Role of the Gastrointestinal Tract in Postprandial Blood Pressure Regulation
Role of the Gastrointestinal Tract in Postprandial Blood Pressure Regulation

A thesis submitted by
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Summary
This thesis presents studies relating to the role of the gastrointestinal tract in postprandial blood pressure regulation. The areas that have been addressed include: (i) the methodological approaches to the evaluation of gastric emptying, blood pressure, splanchnic blood flow, intraluminal manometry and gut hormones and (ii) the pathophysiological mechanisms underlying postprandial hypotension, with a particular focus on ‘gastric’ and ‘small intestinal’ mechanisms and their potential therapeutic relevance. All of the studies have been either published or manuscripts have been prepared for publication.

While scintigraphy represents the ‘gold standard’ for the measurement of gastric emptying, recent studies suggest that three-dimensional (3D) ultrasonography may also allow a precise measure of gastric emptying. Concurrent scintigraphic and ultrasonographic measurements of gastric emptying of liquids were performed in healthy young volunteers. There was a good correlation and agreement between scintigraphic measurements of gastric emptying and 3D ultrasonography after ingestion of both low- and high- nutrient drinks, indicating that 3D ultrasonography, provides a valid measure of gastric emptying of liquid meals in normal subjects.

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20mmHg, occurring within two hours of a meal is now recognised as an important clinical problem, particularly in the elderly and in patients with type 2 diabetes. The mechanisms mediating postprandial hypotension are poorly understood. The effects of variations in concentration of intraduodenal glucose on the magnitude of the fall in
blood pressure were evaluated in healthy elderly subjects. Blood pressure fell, and heart rate and blood glucose increased over time during infusions, however, there was no difference in blood pressure, heart rate or blood glucose concentrations between the study days. These observations suggest that glucose induced postprandial hypotension is a load rather, than concentration, dependent phenomenon.

The effect of meal composition has been reported to influence the hypotensive response to a meal and information relating to the effects of triglyceride and protein on blood pressure is inconsistent. The comparative effects of isocaloric and isovolaemic intraduodenal infusions of glucose, triglyceride and protein on the magnitude of the postprandial fall in blood pressure and rise in heart rate and superior mesenteric artery blood flow were evaluated in healthy elderly subjects. There were comparable falls in systolic blood pressure and rises in heart rate, however, the maximum fall in systolic blood pressure occurred later after triglyceride and protein and the stimulation of superior mesenteric artery blood flow was less after protein. These observations suggest that the relatively slower systolic blood pressure response after triglyceride and protein may potentially reflect the time taken for digestion of triglyceride to free fatty acids and protein to amino acids.

Acarbose is an antidiabetic drug that slows both gastric emptying and small intestinal glucose absorption. The effects of acarbose, on blood pressure, heart rate, gastric emptying of, and the glycaemic, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic-polypeptide (GIP) responses to, an oral sucrose load were
evaluated in healthy elderly subjects. Acarbose attenuated the fall in blood pressure and increase in heart rate induced by oral sucrose. Acarbose slowed gastric emptying and was associated with increased retention in the distal stomach. Stimulation of GLP-1 may contribute to the slowing of gastric emptying and suppression of postprandial glycaemia by acarbose. These findings suggest that acarbose may represent a therapeutic option for the treatment of patients with postprandial hypotension.

Recent studies indicate that gastric distension attenuates the postprandial fall in blood pressure. The effects of gastric distension on blood pressure and heart rate during intraduodenal infusion of glucose at a constant load and concentration were evaluated in healthy elderly subjects. Intragastric administration of water markedly attenuated the falls in systolic and diastolic blood pressure induced by intraduodenal glucose. Heart rate increased, with and without gastric distension, in response to intraduodenal glucose infusion but not after intraduodenal saline infusion. This study suggests that gastric distension may potentially be used as a simple adjunctive treatment in the management of postprandial hypotension.

Studies employing nitric oxide synthase blockers have established, in animals, that nitric oxide mechanisms are important in the regulation of splanchnic blood flow and, hence, may effect postprandial blood pressure. The role of the nitric oxide synthase inhibitor, NG-nitro-L-arginine-methyl-ester (L-NAME), on gastric emptying, postprandial blood pressure, plasma insulin concentration and incretin hormone (ie GIP and GLP-1) release, following an oral glucose load, were evaluated in healthy elderly
subjects. L-NAME attenuated the postprandial fall in blood pressure and increase in heart rate but had no effect on gastric emptying of glucose. L-NAME attenuated the glucose-induced rise in plasma insulin but had no effect on the incretin (GIP and GLP-1) hormone response to oral glucose. The study indicates that the magnitude of the fall in blood pressure and increase in heart rate and stimulation of insulin secretion induced by oral glucose in healthy elderly subjects are mediated by nitric oxide mechanisms by an effect unrelated to changes in gastric emptying, or the secretion of GIP and GLP-1.

Studies utilising 5-hydroxytryptamine (5-HT) infusions in animals have demonstrated regional variations in intestinal blood flow suggesting a role for 5-HT in postprandial haemodynamic responses. The effects of the 5-hydroxytryptamine 3 (5-HT3) antagonist, granisetron, on the blood pressure, heart rate, antropyloroduodenal motility and glycaemic responses to intraduodenal glucose infusion were assessed in healthy elderly subjects. Granisetron had no effect on blood pressure, heart rate or antral and pyloric motor responses but modulated the duodenal motor response, to intraduodenal glucose. This study indicates that while the cardiovascular response to intraduodenal glucose does not appear to be influenced by the stimulation of 5-HT3 receptors, this receptor may be involved in the modulation of the duodenal motor activity.
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 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.

I give consent to this copy of my thesis being made available in the University Library.

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______________________________
Diana Gentilcore

December 2006
Dedication
For my husband Laurie

Your courage is inspirational.
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Publications arising from this thesis


Other publications


Pathophysiology of postprandial hypotension
1.1 Introduction

Postprandial hypotension, which has been 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 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 minutes, after a meal (Jansen and Lipsitz 1995), characteristically with a nadir at 30 - 60 minutes (Aronow and Ahn 1994). In healthy young and elderly individuals, meal ingestion is associated with a prompt rise in heart rate, indicative of normal baroreflex function which serves 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 disturbance and cerebrovascular accidents (Lipsitz et al 1983, Vaitkevicius et al 1991, Jansen and Lipsitz 1995, Aronow and Ahn 1997, Morley 2001, Ahsan Ejaz et al 2006). Current approaches to management 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 ie 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 2005b), while gastric

In this chapter current knowledge of the pathophysiology of postprandial hypotension, with a particular focus on gastric and small intestinal mechanisms and their potential therapeutic relevance 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 represents a substantial cause of both morbidity and mortality (Jansen and Lipsitz 1995, 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 (although in all studies it is high) 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. Jansen and Hoefnagels (1991) have proposed the use of a drink comprising 75g glucose in 300ml water in studies of the epidemiology/pathophysiology of postprandial hypotension (Jansen and Hoefnagels 1991); this has been shown to induce a fall in blood pressure comparable in magnitude to that observed in response to a meal (Jansen et al 1987, Jansen and Hoefnagels 1991).
1.2.1 Prevalence of postprandial hypotension in the healthy elderly

A significant, meal-induced, fall in blood pressure occurs frequently in healthy elderly persons; an initial study of 21 healthy, community-dwelling, elderly subjects reported a mean postprandial reduction in systolic blood pressure of ~ 11 mmHg after a standardised lunch (Lipsitz and Fullerton 1986). Subsequently, it has been reported, in cohorts of 499 (Aronow and Ahn 1994) and 113 (Vaitkevicius et al 1991) subjects, that ~ 24 - 36% of elderly nursing home residents experience a reduction in systolic blood pressure of ≥ 20 mmHg after a standardised carbohydrate meal. In a large study of community-dwelling elderly adults (n = 5888), aged 65 years or greater, systolic blood pressure was significantly less in the first hour (~ 130 mmHg) after a non-standardised meal when compared with 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 consecutive frail geriatric patients admitted to hospital, 57 (67%) experienced a significant fall in postprandial systolic blood pressure of ~ 34 mmHg after a standardised liquid meal (Vloet et al 2005), and in 150 long-term care elderly patients, who were fed either orally, via a nasogastric tube, or using percutaneous endoscopic gastrostomy, 64 (43%) had a decrease in systolic blood pressure of ≥ 20 mmHg after a high carbohydrate meal (Lubart et al 2006).

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 an early study, 20% of 35 patients with long-standing type 2
diabetes, exhibited a fall in systolic blood pressure of > 20 mmHg after a 75g oral glucose load (Sasaki et al 1992). Similar findings were demonstrated in a study of recently diagnosed type 2 diabetics managed by diet alone in which a decrease in mean arterial blood pressure of > 20 mmHg was evident in 7 of 16 subjects (44%) after a 75g glucose drink (Jones et al 1998). While autonomic neuropathy may contribute to the hypotensive response to a meal in type 2 diabetes, postprandial hypotension has been reported in patients without evidence of cardiovascular autonomic dysfunction on formal testing (Jones et al 1998).

1.2.3  Prevalence of postprandial hypotension in patients with other illnesses

Some 82% of patients with Parkinson’s disease experience a significant postprandial fall in blood pressure (Mehagnoul-Schipper et al 2001). In a recent retrospective study of 13 patients with Parkinson’s disease, all had postprandial hypotension on 24 hour ambulatory blood pressure monitoring (Ahsan Ejaz et al 2006). In a study of 10 unselected patients with Alzheimer's disease, a fall in blood pressure of ≥ 20 mmHg after a standard meal was evident in 7 (70%) (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

A strong association between falls and syncope in elderly subjects with postprandial hypotension has been established (Vaitkevicius et al 1991, Aronow and Ahn 1994, Le
Couteur et al 2003) - in 23 - 50% of elderly patients with a history of falls or syncope there is a significant meal-related fall in systolic blood pressure, perhaps particularly in those with hypertension (Jansen et al 1995, Puisieux et al 2000). Postprandial hypotension appears to be an independent predictor of mortality in elderly low-level-care nursing home residents (Fisher et al 2005) - in a group of 179 subjects followed for a period of 4.7 years, the mortality rate in patients with postprandial hypotension was 145.0 per 1000 person-years compared with 98.5 per 1000 person-years in those without postprandial hypotension (Fisher et al 2005). Moreover, 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 ≥ 40 mmHg the mortality rates per 1000 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 currently lacking.

1.3 Factors influencing postprandial blood pressure

Although the aetiology of postprandial hypotension is poorly defined, 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

1.3.1  
**Meal composition**

Of the macronutrients, carbohydrate, particularly in the form of glucose, appears 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 of 10 unselected hypertensive patients aged 70 years or more, there was a reduction in mean arterial blood pressure of ~ 15 mmHg after a glucose drink (75g in 300ml water) (Jansen et al 1990). In contrast, after ingestion of triglyceride (215g cream in 300ml water), protein (75g whey in 300ml water) and water (300ml), blood pressure remained essentially unchanged. The effects of protein (Potter et al 1989, Jansen et al 1990) and triglyceride (Hoeldtke et al 1985, Potter et al 1989, Jansen et al 1990, Waaler and Eriksen 1992, Sidery et al 1993) on blood pressure are controversial and previous observations are inconsistent. As discussed, in 10 elderly hypertensive patients, ingestion of protein had no apparent effect on blood pressure (Jansen et al 1990), however, in another study of 10 healthy elderly subjects, consumption of a high protein mixed meal (53%, chicken) resulted in a significant fall (mean ~ 9 mmHg) in systolic blood pressure (Potter et al 1989). It has recently been reported that ingestion of a high triglyceride drink resulted in a comparable, although delayed, substantial fall (mean ~ 17 mmHg) in systolic blood pressure and rise in heart rate, when compared with an isocaloric glucose (75g in 300ml water) drink in healthy elderly subjects (Visvanathan et al 2006). However, it is unknown whether the observed effects of triglyceride on blood pressure reflect its
slower rate of entry into the small intestine. The effects of intraduodenal, as opposed to intragastric, administration of triglyceride and protein on the postprandial blood pressure response in healthy elderly subjects have been evaluated in the study reported in Chapter 7. The slowing of gastric emptying, stimulation of gastrointestinal hormone release and suppression of energy intake by oral triglyceride are known to be mediated by fatty acids, rather than triglycerides (Feinle et al 2001). It would, accordingly, not be surprising if the hypotensive response to triglyceride is also dependent on lipolysis and this could account for the relative latency in the response. Simple sugars such as fructose (Jansen et al 1987) and xylose (Mathias et al 1989b, Robinson and Potter 1995) appear to have a substantially less effect than glucose on blood pressure. However, ingestion of equal amounts of glucose and sucrose (50g in 300ml water) has been reported to result in comparable decreases in blood pressure, in healthy elderly subjects, with the fall occurring earlier after glucose than 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). In contrast, the glycaemic index ie the magnitude of the increase in blood glucose induced by specific carbohydrates, per se, appears to have little effect on the hypotensive response (Visvanathan et al 2004).

