

**The role of gastric and small
intestinal mechanisms in
postprandial hypotension**

A thesis submitted by

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Thesis abstract

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20 mmHg, within two hours of a meal, leading to syncope, falls, dizziness and angina, occurs frequently in the elderly and is now recognised as an important clinical problem. In healthy young and older individuals, meal ingestion is associated with a rapid rise in heart rate indicative of normal baroreflex function which appears to prevent a significant fall in blood pressure. However, in patients with postprandial hypotension, this response is inadequate to maintain blood pressure. Current approaches to the management of postprandial hypotension are suboptimal and there is a need for novel therapeutic strategies. Recent studies have established that the magnitude of the fall in blood pressure is dependent on the rate of delivery of nutrients from the stomach into the small intestine, while gastric distension attenuates the postprandial fall in blood pressure.

The overall aims of the studies described in this thesis were to investigate the pathophysiology of postprandial hypotension, with the particular focus on gastric and small intestinal mechanisms and their potential therapeutic relevance. In this thesis, studies were carried out in healthy older subjects (age 65 - 80 years) and were designed to evaluate the following: i) the effects of small intestinal glucose load on blood pressure, heart rate and superior mesenteric artery blood flow, ii) the comparative effects of two carbohydrates, glucose and xylose, on blood pressure, heart rate and gastric emptying, iii) the effects of gastric distension, using a barostat, on blood pressure, heart rate and superior mesenteric artery blood flow in response to intraduodenal glucose infusion, iv) the effects of variations in gastric volume, using a barostat, on blood pressure, heart rate and superior

mesenteric artery blood flow during intraduodenal glucose infusion, v) the effects of the oligosaccharide, alpha (α) – cyclodextrin, on blood pressure and heart rate, vi) the effects of acarbose on the blood pressure, heart rate and splanchnic blood flow responses to intraduodenal sucrose. All of the studies have either been published or manuscripts prepared and submitted for publication.

The effects of meal composition on the magnitude of the postprandial fall in blood pressure have been inconsistent. Of the macronutrients, the ingestion of carbohydrate, in particular glucose, was believed to have the greatest effect on blood pressure, with the effects of protein and fat being inconsistent. The fall in blood pressure following intraduodenal glucose appears to be load dependent rather than concentration dependent. A recent study demonstrated that when intraduodenal glucose is administered at a rate of 3 kcal/min, the fall in blood pressure and rise in heart rate were substantially greater compared to a 1 kcal/min glucose infusion. A limitation of this study was that there was no control arm, and because only two intraduodenal glucose loads were evaluated, it could not be determined whether the relationship between the fall in blood pressure and the duodenal glucose load is linear, this was addressed in **Chapter 5**.

There is relatively little information about the effect of different carbohydrates on postprandial blood pressure. Information relating to the effect of xylose on blood pressure is inconsistent, with previous studies showing xylose to have little or no effect. However, in these studies gastric emptying was not measured and it is known that differences in the rate of gastric emptying can affect the magnitude of

the fall in blood pressure. Blood pressure and the rate of gastric emptying of oral glucose and xylose was studied in healthy older subjects in the study reported in **Chapter 6**.

Previous studies have established that the magnitude of the postprandial fall in blood pressure is attenuated by gastric distension, however, it is unknown whether this effect is caused by the change in intragastric pressure (**Chapter 7**) or intragastric volume (**Chapter 8**). Gastric distension at predefined volumes and/or pressures can be achieved using a barostat device. Gastric distension at a pressure of 8 mmHg above minimal distending pressure using a barostat, increased mean arterial pressure, heart rate and total peripheral arterial resistance in healthy subjects. No studies have hitherto evaluated the effects of gastric distension, using a barostat, on the hypotensive response to small intestinal nutrients, and this was addressed in **Chapter 7**.

Intragastric distension with 500 ml water was shown to markedly attenuate the magnitude of the fall in systolic blood pressure in response to intraduodenal glucose. However, a limitation of this study was that during intraduodenal glucose infusion, gastric emptying was markedly attenuated, so that it reached a plateau at 300 ml and little information could be determined in relation to the minimum volume required to attenuate the hypotensive response to glucose, therefore, this was evaluated in **Chapter 8**.

Cyclodextrins inhibit pancreatic amylase activity and are poorly digested in the small intestine. α - and beta (β)- cyclodextrins have been reported to reduce the postprandial glycaemic and insulinaemia responses to a starch meal. However, a limitation of these studies was that the rate of gastric emptying was not measured, hence it remains to be determined whether the observed effects were related to gastric emptying and/or intestinal glucose absorption. In the study reported in **Chapter 9**, the effects of α -cyclodextrin on the rate of gastric emptying and hypotensive response to an oral sucrose drink were evaluated.

Acarbose has been used in the treatment of type 2-diabetes for many years by suppressing postprandial glycaemia and slowing of small intestinal digestion and absorption of carbohydrate. Previous studies have illustrated that acarbose has the capacity to slow gastric emptying and attenuate the hypotensive response to carbohydrate meals. The effects of acarbose on postprandial blood pressure and heart rate when administered intraduodenally i.e. in the absence of an effect on gastric emptying, have not been evaluated. Intraduodenal infusion allows the 'intra-gastric' mechanisms related to changes in gastric emptying to be 'bypassed', which have been evaluated in **Chapter 10**.

Declaration of originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Lora Vanis 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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Lora Vanis

September 2010

Dedication

*To my dear family,
Thank you for your continual love and support,
You have been truly inspirational,
I couldn't have done this without you*

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Publications arising from thesis

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Gentilcore D, **Vanis L**, Teng JC, Wishart JM, Buckley JD, Rayner CK, Horowitz M, Jones KL. “Effects of the oligosaccharide, alpha (a)-cyclodextrin, on gastric emptying of, and the glycemic and blood pressure responses to, oral sucrose in healthy older subjects.” *BJN* (submitted).

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Gentilcore D, **Vanis L**, Wishart JM, Rayner CK, Horowitz M, Jones KL. “Effects of intraduodenal acarbose on blood pressure, heart rate and splanchnic blood flow in healthy older subjects.” *J Hypertens* (submitted).

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1

Pathophysiology of postprandial hypotension

1.1 Introduction

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20 mmHg (Mathias et al. 1989a; Jansen and Hoefnagels 1991; Jansen and Lipsitz 1995) or a reduction in systolic blood pressure to < 90 mmHg when the preprandial blood pressure is > 100 mmHg (Jansen and Lipsitz 1995), within two hours of a meal (Jansen and Lipsitz 1995), occurs frequently and is now recognised as an important clinical problem (Jansen and Lipsitz 1995). The onset of the fall in blood pressure is usually evident almost immediately, but can occur at any time from 15 – 75 min, after a meal (Jansen and Lipsitz 1995), usually with the maximum fall occurring between 30 – 60 min (Aronow and Ahn 1994). In healthy young and older individuals, meal ingestion is associated with a rapid rise in heart rate indicative of normal baroreflex function which appears to prevent a significant fall in blood pressure (Jansen and Lipsitz 1995). However, in patients with postprandial hypotension, this response is inadequate to maintain blood pressure (Jansen and Lipsitz 1995). Postprandial hypotension is associated with significant sequelae, including syncope, falls, weakness, angina, dizziness, visual disturbances and cerebrovascular accidents (Lipsitz et al. 1983; Vaitkevicius et al. 1991; Jansen and Lipsitz 1995; Aronow and Ahn 1997). Current approaches to the management of postprandial hypotension are suboptimal and there is a need for novel therapeutic strategies. Recent studies have established that the magnitude of the fall in blood pressure is dependent on the rate of delivery of nutrients from the stomach into the small intestine i.e. the decrease in blood pressure is greater when gastric emptying is relatively more rapid (Jones et al. 1998; Jones et al. 2001; O'Donovan et al. 2002; Russo et al. 2003; O'Donovan et al. 2005a), while gastric

distension attenuates the postprandial fall in blood pressure (Rossi et al. 1998; Jordan et al. 1999; Jordan et al. 2000; Shannon et al. 2002; van Orshoven et al. 2004; Jones et al. 2005; Gentilcore et al. 2008b).

In this chapter, current knowledge of the pathophysiology of postprandial hypotension, with a particular focus on gastric and small intestinal mechanisms, is reviewed.

1.2 Prevalence and significance of postprandial hypotension

Postprandial hypotension has hitherto received relatively little attention, although it occurs more frequently than orthostatic hypotension (Jansen and Lipsitz 1995; Vloet et al. 2005), and probably represents a substantial cause of both morbidity and mortality (Jansen and Lipsitz 1995; Fisher et al. 2005; Vloet et al. 2005). Those groups most frequently affected are the elderly (Lipsitz et al. 1983) and those with autonomic dysfunction, most often due to diabetes (Mathias et al. 1989a; Mathias 1991; Jansen and Lipsitz 1995) and Parkinson's disease (Micieli et al. 1987; Chaudhuri et al. 1997). There has been some variation in the reported prevalence of postprandial hypotension in these groups, which is likely to reflect, at least in part, differences in the test meals used to evaluate the blood pressure response (Jansen and Lipsitz 1995), as well as heterogeneity in the populations studied. In studies related to the epidemiology/pathophysiology of postprandial hypotension, it has been proposed that a drink comprising 75 g glucose in 300 ml water, should be used routinely. This drink has been shown to induce a fall in

blood pressure, comparable in magnitude to that observed after a meal (Jansen and Hoefnagels 1991).

1.2.1 Prevalence of postprandial hypotension in the elderly

A significant, meal-induced, fall in blood pressure occurs frequently in healthy older persons. In an initial study, involving 21 healthy, community-dwelling older subjects, there was a mean postprandial reduction in systolic blood pressure of ~ 11 mmHg after a standardised lunch (Lipsitz and Fullerton 1986). In cohorts of 499 (Aronow and Ahn 1994) and 113 (Vaitkevicius et al. 1991) older nursing home residents, following a standardised carbohydrate meal, ~ 24 – 36 % experienced a fall in systolic blood pressure of ≥ 20 mmHg. In a very large study of community-dwelling older adults (n = 5888), aged 65 years or older, systolic blood pressure was significantly less in the first hour (~ 130 mmHg) after a non-standardised meal, when compared to measurements obtained immediately (~ 133 mmHg) and at four hours (~ 136 mmHg) after the meal (Smith et al. 2003). More recently, in a study of 85 frail geriatric patients admitted to hospital, 57 (67 %) experienced a significant fall in postprandial systolic blood pressure of ~ 34 mmHg following a standardised liquid meal (Vloet et al. 2005). In 150 long-term care older patients, who were fed either orally, via nasogastric tube, or using percutaneous endoscopic gastrostomy, 64 (43 %) exhibited a decrease in systolic blood pressure of ≥ 20 mmHg after a high carbohydrate meal (Lubart et al. 2006). The prevalence of postprandial hypotension in healthy older individuals and nursing home residents is ~ 25 % and ~ 30 – 40 %, respectively.

1.2.2 *Prevalence of postprandial hypotension in diabetes mellitus*

Information relating to the prevalence of postprandial hypotension in patients with diabetes is limited to two studies, both involving type 2 patients (Sasaki et al. 1992; Jones et al. 1998). In 35 patients with long-standing type 2 diabetes, 7 (20 %) exhibited a fall in systolic blood pressure of > 20 mmHg following a 75 g glucose load (Sasaki et al. 1992), while in 16 recently diagnosed type 2 diabetics managed by diet alone, there was a decrease in mean arterial blood pressure of > 20 mmHg after a 75 g glucose drink, in 7 (44 %) patients (Jones et al. 1998). The prevalence of postprandial hypotension in individuals with diabetes mellitus is ~ 25 %, which is often secondary to autonomic nerve dysfunction.

1.2.3 *Prevalence of postprandial hypotension in patients with other illnesses*

In approximately 82 % of patients with Parkinson's disease there is a significant postprandial fall in blood pressure (Mehagnoul-Schipper et al. 2001). In another study of 13 patients with Parkinson's disease, all had evidence of postprandial hypotension on 24-hour ambulatory blood pressure monitoring (Ejaz et al. 2006). In 10 unselected patients with Alzheimer's disease, 7 exhibited a fall in blood pressure of \geq 20 mmHg following a standard meal (Idiaquez et al. 1997). Postprandial hypotension has also been reported in a high percentage (~ 75 %) of patients with renal failure treated with haemodialysis (Zoccali et al. 1989) and in patients with paraplegia (Catz et al. 1992; Catz et al. 2007).

1.2.4 *Significance of postprandial hypotension*

There is a strong association between falls and syncope in older subjects with postprandial hypotension (Vaitkevicius et al. 1991; Aronow and Ahn 1994; Le Couteur et al. 2003). For example, in 23 – 50 % of older patients with a history of falls or syncope, there is a significant meal-related fall in systolic blood pressure, particularly in those with hypertension (Jansen et al. 1995; Puisieux et al. 2000). Postprandial hypotension appears to be an independent predictor of mortality in older low-level nursing home residents (Fisher et al. 2005). In a study of 179 nursing home residents followed for a total duration of 4.7 years, the mortality rate of those without postprandial hypotension was significantly less at 98.5 per 1000 person-years compared to 145.0 per 1000 person-years in patients with postprandial hypotension (Fisher et al. 2005). Furthermore, the risk of death was related to the magnitude of the postprandial reduction in blood pressure; in patients with postprandial falls in systolic blood pressure of 10 mmHg or less, 11 – 19 mmHg, 20 – 39 mmHg and \geq 40 mmHg, the mortality rates per 100 person-years were 89.1, 116.9, 144.4 and 156.1, respectively (Fisher et al. 2005). Information relating to the impact of postprandial hypotension on mortality in other groups is lacking at present.

1.3 *Factors influencing postprandial blood pressure*

The aetiology of postprandial hypotension is poorly defined, however, a number of factors are known to influence the magnitude of the postprandial fall in blood pressure. These include: meal composition (Jansen et al. 1990; Visvanathan et al. 2004), volume (Vloet et al. 2001) and temperature (Kuipers et al. 1991), the time

of meal ingestion (Kohara et al. 1998; Puisieux et al. 2000), body posture (Mader 1989; Vaitkevicius et al. 1991), various medications (Jansen et al. 1988; Aronow and Ahn 1994; van Kraaij et al. 1999; Mehagnoul-Schipper et al. 2002; Le Couteur et al. 2003) and a number of illnesses related to either age (Jansen et al. 1987; Haigh et al. 1991) and/or disordered autonomic nerve function (Hoeldtke et al. 1986b; Kooner et al. 1989; Mathias et al. 1989a; Mathias et al. 1989b; Hoeldtke et al. 1991; Hirayama et al. 1993a; Lipsitz et al. 1993).

1.3.1 Meal composition

The effects of meal composition on the postprandial fall in blood pressure have been inconsistent (Jansen et al. 1990; Visvanathan et al. 2006; Gentilcore et al. 2008a). Of the macronutrients, ingestion of carbohydrate, in particular glucose, was believed to have the greatest effect on blood pressure (Mathias et al. 1989b; Jansen et al. 1990; Heseltine et al. 1991a; Visvanathan et al. 2005). In an early study by Jansen et al, of 10 unselected hypertensive patients aged 70 years or more, there was a reduction in mean arterial pressure of ~ 15 mmHg following a glucose drink (75 g in 300 ml water), while, fat (215 g cream in 300 ml water), protein (74 g whey in 300 ml) and water (300 ml), had little, or no, effect on blood pressure (Jansen et al. 1990). Studies evaluating the effects of protein (Potter et al. 1989; Jansen et al. 1990) and fat (Hoeldtke et al. 1985; Potter et al. 1989; Jansen et al. 1990; Waaler and Eriksen 1992; Sidery et al. 1993) on blood pressure remain controversial. In 10 healthy older subjects, consumption of a high protein mixed meal (53 % chicken) was reported to result in a significant fall (mean ~ 9 mmHg) in systolic blood pressure (Potter et al. 1989). In 8 healthy

older subjects, intraduodenal infusion of both fat and protein induced comparable and substantial falls in blood pressure when compared to glucose, however, the onset of the hypotensive responses to fat and protein was slower (Gentilcore et al. 2008a). In another study of healthy older subjects, ingestion of a high fat drink resulted in a comparable, albeit delayed substantial fall (mean ~ 17 mmHg) in systolic blood pressure and rise in heart rate, when compared with an isocaloric glucose drink (Visvanathan et al. 2006). The reported effects of fat on blood pressure may potentially reflect its relatively slow rate of gastric emptying, and/or the digestion of triglyceride to fatty acids. The slowing of gastric emptying, stimulation of gastrointestinal hormone release and suppression of energy intake induced by oral fat are mediated by fatty acids, rather than triglycerides (Feinle et al. 2001). Accordingly, it would not be surprising if the hypotensive response to fat is also dependent on lipolysis and this could account for the relative latency in the response to triglyceride.

There is relatively little information about the effect of different carbohydrates on postprandial blood pressure. Simple sugars, such as fructose (Jansen et al. 1987) and xylose (Mathias et al. 1989b; Robinson and Potter 1995), appear to have a substantially reduced effect than glucose on blood pressure. However, in healthy older subjects, the ingestion of equal amounts of glucose and sucrose (50 g in 300 ml water), has been reported to result in comparable decreases in blood pressure, with the fall occurring earlier following glucose compared with sucrose (Visvanathan et al. 2005). Hence, the hypotensive effect of monosaccharides may be dependent on their affinity for the glucose transporter, so that sucrose must be

digested to glucose and fructose to trigger the response (Visvanathan et al. 2005). Information relating to the effect of xylose on blood pressure is inconsistent. Studies in healthy older subjects reported that there was no fall in blood pressure following oral xylose in individuals who had a fall in blood pressure following oral glucose (Robinson et al. 1992; Robinson and Potter 1995). In contrast, Mathias et al. observed a small, transient, fall in blood pressure following oral xylose in patients with autonomic failure, who also exhibited a fall in blood pressure following oral glucose (Mathias et al. 1989b; Mathias 1990). Differences in the rate of small intestinal nutrient delivery may have influenced the findings, as a limitation of these studies is that the rate of gastric emptying of both glucose and xylose was not measured. Gastric emptying of glucose and xylose was quantified in the study described in **Chapter 6**.

1.3.2 *Meal volume/macronutrient load*

The magnitude of the postprandial reduction in blood pressure appears to be greater with increased carbohydrate content (Puvi-Rajasingham and Mathias 1996; Staneczak et al. 2001; Vloet et al. 2001). In 12 older subjects with known postprandial hypotension, both the magnitude and duration of the fall in systolic blood pressure after 200 ml liquid drinks were progressively greater with increased carbohydrate content, with the mean maximum falls in systolic blood pressure ~ 28 mmHg after 25 g, ~ 39 mmHg after 65 g and ~ 40 mmHg after 125 g carbohydrate (Vloet et al. 2001). The study design did not allow for the potential effects of meal carbohydrate concentration to be discriminated from those of the carbohydrate load or the rate of gastric emptying. In another study of

7 subjects with primary autonomic failure, ambulatory blood pressure was measured 30 minutes following the consumption of isocaloric 3 'large' or 6 'small' mixed meals, in the lying, sitting and standing positions (Puvi-Rajasingham and Mathias 1996). The fall in systolic blood pressure was observed to be greater with increased carbohydrate intake at a given meal, i.e. mean systolic blood pressure was less after the 'large', when compared with 'small', meals in the lying (~ 131 vs ~ 151 mmHg), sitting (~ 109 vs ~ 124 mmHg) and standing (~ 89 vs 103 mmHg) postures (Puvi-Rajasingham and Mathias 1996). The use of a mixed meal limits the capacity to discriminate the potential hypotensive effect of carbohydrate from that of other meal constituents. Furthermore, it is difficult to conclude whether these observations reflect differences in the rate of, or duration of, gastric emptying, as it is known that in healthy subjects, following the ingestion of mixed meals of varying sizes, the overall rate of caloric delivery into the small intestine is relatively constant (Puvi-Rajasingham and Mathias 1996).

1.3.3 *Time of meal ingestion*

There is some evidence that the magnitude of the decrease in postprandial blood pressure is greater earlier in the day (Kohara et al. 1998; Puisieux et al. 2000; Vloet et al. 2003). For example, in 156 older patients with a history of falls or syncope, in whom 24-hour ambulatory blood pressure monitoring was performed, the mean decreases in systolic blood pressure, two hours after a standard breakfast, lunch and dinner, were, as expected, greater in those patients with a history of syncope (~ 13 mmHg) or falls (~ 12 mmHg) when compared to the control group (~ 8mmHg) (Puisieux et al. 2000). However, in all patients, the

magnitude of the fall in blood pressure was ~ 2 mmHg greater following breakfast when compared with other meals (Puisieux et al. 2000). Interpretation of these observations are difficult as the study did not employ standardised meals, and the breakfast meal contained a relatively higher amount of carbohydrate.

1.3.4 Meal temperature

Meal temperature may influence postprandial blood pressure (Kuipers et al. 1991). In healthy older subjects, following consumption of a warm (50°C) glucose drink, mean arterial blood pressure initially rose (~ 4 mmHg) and then fell (~ 8 mmHg), but remained essentially unchanged following a cold drink (5°C) (Kuipers et al. 1991). There is evidence that specific warm- and cold- sensitive receptors are present in the gastrointestinal tract and meal temperature is known to affect gastric emptying in healthy young subjects (Sun et al. 1995).

1.3.5 Body posture

Postprandial hypotension is evident in both the supine (Mader 1989) and sitting (Vaitkevicius et al. 1991) positions. In 113 older nursing home residents, there was a greater fall in blood pressure in subjects who were upright during the period immediately following a mixed meal, when compared to those that were bed-bound (Vaitkevicius et al. 1991). It has been suggested that orthostatic hypotension, commonly caused by impaired autonomic function, may contribute to the effect of posture on the postprandial fall in blood pressure (Teramoto et al. 1997). However, in a study involving 50 independent older individuals, this was not the case, in that, orthostatic hypotension was additive to postprandial

hypotension rather than having a synergistic effect (Maurer et al. 2000). Therefore, therapies aimed at reducing orthostatic hypotension may not prove to be effective in the management of postprandial hypotension.

1.3.6 Medication

The relationship between postprandial blood pressure and medication use is poorly characterised, although various medications including diuretics (van Kraaij et al. 1999; Mehagnoul-Schipper et al. 2002), antipsychotics (Aronow and Ahn 1994; Le Couteur et al. 2003), selective serotonin reuptake inhibitors (Le Couteur et al. 2003), angiotensin-converting enzyme inhibitors (Mehagnoul-Schipper et al. 2002), calcium channel blockers (Jansen et al. 1988), nitrates (Aronow and Ahn 1994) and digoxin (Aronow and Ahn 1994), have all been reported to potentiate the postprandial reduction in blood pressure. Studies have evaluated the effects of frusemide, a diuretic, on postprandial blood pressure in older patients already receiving multiple therapies for cardiovascular disease (van Kraaij et al. 1999; Mehagnoul-Schipper et al. 2002). There was a reduction in the postprandial fall in systolic blood pressure from 25 ± 4 to 11 ± 2 mmHg in 13 subjects in whom frusemide was withdrawn over ~ 3 months (van Kraaij et al. 1999). Furthermore, in another study of 11 subjects, an acute dose of frusemide (40 mg orally) resulted in a greater (10 mmHg) reduction in systolic blood pressure in response to a carbohydrate liquid meal (292 kcal) (Mehagnoul-Schipper et al. 2002). Given that many cardiovascular and psychotropic drugs have hypotensive properties, the administration of these medications in conjunction with a meal has the potential to potentiate the postprandial fall in blood pressure (Jansen and Lipsitz 1995). These

effects may relate to changes in splanchnic blood flow, potentially with serotonergic compounds (Hansen et al. 1998).

1.3.7 Other illnesses

Ageing is known to be associated with impaired postprandial blood pressure regulation (Fagan et al. 1986; Kohara et al. 1998), although, recent studies have provided evidence that age-related illness, and not healthy ageing alone, is pivotal to the development of postprandial hypotension (Oberman et al. 2000; Smith et al. 2003). The prevalence of postprandial hypotension is increased in individuals who are hypertensive (Jansen et al. 1987; Haigh et al. 1991; Jansen and Lipsitz 1995), including those with isolated systolic hypertension, which occurs frequently in the elderly (Grodzicki et al. 1998). In hypertensive patients, it has been suggested that the increase in splanchnic blood flow following a meal is reduced, effectively as a result of diminished baroreceptor function (Jansen et al. 1987; Grodzicki et al. 1998).

Postprandial hypotension has also been observed in patients with various causes of autonomic dysfunction (Jansen and Lipsitz 1995). In 10 middle-aged subjects with primary autonomic failure, there was a mean fall in systolic blood pressure of ~ 49 mmHg following a standard meal (Robertson et al. 1981). In 10 unselected patients with multiple system atrophy and postprandial hypotension following a 75 g glucose drink (225 ml water), there was a mean decrease in blood pressure of ~ 22 mmHg (Hirayama et al. 1993a), while in 10 older patients

with Parkinson's disease, there was a mean fall in postprandial systolic blood pressure of ~ 27 mmHg following a standardised lunch (Loew et al. 1995).

1.4 Pathophysiology of postprandial hypotension

The pathophysiological mechanisms underlying postprandial hypotension are poorly understood, however, a number of inter-related factors are known to influence the magnitude of the fall in blood pressure. These include: splanchnic blood flow, small intestinal nutrient exposure, gastric distension and neural and hormonal mechanisms.

1.4.1 Splanchnic blood flow

Following meal consumption, there is a substantial increase in splanchnic blood volume (~ 20 % of total blood volume), with an approximate doubling of superior mesenteric artery flow that is associated with reductions in total systemic vascular resistance and skeletal muscle blood flow (Jansen and Lipsitz 1995). In healthy young and older individuals, the magnitude of the postprandial rises in mesenteric blood flow are comparable, despite a greater fall in blood pressure in older subjects, indicative of inadequate cardiovascular adjustment for the shift of blood volume into the splanchnic system (Lipsitz et al. 1993; Sidery et al. 1993). Octreotide, a somatostatin analogue, has been shown to reduce splanchnic blood pooling (Jansen and Hoefnagels 1991; Jansen and Lipsitz 1995), and attenuate the postprandial fall in blood pressure (Jansen and Hoefnagels 1991) as demonstrated in 10 hypertensive older subjects. When given as a single subcutaneous injection, octreotide (50 µg), completely prevented a fall in blood pressure following a 75 g

glucose drink (**Figure 1.1**) (Jansen et al. 1989a). Octreotide may reduce splanchnic blood flow by a direct action on smooth muscle, or by causing redistribution of blood into the central circulation (Jansen et al. 1989a; Jansen and Hoefnagels 1991). In the majority of previous studies, the effects of nutrients on splanchnic blood flow have been evaluated after oral administration (Moneta et al. 1988; Qamar and Read 1988; Sieber et al. 1992; Waaler and Eriksen 1992; Sidery et al. 1993; Sidery et al. 1994), with only one study investigating the effect of intraduodenal infusion (Gentilcore et al. 2008a). In this latter study involving 8 healthy older subjects, superior mesenteric artery blood flow was shown to increase following isocaloric intraduodenal infusions of glucose, fat and protein, but, the increase in response to protein was less than that of glucose or fat (Gentilcore et al. 2008a). The effect of the intraduodenal glucose load on splanchnic blood flow is not known and this issue is addressed in the study reported in **Chapter 5**.

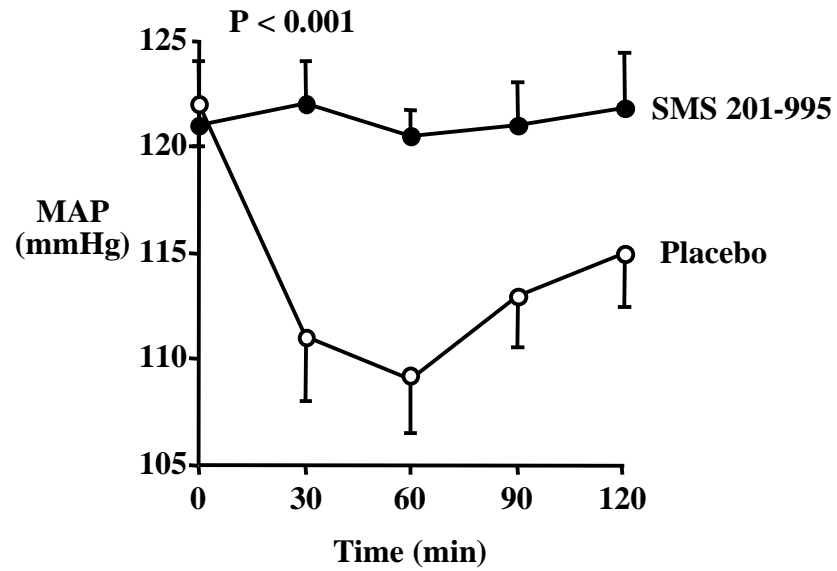


Figure 1.1: Effect of 50 μg subcutaneous octreotide on mean arterial blood pressure after 75 g glucose in hypertensive elderly subjects ($n = 10$). Data are mean \pm SEM. From Jansen et al., (Jansen et al. 1989a).

1.4.2 *Small intestinal nutrient exposure*

In healthy subjects, gastric emptying of most liquid or 'liquefied' nutrients, including glucose, occurs at an overall rate of 1 – 3 kcal/min, following an initial emptying phase that may be slightly faster or slower (Horowitz et al. 1996). This tight regulation occurs primarily as a result of inhibitory feedback arising from receptors located throughout the small intestine (Lin et al. 1989; Horowitz et al. 2001; O'Donovan et al. 2005b). Digestible solids are initially ground into small particles before emptying commences (Meyer et al. 1976). The rate of gastric emptying of carbohydrate has been shown to influence blood pressure. Jones et al initially reported in a cross-sectional study of healthy 'young' and 'older' normal subjects and patients with type 2 diabetes, that the fall in blood pressure following the ingestion of 75 g glucose in 350 ml water was greater in patients with type 2 diabetes, when compared to the normal subjects (**Figure 1.2a**) (Jones et al. 1998), and in the latter group, the magnitude of this fall was directly related to the rate of gastric emptying; so that the fall in blood pressure was greater when gastric emptying was relatively more rapid (**Figure 1.2b**) (Jones et al. 1998). In a subsequent study in healthy older subjects, intraduodenal glucose administered at a rate of 3 kcal/min, resulted in a fall in blood pressure (within 15 minutes) and rise in heart rate that was substantially greater compared to a 1 kcal/min glucose infusion (**Figure 1.3**) (O'Donovan et al. 2002), while the 1 kcal/min infusion appeared to have no effect on blood pressure. A limitation of this study was that there was no 'control' arm, and the relationship between the fall and the glucose load could not be defined (**Chapter 5**). The fall in blood pressure following oral glucose appears to be mediated by the glucose concentration and not its

osmolality. In a study in healthy older subjects, glucose was infused intraduodenally at a constant rate of 3 kcal/min but with varying glucose concentrations (4.1 %, 8.3 % and 16.7 %) (Gentilcore et al. 2006b) and the magnitude of the fall in blood pressure and rise in heart rate was comparable at all concentrations. It is likely that a threshold rate of duodenal glucose delivery of ≥ 1 kcal/min and ≤ 3 kcal/min, is required for a fall in blood pressure to occur in healthy older subjects, but remains to be determined. This issue is discussed in **Chapter 5**.

NOTE:
This figure is included on page 19
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 1.2: a) Change in mean arterial pressure (MAP) from baseline after a 75 g glucose load in patients with type 2 diabetes mellitus (n = 16), and in 'young' (n = 10) and 'older' (n = 9) normal subjects, data are mean \pm SEM and b) relationship between the area under the change in MAP curve between t = 0 - 15 mins and the 50 % emptying time (T50) of 75 g glucose in type 2 diabetic patients. Data from Jones et al., (Jones et al. 1998).

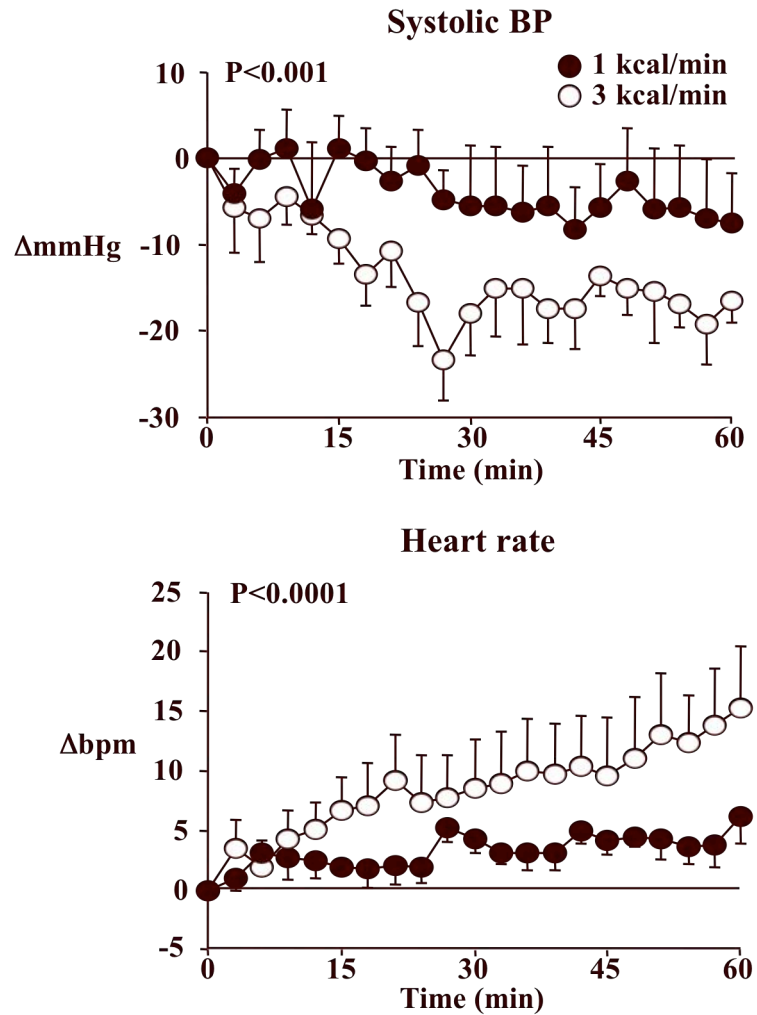


Figure 1.3: Effects of intraduodenal glucose at a rate of either 1 kcal/min or 3 kcal/min on the change from baseline in a) systolic blood pressure and b) heart rate in healthy older subjects (n = 8). Data are mean \pm SEM. Data from O'Donovan et al., (O'Donovan et al. 2002).

The concept of slowing gastric emptying/small intestinal nutrient glucose exposure, by dietary or pharmacological means, to reduce the hypotensive response to carbohydrate has recently been explored with positive outcomes in both healthy older subjects and patients with type 2 diabetes. Guar gum, a naturally occurring, gel-forming carbohydrate of vegetable origin, slows both gastric emptying and small intestinal glucose absorption (Meyer et al. 1988); the latter probably reflecting changes in the viscosity and distribution of intraluminal content (Blackburn et al. 1984a), as well as increased, nutrient-mediated, small intestinal feedback (Meyer et al. 1988). The addition of 9 g guar gum to a 50 g glucose drink (made up to 300 ml with water), has been shown to slow gastric emptying, reduce blood glucose concentrations and attenuate the fall in systolic blood pressure, in both healthy older subjects (Jones et al. 2001) (**Figure 1.4a**) and patients with uncomplicated type 2 diabetes (**Figure 1.4b**) (Russo et al. 2003). In healthy older subjects, the mean fall in systolic blood pressure was reduced from ~ 8 mmHg to ~ 4 mmHg (Jones et al. 2001). In these two studies, there was little difference in the rate of gastric emptying of the two drinks in the first ~ 30 min, suggesting that the effect of guar on blood pressure is related primarily to changes in small intestinal glucose absorption, rather than a slower rate of glucose delivery to the small intestine (Jones et al. 2001). This concept is supported in a study in healthy older subjects which demonstrated that when glucose is infused intraduodenally at 3 kcal/min with or without guar, the presence of guar attenuated the fall in systolic blood pressure and rise in heart rate (O'Donovan et al. 2005a).

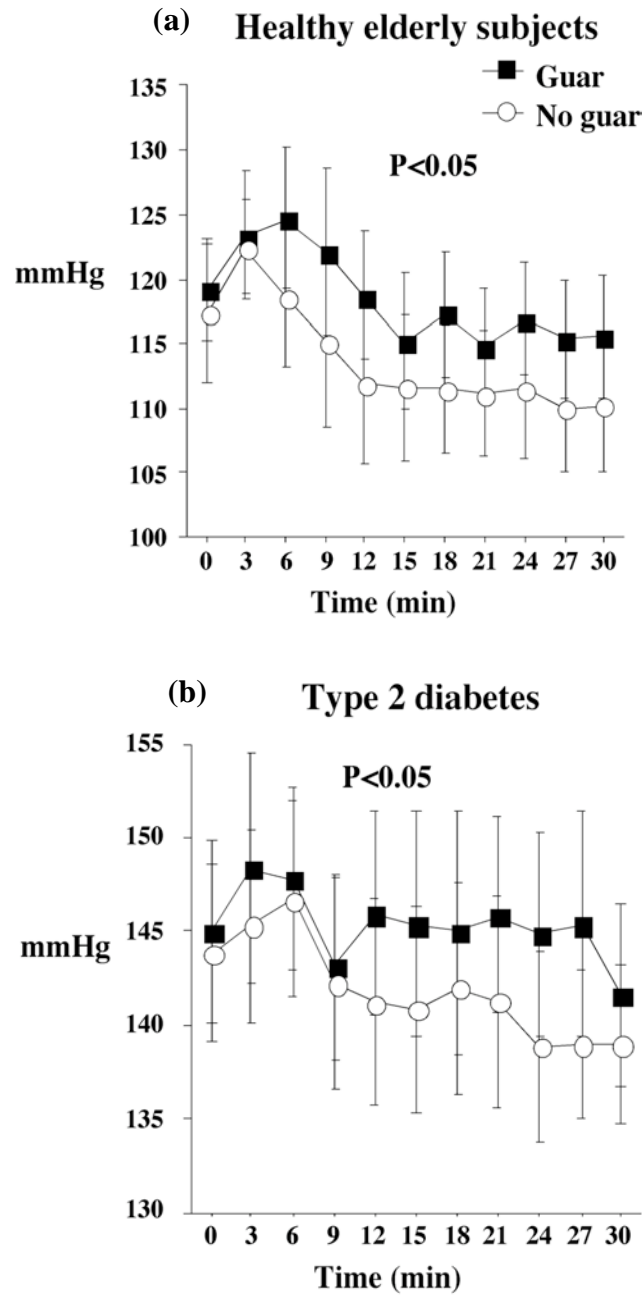


Figure 1.4: Systolic blood pressure after ingestion of 50 g glucose with or without 9 g guar gum in a) healthy older subjects ($n = 10$) and b) patients with type 2 diabetes ($n = 11$). Data are mean \pm SEM. Data from Jones et al. (Jones et al. 2001) and Russo et al. (Russo et al. 2003).

Acarbose has been used in the treatment of type 2 diabetes for many years and may also prevent the development of type 2 diabetes in people with impaired glucose tolerance, as well as induce weight loss (Chiasson et al. 2003). Acarbose reduces postprandial glycaemia without affecting fasting blood glucose; the suppression of postprandial glycaemia has been attributed to the slowing of small intestinal digestion and absorption of carbohydrate, as a result of inhibition of alpha (α)-glucosidase (Chiasson et al. 2003). Two studies involving healthy young subjects have demonstrated that acarbose has the capacity to slow gastric emptying (Ranganath et al. 1998; Enc et al. 2001). In three case studies involving patients with type 1 (Maule et al. 2004) and type 2 diabetes (Sasaki et al. 2001; Yamamoto et al. 2006), acarbose in a dose of 150 mg/day and 300 mg/day respectively, was shown to attenuate the fall in systolic blood pressure and apparently alleviate the symptoms associated with postprandial hypotension. Similar outcomes were also observed with 100 mg/day and 150 mg/day acarbose respectively, in studies involving patients with type 2 diabetes (Brooks et al. 1998), healthy older subjects (Gentilcore et al. 2005a), older subjects with severe autonomic failure (Shibao et al. 2007) and older subjects with postprandial hypotension (Jian and Zhou 2008). In the study involving healthy older subjects, acarbose slowed gastric emptying of oral sucrose, however, this was evident after ~ 90 min, whereas the fall in systolic blood pressure and rise in heart rate occurred well before that time (Gentilcore et al. 2005a). Accordingly, the dominant effects of acarbose on blood pressure and heart rate are most likely to relate to the slowing of small intestinal sucrose digestion and/or absorption (Gentilcore et al. 2005a). The effects of acarbose on blood pressure and heart rate when

administered intraduodenally have not been evaluated. This would allow the 'intra-gastric' mechanisms related to changes in gastric emptying to be 'bypassed' and is addressed in the study reported in **Chapter 10**. It should be recognised that acarbose would not be expected to affect blood pressure, or slow gastric emptying, in response to carbohydrate that does not require digestion by disaccharides, such as glucose. Cyclodextrins are oligosaccharides that inhibit pancreatic amylase activity, are poorly digested in the small intestine, and inhibit the hydrolysis of complex carbohydrates (Buckley et al. 2006). α - and beta (β)-cyclodextrins are inexpensive and diminish the glucose (Raben et al. 1997; Buckley et al. 2006), insulin (Raben et al. 1997) and glucose-dependent insulinotropic-polypeptide (GIP) (Raben et al. 1997) responses to starchy meals markedly, probably as a result of slowing gastric emptying and digestion, although, this has not been evaluated. This was addressed in the study described in **Chapter 9**.

1.4.3 *Gastric distension*

Recent studies indicate that 'intra-gastric' mechanisms, related to gastric distension, attenuate the postprandial fall in blood pressure (Rossi et al. 1998; Jordan et al. 1999; Jordan et al. 2000; Shannon et al. 2002; van Orshoven et al. 2004; Jones et al. 2005; Gentilcore et al. 2008b). Consumption of water has been shown to increase blood pressure in healthy older subjects (Jordan et al. 1999; Jordan et al. 2000), patients with multiple system atrophy and pure autonomic failure (Jordan et al. 1999; Jordan et al. 2000; Cariga and Mathias 2001). In patients with autonomic failure, drinking 480 ml of water immediately prior to the

consumption of a high carbohydrate meal, attenuated the postprandial fall in blood pressure (**Figure 1.5**), however, the rate of gastric emptying was not measured in this study (Shannon et al. 2002). There is also indirect evidence that the site of gastric distension, i.e. proximal or distal, may be important (Rossi et al. 1998; Jones et al. 2005). In another study of healthy older subjects, the fall in blood pressure was shown to be less when glucose drinks of the same concentration (12.5 %) were ingested at a higher volume (600 ml vs 200 ml), suggesting an effect of gastric distension (Jones et al. 2005). However, as the drinks were given orally, differences in gastric emptying may have contributed to the findings. In a subsequent study, the effects of gastric distension with intragastric water, during intraduodenal glucose infusion at a constant load and concentration was evaluated in healthy older subjects and intragastric distension with 500 ml water was shown to markedly attenuate the fall in systolic blood pressure in response to intraduodenal glucose (**Figure 1.6**) (Gentilcore et al. 2008b). A limitation of this study was that during intraduodenal glucose infusion, gastric emptying was slowed, so that it reached a plateau at 300 ml (Gentilcore et al. 2008b) – precluding definition of the minimum volume required to attenuate the hypotensive response to glucose, which represents the focus of the study reported in **Chapter 8**.

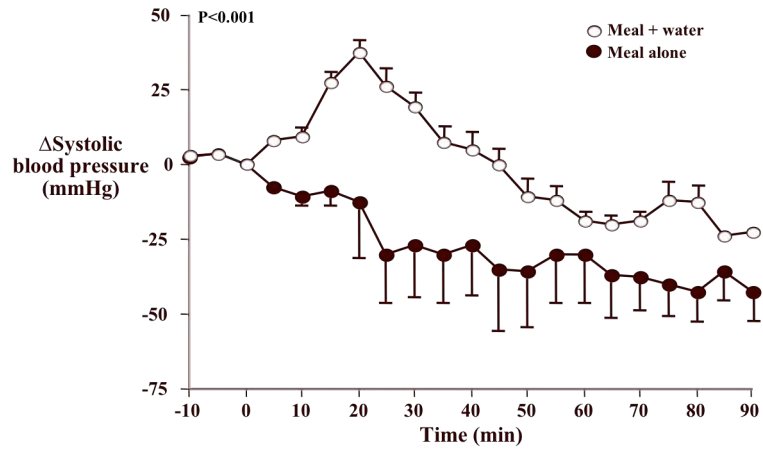


Figure 1.5: The effect of ingestion of 480 ml water prior to a meal on the change in systolic blood pressure in patients with autonomic failure (n = 11). Data are mean \pm SEM. Data from Shannon et al., (Shannon et al. 2002).

