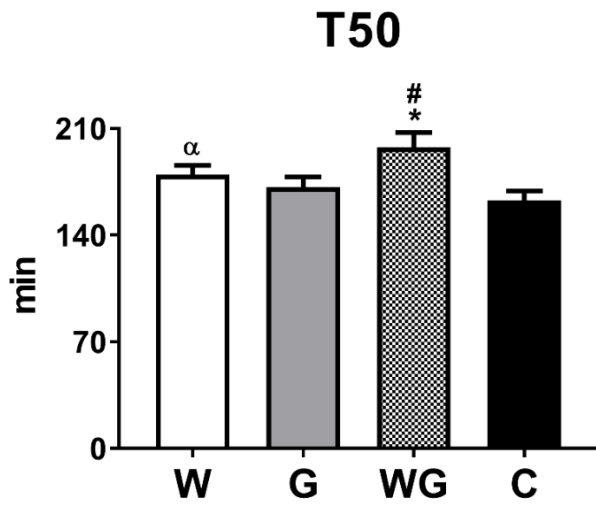


Figure 2: Effects of preloads whey (W), guar (G), whey/guar (WG), or control (C) on gastric emptying half time (T50) of a high carbohydrate meal in 21 patients with type 2 diabetes. One-factor ANOVA was used to determine statistical difference. Post hoc comparisons were adjusted by Bonferroni-Holm correction. * $P < 0.05$ W vs C. # $P < 0.0001$ WG vs. C, $\delta P < 0.05$ WG vs G. Data are mean values \pm SEM.

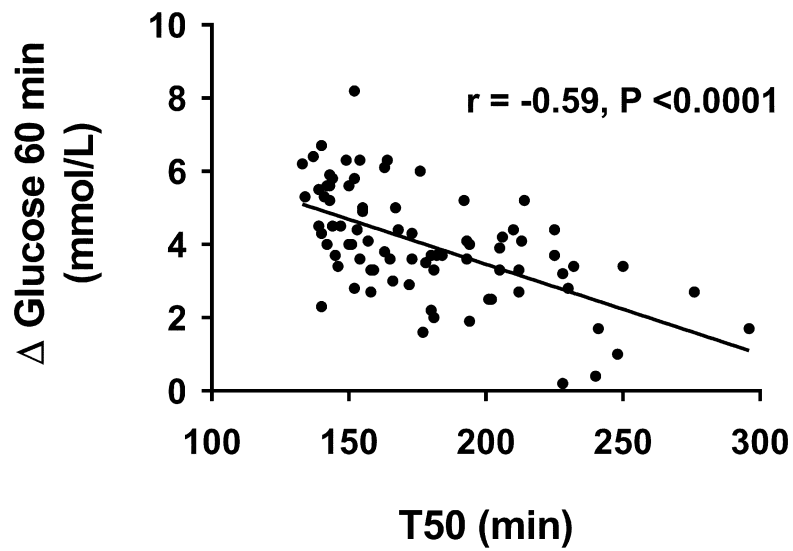


Figure 3: Relationship between change in blood glucose at 60 min and gastric emptying (T50) in a pooled analysis of all subjects on all four study days, evaluated using the Pearson correlation.

CHAPTER 5 - A WHEY/GUAR ‘PRELOAD’ IMPROVES POSTPRANDIAL GLYCAEMIA AND HBA1C IN TYPE 2 DIABETES - A 12-WEEK, SINGLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED TRIAL

5.1 Summary

In acute studies, low-dose whey (17g) together with guar (5g) when given as a preload slows gastric emptying and reduces postprandial glycaemia in subjects with type 2 diabetes. We evaluated the effects of 12 weeks' treatment with a whey/guar preload on gastric emptying, postprandial glycaemia, and glycated haemoglobin (HbA1c), in type 2 diabetes. 79 patients with type 2 diabetes managed on diet or metformin (HbA1c $6.6\pm 0.1\%$ ($49\pm 0.7\text{mmol/mol}$), were randomized, in single-blind fashion, to receive 150mL flavoured preloads, containing either 17g whey protein plus 5g guar, or flavoured placebo, 15 min before two meals each day for 12 weeks. Blood glucose and gastric emptying (breath test) were measured before and after a mashed potato meal at baseline (without preload), and after the preload at the beginning (week 1) and end (week 12) of treatment. HbA1c, energy intake, weight, and body composition were also evaluated. Gastric emptying was slower ($P<0.01$) and postprandial blood glucose lower ($P<0.05$) with the whey/guar preload compared to placebo, and the magnitude of reduction in glycaemia was related to the rate of gastric emptying at both week 1 ($r = -0.54$, $P<0.001$) and week 12 ($r = -0.54$, $P<0.0001$). At the end of treatment, there was a 0.1% reduction in HbA1c in the whey/guar group compared to placebo ($6.6\pm 0.05\%$ ($49\pm 1.0\text{mmol/mol}$) versus $6.7\pm 0.05\%$ ($50\pm 0.8\text{mmol/mol}$), $P<0.05$). There were no differences in energy intake, body weight, or lean or fat mass between the groups. In conclusion, patients with well-controlled type 2 diabetes, 12 weeks' treatment with a low-dose whey/guar preload, taken twice daily before meals, has sustained effects to slow gastric emptying and reduce

postprandial blood glucose, associated with a modest reduction in HbA1c, without causing weight gain.

5.2 Introduction

For the majority of people with type 2 diabetes who have relatively good overall glycaemic control (HbA1c \leq 7.9%), postprandial glycaemia predominates over fasting blood glucose in contributing to HbA1c (Monnier, Lapinski et al. 2003; Riddle, Umpierrez et al. 2011). Indeed, a ‘target’ HbA1c of \leq 7% is difficult to achieve without minimizing postprandial glycaemic excursions (Inzucchi, Bergenstal et al. 2012; Monnier 2000), and a deterioration in postprandial glycaemic control typically precedes any substantial elevation in fasting blood glucose (Monnier, Colette et al. 2007). Glucose fluctuations induce vascular endothelial injury (Murata, Adachi et al. 2017), and interventions that reduce glycaemic variability have been associated with improvements in cardiovascular risk markers (Investigators 2016). Therefore, strategies to control postprandial glycaemia, independent of HbA1c, may be important in improving cardiovascular outcomes (Monnier 2000; Standl, Schnell et al. 2011).