1.3.2 Meal volume

The magnitude of the postprandial fall in systolic blood pressure appears to be greater with increased meal carbohydrate content (Puvi-Rajasingham and Mathias 1996, Staneczek et al 2001, Vloet et al 2001). In 12 elderly subjects with known postprandial
hypotension, both the magnitude and duration of the fall in systolic blood pressure after 200ml liquid drinks were progressively greater with increased carbohydrate content (Vloet et al. 2001); the mean maximum falls in systolic blood pressure were ~ 28 mmHg after 25g, ~ 39 mmHg after 65g and ~ 40 mmHg after 125g, carbohydrate (Vloet et al. 2001). It should be recognised that the design of this study precluded discrimination of the potential effects of meal carbohydrate concentration from those of the carbohydrate load. In another study of 7 subjects with primary chronic autonomic failure, ambulatory blood pressure was measured in the lying, sitting and standing positions 30 minutes after the consumption of either 3 ‘large’ or 6 ‘small’ mixed meals; total energy uptake per day was identical (Puvi-Rajasingham and Mathias 1996). Mean systolic blood pressure was less after the ‘large’, when compared with the ‘small’, meals in the lying (~ 131 vs ~ 151 mmHg), sitting (~ 109 vs ~ 124 mmHg) and standing (~ 89 vs ~ 103 mmHg) positions, suggesting that the fall in blood pressure is greater with increased carbohydrate intake at a given meal (Puvi-Rajasingham and Mathias 1996). The use of a mixed meal in this study, however, precludes discrimination of the potential hypotensive effect of carbohydrate from those of other meal constituents. Moreover, as will be discussed, it is difficult to conclude whether these observations are attributable to differences in the rate of, or duration of, gastric emptying; it is known that in healthy subjects after ingestion of mixed meals of varying sizes the rate of nutrient delivery to the duodenum (ie kcal/min) is relatively constant (Puvi-Rajasingham and Mathias 1996).
1.3.3 Meal temperature

Meal temperature may influence postprandial blood pressure (Kuipers et al. 1991). In healthy elderly subjects (Kuipers et al. 1991), mean arterial blood pressure fell (~8 mmHg) after a warm (50°C) glucose drink whereas, after an initial rise (~4 mmHg), blood pressure remained relatively unchanged after a cold (5°C) drink (Kuipers et al. 1991). There is evidence of specific warm- and cold- sensitive receptors in the gastrointestinal tract and meal temperature is known to affect gastric emptying in healthy younger subjects (Sun et al. 1995).

1.3.4 Time of meal ingestion

There is some evidence that the magnitude of the decrease in postprandial blood pressure is greater when meals are eaten earlier in the day (Kohara et al. 1998, Puisieux et al. 2000, Vloet et al. 2003). Twenty-four hour ambulatory blood pressure monitoring was performed in 156 elderly patients who had experienced falls or syncope (Puisieux et al. 2000). Not surprisingly, the mean decreases in systolic blood pressure (two hours after a standard breakfast, lunch and dinner) were significantly greater in those patients with a history of syncope (~13 mmHg) or falls (~12 mmHg) when compared to the control group (~8 mmHg), but in all patients, the magnitude of the fall was greater by ~2 mmHg after breakfast (Puisieux et al. 2000). It should be recognised that because this study did not employ standardised meals, interpretation of the findings is difficult and ingestion of a relatively higher amount of carbohydrate with the breakfast meal may potentially account for the observation.
1.3.5 **Body posture**

While postprandial hypotension is evident in both the supine (Mader 1989) and sitting positions (Vaitkevicius et al 1991), in a study of 113 elderly nursing home residents there was a more marked fall in blood pressure in subjects who were upright during the period immediately following a mixed meal when compared to those who 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, a study involving 50 independent elderly persons indicates that this is not the case and that orthostatic hypotension is additive to postprandial hypotension, rather than synergistic (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 defined, 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 re-uptake drugs (Le Couteur et al 2003), angiotensin-converting enzyme (ACE) 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 been reported to increase the fall in postprandial blood pressure. Two studies have evaluated the effects of the diuretic, frusemide, on postprandial blood
pressure in elderly patients receiving multiple therapies for cardiovascular disease (van Kraaij et al 1999, Mehagnoul-Schipper et al 2002). In a group of 13 subjects, the withdrawal of frusemide over ~ 3 months was associated with a reduction in the postprandial fall in systolic blood pressure from 25 ± 4 to 11 ± 2 mmHg (van Kraaij et al 1999). In another study of 11 subjects, an acute dose of frusemide (40mg orally) increased the magnitude of the fall in systolic blood pressure, in response to a 292 kcal carbohydrate liquid meal by ~ 10 mmHg (Mehagnoul-Schipper et al 2002). In contrast, in the same group, chronic therapy with frusemide (40 mg/day for two weeks) had no effect on postprandial blood pressure (Mehagnoul-Schipper et al 2002). There is also an association between a number of other cardiovascular, as well as psychotropic, drugs with postprandial hypotension (Aronow and Ahn 1994). It is well recognised that many cardiovascular and psychotropic drugs have hypotensive properties thus, the administration of such medications with a meal could potentially exacerbate any postprandial falls in blood pressure (Jansen and Lipsitz 1995) moreover, serotonin has been implicated as an important regulator of splanchnic blood flow (Hansen et al 1998).

1.3.7 Other illnesses

While it has been reported that ageing is associated with impaired postprandial blood pressure regulation (Fagan et al 1986, Kohara et al 1998), recent studies suggest that age-related illness, and not healthy ageing, is pivotal to the development of postprandial hypotension (Oberman et al 2000, Smith et al 2003). The prevalence of postprandial hypotension is increased in people who are hypertensive (Jansen et al 1987, Haigh et al 1991, Jansen and Lipsitz 1995), including those with isolated systolic hypertension
(Grodzicki et al 1998). It has been suggested that in hypertensive patients, the increase in splanchnic blood flow after a meal is less effectively counter-balanced as a result of diminished baroreceptor function (Jansen et al 1987, Grodzicki et al 1998).

Postprandial hypotension has also been observed in patients with diverse causes of autonomic dysfunction (Jansen and Lipsitz 1995). Mean reductions in systolic blood pressure of ~ 49 mmHg were evident in 10 middle-aged subjects with primary autonomic failure after a standard meal (Robertson et al 1981). Similarly, in a study of 10 unselected patients with multiple system atrophy and postprandial hypotension, blood pressure decreased by a mean of ~ 22 mmHg after a 75g glucose (in 225ml water) drink (Hirayama et al 1993a) and in ten elderly patients with Parkinson's disease, there was a mean fall in postprandial systolic blood pressure of ~ 27 mmHg after a standardised lunch (Loew et al 1995).

1.4 Pathophysiology of postprandial hypotension

The pathophysiological mechanisms underlying postprandial hypotension remain poorly defined, however, a number of interrelated factors, including splanchnic blood flow, gastric distension, small intestinal nutrient delivery and neural and hormonal mechanisms, are thought to play a role.

1.4.1 Splanchnic blood flow

Following a meal, 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). The magnitude of the postprandial increase in mesenteric blood flow is comparable in healthy young and old individuals, despite the greater fall in blood pressure in the latter group, indicating that there is inadequate cardiovascular adjustment for the shift of blood volume into the splanchnic system (Lipsitz et al 1993, Sidery et al 1993). The somatostatin analogue, octreotide, which is known to reduce splanchnic blood pooling (Jansen and Hoefnagels 1991, Jansen and Lipsitz 1995), markedly attenuates the postprandial fall in blood pressure (Jansen and Hoefnagels 1991) - in 10 hypertensive elderly subjects, a single subcutaneous injection of octreotide (50µg) completely prevented a fall in blood pressure after a 75g glucose drink (Jansen et al 1989b). 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 1989b, 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). Hence, the impact of ‘intragastric’ mechanisms on the outcome of these studies is uncertain. In the study reported in Chapter 7, the author has evaluated the effects of intraduodenal glucose, triglyceride and protein on blood pressure and superior mesenteric artery blood flow in healthy elderly subjects.
1.4.2  **Gastric distension**

Gastric distension appears to attenuate the postprandial fall in blood pressure (Rossi et al 1998, Jordan et al 1999, Jordan et al 2000, Cariga and Mathias 2001, Shannon et al 2002, Jones et al 2005). In healthy, young subjects, proximal gastric distension with a barostat device has been reported to increase blood pressure, heart rate and muscle sympathetic nerve activity - the so-called ‘gastrovascular reflex’ (Rossi et al 1998). Similarly, consumption of water has been shown to increase blood pressure in healthy elderly subjects (Jordan et al 1999, Jordan et al 2000), as well as patients with multiple system atrophy and pure autonomic failure (Jordan et al 1999, Jordan et al 2000, Cariga and Mathias 2001). Furthermore, in patients with autonomic failure, drinking 480ml of water immediately prior to ingestion of a high carbohydrate meal, was shown to markedly attenuate the postprandial fall in blood pressure (Shannon et al 2002) (Figure 1.1). There is also indirect evidence that the site of gastric distension, ie proximal or distal, may be important (Rossi et al 1998, Jones et al 2005). In a recent study of healthy elderly subjects, the fall in blood pressure was less when glucose drinks of the same concentration (12.5%) were ingested at a higher volume (600ml vs 200ml) (Jones et al 2005). However, as the drinks were given orally, differences in delivery of glucose to the small intestine may potentially have contributed to the outcome. In the study reported in Chapter 9, the effects of gastric distension during intraduodenal infusion of glucose at a constant load and concentration on blood pressure in healthy elderly subjects were evaluated.
1.4.3 Small intestinal nutrient exposure

In normal subjects gastric emptying of liquid or ‘liquefied’ nutrients, including glucose, occurs at an overall rate of ~1 - 3 kcal/min, after 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 1976b). The rate of gastric emptying of carbohydrate has been shown to influence blood pressure. In an initial study, a relationship was evident between the magnitude of the fall in mean arterial blood pressure and gastric emptying of glucose in type 2 diabetic patients; so that the fall in blood pressure was greater when gastric emptying was relatively more rapid (Jones et al 1998). A subsequent study demonstrated in healthy elderly subjects, that intraduodenal glucose infusion resulted in falls in blood pressure (within 15 minutes) and rises in heart rate that were substantially greater when the infusion rate was ~3 kcal/min when compared to ~1 kcal/min (O'Donovan et al 2002) (Figure 1.2). While, this latter study design excluded the potential effects of gastric distension on blood pressure, it did not allow the potential effects of the small intestinal glucose load and concentration to be distinguished. This issue is addressed in the study reported in Chapter 6.

In view of the above observations 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 positive outcomes. These
studies have hitherto involved healthy elderly 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 slowing of gastric emptying probably reflects changes in the viscosity and distribution of gastric contents (Blackburn et al 1984a) as well as increased, nutrient-mediated, small intestinal feedback (Meyer et al 1988). In healthy elderly subjects (Jones et al 2001) and uncomplicated type 2 diabetic patients (Russo et al 2003) the addition of 9g guar gum to a 50g (in 300ml water) glucose drink predictably slowed gastric emptying and reduced blood glucose concentrations, and was also shown to attenuate the fall in systolic blood pressure (Jones et al 2001); in the healthy subjects the fall in systolic blood pressure was reduced from a mean of ~ 8 mmHg to ~ 4 mmHg (Jones et al 2001). It is of interest that in these two studies (Jones et al 2001, Russo et al 2003) there was little difference in gastric emptying of the two drinks in the first ~ 30 minutes, suggesting that the beneficial effect of guar, is related primarily to changes in small intestinal glucose exposure, rather than a slower rate of glucose delivery to the small intestine (Jones et al 2001). This is supported by a study in healthy elderly subjects which compared the effects of intraduodenal glucose infusions at ~ 3 kcal/min with or without guar (O'Donovan et al 2005b) and demonstrated that guar attenuated the magnitude of the fall in systolic blood pressure and rise in heart rate (O'Donovan et al 2005b) (Figure 1.3).

As discussed in Chapter 8, 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 by acarbose has been attributed to the slowing of small intestinal digestion and absorption of carbohydrate as a result of inhibition of α-glucosidase (Chiasson et al 2003). However, two studies (Ranganath et al 1998, Enç et al 2001) involving healthy young subjects indicate that acarbose has the capacity to slow gastric emptying. There is no information about the effect of acarbose on intragastric meal distribution. In three case studies, involving patients with type 1 (Maule et al 2004) and type 2 (Sasaki et al 2001, Yamamoto et al 2006) diabetes, acarbose (150 mg/day and 300 mg/day, respectively) was shown to attenuate the fall in systolic blood pressure and alleviate the symptoms associated with postprandial hypotension. More recently, despite the expectation that the α-glucosidase inhibitor, voglibose, would not be expected to be of benefit following glucose ingestion, 200µg of voglibose was reported to significantly reduce the magnitude of the fall in blood pressure (by a mean of ~21 mmHg) induced by 75g oral glucose in a cohort of patients with Parkinson’s disease, multiple system atrophy, diabetes mellitus and a healthy elderly subject (Maruta et al 2006). The authors of this latter study (Maruta et al 2006) proposed that these effects reflected a reduction in splanchnic blood pooling due to the inhibition of the release of gastrointestinal peptides, including neurotensin. It should, however, be recognised that the role of neurotensin in postprandial blood pressure regulation remains controversial (Hoeldtke et al 1986b, Mathias et al 1989a, Mathias et al 1989b, Raimbach et al 1989, Jansen and Lipsitz 1995) and that splanchnic blood flow was not measured, hence, interpretation of the observations is speculative. It has also
been suggested that the effect of acarbose to attenuate the reduction in postprandial blood pressure may reflect the concomitant decrease in the blood glucose response and, hence, inhibition of postprandial secretion of insulin (Sasaki et al 2001, Yamamoto et al 2006). The effects of α-glucosidase inhibitors on blood pressure and heart rate may also reflect other effects secondary to the slowing of small intestinal digestion and/or absorption. As will be discussed, stimulation of glucagon-like peptide-1 (GLP-1) may contribute to the effects of α-glucosidase inhibitors on postprandial blood pressure. The relationship between the effects of acarbose on blood pressure and gastric emptying after an oral sucrose load have been evaluated in the study reported in Chapter 8.

1.4.4 Neural mechanisms

1.4.4.1 Sympathetic nerve activity

In healthy, young subjects, the 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 the elderly (Fagius et al 1996) and patients (Hakusui et al 1991) with postprandial hypotension, the latter response is known to be attenuated (Fagius et al 1996); this probably reflects 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, Lingenfelser et al 1997, Su et al 2001). The role of nitric oxide mechanisms is optimally addressed by the use of specific inhibitors of its production, such as NG-mono-methyl-L-arginine (L-NMMA) or NG-nitro-L-arginine-methyl-ester (L-NAME) (Allescher et al 1992, Sarna et al 1993, Orihata and Sarna 1994, Hallgren et al 1995, Lingenfelser et al 1997, Su et al 2001). The effects of nitric oxide synthase inhibition on gastric emptying in humans have been assessed in two studies with inconsistent observations (Konturek et al 1999, Hirsch et al 2000). The role of nitric oxide mechanisms in mediating the hypotensive response to oral glucose, and the effects of L-NAME on gastric emptying, in healthy elderly subjects have been evaluated in the study reported in Chapter 10. Studies in animals also indicate that nitric oxide mechanisms are important in the regulation of splanchnic blood flow eg in pigs L-NMMA attenuates the increase in mesenteric blood flow after a meal (Alemany et al 1997). Hence, although the role of nitric oxide in the regulation of splanchnic blood flow in humans has not been evaluated, this represents a probable mechanism.