NOTE:
This figure is included on page 27
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 1.6: Effect of 500 ml of intragastric water on the change in systolic blood pressure in response to intraduodenal glucose in healthy older subjects (n = 8). Data are mean \pm SEM. Data from Gentilcore et al., (Gentilcore et al. 2008b).

The stomach can be distended at predefined volumes and pressures using a barostat device (Rossi et al. 1998; van Orshoven et al. 2004). In healthy young subjects, proximal gastric distension with a barostat increases blood pressure, heart rate and muscle sympathetic nerve activity – the so-called ‘gastrovascular reflex’ (Rossi et al. 1998). Van Orshoven et al. compared the effects of gastric distension, by ‘stepwise’ increases in gastric pressure with a barostat device, in healthy young and older subjects (van Orshoven et al. 2004). At an intragastric pressure of 8 mmHg, the older subjects exhibited a greater increase in mean arterial pressure, heart rate and total peripheral resistance; a slight increase in cardiac output, was evident in both groups (van Orshoven et al. 2004). Step-wise gastric distension induced an increase in muscle sympathetic nerve activity in both groups, but the increase in the older group was less (van Orshoven et al. 2004). The cardiovascular response to intraduodenal glucose, in the absence of gastric distension, has been investigated in healthy young and older subjects, as well as two patients with postprandial hypotension (van Orshoven et al. 2008). In both healthy young and older subjects, intraduodenal glucose induced a fall in blood pressure, rise in heart rate and increase in muscle sympathetic nerve activity, however, the fall was less in the young compared to the older subjects (van Orshoven et al. 2008). The patients with postprandial hypotension also exhibited a significant decrease in blood pressure in response to intraduodenal glucose that was substantially greater than both healthy young and older control subjects; in one patient, the fall in blood pressure, was ~ 92 mmHg at 45 minutes, and dizziness, fatigue and a brief loss of consciousness resulted (van Orshoven et al. 2008), suggesting the importance of gastric distension as a protective

mechanism in postprandial hypotension. The cardiovascular responses to intraduodenal glucose, in the presence of gastric distension using a barostat was evaluated in the studies reported in **Chapters 7** and **8**. While previous studies have established that the magnitude of the postprandial fall in blood pressure is attenuated by gastric distension, it is not known whether this reflects the change in intragastric pressure and/or intragastric volume. Furthermore, the effects of gastric distension, using a barostat, on splanchnic blood flow have hitherto not been investigated. These latter issues are addressed in the studies reported in **Chapters 7** and **8**.

1.4.4 *Neural mechanisms*

Little information is known about the neural pathways that mediate the postprandial fall in blood pressure. Those implicated include: changes in sympathetic nerve activity following glucose consumption, the neurotransmitter nitric oxide, as it plays a role in the regulation of gastric motility, and 5-hydroxytryptamine (5-HT) mechanisms in the regulation of splanchnic blood flow.

1.4.4.1 *Sympathetic nerve activity*

In healthy young subjects, ingestion of glucose is associated with increases in systemic vascular resistance (Kooner et al. 1989; Puisieux et al. 2000), heart rate (Jansen and Lipsitz 1995; Puisieux et al. 2000), stroke volume and cardiac output (Jansen and Lipsitz 1995). There are also rises in plasma norepinephrine, plasma renin and muscle sympathetic nerve activity (Jansen and Lipsitz 1995). In healthy

older subjects (Fagius et al. 1996) and patients with postprandial hypotension (Hakusui et al. 1991), the latter response is known to be attenuated (Fagius et al. 1996) probably reflecting a reduction in the muscle sympathetic nerve activity response to gastric distension, rather than to small intestinal nutrients (van Orshoven et al. 2004).

1.4.4.2 Nitric oxide

Nitric oxide is an important neurotransmitter in the gastrointestinal tract (Allescher et al. 1992; Sarna et al. 1993; Orihata and Sarna 1994; Hallgren et al. 1995; Lingenfelter et al. 1997; Su et al. 2001). It is the major non-adrenergic, non-cholinergic neurotransmitter (Takahashi 2003), and appears to act as the final common pathway in mediating enteric smooth muscle relaxation. Nitric oxide is a short acting gas which acts as a vasodilator and is released endogenously by action of nitric oxide synthase on L-arginine (Moncada and Higgs 1993; Su et al. 2001). The role of nitric oxide mechanisms is addressed optimally by the use of specific inhibitors of its production, such as NG-monomethyl-L-arginine (L-NMMA) and NG-nitro-L-arginine-methyl-ester (L-NAME) (Su et al. 2001).

Nitric oxide mechanisms appear to play a role in the regulation of gastric motility in humans (Russo et al. 1999), although the outcome of previous studies is inconsistent. Konturek et al. reported that an infusion of L-NMMA reduces antral motor activity in healthy young volunteers (Konturek et al. 1999), while Sun et al. (Sun et al. 1998) and others (Kuiken et al. 2002) have found no effect of either L-NAME (Sun et al. 1998) or L-NMMA (Kuiken et al. 2002) infusions on

antropyloroduodenal motility in healthy young subjects. The effects of nitric oxide inhibition on gastric emptying in humans have been assessed in a number of studies (Konturek et al. 1999; Hirsch et al. 2000; Gentilcore et al. 2005b; Kuo et al. 2009). In healthy young subjects, an infusion of L-NMMA slowed gastric emptying (Konturek et al. 1999). A recent study established that the fall in blood pressure and rise in heart rate and stimulation of insulin secretion induced by oral glucose in healthy older subjects are mediated by nitric oxide mechanisms by an effect apparently unrelated to changes in gastric emptying, or the secretion of the incretin hormones GIP and glucagon-like peptide-1 (GLP-1), i.e. L-NAME attenuated the fall in systolic blood pressure and rise in heart rate induced by oral glucose with minimal effect on gastric emptying (Gentilcore et al. 2005b). There are a number of potential pathways by which nitric oxide mechanisms could influence the cardiovascular responses to oral glucose and changes in splanchnic blood flow are likely to be significant. There are compelling data from animal studies, that nitric oxide is important in the regulation of splanchnic blood flow (Alemany et al. 1997; Matheson et al. 1997) but this has not been evaluated in humans. In pigs, L-NMMA attenuates the increase in mesenteric blood flow following a meal (Alemany et al. 1997), and in rats, intestinal arteriolar distension induced by topical application of glucose is blocked by L-NAME (Matheson et al. 1997). It is possible that the effects of nitric oxide blockade on oral glucose-induced insulin release related to changes in splanchnic blood flow, GLP-1 and GIP (Jansen et al. 1989b).

1.4.4.3 5-hydroxytryptamine

There is evidence that 5-HT mechanisms play a role in the regulation of splanchnic blood flow (Zinner et al. 1983). Recent, invitro studies have demonstrated that 5-HT is released from BON cells, derived from human enterochromaffin cells that act as 'glucose sensors' in the small intestine, in the presence of D-glucose, galactose, α -D-glucopyranoside (aMG) (Kim et al. 2001a; Kim et al. 2001b). The release of 5-HT by D-glucose is also known to slow gastric emptying by activation of extrinsic vagal afferent pathways containing 5-hydroxytryptamine 3 (5-HT₃) receptors (Sanger 1996; Kim et al. 2001a; Raybould et al. 2003). In the canine model, 'low' and 'high' exogenous infusions of serotonin have been reported to increase gastrointestinal blood flow by acting as a vasodilator; this effect is not associated with any changes in either cardiac output, or mean arterial pressure (Hoeldtke et al. 1986a). A recent study in healthy older subjects demonstrated that intravenous granisteron, a 5-HT₃ antagonist, had no effect on the fall in blood pressure and rise in heart rate induced by to intraduodenal glucose in healthy older subjects (Gentilcore et al. 2007).

1.4.5 Humoral mechanisms

While there is no convincing evidence that any single peptide plays a dominant role in postprandial hypotension, various vasoactive peptides released from the small intestine in response to ingestion of food have been implicated (Jansen and Hoefnagels 1991). These include: insulin, incretin hormone GLP-1, vasoactive intestinal polypeptide (VIP) substance P, calcitonin gene-related peptide (CGRP)

and neurotensin (Jansen and Hoefnagels 1991; Mathias 1991; Jansen and Lipsitz 1995).

1.4.5.1 Insulin

Insulin has been implicated in postprandial hypotension, primarily because of its known vasodilatory properties (Jansen and Hoefnagels 1991), capacity to antagonise sympathetic function (Jansen et al. 1990) and the potent effects of oral glucose, a major insulin secretagogue, on blood pressure (Jansen and Lipsitz 1995). However, intravenous glucose, which is a substantial stimulus to insulin secretion, does not affect blood pressure in older individuals (Jansen and Hoefnagels 1987; Jansen and Hoefnagels 1991). Furthermore, postprandial hypotension occurs in patients with type 1 diabetes, who are by definition insulin deficient. These latter observations argue against a major pathophysiological role for insulin (Maule et al. 2004).

1.4.5.2 Glucagon-like peptide-1

GLP-1, released from L-cells in the distal small intestine (Holst and Gromada 2004), has a number of properties – it modulates gastric motility to slow gastric emptying (Meier et al. 2003; Schirra et al. 2006), enhances glucose-dependent insulin release (Holst and Gromada 2004) and suppresses glucagon secretion (Meier et al. 2003). In humans (Edwards et al. 1998) and animals (Barragan et al. 1996), exogenous administration of GLP-1 increases blood pressure and heart rate. Conversely, studies using GLP-1 agonists (exenatide and liraglutide) in the management of type 2 diabetes suggest that long-term use may be associated with

a reduction in blood pressure (White 2009), although, these measurements were not assessed postprandially. As discussed (**section 1.4.2**), the α -glucosidase inhibitor, acarbose, which is used frequently in the management of patients with type 2 diabetes, slows gastric emptying and attenuates the fall in blood pressure induced by oral sucrose, in healthy older subjects and the slowing of gastric emptying is temporally associated with the secretion of GLP-1 (Gentilcore et al. 2005a). The stimulation of GLP-1 secretion by acarbose is substantial and presumably reflects the presence of undigested carbohydrate in the small intestine. Hence, studies evaluating the role of GLP-1 (both physiologically and pharmacologically) in postprandial hypotension are warranted.

1.4.5.3 *Vasoactive intestinal polypeptide, substance P, calcitonin gene-related peptide*

The role of VIP, a vasodilator, is unclear, as plasma levels of this polypeptide are unchanged pre- and postprandially (following oral glucose) in patients with autonomic neuropathy and in older individuals (Jansen et al. 1990). It has been suggested that VIP may act as a neurotransmitter, but not as a circulating peptide (Jansen et al. 1990). Furthermore, administration of octreotide does not affect either pre- or postprandial levels of VIP (Hoeldtke et al. 1986b), arguing against a role for VIP in postprandial blood pressure reduction. Substance P, the most powerful vasodilator, which like VIP may also function as a neurotransmitter (Jansen et al. 1990), is found in the brain and in the nerves supplying organs (Jansen et al. 1990), and also does not appear to be influenced by oral glucose or meal ingestion (Onrot et al. 1985; Hoeldtke et al. 1986b). In contrast, plasma

CGRP increases following oral glucose and has been reported to be associated with blood pressure reductions in older subjects (Edwards et al. 1996). In this latter study, older subjects who experienced decreases in blood pressure of > 15 mmHg, had greater increments in CGRP when compared with young- and middle-aged subjects (Edwards et al. 1996). While these preliminary observations implicate CGRP as a mediator of postprandial hypotension, further studies are required.

1.4.5.4 Neurotensin

Information relating to the effects of neurotensin on postprandial blood pressure is inconsistent. In 12 selected patients with autonomic neuropathy and postprandial hypotension, there was an increase (mean ~ 29 pmol/L) in plasma neurotensin after a meal when compared with normal subjects (Mathias et al. 1989a). Increases in plasma neurotensin levels were observed in 6 selected patients with autonomic neuropathy and postprandial hypotension following both oral glucose and xylose, however, decreases in postprandial blood pressure were only evident following the ingestion of glucose (Mathias et al. 1989b). In contrast, in 8 selected patients with postprandial hypotension and orthostatic hypotension, plasma neurotensin levels remained unchanged after the ingestion of a meal, when compared with basal levels (Hoeldtke et al. 1986b). Therefore, the role of neurotensin in postprandial hypotension remains uncertain.

1.5 Conclusions

This chapter has reviewed the literature in relation to the pathophysiology of postprandial hypotension, with the particular focus on gastric and small intestinal mechanisms. In this thesis, studies were designed to evaluate the following:

- 1) the effects of the small intestinal glucose load on blood pressure, superior mesenteric artery blood flow, glycaemia and GLP-1 release in healthy older subjects (**Chapter 5**).
- 2) the comparative effects of two carbohydrates, glucose and xylose, on blood pressure, gastric emptying and incretin hormones, in healthy older subjects (**Chapter 6**).
- 3) the effects of gastric distension, on blood pressure and superior mesenteric artery blood flow responses to intraduodenal glucose infusion in healthy older subjects (**Chapter 7**).
- 4) the effects of variations in intragastric volume, using a barostat, on blood pressure, heart rate and superior mesenteric artery blood flow during intraduodenal glucose infusion in healthy older subjects (**Chapter 8**).
- 5) the effects of the oligosaccharide, α – cyclodextrin, on blood pressure and heart rate in healthy older subjects (**Chapter 9**).

- 6) the effects of intraduodenal acarbose on blood pressure, heart rate and splanchnic blood flow in healthy older subjects (**Chapter 10**).

2

Regulation of blood pressure

2.1 Introduction

Blood pressure, which can be defined as the pressure or force exerted by blood on the wall of any blood vessel (Sherwood 2001; Tortora and Derrickson 2006), is dependent on the volume of blood contained within the vessel, as well as the compliance of the vessel's walls (Sherwood 2001). As blood pressure is directly proportional to the volume of blood in the cardiovascular system (Tortora and Derrickson 2006), any increase or decrease in blood volume from the normal level (~ 5 litres) results in an increase, or decrease. Systolic blood pressure is defined as the maximum pressure exerted in arteries when blood is ejected into them during systole, i.e. when the ventricles are contracting, and averages 120 mmHg in healthy adults (Sherwood 2001), while diastolic blood pressure is defined as the minimum pressure within the arteries when the ventricles are relaxing and hence, blood is draining into the vessels, and averages 80 mmHg (Sherwood 2001). In this chapter, current knowledge of the factors that are known to influence blood pressure are discussed, with a focus on the mechanisms that modulate cardiac output, peripheral resistance and blood volume.

2.2 Maintenance of blood pressure

The maintenance of a steady flow of blood from the heart, around the body, is pivotal to effective organ function, and is dependent on the heart, blood vessels, and kidneys, as well as central nervous system control. Among the homeostatic mechanisms that regulate cardiovascular dynamics, those that maintain blood pressure are central. **Figure 2.1** summarises the pathways that are involved in the

maintenance of blood pressure, which include: cardiac output, peripheral resistance, regulatory centres and hormones.

2.2.1 *Cardiac output*

Changes in cardiac output resulting from alterations in cardiac function and/or venous return, are common, reflecting changes in blood volume (Marieb 2001).

Cardiac output - the amount of blood ejected by the left ventricle into the aorta every minute (Marieb 2001; Sherwood 2001; Tortora and Derrickson 2006) - is increased when there is an increase in stroke volume (i.e. the volume of blood pumped out of each ventricle with each contraction of the heart) or heart rate, and leads to an increase in blood pressure (Tortora and Derrickson 2006). Conversely, a reduction in cardiac output decreases blood pressure (Tortora and Derrickson 2006).

2.2.2 *Peripheral resistance*

Peripheral resistance is regulated primarily through changes in arteriolar constriction (Marieb 2001) and refers to the resistance to blood flow imposed by the force of friction between blood and its vessel walls (Marieb 2001). It is related to the blood vessel diameter (Tortora and Derrickson 2006), as seen during vasodilation which occurs after meal consumption, which promotes an increase in splanchnic blood flow, and leads to a decrease in mean arterial pressure (Jansen and Lipsitz 1995) (as discussed in **Chapter 1.4.1**). In addition, peripheral resistance is dependent on the viscosity of blood. When viscosity increases (e.g. in conditions with an unusually high number of red blood cells), or decreases (e.g. in

conditions with a depletion of red blood cells), there is a consequent increase, or decrease, in blood pressure (Tortora and Derrickson 2006). The smaller, or larger the diameter of blood vessels, in particular the arterioles (which have a considerable amount of smooth muscle in their walls, and, are therefore, able to change their diameter), the greater or less resistance to blood flow resulting in an increase or decrease in blood pressure (Marieb 2001; Tortora and Derrickson 2006).

2.3 Regulatory centres/pathways

The neural control of peripheral resistance is directed at two main goals - to alter blood distribution to respond to specific demands, and maintain adequate mean arterial pressure by altering blood vessel diameter. Under conditions of low blood volume, all vessels, except those supplying the heart and brain, are constricted to allow as much blood as possible to flow to those vital organs. Most neural controls operate via reflex arcs chiefly involving baroreceptors and the associated afferent fibres, vasomotor centre of the medulla, vasomotor fibres and vascular smooth muscle. Occasionally, inputs from chemoreceptors and higher brain centres influence the neural control mechanism.

2.3.1 Vasomotor centre

The vasomotor centre is a cluster of neurons found within the medulla (Tortora and Derrickson 2006), the function of which is to modulate the diameter of blood vessels, particularly arterioles (Marieb 2001; Tortora and Derrickson 2006). When stimulated, the centre sends impulses to the smooth muscle in arteriole walls

resulting in a constant state of vasoconstriction (Marieb 2001; Tortora and Derrickson 2006). This vasoconstriction assists in the maintenance of peripheral resistance and blood pressure. The vasomotor centre induces vasoconstriction by increasing the number of sympathetic impulses (Tortora and Derrickson 2006). Conversely, vasodilation occurs when the number of sympathetic impulses is less than normal (Tortora and Derrickson 2006).

When blood supply to the medulla is inadequate, there is a consequent deficiency in oxygen and accumulation of carbon dioxide in cerebral tissue (Marieb 2001). Excess carbon dioxide in the blood acts as a direct stimulus to the vasomotor centre which increases sympathetic nerve activity, causing vasoconstriction and an increase in blood pressure (Marieb 2001).

2.3.2 *Cardiovascular control centre*

The cardiovascular control centre is found in the medulla within the brain stem and is responsible for activating parasympathetic nerves which slow the heart rate, decrease cardiac output and reduce blood pressure (Marieb 2001).

2.3.3 *Baroreceptors*

Baroreceptors are pressure receptors located in the walls of carotid sinus, at the junction of the bifurcation of the common carotid artery and in the aortic arch (Sherwood 2001; Tortora and Derrickson 2006). When there is an increase in blood pressure, the baroreceptors are stimulated and send impulses to the cardiovascular control centre which lead to a reduction in heart rate, and the

vasomotor centre to inhibit vasoconstriction of arterioles (Marieb 2001). Conversely, a reduction in blood pressure leads to decreased baroreceptor activity resulting in increased sympathetic, but decreased parasympathetic, outflow with blood pressure being restored to normal levels (Marieb 2001).

2.3.4 Chemoreceptors

Chemoreceptors are found near, or on, the aorta and carotid sinus and are sensitive to low oxygen, high carbon dioxide and decreased pH levels in arterial blood (Marieb 2001; Sherwood 2001; Tortora and Derrickson 2006). When relative hypoxia, (a deficiency in oxygen), hypercapnia, (an excess of carbon dioxide), or a decrease in arterial blood pH are detected, the fibres of chemoreceptors are stimulated to send impulses to the vasomotor centre which stimulate vasoconstrictor sympathetic nerves to restore blood pressure (Marieb 2001; Tortora and Derrickson 2006).

2.3.5 Higher brain centres

Cardiovascular responses associated with behaviours and emotions are mediated through the cerebral cortex – hypothalamic pathway (Sherwood 2001). During intense anger, the cerebral cortex stimulates the vasomotor centre to send sympathetic impulses to arterioles leading to an increase in blood pressure (Tortora and Derrickson 2006). In contrast, when an individual is emotionally upset, impulses arising from the higher brain centres lead to a decrease in sympathetic nerve stimulation producing vasodilation and consequently, a decrease in blood pressure (Tortora and Derrickson 2006).

2.4 Other mediators

As described above, changes in oxygen and carbon dioxide regulate blood pressure via the chemoreceptor reflexes. However, there are a number of other blood-borne chemicals that influence blood pressure by acting directly on vascular smooth muscle, or on the vasomotor centre.

2.4.1 Hormones

A number of hormones affect blood pressure by causing vasoconstriction of arterioles. Adrenaline and noradrenaline produced by the adrenal medulla, increase cardiac contractility and rate, as well as contracting abdominal and cutaneous arterioles (Marieb 2001; Tortora and Derrickson 2006). Antidiuretic hormone, or vasopressin, is produced by the hypothalamus and released from the neurohypophysis (the posterior lobe of the pituitary gland) and causes vasoconstriction of arterioles (Tortora and Derrickson 2006). Angiotensin II raises blood pressure by stimulating the secretion of aldosterone from the adrenal cortex, which modulates renal handling of sodium and water, and leads to vasoconstriction via to the release of renin (Marieb 2001; Tortora and Derrickson 2006). Endothelin, released from endothelial cells that line the lumen of all blood vessels, causes arteriolar smooth muscle contraction and is one of the most potent vasoconstrictors hitherto identified (Sherwood 2001).

2.4.2 Neural control of blood flow

Sympathetic vasoconstrictor nerves, which are particularly dense in the vasculature of the gastrointestinal tract, skin and skeletal muscle, release

noradrenaline and regulate peripheral resistance (Marieb 2001). Nerves stimulated in response to fall in blood pressure cause blood flow to be diverted away from temporarily dispensable areas in an attempt to maintain perfusion of the coronary and cerebral circulations (Marieb 2001). Sympathetic vasodilator nerves, found in the skin, are activated in response to increasing body temperature (Marieb 2001). When excess body heat is detected, arteriolar smooth muscle relaxes resulting in vasodilation, an increase in cutaneous blood flow and heat loss (Marieb 2001; Sherwood 2001).

2.4.3 *Autoregulation*

Autoregulation refers to the local adjustment of blood flow to meet the changing needs of different body tissues (Marieb 2001; Tortora and Derrickson 2006) and is dependent on local changes in vascular resistance, which compensate for changes in pressure (Marieb 2001; Tortora and Derrickson 2006). Vasodilator substances, or metabolites are produced within body tissues when the supply of oxygen falls below its consumption (Marieb 2001). These metabolites, which are thought to include potassium ions, hydrogen ions, carbon dioxide, lactic acid, adenosine (Marieb 2001; Tortora and Derrickson 2006) and nitric oxide (Marieb 2001), decrease local vascular resistance and increase tissue blood flow by dilating local arterioles and causing relaxation of precapillary sphincters, thereby restoring oxygen levels to normal (Tortora and Derrickson 2006).

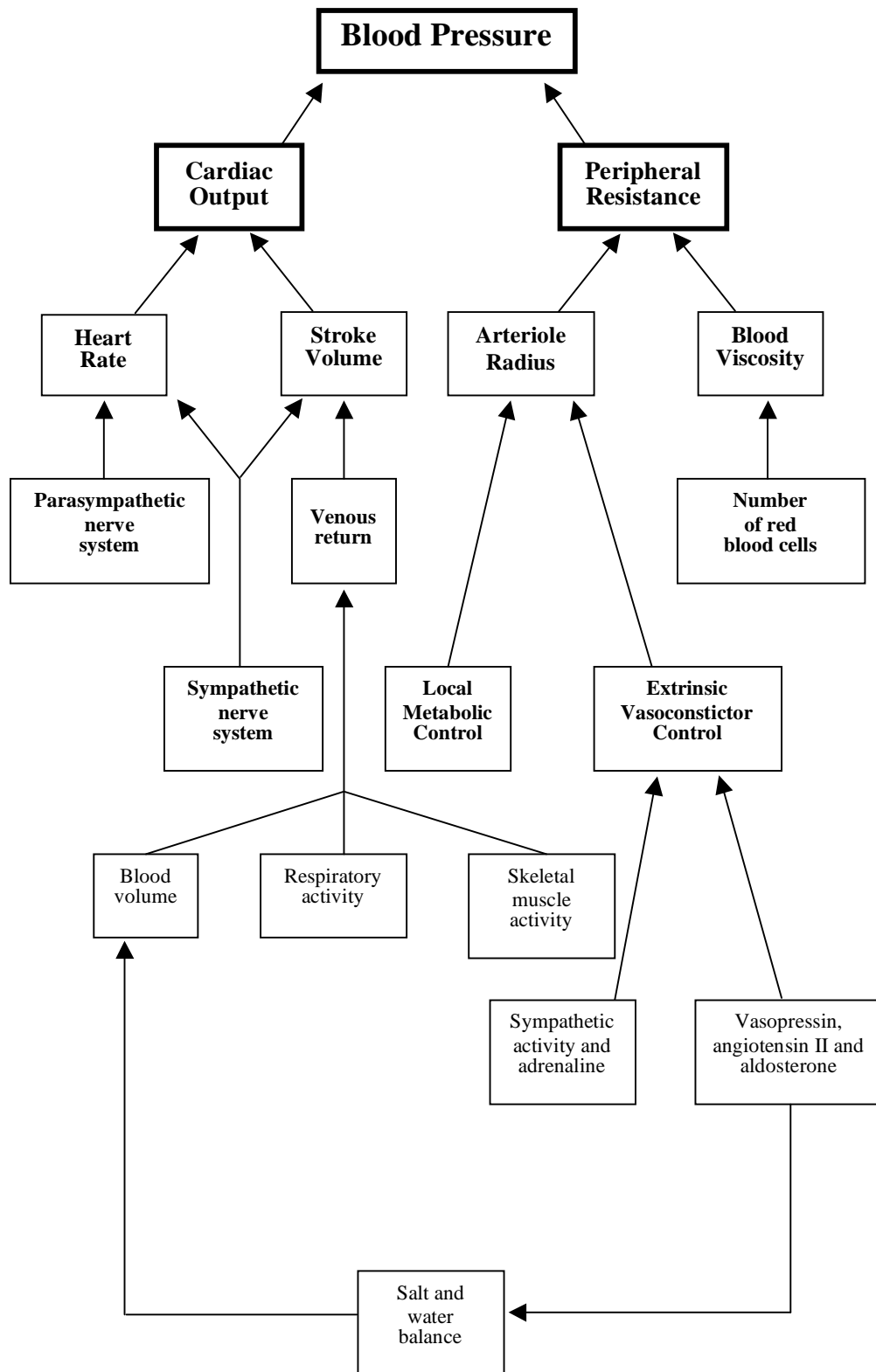


Figure 2.1: A summary of the pathways involved in the maintenance of blood pressure

2.5 Conclusions

This chapter presented a brief summary of the mechanisms involved in blood pressure regulation, which is dependent on the control of its two main determinants, cardiac output and peripheral resistance. While cardiac output is dependent on both stroke volume and heart rate, peripheral resistance is determined primarily by the degrees of arteriolar vasoconstriction. Together, with other factors that act directly on the blood vessels, short- and long- term regulation of blood pressure is achieved.

3

Management of postprandial hypotension

3.1 Introduction

Current approaches to the management of postprandial hypotension are suboptimal hence, there is a need to establish novel therapeutic strategies. The current management of postprandial hypotension includes dietary modifications (i.e. meal composition, volume and timing) (Puvi-Rajasingham and Mathias 1996), maintenance of adequate fluid intake (Jansen and Lipsitz 1995), non-nutrient gastric distension (Shannon et al. 2002), modification of medications (Jansen and Lipsitz 1995), acarbose, and somatostatin analogues, such as octreotide (Hoeldtke et al. 1986b). Recent studies in healthy elderly subjects and patients with type 2 diabetes, suggest that therapies targeted at the slowing of gastric emptying and small intestinal nutrient exposure (Jones et al. 2001; Russo et al. 2003; Gentilcore et al. 2005a; O'Donovan et al. 2005b) may prove effective.

3.2 Non-pharmacological interventions

3.2.1 Dietary modifications

It has been suggested that patients with postprandial hypotension should consume smaller and more frequent meals to minimise falls in blood pressure following meal ingestion (Waler et al. 1991, Puvi-Rajasingham and Mathias 1996, Vloet et al. 2001). Waler et al. conducted a study in four healthy younger individuals who consumed two 'smaller' meals and two 'larger' meals (calculated according to body surface area), on four separate occasions (Waler et al. 1991). The 'larger' meal induced a greater increase in cardiac output with a greater reduction in peripheral vascular resistance and postprandial fall in mean arterial pressure. Similarly, Puvi-Rajasingham et al. (Puvi-Rajasingham and Mathias 1996) found,

in seven subjects with autonomic failure, that when meals were consumed as three 'larger' meals compared with six 'smaller' meals, with an identical daily caloric intake (2.5 MJ per study day), the magnitude of the fall in systolic and diastolic blood pressure was greater following the 'larger' meals when compared with the 'smaller' meals, suggesting the fall in blood pressure is greater with increased carbohydrate loads (Puvi-Rajasingham and Mathias 1996). Furthermore, Vloet et al. (Vloet et al. 2001) reported in older subjects with a history of postprandial hypotension, that following low-, normal-, and high carbohydrate liquid meals, greater falls in blood pressure with increasing carbohydrate load (Vloet et al. 2001). The latter may be attributable to a greater duration of small intestinal nutrient exposure although gastric emptying was not measured in this study.

The effects of different nutrients on the postprandial fall in blood pressure are inconsistent (Jansen et al. 1990; Visvanathan et al. 2006; Gentilcore et al. 2008a). Of the macronutrients, ingestion of carbohydrate, in particular, glucose, was believed to have the greatest hypotensive effect when compared to fat, protein and water (Jansen et al. 1990). As such, many studies related to postprandial hypotension have employed carbohydrate meals. It may be appropriate to recommend a reduction in the intake of those sugars which have the affinity for the glucose transporter (e.g. glucose and sucrose), but there is little information about the effect of different carbohydrates on postprandial blood pressure. Previous studies in healthy older subjects have shown the decrease in blood pressure following the consumption of a 50 g oral glucose and sucrose drinks is greater than fructose (Visvanathan et al. 2005). However, the use of fructose in

the diet remains controversial, as increased consumption of fructose may result in adverse gastrointestinal effects, such as abdominal pain, loose stools and flatulence (Beyer et al. 2005). High fructose diets have also been shown to have the potential to lead to hypertension, elevated plasma triglycerides, insulin resistance and hyperinsulinaemia in dogs (Martinez et al. 1994), as well as cardiac hypertrophy (Kamide et al. 2002), reduced baroreflex sensitivity (Miller et al. 1999) and renal damage (Sanchez-Lozada et al. 2007) in rats. Information relating to the effect of xylose; a monosaccharide, derived from wood sugar, on blood pressure is inconsistent. Studies have reported no fall in blood pressure after oral xylose (42 g in 100 ml water (Robinson et al. 1992) and 0.83 g/kg body weight (Robinson and Potter 1995)) in healthy elderly people who did have falls in blood pressure following oral glucose, whereas studies by Mathias et al. (Mathias et al. 1989b; Mathias 1990) are indicative of a small, transient fall in blood pressure following an oral xylose load (0.83 g/kg body weight dissolved in 250 ml water). A limitation of these studies (Mathias et al. 1989b; Mathias 1990; Robinson et al. 1992; Robinson and Potter 1995) is that the rate of gastric emptying of both glucose and xylose was not measured, and differences in the rate of nutrient delivery into the small intestine may have influenced their observations given that the rate of gastric emptying is known to affect the magnitude of the fall in blood pressure (Jones et al. 1998). A study in monkeys has shown that gastric emptying of xylose occurs in the same fashion to that of glucose; an overall linear pattern, and more slowly with increasing concentration (Moran and McHugh 1981). However, a study in healthy male subjects reported that xylose markedly prolonged gastric emptying when compared to the same amount of glucose

(Shafer et al. 1985). The effects of xylose on postprandial blood pressure form the focus of the study reported in **Chapter 6**.

3.2.1.1 Slowing of gastric emptying and small intestinal nutrient exposure

The rate of gastric emptying of carbohydrate has been shown to influence blood pressure. As discussed in **Chapter 1.4.2**, in an initial study in type 2 diabetic patients, the magnitude of the fall in mean arterial blood pressure after an oral glucose load was shown to be related to the rate of gastric emptying of glucose, so that the fall in blood pressure was greater when gastric emptying was relatively more rapid (Jones et al. 1998). Furthermore, in healthy older subjects, intraduodenal glucose infusion resulted in a fall in blood pressure and rise in heart rate (within 15 minutes) that were significantly greater when the infusion rate was 3 kcal/min when compared to 1 kcal/min (O'Donovan et al. 2002).

The concept that slowing gastric emptying/small intestinal glucose exposure by dietary or pharmacological means would reduce the hypotensive response to carbohydrate has been explored, with favourable outcomes. As discussed in **Chapter 1.4.2**, guar gum, a naturally occurring, gel-forming polysaccharide (Blackburn et al. 1984b), slows gastric emptying and small intestinal glucose absorption in humans (Holt et al. 1979; Blackburn et al. 1984b; French and Read 1994). While the use of guar gum may represent a simple and inexpensive treatment option of postprandial hypotension, it is unpalatable. Pectin (Lawaetz et al. 1983), locust bean gum (Darwiche et al. 2003), psyllium (Washington et al. 1998), fermented milk (Sanggaard et al. 2004) and sodium propionate (Darwiche

et al. 2001) have also been shown to slow gastric emptying and small intestinal nutrient absorption, apparently primarily by increasing meal viscosity. Studies in patients with postprandial hypotension are warranted to determine their efficacies.

Administration of a fat 'preload' prior to carbohydrate ingestion has also been suggested as an approach to the management of postprandial hypotension (Gentilcore et al. 2006a). In patients with type 2 diabetes, an olive oil 'preload' (30 ml) consumed 30 minutes prior to a mashed potato meal, markedly slowed the rate of gastric emptying when compared to a water preload, attenuating both postprandial blood glucose and blood pressure (Gentilcore et al. 2006a). In contrast, when patients with type 2 diabetes, consumed a meal containing both fat and carbohydrate with orlistat, a lipase inhibitor, which reduces the digestion, and hence absorption of fat in the small intestine, the magnitude of the fall in systolic blood pressure induced by the high-fat/carbohydrate meal was potentiated (O'Donovan et al. 2004a).

As discussed in **Chapter 1.4.2**, acarbose, an α -glucosidase inhibitor, has been used in the treatment of type 2 diabetes for many years based on the concept that it delays small intestinal digestion and absorption of complex carbohydrates leading to a reduction in postprandial glycaemia. More recently acarbose has been shown to slow gastric emptying (Gentilcore et al. 2005a). In healthy older subjects 100 mg of acarbose has been shown to attenuate the decrease in systolic blood pressure and rise in heart rate induced by oral sucrose (100 g in 300 ml water) (Gentilcore et al. 2005a), and in healthy older subjects with severe autonomic

failure, 100 mg of acarbose given 20 minutes prior to a standardised meal attenuated the postprandial fall in systolic blood pressure (Shibao et al. 2007). More recently, in 43 older subjects with postprandial hypotension the decrease in systolic blood pressure was attenuated when 50 mg of acarbose was given with a standard semi-liquid meal at breakfast, lunch and dinner (Jian and Zhou 2008). The effects of acarbose on blood pressure and heart rate when administered intraduodenally have not been evaluated. Intraduodenal infusion will allow the 'intra-gastric' mechanisms related to both gastric emptying and distension to be 'bypassed'. This issue forms the focus of the study reported in **Chapter 10**. However, the use of acarbose is frequently associated with gastrointestinal side effects including: flatulence, diarrhoea and abdominal pain, limiting its tolerability (Chiasson et al. 2002). Cyclodextrins are oligosaccharides that inhibit pancreatic amylase activity, are poorly digested in the small intestine, and inhibit the hydrolysis of complex carbohydrates (Buckley et al. 2006). Alpha (α)- and beta (β)- cyclodextrins are inexpensive and diminish glucose (Raben et al. 1997; Buckley et al. 2006), insulin (Raben et al. 1997) and glucose-dependent insulinotropic-polypeptide (GIP) responses (Raben et al. 1997) to a starch meal markedly, probably as a result of slowing gastric emptying, although this has not been evaluated. In a study of 11 healthy young male subjects, peak blood glucose and plasma insulin concentrations were 65 % and 25 % lower respectively, while glucagon-like peptide-1 (GLP-1) levels remained essentially unchanged, following a potato starch meal enriched with 2 % β -cyclodextrin (Raben et al. 1997). In 10 healthy subjects, the area under the plasma blood glucose curve was reduced by 50 % after the consumption of white rice containing 5 and 10 g of α -

cyclodextrin (Buckley et al. 2006). α - and β - cyclodextrins have the potential as treatment strategies for postprandial hypotension by both slowing gastric emptying and decreasing small intestinal carbohydrate digestion and absorption. The effects of α -cyclodextrin on blood pressure, gastric emptying and glycaemia in healthy older subjects will be discussed in **Chapter 9**.

3.2.1.2 *Gastric distension*

Recent studies indicate that gastric distension attenuates the postprandial fall in blood pressure. In older patients with postprandial hypotension and in patients with autonomic failure, consumption of water prior to a meal has shown promise as a treatment (Jordan et al. 1999; Jordan et al. 2000; Shannon et al. 2002; Jones et al. 2005), probably by increasing gastric distension (Jones et al. 2005). Rapid ingestion (i.e. < 5 minutes) of water in volumes as low as 120 ml may be associated with a substantial pressor effect, in subjects with autonomic failure (Shannon et al. 2002). Shannon et al. reported in patients with autonomic failure, that when the consumption of water was increased to 480 ml immediately prior to a high carbohydrate meal, the magnitude of the fall in systolic blood pressure was substantially less (by a mean of ~ 36 mmHg), when compared to the consumption of the meal alone (Shannon et al. 2002). In patients with multiple system atrophy, consumption of 350 ml water prior to breakfast attenuated the fall in systolic blood pressure (Deguchi et al. 2007). In healthy older subjects, consumption of a 600 ml glucose drink, induces an immediate (t = 0 – 3 min) rise in systolic blood pressure (by a mean of ~ 8 mmHg), that is not evident following consumption of a 200 ml glucose drink (Jones et al. 2005). Furthermore, in this study, in the first 30

minutes, regardless of glucose content, systolic blood pressure correlated with the volume in both proximal and total stomach, but not in the distal stomach (Jones et al. 2005), suggesting that the region of gastric distension may be important, although this has not been formally evaluated. Gentilcore et al. reported in healthy older subjects, that intragastric distension with 500 ml water markedly attenuates the magnitude of the fall in systolic blood pressure in response to intraduodenal glucose at 3 kcal/min, while systolic blood pressure increased during intraduodenal saline infusion (Gentilcore et al. 2008b). However, a limitation of this study was that distension with 500 ml intragastric water could not be maintained throughout the entire duration of the intraduodenal glucose infusion, due to gastric emptying (Gentilcore et al. 2008b). While drinking two glasses of water (~ 500 ml) prior to a meal, could be regarded as a simple adjunctive treatment strategy in patients with postprandial hypotension, this may not always be feasible in the older population because of the substantial volume, as well as the potential risk of water intoxication (Shannon et al. 2002). Accordingly, studies are required to evaluate the effects of different regions of gastric distension, and to determine the minimum volume required to attenuate the hypotensive response to a meal. The latter issue forms the focus of the study reported in **Chapter 8**.

3.2.1.3 *Adequate fluid intake*

Maintenance of intravascular volume is crucial, as dehydration in older patients facilitates postprandial hypotension (Jansen and Lipsitz 1995). Adequate fluid and salt uptake are essential for the maintenance of adequate blood circulation (O'Mara and Lyons 2002). In 24 patients with heart failure, the initiation of

frusemide decreased postprandial systolic blood pressure, thereby worsening symptoms referable to postprandial hypotension (Mehagnoul-Schipper et al. 2002). Accordingly, in some patients, discontinuation of diuretic therapy, particularly frusemide, may improve postprandial blood pressure homeostasis with the preservation of adequate hydration (van Kraaij et al. 1999; Mehagnoul-Schipper et al. 2002).

3.2.1.4 Exercise

Exercise immediately following a meal has been suggested as a treatment strategy for postprandial hypotension, as it increases blood pressure, heart rate and cardiac output in healthy subjects (Puvi-Rajasingham et al. 1997). Obmerman et al. (Oberman et al. 1999) evaluated the mean arterial blood pressure following a meal, with or without exercise, in 14 frail older subjects (mean age 88 ± 7 years), who were semi-dependent ambulatory nursing home residents and reported that following exercise (walking for 10 minutes at their own pace), mean arterial blood pressure increased by ~ 18 mmHg above pre-exercise values (Oberman et al. 1999). In 10 healthy older subjects, aerobic exercise, comprising 20 minutes of cycling on a stationary bike, immediately after the consumption of a 75 g glucose drink, resulted in an immediate increase in both systolic blood pressure and heart rate (Gentilcore et al. 2008c). However, in both studies, these increases were only sustained for the duration of the exercise, and not during subsequent rest and in many patients with postprandial hypotension, exercise may not be a practical option.

3.3 Pharmacological interventions

3.3.1 *Octreotide*

The somatostatin analogue, octreotide, inhibits the secretion of almost all gastrointestinal hormones and may modulate splanchnic blood flow (Jansen and Hoefnagels 1991; Jansen and Lipsitz 1995), as discussed in **Chapter 1.4.1**; the latter is likely to be the major mechanism by which somatostatin attenuates postprandial hypotension. In older subjects (Hoeldtke et al. 1986b; Jansen et al. 1989a; Jansen et al. 1989b) and in patients with autonomic failure (Hoeldtke et al. 1986a; Raimbach et al. 1989) a single subcutaneous dose of octreotide has been shown to attenuate the postprandial fall in blood pressure. In 8 subjects with postprandial hypotension, a subcutaneous dose of octreotide (0.4 µg/kg), increased blood pressure by a mean of ~ 35 mmHg within 60 minutes of breakfast (Hoeldtke et al. 1986b). While octreotide is highly effective in attenuating postprandial hypotension, octreotide and other long acting somatostatin analogues, are associated with the limitations of high cost, the requirement of subcutaneous injections and a relatively high prevalence of adverse effects, including abdominal pain and diarrhoea (Jansen et al. 1989a; Jansen et al. 1989b). For these reasons, octreotide is currently only recommended in severely affected symptomatic patients (Jansen and Lipsitz 1995).

3.3.2 *Antihypertensive therapy*

Antihypertensive therapy may favourably affect the regulation of postprandial blood pressure, as the magnitude of the fall in postprandial blood pressure is greater when baseline blood pressure is higher. In 17 older subjects, with a history

of either stable angina or myocardial infarction, chronic treatment with nocardipine (60 mg/day) and isosorbide dinitrate (60 mg/day), for 3 weeks, attenuated the postprandial fall in systolic blood pressure (Connelly et al. 1995). This improvement in postprandial blood pressure, however, most likely represents the effect of nocardipine and isosorbide dinitrate on pre-meal blood pressure (Connelly et al. 1995), and, consequently, the magnitude of the postprandial fall in blood pressure. Further information is required about the role of antihypertensive therapy in the management of postprandial hypotension.

3.3.3 *Acarbose*

As discussed in **Chapter 3.2.2**, acarbose may be effective in the treatment of postprandial hypotension. Although acarbose may represent a therapeutic option for the treatment of postprandial hypotension, it is only effective after meals containing complex carbohydrate, and not glucose (Gentilcore et al. 2005a; Shibao et al. 2007; Jian and Zhou 2008).