While lifestyle measures are advocated as the first step in managing type 2 diabetes (Inzucchi, Bergenstal et al. 2012), nutritional therapies generally have relatively modest benefits over sustained periods, in part due to poor adherence (Layman, Clifton et al. 2008). It is well established that the rate of gastric emptying, which exhibits substantial inter-individual variation in health (Collins, Horowitz et al. 1983), and even more so in type 2 diabetes (Horowitz, Harding et al. 1989), plays a major role in determining the early postprandial glycaemic response, and that interventions that slow gastric emptying can reduce postprandial glycaemic excursions (Gonlanchanvit, Hsu et al. 2003). Gastric emptying is regulated by inhibitory feedback arising from the interaction of nutrients with the small intestine, mediated in part by the secretion of gut hormones. A simple dietary strategy that slows gastric emptying, in order to reduce postprandial glycaemia, would therefore be appealing.

Whey protein, a by-product of the cheese-making process, and has been shown to enhance satiety and reduce food intake at a subsequent meal (Akhavan, Luhovyy et al. 2010; Zafar, Waslien et al. 2013), an effect probably mediated by gut hormones including glucagon-like peptide (GLP)-1 and cholecystokinin (CCK) (Hall, Millward et al. 2003). We have shown, in patients with type 2 diabetes managed by diet, that 55g whey protein, given as a preload 30 minutes before a potato meal, slows gastric emptying, markedly reduces postprandial glucose excursions (by ~3mmol/L), and stimulates GLP-1 and insulin release. The slowing of gastric emptying and reduction in glycaemia were less marked when the same load of whey was ingested with the meal (Ma, Stevens et al. 2009), establishing that the timing of protein ingestion in relation to the meal may be important. Similar acute findings have been reported when a preload incorporating low-dose whey (17g) together with guar (5g) was given in subjects with type 2 or pre-diabetes (Clifton, PM, Galbraith et al. 2014). Guar, a viscous polysaccharide, can itself reduce postprandial glycaemic excursions by slowing both gastric emptying and glucose absorption in the small intestine (Russo, Stevens et al. 2003).

The aims of this study were to determine the effects of a twice daily low dose whey/guar preload taken 15 minutes before breakfast and dinner over 12 weeks, in patients with type 2 diabetes and relatively good glycaemic control treated with diet or metformin alone. The primary outcome was a reduction in HbA1c, independent of weight and body composition. Secondary outcomes included acute and sustained changes in the rate of gastric emptying, postprandial glycaemia, and body weight and composition at the conclusion of the 12 week intervention.

5.3 Research design and methods

5.3.1 Subjects

Participants were recruited by advertisement and screened for eligibility. Inclusion criteria included males or females aged 18-75 years with a diagnosis of type 2 diabetes by ADA criteria managed by diet or metformin alone, and HbA1c $\geq 6.0\%$ and $\leq 7.9\%$ at the time of screening. Exclusion criteria included the presence of significant gastrointestinal symptoms, a history of gastrointestinal disease including known gastroparesis, bariatric surgery or requirement for medications known to affect gastrointestinal function or appetite, and kidney or liver disease. All subjects were also required to be of a stable weight with body mass index (BMI) 19- 40 kg/m² and were excluded if there was $\geq 3\%$ change in weight during the previous 12 months, an inability to maintain a regular meal pattern, or habitual omission of breakfast. Participants were also excluded if they were considered unlikely to comply with the study protocol. The Royal Adelaide Hospital Human Research and Ethics Committee approved the study, and all participants provided written informed consent. The trial was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614001131640).

5.3.2 Protocol

Participants were randomized, in single-blind fashion, using Research Randomizer software (<https://www.randomizer.org>), by an investigator not involved in the assessments, to receive ‘shakes’ containing either 17g whey plus 5g guar, or placebo, as a ‘preload’, 15 min before breakfast and the evening meal each day for 12 weeks. No other specific dietary advice was given. Participants were studied on 3 occasions: at baseline, on the first day (week 1) and last day (week 12) of treatment. Participants were asked to refrain from strenuous physical activity for 24 h before each study day. Each individual was provided with a standardized evening meal (energy content 592kcal) consisting of beef lasagne (McCain Foods, Australia.) to be consumed with bread, a non-alcoholic beverage, and one piece of fruit at 1900h on the evening

before each study. Participants were then instructed to abstain from all food and nutrient beverages, but were allowed water until midnight, before attending the laboratory at 0800h. On arrival, an intravenous cannula was inserted into a forearm vein for repeated blood sampling. At baseline, no preload was given, but at weeks 1 and 12, a preload was given at $t = -15$ min, followed by a standardized semi-solid meal that was consumed within five minutes ($t = 0 - 5$ min). The meal consisted of 65g powdered potato (Deb; Unilever Australia) and 20g glucose, reconstituted with 200mL water and one egg yolk containing 100 μ L 13 C-octanoic acid (368.5kcal: 61.4g carbohydrate, 7.4g protein and 8.9g fat). Breath samples were collected immediately before, and every 5 minutes after, meal ingestion in the first hour and every 15 minutes for a further 3 hours for the measurement of gastric emptying. Venous blood samples (~15 mL) were taken immediately before the preload ($t = -20$ min), and at $t = 0, 15, 30, 60, 90, 120, 180$ and 240 min for measurements of blood glucose, insulin, total GLP-1 and glucagon. Blood samples were placed in ice-chilled EDTA tubes and were centrifuged at 3200 rpm for 15 min. Plasma was separated and stored at -80°C for subsequent analysis. At the same intervals used for blood sampling, appetite and gastrointestinal sensations were assessed using validated 100 mm visual analog scales (Sepple & Read 1989). Body composition was measured by dual energy X-ray absorptiometry (DEXA) at the beginning and end of treatment. Participants were provided with 2 weeks' supply of preload packages commencing on the first day of treatment, and were required to complete a 3-day weighed food record prior to commencing the intervention, and every 2 weeks throughout the intervention period. Participants attended the laboratory every 2 weeks during the intervention (i.e. weeks 2, 4, 6, 8 and 10), when they returned all used and remaining preload packages, submitted their diet diary, and received a further 2 weeks' supply of preload packages. In the intervening weeks (i.e. weeks 1, 3, 5, 7, 9 and 11) an investigator telephoned each subject to reinforce adherence to the preload regimen.