1.4.4.3 **5-hydroxytryptamine**

There is evidence that 5-hydroxytryptamine (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, \(\alpha\)-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 also 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). The potential role of 5-HT, particularly 5-HT3 mechanisms, in the regulation of postprandial blood pressure has hitherto not been evaluated. This issue forms the focus of the study reported in Chapter 11.

1.4.5 \textit{Hormonal mechanisms}

While there is no convincing evidence that any single peptide plays a major role in postprandial hypotension, various vasoactive peptides released from the small intestine in response to the ingestion of food have been implicated (Jansen and Hoefnagels 1991). These include neurotensin, insulin, vasoactive intestinal polypeptide (VIP), substance P, calcitonin gene-related peptide (CGRP) and the incretin hormone, GLP-1 (Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995).

1.4.5.1 \textit{Insulin}

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

1.4.5.2 Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1), which is 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. A recent study in healthy young male subjects has shown that the GLP-1 receptor antagonist, exendin (9-39) amide, inhibits the suppression of antral and stimulation of pyloric motility by intraduodenal glucose which as discussed, may contribute to slowing of gastric emptying (Schirra et al 2005). The effects of acarbose on the GLP-1 response to oral sucrose and its potential effects on postprandial blood pressure and gastric emptying are discussed in the study reported in Chapter 8.
1.4.5.3 **Vasoactive intestinal polypeptide, substance P, calcitonin gene-related peptide**

Plasma levels of vasoactive intestinal polypeptide (VIP), a known vasodilator, are not affected by oral glucose in either patients with autonomic neuropathy or the elderly (Jansen et al 1990). Furthermore, administration of octreotide does not affect either pre- or postprandial levels of VIP (Hoeldtke et al 1986b), also 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), also does not appear to be influenced by oral glucose or meal ingestion (Onrot et al 1985, Hoeldtke et al 1986b). In contrast, plasma calcitonin gene-related peptide (CGRP), increases following oral glucose and has been reported to be associated with blood pressure reductions in elderly subjects (Edwards et al 1996). In this study, elderly 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 remains controversial. 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); similar effects were observed in 6 selected patients with autonomic neuropathy and
postprandial hypotension after an oral glucose (1 g/kg) drink (Mathias et al 1989b). In contrast, in 8 selected patients with postprandial hypotension and orthostatic hypotension, plasma neurotensin levels remained unchanged after 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 Management of postprandial hypotension

Current strategies for the management of postprandial hypotension include dietary modifications (ie 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), exercise after a meal (Oberman et al 1999), ingestion of caffeine (Lipsitz et al 1994), modification in medications (Jansen and Lipsitz 1995) and the use of the somatostatin analogues, such as octreotide (Table 1.2) (Hoeldtke et al 1986b). As discussed, recent studies suggest that therapies targeted at slowing of gastric emptying (Jones et al 2001) and small intestinal nutrient exposure (O'Donovan et al 2005b) may prove effective, based on acute studies in unselected healthy elderly subjects and patients with type 2 diabetes. Current therapies focus on minimising the magnitude of the postprandial fall in blood pressure with a view to alleviating symptoms such as syncope, dizziness, falls and weakness (Jansen and Lipsitz 1995). The effect of treatments on the duration of the hypotensive response has received less attention.
1.5.1 Non-pharmacological approaches

1.5.1.1 Dietary modifications

It has been suggested that patients with postprandial hypotension should consume small, frequent and low carbohydrate meals (Waaler et al 1991, Puvi-Rajasingham and Mathias 1996). As discussed, when 7 subjects with primary autonomic failure, consumed 3 ‘large’, compared with 6 ‘small’ meals with an identical daily caloric intake, systolic blood pressure was less following the ‘larger’ meals compared to the ‘smaller’ meals (Puvi-Rajasingham and Mathias 1996). This is likely to be attributable to a greater duration of small intestinal nutrient exposure and, possibly, more rapid gastric emptying, as a higher intragastric volume, per se, should favour an increase, rather than a reduction, in blood pressure. While the relative effects of different macronutrients on blood pressure are poorly defined, based on current knowledge it seems appropriate to recommend a reduction in intake of those sugars which have an affinity for the glucose transporter (eg glucose and sucrose that has been digested to glucose and fructose). Hence, adjusting the carbohydrate content and volume of meals, as well as replacing glucose and sucrose with fructose, may provide a simple approach to the management of postprandial hypotension. However, studies evaluating the effects of specific dose, type and size of carbohydrate meals, the duration of associated cardiovascular effects and the interactions of carbohydrates with other nutrients are required before more specific recommendations can be made.
1.5.1.2 Adequate fluid intake

Adequate fluid and salt uptake are essential to the maintenance of intravascular blood volume and dehydration may lead to postprandial hypotension (Jansen and Lipsitz 1995). Accordingly, in some patients discontinuation of diuretic therapy, particularly frusemide, may result in improved postprandial blood pressure homeostasis (van Kraaij et al 1999, Mehagnoul-Schipper et al 2002).

1.5.1.3 Non-nutrient gastric distension

As discussed, consumption of water before a meal, has been shown to attenuate postprandial falls in blood pressure in elderly patients with postprandial hypotension and patients with autonomic failure (Jordan et al 1999, Jordan et al 2000, Shannon et al 2002, Jones et al 2005), probably as a result of increase in gastric distension (Jones et al 2005). Although rapid (ie < 5 minutes) ingestion of water in volumes as low as 120ml may be associated with a substantial pressor effect (Shannon et al 2002), in this latter study when the volume of water was increased to 480ml, the magnitude of the elevation in systolic blood pressure was substantially greater (by a mean of ~ 36 mmHg) (Shannon et al 2002). In another study, consumption of a 600ml glucose drink induced an immediate increase in systolic blood pressure (by a mean of ~ 8 mmHg), but there was no change in blood pressure after a 200ml drink (Jones et al 2005). Accordingly, while further studies are required, including those to evaluate the effect of chronic gastric distension, drinking ~ 500ml of water before a meal should be regarded as a simple adjunctive treatment strategy in patients with postprandial hypotension, although
this may not be always feasible in the elderly because of the substantial volume, as well as potential risk of water intoxication (Shannon et al 2002).

1.5.1.4 **Exercise**

Exercise immediately after a meal is associated with increases in blood pressure, heart rate and cardiac output. In a study of 32 frail elderly subjects (mean age 88 ± 7 years), the effect of walking after completion of breakfast was evaluated (Oberman et al 1999) and mean arterial blood pressure was shown to increase by ~ 18 mmHg above pre-exercise values. However, this increase was sustained only for the duration of the exercise and in many patients with postprandial hypotension exercise is not a practical option.

1.5.1.5 **Slowing of gastric emptying and small intestinal nutrient exposure**

Gastric emptying (and hence the rate of small intestinal nutrient exposure) can be slowed by dietary modification in a number of ways. Triglyceride retards gastric emptying markedly as a result of its high caloric density (Horowitz et al 1993b). For example, in healthy subjects, the addition of corn oil to a meal of mashed potato delays gastric emptying, thereby reducing postprandial blood glucose and insulin concentrations (Welch et al 1987). Accordingly, the potential beneficial effects of triglyceride on postprandial blood pressure warrant investigation.

As discussed, guar slows gastric emptying, oral glucose absorption and decreases the magnitude of the fall in systolic blood pressure after oral glucose in both healthy,
elderly subjects (Jones et al 2001) and patients with type 2 diabetes (Russo et al 2003). However, while the use of guar may be simple and inexpensive, 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 by increasing meal viscosity and studies in patients with postprandial hypotension are indicated to determine the magnitude of their effect.

1.5.2 Pharmacological interventions

1.5.2.1 Caffeine
Caffeine has been frequently recommended as a treatment for postprandial hypotension (Onrot et al 1985, Heseltine et al 1991b). However, information relating to its efficacy is inconsistent (Lipsitz et al 1994). When administered immediately before, or after, a meal, caffeine (200 - 250mg) has been reported to attenuate the postprandial fall (by ~ 9 - 12 mmHg) in blood pressure in both patients with autonomic neuropathy and healthy elderly subjects (Onrot et al 1985, Heseltine et al 1991b). However, in other studies of comparable doses caffeine had no effect (Barakatm 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) but these responses may be blunted in individuals with a chronic caffeine intake of ≥ 300 mg/day (ie ~ five or more cups of instant coffee/day) (Izzo et al 1983). Accordingly, there is little evidence to base recommendation for the use of caffeine as a treatment of postprandial hypotension.
1.5.2.2 Medication usage

Antihypertensive therapy may favourably affect the regulation of postprandial blood pressure. In 17 elderly subjects, chronic treatment with nicardipine (60 mg/day) and isosorbide dinitrate (60 mg/day) for three weeks attenuated postprandial decreases in systolic blood pressure (Connelly et al 1995). This observed improvement in postprandial blood pressure, however, most likely represents the effects of nicardipine and isosorbide dinitrate in the reduction of premeal blood pressure (Connelly et al 1995) and, consequently, the magnitude of postprandial blood pressure reductions rather than the prevention of the development of postprandial hypotension. The effect of antihypertensive therapy in the management of postprandial blood pressure warrants further investigation.

Studies in patients with type 1 (Maule et al 2004) and type 2 (Sasaki et al 2001, Yamamoto et al 2006) diabetes, neurologic patients and healthy subjects (Maruta et al 2006) support the concept that the α-glucosidase inhibitors, acarbose and voglibose, are effective in the treatment of postprandial hypotension - eg acarbose in a dose of 300 mg/day was apparently effective in a 58 year old insulin-dependent type 2 diabetic with severe postprandial hypotension; the magnitude of the fall in systolic blood pressure after breakfast and dinner was reduced from ~ 45 mmHg to ~ 18 mmHg (Sasaki et al 2001). Although α-glucosidase inhibitors may represent a therapeutic option for the treatment of patients with postprandial hypotension, they are likely to only be effective after meals containing complex carbohydrate (Sasaki et al 2001, Maule et al 2004, Yamamoto et al 2006), although, as discussed, a recent study (Maruta et al 2006)
suggests that voglibose may attenuate the fall in blood pressure induced by glucose. Furthermore, their use is frequently associated with adverse gastrointestinal symptoms including flatulence, abdominal distension and diarrhoea (Vichayanrat et al 2002, Chiasson et al 2003). There is also no information as to whether the effects of acarbose or voglibose are sustained during chronic administration.

1.5.2.3 **Somatostatin analogues**

As discussed, the somatostatin analogue, octreotide, inhibits the secretion of almost all gastrointestinal hormones and may modulate splanchnic blood flow; the latter is likely to be the major mechanism by which somatostatin attenuates postprandial hypotension (Jansen and Hoefnagels 1991, Jansen and Lipsitz 1995). A single subcutaneous dose of octreotide has been shown to attenuate the postprandial fall in blood pressure in elderly subjects (Hoeldtke et al 1986b, Jansen et al 1989a, Jansen et al 1989b) and patients with autonomic failure (Hoeldtke et al 1986a, Raimbach et al 1989). For example, 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). However, octreotide, and other long acting somatostatin analogues, are associated with the limitations of high cost, 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 should only be used in severely affected symptomatic patients (Jansen and Lipsitz 1995).
1.5.2.4 Other medications

Studies investigating the use of other therapies in the treatment of postprandial hypotension have yielded inconsistent observations and many did not include patients with postprandial hypotension. Nitrendipine (Jansen et al 1988), midodrine and denopamine (Hirayama et al 1993b) and vasopressin (Hakusui et al 1991), taken alone or in combination, may attenuate postprandial blood pressure reductions; the rationale for their use includes the inhibition of portal blood flow and an increase cardiac output and systemic vascular resistance (Hirayama et al 1993b). In hypertensive elderly patients, 12 weeks of treatment with the calcium antagonist nitrendipine (20mg) reduced falls in postprandial blood pressure after an oral glucose load from ~ 13 mmHg to ~ 7 mmHg (Jansen et al 1988). Combination therapy with midodrine (α1-agonist) (4mg) and denopamine (β1-agonist) (10mg) reduced the fall in blood pressure (from ~ 28 mmHg to ~ 11 mmHg) after an oral glucose load in 5 patients with autonomic dysfunction (Hirayama et al 1993b); midodrine (5mg) had similar effects when used in combination with octreotide in patients with autonomic dysfunction (Hoeldtke et al 1998). Chronic use of midodrine is associated with a high prevalence of adverse effects including urinary urgency and supine hypertension (Hoeldtke et al 1998). In patients with postprandial hypotension, acute intravenous administration of vasopressin (0.3 U/min) prevented the fall in blood pressure after an oral glucose load (Hakusui et al 1991). However, the requirement of intravenous injections limits its use in patients with postprandial hypotension. An animal study (Alemany et al 1997) indicates that nitric oxide synthase inhibitors may affect the regulation of splanchnic blood flow and, hence, postprandial hypotension. However, the requirement for intravenous administration
limits the potential of such agents. Additional studies are indicated to evaluate the effectiveness of potential treatment strategies for the management of postprandial hypotension.

1.6 Conclusions

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

1) the effects of variations in concentration of intraduodenal infusion of glucose on the magnitude of the fall in blood pressure in healthy elderly subjects (Chapter 6).

2) the comparative effects of intraduodenal infusion of glucose, triglyceride and protein on the magnitude of the postprandial fall in blood pressure and rise in heart rate and superior mesenteric artery blood flow in healthy elderly subjects (Chapter 7).

3) the effects of acarbose, on blood pressure, heart rate, gastric emptying of, and the glycaemic, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependant insulinotropic-polypeptide (GIP) responses to, an oral sucrose load in healthy elderly subjects (Chapter 8).
4) the effects of gastric distension on blood pressure and heart rate during intraduodenal infusion of glucose at a constant load and concentration in healthy elderly subjects (Chapter 9).