3.3.4 *Glucagon-like peptide-1*

As discussed in **Chapter 1.4.5.2**, GLP-1 may be beneficial in postprandial hypotension. Little is known about the effect of GLP-1 on blood pressure regulation, however, studies in humans (Edwards et al. 1998) and animals (Barragan et al. 1996), indicate that exogenous administration of GLP-1 increases blood pressure and heart rate, these effects being blocked by the specific antagonist, exendin- (9-39) (Barragan et al. 1996).

In rats, both GLP-1 (7-36) amide and the GLP-1 analogue, exendin-4, resulted in a dose-dependent increase in blood pressure and heart rate, although the effect of exendin-4 was more sustained, presumably due to its longer half-life compared to GLP-1 (7-36) (Barragan et al. 1996). Another study in rats found that GLP-1 (1-37) produced a moderate increase in blood pressure, however, a greater effect was observed with GLP-1 (7-36) (Barragan et al. 1994). In healthy elderly subjects the α -glucosidase inhibitor, acarbose, induces an increase in the secretion of GLP-1, and this is temporally associated with the slowing of gastric emptying and attenuation of the fall in postprandial blood pressure (Gentilcore et al. 2005a). The stimulation of GLP-1 secretion by acarbose is substantial and presumably reflects the presence of undigested carbohydrate in the distal small intestine.

GLP-1 agonists e.g. (exenatide and liraglutide), have been developed for the management of type 2 diabetes. These analogues are resistant to degradation by dipeptidyl peptidase IV (DPP-IV) that rapidly degrades endogenous GLP-1. Studies using exenatide and liraglutide suggest that long-term use may be associated with an overall reduction in blood pressure (Fineman et al. 2003; Nauck and Meier 2005; Klonoff et al. 2008; Garber et al. 2009), however, these measurements have not been taken postprandially. As discussed, a reduction in baseline blood pressure may potentially be beneficial in patients with postprandial hypotension. Hence GLP-1, may potentially have a dual method of action in reducing postprandial blood pressure. Given it's relatively short half-life due to degradation, DPP-IV inhibitors have been developed. The effects of GLP-1

analogues, as well as DPP-IV inhibitors on postprandial blood pressure have hitherto not been evaluated.

3.3.5 Nitric oxide inhibitors

As discussed in **Chapter 1.4.4.2**, nitric oxide mechanisms may play a role in postprandial hypotension. A recent study in 8 healthy older subjects, investigated the blood pressure and heart rate responses following a 300 ml glucose drink containing 50 g glucose with or without an intravenous infusion of NG-nitro-arginine-methyl-ester (L-NAME) prior to consumption of the drink (Gentilcore et al. 2005b). Blood pressure fell, and heart rate increased during intravenous saline infusion, however, these effects were attenuated by L-NAME (Gentilcore et al. 2005b). Therefore, this study illustrated that the fall in blood pressure, increase in heart rate and stimulation of insulin secretion induced by oral glucose in healthy older subjects are mediated by nitric oxide mechanisms by an effect unrelated to changes in gastric emptying, or the secretion of the incretin hormones GIP and GLP-1 (Gentilcore et al. 2005b). There are a number of potential pathways by which nitric oxide mechanisms could influence the cardiovascular response to oral glucose and changes in splanchnic blood flow are likely to be important. There are compelling data from animal studies (Alemany et al. 1997; Matheson et al. 1997) that nitric oxide mechanisms are important in the regulation of splanchnic blood flow. In pigs, NG-monomethyl-L-arginine (L-NMMA) attenuates the increase in mesenteric blood flow after a meal (Alemany et al. 1997), and in rats intestinal arteriolar distension induced by topical application of glucose is blocked by L-NAME (Matheson et al. 1997). It is possible that the effects of nitric oxide

blockade on glucose-induced insulin release are also mediated by changes in splanchnic blood flow perhaps via changes in the secretion of so-called incretin hormones, GLP-1 and GIP (Jansen et al. 1989b). The effects of nitric oxide mechanisms on splanchnic blood flow in humans have not yet been evaluated.

3.3.6 Caffeine

Information relating to the efficacy of caffeine as a treatment for postprandial hypotension is inconsistent (Onrot et al. 1985; Heseltine et al. 1991b; Lipsitz et al. 1994). In patients with autonomic neuropathy and healthy older subjects, consumption of caffeine (200 – 250 mg) immediately following a standardised meal, has been reported to attenuate the postprandial fall in blood pressure (by ~ 9 – 12 mmHg) (Onrot et al. 1985; Heseltine et al. 1991b). However, other studies have reported that caffeine in a comparable dose had no effect (Barakat et al. 1993; Lipsitz et al. 1994). The cardiovascular effects of caffeine include increases in cardiac output, vascular resistance and blood pressure (Lipsitz et al. 1994), however, these responses may be blunted in individuals with a chronic caffeine intake of ≥ 300 mg (~ 5 cups of instant coffee/day) (Izzo et al. 1983). Accordingly, there is little information on which to base recommendations for the use of caffeine as treatment of postprandial hypotension.

3.3.7 Other pharmacological agents

Studies investigating the use of other therapies in the treatment of postprandial hypotension have generated inconsistent observations and many did not include patients with postprandial hypotension. Taken alone, or in combination,

nitrendipine (calcium antagonist) (Jansen et al. 1988), midodrine (α 1- agonist), denopamine (β 1- agonist) (Hirayama et al. 1993b) and vasopressin (Hakusui et al. 1991), have been suggested as potential treatments for postprandial hypotension. The rationale for their use includes the inhibition of portal blood flow and an increase in cardiac output and systemic vascular resistance (Hirayama et al. 1993b). In hypertensive older patients, 12 weeks' treatment with nitrendipine 20 mg/day was reported to reduce the fall in blood pressure following an oral glucose load from ~ 13 mmHg to ~ 7 mmHg. In another study, combination therapy with midodrine (4 mg) and denopamine (10 mg) reduced the fall blood pressure following an oral glucose load (from ~ 28 mmHg to ~ 11 mmHg) in 5 patients with autonomic dysfunction and in patients with autonomic dysfunction, midodrine (5 mg) had comparable effects when used in combination with octreotide (Hoeldtke et al. 1998). The chronic administration of midodrine is associated with a high prevalence of adverse effects including urinary urgency and supine hypertension (Hoeldtke et al. 1998). Acute, intravenous administration of vasopressin (0.3 U/min) has been reported to prevent the fall in blood pressure following an oral glucose load in patients with postprandial hypotension (Hakusui et al. 1991). However, the requirement of intravenous injections limits its use in such patients. In order to recommend these agents as effective treatment strategies for the management of postprandial hypotension, additional studies are required to evaluate their effectiveness.

3.4 Conclusion

This chapter has discussed the potential treatment options for postprandial hypotension. Some have produced modest and inconsistent effects, and in regards to pharmacotherapies, a number have adverse effects. Current management options for postprandial hypotension are less than optimal, and further studies exploring potential therapies are needed. Studies in this thesis are designed to investigate possible treatment options for postprandial hypotension, targeted at: slowing gastric emptying and small intestinal nutrient exposure, gastric distension and the effects of various carbohydrates.

4

Common methodologies

4.1 Introduction

The purpose of this chapter is to provide a brief overview and description of the techniques that were employed in the studies presented in this thesis. All of the techniques are well established, have been utilised extensively by our research group and have been considered ethically acceptable.

4.2 Subjects

Healthy older male and female subjects, aged 65 – 80 years old, who were of normal body weight for their height (body mass index (BMI) 19 – 30 kg/m²) were studied. These subjects were recruited from an existing pool of volunteers, or through the use of flyers placed around the Royal Adelaide Hospital.

4.2.1 Inclusion/Exclusion criteria

4.2.1.1 Inclusion criteria

- Male or female subjects aged between 65 – 80 years
- BMI 19 – 30 kg/m²

4.2.1.2 Common exclusion criteria

- Subjects with a history of diabetes mellitus (or fasting plasma glucose levels > 7.0 mmol/L), severe respiratory, cardiovascular, hepatic and/or renal disease, chronic alcohol abuse or epilepsy
- Subjects requiring medication that may influence blood pressure or gastrointestinal function

- Subjects with a past history of gastrointestinal disease, including known gastroparesis, significant upper gastrointestinal symptoms, or previous gastric surgery
- Subjects with severe nasal septum deviation
- Smoking > 10 cigarettes/day
- Alcohol consumption > 20g/day

4.2.1.3 Additional exclusion criteria

Subjects in the study described in Chapter 10 were also excluded when:

- Liver function tests were outside the following ranges:
 - Alanine aminotransferase (ALT) 0 - 55 U/L
 - Alkaline phosphatase 30 - 110 U/L
 - Aspartate transaminase 0 - 45 U/L
 - Bilirubin 6 - 24 mmol/L
- Creatinine clearance < 50 ml/min and/or serum creatinine > 0.12 mmol/l

Calculated creatinine clearance was determined as follows:

$$Cr\ clearance = [140 - age\ (years) \times weight\ (kg)] / [0.814 \times serum\ creatinine\ (\mu mol/L)]$$

(For female subjects: (Cr clearance x 0.85))

- Known hypersensitivity to acarbose or to any of the inactive constituents in the tablet (i.e. cellulose, corn starch, anhydrous colloidal silica and magnesium stearate)

- Inflammatory bowel disease (e.g. ulcerative colitis and Crohn's disease), intestinal obstruction (or predisposition including ileus), conditions aggravated by formation of intestinal gas (e.g. Roemheld's syndrome, major hernia, intestinal obstruction, ulcer) and gastrointestinal malabsorption syndromes.
- Medications including neomycin, cholestyramine, digoxin, intestinal adsorbents, diuretics, corticosteroids, oestrogens, phenothiazines, thyroid products, phenytoin, nicotinic acid, sympathomimetics, isoniazid, digestive enzymes (e.g. amylase, pancreatin) and sucrose (including food sources).

4.3 Human ethics approval

All protocols were approved by the Human Ethics Committee (**Chapters 5 – 10**) and (if applicable) the Investigational Drug Sub-Committee (**Chapters 9 and 10**) of the Royal Adelaide Hospital, prior to recruitment of the subject. A 'Notification of intent to supply unapproved therapeutic goods under the Clinical Trial Notification Scheme' form was lodged with the Australian Government Department of Health and Aging, Therapeutic Goods Administration, prior to the commencement of the study (**Chapter 10**). Each subject provided written, informed consent prior to their involvement in the study and all experiments were carried out in accordance with the Declaration of Helsinki.

4.4 Blood pressure and heart rate

Blood pressure (systolic and diastolic) and heart rate were measured every 3 minutes in all studies using an automated, oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA). 'Baseline' blood pressure and heart rate, i.e. 't = 0 min', were calculated as the mean of the measurements taken at t = -9, -6 and -3 min. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

4.5 Intraduodenal infusion

In **Chapters 5, 7, 8** and **10** intraduodenal infusions were performed using a 17 channel manometric silicone-rubber catheter (~ 4 mm diameter) (Dentsleeve International Ltd, Mui Scientific, Ontario, Canada) that was introduced via an anaesthetised nostril into the stomach (O'Donovan et al. 2002). The catheter was allowed to pass from the stomach into the duodenum by peristalsis. As shown in **Figure 4.1**, the catheter consisted of 16 side holes (spaced 1.5 cm apart) and an infusion channel with a port located ~ 10 cm distal to the pylorus (i.e. in the duodenum). Six side-holes (channels 1 – 6) were positioned in the duodenum, ~ 2.5cm proximal to the pylorus, a 4.5 cm sleeve sensor (channel 7), with two side holes (channel 8 and 9) on the back of the sleeve, was positioned across (straddling) the pylorus, and seven channels (channels 10 – 16) were positioned in the antrum. The correct positioning of the catheter was maintained using a saline-filled, reference electrode (20 gauge intravenous cannula) inserted subcutaneously into the subject's forearm, to enable measurement of antroduodenal transmucosal

potential difference (TMPD) (Heddle et al. 1988a; Heddle et al. 1988b) across the antrum (~ 40 mV) and the duodenum (~ 0 mV).

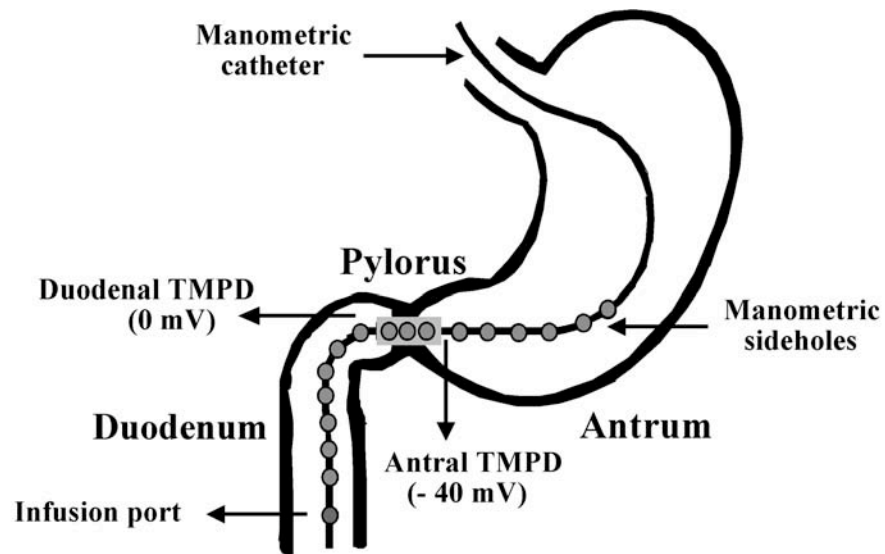


Figure 4.1: Schematic representation of the manometric silicone-rubber catheter used for intraduodenal nutrient infusion (**Chapters 5, 7, 8 and 10**).

4.6 Splanchnic blood flow

In **Chapters 5, 7, 8** and **10**, superior mesenteric artery (SMA) blood flow was measured by Duplex ultrasonography (i.e. B-mode and Doppler imaging) using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, NSW, Australia). For data acquisition, subjects were scanned using a 3.5 C broad spectrum 2.5 – 4 MHz convex transducer (Perko 2001) that was positioned just below the xiphoid process, manoeuvred slightly to the left to locate the abdominal aorta and then moved inferiorly so that the coeliac trunk and SMA could be visualised (Perko 2001). Recordings of peak velocity, end-diastolic velocity and time averaged mean velocity were acquired from Pulsed Doppler waveform complexes. Measurements of the SMA blood flow were performed 2 – 3 cm distal to the aortic origin (Jager et al. 1986; Sieber et al. 1992; Perko et al. 1996; Perko 2001) at an insonation angle of 60° (Perko et al. 1996; Perko 2001). The cross-sectional diameter of the SMA was measured in a longitudinal plane, using manually operated on-screen callipers (Sidery and Macdonald 1994; Sidery et al. 1994; Perko 2001) and this value was utilised in subsequent calculations of blood flow. Blood flow was calculated instantaneously using the time-averaged mean velocity and cross-sectional diameter of the SMA.

4.7 Gastric emptying

Several techniques can be used for the measurement of gastric emptying. In **Chapter 6**, 3D ultrasonography and in **Chapter 9**, scintigraphy was used, as described below.

4.7.1 Scintigraphy

Scintigraphy is the 'gold standard', and most commonly used method, for the evaluation of gastric emptying and intragastric meal distribution. The rate at which a radiolabelled meal empties from the stomach can be quantified over time by acquiring images on a computer via a gamma camera (Collins et al. 1983). In the study described in **Chapter 9**, radioisotopic data were acquired with the subject seated with their back against a gamma camera (Genie, GE Healthcare Technologies, Milwaukee, WI, USA) at 1-minute frames for the first 60 minutes and 3-minute frames thereafter for 60 – 300 minutes after ingestion of the drink labelled with ^{99m}Tc sulphur colloid (Jones et al. 1998). Time zero was defined as the time of completion of the drink. Upon completion of data acquisition, a 60 second left lateral image was taken with the subject seated with their left side against the gamma camera; based on a lateral image, correction factors were applied for γ -ray tissue attenuation (Collins et al. 1983). Data were also corrected for subject movement and radionuclide delay (Collins et al. 1983). Regions-of-interest were drawn around the stomach and gastric emptying curves (expressed as % retention over time) were derived (Jones et al. 1998) (**Figure 4.2**). The time taken for 50 % of the drink to empty (T50) was also determined (Collins et al. 1983) and the lag phase (the time period between meal ingestion and the initiation of emptying) was defined visually as the time before any of the radioactivity had entered the proximal small intestine (Collins et al. 1983).



Figure 4.2: Posterior scintigraphic image depicting total stomach region-of-interest (**Chapter 9**). From Gentilcore et al. (Gentilcore et al. 2006c).

4.7.2 *Ultrasonography*

While scintigraphy is regarded as the ‘gold standard’, it is non-invasive and relatively easy to perform, it is associated with a radiation burden (Hveem et al. 1996; Gilja et al. 1999). Furthermore, scintigraphy is not always readily available and is relatively expensive due to its dependence on specialised equipment (Hveem et al. 1996; Gilja et al. 1999). Another limitation of scintigraphy is that most gastric emptying studies only measure emptying of the meal post-ingestion (O'Donovan et al. 2005b) and assume that the fasting gastric content is small and that no emptying of the stomach contents occurs during meal ingestion (Tougas et al. 2000; Berry et al. 2003). Three-dimensional (3D) ultrasonography can be used as an accurate measure of the gastric emptying of liquids. This technique that has been validated against the ‘gold standard’ scintigraphy, as an accurate measure of the gastric emptying of liquids in healthy subjects (Gentilcore et al. 2006c) and in patients with diabetic gastroparesis (*unpublished*), and allows the evaluation of total, proximal and distal gastric volumes (i.e. gastric emptying and intragastric meal distribution). 3D ultrasonography is non-invasive, is associated with low cost, and does not entail a radiation burden (Hveem et al. 1996; Gilja et al. 1999). Ultrasonography also has the advantage of measuring total volume of the stomach, i.e. fasting and postprandial volumes and gastric secretions that are produced in response to the drink (Holt et al. 1986). 3D ultrasonography, however, does have some limitations. Intragastric air, particularly in the fundus (Gilja et al. 1997), has the potential to limit visualisation of the gastric outline, which may also be influenced by posture (Holt et al. 1980; Bolondi et al. 1985; Gilja et al. 1997), although previous studies have indicated that this is not a major

issue (Gilja et al. 1995; Gilja et al. 1996; Scheffer et al. 2004; Mundt et al. 2005), the technique is also user dependent requiring an experienced technician (De Schepper et al. 2004) and magnetic interference can cause spatial distortion of images (Gilja et al. 1997). Furthermore, 3D ultrasonography can only be utilized to measure the emptying of liquids; although particulate matter can be visualised on the scans, 3D ultrasonography cannot discriminate between the solid and liquid components of a meal (Holt et al. 1986; Hveem et al. 1996).

3D ultrasonography measurements (**Chapter 6**) were performed using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, NSW, Australia) with TruScan Architecture (i.e. built-in magnetically sensed 3D). For 3D positioning and orientation measurement (POM), a transmitter was placed close to the subject and a snap-on sensor attachment to a 3.5 C broad spectrum 2.5 – 4 MHz convex transducer (Tefera et al. 2002). As the transmitter produces a spatially varying magnetic field, which is distorted by ferrous and conductive metals, all metal objects were removed from the subject and from the area directly between the POM transmitter and sensor (Liao et al. 2004). The POM transmitter was placed close (approximately 20 – 30 cm) (Gilja et al. 1997) to the left side of the subject, at the level of the stomach, so that the subject was positioned between the ultrasound scanner and the transmitter. 3D sweeps of the total stomach were taken to evaluate total gastric volume. For 3D data acquisition, subjects were instructed to hold their breath at the end on inspiration (Liao et al. 2004), not move, and the stomach was scanned by a continuous translational movement along its long axis, starting proximally at the left subcostal margin, where the

transducer was tilted cranially to image the superior part of the stomach (Gilja et al. 1997), and moving distally to the gastroduodenal junction (Gilja et al. 1997; Poitras et al. 1997), to produce transverse sections of the entire stomach. The total scanning time was approximately 10 seconds. When gastric contractions were observed, the acquisition was paused until the contraction wave passed.

The raw data (original scan planes) were copied to CD-ROM and then transferred to a windows workstation. These images were used for 3D reconstructions of the stomach using EchoPAC-3D software® (GE Vingmed Sound, Horten, Norway) (Tefera et al. 2002). The proximal and distal gastric segments were separated by vertically slicing the 3D stomach reconstruction from the incisura angularis at the lesser gastric curvature sagittally towards the greater curvature (**Figure 4.3**). Total, proximal and distal gastric volumes were derived at each time point and expressed as percentages of volumes at $t = 0$ min (volume immediately following drink consumption), with total gastric volume at $t = 0$ min defined as 100 %. Gastric emptying profiles were constructed, and the time at which 50 % of the meal had emptied from the stomach (50 % gastric emptying time (T50)) was derived.

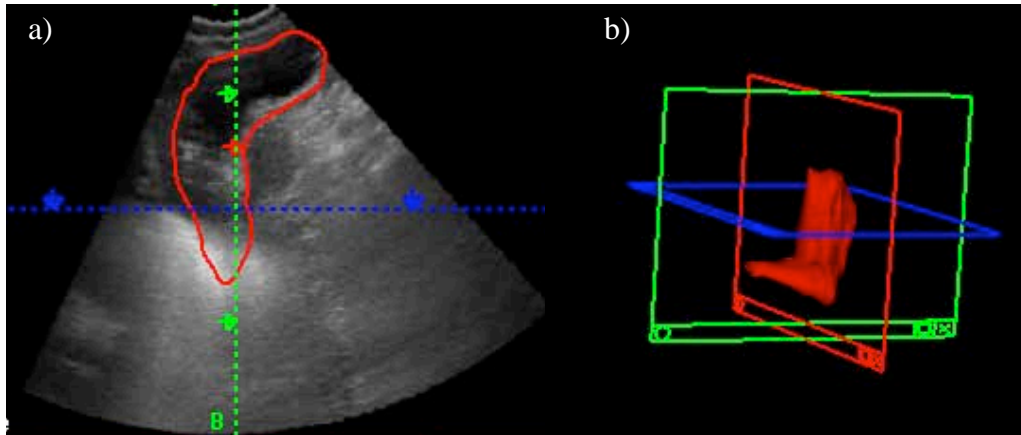


Figure 4.3: a) Ultrasonographic image of the stomach, following 3D reconstruction, demonstrating region-of-interest; and b) 3D reconstructed volumetric image of the stomach (**Chapter 6**). From Gentilcore et al., (Gentilcore et al. 2006c).

4.8 Gastric distension

The electronic barostat device is an instrument used to measure intragastric pressure and volume (Azpiroz and Malagelada 1985). The barostat consists of a strain-gauge linked by an electronic relay to an air aspiration/injection system (**Figure 4.4**). Both the strain-gauge and injection system are connected to an ultrathin, polyethylene bag by means of a single-lumen polyvinyl catheter. When the stomach contracts, the barostat aspirates air from the bag to maintain the pre-set pressure within the bag; when the stomach relaxes, air is injected into the bag (Azpiroz and Malagelada 1985). In addition to maintaining specified pressure within the bag, the barostat is also capable of performing gastric distensions at specific volumes.

In **Chapters 7** and **8**, subjects were required to swallow an orogastric catheter (4 mm outer, 2 mm inner diameter; Tygon® tubing, Sainring Gobain Performance Plastics, OH, USA), which had an ultrathin, flaccid, polyethylene bag (capacity 1200 ml: **Chapter 7**; capacity 600 ml; **Chapter 8**), tightly wrapped around its distal end (Feinle et al. 2000). The catheter was initially advanced to ~ 50 cm. The proximal end of the catheter was then connected via a three-way tap to the measurement and balloon ports of a gastric barostat (Distender Series II™, G & J Electronics Inc, Toronto, Ontario, Canada). To position the bag in the fundus of the stomach correctly, the bag was unfolded by inflating it manually with 400 ml of air, then pulling it back gently until its passage was restricted by the lower oesophageal sphincter and then pushing it aborally by 2 cm (Feinle et al. 2000). The bag was then deflated. In **Chapter 7**, the minimal distending pressure (MDP)

was determined by increasing the pressure in the barostat bag in 1 mmHg increments. The MDP was defined as the lowest pressure level that provided a mean intra-bag volume of 30 ml (Azpiroz and Malagelada 1985) necessary to overcome intra-abdominal pressure. Intra-bag pressures and volumes were digitised and recorded on a computer-based system running commercially available software (Protocol Plus™, G&J Electronics, Toronto, Ontario, Canada), and stored for subsequent analysis.

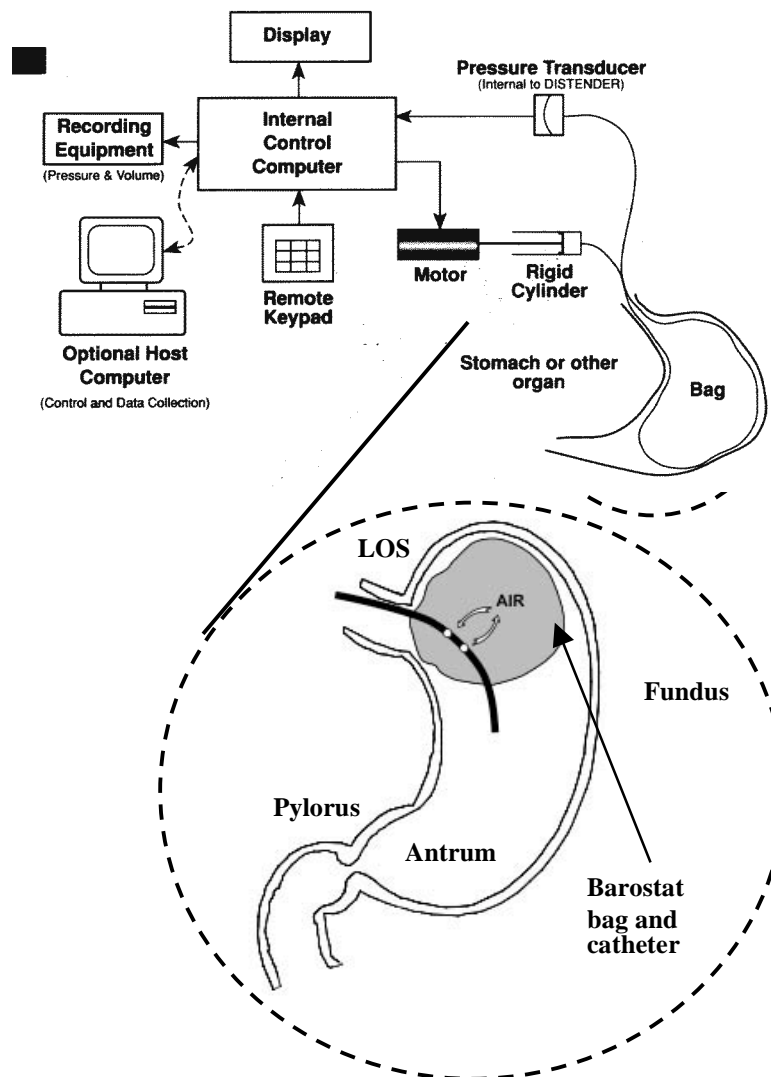


Figure 4.4: Schematic representation of the barostat bag and recording equipment used for gastric distension (**Chapter 7** and **8**).

4.9 Blood sampling

In the studies described in **Chapters 5 – 10**, an intravenous cannula was inserted into an antecubital vein for blood sampling. Venous blood samples (~ 10 ml) were taken during the studies at specific time points as described in individual chapters, for the determination of blood glucose and serum hormone concentrations.

4.9.1 Blood glucose concentrations

Blood glucose (mmol/L) was measured immediately using a portable blood glucose meter (Medisense Precision Q·I·D™ System, Abbott Laboratories, Medisense Products Inc., Bedford, MA, USA) (Jones et al. 1998). This technique has a coefficient of variation of 2.1 – 5.6 %. The accuracy of this method has been confirmed using the hexokinase technique (Horowitz et al. 1991).

4.9.2 Gastrointestinal hormone concentrations

Blood samples for the determination of serum glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were collected in ice-chilled ethylene-diamine-tetra-acetic acid (EDTA) tubes containing 400 µl whole blood protease inhibitor, aprotinin (Trasylol®; Bayer Australia Ltd., Pymble NSW, Australia). Blood samples for the determination of serum insulin were collected in Serum Z tubes containing clotting beads. Serum was separated by centrifugation (3200 rpm, 15 min, 4°C) within 30 minutes of collection and stored at -80°C until analysed.

4.9.2.1 *Serum insulin concentrations*

Serum insulin (mU/L) was measured using an ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, TX, USA). The intraassay coefficient of variation was 2.6 % and the interassay coefficient of variation 6.2 %. The sensitivity of the assay was 0.26 mU/L (O'Donovan et al. 2004b).

4.9.2.2 *Serum glucagon-like peptide (GLP-1) concentrations*

Serum GLP-1 (pmol/L) was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). Minimum detectable limit was 3 pmol/L, intra- and inter-assay coefficient of variation were 6.7 % and 7.8 %, respectively.

4.9.2.3 *Serum glucose-dependent insulinotropic polypeptide (GIP) concentrations*

Serum GIP (pmol/L) was measured by radioimmunoassay with some modifications to the original method (Wishart et al. 1992). The standard curve was prepared in buffer rather than extracted charcoal-stripped serum and the radioiodinated label was supplied by Perkin Elmer, (Boston, MA, USA). The sensitivity of the assay was 2 pmol/L, and both the intra- and inter-assay coefficient of variation were 11.2 % and 11.6%, respectively.

4.10 Cardiovascular autonomic nerve function

Autonomic nerve function was evaluated in all older subjects using standardised cardiovascular relax tests (Ewing and Clarke 1982; Piha 1991). Parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during

deep breathing and the response to standing (“30 : 15” ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according the age-adjusting predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

4.11 Questionnaires

4.11.1 Visual analogue scale questionnaires

Perceptions of appetite, including hunger and desire-to-eat, as well as gastrointestinal symptoms, nausea and fullness, were measured in **Chapter 8**, using a validated visual analogue (VAS) questionnaire (**Appendix I**). Other perceptions, including anxiety, drowsiness and dizziness, were assessed to distract the subjects from the main purpose of the questionnaire, but were not evaluated formally. Each VAS consisted of a 100 mm horizontal line, where 0 mm represented ‘sensation not felt at all’ and 100 mm ‘sensation was felt the greatest’. Subjects were asked to place a vertical mark on the 100 mm line to indicate how they felt at a particular point in time (Parker et al. 2004).

4.11.2 Likert scale questionnaire

In **Chapter 7**, perceptions of fullness, nausea and bloating were assessed using a 7-point Likert scale (Rossi et al. 1998) (**Appendix II**). Subjects were asked to quantify these sensations on a scale from 1 (no sensation) to 7 (unbearable

sensation) (Rossi et al. 1998; van Orshoven et al. 2004), immediately prior to commencing the distension and during the last minute of each distension step.

4.12 Statistical analysis

Statistical analyses performed in each study are described in detail in each of the individual chapters. Data were analysed using commercially available statistical software (i) SPSS Version 16.0 (SPSS INC, Chicago, IL, USA), (ii) SuperANOVA Version 1.1 (Abacus Concepts Inc, Berkely, CA, USA) or (iii) Statview Version 5 (SAS Institute Inc., NC, USA). In accordance with appropriate statistical practise, significant effects have been reported as treatment interaction (for area under the curve) and treatment x time interactions, treatment and/or time effects, in this hierarchy, i.e. by definition, when a treatment effect is reported, no treatment x time interaction is evident. In all studies, data are presented in figures as mean values \pm standard error of the mean (SEM) or absolute values \pm SEM and a P value < 0.05 was considered significant in all analyses. Relationships between individual parameters were assessed using linear regression analysis. Power calculations were based on data from previous studies and the number of subjects studied, and resulted in an 80% power to detect a statistical difference between study days.

4.13 Conclusion

This chapter has presented a brief overview of the techniques used in the studies presented in this thesis. More detail is provided in the methods section of individual chapters (**Chapter 5 – 10**).

5

Effects of small intestinal glucose load on blood pressure, splanchnic blood flow, glycaemia and GLP-1 release in healthy older subjects

5.1 Summary

Postprandial hypotension is an important clinical problem, particularly in the elderly. The magnitude of the fall in blood pressure is related to small intestinal glucose delivery and, possibly, changes in splanchnic blood flow and the release of glucagon-like peptide-1 (GLP-1). The primary aim of this study was to determine in healthy older subjects the effect of variations in small intestinal glucose load on BP, superior mesenteric artery (SMA) blood flow, and GLP-1 release. Twelve subjects (6M,6F; age 65 – 76 years) had concurrent measurements of BP (DINAMAP) and SMA blood flow (Doppler ultrasound), on four separate occasions, in double-blind, randomised order. On each day, subjects were intubated via an anaesthetised nostril, with a nasoduodenal catheter, and received an intraduodenal infusion of either saline ('S') (0.9 %) or glucose at a rate of 1, 2 or 3 kcal/min ('G1', 'G2', 'G3', respectively), for 60 min ($t = 0 - 60$ min), followed by intraduodenal saline for a further 60 min ($t = 60 - 120$ min). Between $t = 0 - 60$ min, there were falls in systolic and diastolic blood pressure following 'G2' and 'G3' ($P = 0.003$ and $P < 0.001$, respectively), but no change during 'S' or 'G1'. SMA blood flow increased slightly during 'G1' ($P = 0.01$), and substantially during 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$), but not during 'S'. The GLP-1 response to 'G3' was much greater ($P < 0.001$) than to 'G2' and 'G1'. In conclusion, the relationship between duodenal glucose delivery and both the hypotensive response and increase in SMA blood flow is non-linear in healthy older subjects; the duodenal glucose load needs to be greater than 1 kcal/min to elicit a significant fall in blood pressure, while the response may be maximal at a

rate of 2 kcal/min. These observations have implications for the therapeutic strategies to manage postprandial hypotension by modulating gastric emptying.

5.2 Introduction

The magnitude of the fall in blood pressure induced by enteral glucose in both healthy older subjects (Jones et al. 2001; O'Donovan et al. 2002) and patients with type 2 diabetes (Jones et al. 1998; Russo et al. 2003) is influenced by the small intestinal glucose load and apparently, not its osmolarity (Gentilcore et al. 2006b). When glucose was administered intraduodenally to healthy older subjects, at rates within the normal physiological range of gastric emptying (Brener et al. 1983), the fall in blood pressure was much greater in response to 3 kcal/min when compared to 1 kcal/min, with no change from baseline in response to 1 kcal/min, suggesting a threshold for the hypotensive response that is between 1 and 3 kcal/min (O'Donovan et al. 2002). A limitation of this study was that there was no control arm. Hence, it could not be determined whether the 1 kcal/min infusion had any effect on blood pressure. Moreover, because only two intraduodenal glucose loads were evaluated, it could not be determined whether the relationship between the fall in blood pressure and the duodenal glucose load is linear, or not. Both of these issues are of relevance to the development of therapeutic strategies designed to manage postprandial hypotension by modulating gastric emptying.

Following a meal, there is a substantial increase in splanchnic blood flow (~ 20 % of total blood volume) with an approximate doubling of superior mesenteric artery (SMA) blood flow (Jansen and Lipsitz 1995). In healthy older subjects, small

intestinal carbohydrate infusion at a rate of 3 kcal/min increases SMA flow and the magnitude of the increase is related directly to the fall in blood pressure (Gentilcore et al. 2008a), indicative of a causal association. The relationship between the small intestinal glucose load and SMA blood flow has hitherto not been evaluated.

The rate of gastric emptying is a major determinant of the glycaemic response to carbohydrate (Horowitz et al. 1993a) as well as the release of the so-called 'incretin' hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (Horowitz et al. 1993a; Jones et al. 1996; Nauck et al. 1997), in both healthy young adults (Pilichiewicz et al. 2007) and patients with type 2 diabetes (Ma et al. 2009). In both groups, the relationship between glycaemia and GLP-1 release with small intestinal glucose delivery is non-linear, so that there is relatively little difference in the glycaemic responses to intraduodenal glucose loads of 2 kcal/min and 4 kcal/min, which is attributable to a much greater GLP-1 (and hence insulin) response to the latter. The relationship between glycaemia and GLP-1 release with small intestinal glucose load has hitherto not been evaluated in apparently healthy older subjects, in whom glucose intolerance occurs frequently. There is also evidence that GLP-1 may increase blood pressure (Edwards et al. 1998), and we have reported that the attenuation of the fall in blood pressure induced by oral sucrose, using the alpha-glucosidase inhibitor, acarbose, which has been recently advocated for the use in the management of postprandial hypotension, is associated temporally with the

secretion of GLP-1 (Gentilcore et al. 2005a). Hence, GLP-1 may attenuate the hypotensive response to nutrients.

The primary aim of this study was to determine in healthy older subjects the effects of variations in small intestinal glucose load on blood pressure, SMA blood flow and GLP-1 release.

5.3 Research design and protocol

5.3.1 Subjects

Twelve healthy older subjects (6 male and 6 female) with a mean age of 68.7 ± 1.0 years (range: 65 - 76 years) and body mass index (BMI) of 23.9 ± 0.7 kg/m² (range: 20.4 – 27.4 kg/m²), recruited by advertisement, were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, or were taking medication known to influence blood pressure or gastrointestinal function.

5.3.2 Experimental protocol

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

Each subject was studied on four occasions, separated by a minimum of three days, in randomised, double-blind order. On each day, subjects attended the University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital, at 0800h following an overnight fast (10h for solids; 8h for liquids). At that time, a silicone-rubber catheter (external diameter ~ 4 mm) (Dentsleeve International Ltd, Mui Scientific, Ontario, Canada) was introduced into the stomach via an anaesthetised nostril (O'Donovan et al. 2002). The assembly included an infusion channel (internal diameter ~ 1 mm) and was positioned so that the infusion port was located ~ 10 cm distal to the pylorus (i.e. in the duodenum), as well as two other channels that were positioned in the antrum (2.5 cm proximal to the pylorus) and duodenum (2.5 cm distal to the pylorus) respectively, and were perfused with 0.9 % saline. The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference (TMPD) between the antral (-40 mV) and the duodenal (0 mV) channel (Heddle et al. 1989). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (Heddle et al. 1989).

Once the tube was positioned correctly, an automated blood pressure cuff was placed around the left arm (O'Donovan et al. 2002), and a 30-minute blood pressure stabilisation period occurred. At $t = 0$ min, the subject received, for 60 minutes (i.e. between $t = 0 - 60$ min), an intraduodenal infusion of either: (i) 1 kcal/min glucose ('G1'), (ii) 2 kcal/min glucose ('G2'), (iii) 3 kcal/min glucose ('G3') or (iv) saline (0.9 %) ('S'), at a rate of 5ml/min, in randomised order. On

all four days, isovolumetric saline was infused between $t = 60 - 120$ min (O'Donovan et al. 2002). Intraduodenal infusions were performed using a volumetric infusion pump (Imed Gemini PC-1, San Diego, CA, USA). At $t = 120$ min, the catheter was removed, the subject given a light meal and then allowed to leave the laboratory. On one of the study days cardiovascular autonomic nerve function was evaluated immediately after the completion of the study (Ewing and Clarke 1982; Piha 1991).

5.3.3 *Measurements*

5.3.3.1 *Blood pressure and heart rate*

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at $t = -9, -6$ and -3 min prior to commencement of the intraduodenal infusions and then every 3 minutes between $t = 0 - 120$ min (O'Donovan et al. 2002). 'Baseline' blood pressure and heart rate, i.e. ' $t = 0$ min', were calculated as the mean of measurements taken at $t = -9, -6$ and -3 min. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

5.3.3.2 *Splanchnic blood flow*

SMA blood flow was measured by Duplex ultrasonography (i.e. B-mode and Doppler imaging) using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, Australia) (Perko 2001). Subjects were scanned using a

3.5 C broad spectrum 2.5 - 4 MHz convex transducer before ($t = -2$ min) the commencement of the intraduodenal infusion, and then at 15-minute intervals between $t = 0 - 120$ min (Gentilcore et al. 2008a). Blood flow (ml/min) was calculated instantaneously using the formula: $\pi \times r^2 \times \text{TAMV} \times 60$, where r = the radius of the SMA and TAMV is the time-averaged mean velocity (Perko 2001).

5.3.3.3 Blood glucose, serum insulin and serum GLP-1 concentrations

Venous blood samples were obtained prior to the commencement of the intraduodenal infusion (i.e. $t = -2$ min) and at 15-minute intervals between $t = 0 - 120$ min. Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Precision Q-I-D™ System, Abbott Laboratories, Medisense Products Inc, Bedford, MA, USA). Serum was separated by centrifugation at 3200 rpm for 15 minutes at 4°C within 30 minutes of collection at stored at -70°C until analysed. Serum insulin (mU/L) was measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc, Webster, TX, USA). The sensitivity of the assay was 0.26 mU/L and the coefficient of variation was 2.6 % within assays and 6.2 % between assays. Serum GLP-1 (pmol/L) was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). Minimum detectable limit was 3 pmol/L, intra- and inter-assay coefficient of variation were 6.7 % and 7.8 %, respectively.

5.3.4 *Assessment of cardiovascular autonomic nerve function*

Autonomic nerve function was assessed using standardized cardiovascular reflex tests (Ewing and Clarke 1982; Piha 1991). Parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during deep breathing and the response to standing (“30 : 15” ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

5.3.5 *Statistical analysis*

Systolic and diastolic blood pressure and heart rate were analysed and presented as changes from baseline. SMA blood flow, blood glucose, serum insulin and serum GLP-1 were analysed and presented as absolute values. One-way ANOVA was used to analyse the effects of ‘time’ on the change from baseline values for systolic and diastolic blood pressure and heart rate, and absolute values of SMA blood flow, blood glucose, serum insulin and serum GLP-1 concentrations. The maximum fall in systolic and diastolic blood pressure and rise in heart rate, SMA blood flow, blood glucose, serum insulin and serum GLP-1 were defined as the greatest change from baseline in each subject at any given time point for each treatment. Areas under the curve (AUC) were calculated using the trapezoidal rule, and analysed by one-way ANOVA, to evaluate a treatment effect between $t = 0 - 60$ min for systolic and diastolic blood pressure and heart rate, between $t = -$

2 – 60 min for SMA blood flow, and between $t = -2 - 120$ min for blood glucose, serum insulin and serum GLP-1. We calculated that a minimum of eight subjects would be required to detect a mean difference in systolic blood pressure of ~ 10 mmHg with the power of 0.80, and at a significance level of $P < 0.05$ (O'Donovan et al. 2002). All analyses were performed using SPSS version 16.0.2 (SPSS Inc, Chicago, IL, USA). Systolic and diastolic blood pressure and heart rate data are shown as changes from baseline and mean values \pm standard error of the mean (SEM). SMA blood flow, blood glucose, serum insulin and serum GLP-1 data are shown as absolute mean values \pm SEM. A P value < 0.05 was considered significant in all analyses.

5.4 Results

The studies were well tolerated, and there were no adverse events. The mean score for autonomic nerve function was 0.75 (range: 0 - 2), i.e. no subject suffered from autonomic nerve dysfunction. One subject exhibited postprandial hypotension (i.e. a fall in systolic blood pressure > 20 mmHg sustained for at least 30 minutes), during 'G2'.

5.4.1 *Systolic and diastolic blood pressure and heart rate (Figure 5.1 a, b & c)*

There was no difference in baseline ($t = 0$ min) blood pressure or heart rate between the four days ('S' vs. 'G1' vs. 'G2' vs. 'G3'): systolic blood pressure (122 ± 5 mmHg vs. 118 ± 4 mmHg vs. 125 ± 5 mmHg vs. 122 ± 5 mmHg; $P = 0.16$); diastolic blood pressure (70 ± 2 mmHg vs. 67 ± 2 mmHg vs. 68 ± 2 mmHg

vs. 69 ± 2 mmHg; $P = 0.17$), and heart rate (57 ± 2 bpm vs. 61 ± 3 bpm vs. 57 ± 2 bpm vs. 59 ± 3 bpm; $P = 0.09$).