Whey/Guar Preload

The shakes consisted of whey/guar preload powder (20g whey protein concentrate, equating to 17g whey protein, plus 5g high molecular weight guar, and non-caloric vanilla, coffee or gazpacho flavouring; Omniblend Innovation, Australia) dissolved in 150mL water (total energy content 90kcal). The placebo consisted of 10mL raspberry flavouring (sweetened with sucralose, calcium cyclamate, and acesulfame potassium) dissolved in 150mL water (total energy content 0kcal). Each participant was provided with a shaker cup and instructed to shake the contents for 7 seconds, and then consume the preload immediately. On the study days, the preloads were prepared by the investigators using identical methods.

5.3.3 Measurements

5.3.3.1 HbA1c concentrations

Glycated haemoglobin (HbA1c) was measured from a fasting blood sample at weeks 1 and 12 (high performance liquid chromatography, Bio-Rad Variant II, with intra-assay CVs 1.5% at a HbA1c of 5.3%, and CV 1.2% at HbA1c 11.1%).

5.3.3.2 Gastric emptying

Gastric emptying of the potato meal, labelled with ^{13}C -octanoic acid, was assessed by excretion of $^{13}\text{CO}_2$ in the breath, measured by non-dispersive infrared spectrometer (FANci2, Fischer ANalysen Instrumente, Germany). The gastric half-emptying time (T50) was calculated using the formula described by Ghooos et al (Ghooos, Y. F., Maes, B. D. et al. 1993). This method has been validated against scintigraphy for the measurement of gastric emptying (Chew, Bartholomeusz et al. 2003).

5.3.3.3 Plasma GLP-1, insulin and glucagon and blood glucose concentrations

Plasma total GLP-1 was measured by radioimmunoassay (GLPIT-36HK; Millipore, Billerica, MA), with sensitivity of 3 pmol/L, and intra- and inter-assay CVs of 5.7% and 8.8% respectively. Plasma insulin was measured by ELISA immunoassay (catalogue no. 10-1113; Merckodia, Uppsala, Sweden), with sensitivity of 1.0 mU/L and intra- and inter-assay CVs of 1.7% and 5.8%. Plasma glucagon was measured by radioimmunoassay (GL-32K; Millipore, Billerica, MA), with sensitivity of 20 pg/mL, and inter- and intra-assay CVs of 10% and 3.1%. Blood glucose concentrations were measured using a glucometer (Optium Xceed, Abbott Laboratories, USA).

5.3.3.4 Appetite perceptions

Symptoms of hunger, desire to eat, projected food consumption, and fullness were assessed before and after the test meal using validated 100mm visual analog scales (VAS) (Parker, Sturm et al. 2004).

5.3.3.5 Weight, height and body composition

Height was measured without shoes at baseline using a stadiometer. Participants were weighed at weeks 1 and 12, while wearing light clothing without shoes, using calibrated digital scales. Body composition was assessed using DEXA (MedixDR, Medilink, France), using a 2mm X 2mm resolution for the whole-body scan. The CVs for lean mass and total body fat were <1.0%.

5.3.3.6 Energy intake

Dietary composition was assessed using 3-day weighed food records (two week days and one weekend day) at baseline and every 2 weeks during the intervention. All food and beverages

consumed were recorded, including preloads, and the energy and macronutrient intake subsequently calculated (Foodworks Professional Edition, version 8, Xyris Software, Australia).

5.3.4 Statistical analysis

Changes in HbA1c were analysed using analysis of covariance (ANCOVA) with baseline HbA1c included as a covariant; other data were evaluated by 2-way repeated measures analysis of variance (ANOVA) using treatment and time as factors, and unpaired t tests, and are shown as means \pm SEM. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed if ANOVAs revealed significant interactions. Incremental areas under the curves (iAUC) for blood glucose concentrations were calculated using the trapezoidal rule (Wolever 2004). The distributions of sex and metformin use in each group were compared using Fisher's exact test. Relationships between change in blood glucose from baseline and gastric half-emptying time (T50) were evaluated using the Pearson correlation coefficient. Analyses were performed using GraphPad Prism 7.0 (Graph Pad Software, USA); $P < 0.05$ was considered statistically significant. Initial sample size calculation indicated that 240 patients were needed to provide 80% power to detect a 0.5% difference in HbA1c assuming a standard deviation of 1.2% (Fonseca, Alvarado-Ruiz et al. 2012). This calculation was revised after the first 47 volunteers completed the study when it was recognized that the standard deviation of HbA1c was less than expected (0.51%), so that a sample size of 70 would provide 90% power. Data are shown as means \pm SEM. A per protocol analysis was conducted, in order to determine the effects of the preload treatment when taken in an optimal manner, for this proof-of-concept study.

5.4 Results

5.4.1 Baseline characteristics

Of the 404 people who responded to advertisements, 165 were screened, and 97 satisfied the inclusion criteria. Figure 1 outlines the recruitment and withdrawal of participants. Seventy-nine participants (37 in the whey/guar group and 42 in the placebo group) completed the study (44 male; age 64 ± 0.7 years; BMI 29.8 ± 0.6 kg/m²; HbA1c $6.6\pm 0.1\%$ (49 ± 0.7 mmol/mol); 40 managed by diet alone and 39 by diet and metformin). Four participants in the whey/guar group withdrew within the first half of the intervention period due to diarrhoea that resolved when the preloads were withheld. Baseline characteristics of each group are shown (Table 1); none of these differed between the whey/guar and placebo group.

5.4.2 Adherence and adverse effects

There was excellent adherence with both preloads, without any difference between whey/guar ($94\pm 1\%$ of scheduled preloads consumed) and placebo ($93\pm 1\%$, $P = 0.4$). Mild diarrhoea was reported by 3 participants in the placebo group and 4 in the whey/guar group. Two participants in the whey/guar group reported flatus; otherwise, the preloads were well tolerated. One subject randomized to whey/guar had incomplete blood sampling at the baseline visit due to difficulty with venous access; his results were excluded from blood glucose and plasma hormone analyses. Six participants in the placebo group, and 2 in the whey group, were excluded from gastric emptying analyses due to inadequate collection of breath samples.