5) the role of the nitric oxide synthase inhibitor, L-NAME, on gastric emptying, postprandial blood pressure, plasma insulin concentration and incretin hormone (ie GIP and GLP-1) release, following an oral glucose load, in healthy elderly subjects (Chapter 10).

6) the effects of the 5-hydroxytryptamine 3 (5-HT3) antagonist, granisetron, on the blood pressure, heart rate, antropyloroduodenal motility and glycaemic responses to intraduodenal glucose infusion in healthy elderly subjects (Chapter 11).
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<th>Increases fall in blood pressure</th>
<th>Attenuates fall in blood pressure</th>
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<tr>
<td><strong>Physiological</strong></td>
<td><strong>Physiological</strong></td>
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<td>high meal glucose content</td>
<td>higher meal volume/consumption of water</td>
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<td>meal consumption in the morning</td>
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<td>higher meal temperature</td>
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<td>upright posture</td>
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<td><strong>Drugs</strong></td>
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<td>drugs (eg diuretics, antipsychotics, selective serotonin re-uptake inhibitors, ACE inhibitors, calcium channel blockers, nitrates, digoxin)</td>
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**Table 1.1:** Factors which may influence the hypotensive response to a meal.
Figure 1.1: Change in systolic blood pressure in 7 patients with primary autonomic failure following ingestion of a standard meal. The effect of 480ml of water taken just before the meal (□) is compared with the effect of the meal alone (●). Oral water substantially attenuated the hypotensive effect of food. Data are mean values ± SEM. Adapted from Shannon et al (2002).
Figure 1.2: Effects of intraduodenal glucose infusion at a rate of either ~1 kcal/min (●) or ~3 kcal/min (○) on (a) systolic blood pressure and (b) heart rate in 8 healthy elderly subjects. Data are presented as changes from baseline and are mean values ± SEM. P < 0.0001 (~3 kcal/min vs 1 kcal/min). Adapted from O'Donovan et al (2002).
Figure 1.3: Effects of intraduodenal glucose at a rate of ~ 3 kcal/min without (●) or with 4g guar gum on (○) on (a) systolic blood pressure and (b) heart rate in 8 healthy elderly subjects. Data are presented as changes from baseline and are mean values ± SEM. P < 0.05 (glucose - only infusion vs glucose and guar infusion over time). Adapted from O'Donovan et al (2005b).
<table>
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<th><strong>Non - pharmacological</strong></th>
<th><strong>Effectiveness of treatment</strong></th>
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<td>maintenance of adequate fluid intake</td>
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<td>water drinking before a meal</td>
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<tr>
<td>reduction in the volume and carbohydrate content of meals</td>
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<tr>
<td>substitution of fructose for glucose and sucrose</td>
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<tr>
<td>slowing of gastric emptying and small intestinal nutrient exposure</td>
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<td>exercise after a meal</td>
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| **Pharmacological**                                                          |                               |
| caffeine                                                                      | ✗                              |
| octreotide (somatostatin analogues)                                           | ✔                              |
| acarbose                                                                      | ✗                              |
| nicardipine, isosorbide dinitrate, nitrendipine, midodrine, denopamine and vasopressin | ✗                              |

**Table 1.2:** Potential treatment strategies for postprandial hypotension.
Blood pressure regulation
2.1 Introduction

Blood pressure can be defined as the pressure or force exerted by blood on the wall of any blood vessel (Tortora and Anagnostakos 1987, Sherwood 2001). It is dependent on the volume of blood contained within the vessel as well as the compliance of the vessel walls (Sherwood 2001). The maximum pressure exerted in arteries when blood is ejected into them during systole (ie when the ventricles are contracting) is known as systolic blood pressure and averages 120 mmHg (Sherwood 2001). In contrast, the minimum pressure within the arteries when the ventricles are relaxing and, hence, blood is draining into vessels, is the diastolic pressure and averages 80 mmHg (Sherwood 2001). In this chapter, current knowledge of the factors that are known to influence the regulation of blood pressure (Figure 2.1), with a particular focus on the mechanisms which alter cardiac output, peripheral resistance and blood volume are discussed.

2.2 Cardiac output

Changes in cardiac output, result from either changes in cardiac function or venous return commonly as a consequence of changes in blood volume (McGeown 2002). Cardiac output is the amount of blood ejected by the left ventricle into the aorta every minute (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996, Sherwood 2001). If cardiac output is increased due to an increase in stroke volume (ie the volume of blood pumped out of each ventricle with each contraction of the heart) or heart rate, blood pressure also increases (Tortora and Anagnostakos 1987). Conversely, a decrease in cardiac output results in a decrease in blood pressure (Tortora and Anagnostakos 1987).
2.3 Peripheral resistance

Peripheral resistance is regulated through changes in arteriolar constriction (McGeown 2002) and refers to the resistance to blood flow imposed by the force of friction between blood and its vessel walls (Thibodeau and Patton 1996). It is related to the viscosity of blood and blood vessel diameter (Tortora and Anagnostakos 1987) such that in cases where the viscosity of blood increases (eg in conditions with an unusually high number of red blood cells) or decreases (eg in conditions with a depletion of red blood cells) there is a subsequent increase or decrease in blood pressure. 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 therefore are able to change their diameter, the greater or less resistance to blood flow resulting in an increase or decrease in blood pressure (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996).

2.4 Blood volume

Blood pressure is directly proportional to the volume of blood in the cardiovascular system (Tortora and Anagnostakos 1987) such that any increase or decrease in blood volume from the normal level (ie ~ 5 litres) results in an increase or decrease in blood pressure.

2.5 Vasomotor centre

The vasomotor centre is a cluster of neurons found within the medulla (Tortora and Anagnostakos 1987), the function of which is to control the diameter of blood vessels,
in particular arterioles (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996). When stimulated, the centre sends impulses to the smooth muscle in arteriole walls resulting in a constant state of vasoconstriction (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996, McGeown 2002). This vasoconstriction aids in the maintenance of peripheral resistance and blood pressure. The vasomotor centre induces vasoconstriction by increasing the number of sympathetic impulses above normal (Tortora and Anagnostakos 1987). Conversely, vasodilation occurs when the number of sympathetic impulses are below normal (Tortora and Anagnostakos 1987).

### 2.6 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, decrease cardiac output and reduce blood pressure (McGeown 2002).

### 2.7 Baroreceptors

Baroreceptors are pressure receptors located in the walls of the carotid sinus, at the junction of the bifurcation of the common carotid artery, and in the aortic arch (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996, Sherwood 2001, McGeown 2002). An increase in blood pressure stimulates these baroreceptors to send impulses to the cardiovascular control centre to reduce heart rate, and the vasomotor centre, to inhibit vasoconstriction of arterioles (Thibodeau and Patton 1996). Conversely, a reduction in blood pressure leads to reduced baroreceptor activity resulting in increased
sympathetic, but decreased parasympathetic, outflow with blood pressure being restored to normal levels (McGeown 2002).

2.8 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 (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996, Sherwood 2001, McGeown 2002). When a deficiency of oxygen (hypoxia), an excess of carbon dioxide (hypercapnia) or decreased arterial blood pH is detected, the fibres of chemoreceptors are stimulated to send impulses to the vasomotor centre which stimulate vasoconstrictor sympathetic nerves in an attempt to restore blood pressure (Tortora and Anagnostakos 1987, Thibodeau and Patton 1996, McGeown 2002).

2.9 Cerebral ischaemia

When blood supply to the medulla becomes inadequate, this leads to a deficiency in oxygen and accumulation of carbon dioxide in cerebral tissue (Thibodeau and Patton 1996, McGeown 2002). 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 (Thibodeau and Patton 1996, McGeown 2002).
2.10 Higher brain centres
Cardiovascular responses associated with behaviours and emotions are mediated through the cerebral cortex - hypothalamic pathway (Sherwood 2001). For example, during intense anger, the cerebral cortex stimulates the vasomotor centre to send sympathetic impulses to arterioles causing an increase in blood pressure (Tortora and Anagnostakos 1987). In contrast, when an individual is emotionally upset, impulses from the higher brain centres leads to a decrease in sympathetic nerve stimulation producing vasodilation and, consequently, a decrease in blood pressure (Tortora and Anagnostakos 1987).

2.11 Hormones
There are a number of hormones that affect blood pressure by causing vasoconstriction of arterioles. Epinephrine and norepinephrine (noradrenaline) produced by the adrenal medulla cause an increase in cardiac contractility and rate and vasoconstriction of abdominal and cutaneous arterioles (Tortora and Anagnostakos 1987, McGeown 2002). The Antidiuretic Hormone (ADH) or vasopressin is produced by the hypothalamus and released from the neurohypophysis (ie the posterior lobe of the pituitary gland) causing vasoconstriction of arterioles (Tortora and Anagnostakos 1987). Angiotensin II raises blood pressure by stimulating the secretion of aldosterone, which is responsible for increasing sodium ion concentration and water reabsorption, from the adrenal cortex and causing vasoconstriction due to the release of renin (Tortora and Anagnostakos 1987, McGeown 2002). Endothelin, that is 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 yet identified (Sherwood 2001).

2.12 Autoregulation

Autoregulation refers to the local adjustment of blood flow to meet the changing needs of different body tissue (Tortora and Anagnostakos 1987, McGeown 2002). This autoregulation is dependent on local changes in vascular resistance which compensate for changes in pressure (Tortora and Anagnostakos 1987, McGeown 2002). For example, vasodilator substances or metabolites are thought to be produced within body tissue when the supply of oxygen falls below its consumption (McGeown 2002). These metabolites which are thought to include potassium ions, hydrogen ions, carbon dioxide, lactic acid, adenosine (Tortora and Anagnostakos 1987, McGeown 2002) and nitric oxide (Thibodeau and Patton 1996) (endothelial-derived relaxing factor) (Sherwood 2001), decrease local vascular resistance and increase blood flow in tissue by dilating local arterioles and causing relaxation of precapillary sphincters, thereby restoring oxygen levels to normal (Tortora and Anagnostakos 1987).

2.13 Nervous control of blood flow

Sympathetic vasoconstrictor nerves which are particularly dense in the vasculature of the gastrointestinal tract, skin and skeletal muscle, release norepinephrine and regulate peripheral resistance (McGeown 2002). Nerves stimulated in response to a 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 (McGeown
Sympathetic vasodilator nerves are thought to be found in the skin and activation of these nerves is usually in response to increasing body temperature (McGeown 2002). When excess body heat is detected, arteriolar smooth muscle relax resulting in vasodilation, an increase in cutaneous blood flow and heat loss (Sherwood 2001, McGeown 2002).

2.14 Conclusions

This chapter has presented a brief summary of the literature in relation to the mechanisms involved in blood pressure regulation. The regulation of blood pressure depends on control of its two main determinants, cardiac output and peripheral resistance. While control of cardiac output is dependent on stroke volume and the regulation of heart rate, peripheral resistance is determined primarily by the degree of arteriolar vasoconstriction. Together, with other factors that act directly on the blood vessels, short- and long-term regulation of blood pressure is achieved.
Figure 2.1: A summary of the main controls that affect arterial blood pressure. Adapted from McGeown (2002).
Gastric motor function
3.1 Introduction

The essential functions of the stomach are to receive, mix and empty nutrients from the stomach into the small intestine for optimal digestion and absorption. The purpose of this chapter is to review the current knowledge of gastric motor function, with a focus on the role of the proximal and distal stomach on the normal physiology and patterns of gastric emptying. The factors influencing the regulation of gastric emptying are also discussed.

3.2 Physiology of normal gastric emptying

Gastric emptying can be considered in three phases which are closely related: (i) initial storage of ingested food, (ii) mixing with gastric secretions and grinding of food to particles 1 - 2mm in size, and (iii) the controlled delivery of chyme into the small intestine. Understanding of the mechanical factors involved in gastric emptying remains far from complete (Horowitz and Dent 1991, Rayner and Horowitz 2005); this is indicative of the technical difficulties associated with the assessment of human gastric motility. Gastric emptying is, predominantly a pulsatile, rather than a continuous, process; most liquefied chyme enters the small intestine as a succession of small gushes (King et al 1984, Malbert and Ruckebusch 1991, Malbert and Mathis 1994). Both antegrade and retrograde flow occurs and there is considerable variation in the characteristics of individual flow pulses (Hausken et al 1992), reflecting the integration of motor activity in the proximal stomach, antrum, pylorus and proximal small intestine (Horowitz et al 1994, Koch 1999) (Figure 3.1).
3.2.1 Role of the proximal stomach

Ingested food is initially stored in the proximal region of the stomach. During swallowing, vagally mediated transient ‘receptive’ relaxation is followed by a more prolonged proximal gastric relaxation, known as ‘accommodation’. Accordingly, an increase in gastric volume does not usually result in a substantial rise in intragastric pressure (Azpiroz and Malagelada 1987).

3.2.2 Role of the distal stomach

The distal stomach plays a major role in grinding and breakdown of solid food into small particles as well as the delivery of chyme into the small intestine (Horowitz et al 2001). The contractile activity of the distal stomach is controlled by electrical signals generated by pacemaker cells located on the greater curvature, which discharge at a rate of about three per minute (Figure 3.2). The so-called interstitial cells of Cajal, found in the circular and longitudinal muscle layers within the gastric wall, may be responsible for the initiation of slow waves (Der-Silaphet et al 1998). Distal stomach contractile activity is always associated with gastric slow wave; however, the slow wave persists in the absence of gastric contractile activity (Horowitz et al 2001). In humans, the velocity of contractions increases from proximal to distal stomach: in the mid-corpus contractions have a velocity of approximately 0.5 cm/second which increases to 4 cm/second in the terminal antrum (Hinder and Kelly 1977).
3.2.3 **Role of the pylorus**

The pylorus exhibits both tonic and phasic contractile activity, occurring over a narrow zone (approximately 2mm), either in isolation or in temporal association with antral contractions, and functions as a brake ie transpyloric flow can only occur when the pylorus is open (Horowitz et al 1994) and the mechanical determinants of individual episodes are unclear (Horowitz et al 2001). Flow may reflect a local increase in the antroduodenal pressure difference due to peristaltic antral contractions, or be associated with a common cavity pressure difference between the distal antrum and proximal duodenum during periods of relative antral quiescence (Rayner et al 2001); it has been suggested that the latter may play a primary role (Indireshkumar et al 2000). Duodenal contractions may promote or retard gastric emptying.