Between $t = 0 - 60$ min, there was a fall in systolic blood pressure during 'G2' ($P < 0.001$) and 'G3' ($P = 0.003$), but not during 'S' ($P = 0.25$) or 'G1' ($P = 0.74$). The maximum falls in systolic blood pressure from baseline during 'G2' (15 ± 2 mmHg), and 'G3' (12 ± 2 mmHg), were not different ($P = 0.18$). There was a treatment effect ($P = 0.001$) for the AUC ($t = 0 - 60$ min) for the change in systolic blood pressure, so that systolic blood pressure was less during 'G2' ($P = 0.007$) and 'G3' ($P = 0.002$), but not during 'G1' ($P = 0.17$), compared with 'S'. Similarly, the fall in systolic blood pressure was greater during 'G2' ($P = 0.03$) and 'G3' ($P = 0.005$) compared with 'G1', but there was no significant difference between 'G2' and 'G3' ($P = 0.51$). At $t = 120$ min, systolic blood pressure was not different from baseline after 'G2' (122 ± 4 mmHg; $P = 0.94$), but was greater after 'S' (127 ± 6 mmHg; $P = 0.04$) and 'G1' (126 ± 5 mmHg; $P = 0.004$), and tended to be less following 'G3' (118 ± 4 mmHg; $P = 0.09$).

Between $t = 0 - 60$ min, there was a fall in diastolic blood pressure during 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$), but not during 'S' ($P = 0.79$) or 'G1' ($P = 0.18$). The maximum falls in diastolic blood pressure from baseline during 'G2' and 'G3' (11 ± 1 mmHg and 12 ± 1 mmHg, respectively), with no difference between the two ($P = 0.46$). There was a treatment effect ($P < 0.001$) for the AUC ($t = 0 - 60$ min) for the change in diastolic blood pressure, so that diastolic blood pressure was less during both 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$), and tended to be less

during 'G1' ($P = 0.06$), compared with 'S'. Similarly, the fall in diastolic blood pressure was greater during 'G2' ($P = 0.002$) and 'G3' ($P < 0.001$) compared with 'G1', while there was no difference between 'G2' and 'G3' ($P = 0.21$). At $t = 120$ min, diastolic blood pressure was not different from baseline after 'S' (70 ± 3 mmHg; $P = 0.95$) and 'G3' (68 ± 3 mmHg; $P = 0.22$), but was greater after 'G1' (69 ± 2 mmHg; $P < 0.05$) and 'G2' (71 ± 2 mmHg; $P = 0.02$).

Between $t = 0 - 60$ min, there was a rise in heart rate during 'G2' ($P = 0.03$) and 'G3' ($P < 0.001$), but not during 'S' ($P = 0.28$) or 'G1' ($P = 0.45$). The maximum rise in heart rate from baseline was slightly greater ($P = 0.05$) after 'G3' (15 ± 4 bpm) compared to 'G2' (11 ± 3 bpm). There was a treatment effect ($P = 0.001$) for the AUC ($t = 0 - 60$ min) for the change in heart rate, so that heart rate was greater during 'G2' ($P = 0.01$) and 'G3' ($P = 0.01$), but not during 'G1' ($P = 0.67$), compared with 'S'. Heart rate was greater during 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$) compared with 'G1', with no difference between 'G3' and 'G2' ($P = 0.17$). At $t = 120$ min, heart rate did not differ from baseline after 'S' (60 ± 2 bpm; $P = 0.16$), 'G1' (60 ± 3 bpm; $P = 0.92$), and 'G2' (58 ± 2 bpm; $P = 0.46$), but was higher following 'G3' (64 ± 2 bpm; $P = 0.02$).

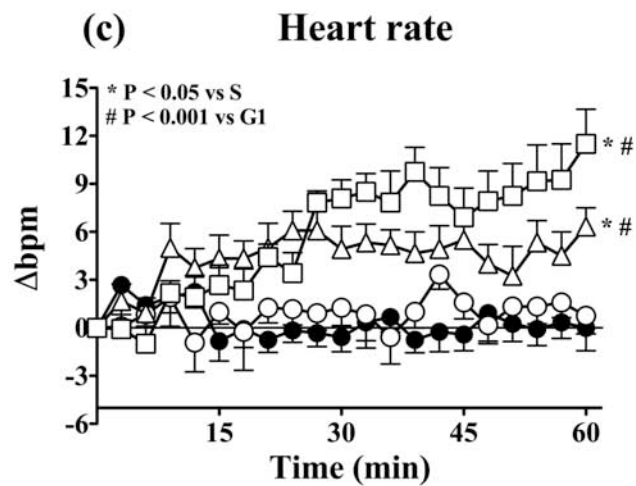
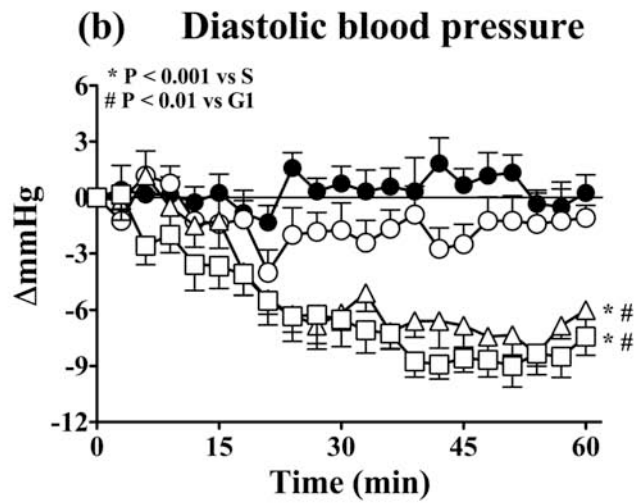
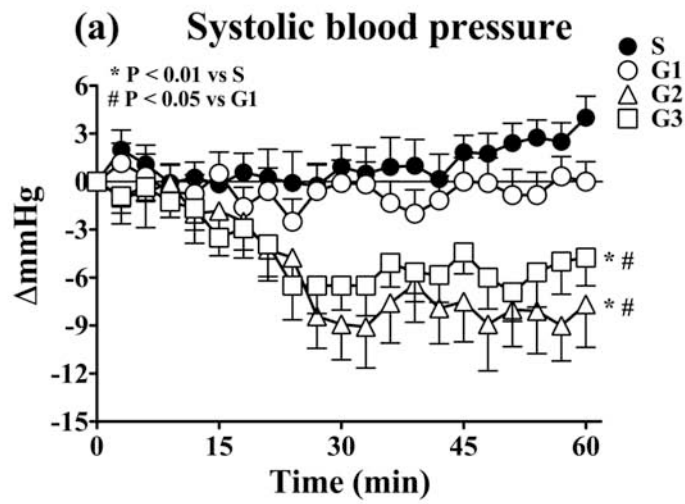


Figure 5.1: Change in (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate, from baseline, in response to intraduodenal saline (●) and glucose at a rate of either 1 kcal/min (○), 2 kcal/min (△) or 3 kcal/min (□), in healthy older subjects. Data are mean values \pm SEM (n = 12). Systolic blood pressure treatment effect: * P < 0.01 'S' compared with 'G2' and 'S' compared 'G3', # P < 0.05 'G1' compared with 'G2' and 'G1' compared with 'G3'; Diastolic blood pressure treatment effect: * P < 0.001 'S' compared with 'G2' and 'S' compared 'G3', # P < 0.01 'G1' compared with 'G2' and 'G1' compared with 'G3'; Heart rate: * P < 0.05 'S' compared with 'G2' and 'S' compared 'G3', # P < 0.001 'G1' compared with 'G2' and 'G1' compared with 'G3'.

5.4.2 SMA blood flow (Figure 5.2)

There was no difference ($P = 0.73$) in baseline ($t = -2$ min) SMA blood flow between the four days ('S' vs. 'G1' vs. 'G2' vs. 'G3'): 916 ± 50 ml/min vs. 977 ± 74 ml/min vs. 927 ± 69 ml/min vs. 965 ± 55 ml/min, ($P = 0.73$).

Between $t = -2 - 60$ min, there was a rise in SMA blood flow during 'G1' ($P = 0.01$), 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$), but no overall change during 'S' ($P = 0.15$). The peak SMA blood flow during 'G1' (1428 ± 107 ml/min) was greater than 'S' ($P = 0.04$); the peak SMA flow during 'G2' (2144 ± 133 ml/min) was greater than 'S' ($P < 0.001$) and 'G1' ($P < 0.001$), and the peak SMA flow during 'G3' (2797 ± 184 ml/min) was greater than 'S' ($P < 0.001$), 'G1' ($P < 0.001$) and 'G2' ($P = 0.001$). There was a treatment effect ($P < 0.001$) for the AUC ($t = -2 - 60$ min) of SMA blood flow, so that SMA blood flow was greater during 'G1' compared with 'S' ($P = 0.04$), during 'G2' compared with 'S' ($P < 0.001$) and 'G1' ($P < 0.001$), and during 'G3' compared with 'S' ($P < 0.001$), 'G1' ($P < 0.001$) and 'G2' ($P < 0.001$). At $t = 120$ min, SMA blood flow did not differ from baseline after 'S' (941 ± 60 ml/min; $P = 0.38$), 'G1' (903 ± 68 ml/min; $P = 0.29$), and 'G2' (927 ± 69 ml/min; $P = 0.99$), but was greater than baseline following 'G3' (1125 ± 87 ml/min; $P = 0.04$).

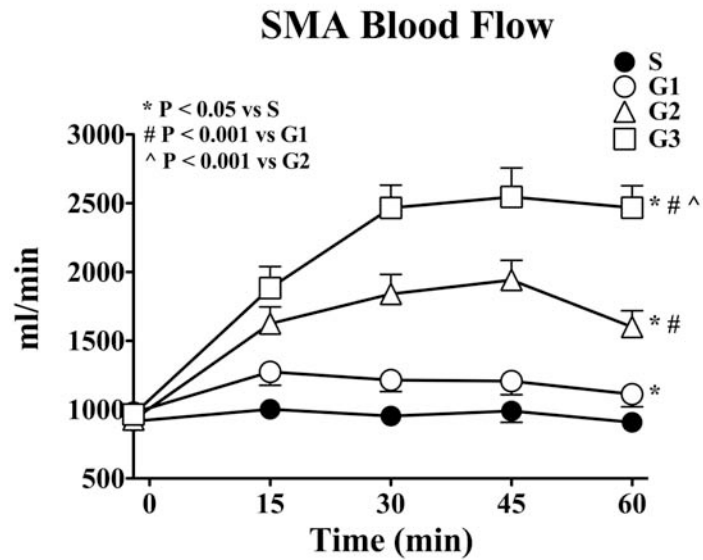


Figure 5.2: Effects of intraduodenal saline (●) and glucose at a rate of either 1cal/min (○), 2 kcal/min (△) or 3 kcal/min (□) on SMA blood flow, in healthy older subjects. Data are mean values \pm SEM (n = 12). * $P < 0.05$ 'S' compared with 'G1', 'S' compared 'G2', and 'S' compared with 'G3', # $P < 0.001$ 'G1' compared with 'G2' and 'G1' compared with 'G3', ^ $P < 0.001$ 'G2' compared with 'G3'.

5.4.3 **Blood glucose, serum insulin and serum GLP-1 (Figures 5.3a, b & c)**

There was no difference ($P = 0.65$) in baseline ($t = -2$ min) blood glucose between the four days ('S' vs. 'G1' vs. 'G2' vs. 'G3'): 6.2 ± 0.1 mmol/L vs. 6.2 ± 0.2 mmol/L vs. 6.2 ± 0.1 mmol/L vs. 6.0 ± 0.1 mmol/L, ($P = 0.65$). Between $t = -2 - 120$ min, there was an increase in blood glucose during 'G1' ($P < 0.001$), 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$), but not during 'S' ($P = 0.22$). Maximum blood glucose during 'G1' (8.9 ± 0.3 mmol/L) was greater than 'S' (6.4 ± 0.1 mmol/L) ($P < 0.001$); maximum blood glucose during 'G2' (11.1 ± 0.3 mmol/L) was greater than 'S' ($P < 0.001$) and 'G1' ($P < 0.001$), and maximum blood glucose during 'G3' (12.2 ± 0.5 mmol/L) was greater than 'S' ($P < 0.001$), 'G1' ($P = 0.001$), and 'G2' ($P = 0.02$). There was a treatment effect ($P < 0.001$) for the AUC ($t = -2 - 120$ min) for the change in blood glucose, so that the rise in blood glucose during 'G1', 'G2' and 'G3' was greater compared with 'S' ($P \leq 0.001$, for all). Similarly, the rises in blood glucose during 'G2' ($P < 0.001$) and 'G3' ($P = 0.005$) were greater compared with 'G1', while there was no difference between 'G2' and 'G3' ($P = 0.32$). At $t = 120$ min, blood glucose did not differ from baseline after 'G1' (6.2 ± 0.2 mmol/L; $P = 0.83$), 'G2' (6.6 ± 0.3 mmol/L; $P = 0.30$), and 'G3' (5.6 ± 0.8 mmol/L; $P = 0.56$), but was less following 'S' (5.9 ± 0.1 mmol/L; $P = 0.01$).

There was no difference ($P = 0.31$) in baseline ($t = -2$ min) serum insulin between the four days ('S' vs. 'G1' vs. 'G2' vs. 'G3'): 7.3 ± 0.8 mU/L vs. 6.9 ± 1.3 mU/L vs. 6.9 ± 0.9 mU/L vs. 6.1 ± 0.6 mU/L, ($P = 0.31$). Between $t = -2 - 120$ min,

there was an increase in serum insulin during 'G1' ($P < 0.001$), 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$), but no change during 'S' ($P = 0.40$). Maximum serum insulin during 'G1' (21.0 ± 2.8 mU/L) was greater than 'S' (10.1 ± 1.9 mU/L) ($P = 0.001$); maximum serum insulin during 'G2' (68.4 ± 11.7 mU/L) was greater than 'S' ($P < 0.001$) and 'G1' ($P < 0.001$), and maximum serum insulin during 'G3' (155.8 ± 25.9 mU/L) was greater than 'S' ($P < 0.001$), 'G1' ($P < 0.001$), and 'G2' ($P = 0.001$). There was a treatment effect ($P < 0.001$) for the AUC ($t = -2 - 120$ min) for the change in serum insulin, so that the increase in serum insulin during 'G1', 'G2' and 'G3' was greater compared with 'S' ($P \leq 0.001$, for all). Similarly, the increase in serum insulin during 'G2' and 'G3' was greater compared with 'G1' ($P < 0.001$, for both) and greater during 'G3' compared with 'G2' ($P < 0.001$). At $t = 120$ min, serum insulin did not differ from baseline following 'G1' (8.1 ± 1.3 mU/L; $P = 0.17$), was less than baseline following 'S' (5.7 ± 0.7 mU/L; $P = 0.005$), and greater following 'G2' (10.8 ± 1.5 mU/L; $P = 0.007$) and 'G3' (18.1 ± 2.7 mU/L; $P = 0.001$).

There was no difference ($P = 0.31$) in baseline ($t = -2$ min) serum GLP-1 between the four days ('S' vs. 'G1' vs. 'G2' vs. 'G3'): 14.4 ± 1.2 pmol/L vs. 14.5 ± 1.4 pmol/L vs. 15.8 ± 1.7 pmol/L vs. 15.9 ± 1.8 pmol/L, ($P = 0.23$). Between $t = -2 - 120$ min, there was an increase in serum GLP-1 during 'G1' ($P < 0.001$), 'G2' ($P < 0.001$) and 'G3' ($P < 0.001$) but no change during 'S' ($P = 0.17$). Maximum serum GLP-1 during 'G1' (19.4 ± 2.1 pmol/L) was not greater than 'S' (18.4 ± 2.0 pmol/L) ($P = 0.58$); maximum serum GLP-1 during 'G2' (28.3 ± 3.4 pmol/L) was greater than 'S' ($P = 0.004$) and 'G1' ($P = 0.01$), and maximum serum GLP-1

during 'G3' (62.0 ± 5.7 pmol/L) was greater than 'S' ($P < 0.001$), 'G1' ($P < 0.001$), and 'G2' ($P < 0.001$). There was a significant treatment effect ($P < 0.001$) for the AUC ($t = -2 - 120$ min) for the change in serum GLP-1, so that the rise in serum GLP-1 during 'G2' ($P = 0.02$) and 'G3' ($P \leq 0.001$) was greater, and tended to be greater during 'G1' (0.07), compared with 'S'. Similarly, the increase in serum GLP-1 during 'G2' ($P = 0.005$) and 'G3' ($P \leq 0.001$) was greater compared with 'G1', and during 'G3' compared with 'G2' ($P \leq 0.001$). At $t = 120$ min, serum GLP-1 did not differ from baseline following 'S' (15.5 ± 1.6 pmol/L; $P = 0.21$), 'G1' (13.7 ± 1.7 pmol/L; $P = 0.63$), 'G2' (12.7 ± 1.4 pmol/L; $P = 0.13$) or 'G3' (20.0 ± 2.2 pmol/L; $P = 0.13$).

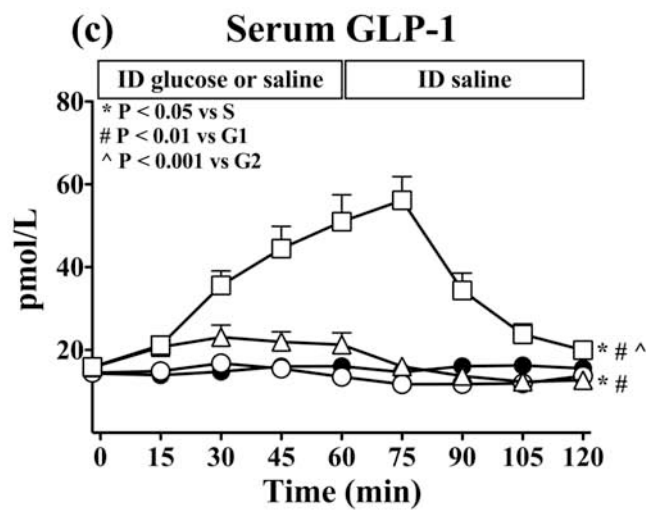
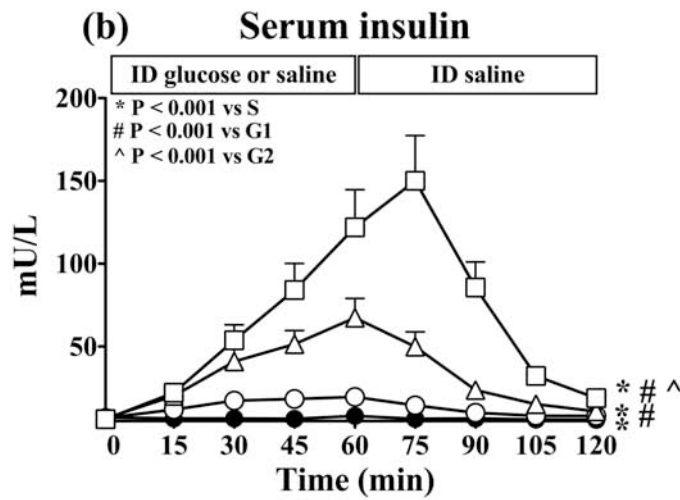
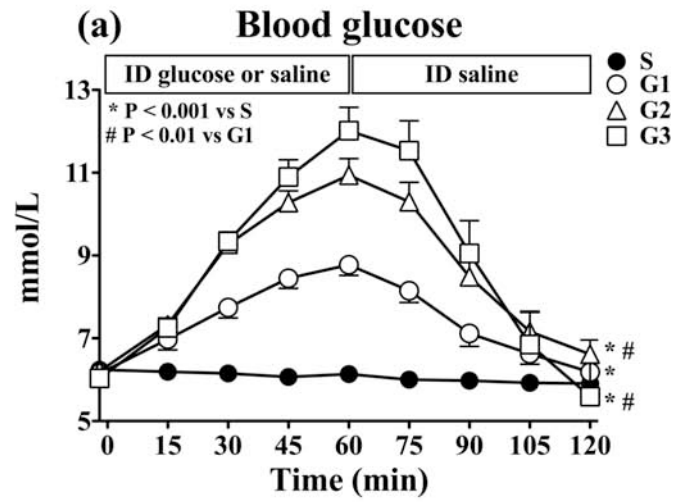


Figure 5.3: Effects of intraduodenal saline (●) and glucose at a rate of either 1 kcal/min (○), 2 kcal/min (△) or 3 kcal/min (□) on (a) blood glucose concentration, (b) serum insulin concentration and (c) serum GLP-1 concentration in healthy older subjects. Data are mean values \pm SEM (n = 12). Blood glucose * P < 0.001 'S' compared with 'G1', 'S' compared with 'G2' and 'S' compared with 'G3', # P < 0.01 'G1' compared with 'G2' and 'G1' compared with 'G3'; Serum insulin * P < 0.001 'S' compared with 'G1', 'S' compared with 'G2' and 'S' compared with 'G3', # P < 0.001 'G1' compared with 'G2' and 'G1' compared with 'G3', ^ P < 0.001 'G2' compared with 'G3'; Serum GLP-1 * P < 0.05 'S' compared with 'G1', 'S' compared with 'G2' and 'S' compared with 'G3', # P < 0.01 'G1' compared with 'G2' and 'G1' compared with 'G3', ^ P < 0.001 'G2' compared with 'G3'.

5.5 Discussion

This study establishes in healthy older subjects that the relationship between the magnitude of the fall in blood pressure in response to intraduodenal glucose infusion is non-linear – intraduodenal glucose at a rate of 1 kcal/min has no effect on blood pressure, whereas there was no difference in the hypotensive responses to infusions of 2 kcal/min and 3 kcal/min. In contrast, the 1 kcal/min glucose infusion resulted in a slight increase in SMA blood flow, and the stimulation of SMA blood flow by the 3 kcal/min infusion was greater than the 2 kcal/min infusion. The GLP-1 responses are consistent with those observed in healthy young subjects (Pilichiewicz et al. 2007) and patients with diet-controlled type 2 diabetes (Ma et al. 2009). As with blood pressure, the GLP-1 response was non-linear, with substantially greater stimulation of GLP-1 by the 3 kcal/min infusion. This is likely to account for the greater insulinaemic response and, potentially, the lack of difference in the blood pressure responses to the 2 kcal/min and 3 kcal/min infusions.

In healthy older subjects, the fall in systolic blood pressure and rise in heart rate following glucose are known to be load - (O'Donovan et al. 2002; Jones et al. 2005), rather than concentration - (Gentilcore et al. 2006b) dependent, so that the hypotensive response to intraduodenal glucose infusion at a rate of 3 kcal/min is much greater than to 1 kcal/min (O'Donovan et al. 2002), whereas the response to 3 kcal/min infusions of varying osmolarity is not different (Gentilcore et al. 2006b). We have now shown that intraduodenal glucose at a rate of 1 kcal/min has no effect on blood pressure in this group i.e. we would anticipate that if

gastric emptying occurred at a rate of 1 kcal/min, or less, there would be no fall in blood pressure, particularly as gastric distension attenuates the postprandial fall in blood pressure (Rossi et al. 1998; Shannon et al. 2002; Gentilcore et al. 2008b). That the falls in blood pressure and rise in heart rate induced by 2 kcal/min and 3 kcal/min intraduodenal glucose, were comparable, indicates that the response to the 2 kcal/min infusion was close to maximal and establishes that the response is non-linear within the physiological range for gastric emptying (Brener et al. 1983).

Previous studies by our group have shown that SMA blood flow increases during intraduodenal nutrient infusion (Gentilcore et al. 2008a; Gentilcore et al. 2009). In healthy older subjects, when glucose is infused intraduodenally at a rate of 3 kcal/min, there is a prompt rise in SMA blood flow (Gentilcore et al. 2008a). Similarly, intraduodenal infusion of sucrose at a rate of 3 kcal/min is associated with a fall in blood pressure and an increase in SMA blood flow, which are related (Gentilcore et al. 2010). The current study establishes that intraduodenal glucose infused at rates of 1, 2 and 3 kcal/min increases SMA blood flow from ~ 15 min, and that, in contrast to blood pressure, there is a modest response to the 1 kcal/min infusion, and the response to the 3 kcal/min infusion is substantially greater than to 2 kcal/min, so that the maximum response remains uncertain. It is not surprising that the infusion of intraduodenal saline did not affect SMA blood flow, as nutrients are required to elicit this response (Gentilcore et al. 2008a).

Previous studies have evaluated the effects of different intraduodenal glucose loads on blood glucose, serum insulin and serum GLP-1 concentrations, in healthy young subjects (Pilichiewicz et al. 2007), and patients with type 2 diabetes (Ma et al. 2009). In these groups, in response to infusion of glucose at 1, 2 or 4 kcal/min for 120 minutes, there is little, if any, difference between the glycaemic responses to 2 kcal/min and 4 kcal/min glucose, which is attributable to the substantially greater GLP-1 and insulin responses to the latter (Pilichiewicz et al. 2007; Ma et al. 2009). Hence, the current observations in healthy older subjects illustrate that there is a rise in blood glucose following 1, 2 and 3 kcal/min intraduodenal glucose infusion, the responses to 2 kcal/min and 3 kcal/min are comparable and the insulin and GLP-1 responses to the latter are much greater, is not surprising. The implication is that if gastric emptying of carbohydrate does not exceed 1 kcal/min, there will only be a modest glycaemic response and that the incretin (GLP-1) effect is of greater relevance when rates of small intestinal glucose entry are higher.

Studies in humans (Edwards et al. 1998) and animals (Barragan et al. 1996) indicate that acute administration of exogenous GLP-1 increases blood pressure. The alpha glucosidase inhibitor, acarbose, appears to be useful in the management of postprandial hypotension (Sasaki et al. 2001; Gentilcore et al. 2005a; Jian and Zhou 2008) and we have reported that acarbose, which is used frequently in patients with type 2 diabetes, slows gastric emptying and attenuates the fall in blood pressure induced by oral (Gentilcore et al. 2005a) and intraduodenal (Gentilcore et al. 2010) sucrose in healthy older subjects, and the former effect is

temporally associated with a marked stimulation of endogenous GLP-1, supporting a potential role for GLP-1 in reducing postprandial hypotension. We would speculate that the increased GLP-1 response for the 3 kcal/min compared to the 2 kcal/min duodenal glucose infusion may account for the absence of differential effects on blood pressure. Conversely, studies using GLP-1 agonists (exenatide and liraglutide) in the management of type 2 diabetes suggest that long-term use may be associated with a fall in blood pressure, but the latter was not measured postprandially. While insulin has vasodilatory properties (Jansen and Hoefnagels 1991), both glucose and insulin are unlikely to play a major role in postprandial hypotension, given that intravenous glucose has little, if any, effect on blood pressure (Jansen and Hoefnagels 1987; Jansen and Hoefnagels 1991), and postprandial hypotension occurs in type 1 patients who are insulin-deficient (Jansen and Hoefnagels 1990). In the current study, the insulinaemic response to the 3 kcal/min infusion was much higher than the 2 kcal/min, but there was no difference in the hypotensive response.

It should be recognised that we studied healthy older subjects and evaluated the responses to intraduodenal, rather than intragastric, glucose loads. This approach ‘bypassed’ the potential effects of gastric distension. Preliminary studies suggest that patients with postprandial hypotension may be more ‘sensitive’ to small intestinal nutrients i.e. the hypotensive response to a given intraduodenal glucose load is substantially greater (van Orshoven et al. 2008), and it will be important to define thresholds and load-responses in this group given the therapeutic implications.

In summary, in healthy older subjects, the fall in systolic blood pressure induced by intraduodenal glucose is non-linear, so that at a rate of gastric emptying or small intestinal nutrient delivery of approximately 1 kcal/min, there is likely to be minimal postprandial fall in blood pressure.

6

Comparative effects of glucose and xylose on blood pressure, gastric emptying and incretin hormones in healthy older subjects

6.1 Summary

Postprandial hypotension is an important disorder for which current management is suboptimal. In healthy older subjects, oral and small intestinal administration of glucose decreases blood pressure, and the magnitude of the reduction is dependent on the rate of entry of glucose into the small intestine and, possibly, the release of glucagon-like peptide-1 (GLP-1). There is little information about the effects of other carbohydrates, particularly those that are poorly absorbed, on blood pressure. The aim of this study was to compare the effects of drinks containing, xylose, glucose or water alone, on blood pressure, gastric emptying, incretin hormone secretion, glycaemia and insulinaemia in healthy older subjects. Eight healthy older subjects (age 65 - 75yr) had simultaneous measurements of blood pressure (DINAMAP), gastric emptying (3D ultrasound), blood glucose, serum insulin, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), on three separate occasions, in double blind, randomised order. On each day, subjects consumed a 300 ml drink of water, glucose (50 g) or d-xylose (50 g). Glucose ($P = 0.02$), but not xylose ($P = 0.63$), was associated with a fall in blood pressure. There was no difference in gastric emptying of glucose and xylose ($P = 0.47$); both emptied slower than water ($P < 0.001$). Xylose had minimal effects on blood glucose, serum insulin or serum GIP, but was more potent than glucose in stimulating GLP-1 ($P = 0.002$). In conclusion, in healthy older subjects, xylose empties from the stomach at the same rate as glucose but has no effect on blood pressure, possibly because it is a potent stimulus for GLP-1 release. Xylose may be considered as an alternative sweetener to glucose in the management of postprandial hypotension.

6.2 Introduction

The onset of the fall in blood pressure is usually evident soon after a meal, with a maximum response at 30 - 60 minutes (Jansen and Lipsitz 1995), suggesting a relationship to the delivery of nutrients to the small intestine which has proven to be the case. When glucose is administered intraduodenally in healthy older subjects at rates of 1 kcal/min or 3 kcal/min (O'Donovan et al. 2002), i.e. within the normal physiological range of gastric emptying (Brener et al. 1983), the fall in blood pressure is much greater in response to 3 kcal/min when compared to 1 kcal/min. In contrast, gastric distension, probably even at low volumes, attenuates the fall in blood pressure (Rossi et al. 1998; Shannon et al. 2002; Gentilcore et al. 2008b). Ingestion of carbohydrate, particularly glucose, was believed to have the greatest suppressive effect on blood pressure (Jansen et al. 1990) when compared to fat and protein (Jansen et al. 1990), but recent studies by our group have shown that oral (Visvanathan et al. 2006) and intraduodenal (Visvanathan et al. 2006; Gentilcore et al. 2008a) infusion of fat, protein and glucose (Gentilcore et al. 2008a) induce comparable falls in blood pressure, although the hypotensive response to glucose occurs earlier than with fat or protein (Visvanathan et al. 2006; Gentilcore et al. 2008a). There is little information about the effect of different carbohydrates on postprandial blood pressure, particularly those that are absorbed more slowly than glucose. Xylose is a poorly absorbed pentose, commonly found in plant cell walls, which is used as a food additive to produce a 'savory' flavour (Arnoldi et al. 1997). Information relating to the effects of xylose on blood pressure is inconsistent. It has been reported that there is no fall in blood pressure after oral xylose in amounts of 42 g (Robinson et al. 1992) and

0.83 g/kg body weight (Robinson and Potter 1995) in healthy older subjects who exhibited a fall in blood pressure following oral glucose, whereas Mathias et al. (Mathias et al. 1989b; Mathias 1990) suggested that there is a small fall in blood pressure following oral xylose. A limitation of these studies (Mathias et al. 1989b; Mathias 1990; Robinson et al. 1992; Robinson and Potter 1995) was that gastric emptying of glucose and xylose was not measured and differences in the rate of carbohydrate delivery into the small intestine may have, accordingly, influenced the observations. In monkeys, gastric emptying of xylose apparently occurs in a similar fashion to that of glucose; i.e. in an overall linear pattern and more slowly with increasing concentration, presumably as a result of inhibitory feedback arising from the small intestine (Moran and McHugh 1981). In contrast, in humans, xylose (25 g) has been reported to prolong gastric emptying markedly when compared with the same amount of glucose (Shafer et al. 1985).

The ‘incretin hormones’, glucose-dependant insulinotropic-polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are responsible for the substantially greater insulin response to oral compared to isoglycaemic intravenous glucose loads (Holst and Gromada 2004). GLP-1 is secreted by L-cells located predominately in the distal small intestine and colon, and suppresses glucagon secretion, as well as stimulating glucose-dependent insulin secretion, while GIP is released from the K-cells, which are located predominantly in the proximal small intestine (Holst and Gromada 2004; Schirra et al. 2006). Recent observations suggest the GLP-1 may have a protective role in postprandial hypotension. In humans (Edwards et al. 1998) and animals (Barragan et al. 1996), exogenous administration of GLP-1

may increase blood pressure. We have reported that the α -glucosidase inhibitor, acarbose, which is used frequently in the management of type 2 diabetes, attenuates the fall in blood pressure induced by oral sucrose in healthy older subjects, slows gastric emptying and markedly stimulates the secretion of GLP-1 (Gentilcore et al. 2005a), the latter effect presumably reflecting the presence of carbohydrate in the small intestine. In dogs, there was no increase in the release of GLP-1 following an infusion of xylose into an ileal loop (Shima et al. 1990). The effects of carbohydrate on GLP-1 secretion may, however, be species-dependent (Baggio and Drucker 2007) and there is no information about the effects of xylose on GIP and GLP-1 in humans.

The aims of the current study were to determine the effects of oral xylose on blood pressure, gastric emptying and incretin hormone secretion, when compared to oral glucose and water, in healthy older subjects.

6.3 Research design and methods

6.3.1 Subjects

Eight healthy older subjects (six male and two female), with a median age of 70.5 years (range: 65 - 75 years) and body mass index (BMI) of 23.5 kg/m² (range: 20.4 - 27.1 kg/m²), were recruited by advertisement. All were non-smokers and none had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal, hepatic or cardiac disease, intake of > 20 g alcohol/day, or was taking medication known to influence blood pressure or gastrointestinal function.

6.3.2 *Experimental protocol*

The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written, informed consent. All experiments were carried out in accordance with the Declaration of Helsinki.

Each subject was studied on three occasions, in randomised double-blind order; each study day was separated by a minimum of three days. On each day, the subject attended the laboratory at 0800h following an overnight fast (10h for solids; 8h for liquids). An intravenous cannula was placed in a left antecubital vein for blood sampling and an automated blood pressure cuff positioned around the right arm for measurement of blood pressure and heart rate. Each subject was then allowed to rest, seated in a chair, for about 30 minutes. At $t = -2$ min, the subject consumed a 300 ml drink comprising either: i) water (50 ml low joule lemon cordial (Bickford's, Adelaide, Australia) + 250 ml water) – 'W' ii) 50 g glucose monohydrate (dissolved in 50 ml low joule lemon cordial + 155 ml water + 80 ml hypertonic saline (3 %)) – 'G' or iii) 50 g d-xylose (dissolved in 50 ml low joule lemon cordial + 235 ml water) – 'X', within 2 minutes. Both carbohydrate drinks were isocaloric, (~ 187 kcal) and iso-osmolar (~ 1350 mOsmol). Gastric emptying, blood pressure (systolic and diastolic) and heart rate were then measured for 120 minutes. On one day, cardiovascular autonomic nerve function was evaluated immediately after the completion of the study (Ewing and Clarke 1982; Piha 1991).

6.3.3 *Measurements*

6.3.3.1 *Blood pressure and heart rate*

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) prior to the consumption of the drink and then every 3 minutes between $t = 0 - 120$ min (O'Donovan et al. 2002). 'Baseline' blood pressure and heart rate, i.e. 't = 0 min', were calculated as the mean of measurements taken at $t = -9, -6$ and -3 min. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes. (Jansen and Lipsitz 1995)

6.3.3.2 *Gastric emptying*

Gastric emptying was assessed using 3D ultrasonography, using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, Australia) with TruScan Architecture (i.e. built-in magnetically sensed 3D (Gentilcore et al. 2006c). For 3D positioning and orientation measurement (POM), a transmitter was placed close to the subject and a snap-on sensor attached to a 3.5 C broad spectrum 2.5 - 4 MHz convex transducer (Tefera et al. 2002; Gentilcore et al. 2006c). As the transmitter produces a spatially varying magnetic field, and ferrous and conductive metals distort the magnetic field, all metal objects were removed from the subject and from the area directly between the POM transmitter and sensor (Liao et al. 2004). The POM transmitter was placed behind (~ 10 cm) the subject (Gilja et al. 1997), at the level of the stomach, so that the subject was positioned between the ultrasound scanner and the transmitter. For 3D data

acquisition, the subject was scanned at $t = -2$ min, $t = 0$ min (i.e. immediately following drink consumption) and then at 15-minute intervals between $t = 0 - 120$ min. A region-of-interest was drawn around the total stomach and the volume of the drink in the total stomach was derived and expressed as a percentage of the original volume at $t = 0$ min (i.e. 100 %) (Gentilcore et al. 2006c). Gastric emptying curves (expressed as % retention over time) were derived for the total stomach at $t = 0, 15, 30, 45, 60, 75, 90, 105$ and 120 min. The 50 % gastric emptying time (T50) was also determined.

6.3.3.3 *Blood glucose, serum insulin, GLP-1 and GIP concentrations*

Venous blood samples were obtained prior to consumption of the drink (i.e. $t = -2$ min) and at 15-minute intervals between $t = 0 - 120$ min. Blood glucose concentrations (mmol/L) were determined immediately using a portable blood glucose meter (Medisense Precision Q-I-D™ System, Abbott Laboratories, Medisense Products Inc, Bedford, MA, USA).

Serum was separated by centrifugation at 3200 rpm for 15 min at 4°C within 30 minutes of collection and stored at -70°C until analysed. Serum insulin (mU/L) was measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc, Webster, TX, USA). The sensitivity of the assay was 0.26 mU/L and the coefficient of variation was 2.6 % within assays and 6.2 % between assays (O'Donovan et al. 2004b).

Serum GLP-1 (pmol/L) was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). The minimum detection limit was 3 pmol/L, the intraassay coefficient of variation was 6.7 % and the interassay coefficient was 7.8 %.

Serum GIP (pmol/L) was measured by radioimmunoassay with some modifications to the original method (Wishart et al. 1992). The standard curve was prepared in buffer rather than extracted charcoal-stripped serum and the radioiodinated label was supplied by Perkin Elmer (Boston, MA, USA). The minimum detection limit of the assay was 2 pmol/L, and both the intra- and interassay coefficients of variation were 11.2 % and 11.6 %, respectively.

6.3.4 *Assessment of cardiovascular autonomic nerve function*

Autonomic nerve function was assessed using standardised cardiovascular reflex tests (Ewing and Clarke 1982; Piha 1991). In brief, parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during deep breathing and the response to standing ("30 : 15" ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

6.3.5 *Statistical analysis*

Systolic and diastolic blood pressure and heart rate were expressed as changes from baseline. Gastric emptying, blood glucose, serum insulin, GLP-1 and GIP concentrations were analysed as absolute values. One-way ANOVA was used to analyse the effects of 'time' on gastric emptying, systolic and diastolic blood pressure, heart rate, blood glucose, serum insulin, GLP-1 and GIP concentrations. The maximum fall in systolic and diastolic blood pressure, and maximum rise in heart rate, were defined as the greatest change from baseline in each subject at any given time point for each treatment. For blood glucose, serum insulin, GLP-1 and GIP concentrations, the peak absolute value was analysed in each subject at any given time point for each treatment. Areas under the curve (AUC), between $t = 0 - 120$ min were calculated using the trapezoidal rule and analysed by one-way ANOVA, to evaluate a 'treatment' effect for gastric emptying, systolic and diastolic blood pressure and heart rate and between $t = -2 - 120$ min for blood glucose, serum insulin, GLP-1 and GIP concentrations. All analyses were performed using SPSS version 16.0.2 (SPSS Inc, Chicago, IL, USA). Data are shown as changes from baseline and mean values \pm standard error of the mean (SEM), unless stated otherwise. The number of subjects studied was based on power calculations derived from our previous work; the sample size of 8 subjects was calculated to have 80 % power at the $P = 0.05$ significance level to detect a difference in maximum fall in systolic blood pressure between glucose and xylose of 7.3 mmHg (Visvanathan et al. 2005). A P value < 0.05 was considered significant in all analyses.

6.4 Results

The studies were well tolerated and there were no adverse events. No subject had definite autonomic neuropathy (mean score 0.63, range: 0-2), or had PPH.

6.4.1 *Systolic and diastolic blood pressure and heart rate (Figure 1a, b and c)*

There was no difference in baseline ($t = 0$ min) blood pressure, or heart rate between the three days: systolic blood pressure ('W' 118 ± 6 mmHg vs. 'G' 120 ± 7 mmHg vs. 'X' 119 ± 6 mmHg; $P=0.44$); diastolic blood pressure ('W' 70 ± 3 mmHg vs. 'G' 71 ± 3 mmHg vs. 'X' 70 ± 3 mmHg; $P=0.40$), and heart rate ('W' 58 ± 2 bpm vs. 'G' 59 ± 3 bpm vs. 'X' 59 ± 3 bpm; $P=0.79$).

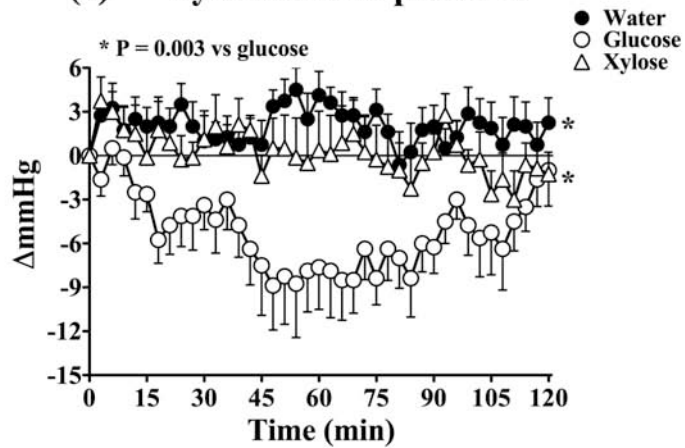
Between $t = 0 - 120$ min, there was a fall in systolic blood pressure during 'G' ($P = 0.02$) and no change during 'W' ($P = 0.71$) or 'X' ($P = 0.63$). There was a treatment effect ($P < 0.001$) for the AUC of the change in systolic blood pressure between $t = 0 - 120$ min, so that systolic blood pressure was less during 'G' when compared with 'W' and 'X' ($P = 0.003$ for both), without any difference between 'W' and 'X' ($P = 0.19$). During 'G' the maximum fall in blood pressure was 15 ± 3 mmHg occurring at 64 ± 9 min. At $t = 120$ min, systolic blood pressure was not different from baseline after 'W' (121 ± 6 mmHg; $P=0.23$), 'G' (119 ± 6 mmHg; $P=0.69$), or 'X' (118 ± 5 mmHg; $P=0.43$).

Between $t = 0 - 120$ min, there was a fall in diastolic blood pressure during 'G' ($P = 0.003$), and no change during 'W' ($P = 0.88$) or 'X' ($P = 0.26$). There was a

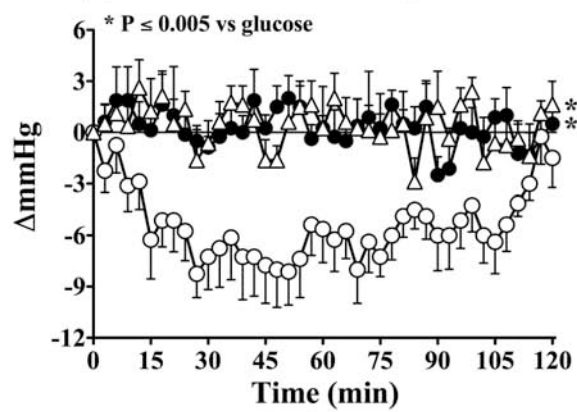
treatment effect ($P < 0.001$) for the AUC of the change in diastolic blood pressure between $t = 0 - 120$ min, so that diastolic blood pressure was less during 'G' when compared with 'W' ($P = 0.002$) and 'X' ($P = 0.005$), without any significant difference between 'W' and 'X' ($P = 0.92$). During 'G' the maximum fall in blood pressure was 13 ± 2 mmHg occurring at 56 ± 11 min. At $t = 120$ min, diastolic blood pressure was not different from baseline after 'W' (70 ± 2 mmHg; $P = 0.58$), 'G' (70 ± 3 mmHg; $P = 0.41$) or 'X' (72 ± 3 mmHg; $P = 0.27$).

Between $t = 0 - 120$ min, there was no significant change in heart rate during 'W' ($P = 0.22$), 'G' ($P = 0.28$) or 'X' ($P = 0.19$). At $t = 120$ min, heart rate was not significantly different from baseline after 'W' (58 ± 3 bpm; $P = 0.77$), 'G' (60 ± 3 bpm; $P = 0.63$) or 'X' (65 ± 5 bpm; $P = 0.13$).