5.4.3 Blood glucose and HbA1c concentrations

Fasting blood glucose concentrations did not differ between the whey/guar and placebo groups at either baseline, week 1 or week 12. Postprandial blood glucose concentrations were similar

in both groups during the baseline study, and were lower after the whey/guar than placebo preload at both week 1 and week 12 ($P < 0.05$, treatment x time interaction), with significantly lower values at $t = 30$ and $t = 60$ for both (all $P < 0.05$), and ~ 2 mmol/L reduction in peak blood glucose (placebo 13.2 ± 0.4 vs whey/guar 11.2 ± 0.4 mmol/L; $P < 0.05$) (Figure 2).

At the end of treatment, the difference in HbA1c between whey/guar and placebo groups was 0.1% (placebo $6.7 \pm 0.05\%$ [50 ± 0.8 mmol/mol] vs whey/guar $6.6 \pm 0.05\%$ [49 ± 1.0 mmol/mol]; $P < 0.05$).

5.4.4 Gastric emptying

Gastric half-emptying time (T50) at baseline did not differ between the groups. Gastric emptying was slower with the whey/guar preload compared to placebo ($P < 0.01$, treatment x time interaction), such that the T50 was greater in the whey/guar group at both week 1 (placebo 167 ± 5 min vs whey/guar 189 ± 7 min, $P < 0.05$) and week 12 (placebo 161 ± 5 min vs whey/guar 182 ± 7 min, $P < 0.05$) (Figure 3). There was no difference in T50 between week 1 and week 12 within the whey/guar group.

5.4.5 Correlation between T50 and change in blood glucose at $t = 60$ min

In a pooled analysis of all subjects, there was a relationship between the change in blood glucose between $t = 0$ to 60 min and T50 at both week 1 ($r = -0.54$, $P < 0.0001$) and week 12 ($r = -0.54$, $P < 0.0001$), such that when gastric emptying was slower, the rise in postprandial glucose was less (Figure 4).

5.4.6 Plasma GLP-1, insulin and glucagon concentrations

Fasting GLP-1 concentrations were not different between the groups at baseline ($P = 0.7$), week 1 ($P = 0.9$) or week 12 ($P = 0.6$). Postprandial GLP-1 responses at baseline were similar

($P = 0.4$), and were lower after the whey/guar preload than placebo at week 1 and week 12 (both $P < 0.0001$, treatment x time interaction), with a significant difference at $t = 30$ min ($P < 0.05$) for both. There was a modest increment in plasma GLP-1 in advance of the test meal at week 1 after the whey/guar preload (0.6 ± 0.5 pmol/L) but not placebo (-1.2 ± 0.6 pmol/L; $P < 0.05$), but this was not apparent at week 12 (Figure 2).

Fasting insulin concentrations did not differ between the groups at baseline ($P = 0.7$), week 1 ($P = 0.7$), and week 12 ($P = 0.6$). Postprandial insulin also did not differ significantly between the groups at baseline ($P = 0.07$), week 1 ($P = 0.06$), or week 12 ($P = 0.15$). There was a small increase in insulin in advance of the test meal after the whey/guar preload that was greater than for placebo at week 1 (2.0 ± 0.3 vs 0.9 ± 0.2 mU/L; $P < 0.05$), and week 12 (2.3 ± 0.6 vs 0.3 ± 0.2 mU/L, $P < 0.05$) (Figure 2).

Fasting glucagon concentrations were not different between the groups at baseline ($P = 0.7$), week 1 ($P = 0.7$), or week 12 ($P = 0.8$). Postprandial glucagon concentrations were greater after the whey/guar preload than placebo at week 1 ($P < 0.05$, treatment effect; $P < 0.0001$, treatment x time interaction) and at week 12 ($P < 0.05$, treatment effect; $P < 0.0001$, treatment x time interaction), with significant differences between $t = 15$ to $t = 60$ min (all $P < 0.05$). There was a small increase in glucagon in advance of the test meal after the whey/guar preload that was greater than for placebo at week 1 (12.0 ± 2.3 vs 1.0 ± 1.2 pg/mL; $P < 0.05$), and week 12 (9.4 ± 1.9 vs 2.0 ± 1.1 pg/mL; $P < 0.05$) (Figure 2).

5.4.7 Weight and body composition

No change in weight was observed between week 1 and week 12 for the whey/guar group (0.2 ± 0.2 kg) or placebo group (-0.4 ± 0.2 kg), and there was no difference between the groups ($P=0.5$, 2-way ANOVA). There was a small reduction in fat mass in both the whey/guar ($-1.7 \pm 0.6\%$; $P < 0.05$) and placebo ($-1.9 \pm 0.6\%$; $P < 0.05$) groups, without any difference between

the treatments ($P = 0.8$, 2-way ANOVA). Lean mass increased in both the whey/guar ($1.6 \pm 0.6\%$; $P < 0.05$) and placebo ($1.8 \pm 0.6\%$; $P < 0.05$) groups, without any difference between groups ($P = 0.8$).

5.4.8 Appetite Perceptions

Fasting scores for hunger, desire to eat, projected consumption, and fullness did not differ between the groups at baseline, week 1 or week 12. After the meal, there were no differences in any of these sensations between the two groups at any of the three visits, nor was there any difference in any score in advance of the meal after either preload (data not shown).

5.4.9 Energy Intake

Total protein intake during weeks 1 and 12 was greater than at baseline in the whey/guar group ($P < 0.05$) but not with placebo. Total energy intake differed over time ($P < 0.05$), such that energy intake increased from baseline in the whey/guar group; however, there was no overall difference in energy intake between the groups, nor were there significant differences in carbohydrate or fat intake at baseline, week 1 or week 12 (Table 2).

5.5 Discussion

This study has evaluated the effect of 12 weeks' treatment with a low dose whey/guar preload, taken twice daily before breakfast and dinner in patients with well controlled type 2 diabetes, managed by diet or metformin only. The whey/guar preload had a sustained effect to slow gastric emptying and substantially reduce postprandial blood glucose, associated with a reduction in HbA1c (0.1%). This inherently simple dietary strategy was well tolerated, as indicated by the low dropout rate. Four subjects in the whey/guar group withdrew due to

diarrhoea, which could possibly have been an effect of guar, while no subjects withdrew from the placebo arm.