3.2.4 **Migrating motor complex**

Normal fasting antral motility is cyclical and has been termed the migrating motor complex (MMC). The MMC encompasses three phases with a total cycle time of about 100 minutes. Phase 1 (about 40 minutes) is motor quiescence, phase 2 (approximately 50 minutes) is characterized by irregular contractions and phase 3 (5 - 10 minutes) regular, high amplitude contractions at the maximal rate of 3 / minute (Horowitz et al 2001). During late phase 2, and phase 3, larger indigestible particles are emptied from the stomach into the small intestine. The MMC is interrupted by food ingestion; ‘fed motility’ is associated with increases in the tonic activity of the proximal stomach and irregular contractile activity in the antrum (Lee et al 2002) (Figure 3.3).
3.2.5 Patterns of gastric emptying

Overall patterns of gastric emptying are critically dependent on the physical and chemical composition of a meal; solids, semi-solids, nutrient liquids and non-nutrient liquids empty from the stomach at different rates (Horowitz and Dent 1991).

3.2.5.1 Solids

A lag phase (usually 20 - 40 minutes in duration for solids) occurs before emptying of digestible solids commences; the following emptying phase approximates a linear pattern (Figure 3.4), at least for the majority of emptying (Collins et al 1991). During the lag phase, solids move from the proximal into the distal stomach and are ground into small particles.

3.2.5.2 Liquids

Emptying of high nutrient liquids also approximates a linear pattern, after an initial emptying phase that may be somewhat faster (Horowitz and Dent 1991), but there is minimal, if any, lag phase. In contrast, emptying of non-nutrient liquids approximates a mono-exponential pattern (Figure 3.4) and is influenced by both intragastric volume and posture (Collins et al 1991).

When liquids and solids are consumed together, liquids empty preferentially (approximately 80% before solids start to empty) and the presence of a solid meal slows emptying of a simultaneously ingested liquid (Horowitz et al 1989b). Hence, the stomach can to some extent, regulate gastric emptying of solids and liquids separately.
3.3 Regulation of gastric emptying

Several factors are responsible for the regulation of gastric emptying, the most important of which, is small intestinal feedback. Feedback inhibition is generated by the interaction of nutrients (as well as pH, osmolality and distension) with receptors distributed throughout the small intestine. As a consequence of this interaction emptying of nutrient-containing meals approximates a rate of ~ 1 - 3 kcal/minute (Brener et al 1983, Lin et al 1989, Horowitz et al 1993a). There are ‘specific’ small intestinal receptors for various nutrients (eg glucose, fatty acids and amino acids), with regional variations in receptor number and type (Horowitz et al 2001). The magnitude of small intestinal feedback is dependent on both the length and region of small intestine exposed to nutrients (Lin et al 1989) and is influenced by prior nutrient exposure (Horowitz et al 1996). Of the nutrients, triglyceride is the most potent at slowing gastric emptying (Lin et al 1996). Intraduodenal infusion of lipid slows gastric emptying and is associated with suppression of antral pressure waves, stimulation of pressure waves localised to the pylorus and a reduction in the tone of the proximal stomach (Heddle et al 1989). Caeco-ileal reflux may also potentially contribute to the regulation of gastric emptying (Cuche and Malbert 1999). The extent of small intestinal feedback is also mediated by both neural and hormonal mechanisms; the latter may include cholecystokinin, glucagon-like peptide-1 (GLP-1), peptide Y-Y and amylin. Triglycerides must be digested to fatty acids by lipase to stimulate small intestinal mechanisms which slow gastric emptying. For example, in patients, with cystic fibrosis who suffer from exocrine pancreatic insufficiency, gastric emptying of high triglyceride
meals is abnormally rapid because of diminished small intestinal feedback (Carney et al 1995).

3.4 Conclusions

This chapter has reviewed the current knowledge of gastric motor function, with a focus on the role of the proximal stomach, which involves the storage of food, and the distal stomach which plays an important role in the grinding and delivery of food to the small intestine. While patterns of gastric emptying are essentially dependent on the physical and chemical composition of a meal, the regulation of gastric emptying is primarily dependent on small intestinal feedback, the magnitude of which is influenced by a number of factors including the length and region of the small intestine exposed to nutrients.
Figure 3.1: Motor effects during normal gastric emptying. The fundus relaxes to accommodate the meal, while the antrum grinds and sieves solids, pumping the resultant chyme into the duodenum against resistance generated by phasic and tonic contractions. The presence of nutrients in the small intestine generates neurohumoral feedback on gastric motor function, enhancing fundic relaxation and pyloric contraction, while suppressing antral motility, with the net effect of slowing further emptying to a closely regulated rate. Adapted from Rayner and Horowitz (2005).
Figure 3.2: Gastric pacesetter potentials or slow waves originate from the pacemaker area. Pacesetter potentials travel in a circumferential and aboral direction at a rate of approximately 3 cycles per minute. The cutaneous recorded electrogastrogram (EGG) shows a 3 cycles per minute. The fundus has no rhythmic electrical activity. Adapted from Koch (1999).
**Figure 3.3:** Example of manometric recordings during infusion of saline and glucose in a healthy young subject. Saline infusion did not change antropyloroduodenal motility (A). During glucose infusion (B) the number of isolated pyloric pressure waves increased and antral motility was inhibited. A3 and A4, antral channels; S1 and S2, channels along the sleeve; D1 and D2, duodenal channels. Adapted from Verhagen et al (1999).
Figure 3.4: Scintigraphic gastric emptying curves for solid, semi-solid/nutrient liquid and non-nutrient liquid. Solids and semi-solids/nutrient liquids empty in a linear fashion following a lag phase, while non-nutrient liquids empty in a mono-exponential manner, with minimal lag.
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 procedures, with the exception of three-dimensional (3D) ultrasonography (Chapter 5), are well established, have been utilised extensively and are recognised as acceptable assessment methods.

4.2 Ethics and subject approval

All protocols were approved by the Human Research Ethics Committee (Chapters 5 - 11) and the Investigational Drug Sub-committee (Chapters 8, 10 and 11) of the Royal Adelaide Hospital, prior to recruitment of subjects. Each subject provided written, informed consent prior to their involvement in the study. All experiments were carried out in accordance with the Declaration of Helsinki.

4.3 Blood pressure and heart rate

Blood pressure (systolic and diastolic) and heart rate were measured in all studies using an automated, oscillometric blood pressure monitor. Postprandial hypotension was defined as a postprandial fall in systolic blood pressure of ≥ 20mmHg that was sustained for at least thirty minutes (Jansen and Lipsitz 1995).

4.4 Gastric emptying

Several techniques can be used to measure gastric emptying, of which scintigraphy is the ‘gold standard’ (Collins et al 1983, Collins et al 1991, Horowitz and Dent 1991,
Malbert and Mathis 1994, Maurer and Parkman 2006). In the studies performed by the author, gastric emptying was assessed using scintigraphy, two-dimensional (2D) and 3D ultrasonography, as described below.

4.4.1 Scintigraphy

In the studies described in Chapters 5, 8 and 10, radioisotopic data were acquired with the subject seated with their back against a gamma camera (GEnie, GE Healthcare Technologies, Milwaukee, WI, USA) at 60 second frames for the first 60 minutes and three minute frames thereafter, for 60 - 180 minutes after ingestion of a drink labelled with \(^{99m}\)Technetium (Tc) sulphur colloid (Jones et al 1998). Time zero was defined as the time of completion of the drink. Upon completion of the data acquisition, a 60 second left lateral image was taken with the subject seated with their left side against the gamma camera; based on the lateral image, correction factors were derived for \(\gamma\)-ray tissue attenuation (Collins et al 1983). Data were also corrected for subject movement and radionuclide decay (Collins et al 1983). Regions-of-interest were drawn around the stomach and gastric emptying curves (expressed as % retention over time) derived (Jones et al 1998). The time taken for 50% of the drink to empty (T50) was also determined (Collins et al 1983). The lag phase was defined visually as the time before any of the radioactivity had entered the proximal small intestine (Collins et al 1983).
4.4.2 Ultrasonography

4.4.2.1 Two-dimensional (2D) imaging

Antral area measurements (Chapter 9) were performed by real-time ultrasonography using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, NSW, Australia). Subjects were scanned using a 3.5C broad spectrum 2.5 - 4 MHz convex transducer (Jones et al 1997). With the subject seated, the transducer was positioned vertically, in the region of the umbilicus, to visualise the antrum in cross-section with the superior mesenteric vein and the abdominal aorta as landmarks (Hveem et al 1996) (Figure 4.1). Antral area (cm$^2$) was measured using manually operated on-screen callipers. The circumference of the antrum was outlined and the area measured during the fasting state was subtracted from subsequent measurements performed after the test drink. Gastric emptying was expressed at any time point as:

\[
\text{Retention (\%)} = \frac{\text{AA}(t) - \text{AA}(f)}{\text{AA}(\text{max}) - \text{AA}(f)} \times 100
\]

where \(\text{AA}(t)\) = antral area measured at any given time point, \(\text{AA}(f)\) = fasting antral area and \(\text{AA}(\text{max})\) = maximum antral area recorded after drink ingestion (Hveem et al 1996).

4.4.2.2 Three-dimensional (3D) imaging

3D ultrasonography measurements (Chapter 5) were performed using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, NSW, Australia) with
TruScan Architecture (ie built-in magnetically sensored 3D). For 3D positioning and orientation measurement (POM), a transmitter was placed close to the subject and a snap-on sensor attached to a 3.5C 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 - 30cm) (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.

For 3D data acquisition, subjects were instructed to hold their breath at the end of 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 about ten seconds. When gastric contractions were observed, the acquisition was paused until the contraction wave had passed. The stored images were copied to CD-ROM and then transferred to a windows workstation. Data processing and volume estimation were performed with the use of EchoPAC-3D software® (GE Vingmed Sound, Horten, Norway), as described (Tefera et al 2002).
4.5 Intraduodenal infusion

In Chapters 6, 7, 9 and 11 intraduodenal infusions were performed using a 17 channel manometric silicone-rubber catheter (~ 4mm diameter) (Dentsleeve International Ltd, Mui Scientific, Ontario, Canada) that was introduced into the stomach via an anaesthetised nostril (O'Donovan et al 2002). The catheter was allowed to pass through the stomach and into the duodenum by peristalsis. As depicted in Figure 4.2, this catheter consisted of 16 side holes (spaced 1.5cm apart) and an infusion channel with a port located ~ 10cm distal to the pylorus (ie in the duodenum). Six side-holes (channels 1 - 6) were positioned in the antrum, ~ 2.5cm proximal to the pylorus, a 4.5cm sleeve sensor (channel 7), with two channels (channels 8 and 9) on the back of the sleeve, was positioned across the pylorus, and seven channels (channels 10 - 16) were positioned in the duodenum. The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained using a saline-filled, reference electrode (20 gauge intravenous cannula) inserted subcutaneously into the subject’s forearm to enable measurement of the antroduodenal transmucosal potential difference (TMPD) (Heddle et al 1998a, Heddle et al 1988c) across the antrum (~40mV) and duodenum (~0mV). In Chapter 10, a silicone tube of smaller diameter (ie 0.4mm internal) was attached to the manometric catheter, just above the level of channel 1, for intragastric infusion of water. The catheter and tube assembly were introduced into the stomach via an anaesthetised nostril and correct positioning was determined by measuring the TMPD, as described previously.
4.6 **Intraluminal pressures**

Intragastric pressures and contractions were measured (Chapter 11) with antropyloroduodenal manometry. Subjects were intubated with a multi-lumen catheter as described in section 4.5 and the position of the catheter was maintained accurately by monitoring the TMPD between the stomach and duodenum. The manometric channels were perfused with degassed, distilled water, except the TMPD channels which were perfused with degassed saline (0.9%), at 0.15 ml/min (Heddle et al 1989). The catheter design allowed antral, pyloric and duodenal pressures to be recorded simultaneously, via the side-holes (Heddle et al 1988a), with only gastric contractions which resulted in the total occlusion of the lumen of the side-holes accepted. The sleeve sensor was utilised to record pyloric pressure waves as the structure and movement of the pylorus renders positioning of a single side-hole extremely difficult (Heddle et al 1988c). Phasic pressure waves in the antrum and pylorus were defined by pressure increases which lasted 1 - 20 seconds and had an amplitude of > 10 mmHg, with a minimum interval of fifteen seconds between peaks. Phasic pressure waves in the duodenum were defined as those having an amplitude of > 10 mmHg, with a minimal interval of 3 seconds between peaks. Antropyloroduodenal pressure wave sequences were defined as two or more temporally-related pressure waves with onsets within ± 5 seconds (in the antrum), or ± 3 seconds (in the duodenum) of each other (Samsom et al 1998). Manometric pressures were digitised and recorded on a computer-based system running commercially available software (Flexisoft®, Version 3, Associate Professor GS Hebbard, Royal Melbourne Hospital, Melbourne, VIC, Australia, written in Labview 3.1.1 (National Instruments)), and stored for subsequent analysis. Basal pyloric pressure
(tone) was determined by subtracting the mean basal pressure recorded at the most distal antral side-hole from the mean basal pressure recorded at the sleeve, using custom-written software (MAD, Professor Charles Malbert, Institut National de la Recherche Agronomique (INRA), Rennes, France) (Heddle et al 1988c).

4.7 Splanchnic blood flow

In Chapter 7, superior mesenteric artery blood flow was measured by Duplex ultrasonography (ie 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 3.5C broad spectrum 2.5 - 4 MHz convex transducer (Perko 2001) that was positioned just below the xiphoid process, manoeuvred slightly to the left to visualise the abdominal aorta and then moved inferiorly so that the coeliac trunk and superior mesenteric artery could be seen (Perko 2001). Subjects were instructed to hold their breath at the end of inspiration (Sieber et al 1992, Sidery and Macdonald 1994) and recordings of peak systolic velocity, end-diastolic velocity and time averaged mean velocity were acquired from Pulsed Doppler waveform complexes. Measurements of the superior mesenteric artery blood were performed 2 - 3cm distal to its aortic origin (Jager et al 1986, Sieber et al 1992, Perko et al 1996, Perko 2001) at an angle of insonation of 60° (Perko et al 1996, Perko 2001). The cross-sectional diameter of the superior mesenteric artery 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
(ml/min) was calculated instantaneously using the time-averaged mean velocity and cross-sectional diameter of the superior mesenteric artery.