(a) Systolic blood pressure



(b) Diastolic blood pressure



(c) Heart rate

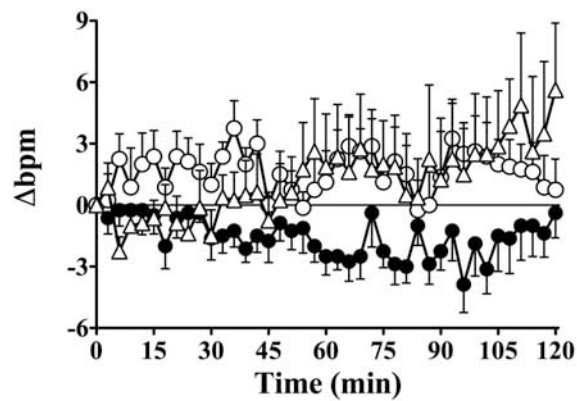


Figure 6.1: Change in a) systolic blood pressure, b) diastolic blood pressure and c), heart rate, from baseline in response to oral: water (●), glucose (○), and xylose (△). Data are mean values \pm SEM (n = 8). Systolic blood pressure treatment effect: * P = 0.003 'G' compared with 'W' and 'X'. Diastolic blood pressure treatment effect: * P \leq 0.005 'G' compared with 'W' and 'X'.

6.4.2 Gastric emptying (Figure 2)

There was a significant treatment effect ($P < 0.001$) for the AUC for gastric emptying between $t = 0 - 120$ min. 'W' emptied in an overall exponential, and more rapid fashion when compared with 'G' and 'X', which emptied linearly and more slowly ($P < 0.001$ for both), with no significant difference between 'G' and 'X' ($P = 0.47$). The T50 of 'W' ($t = 19 \pm 3$ min) was less than 'G' ($t = 75 \pm 7$ min) and 'X' ($t = 75 \pm 8$ min) ($P < 0.001$).

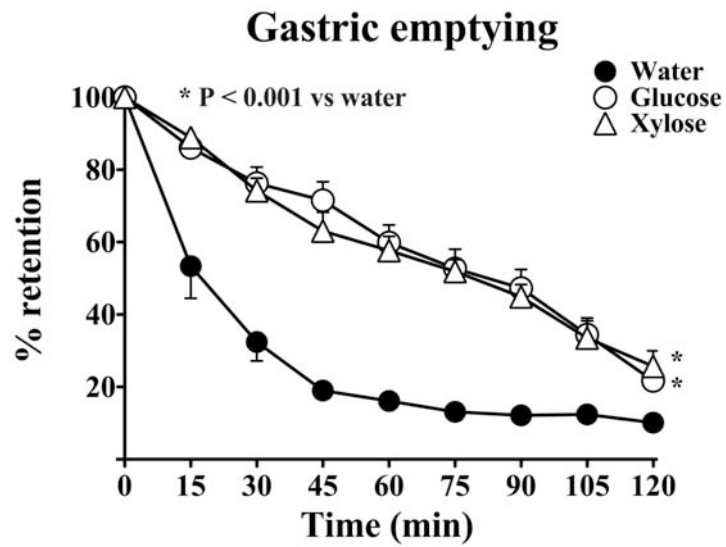


Figure 6.2: Gastric emptying of water (●), glucose (○), and xylose (△). Data are mean values \pm SEM (n = 8). Treatment effect of the AUC: * P < 0.001 'W' compared with 'G' and X'.

6.4.3 *Blood glucose, serum insulin, GLP-1 and GIP (Figure 3a, b, c and d)*

There was no difference in baseline ($t = -2$ min) blood glucose between the three days ('W' vs. 'G' vs. 'X'): 6.2 ± 0.2 mmol/L vs. 6.2 ± 0.2 mmol/L vs. 6.1 ± 0.2 mmol/L; $P = 0.89$. Between $t = -2 - 120$ min, there was a rise in blood glucose during 'G' ($P < 0.001$), and a slight rise following 'X' ($P = 0.03$), but no change during 'W' ($P = 0.50$). There was a significant treatment effect ($P < 0.001$) for the AUC of the blood glucose concentration between $t = -2 - 120$ min, so that the magnitude of the rise in blood glucose was much greater during 'G' compared with both 'W' ($P \leq 0.001$) and 'X' ($P \leq 0.001$). During 'G' peak blood glucose was 10.2 ± 0.6 mmol/L at 53 ± 8 min. At $t = 120$ min, blood glucose concentrations were not different from baseline after 'W' (6.1 ± 0.1 mmol/L; $P = 0.58$), 'G' (6.8 ± 0.5 mmol/L; $P = 0.33$), but were slightly higher after 'X' (6.5 ± 0.2 mmol/L; $P = 0.03$).

There was no difference in baseline ($t = -2$ min) serum insulin between the three days ('W' vs. 'G' vs. 'X'): 8.7 ± 1.3 mU/L vs. 8.5 ± 1.1 mU/L vs. 8.4 ± 1.6 mU/L; $P = 0.88$. Between $t = -2 - 120$ min, there was a rise in serum insulin during 'G' ($P < 0.001$), a trend for a fall during 'W' ($P = 0.06$) and no change during 'X' ($P = 0.18$). There was a significant treatment effect ($P < 0.001$) for the AUC of the serum insulin between $t = -2 - 120$ min, so that the magnitude of the rise in serum insulin was much greater during 'G' compared with 'W' and 'X' ($P < 0.001$ for both), without any significant difference between 'W' compared with 'X' ($P = 0.13$). At $t = 120$ min, serum insulin concentrations were not different

from baseline after 'X' (8.0 ± 1.6 mU/L; $P = 0.63$), slightly lower following 'W' (7.3 ± 1.0 mU/L; $P = 0.03$), and substantially higher after 'G' (42.8 ± 10.1 mU/L; $P = 0.009$).

There was no significant difference in baseline ($t = -2$ min) serum GLP-1 between the three days ('W' vs. 'G' vs. 'X'): 16.6 ± 2.3 pmol/L vs. 13.8 ± 1.4 pmol/L vs. 18.9 ± 3.3 pmol/L; $P = 0.08$. Between $t = -2 - 120$ min, there was a rise in serum GLP-1 during 'G' ($P = 0.01$) and 'X' ($P < 0.001$), but no change during 'W' ($P = 0.39$). There was a significant treatment effect ($P \leq 0.001$) for the AUC of the serum GLP-1 concentration between $t = -2 - 120$ min, so that the magnitude of the rise in serum GLP-1 was much greater during 'X' compared with 'W' ($P \leq 0.001$) and 'G' ($P = 0.002$), with a trend for a difference between 'G' compared with 'W' ($P = 0.07$). During 'G', peak GLP-1 was 30.5 ± 4.6 pmol/L at 26 ± 5 min and during 'X', 42.0 ± 4.0 pmol/L at 48 ± 5 min ($P < 0.05$ for peak and $P < 0.01$ for time to peak). At $t = 120$ min, serum GLP-1 concentrations were not different from baseline after 'W' (15.7 ± 1.2 pmol/L; $P = 0.15$) and 'G' (12.3 ± 1.0 pmol/L; $P < 0.001$), but higher following 'X' (27.2 ± 1.8 pmol/L; $P = 0.002$).

There was no significant difference in baseline ($t = -2$ min) serum GIP between the three days ('W' vs. 'G' vs. 'X'): 17.3 ± 1.3 pmol/L vs. 18.4 ± 1.6 pmol/L vs. 18.9 ± 1.6 pmol/L; $P = 0.30$. Between $t = -2 - 120$ min, there was a prompt rise in serum GIP during 'G' ($P < 0.001$), and a fall, albeit minor, during 'W' and 'X' ($P < 0.001$ for both). There was a significant treatment effect ($P \leq 0.001$) of the AUC for the serum GIP concentration between $t = -2 - 120$ min, so that the magnitude

of the rise in serum GIP was much greater during 'G' compared with 'W' and 'X' ($P \leq 0.001$ for both), without any difference between 'W' compared with 'X' ($P = 0.41$). During 'G' peak GIP was 61.0 ± 8.0 pmol/L at 56 ± 11 min. At $t = 120$ min, serum GIP concentrations were not different from baseline after 'W' (15.7 ± 2.0 pmol/L; $P = 0.15$), less following 'X' (16.2 ± 1.1 pmol/L; $P = 0.02$), and greater after 'G' (48.9 ± 4.7 pmol/L; $P < 0.001$).

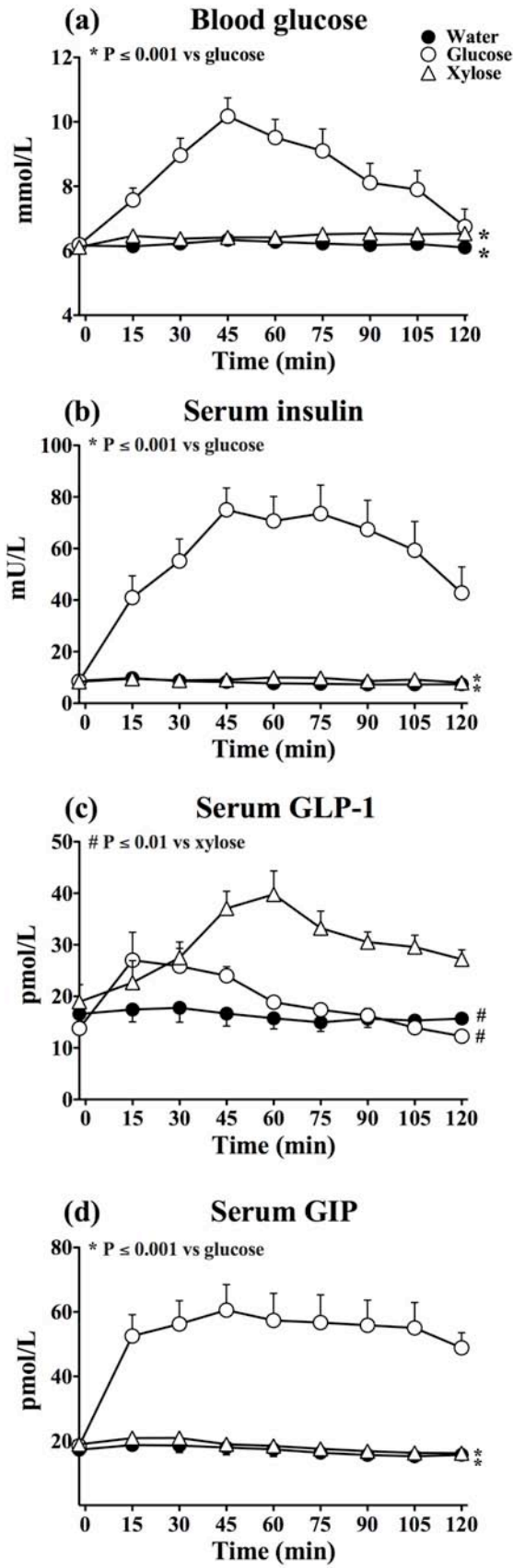


Figure 6.3: Change in a) blood glucose, b) serum insulin, c) serum GLP-1 and d) serum GIP in response to oral: water (●), glucose (○), and xylose (△). Data are mean values \pm SEM (n = 8). Blood glucose treatment effect: $P \leq 0.001$ 'G' compared with 'W', and 'X'. Serum insulin treatment effect: $P \leq 0.001$ 'G' compared with 'W', and 'X'. Serum GLP-1 treatment effect: $P \leq 0.01$ 'X' compared with 'W', and 'G'. Serum GIP treatment effect: $P \leq 0.001$ 'G' compared with 'W', and 'X'.

6.5 Discussion

This study indicates that oral d-xylose (50 g), unlike glucose, has no effect on blood pressure in healthy older subjects despite emptying from the stomach at a comparable rate. Xylose is also more potent than glucose in stimulating GLP-1, but has no effect on GIP, and minimal effect on glycaemia and insulinaemia, at least during euglycaemia.

The current study confirms that oral glucose induces a substantial fall (15 ± 3 mmHg) in systolic blood pressure in healthy older subjects studied under resting conditions. Previous studies relating to the effects of xylose on blood pressure are inconsistent (Mathias et al. 1989b; Mathias 1990; Robinson et al. 1992; Robinson and Potter 1995), but gastric emptying was not measured in any of these studies, and may have potentially accounted for the observations, given that the rate of nutrient delivery into the small intestine affects the fall in blood pressure both as a result of gastric distension (Jones et al. 1998; Gentilcore et al. 2008b) and the exposure of small intestine to nutrients (O'Donovan et al. 2002).

Our study establishes that glucose and xylose empty from the stomach at a comparable rate with an overall linear pattern that is substantially slower than water, which empties exponentially, consistent with a previous animal (primate) study (Moran and McHugh 1981). Hence, gastric emptying does not account for the different effects of glucose and xylose on blood pressure. The regulation of gastric emptying of nutrients arises predominantly as a result of inhibitory feedback from receptors in the small intestine, the magnitude of which is

dependent on the length and, possibly, region (Lin et al. 1989) of small intestine exposed, as influenced by the energy load. Accordingly, it appears that the magnitude of this inhibitory feedback is comparable for xylose and glucose, although the mechanism(s) which account for this feedback may differ (Moran and McHugh 1981). In healthy adult males it was reported that xylose in a dose of 25 g in 50 ml water, given immediately after the consumption of a scrambled egg meal, markedly prolonged gastric emptying, when compared with the same amount of glucose (Shafer et al. 1985). Differences in the rate of gastric emptying of xylose between these studies, possibly influenced by the xylose dose, may account for the discrepant observations.

While it is clear that differences in gastric emptying do not account for the substantial, differential effects of xylose and glucose on blood pressure, the two sugars had discrepant effects on glycaemia, insulinaemia and the secretion of the incretin hormones, GIP and GLP-1, which, accordingly, warrant consideration. It is well documented, and confirmed in this study that xylose has minimal, if any, effect on plasma glucose or insulin (Mathias et al. 1989b; Mathias 1990; Robinson and Potter 1995). However, both hyperglycaemia and hyperinsulinaemia are unlikely to play a major role in postprandial hypotension e.g. intravenous glucose has little, if any, effect on blood pressure. The comparative effects of xylose and glucose on splanchnic blood flow remain to be determined and it is possible that the relatively poorly absorbed xylose induces a lesser increase. This is the first evaluation of the effect of xylose on the release of GLP-1 and GIP – that xylose had no effect on GIP is predictable, given that the

secretion of GIP occurs predominantly in the proximal small intestine and, in the case of carbohydrate, appears to be dependent on an affinity for the transporter, SGLT-1 (Baggio and Drucker 2007). There is also no evidence that GIP affects blood pressure. It has been reported that xylose has no effect on GLP-1 secretion in dogs (Shima et al. 1990) although xylose apparently stimulates the release of glucagon-like immunoreactivity in the canine intestine (Marco et al. 1970). Our study establishes that xylose is a potent stimulant of GLP-1 in humans - the sustained stimulation is likely to reflect the delay in intestinal absorption when compared to glucose, so that the distal small intestine is exposed; the initial stimulation appeared similar to that induced by glucose. It is not surprising that the stimulation of GLP-1 by xylose was not associated with a substantial increase in serum insulin in this study, as the insulinotropic property of GLP-1 is known to be glucose dependent i.e. GLP-1 has little, if any, effect on insulin during euglycaemia (Holst and Gromada 2004). It is, accordingly probable that xylose will stimulate insulin in type 2 patients during hyperglycaemia by increasing GLP-1. The stimulation of GLP-1 secretion by xylose may also be of relevance to the use of DPP-IV inhibitors and GLP-1 analogues in the management of type 2 diabetes (Khoo et al. 2009). As discussed, this stimulation of GLP-1 may account for the absence of any fall in blood pressure. We studied a small number of subjects precluding assessment of meaningful correlations. Further studies are required to address this issue, including the effects of different xylose loads. Given that GLP-1 plays a physiological role to slow gastric emptying (Deane et al. 2010), it is perhaps surprising that xylose did not empty from the stomach slower than glucose. However, it should also be recognised that glucose ingestion

increased the blood glucose concentration substantially, whereas xylose did not, and elevations of blood glucose, even within normal postprandial range, slow gastric emptying (Schvarcz et al. 1997). It is also not known whether the presence of xylose in a glucose drink, could attenuate the fall in blood pressure. Furthermore, the effects of xylose in patients with postprandial hypotension remain to be determined. In considering the potential dietary use of xylose, it should be recognised that while xylose is palatable, it is relatively expensive. In view of our observations, it would be of interest to evaluate the effects of the related pentose sugar, xylitol (Shafer et al. 1987), which is considerably cheaper.

In summary, in healthy older subjects, oral xylose, unlike glucose in a dose of 50 g, has no effect on blood pressure, despite emptying from the stomach at a comparable rate to glucose and is a potent stimulant of GLP-1 secretion. These observations suggest that xylose may represent an alternative sweetener to glucose, in the management of postprandial hypotension.

7

Effects of gastric distension on blood pressure and superior mesenteric artery blood flow responses to intraduodenal glucose in healthy older subjects

7.1 Summary

Postprandial hypotension occurs frequently in the elderly and is associated with increased morbidity and mortality. Recent studies suggest that gastric distension may attenuate the postprandial fall in blood pressure. Splanchnic blood flow may also influence postprandial blood pressure. The study aim was to determine the effects of non-nutrient gastric distension, using a barostat, on blood pressure, heart rate and superior mesenteric artery (SMA) flow responses to intraduodenal glucose in healthy older subjects. 8 subjects (6M,2F; age 65 - 75yr) had measurements of blood pressure and heart rate (automated device) and SMA flow (Doppler ultrasound) after an overnight fast, on 4 separate days in random order. SMA blood flow was calculated instantaneously using the radius of the SMA and time-averaged mean velocity. On each day, subjects were intubated with a nasoduodenal catheter incorporating a duodenal infusion port. On 2 of the 4 days they were intubated orally with a second catheter, incorporating a barostat bag, positioned in the fundus; the barostat was set at 8 mmHg above minimal distending pressure. Each subject received a 60 min ($t = 0 - 60$ min) intraduodenal infusion of either glucose (3 kcal/min) or saline (0.9 %), therefore, the 4 study conditions were intraduodenal glucose + barostat ('glucose + distension'), intraduodenal saline + barostat ('saline + distension'), intraduodenal glucose ('glucose') and intraduodenal saline ('saline'). Systolic and diastolic blood pressure fell during 'glucose' when compared with 'saline' ($P = 0.05$ and $P = 0.003$, respectively) and 'glucose + distension' ($P = 0.01$ and $P = 0.05$, respectively), and increased during 'saline + distension' when compared with 'saline' ($P = 0.04$ and $P = 0.006$, respectively). The maximum changes in systolic

blood pressure were -14 ± 5 mmHg, $+11 \pm 2$ mmHg, -3 ± 4 mmHg and $+15 \pm 3$ mmHg, for 'glucose', 'saline', 'glucose + distension' and 'saline + distension', respectively. There was an increase in heart rate during 'glucose' and 'glucose + distension' (maximum rise 14 ± 2 bpm and 14 ± 3 bpm, respectively), but not 'saline' or 'saline + distension'. SMA flow increased during 'glucose' and 'glucose + distension' (2388 ± 365 ml/min and 1673 ± 187 ml/min, respectively) but not during 'saline' and tended to decrease during 'saline + distension' (821 ± 115 ml/min and 864 ± 116 ml/min, respectively). In conclusion, gastric distension has the capacity to abolish the fall in blood pressure, and attenuate the rise in SMA flow, induced by intraduodenal glucose in healthy older subjects.

7.2 Introduction

While the mechanisms underlying postprandial hypotension are poorly defined, the rate of small intestinal nutrient delivery, splanchnic blood flow and neural and hormonal mechanisms are important (Mathias et al. 1989a; Jansen and Hoefnagels 1991; Mathias 1991; Jansen and Lipsitz 1995). A series of studies performed by our group, primarily in healthy older subjects, has established that the postprandial fall in blood pressure is triggered by the interaction of nutrients (fat, carbohydrate or protein) with the small intestine, presumably as a result of both neural and humoral mediators (Jones et al. 1998; O'Donovan et al. 2002; Gentilcore et al. 2008a). When gastric emptying is relatively more rapid, the magnitude of the fall in blood pressure is greater (Jones et al. 1998). In contrast, intragastric mechanisms, related to gastric distension, attenuate the postprandial fall in blood pressure (Rossi et al. 1998; Jordan et al. 2000; Shannon et al. 2002;

van Orshoven et al. 2004; Jones et al. 2005; Gentilcore et al. 2008b; van Orshoven et al. 2008). For example, consumption of water increases systolic blood pressure in healthy older subjects (Jordan et al. 2000) and patients with autonomic failure (Jordan et al. 2000; Shannon et al. 2002), and attenuates the hypotensive response to a meal (Shannon et al. 2002). In healthy older subjects, the magnitude of the fall in systolic blood pressure is greater when glucose is ingested at a smaller volume (200ml when compared with 600ml), but the same glucose concentration (Jones et al. 2005). Intra-gastric administration of 500ml of water markedly attenuates the fall in systolic blood pressure induced by intraduodenal glucose (Gentilcore et al. 2008b), and when glucose is infused directly into the proximal small intestine at a rate comparable to gastric emptying of oral glucose, the magnitude of the fall in blood pressure is greater (Gentilcore et al. 2009). Inherent limitations in these studies are that distension, with liquid nutrient or non-nutrient, cannot be well quantified, nor sustained, i.e. with intra-gastric administration gastric distension decreases as gastric emptying proceeds (Gentilcore et al. 2008b), and if the distension stimulus includes nutrients, these may induce a fall in blood pressure (Gentilcore et al. 2008b). Moreover, the pressor effect of water may, in part, be mediated by its hyposmolarity (Shannon et al. 2002).

Longhurst et al. demonstrated in anaesthetised cats, that gastric distension induces a sympathoexcitatory reflex leading to an increase in blood pressure (Longhurst et al. 1981). Gastric distension at predefined volumes and/or pressures can be achieved using a barostat device (Rossi et al. 1998; van Orshoven et al. 2004). In healthy, young adults, proximal gastric distension with a barostat has been shown

to increase blood pressure, heart rate and muscle sympathetic nerve activity, the so-called 'gastrovascular reflex' (Rossi et al. 1998). In a study comparing healthy young and older subjects, gastric distension at a pressure of 8 mmHg above minimal distending pressure (MDP) using a barostat, increased mean arterial pressure, heart rate and total peripheral arterial resistance more in the older subjects, while in both groups there was a slight rise in cardiac output (van Orshoven et al. 2004). No studies have hitherto evaluated the effects of gastric distension, using a barostat, on the hypotensive response to small intestinal nutrients.

Meal ingestion (Gentilcore et al. 2009) and small intestinal nutrient infusion (Gentilcore et al. 2008a) increase superior mesenteric artery (SMA) blood flow, which can be measured by Doppler techniques (Perko 2001). There is little information about the effects of gastric distension on SMA blood flow, with the majority of these studies in animals (Vacca et al. 1996; Molinari et al. 2003), and only one study in humans (Gentilcore et al. 2009). The outcome of these studies is inconsistent, with no effect (Vacca et al. 1996), increases (Longhurst and Ibarra 1984; Vacca et al. 1996; Gentilcore et al. 2009) or decreases (Vacca et al. 1996; Molinari et al. 2003) in SMA blood flow, being reported.

The aims of this study were to determine the effects of gastric distension with a barostat on blood pressure, heart rate and SMA blood flow responses to intraduodenal glucose infusion in healthy older subjects. The broad hypothesis

was that gastric distension would attenuate the hypotensive, and increase SMA blood flow, responses induced by intraduodenal glucose.

7.3 Research design and methods

7.3.1 Subjects

Eight healthy older subjects (six male and two female), with a median age of 70.5 years (range: 65 - 75 years) and body mass index (BMI) of 23.5 kg/m² (range: 20.4 - 27.1 kg/m²), recruited by advertisement, were enrolled in the study. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal, hepatic or cardiac disease, intake of > 20 g alcohol/day, epilepsy, nor was taking medication known to influence blood pressure or gastrointestinal function.

7.3.2 Experimental protocol

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

Each subject was studied on four occasions in randomised order; each study was separated by a minimum of three days. On each day, the subject attended the University of Adelaide, Discipline of Medicine, at the Royal Adelaide Hospital, at 0800h following an overnight fast (10h for solids; 8h for liquids). A silicone-rubber catheter (external diameter ~ 4 mm; Dentsleeve International Ltd, Mui

Scientific, Mississauga, Canada) was introduced into the stomach via an anaesthetized nostril (O'Donovan et al. 2002). The assembly included an infusion channel (internal diameter ~ 1 mm) and was positioned so that the infusion port was located ~ 10 cm distal to the pylorus (i.e. in the duodenum). Two other channels were positioned in the antrum (2.5 cm proximal to the pylorus) and duodenum (2.5 cm distal to the pylorus), respectively, and were perfused with 0.9 % saline. The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference (TMPD) at the antral (-40 mV) and duodenal (0 mV), channels (Hedde et al. 1989). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (Hedde et al. 1989).

On two of the four study days, the subject swallowed a single-lumen polyvinyl orogastric catheter (outer diameter 4 mm, inner diameter 2 mm; Tygon® Tubing, Saint Gobain Performance Plastics, Akron, OH, USA), which had an ultrathin, flaccid polyethylene bag (capacity 1200 ml), tightly wrapped around the distal end. The proximal end of the catheter was connected via a three-way tap to a gastric barostat (Distender Series II™, G & J Electronics Inc, Ontario, Canada). The bag was unfolded by inflation with 400 ml of air, while ensuring that the pressure did not exceed 20 mmHg, and adjusted to be positioned in the proximal stomach, just below the diaphragm. The bag was then deflated, and the barostat assembly was fixed in this position by taping it to the skin of the cheek (Rossi et al. 1998; Feinle et al. 2000; van Orshoven et al. 2004).

The bag was then inflated in steps of 1 mmHg, at 5-minute intervals, in order to determine the MDP, which represents the minimal pressure required to overcome the intra-abdominal pressure, defined as the pressure required to achieve a volume > 30 ml in the bag (Feinle et al. 2000). The stomach was then distended using a single 'staircase' protocol, in which intragastric pressure was increased by 2 mmHg every 3 minutes, in four steps, to achieve a distension of 8 mmHg above MDP (Whitehead and Delvaux 1997). The intraballoon volume was recorded at 3-minute intervals commencing immediately prior to the intraduodenal infusion. Perceptions of fullness, nausea and bloating were assessed using a 7-point Likert scale (Rossi et al. 1998), commencing immediately prior to the distension and during the last minute of each distension step. The subject was asked to quantify these sensations on a scale from 1 (no sensation) to 7 (unbearable sensation) (Rossi et al. 1998; van Orshoven et al. 2004).

After the catheters were positioned correctly, and stepwise distension completed, at $t = 0$ min, the subject received either (i) an intraduodenal infusion of glucose (3 kcal/min) ('glucose'), (ii) intraduodenal infusion of saline (0.9 %) ('saline'), (iii) intraduodenal infusion of glucose (3 kcal/min) with intraballoon pressure set to 8 mmHg above MDP ('glucose + distension'), or (iv) intraduodenal infusion of saline with intraballoon pressure set to 8 mmHg above MDP ('saline + distension'), for 60 minutes (i.e. between $t = 0 - 60$ min). The barostat bag was deflated at $t = 60$ min. Between $t = 60 - 120$ min, saline (0.9 %) was infused intraduodenally at an identical rate (O'Donovan et al. 2002). Intraduodenal infusions were performed using a volumetric infusion pump (Imed Gemini PC-1:

IMED Corp, San Diego, CA, USA). An intravenous cannula was positioned in a left antecubital vein for blood sampling, and an automated blood pressure cuff around the right arm. Each subject remained in a supine position while blood sampling, and measurements of blood pressure, heart rate and SMA blood flow were performed. At $t = 120$ minutes the catheters were removed, the subject given a light meal and then allowed to leave the laboratory. On one day, cardiovascular autonomic nerve function was evaluated immediately after the completion of the study (Ewing and Clarke 1982; Piha 1991).

7.3.3 *Measurements*

7.3.3.1 *Blood pressure and heart rate*

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at $t = -9, -6$ and -3 min prior to commencement of the intraduodenal infusions and then every 3 minutes between $t = 0 - 120$ min (O'Donovan et al. 2002). 'Baseline' blood pressure and heart rate, i.e. ' $t = 0$ min', were calculated as the mean of measurements taken at $t = -9, -6$ and -3 min. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

7.3.3.2 *Splanchnic blood flow*

SMA blood flow was measured by Duplex ultrasonography (i.e. B-mode and Doppler imaging) using a Logiq™ 9 ultrasonography system (GE Healthcare

Technologies, Sydney, Australia) (Perko 2001). The subject was scanned using a 3.5 C broad spectrum 2.5 - 4 MHz convex transducer before ($t = -2$ min) the commencement of the intraduodenal infusion, and then at 15-minute intervals between $t = 0 - 120$ min. Blood flow (ml/min) was calculated instantaneously using the formula: $\pi \times r^2 \times \text{TAMV} \times 60$, where r = the radius of the SMA and TAMV is the time-averaged mean velocity (Perko 2001; Gentilcore et al. 2008a).

7.3.3.3 Blood glucose concentrations

Venous blood samples were obtained prior to the commencement of the intraduodenal infusion (i.e. $t = -2$ min) and at 15-minute intervals between $t = 0 - 120$ min. Blood glucose concentrations (mmol/L) were determined immediately using a portable blood glucose meter (Medisense Precision Q·I·D™ System, Abbott Laboratories, Medisense Products Inc, Bedford, MA, USA).

7.3.3.4 Perceptions of distension

Perceptions of nausea, bloating and fullness were assessed by a Likert scale between 1 (no sensation) and 7 (unbearable sensation) (Rossi et al. 1998), during stepwise distension.

7.3.4 Assessment of cardiovascular autonomic nerve function

Autonomic nerve function was assessed using standardised cardiovascular reflex tests (Ewing and Clarke 1982; Piha 1991). Parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during deep breathing and the response to standing ("30 : 15" ratio). Sympathetic function was assessed

by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

7.3.5 *Statistical analysis*

Systolic and diastolic blood pressure, heart rate and perception scores were analysed as changes in absolute values from baseline. Intra-gastric volume, SMA blood flow and blood glucose concentrations were analysed as absolute values. One-way ANOVA was used to analyse the effects of 'time' on intra-gastric volume, systolic and diastolic blood pressure, heart rate, SMA blood flow, blood glucose concentrations and perception scores. The maximum change in systolic and diastolic blood pressure, heart rate, SMA blood flow and blood glucose concentrations were defined as the greatest change from baseline in each subject at any given time point for each treatment. Areas under the curve (AUC), were calculated using the trapezoidal rule, and analysed by one-way ANOVA, to evaluate a 'treatment' effect between $t = 0 - 60$ min for systolic and diastolic blood pressure and heart rate, between $t = -2 - 60$ min for SMA blood flow and blood glucose concentrations. All analyses were performed using SPSS version 16.0.2 (SPSS Inc, Chicago, IL, USA). Systolic and diastolic blood pressure and heart rate data are shown as change from baseline and mean values \pm standard error of the mean (SEM). Intra-gastric volume, SMA blood flow and blood glucose are shown as absolute mean values \pm SEM. The number of subjects studied was

based on power calculations derived from our previous work (Gentilcore et al. 2008b), with systolic blood pressure as the primary endpoint. A P value < 0.05 was considered significant in all analyses.

7.4 Results

The studies were well tolerated and there were no adverse events. No subject had definite autonomic neuropathy (mean score 0.9, range: 0 - 2). One subject had postprandial hypotension (i.e. a fall in systolic blood pressure > 20 mmHg that was sustained for at least 30 minutes) following 'glucose'. In one of the remaining eight subjects, SMA blood flow measurements could not be obtained adequately during the 'glucose + distension' visit because the blood vessel was obscured by abdominal gas; these data were, accordingly, not included.

7.4.1 *Intraballoon volume and pressure during gastric distension*

(Figure 7.1)

There was no significant difference in baseline (t = -2 min) intraballoon volume between the two days: 'glucose + distension' 443 ± 60 ml vs. 'saline + distension' 410 ± 80 ml (P = 0.59). Between t = 0 - 60 min, there was a prompt rise in intraballoon volume during both 'glucose + distension' (P < 0.001) and 'saline + distension' (P = 0.002), with a plateau from ~ 15 min. There was a trend (P = 0.07) for the AUC for intraballoon volume to be greater during 'glucose + distension' than 'saline + distension'. At t = 60 min, intragastric volume was greater than baseline after 'glucose + distension' (790 ± 71 ml; P = 0.001) and

tended to be greater after 'saline + distension' (637 ± 80 ml; $P = 0.07$) without any difference between them.

During distension, MDP ranged between 3 - 6 mmHg, so that pressures within the barostat bag ranged from 11 - 14 mmHg (MDP + 8mmHg). During the intraduodenal infusions, barostat bag volumes ranged from 300 - 950 ml.

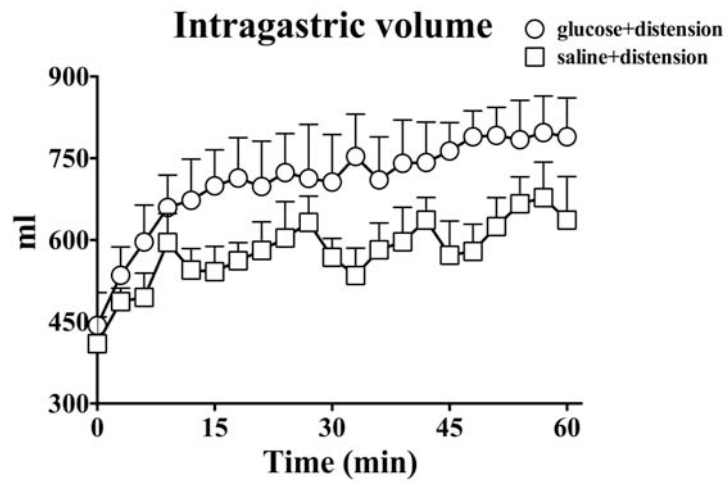


Figure 7.1: Intrabag (barostat) volumes during ‘glucose + distension’ (○), ‘saline + distension’ (□). Data are mean values \pm SEM (n = 8).

7.4.2 *Systolic and diastolic blood pressure and heart rate (Figure 7.2a, b & c and Figure 7.3)*

There was no significant difference in baseline (t = 0 min) blood pressure or heart rate between the four days: systolic blood pressure ('glucose' 119 ± 7 mmHg vs. 'saline' 117 ± 5 mmHg vs. 'glucose + distension' 121 ± 5 mmHg vs. 'saline+distension' 122 ± 5 mmHg; $P = 0.52$); diastolic blood pressure ('glucose' 69 ± 3 mmHg vs. 'saline' 68 ± 2 mmHg vs. 'glucose + distension' 71 ± 2 mmHg vs. 'saline + distension' 69 ± 2 mmHg; $P = 0.43$), and heart rate ('glucose' 58 ± 3 bpm vs. 'saline' 59 ± 3 bpm vs. 'glucose + distension' 59 ± 3 bpm vs. 'saline + distension' 60 ± 2 bpm; $P = 0.45$).

Between t = 0 – 60 min, there was a substantial fall in systolic blood pressure during 'glucose' ($P < 0.05$), no overall change during 'saline' ($P = 0.19$) or 'glucose + distension' ($P = 0.20$), and a rise during 'saline + distension' ($P = 0.008$). The maximum fall in systolic blood pressure from baseline during 'glucose' was 14 ± 5 mmHg, while there was minimal change following 'glucose + distension' (-3 ± 4 mmHg) and a maximum increase of $+11 \pm 2$ mmHg and $+15 \pm 3$ mmHg during 'saline' and 'saline + distension', respectively. There was a significant treatment effect ($P = 0.01$) for the AUC for the change in systolic blood pressure between t = 0 – 60 min. Systolic blood pressure was less during 'glucose' when compared with 'saline' ($P = 0.05$), 'glucose + distension' ($P = 0.01$) and 'saline + distension' ($P = 0.03$). Systolic blood pressure was greater during 'saline + distension' when compared with 'saline' ($P = 0.04$), with no significant difference between 'glucose + distension' and 'saline + distension' (P

= 0.56). At $t = 120$ min, systolic blood pressure was not different from baseline after 'glucose' (116 ± 5 mmHg; $P = 0.40$), 'saline' (123 ± 6 mmHg; $P = 0.26$), and 'glucose + distension' (125 ± 5 mmHg; $P = 0.15$) but was greater after 'saline + distension' (132 ± 7 mmHg; $P = 0.01$).

Between $t = 0 - 60$ min, there was a substantial fall in diastolic blood pressure during 'glucose' ($P = 0.001$), a slight fall during 'glucose + distension' ($P = 0.02$), a rise during 'saline + distension' ($P = 0.02$), and no overall change during 'saline' ($P = 0.48$). The maximum fall in diastolic blood pressure from baseline during 'glucose' was 12 ± 2 mmHg (at $t = 43 \pm 5$ min) and during 'glucose + distension' was 9 ± 1 mmHg (at $t = 45 \pm 5$ min). There was a significant treatment effect ($P < 0.001$) for the AUC for the change in diastolic blood pressure between $t = 0 - 60$ min. The magnitude of the fall in diastolic blood pressure was greater during 'glucose' compared with 'saline' ($P = 0.003$), 'glucose + distension' ($P = 0.05$) and 'saline + distension' ($P = 0.002$). Diastolic blood pressure during 'saline + distension' was greater compared with 'saline' ($P = 0.006$) and 'glucose+distension' ($P = 0.01$). At $t = 120$ min, diastolic blood pressure was not significantly different from baseline after 'glucose' (68 ± 3 mmHg; $P = 0.26$), 'glucose + distension' (73 ± 3 mmHg; $P = 0.17$) and 'saline + distension' (71 ± 2 mmHg; $P = 0.28$), but was slightly greater than baseline after 'saline' (71 ± 2 mmHg; $P = 0.03$).

Between $t = 0 - 60$ min, there was a progressive rise in heart rate during 'glucose' ($P < 0.001$) and 'glucose + distension' ($P < 0.001$), but no overall change during

'saline' ($P = 0.42$) or 'saline + distension' ($P = 0.41$). The maximum rise in heart rate from baseline during 'glucose' (14 ± 2 bpm at $t = 45 \pm 4$ min), and 'glucose + distension' (14 ± 3 bpm at 44 ± 5 min), were similar with no significant difference between them ($P = 0.99$). There was a significant treatment effect ($P = 0.002$) for the AUC for the change in heart rate between $t = 0 - 60$ min. The magnitude of the rise in heart rate was greater during 'glucose' compared with 'saline' ($P = 0.005$), but not 'glucose + distension' ($P = 0.94$). Similarly, the magnitude of the increase in heart rate during 'glucose + distension' was greater compared with 'saline + distension' ($P = 0.02$). There was no difference in heart rate following 'saline' compared with 'saline + distension' ($P = 0.43$). At $t = 120$ min, heart rate was not significantly different from baseline after 'saline' (60 ± 3 bpm; $P = 0.56$) and 'saline + distension' (61 ± 3 bpm; $P = 0.13$), but higher than baseline following 'glucose' (56 ± 3 bpm; $P = 0.007$) and 'glucose + distension' (64 ± 2 bpm; $P = 0.02$).

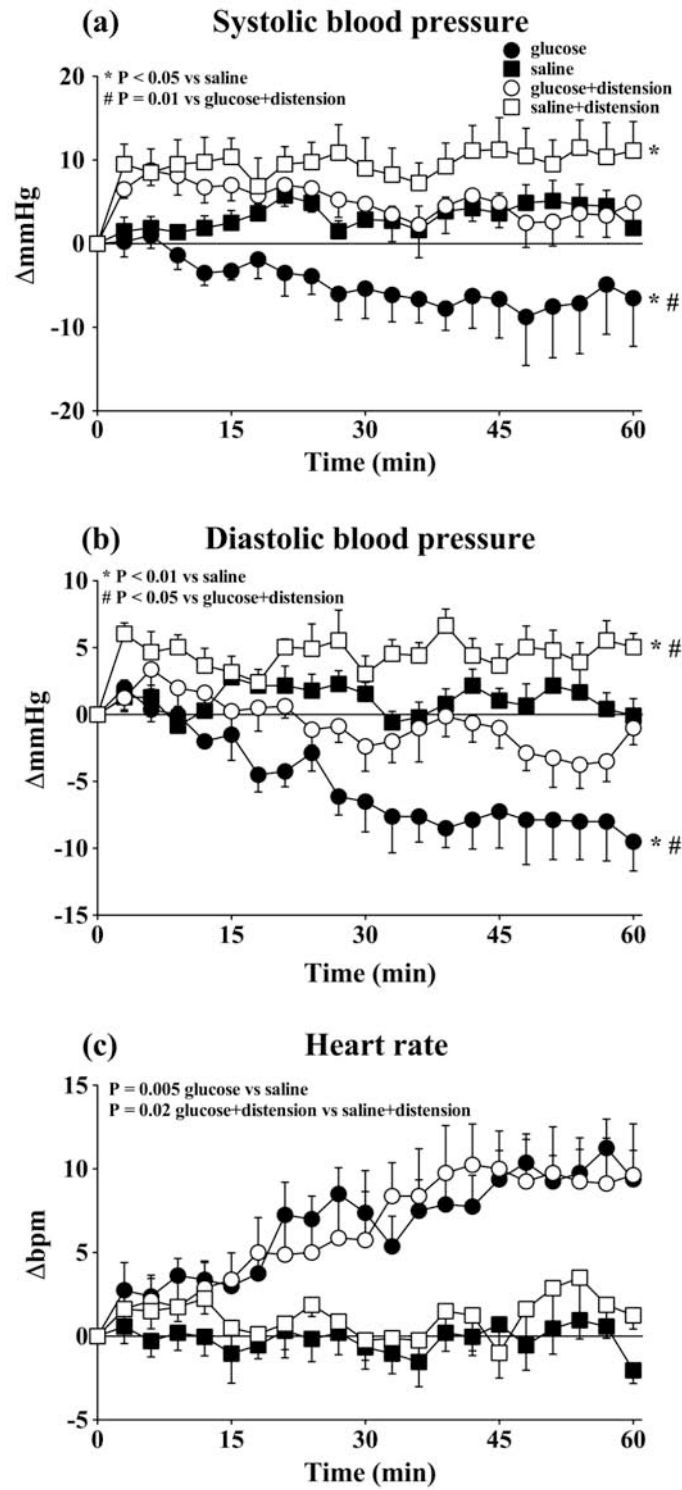


Figure 7.2: Change in a) systolic blood pressure, b) diastolic blood pressure c), heart rate, from baseline and in response to ‘glucose’ (●), ‘saline’ (■), ‘glucose + distension’ (○), and ‘saline + distension’ (□). Data are mean values \pm SEM (n = 8). Systolic blood pressure treatment effect: * P < 0.05 ‘saline’ compared with ‘glucose’ and ‘saline’ compared with ‘saline + distension’; # P = 0.01 ‘glucose’ compared with ‘glucose + distension’. Diastolic blood pressure treatment effect: * P < 0.01 ‘saline’ compared with ‘glucose’ and ‘saline’ compared with ‘saline + distension’; # P < 0.05 ‘glucose + distension’ compared with ‘glucose’ and ‘glucose + distension’ compared with ‘glucose’. Heart rate treatment effect: P = 0.005 ‘saline’ compared with ‘glucose’; P = 0.02 ‘saline + distension’ compared with ‘glucose + distension’. SMA blood flow effect: * P = 0.001 ‘saline’ compared with ‘glucose’; # P = 0.03 ‘glucose + distension’ compared with ‘glucose’.