The observed reduction of up to ~2mmol/l in postprandial blood glucose is substantial and comparable to the effect of many oral glucose lowering drugs (Carroll, Izard et al. 2002; Wu, Bound et al. 2013). Whilst the high carbohydrate test meal used at the laboratory evaluations may not be typical of the patients' usual diet, the fact that we were able to demonstrate a reduction in HbA1c suggests that lowering of glycaemia was achieved throughout the 12-week intervention under free-living conditions. It is not surprising that the reduction in HbA1c was small, given the excellent glycaemic control in the majority of participants at baseline (HbA1c ~6.5%), and the fact that the reduction in HbA1c achieved with any glucose-lowering therapy is related to baseline HbA1c (DeFronzo, Stonehouse et al. 2010).

Gastric emptying is a major determinant of postprandial glycaemia, accounting for ~35% of the variance in glucose excursions after ingestion of oral glucose in both health (Horowitz, Edelbroek et al. 1993) and type 2 diabetes (Jones, Horowitz et al. 1996), consistent with the findings in our study. Both whey protein and guar gum are associated with slowing of gastric emptying (Russo, Stevens et al. 2003), and a low dose combination of these had previously been shown to reduce postprandial glycaemia acutely (Clifton, PM, Galbraith et al. 2014). In our study, the same combination whey/guar preload was associated with slowing of gastric emptying and this effect was sustained at the end of 12 weeks' treatment. Moreover, there was a strong relationship between the magnitude of the postprandial increment in blood glucose and the rate of gastric emptying, consistent with the concept that slowing of gastric emptying is key to the reduction in postprandial glycaemia (Rayner, Samsom et al. 2001).

Whey protein is an important source of essential and branched chain amino acids that have potent insulinotropic properties (Holmer-Jensen, Hartvigsen et al. 2012). The incretin hormones, GLP-1 and GIP, are known to be stimulated by whey protein (Bowen, Noakes et al. 2007; Frid, Nilsson et al. 2005; Hall, Millward et al. 2003; Nilsson, Holst et al. 2007) and

account for the ‘incretin effect’, the phenomenon by which insulin secretion is augmented when glucose is given enterally when compared to an isoglycaemic intravenous glucose infusion (Elrick, Stimmler et al. 1964). Whey protein ingestion also increases plasma glucagon concentrations (Hutchison, Piscitelli et al. 2015; Ma, Stevens et al. 2009). Conversely, guar gum is associated with decreased plasma concentrations of GLP-1 and insulin, and neutral effects on glucagon (O'Donovan, D, Feinle-Bisset et al. 2005; Trinick, Laker et al. 1986). In response to the whey/guar preload, we observed a postprandial increase in glucagon, and decrease in GLP-1, with neutral effects on insulin concentrations. The reduction in postprandial GLP-1 is likely related to the effects of guar gum to slow gastric emptying and modify small intestinal nutrient absorption, while the rise in glucagon may be secondary to the effects of whey and the concomitant increase in circulating amino acids (Calbet & MacLean 2002). It is noteworthy that the net effect of the whey/guar preload was to lower postprandial blood glucose, despite the observed rise in plasma glucagon.

Overall energy intake during the intervention, assessed by 3-day weighed food records, showed no difference between the two preloads, despite the increase in protein intake observed in the whey/guar group accounted for by ingestion of the preload. Weight remained stable, even in the whey/guar group where the use of preloads entailed an extra 180kcal per day, with both groups demonstrating a reduction in fat mass and an increase in lean mass, albeit modest, without any difference between them. Therefore, it appears likely that the whey/guar preload did enhance satiety under free-living conditions, and that participants in this group ‘compensated’ for the increased protein load (Akhavan, Lohovyy et al. 2010).

Limitations of our study include the use of a breath test to measure gastric emptying, rather than the gold standard technique of scintigraphy. The stable isotope test is generally considered an acceptable alternative, and has the advantages of convenience and lack of radiation exposure; it was more feasible than scintigraphy in our study, given the number of patients involved. We also did not measure the effect of the whey/guar preload on other gut

hormones that influence gastric emptying and/or appetite, such as GIP, CCK, PYY or ghrelin. Although our inclusion criteria targeted patients across the range of HbA1c between 6% and 8%, participants in our study had a mean HbA1c of ~ 6.5%, and were treated with diet or metformin alone. Therefore our results cannot be extrapolated to patients with diabetes and higher HbA1c, and those managed with other diabetic medications. It is likely that patients with a higher HbA1c (e.g. 7.5 - 8%) would achieve a greater HbA1c reduction with this intervention. However, approaches such as this that specifically target postprandial glycaemia are less likely to be effective in patients with poor glycaemic control (e.g. HbA1c \geq 9%), where fasting blood glucose makes the predominant contribution to HbA1c (Monnier, Lapinski et al. 2003). We acknowledge that un-blinding of the study after 47 cases also represents a limitation, but it had the pragmatic outcome of indicating that the sample size could be reduced. Our study design did not allow us to ascertain the relative therapeutic effects from each component of the preload, and it is possible that other combinations may have greater effects to lower glycaemia, particularly given the rise in glucagon observed in this study. Clearly, these concepts warrant further evaluation, including longer-term studies in other diabetes sub-groups.

We conclude that, in patients with well controlled type 2 diabetes, 12 weeks' treatment with a low dose whey/guar preload taken twice daily before meals has sustained effects to slow gastric emptying and reduce postprandial glycaemia, associated with a modest reduction in HbA1c, without inducing weight gain.

Acknowledgements and conflicts of interest

Omniblend Innovation (Victoria, Australia) supplied the whey/guar and placebo preloads. However, the study was investigator-initiated, and Omniblend had no role in design, analysis of results, writing of the manuscript, or the decision to publish.