### 4.8 Blood glucose concentrations

Blood glucose concentrations were 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). The accuracy of this method has been confirmed using the hexokinase technique (Horowitz et al 1991). In Chapter 7, blood glucose was determined by the hexokinase technique using an Olympus 5400 analyser at the Institute of Medical and Veterinary Science (IMVS) Laboratory, Adelaide, South Australia.

### 4.9 Gastrointestinal hormone concentrations

Blood samples for determination of plasma insulin, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were collected in ice-chilled ethylene-diamine-tetra-acetic acid (EDTA)-treated tubes containing 400 kIU aprotinin (Trasylol, Bayer Australia Ltd., Pymble, NSW, Australia) per milliliter blood (Chapters 8 and 10). Plasma was separated by centrifugation (3200 rpm, 15 minutes, 4°C) and stored at -70°C for later analysis.

#### 4.9.1 Plasma insulin concentrations

Plasma insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, TX, USA). The sensitivity of the assay was 0.26
mU/L; the intraassay coefficient of variation was 2.6% and the interassay coefficient of variation 6.2% (O'Donovan et al 2004a).

4.9.2  Plasma glucagon-like peptide-1 (GLP-1) concentrations

Plasma GLP-1 concentrations were measured by radioimmunoassay using an adaptation (Wishart et al 1998) of a previously established method (Orskov et al 1991). Ethanol extraction of plasma samples was undertaken using an antibody supplied by Professor SR Bloom (Hammersmith Hospital, London, UK) that did not cross-react with glucagon, GIP, or other gut or pancreatic peptides and has been demonstrated by chromatography to measure intact GLP-1(7-36) amide. The intraassay coefficient of variation was 17%, and the interassay coefficient of variation was 18%. Sensitivity was 1.5 pmol/L.

4.9.3  Plasma glucose-dependent insulino tropic polypeptide (GIP) concentrations

Plasma GIP was measured by radioimmunoassay, the details of which have been published previously (Wishart et al 1992). The minimum detectable limit was 2 pmol/L and both intra- and interassay coefficients of variation were 15%.

4.10  Cardiovascular autonomic nerve function

Autonomic nerve function was evaluated 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 were 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).

4.11 Statistical analysis

In all studies, data are presented as mean values ± standard error of the mean (SEM) and a P value < 0.05 was considered significant in all analyses. Power calculations were based on data from previous studies and the number of subjects studied, resulted in an 80% power to detect a difference between the study days.

4.12 Conclusions

This chapter has presented a brief overview of the techniques used in the studies presented in this thesis. With the exception of 3D ultrasonography, the methods discussed have all been validated previously. While scintigraphy is currently considered the ‘gold standard’ for the assessment of gastric emptying, 3D ultrasonography has the potential to allow a precise measure of gastric emptying, by utilising a direct method of volume determination. This issue forms the focus of the study reported in Chapter 5.
Figure 4.1: Parasagittal ultrasonography image of the antrum.
Figure 4.2: Diagram of the multi-lumen catheter used in intraduodenal nutrient infusion (Chapters 6, 7, 9 and 11), intragastric infusion (Chapter 9) and measurement of intraluminal pressures (Chapter 11). TMPD, transmucosal potential difference.
Measurement of gastric emptying of low- and high-nutrient liquids using 3D ultrasonography and scintigraphy in healthy subjects
5.1 Summary

Scintigraphy represents the ‘gold standard’ for the measurement of gastric emptying. Recent studies suggest that three-dimensional (3D) ultrasonography may allow a precise measure of gastric emptying, given the capacity for accurate volume calculations of the stomach. The aim of this study was to compare measurements of gastric emptying of both low- and high- nutrient drinks by 3D ultrasonography with scintigraphy. Ten healthy young subjects (six male and four female, aged 18 - 35 years) were studied on two days. Concurrent measurements of gastric emptying by scintigraphy and 3D ultrasonography were performed after ingestion of 500ml beef soup (12 kcal) or 300ml dextrose (25% w/v) (314 kcal) labelled with 20MBq $^{99m}$Tc-sulphur colloid. There was no significant difference between scintigraphic and ultrasonographic 50% emptying times (T50’s) (soup: $27.7 \pm 4.8$ minutes vs $23.8. \pm 4.8$ minutes; dextrose: $122.2 \pm 13.3$ minutes vs $131.9 \pm 10.2$ minutes). There was a close correlation between scintigraphic and ultrasonographic T50’s for both soup ($r = 0.92, P = 0.0005$) and dextrose ($r = 0.88, P = 0.0007$). For the T50’s, the limits of agreement were $-15.2$ minutes and $+8.1$ minutes for the soup (mean difference $-3.6$ minutes) and $-35.3$ minutes and $+47.6$ minutes for dextrose (mean difference $+6.2$ minutes). In conclusion, 3D ultrasonography provides a valid measure of gastric emptying of liquid meals in healthy subjects.

5.2 Introduction

Scintigraphy is generally regarded as the ‘gold standard’ for the measurement of gastric emptying in both clinical and research studies (Collins et al 1983, Collins et al 1991,
Horowitz and Dent 1991, Malbert and Mathis 1994). While this technique is non-invasive and relatively easy to perform, the associated radiation burden (ie equivalent to approximately 0.5 mSv per study with ingestion of a liquid meal labelled with 20MBq $^{99m}$Tc sulphur colloid) limits its use (Hveem et al 1996, Gilja et al 1999a). 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 1999a). Ultrasonography has been used to measure gastric emptying (Holt et al 1980, Bateman and Whittingham 1982, Bolondi et al 1985, Holt et al 1986, Marzio et al 1989, Duan et al 1993, Ricci et al 1993), is non-invasive, associated with low cost, does not entail a radiation burden, and is widely available (Hveem et al 1996, Gilja et al 1999a).

Two-dimensional (2D) ultrasonography techniques have been used to quantify antral wall motion (Holt et al 1980, Vogelberg et al 1987, Hausken et al 1991), short-term patterns of transpyloric flow (King et al 1984, Hausken et al 1992, Brown et al 1993) and gastric emptying. The latter is evaluated indirectly by determining changes in 2D antral cross-sectional area (Hausken and Berstad 1992, Hausken et al 1993, Hveem et al 1996) or diameter (Holt et al 1986, Bergmann et al 1992). A previous study demonstrated that 2D ultrasonography measurements of antral area, are comparable in sensitivity to scintigraphy, in quantifying emptying of both low- and high- nutrient drinks (Hveem et al 1996). In healthy young subjects, there was a close correlation and good agreement between scintigraphic and ultrasonographic 50% emptying times and a close concordance between the content of the distal stomach (expressed as a % of its maximum) measured scintigraphically and ultrasonographic 50% emptying time.
(Hveem et al 1996). The measurement of gastric emptying using 2D ultrasonography techniques is reliant on the concept that the retention of a nutrient-containing drink in the distal stomach is related to the rate of emptying from the total stomach (Horowitz et al 1993a), so that a decline in antral area over time is indicative of more rapid gastric emptying. However, in patients with gastroparesis (as occurs in longstanding diabetes (Fraser et al 1993) and patients with functional dyspepsia (Malagelada and Stanghellini 1985, Camilleri et al 1986, Kerlin 1989)), in whom both fasting and postprandial antral hypomotility is frequently diminished (Horowitz and Fraser 1995), the utility of this technique is limited. These patients may retain a greater proportion of a meal in the distal, when compared with the proximal, stomach (Gilja et al 1996a). Hence, measurement of antral area may not reflect true intragastric meal distribution in this situation (Troncon et al 1994). Furthermore, while 2D ultrasonography techniques can be used to estimate volumes in the gastric antrum, using either the sum-of-cylinders method (Whittingham and Bateman 1983, Radberg et al 1989), or algorithms (Bolondi et al 1985), significant errors may be introduced given the inherent geometric assumptions in volume estimations (Gilja et al 1999b).

More recently, 3D ultrasonography has been applied to the measurement of gastric volumes with both in-vitro (Gilja et al 1994, Thune et al 1996, Gilja et al 1997) and in-vivo (Gilja et al 1995a, Tefera et al 2002) validation. Gilja et al (1997), initially evaluated the in-vitro accuracy of 3D ultrasonography in volume estimation of a porcine stomach filled with water and demonstrated a close correlation and agreement between true and estimated volumes. Subsequently, Tefera et al (2002), reported a close
correlation and agreement between true and estimated volumes of a barostat bag filled
with soup and positioned in the proximal stomach, as well as low interobserver
variation using 3D ultrasonography. However, in the latter study it was assumed that a
constant pressure level is maintained in the stomach similar to that within the soup-
filled barostat bag and that the stomach is devoid of wall motion generated by muscular
contractions. Importantly, while these recent studies suggest that 3D ultrasonography
may allow a precise measure of gastric emptying, by utilising a direct method of
volume determination, comparison of this technique with the ‘gold standard’,
scintigraphy, has hitherto not been performed.

In normal subjects, gastric emptying of solid meals (after an initial lag phase)
(Horowitz et al 1994) and high nutrient drinks, approximates an overall linear pattern
(Collins et al 1983, Camilleri et al 1985) with an emptying rate of ~ 1 - 3 kcal/min; this
tight regulation occurs primarily as a result of inhibitory feedback arising from
receptors located throughout the small intestine (Horowitz et al 1994). In contrast, low
nutrient drinks empty more quickly, in a mono-exponential pattern, (Collins et al 1983,
Camilleri et al 1985) as they do not stimulate small intestinal mechanisms which retard
gastric emptying to the same extent (Horowitz and Fraser 1995). In the diagnosis of
gastroparesis, the use of test meals that trigger small intestinal mechanisms, ie high
nutrient liquids or solids, are likely to increase sensitivity.

In this study, measurements of gastric emptying of both low- and high- nutrient drinks
with 3D ultrasonography and scintigraphy were compared in healthy young subjects.
5.3 Research design and methods

Ten healthy young subjects, (six male and four female) with a mean age of 23 years (range: 18 - 35 years) and body mass index (BMI) of 22.5 ± 0.8 kg/m² (range: 18.5 - 25.4 kg/m²), recruited by advertisement, were studied. The author calculated that a minimum of 6 subjects would be required to detect a correlation of 0.95 between the 3D ultrasonographic and scintigraphic techniques with power of 0.80 in a two-sided test, assuming a significance value < 0.05. 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 was taking medication known to influence gastrointestinal function.

5.3.1 Experimental protocol

Each subject underwent measurements of gastric emptying by scintigraphy and 3D ultrasonography at the same time on two occasions, in random order, and separated by at least three days. On each day, subjects attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry, Royal Adelaide Hospital, at either 08.30 hours or 14.00 hours following a fast of at least 14 hours for solids and 12 hours for liquids. In each subject the two studies were performed at the same time of the day. On arrival, subjects were seated with their back against a gamma camera (GEnie, GE Healthcare Technologies, Milwaukee, USA) and at t = -2 minutes, ingested either 500ml (low nutrient) beef soup (12 kcal) (Maggi® beef stock cubes, Nestlé Australia Ltd, North Ryde, NSW, Australia) or 300ml water containing 75g dextrose (25% w/v)
(high nutrient) (314 kcal) labelled with 20MBq \(^{99m}\)Tc sulphur colloid within two minutes.

### 5.3.2 Scintigraphy

Radioisotopic data were acquired for 60 minutes after ingestion of the soup, and for 180 minutes after the dextrose (to ensure that > 50% of the drinks had emptied from the stomach) according to the method described in Chapter 4.4.1. Regions-of-interest were drawn around the total stomach (Figure 5.1) and gastric emptying curves (expressed as % retention over time) derived. The amount of the drink remaining in the total stomach at \(t = 0, 10, 20, 30, 40, 50\) and 60 minutes for soup, and at \(t = 0, 15, 30, 45, 60, 90, 120, 150\) and 180 minutes for dextrose, were calculated and expressed as a percentage of the counts in the total stomach (ie at \(t = 0\) minutes, 100% of the drink was in the total stomach) (Collins et al 1983). The 50% emptying time (ScT50) was also determined (Collins et al 1983). The lag phase was defined visually as the time before any of the radioactivity had entered the proximal small intestine (Collins et al 1983).

### 5.3.3 Ultrasonography

Ultrasonographic measurements were performed with a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, NSW, Australia) using the technique described in Chapter 4.4.2.2. For 3D data acquisition, subjects were scanned immediately before \((t = -5\) minutes\) and after drink ingestion \((t = 0\) minutes\), followed by images at \(t = 10, 20, 30, 40, 50\) and 60 minutes for soup and at \(t = 15, 30, 45, 60, 90, 120, 150\) and 180 minutes for dextrose. The stored images were copied to CD-ROM.
and then transferred to a windows workstation. Data processing and volume estimation were performed with the use of EchoPAC-3D software® (GE Vingmed Sound, Horten, Norway), as described (Figure 5.2) (Tefera et al 2002). The volume of the drink in the total stomach was derived and expressed as a percentage of the original volume at t = 0 minutes in the total stomach, where 100% immediately followed ingestion of the drink (Collins et al 1983). The 50% emptying time (UT50) was also determined (Hausken and Berstad 1992). Visible amounts of air in the stomach were graded as: 1, small amounts of air in the fundus; 2, moderate amounts; and 3, great amounts of air, necessitating exclusion from the study (Tefera et al 2002).