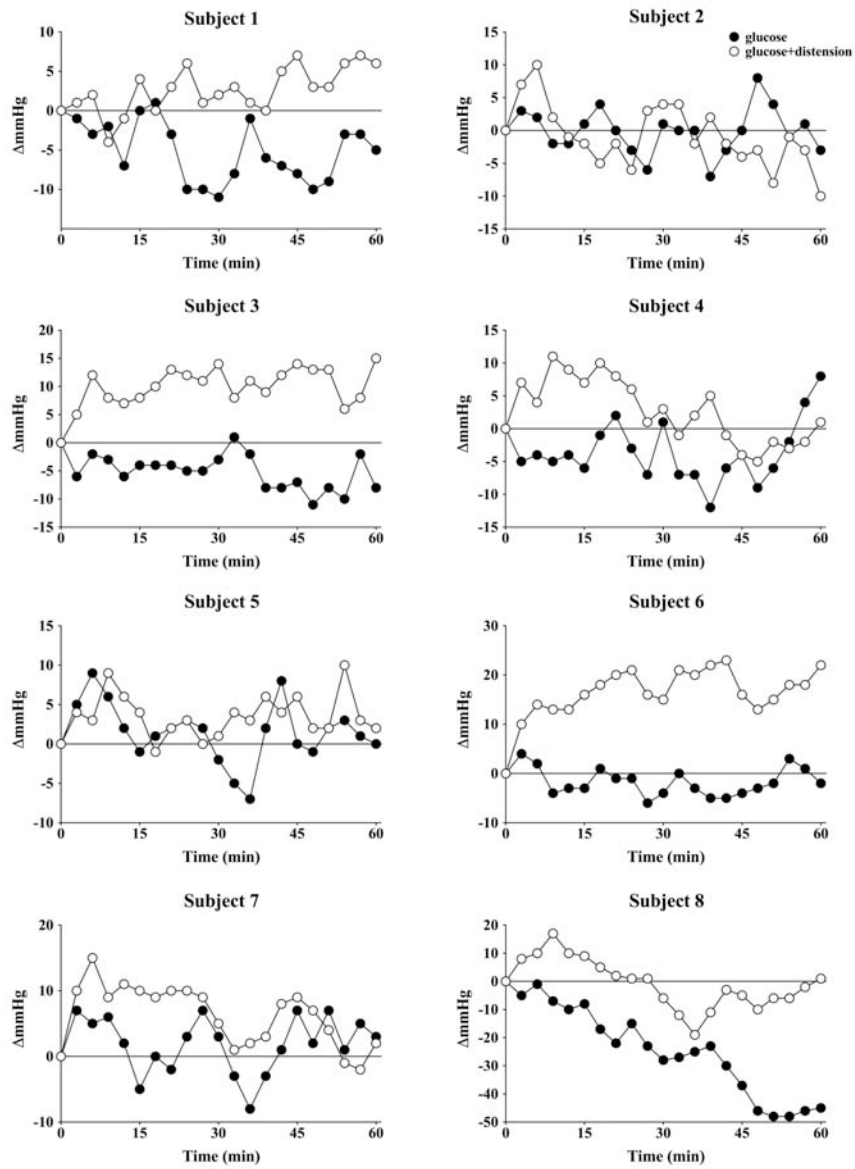


Figure 7.3: Individual data in all eight subjects showing the changes in systolic blood pressure from baseline, in response to ‘glucose’ (●) and ‘glucose + distension’ (○).

7.4.3 SMA blood flow (Figure 7.4)

There was no significant difference in baseline ($t = -2$ min) SMA blood flow between the four days ('glucose' vs. 'saline' vs. 'glucose + distension' vs. 'saline + distension'): 798 ± 132 ml/min vs. 844 ± 91 ml/min vs. 770 ± 121 ml/min vs. 829 ± 120 ml/min; $P = 0.81$.

Between $t = -2 - 60$ min, there was a rise in SMA blood flow during 'glucose' ($P = 0.004$), and 'glucose + distension' ($P = 0.001$), but no overall change during 'saline' ($P = 0.13$), and a trend for a decrease during 'saline + distension' ($P = 0.07$). The maximum rise in SMA blood flow from baseline during 'glucose' (2388 ± 365 ml/min at 43 ± 6 min) was greater ($P = 0.05$) than the maximum rise during 'glucose + distension' (1673 ± 187 ml/min at 41 ± 9 min). There was a significant treatment effect ($P < 0.001$) for the AUC for the change in SMA blood flow between $t = -2 - 60$ min, so that the magnitude of the rise in SMA blood flow was greater during 'glucose' compared with 'saline' ($P = 0.001$), 'glucose + distension' ($P = 0.03$), and 'saline + distension' ($P = 0.001$). There was a trend for a rise in SMA flow during 'glucose + distension' to be greater compared with 'saline + distension' ($P = 0.09$) and no significant difference during 'saline' compared with 'saline + distension' ($P = 0.14$). At $t = 120$ min, SMA blood flow had returned to baseline after 'glucose' (848 ± 134 ml/min; $P = 0.73$), 'saline' (747 ± 116 ml/min; $P = 0.19$), 'glucose + distension' (894 ± 120 ml/min; $P = 0.16$), and 'saline + distension' (839 ± 142 ml/min; $P = 0.92$).

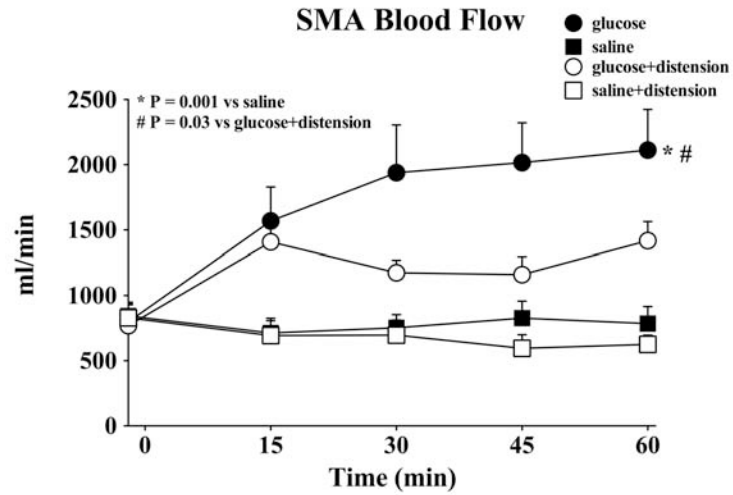


Figure 7.4: Superior mesenteric artery (SMA) blood flow during ‘glucose’ (●) (n = 8), ‘saline’ (■) (n = 8), ‘glucose + distension’ (○) (n = 7), ‘saline + distension’ (n = 8) (□). Data are mean values ± SEM (n = 8). Treatment effect: * P = 0.001 ‘saline’ compared with ‘glucose’; # P = 0.03 ‘glucose’ compared with ‘glucose + distension’.

7.4.4 Blood glucose (Figure 7.5)

There was no significant difference in baseline ($t = -2$ min) blood glucose between the four days ('glucose' vs. 'saline' vs. 'glucose + distension' vs. 'saline + distension'): 6.0 ± 0.2 mmol/L vs. 6.1 ± 0.1 mmol/L vs. 6.1 ± 0.1 mmol/L vs. 6.2 ± 0.1 mmol/L; $P = 0.78$.

Between $t = -2 - 60$ min, there was a progressive rise in blood glucose during 'glucose' ($P < 0.001$), and 'glucose + distension' ($P < 0.001$), but no overall change during 'saline' ($P = 0.55$) or 'saline + distension' ($P = 0.48$). The maximum rises in blood glucose during 'glucose' (11.3 ± 0.7 mmol/L at 56 ± 3 min) and 'glucose + distension' (11.5 ± 0.8 mmol/L at 60 ± 0 min), were not different ($P = 0.69$). There was a significant treatment effect ($P < 0.001$) for the AUC for the blood glucose between $t = -2 - 60$ min. The magnitude of the rise in blood glucose was greater during 'glucose' compared with 'saline' ($P < 0.001$), but not different when compared with 'glucose + distension' ($P = 0.94$). At $t = 120$ min, blood glucose were not different from baseline after 'glucose' (5.8 ± 0.8 mmol/L; $P = 0.74$), 'saline' (6.0 ± 0.2 mmol/L; $P = 0.28$), 'glucose + distension' (6.9 ± 0.8 mmol/L; $P = 0.31$), or 'saline + distension' (6.1 ± 0.1 mmol/L; $P = 0.43$).

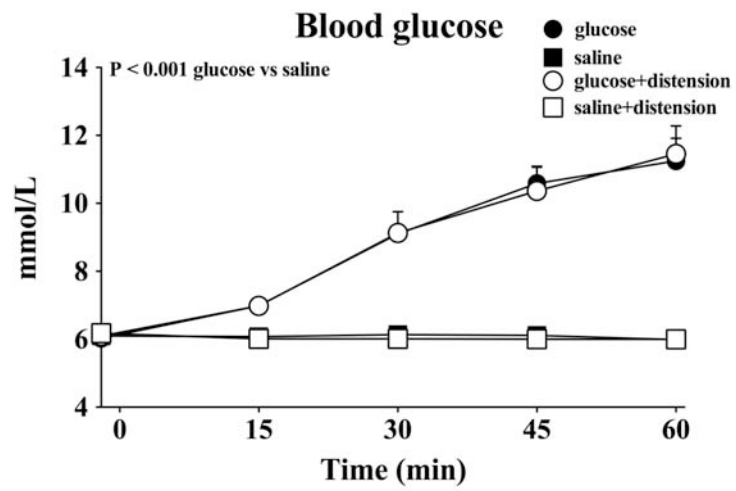


Figure 7.5: Blood glucose during ‘glucose’ (●), ‘saline’ (■), ‘glucose + distension’ (○), ‘saline + distension’ (□). Data are mean values \pm SEM ($n = 8$). Treatment effect: $P < 0.001$ ‘saline’ compared with ‘glucose’.

7.4.5 Perceptions of distension

Baseline (at MDP) perceptions on the two days ('glucose + distension' vs. 'saline + distension') were: nausea (1.3 ± 0.2 vs. 1.1 ± 0.1 ; $P = 0.35$), bloating (1.6 ± 0.4 vs. 1.1 ± 0.1 ; $P = 0.35$) and fullness (1.6 ± 0.5 vs. 1.5 ± 0.4 ; $P = 0.23$). Prior to the glucose infusion, the stepwise distension ('glucose + distension'), there was no change in nausea ($P = 0.21$), or fullness ($P = 0.12$), and a trend for an increase in bloating ($P = 0.08$). On the day that subjects received saline ('saline + distension'), there were no changes in sensations of nausea ($P = 0.42$), bloating ($P = 0.52$) or fullness ($P = 0.25$). There were no differences in perceptions between the two days i.e. 'glucose + distension' and 'saline + distension'.

7.5 Discussion

This study establishes that gastric distension, induced by a barostat, has the capacity to abolish the fall in systolic blood pressure and attenuate the rise in SMA blood flow, but has no effect on the rise in heart rate, induced by intraduodenal glucose infusion at a rate of 3 kcal/min in healthy older subjects. These observations have implications for the non-pharmacological management of postprandial hypotension.

There is increasing evidence that gastric distension plays a protective role in the regulation of postprandial blood pressure (Rossi et al. 1998; Jordan et al. 1999; Jordan et al. 2000; Cariga and Mathias 2001; Shannon et al. 2002; van Orshoven et al. 2004; Jones et al. 2005; Gentilcore et al. 2008b). In a recent study in healthy older subjects, we demonstrated that the hypotensive response to intraduodenal

infusion of glucose at 3 kcal/min was markedly attenuated by the presence of as little as ~ 300 ml of intragastric water, while during intraduodenal saline infusion, the presence of ~ 100 ml increased systolic blood pressure by 6 - 8 mmHg above baseline (Gentilcore et al. 2008b). In that study, gastric distension could not be sustained and it was possible that distension of the small intestine due to gastric emptying of the intragastric water could have influenced the response. In the current study we were able to address these limitations by the use of a gastric barostat (Rossi et al. 1998; van Orshoven et al. 2004). We distended the stomach to a fixed pressure of 8 mmHg above MDP, as this has been shown to be well tolerated (as proved to be the case) and to increase blood pressure in both healthy young (Rossi et al. 1998; van Orshoven et al. 2004) and older subjects (van Orshoven et al. 2004), in the absence of intraduodenal nutrients. In the current study, there was a trend for a rise in blood pressure (~ 12 – 18 mmHg above baseline) during intraduodenal saline when 300 – 950 ml of air was present within the barostat bag, consistent with previous observations (Gentilcore et al. 2008b). It should also be noted that during intraduodenal glucose there was a trend for intraballoon volume to be greater. This is not surprising as intraduodenal carbohydrate is known to be associated with greater gastric relaxation than intraduodenal saline as a result of feedback from small intestinal chemoreceptors (Azpiroz and Malagelada 1985) and acute hyperglycaemia is known to induce proximal stomach relaxation (Hebbard et al. 1996a; Hebbard et al. 1996b; Verhagen et al. 1999). Hence, while the intragastric pressures were matched in both distension experiments, the distending volume should be considered as comparable, rather than identical. It has been reported that consumption of 480 ml

of water increases systolic blood pressure in healthy older subjects, and patients with multiple system atrophy and autonomic failure (Jordan et al. 2000), as well as attenuate the fall in blood pressure following a high carbohydrate meal in patients with autonomic failure (Shannon et al. 2002). Furthermore, in healthy older subjects, the magnitude of the fall in systolic blood pressure is greater when glucose was ingested at a smaller volume (200 ml compared with 600 ml), at the same glucose concentration (Jones et al. 2005). The latter study also provided evidence that proximal, rather than distal, gastric distension, may be primarily responsible for this effect (Jones et al. 2005). If this proves to be the case, it would be possible to modify the intragastric meal distribution and hence, the regional gastric distension by changes in meal composition and/or 'posture' for therapeutic purposes (Horowitz et al. 1993b; Doran et al. 1998).

The normal overall rate of gastric emptying of glucose in healthy young and older subjects is in the range of 1 – 4 kcal/min (Brener et al. 1983; Hunt et al. 1985), and we have shown that in healthy older subjects, administration of intraduodenal glucose at a rate of 3 kcal/min induces a substantially greater fall in blood pressure than 1 kcal/min (O'Donovan et al. 2002; van Orshoven et al. 2008). In contrast, infusion of glucose intraduodenally at 3 kcal/min has minimal effect on blood pressure in young adults (van Orshoven et al. 2008), whereas preliminary data indicate that in patients with postprandial hypotension the response is exaggerated (van Orshoven et al. 2008). The differential response to intraduodenal glucose in healthy young and older subjects, has been attributed to alterations in baroreceptor function (van Orshoven et al. 2008). As in previous studies

(Gentilcore et al. 2008b), the magnitude of the fall in systolic blood pressure during intraduodenal glucose without gastric distension was substantial (14 ± 5 mmHg) and it is, accordingly, remarkable that gastric distension completely abolished the fall. Interestingly, heart rate increased progressively in response to intraduodenal glucose and this was not influenced by gastric distension. The latter observation was surprising and, while it could reflect the greater variability of heart rate, it suggests that this response may not represent an effect of splanchnic vasodilation and a fall in blood pressure, but some form of ‘entero-cardiac’ reflex.

The mechanism(s) mediating the effects of gastric distension on the hypotensive response to intraduodenal glucose remain uncertain and there are a number of possibilities which warrant further exploration. The glycaemic responses to intraduodenal glucose are comparable in studies with and without gastric distension (Gentilcore et al. 2008b). Furthermore, insulin is unlikely to play a major role in postprandial hypotension, since intravenous glucose has little, if any, effect on blood pressure and postprandial hypotension occurs in patients with type-1 diabetes (Mathias 1991; Maule et al. 2004), who are, by definition, insulin deficient. The observed effects of gastric distension in the stimulation of SMA blood flow are of considerable interest, particularly given the paucity of previous information. The current study establishes that gastric distension markedly attenuates the increase in SMA blood flow induced by intraduodenal glucose. In both cases, the pattern of SMA blood flow response differed from that of heart rate – while the increase in heart rate was progressive, whereas SMA blood flow plateaued – during gastric distension, the plateau occurred substantially earlier, a

response which may contribute to the maintenance of blood pressure. While this may suggest the existence of a 'gastro-vascular' reflex in the peripheral, somatic circulation, gastric distension had no effect on SMA blood flow during intraduodenal saline. Further studies are indicated to explore this issue. In the pig, fasting SMA flow has been reported to be decreased (Vacca et al. 1996; Molinari et al. 2003), increased (Vacca et al. 1996) or unchanged (Vacca et al. 1996) by gastric distension, whereas, in the cat, a modest increase has been reported (Longhurst and Ibarra 1984). A recent study by our group reported that the rise in SMA blood flow was greater following oral, compared to, intraduodenal glucose (Gentilcore et al. 2009), which may reflect differences in the method of gastric distension - the barostat distends primarily the proximal stomach, while an intragastric load distends the whole stomach. There is hitherto no information relating to the potential regional effects of gastric distension on SMA flow, which would be of interest. It should be recognised that measurement of SMA blood flow using Doppler ultrasound is affected by the presence of abdominal gas, which may compromise locating and imaging the vessel, thereby degrading image quality, and this may represent an issue with the barostat bag. For logistical reasons we did not perform studies with the barostat bag deflated. The minimal intragastric pressure required to attenuate the fall in blood pressure induced by intraduodenal glucose, and whether the effect is volume and/or pressure dependent, remain to be determined. We elected to study healthy older subjects, not those with known postprandial hypotension given that the latter occurs frequently in this group (Jansen and Lipsitz 1995), which also exhibit a fall in blood pressure in response to intraduodenal glucose; a response that is substantial,

but not dramatic (O'Donovan et al. 2002; Gentilcore et al. 2008a). Preliminary data also indicate that the magnitude of the fall may be precipitous in patients with known postprandial hypotension (van Orshoven et al. 2008). Studies in this latter group are now indicated given that they represent the target population.

In summary, in healthy older subjects, the fall in systolic blood pressure and rise in SMA blood flow induced by intraduodenal glucose, are markedly attenuated by modest gastric distension, supporting the concept that maximising non-nutrient gastric distension may represent a simple approach to the management of postprandial hypotension (for example, consumption of water prior to a meal).

8

Effects of variations in intragastric volume on blood pressure and superior mesenteric artery blood flow during intraduodenal glucose infusion in healthy older subjects

8.1 Summary

Postprandial hypotension occurs frequently and results from the interaction of nutrients with the small intestine. There is evidence that gastric distension may attenuate the postprandial fall in blood pressure and that splanchnic blood flow may influence postprandial blood pressure. The aims of the study were to determine the effects of differences in intragastric volume, on blood pressure and superior mesenteric artery (SMA) blood flow responses to intraduodenal glucose in healthy older subjects. 9 male subjects (age 65 - 75yr) had measurements of blood pressure and heart rate (automated device) and SMA blood flow (Doppler ultrasound) after an overnight fast, on 4 separate days in random order. On each day, subjects were intubated with a nasoduodenal catheter incorporating a duodenal infusion port, and orally with a second catheter, incorporating a barostat bag, which was positioned in the fundus. Each subject then received a 60 min ($t = 0 - 60$ min) intraduodenal infusion of glucose (3 kcal/min) and gastric distension at a volume of either i) 0 ml ('V0'), ii) 100 ml ('V100'), iii) 300ml ('V300') or iv) 500 ml ('V500'). Systolic blood pressure fell ($P < 0.05$) during 'V0', but not 'V100', 'V300', 'V500'. SMA blood flow increased ($P < 0.001$) on all 4 days. There was a significant distension effect for the area under the curve (AUC) for no volume distension ('V0') versus the average of the volume distension ('V100', 'V300' and 'V500') for systolic blood pressure ($P = 0.008$) and diastolic blood pressure ($P = 0.02$) but not for SMA blood flow ($P = 0.16$). There was a relationship between the AUC of the change in systolic blood pressure from baseline with intragastric volume ($r = 0.60$, $P < 0.001$). In conclusion, low volume gastric distension (≤ 100 ml) has the capacity to abolish the fall in blood pressure

induced by intraduodenal glucose in healthy older subjects, without affecting SMA blood flow.

8.2 Introduction

Our previous studies have established that the magnitude of the postprandial fall in blood pressure in response to enteral glucose is related to the rate of nutrient delivery into the small intestine in a load-dependent manner (Jones et al. 1998; O'Donovan et al. 2002)(**Chapter 5**). For example, in healthy older subjects, when glucose is infused intraduodenally at rates within the physiological range for gastric emptying (Brener et al. 1983), infusions at 3 kcal/min and 2 kcal/min induce a greater fall in blood pressure when compared to 1 kcal/min (O'Donovan et al. 2002)(**Chapter 5**). In contrast to the effects of small intestinal nutrients, gastric distension attenuates the postprandial fall in blood pressure (Rossi et al. 1998; Shannon et al. 2002; van Orshoven et al. 2004)(**Chapter 7**). For example, gastric distension with a balloon linked to a barostat has the capacity to increase blood pressure in both healthy young (Rossi et al. 1998) and older (van Orshoven et al. 2004)(**Chapter 7**) subjects. We have recently reported that gastric distension, using a barostat set to a pressure of 8 mmHg above minimal distending pressure (MDP), resulting in intragastric volumes ranging from 400 to 900 ml, markedly attenuates the hypotensive response to a 3 kcal/min intraduodenal glucose infusion (**Chapter 7**). This latter study did not provide information about the minimum volume/pressure required to attenuate the fall in blood pressure, nor the potential relationship between blood pressure with intragastric volume.

Both meal ingestion (Gentilcore et al. 2009) and small intestinal nutrient infusion (Gentilcore et al. 2008a)(**Chapter 5 and 7**) increase SMA blood flow, which may be important in mediating the hypotensive response (Lipsitz et al. 1993) and can be measured by Doppler techniques (Perko 2001). Information about the effects of gastric distension on SMA blood flow is limited. Moreover, the outcome of studies in both animals (Vacca et al. 1996; Molinari et al. 2003), and humans (Gentilcore et al. 2009)(**Chapter 7**), is inconsistent – ranging from no effect (Vacca et al. 1996), to increases (Longhurst and Ibarra 1984; Vacca et al. 1996; Gentilcore et al. 2009) and decreases (Vacca et al. 1996; Molinari et al. 2003)(**Chapter 7**), in SMA blood flow.

The aims of this study were to determine, in healthy older subjects, who received an intraduodenal glucose infusion at 3 kcal/min (i) the minimum volume of gastric distension required to attenuate the fall in blood pressure, (ii) the relationship between blood pressure and intragastric volume, and (iii) to further evaluate the effect of gastric distension on SMA blood flow.

8.3 Research design and methods

8.3.1 Subjects

Nine healthy older males, with a median age of 68.6 years (range: 65 - 75 years) and body mass index (BMI) of 25.4 kg/m² (range: 22.3 – 29.3 kg/m²), recruited by advertisement, were enrolled in the study. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal, hepatic or cardiac disease, intake of > 20 g alcohol/day,

epilepsy, nor was taking medication known to influence blood pressure or gastrointestinal function. Two of the nine subjects had participated in studies involving gastrointestinal intubation previously.

8.3.2 *Experimental protocol (figure 8.1)*

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

Each subject was studied on four occasions in randomised order; each separated by a minimum of three days. On each day, the subject attended the University of Adelaide, Discipline of Medicine, at the Royal Adelaide Hospital, at 0800h following an overnight fast (10h for solids; 8h for liquids) (**Chapter 7**). A silicone-rubber catheter (external diameter ~4 mm; Dentsleeve International Ltd, Mui Scientific, Mississauga, Canada) was introduced into the stomach via an anaesthetised nostril. (O'Donovan et al. 2002) The assembly included an infusion channel (internal diameter ~1 mm) and was positioned so that the infusion port was located ~10 cm distal to the pylorus (i.e. in the duodenum). Two other channels were positioned in the antrum (2.5 cm proximal to the pylorus) and duodenum (2.5cm distal to the pylorus), respectively, and were perfused with 0.9 % saline. The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference (TMPD) at the antral (- 40 mV) and duodenal (0 mV), channels. (Hedde et al. 1989) For this purpose, an

intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode. (Hedde et al. 1989)

The subject also swallowed a single-lumen polyvinyl orogastric catheter (OD 4 mm, ID 2 mm; Tygon® Tubing, Saint Gobain Performance Plastics, Akron, USA), which had an ultrathin, flaccid polyethylene bag (capacity 600 ml), wrapped tightly around the distal end. The proximal end of the catheter was connected via a three-way tap to a gastric barostat (Distender Series II™, G & J Electronics Inc, Ontario, Canada). The bag was unfolded by inflation with 400 ml of air, while ensuring that the pressure did not exceed 20 mmHg, and adjusted to be positioned in the proximal stomach, just below the diaphragm. The bag was then deflated, and the barostat assembly was fixed in this position by taping it to the skin of the cheek (Rossi et al. 1998; Feinle et al. 2000; van Orshoven et al. 2004).

After the catheters were positioned correctly (at $t = -2$ min), gastric distension was performed over 2 minutes to a volume of either i) 0 ml ('V0'), ii) 100 ml ('V100'), iii) 300ml ('V300') or iv) 500 ml ('V500'). After the distension was established (at $t = 0$ min), an intraduodenal glucose infusion (3 kcal/min) was commenced and continued for 60 minutes (i.e. between $t = 0 - 60$ min). At $t = 60$ min the barostat bag was deflated. Between $t = 60 - 120$ min saline (0.9 %) was infused intraduodenally at an identical rate (O'Donovan et al. 2002). Intraballoon pressure and volumes were measured and recorded on a computer-based system running commercially available software (Protocol Plus™, G&J Electronics,

Toronto, Ontario, Canada), and stored for subsequent analysis. Intraduodenal infusions were performed using a volumetric infusion pump (Imed Gemini PC-1: IMED Corp, San Diego, CA, USA). An intravenous cannula was positioned in a left antecubital vein for blood sampling, and an automated blood pressure cuff placed around the right arm. Each subject remained in a supine position while blood sampling, and measurements of blood pressure, heart rate and SMA blood flow were performed. At $t = 120$ min the catheters were removed, the subject given a light meal and then allowed to leave the laboratory. On one day, cardiovascular autonomic nerve function was evaluated immediately after the completion of the study. (Ewing and Clarke 1982; Piha 1991)

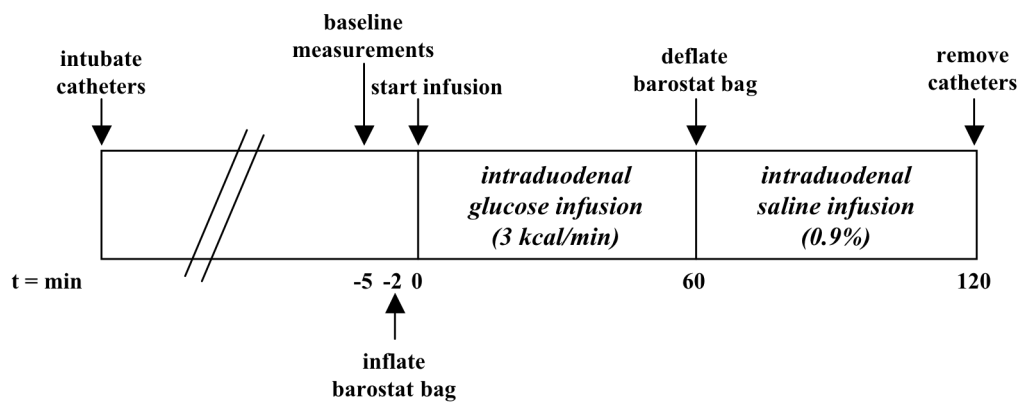


Figure 8.1: Schematic representation of experimental protocol

8.3.3 *Measurements*

8.3.3.1 *Blood pressure and heart rate*

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at $t = -12, -9$ and -6 min prior to commencement of any intervention i.e. gastric distension or intraduodenal infusions and then every 3 minutes between $t = 0 - 120$ min (O'Donovan et al. 2002). 'Baseline' blood pressure and heart rate, i.e. ' $t = -6$ min', were calculated as the mean of measurements taken at $t = -12, -9,$ and -6 min. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

8.3.3.2 *Splanchnic blood flow*

SMA blood flow was measured by Duplex ultrasonography (i.e. B-mode and Doppler imaging) using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, Australia) (Perko 2001). The subject was scanned using a 3.5 C broad spectrum 2.5 - 4 MHz convex transducer before ($t = -5$ min) the commencement of the intraduodenal infusion, and then at 15-minute intervals between $t = 0 - 120$ min. Blood flow (ml/min) was calculated instantaneously using the formula: $\pi \times r^2 \times \text{TAMV} \times 60$, where r = the radius of the superior mesenteric artery and TAMV is the time-averaged mean velocity (Perko 2001; Gentilcore et al. 2008a).

8.3.3.3 *Blood glucose concentrations*

Venous blood samples were obtained prior to the commencement of the intraduodenal infusion (i.e. $t = -5$ min) and then at 15-minute intervals between $t = 0 - 120$ min. Blood glucose concentrations (mmol/L) were determined immediately using a portable blood glucose meter (Medisense Precision Q-I-D™ System, Abbott Laboratories, Medisense Products Inc, Bedford, MA, USA).

8.3.3.4 *Visual analogue scale questionnaires*

Perceptions of gastric distension i.e. nausea and fullness, were measured, using a validated visual analogue (VAS) questionnaire. Other perceptions, including anxiety, drowsiness and dizziness, were assessed to distract the subject from the main purpose of the questionnaire, but were not evaluated formally. Each VAS consisted of a 100 mm horizontal line, where 0 mm represented 'sensation not felt at all' and 100 mm 'sensation was felt the greatest'. Subjects were asked to place a vertical mark on the 100 mm line to indicate how they felt at a particular point in time. Measurements were taken prior to the commencement of the intraduodenal infusion (i.e. $t = -5$ min) and at 15-minute intervals between $t = 0 - 120$ min (Parker et al. 2004).

8.3.4 *Assessment of cardiovascular autonomic nerve function*

Autonomic nerve function was assessed using standardised cardiovascular reflex tests. (Ewing and Clarke 1982; Piha 1991) Parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during deep breathing and the response to standing ("30 : 15" ratio). Sympathetic function was assessed

by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

8.3.5 *Statistical analysis*

Systolic and diastolic blood pressure, heart rate and effects of distension were analysed as changes in absolute values from baseline. Intra-gastric pressure, SMA blood flow and blood glucose were analysed as absolute values. The maximum changes in heart rate, SMA blood flow and blood glucose were defined as the greatest change from baseline in each subject occurring at any time point for each treatment. Differences between baseline levels on each study visit were analysed using mixed models. AUC were calculated using the trapezoidal rule and analysed by a maximum likelihood mixed effects model, including the fixed effect of volume, with Bonferroni-adjusted post-hoc tests following a significant volume effect. A planned contrast of volume of 0 ml versus the average of the volumes 100, 300 and 500 ml was also included in the mixed model to test for an overall effect of distension. Differences between baseline and $t = 60$ mins were analysed using paired t-tests for each treatment. Relationships between intra-gastric volume and AUCs for systolic blood pressure, SMA blood flow and intra-gastric pressure were calculated using within-subject correlations (r). Multiple within-subject regressions were used to assess the independent effects of intra-gastric volume and intra-gastric pressure on the AUC and maximum fall of systolic blood pressure and

intra-gastric pressure. All analyses were performed using SPSS version 16.0.2 and version 17 (SPSS Inc, Chicago, MI, USA). Data are shown as either changes from baseline and mean values \pm standard error of the mean (SEM), or absolute mean value \pm SEM. Based on our previous study by our group, we calculated that a minimum of six subjects would be required to detect a difference of \sim 14 mmHg in systolic blood pressure between 'V0' and 'V500', with the power of 0.80, and at a significance level of $P < 0.05$ (**Chapter 7**). A P value < 0.05 was considered statistically significant in all analyses.

8.4 Results

The studies were generally well tolerated. One subject experienced nausea and subsequently vomited during 'V500', and that study day was, accordingly, excluded in all analyses. No subject had definite autonomic neuropathy (mean score 0.78, range: 0 - 2). No subject had postprandial hypotension (Jansen and Lipsitz 1995).

8.4.1 *Intra-gastric pressure during gastric distension (figure 8.2)*

There was a significant difference in baseline ($t = 0$ min, after gastric distension) intra-gastric pressure between the four days: systolic blood pressure ('V0' 1.7 ± 0.4 mmHg vs. 'V100' 5.2 ± 0.6 mmHg vs. 'V300' 10.3 ± 1.3 mmHg vs. 'V500' 16.0 ± 1.1 mmHg; $P < 0.001$). Between $t = 0 - 60$ min, there was a distension effect ($P < 0.001$) on the intra-gastric pressure AUC for no distension ('V0') versus the average of the volume distensions ('V100', 'V300' and 'V500'). In the mixed model comparing all conditions, there was a significant treatment effect ($P = 0.002$), so that intra-gastric pressure was less during 'V0' when compared with

'V100' ($P = 0.003$), 'V300' ($P < 0.001$) and 'V500' ($P = 0.002$), less during 'V100' compared with 'V300' ($P = 0.001$) and 'V500' ($P = 0.02$), with a trend for a difference between 'V300' compared with 'V500' ($P = 0.10$). At $t = 60$ min, intragastric pressure did not differ from baseline after 'V100' (4.8 ± 0.6 mmHg; $P = 0.47$), was less following 'V300' (6.5 ± 1.0 mmHg; $P = 0.03$), and 'V500' (9.5 ± 1.8 mmHg; $P = 0.002$), but marginally higher following 'V0' (2.8 ± 0.4 mmHg; $P = 0.007$).

8.4.2 *Systolic and diastolic blood pressure and heart rate (figure 8.3 a, b & c)*

There was no difference in pre-distension ($t = -6$ min) blood pressure or heart rate between the four days: systolic blood pressure ('V0' 124 ± 4 mmHg vs. 'V100' 124 ± 5 mmHg vs. 'V300' 122 ± 4 mmHg vs. 'V500' 119 ± 4 mmHg; $P = 0.20$); diastolic blood pressure ('V0' 71 ± 2 mmHg vs. 'V100' 69 ± 3 mmHg vs. 'V300' 71 ± 2 mmHg vs. 'V500' 69 ± 2 mmHg; $P = 0.24$), and heart rate ('V0' 58 ± 3 bpm vs. 'V100' 59 ± 3 bpm vs. 'V300' 58 ± 3 bpm vs. 'V500' 57 ± 3 bpm; $P = 0.79$).

There was no difference in baseline blood pressure or heart rate after distension ($t = 0$ min) between the four days: systolic blood pressure ('V0' 124 ± 4 mmHg vs. 'V100' 125 ± 5 mmHg vs. 'V300' 124 ± 4 mmHg vs. 'V500' 122 ± 4 mmHg; $P = 0.93$); diastolic blood pressure ('V0' 70 ± 3 mmHg vs. 'V100' 70 ± 3 mmHg vs. 'V300' 71 ± 2 mmHg vs. 'V500' 72 ± 3 mmHg; $P = 0.38$), and heart rate ('V0' 59 ± 3 bpm vs. 'V100' 60 ± 3 bpm vs. 'V300' 59 ± 4 bpm vs. 'V500' 57 ± 3 bpm; $P = 0.86$).

Between $t = 0 - 60$ min, there was a distension effect ($P = 0.008$) on the AUC of the change in systolic blood pressure for no distension ('V0') versus the average of the volume distensions ('V100', 'V300' and 'V500'). Between $t = 0 - 60$ min, there was a fall in systolic blood pressure for 'V0' ($P = 0.05$), no change during 'V100' ($P = 0.58$) or 'V300' ($P = 0.11$), and a trend for a rise during 'V500' ($P = 0.10$). In a mixed model comparing all conditions, there was a significant

treatment effect ($P = 0.01$), so that systolic blood pressure was less during 'V0' when compared with 'V300' ($P = 0.008$), and 'V500' ($P = 0.01$), with a trend for a difference when compared with 'V100' ($P = 0.09$). There was no significant difference in systolic blood pressure during 'V300' when compared with 'V100' ($P = 0.36$) or 'V500' ($P = 0.72$) however, there was a trend for a difference in systolic blood pressure between 'V100' and 'V500' ($P = 0.10$). At $t = 60$ min, systolic blood pressure did not differ from baseline after 'V0' (122 ± 4 mmHg; $P = 0.37$), 'V100' (123 ± 5 mmHg; $P = 0.58$), 'V500' (123 ± 6 mmHg; $P = 0.32$) but tended to be greater after 'V300' (126 ± 5 mmHg; $P = 0.07$).

Between $t = 0 - 60$ min, there was a distension effect ($P = 0.02$) on the AUC of the change in diastolic blood pressure for no distension ('V0') versus the average of the volume distension ('V100', 'V300' and 'V500'). In the mixed model comparing all conditions there was a significant treatment effect ($P = 0.01$), so that diastolic blood pressure was less during 'V0' compared with 'V100' ($P = 0.01$), and 'V500' ($P = 0.02$), but not 'V300' ($P = 0.12$). There was no difference in diastolic blood pressure during 'V100' compared with 'V300' ($P = 0.32$), and 'V500' ($P = 0.44$), but a trend for a difference during 'V300' compared with 'V500' ($P = 0.08$). At $t = 60$ min, diastolic blood pressure did not differ from baseline after 'V100' (66 ± 3 mmHg; $P = 0.18$), 'V300' (70 ± 3 mmHg; $P = 0.58$), and 'V500' (68 ± 3 mmHg; $P = 0.42$), but was less following 'V0' (66 ± 3 mmHg; $P = 0.001$).

Between $t = 0 - 60$ min, there was no significant distension effect ($P = 0.11$) on the AUC of the change in heart rate for no distension ('V0') versus the average of the volume distension ('V100', 'V300' and 'V500'). There was a trend for a difference ($P = 0.10$) in the maximum rise in heart rate from baseline during 'V0' (11 ± 2 bpm at 39 ± 5 min), 'V100' (13 ± 2 bpm at 43 ± 6 min), 'V300' (11 ± 2 bpm at 45 ± 4 min), and 'V500' (10 ± 2 bpm at 32 ± 6 min). At $t = 60$ min, heart rate was higher than baseline after 'V0' (65 ± 4 bpm; $P = 0.01$), 'V100' (67 ± 5 bpm; $P = 0.02$), 'V300' (65 ± 4 bpm; $P < 0.001$), and 'V500' (64 ± 4 bpm; $P = 0.003$).

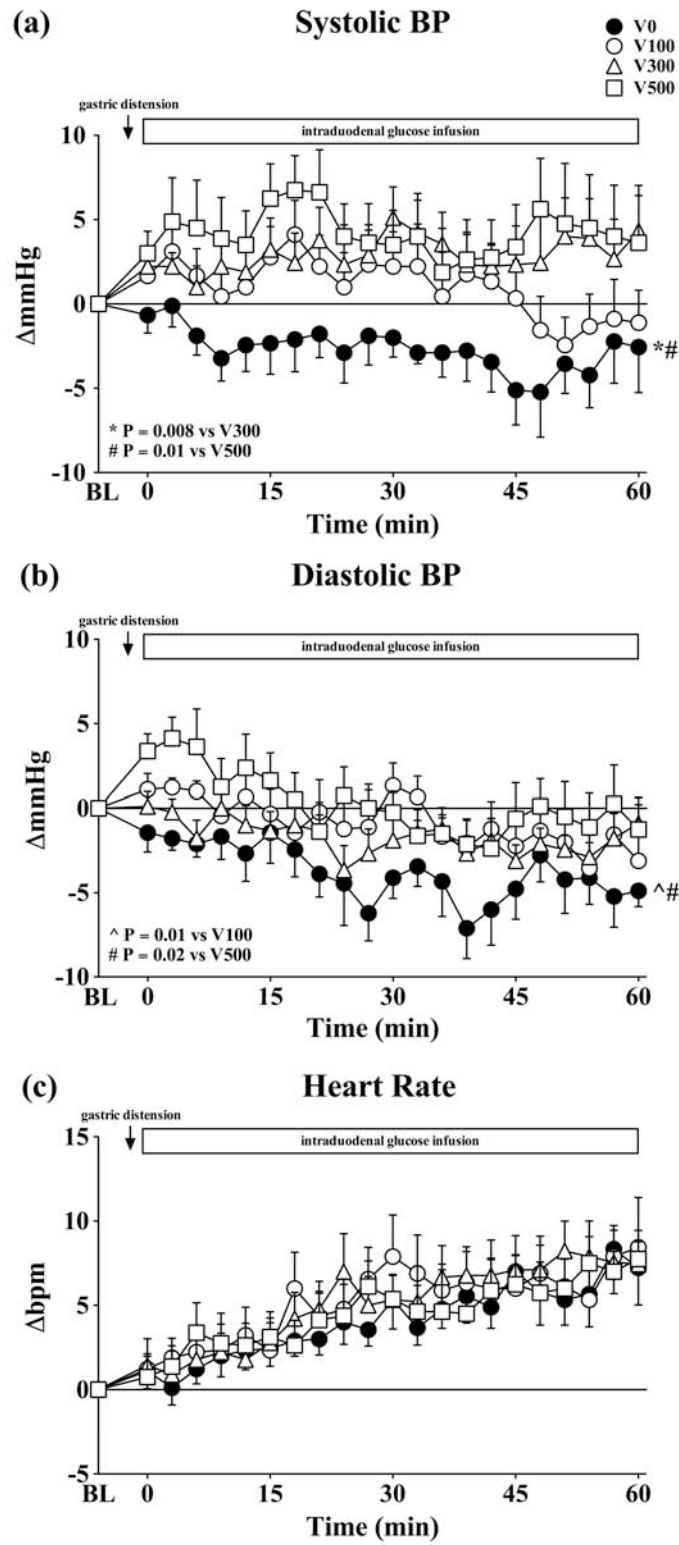


Figure 8.3: Changes in a) systolic blood pressure, b) diastolic blood pressure c), heart rate, from baseline in response to intragastric balloon distensions at volumes of 0, 100, 300 and 500 ml - 'V0' (●), 'V100' (○), 'V300' (△), and 'V500' (□). Data are mean values \pm SEM (n = 9). Systolic blood pressure treatment effect: * P = 0.008 'V0' compared with 'V300', # P = 0.01 'V0' compared with 'V500'; Diastolic blood pressure treatment effect: ^ P = 0.01 'V0' compared with 'V100', # P = 0.02 'V0' compared with 'V500'.

8.4.3 *SMA blood flow (Figure 8.4)*

There was no significant difference in pre-distension ($t = -5$ min) SMA blood flow between the four days ('V0' vs. 'V100' vs. 'V300' vs. 'V500'): 829 ± 78 ml/min vs. 771 ± 62 ml/min vs. 778 ± 112 ml/min vs. 778 ± 72 ml/min; $P = 0.93$.

There was no significant distension effect ($P = 0.16$) on the AUC of SMA blood flow between $t = -5 - 60$ min for no distension ('V0') versus the average of the volume distensions ('V100', 'V300' and 'V500'). There was also no difference ($P = 0.15$) in the maximum SMA blood flow from baseline during 'V0' (2080 ± 228 ml/min), 'V100' (1806 ± 184 ml/min), 'V300' (1748 ± 187 ml/min), and 'V500' (1555 ± 280 ml/min). At $t = 60$ min, SMA blood flow was greater than baseline after 'V0' (1650 ± 159 ml/min; $P < 0.001$), 'V100' (1675 ± 201 ml/min; $P = 0.001$), 'V300' (1517 ± 188 ml/min; $P < 0.001$), and 'V500' (1397 ± 144 ml/min; $P = 0.003$).

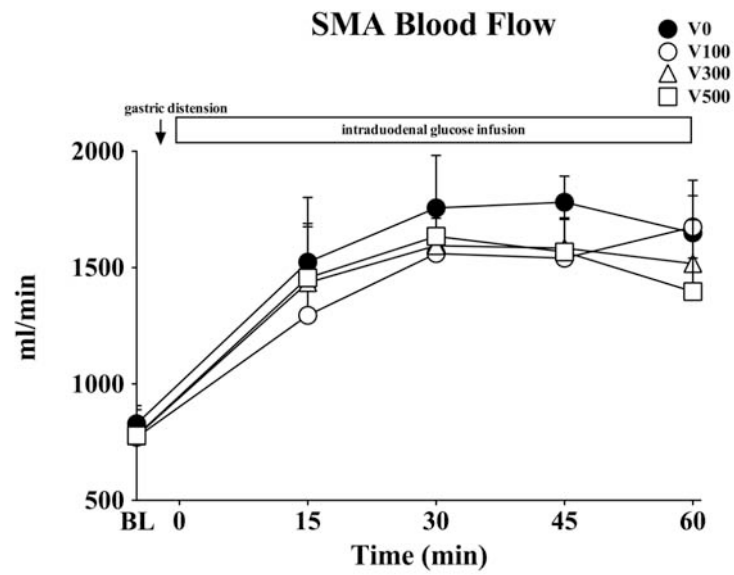


Figure 8.4: Superior mesenteric artery (SMA) blood flow, during intragastric balloon distension at 0, 100, 300 and 500 ml - 'V0' (●), 'V100' (○), 'V300' (△), and 'V500' (□). Data are mean values \pm SEM ($n = 9$).