	Whey/guar (90kcal, 17g protein)	Placebo (0kcal, 0g protein)	P value
Sex (M/F), <i>n</i>	23/14	21/21	ns
Age (years)	64.0 ± 1.1	65.5 ± 1.0	ns
BMI (kg/m ²)	30.3 ± 0.9	29.7 ± 0.7	ns
Duration of diabetes (years)	4.6 ± 0.7	6.4 ± 1.5	ns
HbA _{1c} (%)	6.7 ± 0.1,	6.6 ± 0.1	ns
HbA _{1c} (mmol/mol)	49.5 ± 1.0	48.8 ± 0.8	ns

Table 1 – *Baseline characteristics of the participants. Data are mean values ± SEM.*

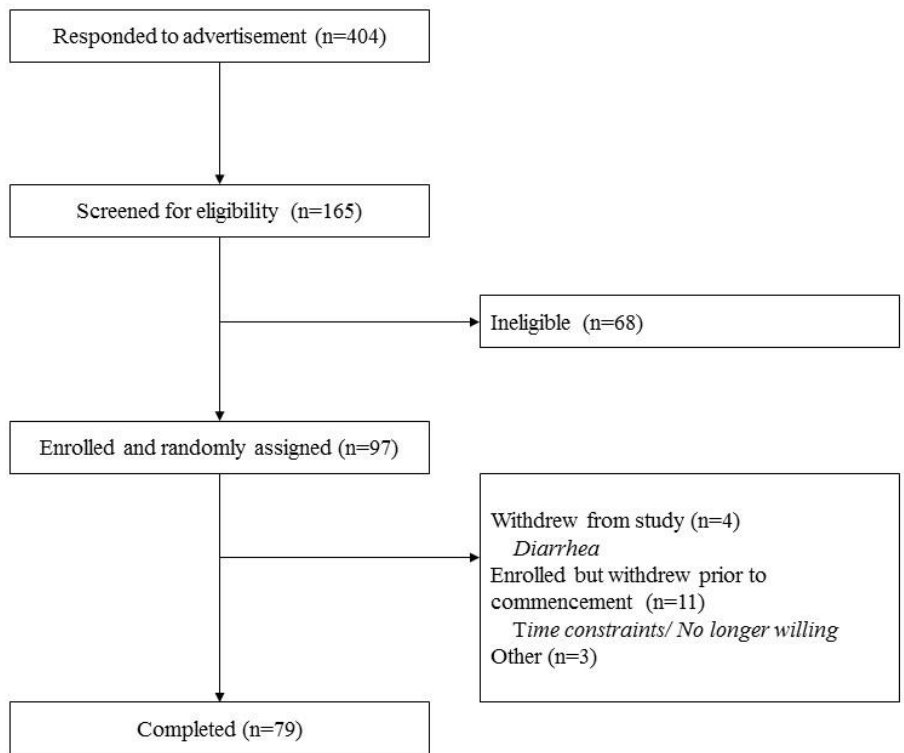


Figure 1. Recruitment and withdrawal of participants

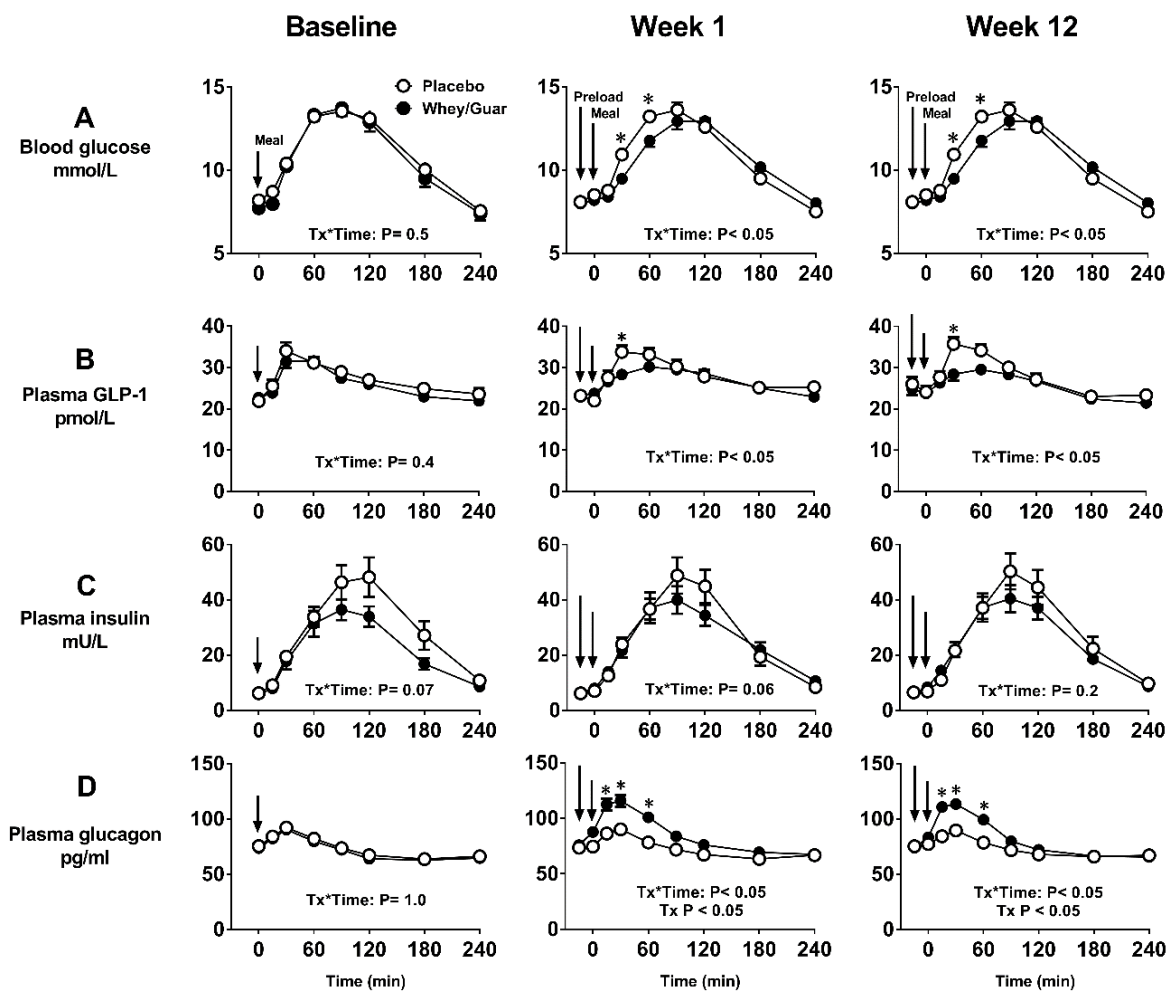


Figure 2. Effect of a preload of whey/guar compared with placebo on blood glucose (A) plasma GLP-1 (B), plasma insulin (C) and plasma glucagon (D) at baseline, week 1 and week 12. 2-way repeated measures ANOVA was used to determine statistical significance. Post hoc comparisons were adjusted by Bonferroni-Holm correction, * $P < 0.05$. Data are mean values \pm SEM.