### 5.3.4 Statistical analysis

Data were evaluated using repeated measures 2 way Analysis of Variance (ANOVA). Non-parametric comparisons between groups (T50, lag phase) were performed by the Wilcoxon signed rank test. Correlations were assessed by linear regression analysis. Limits of agreement analyses were performed according to Bland and Altman (1986), such that the difference between ScT50 and UT50 was plotted against the mean of the two methods (difference plot) (Bland and Altman 1986, Pollock et al 1992) and limits of agreement were defined as acceptable if within the mean (2 standard deviation (SD)) difference (Bland and Altman 1986). All analyses, unless stated otherwise, were performed using Statview (version 5.0; Abacus Concepts, Berkeley, CA, USA) and SuperANOVA (version 1.11, Abacus Concepts, Berkeley, CA, USA). Limits of agreement analyses were performed by a professional statistician using SAS 9.1 (SAS
Institute Inc., Cary, NC, USA). Data are presented as mean values ± standard error of the mean (SEM) and a P value < 0.05 was considered significant in all analyses.

5.4 Results

All subjects tolerated the study well and none had to be excluded because of air pockets in the gastric fundus. All subjects had a small amount of visible air in the gastric fundus (ie grade 1) during both studies and four subjects had moderate amounts of air (ie grade 2) after the dextrose drink. The extent of air pockets in the gastric fundus did not influence the results. In one subject, data from the soup study were not included because of poor image visualisation.

5.4.1 Scintigraphic measurements of gastric emptying

After a short lag phase (soup: 2.8 ± 0.9 minutes vs dextrose: 3.0 ± 1.1 minutes; P = 0.67), gastric emptying of soup approximated a mono-exponential, and dextrose, an overall linear, pattern. Gastric emptying of the soup was more rapid than the dextrose drink (ScT50 - soup: 27.7 ± 4.8 minutes vs dextrose: 122.2 ± 13.3 minutes; P = 0.005).

5.4.2 Relations, and limits of agreement, between scintigraphic and 3D ultrasonography measurements

There was no significant difference in mean fasting volume (soup: 51.8 ± 13.0 ml vs dextrose: 39.9 ± 6.6 ml) before ingestion of the drink (t = -5 minutes). There was no significant difference between the ScT50 and UT50 (soup - scintigraphy: 27.7 ± 4.8 minutes vs ultrasonography: 23.8. ± 4.8 minutes; dextrose - scintigraphy: 122.2 ± 13.3
minutes vs ultrasonography: 131.9 ± 10.2 minutes). Nor were there any differences in the overall curves for gastric emptying between the techniques (Figures 5.3 (a) and 5.3 (b)).

There was a close correlation between the ScT50 and UT50 for both soup (r = 0.92, P = 0.0005) (Figure 5.4 (a)) and dextrose (r = 0.88, P = 0.0007) (Figure 5.4 (b)). For the T50’s, the limits of agreement were -15.2 minutes and +8.1 minutes for the soup (mean difference -3.6 minutes) (Figure 5.5 (a)) and -35.3 minutes and +47.6 minutes for dextrose (mean difference +6.2 minutes) (Figure 5.5 (b)).

5.5 Discussion

This study demonstrates that there is good correlation and agreement between scintigraphic measurements of gastric emptying and 3D ultrasonography after ingestion of both low- and high- nutrients drinks in healthy young subjects.

More recently, 3D ultrasonography has been utilised to image the stomach as it offers high precision and accuracy in volume determination (Gilja et al 1999b). 3D ultrasonography also allows total and partial gastric volumes and intragastric distribution to be evaluated directly (Tefera et al 2002, Scheffer et al 2004, Mundt et al 2005). Hitherto, studies have validated this technique against a porcine model in-vitro (Gilja et al 1997) and a gastric barostat in humans (Tefera et al 2002). In both studies, the 3D ultrasonography technique correlated well and showed excellent agreement with infused volumes (Gilja et al 1997, Tefera et al 2002). In the current study, there was a close correlation and agreement as the scatter of the difference in T50’s between the 3D ultrasonographic and scintigraphic techniques were within the mean ± 2 SD, suggesting that 3D ultrasonography provides a true measurement of gastric emptying of liquids. The concordance between the two methods is yet to be confirmed in patients with disordered gastric emptying, however, the use of a high nutrient drink may potentially be useful in the evaluation of patients with gastroparesis. Furthermore, it should be acknowledged that gender differences were not considered in this study and that ten subjects may well be insufficient to establish an acceptable normal range for measurement of gastric emptying with 3D ultrasonography. These issues represent priorities for future studies. While the most precise tissue attenuation correction for gastric emptying is the geometric mean of the gastric counts from both anterior and posterior images (Christian et al 1980), in this study a method which involves applying a depth correction from a lateral image to the posterior images was utilised (Collins et al 1984). Which, may potentially have contributed to the slight, albeit non-significant,
differences in gastric emptying curves between the scintigraphic and 3D ultrasonographic techniques.

A limitation of scintigraphy is that most gastric emptying studies only measure emptying of the meal post ingestion ie the ‘post fill’ period (O'Donovan et al 2005a); this assumes that fasting gastric content is small and very little, if any, emptying of stomach contents occurs during meal ingestion (Tougas et al 2000, Berry et al 2003). In this current study, there was about 40 - 50ml in the stomach prior to drink ingestion. Kaplan et al (1992), have reported that a large proportion of a drink may empty before ingestion is complete. A recent study (O'Donovan et al 2005a), confirmed that gastric emptying occurs during ingestion of a drink in healthy subjects ie during ‘gastric fill’. Accordingly, in order to accurately compare measurements of gastric emptying by 3D ultrasonography and scintigraphy, the author only considered data acquired during the post ingestion period. In contrast to scintigraphy, 3D ultrasonography has the advantage of measuring total volume of the stomach (ie fasting and postprandial drink volumes and gastric secretions that are produced in response to the drink (Holt et al 1986)). However, 3D ultrasonography also has some limitations - intragastric air, particularly in the fundus which may be influenced by posture (Gilja et al 1997), has the potential to limit the visualisation of the gastric outline (Holt et al 1980, Bolondi et al 1985, Gilja et al 1997), although previous studies indicate that this is not a major issue (Gilja et al 1995b, Gilja et al 1996a, Scheffer et al 2004, Mundt et al 2005). The technique is also user dependent, requires 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 utilised 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). Despite these issues, 3D ultrasonography is superior to 2D ultrasonography as it allows visualisation of anatomical detail (Molin et al 1999) and offers greater accuracy and precision in determining gastric emptying of liquids (Gilja et al 2005), by providing a direct measure of total gastric volume.

In conclusion, this study has demonstrated that 3D ultrasonography, provides a valid measure of gastric emptying of liquids, when compared with the ‘gold standard’ scintigraphy.
Figure 5.1: Posterior scintigraphic image depicting total stomach region of interest.
Figure 5.2: (a) Ultrasonographic image of the stomach, following 3D reconstruction, demonstrating region of interest and (b) 3D reconstructed volumetric image of the stomach.
Figure 5.3: Retention of (a) soup and (b) dextrose in the total stomach as assessed by scintigraphy (●) and 3D ultrasonography (○). Data are mean values ± SEM.
Figure 5.4: Relation between scintigraphic (ScT50) and 3D ultrasonographic (UT50) 50% emptying times for (a) soup and (b) dextrose. Data are mean values ± SEM. The dashed line indicates $r = 1.0$ correlation.
Figure 5.5: Plot displaying the limits of agreement for scintigraphic (ScT50) and 3D ultrasonographic (UT50) 50% emptying times (T50’s) for (a) soup and (b) dextrose.
Effects of intraduodenal glucose concentration on blood pressure and heart rate in healthy elderly subjects
6.1 Summary

Postprandial hypotension, after ingestion of carbohydrate, occurs frequently in the elderly. Recent studies suggest that the magnitude of the fall in blood pressure is related to the rate of glucose entry into the duodenum. It is not known whether this reflects the potential effects of the small intestinal glucose load and/or concentration. The aims of this study were to determine whether the hypotensive and heart rate responses to small intestinal glucose infusion are dependent on the glucose concentration. Eight healthy subjects, aged 65 - 78 years, were studied on three separate occasions, in randomised order on which they received an intraduodenal infusion of 50g glucose in saline (0.9%) made up to either 300ml (16.7%), 600ml (8.3%) or 1200ml (4.1%), at a rate of ~ 3 kcal/min for 60 minutes (t = 0 - 60 minutes), followed by saline (0.9%) for a further 60 minutes (t = 60 - 120 minutes). During the infusions, blood pressure (systolic and diastolic) and heart rate were measured every 3 minutes, and blood glucose concentrations every 15 minutes. Systolic and diastolic blood pressure fell (P < 0.0001), and heart rate and blood glucose increased (P = 0.0001 for both) over time, during all three infusions. Between t = -2 - 120 minutes, there was no difference in systolic blood pressure (P = 0.20), diastolic blood pressure (P = 0.61), or heart rate (P = 0.09) between the study days. There was also no significant difference in the glycaemic response to the infusions. It is concluded that in healthy elderly subjects, the glucose concentration does not affect the blood pressure or heart rate responses to intraduodenal glucose. Accordingly, the magnitude of the postprandial fall in blood pressure induced by oral glucose is likely to be dependent primarily on the small intestinal glucose load.
6.2 Introduction

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20 mmHg, occurring within two hours of a meal (Mathias et al 1989a, Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995), is now recognised as an important clinical problem, particularly in the elderly (Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995) and in patients with autonomic nerve dysfunction (Jansen and Lipsitz 1995). The mechanisms responsible for postprandial hypotension are poorly understood, but impaired regulation of splanchnic blood flow (Puisieux et al 2000), the release of gastrointestinal hormones (Jansen and Hoefnagels 1991) and changes in sympathetic nerve activity (Masuo et al 1996) may be important. Current therapies are sub-optimal (Jansen and Lipsitz 1995).

The fall in blood pressure is known to be dependent on meal composition (Potter et al 1989, Jansen et al 1990); ingestion of carbohydrate, particularly glucose, is said to have the greatest effect on blood pressure (Potter et al 1989, Jansen et al 1990). It has recently been demonstrated, in both healthy elderly subjects (Jones et al 2001, O'Donovan et al 2002) and type 2 diabetic patients (Jones et al 1998, Russo et al 2003), that the magnitude of the hypotensive response to oral glucose is greater when gastric emptying is relatively more rapid. Furthermore, when glucose is infused intraduodenally at a rate of ~ 3 kcal/min, in elderly subjects, the fall in blood pressure and rise in heart rate are substantially greater than observed during a ~ 1 kcal/min infusion (O'Donovan et al 2002). These observations might indicate that the blood pressure response to enteral glucose is dependent on the glucose load (ie g/min)
entering the small intestine, as has been established to be the case for gastric emptying of glucose (McHugh and Moran 1979, Brener et al 1983, Lin et al 1989). However, previous studies (Vloet et al 2001, O'Donovan et al 2002, Russo et al 2003, O'Donovan et al 2005b) do not allow the potential effects of glucose load to be distinguished from those of the glucose concentration. For example, in the study by O'Donovan et al (2002), the duodenal glucose loads of ~ 1 and 3 kcal/min were achieved by infusing, respectively, 8.3% and 25% glucose at a rate of ~ 3 ml/min. That is, both the caloric load and the glucose concentration were raised in parallel. In a recent study (Jones et al 2005) both the glucose content and volumes of orally ingested drinks were varied. While the results indicated that postprandial hypotensive responses were the same after drinks of low or high concentrations, as long as the load (g/min) entering the duodenum was constant, the interpretation of these data was complicated by counter-regulation induced by large meal volumes (gastric distension). The author, therefore, undertook the present study utilising duodenal perfusion to eliminate potential effects of gastric distension.

### 6.3 Research design and methods

Eight healthy elderly subjects, recruited by advertisement (five female and three male) with a median age of 71 years (range: 65 - 78 years) and median body mass index (BMI), 26.5 kg/m² (range: 22.2 - 29.2 kg/m²), were studied. All subjects were non-smokers and none had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal or cardiac disease, chronic alcohol abuse or epilepsy, or were taking medication known to influence blood pressure or gastrointestinal function.
Cardiovascular autonomic nerve function was evaluated (Ewing and Clarke 1982, Piha 1991) in each subject during one of the visits.

### 6.3.1 Experimental protocol

Each subject underwent three studies in randomised order; each study was separated by at least three days. Subjects attended the University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital, at 08.30 hours following a fast (10.5 hours for solids; 8.5 hours for liquids). At that time, a silicone-rubber catheter (~ 4mm diameter) was introduced into the stomach via an anaesthetised nostril (O'Donovan et al 2002). The catheter included an infusion channel with a port located ~ 10cm distal to the pylorus (ie in the duodenum) and two other channels, positioned in the antrum (2.5cm proximal to the pylorus) and duodenum (2.5cm distal to the pylorus). The latter two channels were perfused with normal saline (0.9%). A saline-filled, reference electrode (20 gauge intravenous cannula) was inserted subcutaneously into the subject’s forearm to enable measurement of the antroduodenal transmucosal potential difference (TMPD) (O'Donovan et al 2002). The tip of the catheter was allowed to pass into the duodenum by peristalsis, which took between 30 and 205 minutes. An intravenous cannula was positioned in a right antecubital vein for blood sampling, and an automated blood pressure cuff placed on the left arm (O'Donovan et al 2002). Once intubated, subjects were studied in the recumbent position. Approximately 30 minutes after the tube was in position (at t = 0 minutes) an intraduodenal infusion of 50g glucose dissolved in saline (0.9%) for a total volume of either 300ml (16.7%), 600ml (8.3%) or 1200ml (4.1%), was commenced and maintained at a rate of 5 ml/min, 10 ml/min and 20 ml/min
respectively, for 60 minutes ie in all cases the energy delivery was \(\sim 3\) kcal/min. Between \(t = 60 - 120\) minutes saline (0.9%) was infused intraduodenally, at 5 ml/min, 10 ml/min or 20 ml/min. At \(t = 120\) minutes the catheter, intravenous cannula and reference electrode were removed; the subject was then given a light meal and allowed to leave the laboratory. All solutions were infused at room temperature ie \(\sim 22^\circ\)C.