8.4.4 *Blood glucose (Figure 8.5)*

There was no significant difference in pre-distension ($t = -5$ min) blood glucose between the four days ('V0' vs. 'V100' vs. 'V300' vs. 'V500'): 5.4 ± 0.2 mmol/L vs. 5.2 ± 0.2 mmol/L vs. 5.4 ± 0.2 mmol/L vs. 5.3 ± 0.1 mmol/L; $P = 0.47$.

There was no significant distension effect ($P = 0.99$) on the AUC of blood glucose between $t = -5 - 60$ min for no distension ('V0') versus the average of the volume distension ('V100', 'V300' and 'V500'). There was also no difference ($P = 0.30$) in the maximum blood glucose during 'V0' (9.8 ± 0.3 mmol/L), 'V100' (10.3 ± 0.6 mmol/L), 'V300' (10.0 ± 0.6 mmol/L), and 'V500' (9.9 ± 0.5 mmol/L). At $t = 60$ min, blood glucose was greater than baseline after 'V0' (9.7 ± 0.3 mmol/L; $P < 0.001$), 'V100' (10.3 ± 0.6 mmol/L; $P < 0.001$), 'V300' (10.0 ± 0.6 mmol/L; $P < 0.001$) and 'V500' (9.9 ± 0.5 mmol/L; $P < 0.001$).

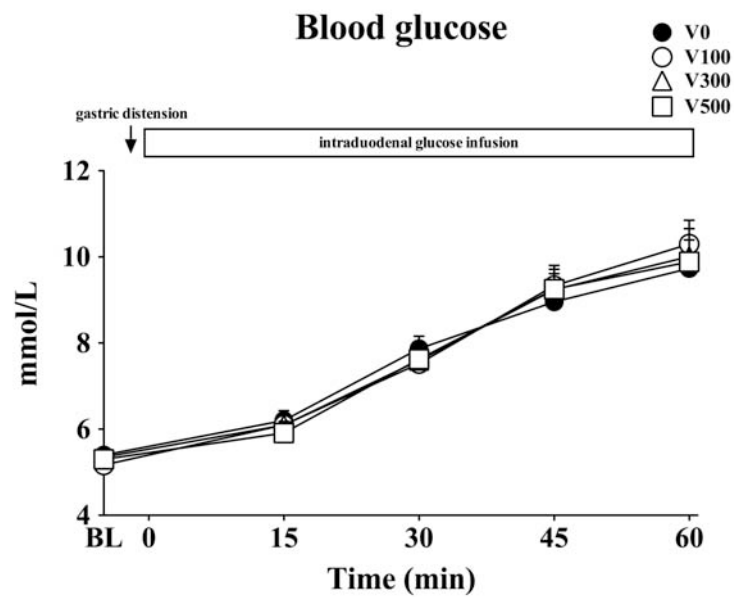


Figure 8.5: Blood glucose during intragastric balloon distension at 0, 100, 300 and 500 ml - 'V0' (●), 'V100' (○), 'V300' (△), and 'V500' (□). Data are mean values \pm SEM (n = 9)

8.4.5 *Perceptions of distension (Figure 8.6 a & b)*

There was no significant difference at pre-distension ($t = -5$ min) for the perceptions of distension between the four days: nausea ('V0' 3 ± 1 mm vs. 'V100' 5 ± 4 mm vs. 'V300' 2 ± 1 mm vs. 'V500' 2 ± 1 mm; $P = 0.43$); fullness ('V0' 13 ± 6 mm vs. 'V100' 8 ± 4 mm vs. 'V300' 6 ± 2 mm vs. 'V500' 10 ± 6 mm; $P = 0.71$).

Between $t = -5 - 60$ min, there was no distension effect ($P = 0.89$) on the AUC of the scores for nausea for no distension ('V0') versus the average of the volume distensions ('V100', 'V300' and 'V500'). At $t = 60$ min, scores for nausea did not differ from baseline after 'V0' (5 ± 4 mm; $P = 0.52$), 'V100' (4 ± 3 mm; $P = 0.26$), 'V300' (7 ± 6 mm; $P = 0.35$), or 'V500' (2 ± 1 mm; $P = 0.70$).

Between $t = -5 - 60$ min, there was no treatment effect ($P = 0.86$) on the AUC of the scores for fullness for no distension ('V0') versus the average of the volume distensions ('V100', 'V300' and 'V500'). At $t = 60$ min, scores for fullness did not differ from baseline after 'V0' (12 ± 5 mm; $P = 0.89$), 'V100' (11 ± 4 mm; $P = 0.66$), 'V300' (19 ± 8 mm; $P = 0.18$), or 'V500' (7 ± 3 mm; $P = 0.66$).

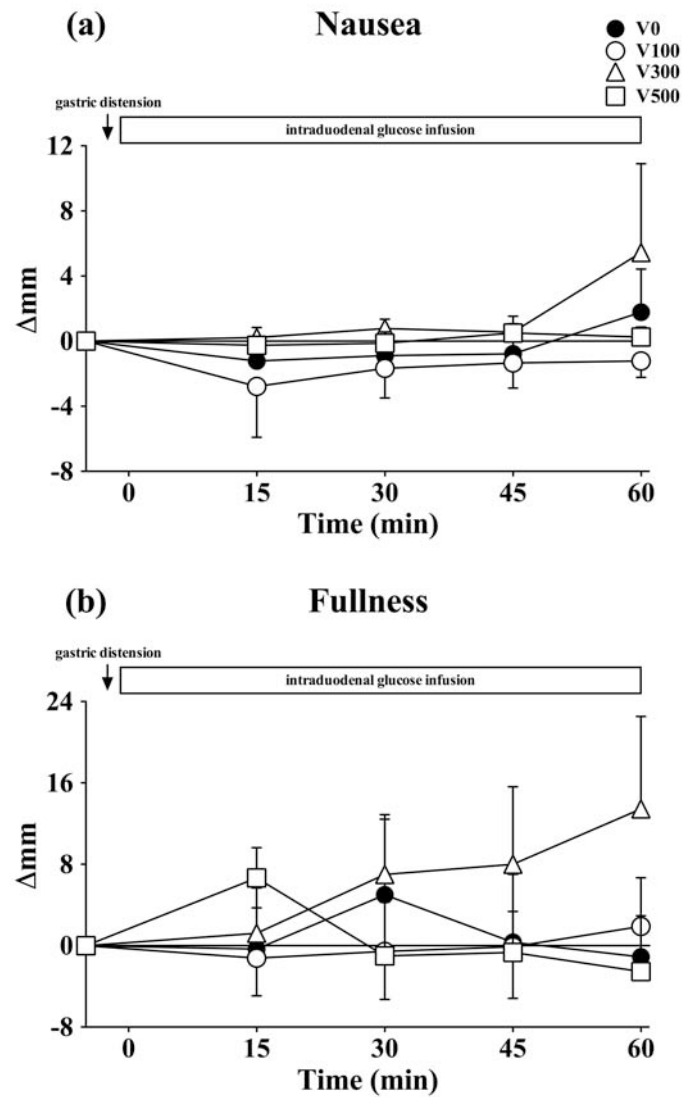


Figure 8.6: Changes from baseline in visual analogue scale scores for the sensation of a) nausea, b) fullness, during intragastric balloon distension at 0, 100, 300 and 500 ml - ‘V0’ (●), ‘V100’ (○), ‘V300’ (△), and ‘V500’ (□). Data are mean values \pm SEM ($n = 9$).

8.4.6 *Within-subjects relationships between blood pressure with intragastric pressure and volume and SMA blood flow*

There was an inverse relationship ($r = -0.44$, $P = 0.01$) between the AUC of the change in systolic blood pressure ($t = 0 - 60$ min) from baseline versus the AUC of SMA blood flow ($t = 0 - 60$ min).

There was a positive relationship ($r = 0.60$, $P = 0.001$) between the AUC of the change in systolic blood pressure ($t = 0 - 60$ min) from baseline versus intragastric volume ($t = 0 - 60$ min). There was a positive correlation ($r = 0.64$, $P < 0.001$) between the AUC of the change in systolic blood pressure ($t = 0 - 60$ min) from baseline with the AUC of intragastric pressure ($t = 0 - 60$ min). Furthermore, there was a significant positive correlation ($r = 0.50$, $P = 0.008$) between the maximum fall in systolic blood pressure with the AUC of intragastric pressure ($t = 0 - 60$ min).

In multiple within-subjects regressions, the AUC for the change in systolic blood pressure ($t = 0 - 60$ min) from baseline demonstrated no significant independent association with either the AUC's for intragastric pressure ($t = 0 - 60$ min) ($P = 0.18$) or intragastric volume ($t = 0 - 60$ min) ($P = 0.63$). There was, however, a significant independent association between the maximum fall in systolic blood pressure and intragastric volume ($P = 0.03$), but not intragastric pressure ($P = 0.46$).

8.5 Discussion

This study has evaluated the effects of gastric balloon distension, at volumes of 100, 300 and 500 ml, on blood pressure and SMA blood flow responses to intraduodenal glucose infusion in healthy older subjects. In the absence of gastric distension, systolic blood pressure fell and SMA blood flow rose. Gastric distension at all volumes prevented the fall in blood pressure and while the magnitude of this attenuation was related to the intragastric volume, the differences in the blood pressure responses to the three distension volumes was not significant. In contrast to its substantial effect on blood pressure, gastric distension had no effect on heart rate or SMA blood flow, although the latter was related to the change in blood pressure. That low volume gastric distension (~ 100 ml) has the capacity to markedly attenuate the fall in blood pressure induced by small intestinal nutrients has substantial implications for the non-pharmacological management of postprandial hypotension.

There is persuasive evidence that gastric distension plays a protective role in the maintenance of postprandial blood pressure (Rossi et al. 1998; Jordan et al. 1999; Jordan et al. 2000; Cariga and Mathias 2001; Shannon et al. 2002; van Orshoven et al. 2004; Jones et al. 2005; Gentilcore et al. 2008b)(**Chapter 7**). Consumption of 480ml of water increases systolic blood pressure in healthy older subjects and patients with multiple system atrophy and autonomic failure (Jordan et al. 2000), as well as attenuates the fall in blood pressure following a high carbohydrate meal in patients with autonomic failure (Shannon et al. 2002). In healthy older subjects, the magnitude of the fall in systolic blood pressure is greater when glucose was

ingested at a smaller volume (200 ml compared with 600 ml), at the same glucose concentration (Jones et al. 2005). We have reported, also in healthy older subjects, that gastric distension, using a barostat balloon set to 8 mmHg above MDP, prevented the fall in blood pressure induced by intraduodenal glucose given at a rate of 3 kcal/min (**Chapter 7**). In this latter study the volume of air within the barostat bag increased progressively from ~ 400 ml to ~ 900 ml (**Chapter 7**). In another study, the hypotensive response to intraduodenal infusion of glucose was markedly attenuated by the presence of as little as ~ 300 ml of intragastric water (Gentilcore et al. 2008b). In the current study, we elected to distend the stomach at fixed volumes of 100, 300 and 500 ml, as these volumes have been well tolerated in previous studies (Gentilcore et al. 2008b)(**Chapter 7**) and can be considered representative of ‘small’ and ‘moderate’ sized meals. The consequent increases in intragastric pressure were sustained and not associated with either nausea or the perception of fullness. The rate of small intestinal glucose infusion (3 kcal/min) is at the upper end of the physiological range for gastric emptying (Brener et al. 1983) and the magnitude of the fall in blood pressure and rise in heart rate observed during the control study was comparable to what has been reported (O'Donovan et al. 2002)(**Chapter 5**).

The mechanism(s) mediating the effects of gastric distension on the hypotensive response to intraduodenal glucose remain uncertain. Given that small intestinal glucose delivery was standardised, the absence of any effect of distension on the rise in blood glucose was predictable. However, elevations in blood glucose and insulin are also unlikely to play a major role in postprandial hypotension, since

intravenous glucose has little, if any, effect on blood pressure and postprandial hypotension occurs in type 1 diabetics, who are, by definition, insulin deficient (Mathias 1991; Maule et al. 2004). An increase in sympathetic activity may potentially account for the effects of gastric distension. Gastric distension has been shown to increase muscle sympathetic nerve activity in both healthy young and older subjects, although the magnitude of the increase was less in the elderly (van Orshoven et al. 2004). In the current study, gastric distension did not affect the stimulation of SMA blood flow by small intestinal glucose. In the pig, fasting SMA flow has been reported to be decreased (Vacca et al. 1996; Molinari et al. 2003), increased (Vacca et al. 1996) or unchanged (Vacca et al. 1996) by gastric distension, whereas, in the cat, a modest increase has been reported (Longhurst and Ibarra 1984). We have reported, in healthy older subjects, that gastric distension with a barostat set to 8 mmHg above MDP, attenuated the rise in SMA blood flow observed in response to an identical intraduodenal infusion (**Chapter 7**). These observations which are apparently discrepant to those in the current study, are likely to reflect methodological differences. In particular, in the previous study (**Chapter 7**) the intragastric volumes resulting from the distension were substantially greater (mean volume ~ 750 ml). It is, accordingly, likely that a 'threshold' volume and/or pressure needs to be exceeded for a reduction in SMA blood flow which is greater than 500 ml. The observed relationship between the magnitude of the fall in blood pressure and rise in SMA blood flow is consistent with previous observations (Gentilcore et al. 2009)(**Chapter 7**) consistent with the concept that the latter is integral to the hypotensive response. Our study does not allow a clear distinction between the potential effects of intragastric volume or

pressure, but based on the outcome of the multiple regression analysis, this suggests that volume may be relatively more important. There is evidence that the site of gastric distension (proximal versus distal) may be important (Rossi et al. 1998; Jones et al. 2005), and given that the positioning of the barostat was proximal in our study, evaluation of the effects of distal stomach would be of interest. It should also be recognised that we studied healthy older subjects, not those with known postprandial hypotension and studies in this group are indicated given that they are the target population.

In summary, in healthy older subjects, the fall in systolic blood pressure induced by intraduodenal glucose, was abolished by low volume gastric distension, supporting the concept that non-nutrient gastric distension represents a simple approach to the management of postprandial hypotension.

9

Effects of the oligosaccharide, alpha (α)-cyclodextrin, on gastric emptying of, and the glycaemic and blood pressure responses to, oral sucrose in healthy older subjects

9.1 Summary

In healthy older subjects the hypotensive and glycaemic responses to carbohydrate-containing meals are dependent on gastric emptying and intestinal absorption; when the latter are slowed, the magnitude of the fall in blood pressure and rise in glucose are attenuated. The oligosaccharide, alpha (α)-cyclodextrin has been reported to diminish the glycaemic response to starch in young adults; this effect has been attributed to inhibition of pancreatic amylase. The aims of this study were to determine the effects of α -cyclodextrin on gastric emptying of, and the glycaemic and blood pressure responses to, an oral sucrose load in healthy older subjects; as sucrose is hydrolysed by intestinal disaccharidases any effect(s) of α -cyclodextrin would not be attributable to amylase inhibition. Ten subjects (7M,3F; age 68 – 76yr) were studied on two days. Gastric emptying, blood glucose, serum insulin, blood pressure (systolic and diastolic) and heart rate were measured after ingestion of a 300 ml drink containing 100 g sucrose, labelled with ^{99m}Tc -sulfur colloid, with or without 10 g α -cyclodextrin. Gastric emptying was slowed slightly by α -cyclodextrin; this effect was evident between $t = 135 - 210$ min and was associated with a slight increase ($P < 0.05$) in retention of the drink in the distal stomach. Following α -cyclodextrin, blood glucose concentrations were slightly less ($P < 0.05$) at $t = 60$ and 75 min, and serum insulin less ($P < 0.0001$) at $t = 90$ and 120 min. α -cyclodextrin had no effect on the magnitude of the fall in blood pressure after the drink. We conclude that a dose of 10 g α -cyclodextrin attenuates the rise in glycaemia and serum insulin and slows gastric emptying of oral sucrose in healthy older subjects, probably as a result of delayed

intestinal carbohydrate absorption. Presumably because this effect is modest, α -cyclodextrin does not attenuate the fall in blood pressure induced by sucrose.

9.2 Introduction

In healthy subjects (Horowitz et al. 1993a) and patients with type 2 diabetes (Jones et al. 1996), the effects of carbohydrate-containing meals on glycaemia and blood pressure are dependent on the rates of gastric emptying and small intestinal carbohydrate absorption. It is now well established that the rate of gastric emptying is a major determinant of postprandial glycaemia in healthy subjects (Horowitz et al. 1993a) and patients with type 1 (Ishii et al. 1994) and type 2 (Jones et al. 1996) diabetes, such that gastric emptying accounts for at least 35 % of the variance in the initial rise in blood glucose, as well as influencing the incretin hormone (glucagon-like peptide-1 (GLP-1) and glucose-dependant insulinotropic polypeptide (GIP)) responses, after oral glucose (Horowitz et al. 1993a; Jones et al. 1996; Nauck et al. 1997). Even relatively minor variations in the rate of gastric emptying can have a marked impact on the glycaemic response to carbohydrate (O'Donovan et al. 2004b), particularly as the relationship of glycaemia with small intestinal carbohydrate delivery is non-linear (Pilichiewicz et al. 2007). Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20 mmHg (Mathias et al. 1989a; Jansen and Hoefnagels 1991; Jansen and Lipsitz 1995) within two hours of a meal (Jansen and Lipsitz 1995), is an important clinical problem (Jansen and Lipsitz 1995) that lacks an effective treatment (**Chapter 3**). In healthy older subjects the magnitude of the fall in blood pressure is dependent on both the rate of delivery of nutrients from the stomach

into the small intestine and carbohydrate digestion i.e. the decrease is less when gastric emptying (Jones et al. 1998; O'Donovan et al. 2002) and/or the digestion/absorption of carbohydrate (Jones et al. 2001; Gentilcore et al. 2005a; O'Donovan et al. 2005a) is relatively slower. The relationship between blood pressure and the rate of gastric emptying is, as with glycaemia, non-linear (**Chapter 5**). Hence, dietary and/or pharmacological interventions which slow gastric emptying and/or carbohydrate absorption have the potential to improve postprandial glycaemic control in patients with type 2 diabetes (Rayner et al. 2001) and be beneficial in the management of postprandial hypotension (Gentilcore et al. 2006d). The α -glucosidase inhibitor, acarbose, has these effects when given with oral sucrose (Gentilcore et al. 2005a), as does the viscous polysaccharide, guar gum, when given with oral glucose (Jones et al. 2001; Russo et al. 2003).

Cyclodextrins are cyclic oligosaccharides composed of six to eight glucose monomers (Raben et al. 1997) that inhibit pancreatic amylase activity (Buckley et al. 2006), are poorly digested in the small intestine (Flourie et al. 1993), and inhibit the hydrolysis of complex carbohydrates (Buckley et al. 2006). Alpha (α)- and beta (β)-cyclodextrins have been reported to reduce the postprandial glycaemic (Raben et al. 1997; Buckley et al. 2006), insulinaemic (Raben et al. 1997) and GIP responses (Raben et al. 1997) to a starch meal. For example, in healthy young males, Raben et al. (Raben et al. 1997) reported that peak blood glucose and plasma insulin and GIP concentrations were less when a potato starch meal was enriched with 2 % β -cyclodextrin. While plasma GLP-1 levels remained

essentially unchanged (Raben et al. 1997). Subsequently, Buckley et al. (Buckley et al. 2006) reported in healthy subjects, that the area under the blood glucose curve after consumption of white rice was reduced by ~ 50 % by 10 g of α -cyclodextrin. A limitation of these studies (Raben et al. 1997; Buckley et al. 2006) was that gastric emptying was not measured, hence, it remains to be determined whether the observed effects on postprandial glycaemia were related to effects on gastric emptying and/or intestinal glucose digestion and absorption. The effect of α -cyclodextrin on the hypotensive response to a meal has, hitherto, not been evaluated.

The aims of this study were to determine the effects of α -cyclodextrin on gastric emptying of, and the glycaemic and blood pressure responses to, an oral sucrose load in healthy older subjects. By selecting sucrose, a disaccharide hydrolysed by the intestine, rather than pancreatic enzymes, any effect(s) of α -cyclodextrin would not be attributable to inhibition of amylase. The broad hypothesis was that α -cyclodextrin would attenuate the glycaemic response by slowing both gastric emptying and intestinal carbohydrate absorption and, thereby, diminish the hypotensive response.

9.3 Research design and methods

9.3.1 Subjects

Ten healthy older subjects, (seven male and three female) with a median age of 70 years (range: 68 - 76 years) and body mass index (BMI) of 26.9 kg/m² (range: 23.2 - 32.4 kg/m²), recruited by advertisement, were studied. All subjects were

non-smokers and none had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, or was taking medication known to influence blood pressure or gastrointestinal function.

9.3.2 *Experimental protocol*

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

Each subject was studied on two occasions, on which they attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry at 0830h following an overnight fast (10.5h for solids; 8.5h for liquids) (Gentilcore et al. 2005a; Gentilcore et al. 2005b; Gentilcore et al. 2006c). A cannula was placed in a left antecubital vein for blood sampling and subjects were seated with their back against a gamma camera with a blood pressure cuff around the right arm. Each subject rested comfortably in the sitting position for about 30 minutes (Gentilcore et al. 2005a; Gentilcore et al. 2005b; Gentilcore et al. 2006c). At $t = -2$ min, subjects consumed a drink comprising 100 g sucrose dissolved in water with a total volume of 300 ml and labelled with 20 MBq ^{99m}Tc -sulfur colloid. On one of the days, 10 g α -cyclodextrin (Cavamax®, Wacker Fine Chemicals, MI, USA) was added to the drink. The two studies were separated by at least seven days and performed in double-blind, randomised order. Gastric

emptying, blood glucose, serum insulin, blood pressure (systolic and diastolic) and heart rate were measured. At $t = 300$ min the intravenous cannula was removed, and soon after this the subject was allowed to leave the laboratory. On one day cardiovascular autonomic nerve function was evaluated after the completion of the gastric emptying measurement (Ewing and Clarke 1982; Piha 1991). On both study days subjects were given a light meal prior to leaving the laboratory.

9.3.3 *Measurements*

9.3.3.1 *Blood pressure and heart rate*

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at $t = -9, -6$ and -3 min prior to ingestion of the drink, and then every three minutes between $t = 0$ and 300 min (Gentilcore et al. 2005a; Gentilcore et al. 2005b). ‘Baseline’ blood pressure and heart rate, i.e. $t = 0$ min, were calculated as the mean of measurements taken at $t = -9, -6$ and -3 min prior to ingestion of the drink. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

9.3.3.2 *Gastric emptying and intragastric distribution*

Subjects consumed the drink within two minutes and the time of drink completion was considered to be $t = 0$ min. Radioisotopic data were acquired for 300 minutes (1-minute frames for the first 60 minutes and 3-minute frames thereafter) (Jones et al. 1998). Data were corrected for subject movement, radionuclide decay and γ -

ray attenuation (Collins et al. 1983). Regions-of-interest were drawn around the total stomach, which was subsequently divided into proximal and distal stomach regions, and gastric emptying curves (expressed as % retention over time) were derived. The amount of the drink remaining in the total, proximal and distal stomach at 15-minute intervals between $t = 0 - 300$ min were calculated. The 50 % gastric emptying time (T50) was also determined (Collins et al. 1983).

9.3.3.3 Blood glucose and serum insulin concentrations

Venous blood samples (~ 7.5ml) were obtained immediately prior to the drink (i.e. $t = -2$ min) and at 15 minute intervals between $t = 0 - 300$ min (Gentilcore et al. 2005a; Gentilcore et al. 2005b). Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Precision Q-I-D™ System, Abbott Laboratories, Medisense Products Inc, Bedford, MA, USA) (Gentilcore et al. 2005a; Gentilcore et al. 2005b). Blood samples for serum insulin were collected in ice-chilled serum tubes with clotting activator and stored at -70°C for subsequent analysis and insulin was measured on samples collected at 30 minute intervals between $t = 0 - 300$ min. Insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc, Webster, TX, USA). Sensitivity was 0.26 mU/L, intraassay coefficient of variation was 2.6 % and interassay coefficient of variation was 6.2 % (O'Donovan et al. 2004b).

9.3.4 Assessment of cardiovascular autonomic nerve function

Autonomic nerve function was assessed using standardised cardiovascular reflex tests (Ewing and Clarke 1982; Piha 1991). Parasympathetic function was

evaluated by the variation (R - R interval) of the heart rate during deep breathing and the heart rate response to standing (“30 : 15”). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

9.3.5 *Statistical analysis*

Data were evaluated using repeated measures two-way Analysis of Variance (ANOVA), with ‘treatment’ and ‘time’ as within subject factors. Gastric emptying and blood glucose and serum insulin concentrations were analysed as absolute values. Systolic and diastolic blood pressure and heart rate were analysed as changes from baseline. Data were analysed from $t = 0 - 300$ min to determine the effects (‘treatment’ and ‘time’) of sucrose and α -cyclodextrin; with the exception of systolic and diastolic blood pressure and heart rate, which were also assessed from $t = 0 - 60$ min, as maximum changes in these parameters were anticipated to occur during this time frame. One-way ANOVA was used to analyse the effects of ‘time’ on blood glucose and serum insulin concentrations and systolic and diastolic blood pressure and heart rate. In all analyses, post-hoc comparisons of adjusted means were performed using Student’s t-tests. Relationships between variables were assessed using linear regression analysis. The maximum rises in blood glucose, serum insulin and heart rate and fall in systolic blood pressure were defined as the greatest changes from baseline in each subject for each

treatment at any given time point. It was calculated that a minimum of five subjects would be required to detect a mean difference in systolic blood pressure of ~ 12 mmHg with power of 0.80, assuming a significance value < 0.05 (Gentilcore et al. 2005a). All analyses were performed using Statview (version 5.0; Abacus Concepts, Berkeley, CA, USA) and SuperANOVA (version 1.11, Abacus Concepts, Berkeley, CA, USA). Data are presented as mean values ± standard error of the mean (SEM). A P value < 0.05 was considered significant in all analyses.

9.4 Results

The studies were well tolerated. Loose stools were reported by three of the ten subjects after *α*-cyclodextrin (i.e. from t = 300 min). In all cases, these symptoms were mild and resolved spontaneously within seven hours of the completion of each study. No subject had definite autonomic neuropathy (median score 1.0; range: 0 - 2). Postprandial hypotension (i.e. a fall in systolic blood pressure ≥ 20 mmHg, sustained for at least 30 minutes) was evident in one subject after *α*-cyclodextrin. No subject reported a difference in the taste of the drink between study days.

9.4.1 *Systolic and diastolic blood pressure and heart rate (Figure 9.1 a, b & c)*

There was no difference in baseline (i.e. t = 0 min) blood pressure or heart rate between the two study days (control vs *α*-cyclodextrin): systolic blood pressure (118 ± 5 mmHg vs 118 ± 5 mmHg; P = 0.91); diastolic blood pressure (70 ± 3

mmHg vs 71 ± 3 mmHg; $P = 0.88$) or heart rate (62 ± 2 bpm vs 63 ± 2 bpm; $P = 0.70$).

Between $t = 0 - 300$ min there was a trend for a fall in systolic blood pressure after control ($P = 0.07$) and a significant fall after α -cyclodextrin ($P < 0.0001$). The maximum falls in systolic blood pressure after control (15 ± 3 mmHg) and α -cyclodextrin (14 ± 3 mmHg) were not significantly different ($P = 0.72$). Between $t = 0 - 60$ min ($P = 0.53$) and $t = 0 - 300$ min ($P = 0.76$), there was no difference in systolic blood pressure between the two study days. At $t = 300$ min, there was no difference in systolic blood pressure from baseline after control ($P = 0.81$) or α -cyclodextrin ($P = 0.13$).

Between $t = 0 - 300$ min there was a fall in diastolic blood pressure after control ($P < 0.003$) but not after α -cyclodextrin ($P = 0.37$). Between $t = 0 - 60$ min ($P = 0.41$) and $t = 0 - 300$ min ($P = 0.87$), there was no significant difference in diastolic blood pressure between the study days. At $t = 300$ min, there was no difference in diastolic blood pressure from baseline after control ($P = 0.96$) or α -cyclodextrin ($P = 0.47$).

Between $t = 0 - 300$ min there was an initial rise in heart rate followed by a decrease after both control ($P < 0.0001$) and α -cyclodextrin ($P < 0.0001$). The maximum increases in heart rate after control (17 ± 3 bpm) and α -cyclodextrin (19 ± 2 bpm) were not different ($P = 0.17$). Between $t = 0 - 60$ min ($P = 0.23$) and $t = 0 - 300$ min ($P = 0.32$), there was no difference in heart rate between the two

study days. At $t = 300$ min, there was a trend for heart rate to be greater than baseline after control ($P = 0.08$) and greater than baseline after α -cyclodextrin ($P = 0.001$).

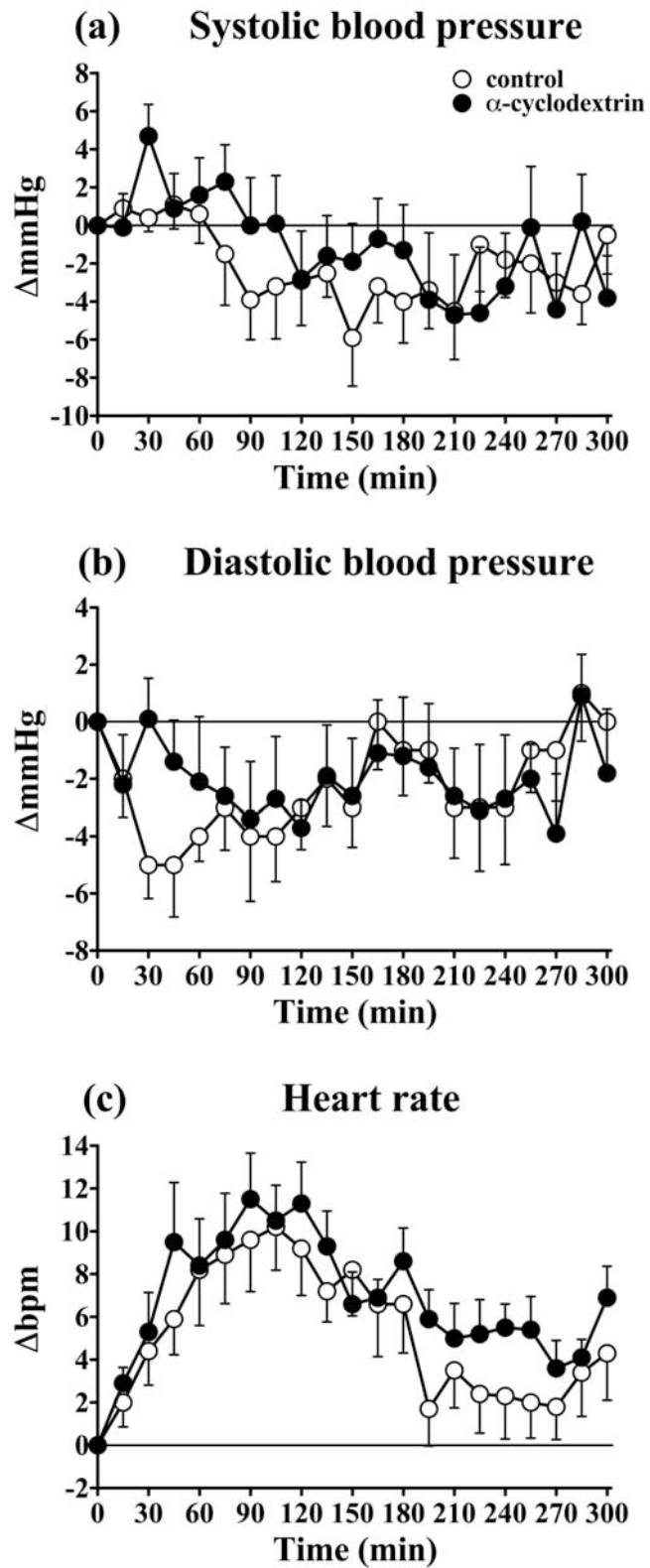


Figure 9.1: Effects of control (○) and α -cyclodextrin (10 g) (●) on changes in (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate from baseline following a 300 ml drink containing 100 g sucrose in ten healthy older subjects. Data are mean values \pm SEM.

9.4.2 Gastric emptying

9.4.2.1 Total stomach

On both study days gastric emptying of sucrose was non-linear and approximated a mono-exponential pattern. There was a significant treatment x time interaction ($P < 0.002$) for gastric emptying so that intragastric content was slightly less ($P < 0.05$) between $t = 135 - 210$ min, after control, when compared to α -cyclodextrin. There was no difference in the T50 between the two days (control: 92 ± 11 min vs α -cyclodextrin: 105 ± 19 min; $P = 0.37$) (**Figure 9.2a**).

9.4.2.2 Intragastric distribution

There was no significant difference in proximal stomach retention between the two study days ($P = 0.14$) (**Figure 9.2b**). In contrast, there was a significant treatment x time interaction for the distal stomach ($P < 0.005$), so that retention of the drink in the distal stomach was slightly greater for control, than for α -cyclodextrin between $t = 75 - 90$ min ($P < 0.05$) (**Figure 9.2c**).

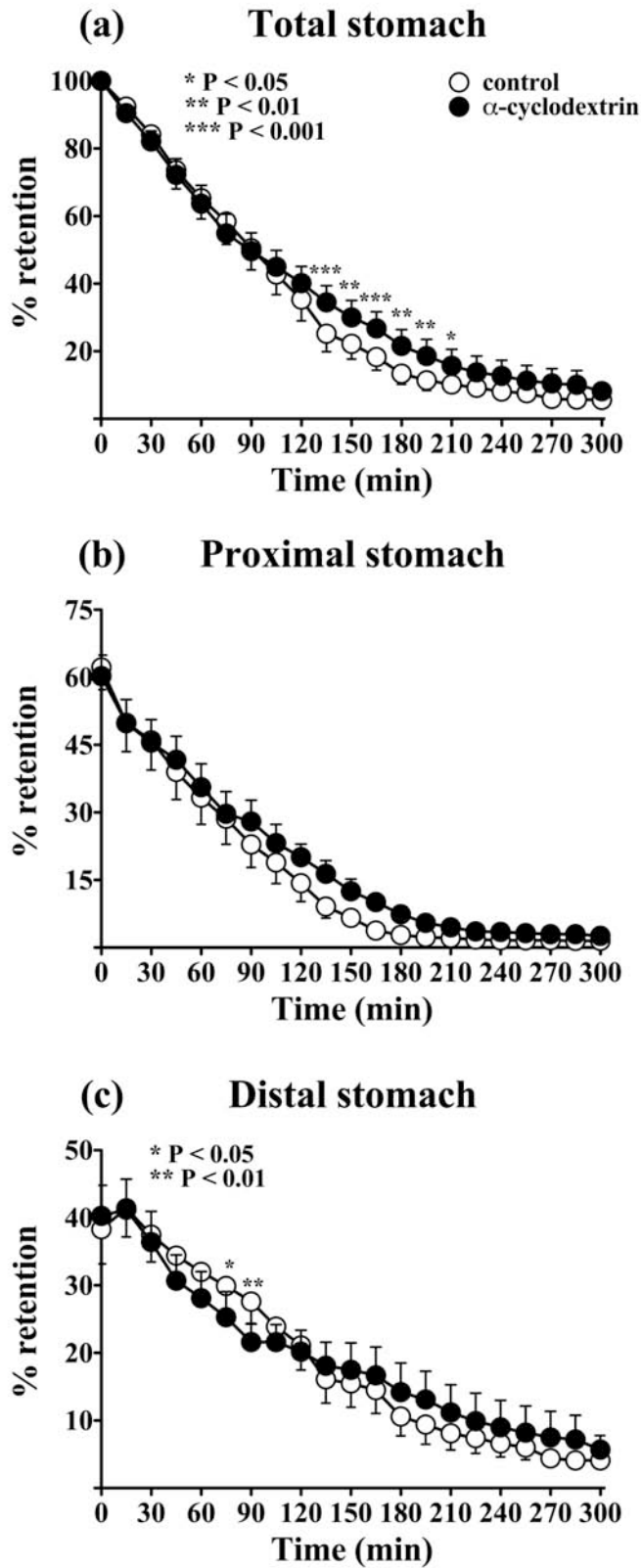


Figure 9.2: Effects of control (○) and α -cyclodextrin (10 g) (●) on gastric emptying (a) total stomach and (b) and (c) intragastric distribution of a 300 ml drink containing 100 g sucrose in ten healthy older subjects. Data are mean values \pm SEM. Total stomach ‘treatment x time’ interaction * P < 0.05, ** P < 0.01, *** P < 0.001; distal stomach ‘treatment x time’ interaction * P < 0.05, ** P < 0.01.

9.4.3 Blood glucose and serum insulin (Figure 9.3a & b)

There was no significant difference in baseline (i.e. $t = -2$ min) blood glucose between the two study days (control vs α -cyclodextrin): 6.3 ± 0.2 mmol/L for both; $P = 0.87$). There was a rise in blood glucose after the drink on both days ($P < 0.0001$ for both), which was evident from $t = 15$ min following both control and α -cyclodextrin ($P = 0.0001$ for both). Peak blood glucose were not significantly different after control (10.3 ± 0.6 mmol/L) versus α -cyclodextrin (10.3 ± 0.5 mmol/L; $P = 0.88$). There was, however, a significant ‘treatment x time’ interaction ($P < 0.0001$) for blood glucose. At $t = 60$ and 75 min, blood glucose was slightly greater ($P < 0.05$) and between $t = 180 - 210$ min slightly less ($P < 0.001$) after control when compared with α -cyclodextrin. At $t = 300$ min, there was a trend for blood glucose to be less than baseline after control ($P = 0.07$) but not after α -cyclodextrin ($P = 0.17$).

There was no significant difference in baseline (i.e. $t = -2$ min) serum insulin between the two study days (control vs α -cyclodextrin): 9.6 ± 2.0 mU/L vs 10.5 ± 2.0 mU/L; $P = 0.47$). There was a rise in serum insulin after the drink on both days ($P < 0.0001$ for both), which was evident from $t = 30$ min (the first time point at which insulin was measured) following control and α -cyclodextrin ($P = 0.0001$ for both). Peak serum insulin were not significantly different after control (120.8 ± 21.6 mU/L) versus α -cyclodextrin (111.0 ± 19.8 mU/L; $P = 0.22$). There was, however, a significant ‘treatment x time’ interaction ($P < 0.0001$) for serum insulin concentrations. At $t = 90$ and 120 min, serum insulin were greater ($P < 0.0001$) after control when compared with α -cyclodextrin. At $t = 300$ min, there

was no difference in serum insulin from baseline after control ($P = 0.26$) or α -cyclodextrin ($P = 0.81$).

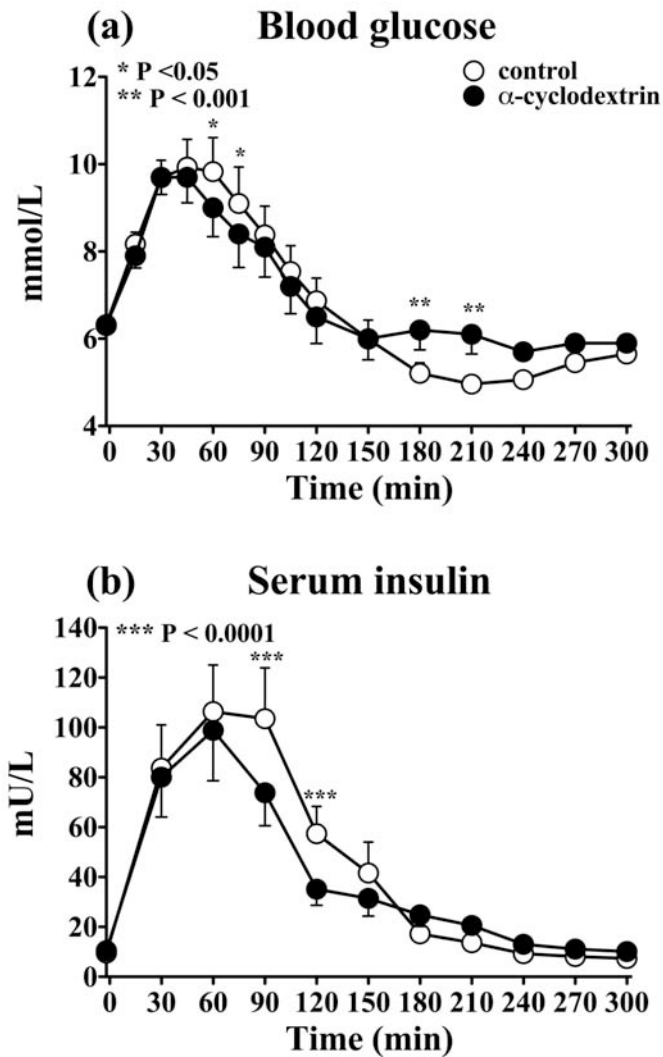


Figure 9.3: Effects of control (○) and α -cyclodextrin (10 g) (●) on (a) blood glucose and (b) serum insulin concentrations following a 300 ml drink containing 100 g sucrose in ten healthy older subjects. Data are mean values \pm SEM. Blood glucose ‘treatment x time’ interaction * P < 0.05, ** P < 0.001; serum insulin ‘treatment x time’ interaction *** P < 0.001.

9.5 Discussion

This study indicates that in healthy older subjects α -cyclodextrin, when given acutely in a dose of 10 g with oral sucrose, has modest effects to decrease the glycaemic and insulinaemic responses and slow gastric emptying but has no effect on the hypotensive response. Given that the digestion of sucrose is not dependent on the action of pancreatic amylase, the study establishes that α -cyclodextrin has the capacity to diminish postprandial glycaemia by effect(s) unrelated to inhibition of amylase.

α -cyclodextrin has been reported to attenuate the blood glucose (Raben et al. 1997; Buckley et al. 2006) and serum insulin (Raben et al. 1997) responses to starch meals, the digestion of which is dependent on amylase. In contrast to what was observed in the current study, these effects were substantial. In the current study when α -cyclodextrin was included in a sucrose drink, there was a slight lowering of blood glucose at 60 and 75 minutes, and blood glucose concentrations were subsequently greater at 180, 195 and 210 minutes; α -cyclodextrin had no effect on the initial rise, or peak blood glucose concentration. Serum insulin was lower with α -cyclodextrin at 90 and 120 minutes. Hence, the observed glucose lowering induced by α -cyclodextrin is not accounted for by stimulation of insulin secretion, but rather the reduction in blood glucose, albeit small, resulted in a lesser insulin response. This would be consistent with previous reports with starch meals that α -cyclodextrin does not stimulate insulin (Buckley et al. 2006) or incretin hormones postprandially (Raben et al. 1997), and given that the sucrose

drink was used, must be indicative of a minor reduction in the rate of small intestinal glucose absorption.

Gastric emptying of glucose and other carbohydrates is regulated at an overall rate of 1 - 4 kcal/min as a result of a length-dependent inhibitory feedback from receptors located throughout the small intestine (Brener et al. 1983; Lin et al. 1989; Moran et al. 2001). In the case of fat, protein and carbohydrate, it appears that this feedback is triggered by the digestion products ie fatty acids, amino acids and monosaccharides (Hunt et al. 1980). The observed effect of α -cyclodextrin to slow gastric emptying, with concomitant changes in intragastric distribution, is novel. Because differences in gastric emptying were only evident after ~ 135 minutes, the modest slowing is unlikely to be mediated by changes in the physical characteristics of the intragastric content (viscosity) and probably reflects an increase in the small intestinal feedback secondary to the exposure of a greater length of small intestine to sucrose. The latter appears to be the primary mechanism by which guar gum (Jones et al. 2001; Russo et al. 2003), and acarbose (Gentilcore et al. 2005a), slow gastric emptying. Given that glucose-lowering occurred before any retardation of gastric emptying, we conclude that the former is likely to reflect primarily a delay in small intestinal carbohydrate absorption, possibly due to the inhibitory effect of α -cyclodextrin on absorption of carbohydrate, as a result of its physical properties, so that carbohydrate is absorbed more distally. The viscous polysaccharide, guar and other fibres (Jenkins et al. 1978; Holt et al. 1979; Torsdottir et al. 1991) have similar effects (Meyer et al. 1988; Jones et al. 2001; Russo et al. 2003). It should, however, be recognised

that even minor perturbations in the rate of gastric emptying can effect postprandial glycaemia (O'Donovan et al. 2004b).