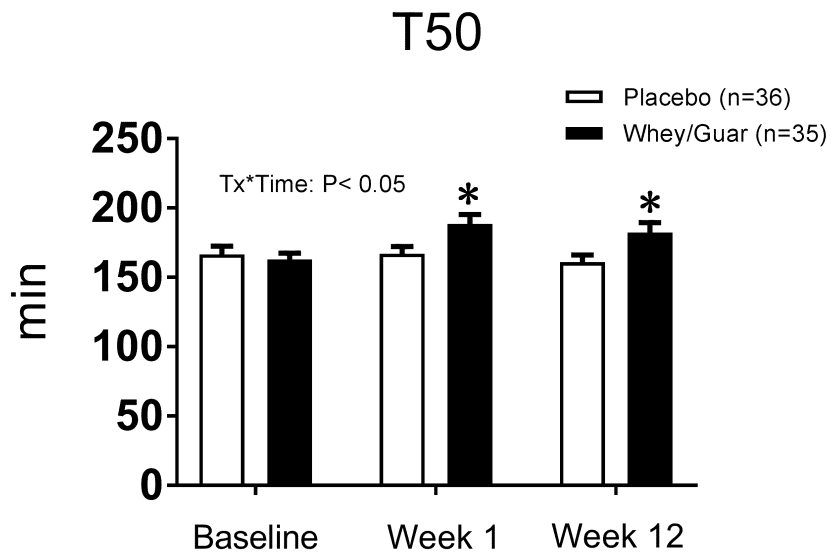


Figure 3. Gastric half-emptying time (T50) at baseline, week 1 and week 12. Two-way repeated measures ANOVA was used to determine statistical significance. Post hoc comparisons were adjusted by Bonferroni-Holm correction, * $P < 0.05$. Data are mean values \pm SEM.

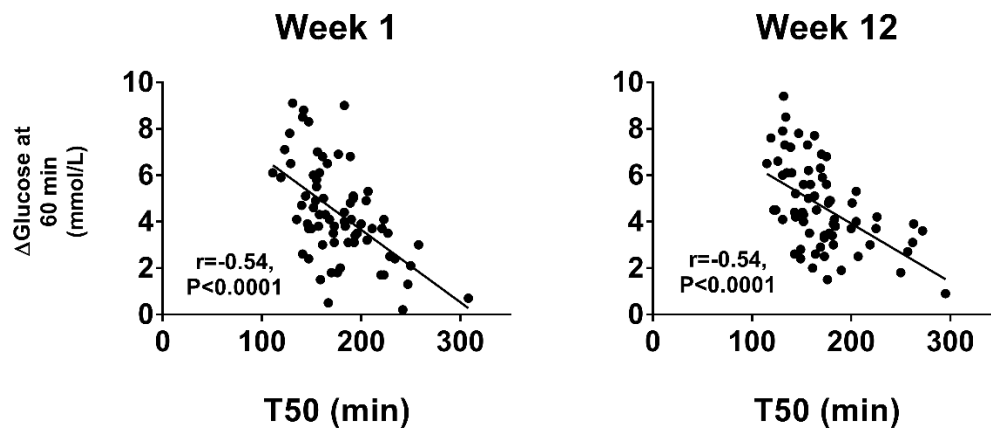


Figure 4. Relationship between change in blood glucose at 60min and gastric emptying (T50) evaluated using the Pearson correlation.

	Baseline		Week 1		Week 12	
	<i>Placebo</i>	<i>Whey/Guar</i>	<i>Placebo</i>	<i>Whey/Guar</i>	<i>Placebo</i>	<i>Whey/Guar</i>
Energy (kcal)	1882 ± 85	1796 ± 83	1958 ± 97	2054 ± 91	1852 ± 82	1901 ± 82
Protein (g)	90 ± 4	90 ± 4	99 ± 5	126 ± 5*	91 ± 4	120 ± 5*
Total fat (g)	73 ± 4	69 ± 4	76 ± 5	76 ± 4	75 ± 6	71 ± 4
Carbohydrate (g)	192 ± 9	173 ± 10	196 ± 10	182 ± 11	175 ± 8	178 ± 11

Table 2: Energy intake from 3-day weighed food record: * treatment x time interaction, $P < 0.05$ (2 way ANOVA).

CHAPTER 6 – CONCLUSIONS

The studies reported in this thesis provide important insights into the role of manipulating the gastrointestinal tract through the use of nutritional preloads to slow gastric emptying and improve postprandial blood glucose for therapeutic gain in type 2 diabetes.

Gastric emptying plays a major role in determining postprandial blood glucose concentrations, particularly the ‘early’ glycaemic response, and that slowing gastric emptying can diminish postprandial glycaemic excursions in health and diabetes (Horowitz, Edelbroek et al. 1993; Jones, Horowitz et al. 1996; Kojecky, Bernatek et al. 2008; Rayner, Samsom et al. 2001). In both type 1 and type 2 diabetes, the inter-individual variation of gastric emptying is predictably greater than in health because of the higher prevalence of abnormal gastric emptying (Horowitz, Harding et al. 1989). In type 1 diabetes, the natural history, even over 25 years, is for gastric emptying to remain stable (Chang, Russo et al. 2012). However, little is known about the intra-individual stability of gastric emptying measurements over time in type 2 diabetes. If modulating the rate of gastric emptying using nutritional preloads is to be developed further as a therapeutic strategy for postprandial glycaemic control, it is important to have an understanding of the natural history of gastric emptying in these patients, which I explored in the study reported in Chapter 2. It was demonstrated that in patients with long-term type 2 diabetes, gastric emptying of solids and liquids does not usually become delayed over time particularly if gastric emptying was initially within the normal range, and abnormally slow gastric emptying of solids may improve. These findings indicate that patients with type 2 diabetes have relatively stable gastric emptying, and would be candidates for dietary therapies that slow gastric emptying, and that patients with initial slow gastric emptying could be re-evaluated later in the course of their management and be considered for such therapies.

There is conflicting information regarding the effect of ageing on gastric emptying (Kuo, Rayner et al. 2007), with reports that emptying is either slowed (Horowitz, Maddern et al. 1984), accelerated (Kupfer, Heppell et al. 1985) or unchanged (Madsen & Graff 2004), but overall there appears to be a modest slowing of gastric emptying associated with healthy ageing (Kao, Lai et al. 1994; Moore, Tweedy et al. 1983). In addition, obese patients are reported to have more accelerated gastric emptying compared with normal weight individuals, as evaluated by either scintigraphy or ¹³C-octanoic breath test (Cardoso-Junior, Coelho et al. ; Gryback, Naslund et al.). Scintigraphy is the gold standard technique used to quantify gastric emptying, however stable isotope breath tests, such as the ¹³C-octanoic acid breath test, have been validated against scintigraphy in both health (Ghoos, Y.F., Maes, B.D. et al. 1993) and type 2 diabetes (Zahn, Langhans et al. 2003; Ziegler, Schadewaldt et al. 1996). In the study reported in Chapter 3, we evaluated the rate of gastric emptying in community-based patients with early type 2 diabetes with relatively good glycaemic control (HbA1c <7.9%) on diet or metformin alone, and compared this with gastric emptying in age- and BMI-matched healthy controls using the ¹³C-octanoic acid breath test. The main observation in this study was that gastric emptying was more rapid in type 2 diabetes. These findings indicate that this group of patients would be suitable for interventions that slow gastric emptying to reduce postprandial glycaemic excursions (Gonlachanvit, Hsu et al. 2003).

The rate of gastric emptying, and the actions of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), are major determinants of postprandial glycaemic excursions (Chang, Rayner et al. 2010; Rayner, Samsom et al. 2001). In type 2 diabetes, the insulintropic effect of GIP is diminished, whereas GLP-1 retains its capacity to stimulate insulin secretion, and also slows gastric emptying (Deane, Nguyen et al. 2010; Nauck, Heimesaat et al. 1993) and suppresses glucagon secretion and energy intake (Wu, Rayner et al. 2016). Accordingly, stimulation of GLP-1 secretion is appealing in the management of type 2 diabetes. We have previously reported that 55g whey protein given 30

min before a high carbohydrate meal as a 'preload' markedly reduced postprandial blood glucose (by ~3mmol/L), increases GLP-1, augments insulin secretion (Fieseler, Bridenbaugh et al. 1995; Ma, Stevens et al. 2009), and slows gastric emptying of the meal in type 2 diabetes. Guar gum, when given with a meal, can decrease postprandial glycaemic excursions by slowing gastric emptying (Russo, Stevens et al. 2003) and inhibiting small intestinal absorption of glucose (Jenkins, Goff et al. 1976; O'Donovan, D, Feinle-Bisset et al. 2005), associated with reduced, rather than increased plasma insulin levels, as well as attenuation of plasma GLP-1. A low dose of whey (17g), when combined with 5g guar and taken 15 minutes before a high carbohydrate test meal, was recently reported to reduce postprandial blood glucose excursions in type 2 diabetes (Clifton, PM, Galbraith et al. 2014). In Chapter 4, I evaluated the comparative acute effects of low-dose whey protein and low-dose guar gum preloads, either alone or in combination, on postprandial glycaemia, GLP-1, insulin, and gastric emptying in patients with type 2 diabetes managed by diet or metformin alone who have relatively good glycaemic control. It was found that both low-dose whey and whey/guar preloads reduced the postprandial glycaemic excursion in response to a high carbohydrate meal, associated with slowing of gastric emptying. The addition of guar to whey did not augment glucose-lowering compared to whey alone, but attenuated the secretion of GLP-1, insulin and glucagon. Either a low-dose whey or whey/guar preload would be effective in reducing postprandial glycaemic excursions in type 2 diabetes.

In Chapter 5, I report on a study evaluating the effects of 12 weeks' treatment with a low-dose whey/guar preload on gastric emptying, postprandial glycaemia, and glycated haemoglobin (HbA1c), in patients with type 2 diabetes and relatively good glycaemic control treated with diet or metformin alone. It was found that a low-dose whey/guar preload had a sustained effect to slow gastric emptying and substantially reduce postprandial blood glucose, associated with a reduction in HbA1c (0.1%) without causing weight gain. The observed reduction of up to ~2mmol/l in postprandial blood glucose is substantial and comparable to the effect of many

oral glucose lowering drugs (Carroll, Izard et al. 2002; Wu, Bound et al. 2013). Our observed reduction in HbA1c was modest, which is likely attributable to our patients having well controlled type 2 diabetes with a mean baseline HbA1c of ~ 6.5%, despite our inclusion criteria targeting patients across the range of HbA1c between 6-8%. It is likely that patients with a higher HbA1c (e.g. 7.5 - 8%) would achieve a greater HbA1c reduction with this intervention. It would be of interest to evaluate, in future longer term studies, the effects low-dose whey/guar preloads in other diabetes sub-groups with higher HbA1c and those managed with other diabetes medications.

SIGNIFICANCE OF THIS PhD PROJECT

For the majority of people with type 2 diabetes who have relatively good overall glycaemic control (HbA1c \leq 7.9%), postprandial glycaemia predominates over fasting blood glucose in contributing to HbA1c (Monnier, Lapinski et al. 2003; Riddle, Umpierrez et al. 2011). Indeed, a 'target' HbA1c of \leq 7% is difficult to achieve without minimizing postprandial glycaemic excursions (Inzucchi, Bergenstal et al. 2012; Monnier 2000). The potential use of dietary manipulations to reduce postprandial glycaemia is intuitively appealing, particularly given the escalation in health care costs with the rising incidence of type 2 diabetes.

My studies have shown that gastric emptying in type 2 diabetes is relatively stable over time, and indeed those with abnormally slow gastric emptying of solids may improve. Moreover, well controlled patients with type 2 diabetes have accelerated gastric emptying compared with age and BMI matched healthy controls. This suggests that interventions that slow gastric emptying to improve postprandial glycaemia are appropriate.

In my acute study evaluating nutritional preloads in type 2 diabetes, I have shown that a low-dose whey/guar preload is effective in reducing postprandial glycaemia and gastric emptying in patients with type 2 diabetes managed by diet or metformin alone. My 12 week randomized,

single blind, placebo-controlled trial in patients with type 2 diabetes managed on diet or metformin showed that a low-dose whey/guar preload has sustained effects to slow gastric emptying, reduce postprandial glycaemia and improve HbA1c. This novel, simple nutritional approach to the management of type 2 diabetes was well tolerated, and could be considered in patients with well controlled type 2 diabetes. I believe that the studies that make up this PhD thesis will be of considerable interest considering the rising burden of diabetes throughout the world.

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