Postprandial hypotension is now recognised as a major cause of morbidity in older subjects and patients with type 1 and 2 diabetes (Mathias 1991; Jansen et al. 1995; Jansen and Lipsitz 1995). In healthy older subjects (Jones et al. 2001; Gentilcore et al. 2005a) and patients with postprandial hypotension (Jian and Zhou 2008), severe autonomic failure (Shibao et al. 2007) and diabetes (Sasaki et al. 2001; Russo et al. 2003; Maule et al. 2004), the postprandial fall in blood pressure is attenuated when gastric emptying and small intestinal carbohydrate absorption are slowed by dietary (e.g. guar) or pharmacological (e.g. acarbose) means. Conversely, gastric distension attenuates the postprandial fall in blood pressure (Rossi et al. 1998; Shannon et al. 2002; Gentilcore et al. 2008b; Gentilcore et al. 2009). The effects of α -cyclodextrin on the hypotensive response to a 'meal' have, to our knowledge, not been evaluated. Although mean systolic blood pressure in our cohort was normal, sucrose induced a substantial (~ 14 mmHg) fall, comparable to that observed previously (Gentilcore et al. 2005a). That α -cyclodextrin had no effect on the hypotensive responses, whereas acarbose did, may be attributable to its more modest effects on both gastric emptying and intestinal glucose absorption. In part, the latter may reflect the marked inhibition of sucrose absorption as a result of disaccharide inhibition and consequent substantial stimulation of GLP-1 by acarbose (Gentilcore et al. 2005a). Guar, which has no effect on disaccharide activity, attenuates the hypotensive response to oral glucose in both healthy older subjects (Jones et al. 2001) and type 2

patients (Russo et al. 2003) substantially, but unlike α -cyclodextrin, has marked effects to slow gastric emptying and intestinal glucose absorption. Hence we conclude that the effects of α -cyclodextrin on intestinal carbohydrate absorption are likely to be modest.

In interpreting our observations, it should be recognised that we utilised a dose (10 g) of α -cyclodextrin that has been reported to reduce the glycaemic response to a carbohydrate (rice) meal in young, healthy subjects and was associated with a few, albeit tolerable, adverse gastrointestinal symptoms including nausea, abdominal bloating and diarrhoea (Buckley et al. 2006). 10 g of α -cyclodextrin has been shown to reduce the blood glucose response to rice to a greater extent than 5 g (Buckley et al. 2006). While it is possible that a higher dose of α -cyclodextrin, may have induced greater effects on glycaemia, gastric emptying and blood pressure, three of the ten subjects reported adverse effects, and it is likely that higher doses would not be well tolerated although this remains to be determined. It is also important not to extrapolate our observations to the effects of α -cyclodextrin on gastric emptying of, and hypotensive response to, starch meals where amylase inhibition is of relevance. We would speculate that gastric emptying of starch may be accelerated initially as a result of reduced intestinal feedback, but the effects on carbohydrate absorption, and hence glycaemia and blood pressure, may be greater.

10

**Effects of intraduodenal acarbose
on blood pressure, heart rate and
splanchnic blood flow in healthy
older subjects**

10.1 Summary

Postprandial hypotension is an important problem, particularly in the elderly, which may be triggered by an increase in splanchnic blood flow. Acarbose attenuates the fall in blood pressure induced by oral sucrose and may be useful in the management of postprandial hypotension. It is uncertain whether this effect reflects slowing of gastric emptying and/or carbohydrate absorption. The aims of this study were to determine the effects of intraduodenal acarbose on blood pressure, heart rate, superior mesenteric artery (SMA) blood flow, glycaemic and insulin responses to intraduodenal sucrose in older subjects and the relationship between changes in blood pressure with superior mesenteric artery blood flow, i.e. any 'gastric' effects of acarbose were excluded. Eight healthy subjects (4M, 4F, age 66 - 77yrs) received an intraduodenal infusion of sucrose (~ 6 kcal/min), with or without acarbose (100 mg), over 60 minutes. Blood pressure, heart rate, SMA blood flow, blood glucose and serum insulin were measured. Acarbose markedly attenuated the falls in systolic ($P < 0.01$) and diastolic ($P < 0.05$) blood pressure and rises in heart rate ($P < 0.05$), SMA blood flow ($P < 0.05$), blood glucose ($P < 0.01$) and serum insulin ($P < 0.05$). The maximum fall in systolic blood pressure and peak SMA blood flow were inversely related on the control day ($r = 0.73$, $P < 0.05$), but not with acarbose ($r = 0.17$, $P = 0.70$). We conclude that the fall in blood pressure induced by intraduodenal sucrose in healthy older subjects is related to the concomitant increase in SMA blood flow and that acarbose markedly attenuates the hypotensive response by slowing carbohydrate absorption and attenuating the rise in splanchnic blood flow.

10.2 Introduction

The alpha (α)-glucosidase inhibitor, acarbose, is used frequently in the management of type 2 diabetes to reduce postprandial glycaemia (Breuer 2003). It has been assumed that the latter effect reflects a delay in small intestinal carbohydrate absorption (Breuer 2003), but studies conducted by ourselves (Gentilcore et al. 2005a) and others (Ranganath et al. 1998; Enc et al. 2001) have demonstrated that acarbose may slow gastric emptying substantially; the latter is a major determinant of the glycaemic response to carbohydrate (Horowitz et al. 1993a; Jones et al. 1996). Acarbose also stimulates the secretion of the incretin hormone, glucagon-like peptide-1 (GLP-1) (Qualmann et al. 1995), and this may contribute to a reduction in glycaemia by stimulating insulin (Holst et al. 1987), suppressing glucagon (Meier et al. 2002), and slowing gastric emptying (Deane et al. 2009). A number of studies (Sasaki et al. 2001; Maule et al. 2004; Gentilcore et al. 2005a; Yamamoto et al. 2006; Shibao et al. 2007; Jian and Zhou 2008) have established that acarbose attenuates the postprandial hypotensive response to carbohydrate meals in patients with (Sasaki et al. 2001; Maule et al. 2004; Yamamoto et al. 2006; Shibao et al. 2007; Jian and Zhou 2008) and without (Gentilcore et al. 2005a) postprandial hypotension. We reported in healthy older subjects that acarbose (100 mg) markedly attenuated the fall in systolic blood pressure induced by oral sucrose (Gentilcore et al. 2005a). More recently, Jian et al. (Jian and Zhou 2008) reported that acarbose (50 mg) attenuated the postprandial falls in blood pressure, after a semi-liquid 'meal' in a cohort of 43 older patients with postprandial hypotension. There are three case reports suggesting that acarbose is beneficial in patients with symptomatic postprandial

hypotension associated with type 1 (Maule et al. 2004) and type 2 (Sasaki et al. 2001; Yamamoto et al. 2006) diabetes, and evidence that another α -glucosidase inhibitor, voglibose, is also effective (Maruta et al. 2006). The effect of acarbose on postprandial blood pressure may relate to prolongation of gastric distension by delaying gastric emptying (Jones et al. 2005; Gentilcore et al. 2008b) and/or slowing of carbohydrate absorption, as a result of inhibition of carbohydrate digestion (Jones et al. 2001; Russo et al. 2003; O'Donovan et al. 2005a) and a reduced rate of delivery of carbohydrate to the small intestine (Jones et al. 1998; O'Donovan et al. 2002). To allow discrimination between these potential mechanisms it would be necessary to infuse carbohydrate directly into the small intestine with and without acarbose, thereby excluding any 'gastric' effect. A substantial effect of acarbose in these circumstances would suggest that inhibition of carbohydrate absorption represents the dominant mechanism.

Following a meal, there is a considerable increase in splanchnic blood volume with an approximate doubling of superior mesenteric artery (SMA) blood flow (Jansen and Lipsitz 1995). The magnitude of the postprandial increase in mesenteric blood flow is comparable in healthy young and older individuals, however, the latter experience a fall in blood pressure, indicative of inadequate cardiovascular adjustment (Lipsitz et al. 1993; Sidery et al. 1993). Somewhat surprisingly the relationship between the postprandial fall in blood pressure with splanchnic blood flow has not been assessed. The beneficial effects of acarbose on the hypotensive response to carbohydrate may reflect changes in the splanchnic

blood flow response to enteral glucose. This possibility has also hitherto not been evaluated.

The aims of this study were to determine in healthy older subjects the effects of intraduodenal acarbose on the blood pressure, heart rate, SMA blood flow, blood glucose and serum insulin responses to an intraduodenal sucrose infusion and to evaluate the relationship between changes in blood pressure and SMA blood flow. The broad hypotheses were that the magnitude of the fall in blood pressure and rise in SMA blood flow induced by intraduodenal sucrose would be related directly and acarbose would attenuate the fall in blood pressure and rise in heart rate induced by intraduodenal sucrose by slowing small intestinal carbohydrate absorption and, thereby, also attenuating the rise in SMA blood flow.

10.3 Research design and methods

10.3.1 Subjects

Eight healthy older subjects, (four male and four female) with a median age of 70 years (range: 66 – 77 years) and body mass index (BMI) of 24.6 kg/m² (range: 20.1 - 28.7 kg/m²), recruited by advertisement, were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, or were taking medication known to influence blood pressure or gastrointestinal function.

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

10.3.2 Experimental protocol

Each subject was studied on two occasions, separated by 3 - 21 days. On each day, subjects attended the University of Adelaide, Discipline of Medicine at the Royal Adelaide Hospital at 0830h following an overnight fast (10.5h for solids; 8.5h for liquids) (Gentilcore et al. 2006b; Gentilcore et al. 2008a). At that time, a silicone-rubber catheter (external diameter ~ 4 mm) (Dentsleeve International Ltd, Mui Scientific, Mississauga, Canada), was introduced into the stomach via an anaesthetised nostril (Gentilcore et al. 2006b; Gentilcore et al. 2008a). The assembly included an infusion channel (internal diameter ~ 1 mm) and was positioned so that the infusion port was located ~ 10 cm distal to the pylorus (i.e. in the duodenum), as well as two other channels that were positioned in the antrum (2.5 cm proximal to the pylorus) and duodenum (2.5 cm distal to the pylorus), respectively, and were perfused with 0.9 % saline. The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference (TMPD) from the antral (- 40 mV) and duodenal (0 mV) channels (Heddle et al. 1989; Gentilcore et al. 2006b; Gentilcore et al. 2008a). For this purpose, a cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (Heddle et al. 1989; Gentilcore et al. 2006b; Gentilcore et al. 2008a). The tip of the catheter

passed into the duodenum by peristalsis, which took between 10 and 180 minutes. Once intubated, the subject rested in the recumbent position. An automated blood pressure cuff (23 - 33 cm Adult Sensa-cuf®, Critikon BP Cuffs, GE Medical Systems, Milwaukee, WI, USA) was placed around the right arm, which was positioned, by the side of the subject (Gentilcore et al. 2006b; Gentilcore et al. 2008a). Approximately 30 minutes after the tube was positioned correctly (i.e. at $t = 0$ min) an intraduodenal infusion of 100 g sucrose dissolved in saline (0.9 %) in a total volume of 300 ml was commenced and maintained at a rate of 5 ml/min for 60 minutes (i.e. ~ 6 kcal/min). On one of the two days, 100 mg acarbose (Bayer Australia Ltd, Pymble, Australia) was added to the intraduodenal infusion, in randomised, double-blind order. Intraduodenal infusions were performed using a volumetric infusion pump (Gemini PC-1; IMED Corp, San Diego, CA, USA). Blood pressure (systolic and diastolic), heart rate and SMA blood flow were measured for 60 minutes. At $t = 60$ min the catheter was removed. On one day, cardiovascular autonomic nerve function was evaluated immediately after the completion of the study (Ewing and Clarke 1982; Piha 1991). On both study days subjects were given a light meal prior to leaving the laboratory.

10.3.3 *Measurements*

10.3.3.1 *Blood pressure and heart rate*

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at $t = -9, -6$ and -3 min prior to commencement of the intraduodenal infusions and, subsequently, every three

minutes between $t = 0 - 60$ min (Gentilcore et al. 2005a; Gentilcore et al. 2006b; Gentilcore et al. 2008a). 'Baseline' blood pressure and heart rate, i.e. $t = 0$ min, were calculated as the mean of measurements taken at $t = -9, -6$ and -3 min prior to the commencement of the intraduodenal infusion. Postprandial hypotension was defined as a fall in systolic blood pressure of ≥ 20 mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

10.3.3.2 Splanchnic blood flow

SMA blood flow was measured by Duplex ultrasonography (i.e. B-mode and Doppler imaging) using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, Australia), as described previously (Perko 2001; Gentilcore et al. 2008a). Each subject was scanned using a 3.5 C broad spectrum 2.5 - 4 MHz convex transducer (Perko 2001; Gentilcore et al. 2008a) at $t = -2$ min and then at 15-minute intervals between $t = 0 - 60$ min. Blood flow (ml/min) was calculated immediately using the formula: $\pi \times r^2 \times \text{TAMV} \times 60$, where r = the radius of the SMA and TAMV is the time-averaged mean velocity (Perko 2001).

10.3.3.3 Blood glucose and serum insulin concentrations

Venous blood samples (~ 7.5 ml) were obtained prior to the commencement of the intraduodenal infusion (i.e. $t = -2$ min) and at 15-minute intervals between $t = 0 - 60$ min (Gentilcore et al. 2005a; Gentilcore et al. 2006b). Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Precision Q·I·D™ System, Abbott Laboratories, Medisense Products Inc, Bedford, MA, USA) (Gentilcore et al. 2005a; Gentilcore et al. 2006b). Blood

samples for serum insulin were collected in ice-chilled serum tubes with clotting activator and stored at -70°C for subsequent analysis. Insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc, Webster, TX, USA) (Gentilcore et al. 2005a). Sensitivity was 0.26 mU/L, intraassay coefficient of variation was 2.6 % and interassay coefficient of variation was 6.2 % (O'Donovan et al. 2005a).

10.3.4 Assessment of cardiovascular autonomic nerve function

Autonomic nerve function was assessed using standardised cardiovascular reflex tests (Ewing and Clarke 1982; Piha 1991). Parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during deep breathing and the heart rate response to standing ("30 : 15" ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal for a total maximum score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

10.3.5 Statistical analysis

Data were evaluated using repeated measures two-way Analysis of Variance (ANOVA), with 'treatment' and 'time' as within subject factors. Systolic and diastolic blood pressure and heart rate were analysed as changes from baseline. SMA blood flow and blood glucose and serum insulin concentrations were analysed as absolute values. Data were analysed from $t = 0 - 60$ min (systolic and

diastolic blood pressure and heart rate) and $t = -2 - 60$ min (SMA blood flow, blood glucose and serum insulin concentrations) to determine the effects ('treatment' and 'time') of sucrose and acarbose. One-way ANOVA was used to analyse the effects of 'time' on systolic and diastolic blood pressure, heart rate, SMA blood flow, blood glucose and serum insulin concentrations. In all analyses, post-hoc comparisons of adjusted means were performed using Student's t-tests. The maximum falls in systolic and diastolic blood pressure and rises in heart rate, blood glucose and serum insulin concentrations were defined as the greatest mean changes from baseline in each subject at any given time point for each treatment. Relationships between variables were assessed using linear regression analysis. Systolic blood pressure in the first 60 minutes after intraduodenal sucrose, without acarbose, were compared to those observed in the first 60 minutes after oral sucrose without acarbose (Gentilcore et al. 2005a). We have reported the effects of acarbose on the hypotensive response to 300 ml water containing 100 g sucrose in eight healthy older subjects (five male and three female with a median age of 71 years, range 65 - 79 years) (Gentilcore et al. 2005a). Data in this group were compared to those obtained in the current study. All analyses were performed using Statview (version 5.0; Abacus Concepts, Berkeley, CA, USA) and SuperANOVA (version 1.11, Abacus Concepts, Berkeley, CA, USA). Data are presented as mean values \pm standard error of the mean (SEM). A P value < 0.05 was considered significant in all analyses.

10.4 Results

The studies were well tolerated. Mild looseness of stools and/or flatulence were reported by all subjects during (two subjects between $t = 30 - 53$ min) and after (eight subjects from $t = 60$ min) acarbose infusion. In all cases the symptoms had resolved within 11 hours. No subjects volunteered gastrointestinal symptoms during the control infusion. No subject had definite autonomic neuropathy; median score 0.6 (range: 0 - 2). No subject had experienced postprandial hypotension on the control day, or after acarbose.

10.4.1 *Systolic and diastolic blood pressure and heart rate (figure 10.1 a, b & c)*

There was no significant difference in baseline (i.e. $t = 0$ min) blood pressure or heart rate between the study days (control vs acarbose): systolic blood pressure (124 ± 6 mmHg vs 126 ± 8 mmHg; $P = 0.59$); diastolic blood pressure (70 ± 3 mmHg vs 72 ± 3 mmHg; $P = 0.39$) and heart rate (58 ± 3 bpm vs 57 ± 3 bpm; $P = 0.42$).

Between $t = 0 - 60$ min there was a fall in systolic blood pressure after control ($P < 0.0003$), but not after acarbose ($P = 0.35$). Systolic blood pressure fell promptly during control and the maximum decrease was 11 ± 2 mmHg. There was a significant 'treatment' effect for systolic blood pressure ($P < 0.01$) between the study days. At $t = 60$ min, there was no significant difference in systolic blood pressure from baseline after control ($P = 0.39$) or acarbose ($P = 0.49$).

When compared to our previous study (Gentilcore et al. 2005a) in the first 60 minutes, systolic blood pressure was lower ($P < 0.0001$) after intraduodenal than oral sucrose without acarbose between $t = 3 - 42$ min and at $t = 57 - 60$ min ($P < 0.01$), and the onset of the fall was earlier.

Between $t = 0 - 60$ min there was a fall in diastolic blood pressure during both control ($P < 0.0001$) and acarbose ($P < 0.03$). The maximum fall in diastolic blood pressure was greater ($P = 0.05$) on the control day (11 ± 1 mmHg) compared with after acarbose (8 ± 2 mmHg). There was a significant 'treatment x time' interaction for diastolic blood pressure ($P < 0.0001$) between the study days. Between $t = 18 - 27$ min and $t = 36 - 60$ min diastolic blood pressure was less ($P < 0.01$) on the control day when compared with acarbose. At $t = 60$ min, diastolic blood pressure was lower than baseline after control ($P = 0.01$) but not significantly different from baseline after acarbose ($P = 0.46$).

Between $t = 0 - 60$ min there was a rise in heart rate during both control ($P < 0.0001$) and acarbose ($P < 0.0001$). The maximum increase in heart rate was greater ($P = 0.002$) on the control day (19 ± 2 bpm) compared with after acarbose (10 ± 2 bpm). There was a significant 'treatment x time' interaction for heart rate ($P < 0.0001$) between the study days. Between $t = 15 - 60$ min heart rate was greater ($P < 0.05$) on the control day compared with acarbose. At $t = 60$ min, heart rate was greater than baseline after control ($P = 0.0002$) and acarbose ($P = 0.0008$).

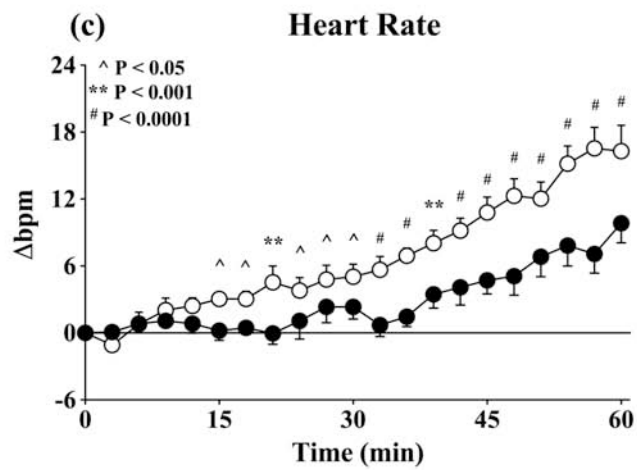
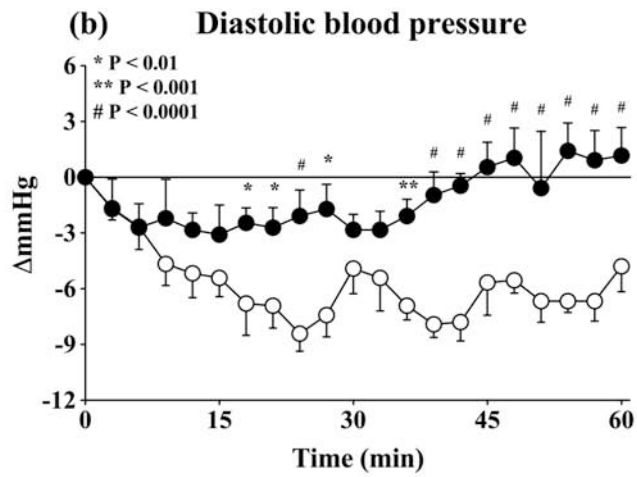
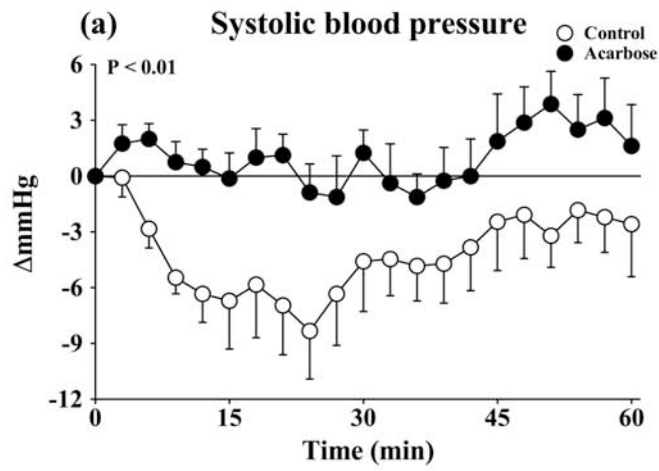


Figure 10.1: Changes in (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate from baseline in response to intraduodenal infusion of control (○) and acarbose (100 mg) (●) (n = 8). Data are mean values ± SEM. Systolic blood pressure ‘treatment x time’ interaction: P < 0.01; diastolic ‘treatment x time’ interaction * P < 0.01, ** P < 0.001, # P < 0.0001; heart rate ‘treatment x time’ interaction ^ P < 0.05, ** P < 0.001, # P < 0.0001.

10.4.2 SMA blood flow (Figure 10.2)

There was no difference in baseline (i.e. $t = -2$ min) SMA blood flow between the two days (control vs acarbose: 886 ± 72 ml/min vs 898 ± 88 ml/min; $P = 0.88$). Between $t = -2 - 60$ min there was a rise in SMA blood flow during both control ($P < 0.0001$) and acarbose ($P < 0.0006$), which was evident from $t = 15$ min during both control ($P = 0.0009$) and acarbose ($P < 0.05$). Peak SMA blood flow was 3000 ± 383 ml/min at 43 ± 5 min and 1782 ± 225 ml/min at 56 ± 3 min ($P = 0.001$) for control and acarbose days, respectively. There was a significant 'treatment x time' interaction ($P < 0.0001$) for SMA blood flow. Between $t = 15 - 60$ min, SMA blood flow was greater ($P < 0.05$) on the control day compared with acarbose. At $t = 60$ min, SMA blood flow was greater than baseline after both control ($P = 0.001$) and acarbose ($P = 0.005$).

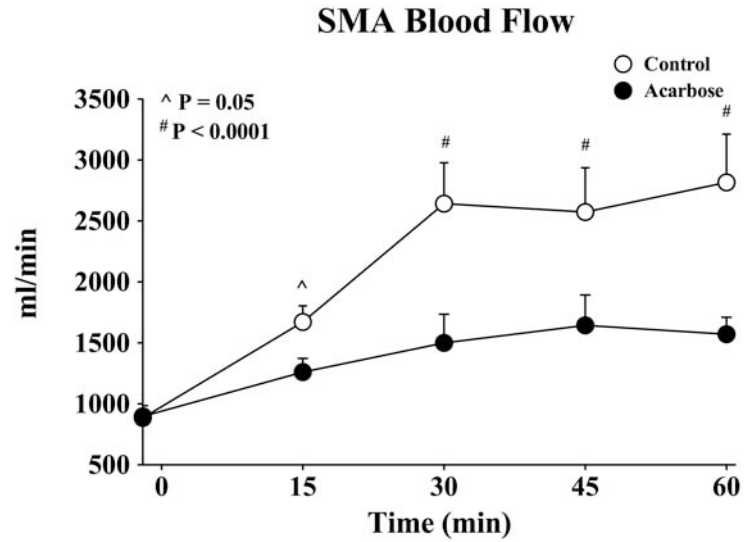


Figure 10.2: Superior mesenteric artery (SMA) blood flow in response to intraduodenal infusion of control (○) and acarbose (100 mg) (●) (n = 8). Data are mean values ± SEM. ‘Treatment x time’ interaction [^] P < 0.05, # P < 0.0001.

10.4.3 Blood glucose and serum insulin (Figures 10.3a & b)

There was no difference in baseline (i.e. $t = -2$ min) blood glucose, or serum insulin between the two days (control vs acarbose): blood glucose (5.9 ± 0.2 mmol/L vs 5.8 ± 0.2 mmol/L; $P = 0.67$) and serum insulin (9 ± 0.9 mU/L vs 10 ± 1.3 mU/L; $P = 0.29$).

Between $t = -2 - 60$ min there was a rise in blood glucose from baseline during control ($P < 0.0001$) and acarbose ($P < 0.0009$), which was significant from $t = 15$ min during control ($P = 0.0007$), and from $t = 30$ min during acarbose ($P = 0.001$). Peak blood glucose were 10.5 ± 0.7 mmol/L at 49 ± 5 min and 7.0 ± 0.2 mmol/L at 45 ± 5 min ($P = 0.0004$) for control and acarbose days, respectively. There was a significant 'treatment x time' interaction ($P < 0.0001$) for blood glucose concentrations. Between $t = 15 - 60$ min blood glucose were much greater ($P < 0.01$) on the control day compared with acarbose. At $t = 60$ min, blood glucose were greater than baseline after both control ($P = 0.0004$) and acarbose ($P = 0.009$).

Between $t = -2 - 60$ min there was a rise in serum insulin from baseline during control and acarbose ($P < 0.0001$ for both) which was significant from $t = 15$ min during control ($P = 0.0004$), and from $t = 30$ min during acarbose ($P = 0.01$). Peak serum insulin were 130 ± 2.8 mU/L at 60 ± 0 min and 31 ± 5.3 mU/L at 53 ± 4 min ($P = 0.0001$) for control and acarbose days, respectively. There was a significant 'treatment x time' interaction ($P < 0.0001$) for serum insulin. Between $t = 15 - 60$ min, serum insulin were much greater ($P < 0.05$) on the control day

compared with acarbose. At $t = 60$ min, serum insulin were greater than baseline after both control ($P = 0.0001$) and acarbose ($P = 0.01$).

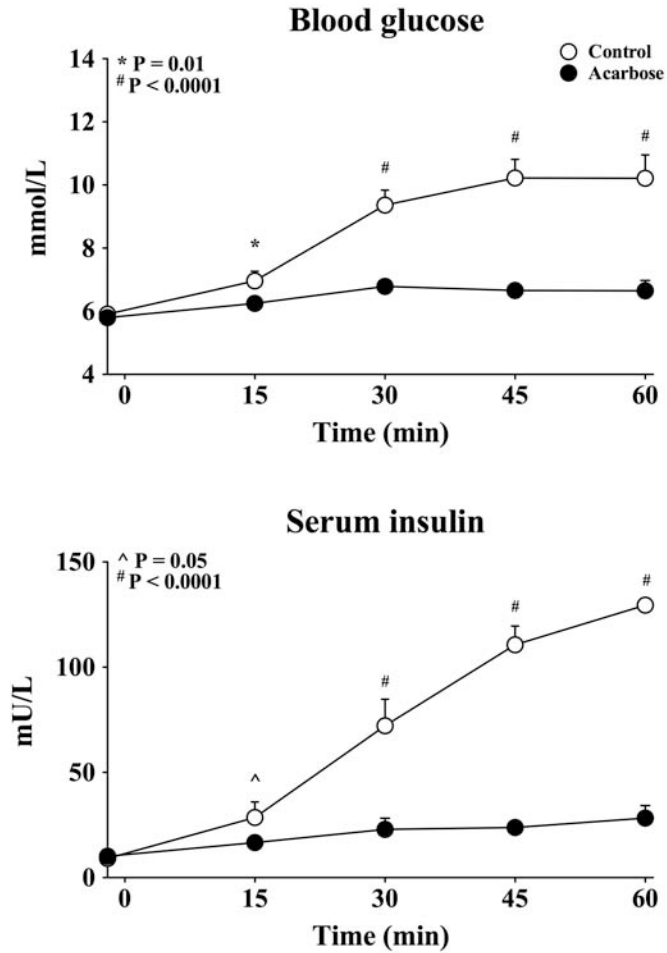


Figure 10.3: (a) Blood glucose and (b) serum insulin concentrations in response to intraduodenal infusion of control (○) and acarbose (100 mg) (●) (n = 8). Data are mean values ± SEM. Blood glucose ‘treatment x time’ interaction * P = 0.01, # P < 0.0001; serum insulin ‘treatment x time’ interaction ^ P < 0.05, # P < 0.0001.

**10.4.4 Relationships between systolic blood pressure SMA blood flow
(figure 10.4 a & b)**

There was a significant relationship between the maximum fall in systolic blood pressure and peak SMA blood flow ($r = -0.73$, $P < 0.04$) on the control day, but not with acarbose ($r = 0.17$, $P = 0.70$).

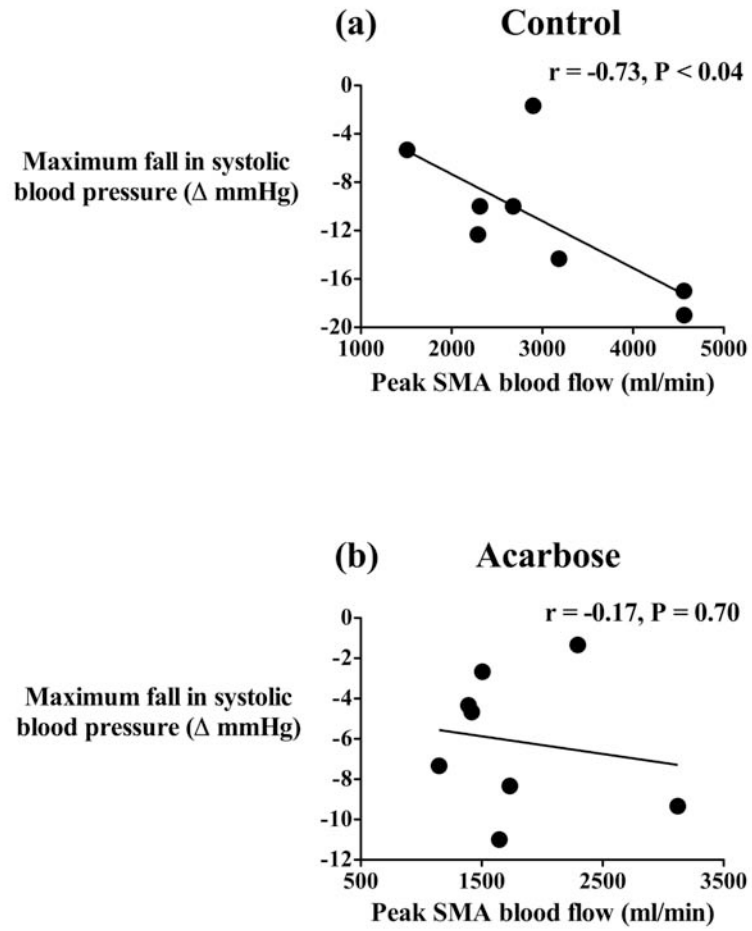


Figure 10.4: Relationship between the maximum fall in systolic blood pressure and peak superior mesenteric artery (SMA) blood flow for (a) control and (b) acarbose (100 mg) (n = 8).

10.5 Discussion

Current management of postprandial hypotension is less than optimal. Whilst strategies involving the use of somatostatin analogues, such as octreotide (Hoeldtke et al. 1986a; Jansen et al. 1989b), caffeine (Onrot et al. 1985; Heseltine et al. 1991b), modifications to medications (Jansen and Lipsitz 1995; Freeman et al. 1996) and various dietary permutations (Waalder et al. 1991; Puvi-Rajasingham and Mathias 1996) have been reported to attenuate postprandial falls in blood pressure, these are all associated with undesirable side effects and/or inconsistent efficacy. Recent observations indicate that slowing the rate of small intestinal nutrient delivery and absorption may represent a novel, and potentially effective, approach to the treatment of postprandial hypotension (Jones et al. 1998; Jones et al. 2001; O'Donovan et al. 2002; Russo et al. 2003; O'Donovan et al. 2005a). Consistent with this concept, oral ingestion of acarbose, which slows gastric emptying (Ranganath et al. 1998; Enc et al. 2001; Gentilcore et al. 2005a) and delays small intestinal absorption of carbohydrate (Chiasson et al. 2002), markedly attenuates the fall in postprandial blood pressure in healthy older subjects (Gentilcore et al. 2005a) and patients with postprandial hypotension (Sasaki et al. 2001; Maule et al. 2004; Yamamoto et al. 2006; Shibao et al. 2007; Jian and Zhou 2008).

In the current study, sucrose and acarbose were infused directly into the small intestine, so that the potential 'gastric' effect of acarbose was eliminated, and acarbose attenuates the hypotensive (systolic and diastolic) response to intraduodenal sucrose. This effect was major - following acarbose, systolic blood

pressure did not decrease. This is despite the fact that sucrose was infused at a rate modestly greater than the normal range of gastric emptying of glucose i.e. 1 – 4 kcal/min (Brenner et al. 1983). In considering the potential mechanisms underlying the effect of acarbose on blood pressure, the concomitant changes in SMA blood flow are of considerable interest. On the control study, there was an inverse relationship between the hypotensive response and the rise in SMA blood flow. While the demonstration of a relationship does not establish a causal association i.e. that the fall in systemic blood pressure represents a response to the increase in splanchnic blood flow, further studies are required. This is to our knowledge the first time that changes in another ‘cardiovascular’ parameter have been shown to account for the postprandial fall in blood pressure. With acarbose, there was virtually no change in SMA blood flow, which is consistent with the concept that increases in splanchnic blood flow are of central importance. The effect of acarbose on SMA blood flow may potentially reflect the delay in carbohydrate absorption and attenuation of the serum insulin response. However, while insulin has vasodilatory properties (Jansen and Hoefnagels 1991), both glucose and insulin are unlikely to play a major role in postprandial hypotension, given that intravenous glucose has little, if any, effect on blood pressure (Jansen and Hoefnagels 1987; Jansen and Hoefnagels 1991) and postprandial hypotension occurs in type 1 patients who are insulin-deficient (Jansen and Hoefnagels 1990). An alternative, and not mutually exclusive possibility is that the substantial stimulation of gut peptides by acarbose, particularly GLP-1 and glucagon-like peptide-2 (GLP-2) (Bremholm et al. 2009) is important, but this remains to be determined. That differences in SMA blood flow were evident within 15 minutes

argues against a role for GLP-1 or GLP-2 given that their initial secretion after nutrient ingestion is modest (Herrmann et al. 1995).

In considering our observations, it should be recognised that by infusing acarbose intraduodenally with sucrose, the inhibition of carbohydrate digestion was maximised - this is attested to by the minimal glycaemic and insulinaemic responses and high prevalence of, albeit mild, adverse effects. While the dose of acarbose (100 mg) is used therapeutically, oral acarbose would not be expected to be as effective and this warrants evaluation. As discussed, the rate of sucrose infusion (~ 6 kcal/min) was supraphysiological (Brener et al. 1983), and it would be of interest to evaluate the effects of other sucrose loads. We have shown that the hypotensive response to small intestinal carbohydrate is related to the load, and not its concentration (Gentilcore et al. 2006b). In our previous study using oral acarbose, the mean rate of gastric emptying of sucrose (100 g) on placebo was 2.1 ± 0.2 kcal/min in healthy older subjects (Gentilcore et al. 2005a). It is accordingly predictable, given that the protective effects of gastric distension were 'bypassed' and the rate of duodenal sucrose delivery was greater, that the onset of the fall in blood pressure was earlier after intraduodenal, than oral, sucrose and the maximum fall was greater. There is, however, evidence that the relationship between the hypotensive response to small intestinal glucose and the glucose load is non-linear i.e. in healthy older subjects, the falls in systolic blood pressure induced by intraduodenal infusions of 2 kcal/min and 3 kcal/min are comparable, while an infusion at the rate of 1 kcal/min has no effect (**Chapter 5**).

In conclusion, our observations establish that, in healthy older subjects, (i) there is a direct relationship between the hypotensive response to small intestinal sucrose and the rise in SMA blood flow and (ii) acarbose markedly attenuates the hypotensive response to intraduodenal sucrose by slowing carbohydrate absorption and thereby attenuating the rise in splanchnic blood flow. The latter observations support the use of acarbose, and presumably other α -glucosidase inhibitors such as voglibose and miglitol, in the management of postprandial hypotension. Studies to evaluate the effects of chronic administration are required.

11

Conclusions

The studies performed in this thesis have provided novel insights into the role of the gastrointestinal tract and small intestinal mechanisms in postprandial blood pressure regulation. Pathophysiological mechanisms underlying postprandial hypotension, an important clinical problem that is a major cause of morbidity and mortality particularly in the elderly, with the focus on gastric and small intestinal mechanisms and their potential therapeutic relevance have been examined in healthy older volunteers.

The study reported in **Chapter 5**, demonstrated that in healthy older subjects, the fall in blood pressure and rise in heart rate, in response to various intraduodenal glucose loads is non-linear. There was a significant fall in systolic and diastolic blood pressure and rise in heart rate following both glucose at 2 kcal/min and 3 kcal/min but no change during saline and glucose at 1 kcal/min. The rise in superior mesenteric artery (SMA) blood flow, blood glucose and serum insulin and serum glucagon-like peptide-1 (GLP-1) was greatest following the 3 kcal/min intraduodenal glucose infusion, when compared to the other glucose infusions. These observations suggest that the rate of gastric emptying or small intestinal nutrient delivery should be slowed to approximately 1 kcal/min to prevent a postprandial fall in blood pressure. However, it is likely that the effects of small intestinal glucose load on postprandial blood pressure would be exacerbated in patients with postprandial hypotension, this issue remains to be formally assessed.

Information relating to the effect of various carbohydrates, in particular, xylose, on blood pressure remains inconsistent, with previous studies showing xylose to have little or no effect (Mathias et al. 1989b; Mathias 1990; Robinson et al. 1992; Robinson and Potter 1995). The effects of oral glucose and xylose on blood pressure, the rate of gastric emptying and the release of incretin hormones, in healthy older subjects were studied in **Chapter 6**. The fall in blood pressure was greater following glucose compared to xylose, while there was no difference in the rate of gastric emptying following glucose and xylose. Xylose had minimal, or no effect on blood glucose, plasma insulin or plasma glucose-dependent insulinotropic polypeptide (GIP), however was more potent than glucose in stimulating GLP-1. These findings suggest that xylose may be considered an alternative sweetener to glucose, for the better management of postprandial hypotension, although, further evaluation is now warranted in patients with postprandial hypotension. It should be recognised that while xylose is palatable, it is relatively expensive, therefore in view of our observations, it would be of interest to evaluate the effects of the related pentose sugar, xylitol, which is considerably cheaper.

Previous studies have established that the magnitude of the postprandial fall in blood pressure is attenuated by gastric distension (Rossi et al. 1998; Shannon et al. 2002; van Orshoven et al. 2004; Gentilcore et al. 2008b), however, it is unknown whether this effect is caused by the change in intragastric pressure (**Chapter 7**) or intragastric volume (**Chapter 8**). In **Chapter 7**, the effects of gastric distension, using a barostat set to 8 mmHg above minimal distending

pressure, on blood pressure, heart rate and SMA blood flow, during intraduodenal glucose in healthy older subjects, were evaluated. The fall in blood pressure following intraduodenal glucose infusion was completely abolished in the presence of gastric distension. Heart rate increased, with and without distension, in response to intraduodenal glucose but not after intraduodenal saline infusion. Furthermore, gastric distension attenuated the rise in SMA blood flow following intraduodenal glucose. Findings from this study support the concept that maximising non-nutrient gastric distension may represent a simple approach to the management of postprandial hypotension, for example, consumption of water prior to a meal.

In **Chapter 8**, the effects of gastric distension, using a barostat at fixed volumes, on blood pressure, heart rate and SMA blood flow, during intraduodenal glucose in healthy older subjects, were evaluated. In the absence of gastric distension, there was a postprandial fall in blood pressure following intraduodenal glucose infusion, and gastric distension at all volumes prevented this fall in blood pressure. Heart rate and blood glucose increased to the same magnitude, with and without distension, in response to intraduodenal glucose. Furthermore, the rise in SMA blood flow was not attenuated during distension. These findings suggest that drinking as little as 100 ml water immediately prior to consumption of a meal can assist in the management of postprandial hypotension. Formal studies to evaluate this hypothesis in patients with postprandial hypotension are required.

In healthy older subjects, the effects of alpha-cyclodextrin on blood pressure, gastric emptying, glycaemia and insulinaemia, in response to oral sucrose, were evaluated in **Chapter 9**. Preliminary data illustrate that alpha-cyclodextrin has a modest effect to decrease glycaemic and insulinaemic responses and slow gastric emptying, but has no effect on the hypotensive response induced by oral sucrose. Further studies evaluating the effects of alpha-cyclodextrin on blood pressure, gastric emptying, glycaemia and insulinaemia, in response to a starch meal, in healthy older subjects or patients with postprandial hypotension are warranted.

The effects of acarbose in healthy older subjects, on postprandial blood pressure, heart rate, SMA blood flow and glycaemia, when administered intraduodenally, were evaluated in **Chapter 10**. Acarbose markedly attenuated the fall in systolic and diastolic blood pressure and rise in heart rate, SMA blood flow, blood glucose and serum insulin, induced by intraduodenal sucrose infusion. These findings suggest that the beneficial effect of acarbose as a therapeutic option for the treatment of patients with postprandial hypotension is not dependent on the rate of nutrient delivery.

The studies presented in this thesis provide fundamental insights into the role of the gastrointestinal tract and small intestine in healthy older subjects. Future studies are warranted in patients with postprandial hypotension, with a focus on these mechanisms.

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Appendix I: Visual Analogue Questionnaire

Visual Analogue Questionnaire

Name (Initials):

Visit:

Time:

Please indicate how you are feeling at this moment by placing a vertical mark at the appropriate point on each horizontal line below. Furthest **LEFT** means you do not feel the sensation. Furthest **RIGHT** means you feel it very much. Please, mark all horizontal lines.

I feel anxious _____

I feel sleepy _____

How strong is your desire to eat?

_____ WEAK STRONG

I feel hungry _____

How much food do you think you could eat?

_____ A small amount A large amount

I feel sick _____

I feel full _____

I feel dizzy _____

I have indigestion _____

My tummy is rumbling _____

I have a headache _____

I feel thirsty _____

If you were given a meal now, would you want to eat it? YES NO

Appendix II: Likert Scale Questionnaire

Name:

Code:

Date: Visit number:

Time:

Please indicate how you are feeling at this moment by placing a **circle** at the appropriate point on each scale below. Please mark all scales.

Nauseated ?

Not at all	Very little	A little	Somewhat	Much	Very much	The most
1	2	3	4	5	6	7

Bloated ?

Not at all	Very little	A little	Somewhat	Much	Very much	The most
1	2	3	4	5	6	7

Full ?

Not at all	Very little	A little	Somewhat	Much	Very much	The most
1	2	3	4	5	6	7

Pressure ?

Not at all	Very little	A little	Somewhat	Much	Very much	The most
1	2	3	4	5	6	7

Discomfort ?

Not at all	Very little	A little	Somewhat	Much	Very much	The most
1	2	3	4	5	6	7

Pain ?

Not at all	Very little	A little	Somewhat	Much	Very much	The most
1	2	3	4	5	6	7