### 6.3.2 Measurement of blood pressure and heart rate

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP, Johnson & Johnson, Tampa, FL, USA) immediately prior to the intraduodenal infusion (ie baseline at \(t = -2\) minutes) and then every three minutes between \(t = 0 - 120\) minutes (O'Donovan et al 2002). Postprandial hypotension was defined as a fall in systolic blood pressure \(\geq 20\) mmHg after glucose that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

### 6.3.3 Measurement of blood glucose concentrations

Venous blood samples (~ 5ml) were obtained immediately prior to commencement of the infusion (\(t = -2\) minutes) and at 15 minute intervals between \(t = 0 - 120\) minutes. Blood glucose concentrations were determined according to the method described in Chapter 4.8.
6.3.4 **Assessment of cardiovascular autonomic nerve function**

On one of the study days, after completion of the intraduodenal infusions, autonomic nerve function was evaluated using standardised cardiovascular reflex tests (Ewing and Clarke 1982, Piha 1991). A description of the method is included in Chapter 4.10.

6.3.5 **Statistical analysis**

Data were evaluated using repeated measures 2 way Analysis of Variance (ANOVA) and are presented as mean values ± standard error of the mean (SEM), unless stated otherwise. Contrasts were used to examine point-by-point comparisons to test pre-planned hypotheses of interest and in the event of a ‘treatment x time’ interaction. All cardiovascular parameters (systolic blood pressure, diastolic blood pressure and heart rate) and changes in blood glucose concentrations were analysed as absolute values. All analyses were performed using Statview (version 5.0; Abacus Concepts, Berkeley, CA, USA) and SuperANOVA (version 1.11, Abacus Concepts, Berkeley, CA, USA). A P value < 0.05 was considered significant in all analyses.

6.4 **Results**

The studies were, in general, well tolerated. No subject had definite autonomic neuropathy; the median score for autonomic nerve dysfunction was 1.0 (range: 0 - 2). Seven of the eight subjects reported diarrhoea after the 1200ml infusion - in all cases the onset of diarrhoea was soon after the end of the infusion (ie t = 120 minutes) and in all cases had resolved within six hours. One subject reported mild nausea on all three study days.
6.4.1 Blood pressure and heart rate

There was no significant difference in baseline (ie t = -2 minutes) blood pressure or heart rate between the three study days (300ml vs 600ml vs 1200ml): systolic blood pressure (131.6 ± 5.8 mmHg vs 135.3 ± 7.8 mmHg vs 130.9 ± 6.0 mmHg); diastolic blood pressure (67.1 ± 3.6 mmHg vs 67.6 ± 4.2 mmHg vs 64.3 ± 4.0 mmHg) and heart rate (63.3 ± 1.7 beats/minute (bpm) vs 63.4 ± 3.2 bpm vs 64.4 ± 3.7 bpm) (Figure 6.1).

Between t = -2 - 120 minutes, there was no significant difference in systolic blood pressure (P = 0.20), diastolic blood pressure (P = 0.61) or heart rate (P = 0.09) over the three study days (Figure 6.1). There was a significant ‘time’ effect for systolic blood pressure, diastolic blood pressure and heart rate (P < 0.0001 for all) between t = -2 - 120 minutes. Systolic and diastolic blood pressure fell, and heart rate increased, on all study days following the glucose infusions. The maximum falls in systolic blood pressure during the glucose infusions were 6.9 ± 1.4 mmHg at 24 minutes, 6.9 ± 2.9 mmHg at 15 minutes and 5.8 ± 3.3 mmHg at 27 minutes, respectively, for the 16.7%, the 8.3%, and the 4.1% glucose solutions. The magnitude of the fall in systolic blood pressure was > 20 mmHg in one subject during the 600ml infusion. On all study days, systolic blood pressure had returned to baseline by 60 minutes (Figure 6.1).

6.4.2 Blood glucose concentrations

There was no significant difference (16.7% vs 8.3% vs 4.1% glucose: 6.2 ± 0.2 mmol/L vs 6.2 ± 0.1 mmol/L vs 6.3 ± 0.2 mmol/L), in baseline blood glucose concentrations between the three study days. Between t = -2 - 120 minutes, there was a rise in blood
glucose (P < 0.0001) on all three study days. Blood glucose concentrations were less than baseline at t = 120 minutes on all three study days (P < 0.004). There was no significant difference in blood glucose concentrations between t = -2 - 120 minutes (P = 0.64) (Figure 6.2).

6.5 Discussion

This study indicates that the blood pressure and heart rate responses to enteral glucose are not influenced by glucose concentrations in the range of 4.1 - 16.7% in healthy elderly subjects. It can, therefore, be inferred from previous studies (O'Donovan et al 2002, Russo et al 2003, Jones et al 2005, O'Donovan et al 2005b) and Vloet et al (2001) that glucose induces postprandial hypotension in a load-dependent manner. The present observations strengthen the findings of a recent study, in healthy elderly subjects, which suggested that the postprandial fall in blood pressure after oral glucose is dependent on the small intestinal load, but not the concentration, of glucose (Jones et al 2005). In this study it was reported that there was no difference in the effects of glucose drinks at different concentrations (ie between 4% - 37%) on postprandial blood pressure when ingested at the same volume (ie 200ml or 600ml) (Jones et al 2005).

The magnitude of the falls in systolic and diastolic blood pressure in response to 200ml glucose drinks are known to be greater with increased carbohydrate content (25g, 75g or 125g) (Vloet et al 2001) and when glucose is infused intraduodenally at a rate of ~ 3 kcal/min when compared to ~ 1 kcal/min (O'Donovan et al 2002). These studies were, however, not designed to discriminate between the potential effects of the small
intestinal glucose load and glucose concentration on blood pressure and heart rate. As gastric distension may attenuate the hypotensive response to glucose (Rossi et al 1998, Shannon et al 2002, Jones et al 2005) glucose was infused directly into the small intestine at concentrations of 4.1%, 8.3% and 16.7% at a constant rate, thus eliminating the potential effects of gastric distension and allowing the effects of glucose concentration, independent of load, to be determined. There was a gradual fall in blood pressure, and a rapid increase in heart rate, during all three infusions; while the magnitude of the responses was substantial, and comparable to those observed previously at loads of ~ 3 kcal/min (O'Donovan et al 2002), there was no difference between them. In interpreting these observations, it should be recognised that small intestinal distension may potentially influence blood pressure and heart rate in a similar manner to gastric distension (Rossi et al 1998, van Orshoven et al 2004). Increases in blood pressure and heart rate have been observed in rats during small intestinal distension resulting in intraluminal pressures of 20 mmHg or higher (Timar-Peregrin et al 2001), as well as in human volunteers in response to painful distension of the sigmoid colon (ie 20 - 70 mmHg) (Ness et al 1990). Accordingly, it is possible that greater distension of the small intestinal wall, as infusion volume increased, attenuated the postprandial hypotensive response to intraduodenal glucose. Arguing against this, Fordtran and Locklear (1966) reported that the duodenum is very permeable to water and, hence, in our study, it is possible that the absorption of glucose occurred in similar volumes on the three study days, thereby excluding the potential effects of small intestinal distension. After ~ 75 minutes, mean blood pressure and heart rate were higher after the 1200ml infusion - while these differences were small and not
significant, they may be attributable to discomfort/anxiety (which were not reported) as seven of the eight subjects experienced diarrhoea soon after the completion of the experiment; saline is well recognised to have cathartic properties.

A number of gastrointestinal responses to glucose and other nutrients have been shown to be primarily dependent on the load and not the concentration. These include the regulation of gastric emptying (McHugh and Moran 1979), pancreatic exocrine secretion (Meyer et al 1976a) and appetite (Meyer et al 1998). Gastric emptying of glucose solutions, in the range of concentrations evaluated in the current study, has been shown to be tightly regulated in both animals (McHugh and Moran 1979) and humans (Brener et al 1983, Hunt et al 1985) so that in a given animal or human the rate at which glucose enters the small intestine is constant at ~ 1 - 3 kcal/min independent of the glucose concentration (Brener et al 1983). This regulation has been shown to reflect small intestinal feedback, which is related to the length of small intestine exposed to glucose (Lin et al 1989). A comparable mechanism leads to the suppression of energy intake by oral glucose (Lin et al 1989). The absence of any difference in the blood glucose response to the three infusions was expected, given the efficiency of small intestinal glucose absorption. It is appropriate to note that intravenous glucose does not affect blood pressure in the elderly (Jansen and Hoefnagels 1991, Jansen and Lipsitz 1995) and that postprandial hypotension occurs in patients with type 1 diabetes, who are by definition insulin deficient (Jansen et al 1990). Hence, rises in blood glucose (O'Donovan et al 2002) and insulin (Jansen et al 1987, Jansen et al 1990) do not appear to play a major role in the hypotensive response to oral glucose.
That the hypotensive response to enteral glucose may depend on the concentration of the duodenal effluent was suggested by the observation that both the fall in blood pressure and rise in heart rate induced by a glucose drink (Jones et al 2001, Russo et al 2003) and intraduodenal glucose (O'Donovan et al 2005b) are attenuated by guar. Guar slows gastric emptying (Meyer et al 1988) and small intestinal glucose absorption (Meyer et al 1988, Jones et al 2001, Russo et al 2003, O'Donovan et al 2005b); while the slowing of gastric emptying probably reflects the higher viscosity of the gastric content, the dominant mechanism is likely to be an increase in small intestinal feedback secondary to the exposure of a greater length of small intestine to glucose (Meyer et al 1988). Guar may also slow glucose absorption by acting as a physical barrier (Blackburn et al 1984b). It has been demonstrated that glucose ‘sensors’ which regulate gastric emptying (Lin et al 1989) and appetite (Meyer et al 1998) are distributed throughout both the proximal and distal small intestine, and that inhibition is dependent on the length of small intestine allowed exposure to glucose (Lin et al 1989). Nevertheless, at some point, as concentration (and thus rate of absorption across the signalling cell) becomes progressively less, a concentration-dependence may appear.

For example, at pH above 2.5 - 3.0 (respectively, for inhibition of gastric emptying (Lin et al 1990) or stimulation of pancreatic bicarbonate secretion (Meyer et al 1970)) and from 3 - 9 mM oleic acid on inhibition of gastric emptying (Lin et al 1990). Accordingly, while it was demonstrated in the present experiments that varying glucose concentrations from 4.1 - 16.7% (233 - 928 mM) does not alter the hypotensive response to ~ 3 kcal/min of glucose, it is nevertheless probable that an effect of concentration would be evident at lower concentrations.
Figure 6.1: Effects of 60 minute intraduodenal glucose infusions (~ 3 kcal/min) in volumes of 300ml (●), 600ml (○) and 1200ml (□) on (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate. Data are mean values ± SEM. P values indicate ‘treatment’ effect.
Figure 6.2: Effects of 60 minute intraduodenal glucose infusions (~ 3 kcal/min) in volumes of 300ml (●), 600ml (∗) and 1200ml (□) on blood glucose concentrations. Data are mean values ± SEM. P value indicates ‘treatment’ effect.
Comparative effects of intraduodenal glucose, triglyceride and protein on blood pressure, heart rate and splanchnic blood flow in healthy elderly subjects
7.1 Summary

Postprandial hypotension represents an important clinical problem in the elderly. Meal composition has been reported to influence the magnitude of the fall in blood pressure after a meal. There is evidence that the greatest falls occur after carbohydrate; information relating to the effects of triglyceride and protein are conflicting. In these studies, nutrients have been given orally and a distinction could accordingly not be made between the intragastric effects of gastric distension and small intestinal nutrient exposure. Changes in splanchnic blood flow are likely to be important in the postprandial fall in blood pressure. The aims of this study were to determine the comparative effects of isocaloric and isovolaemic intraduodenal glucose, triglyceride and protein infusions on blood pressure, heart rate and superior mesenteric artery blood flow in healthy elderly subjects. Eight subjects (four female and four male, aged 68 - 79 years) were studied on four days, in single-blind, randomised order. Subjects received intraduodenal glucose (64g), triglyceride (10% Intralipid®), protein (72g whey) or saline (0.9%) at a rate of 2.7 ml/min for 90 minutes, followed by intraduodenal saline for a further 30 minutes. Blood pressure, heart rate, and superior mesenteric artery blood flow were measured. There were comparable (P = 0.97) maximum falls in systolic blood pressure during glucose (11.7 ± 2.8 mmHg), triglyceride (11.7 ± 4.8 mmHg) and protein (11.0 ± 1.5 mmHg), however, the onset of the maximum fall in response to glucose was earlier (glucose: 18 ± 3.0 minutes vs triglyceride: 46 ± 11.0 minutes; P = 0.02 and vs protein 33 ± 7.0 minutes; P = 0.04). Heart rate increased during glucose, triglyceride and protein (P < 0.0001 for all) and there was no significant difference between nutrients. Superior mesenteric artery blood flow increased after all
of the nutrients (glucose: P < 0.0001, triglyceride: P < 0.001, protein: P < 0.001) and the rise in superior mesenteric artery blood flow was lower after protein when compared with both glucose (P < 0.05) and triglyceride (P < 0.01). Intraduodenal glucose, triglyceride and protein induce a comparable fall in systolic blood pressure and rise in heart rate, but the effects of protein on superior mesenteric artery blood flow may be less than both glucose and triglyceride.

7.2 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) within two hours of a meal (Jansen and Lipsitz 1995), occurs frequently and is now recognised as an important clinical problem particularly in the elderly and in patients with autonomic dysfunction, often secondary to diabetes mellitus (Jansen and Lipsitz 1995, Fisher et al 2005). The onset of the fall in blood pressure is usually evident almost immediately, but can occur at any time from 15 - 75 minutes, after a meal (Jansen and Lipsitz 1995), usually with a nadir at 30 - 60 minutes (Aronow and Ahn 1994). Postprandial hypotension is associated with significant sequelae, including syncope, falls, weakness, angina, dizziness, visual disturbance and cerebrovascular accidents (Lipsitz et al 1983, Vaitkevicius et al 1991, Jansen and Lipsitz 1995, Aronow and Ahn 1997). Current approaches to management are suboptimal.

The mechanisms mediating postprandial hypotension are poorly defined although impaired regulation of splanchnic blood flow, which can be quantified by Doppler
ultrasonography, has been identified as a possible pathophysiological mechanism (Jansen and Lipsitz 1995). It has been established that the magnitude of the fall in blood pressure is dependent on meal composition; ingestion of carbohydrate, particularly glucose, has the greatest effect on blood pressure (Jansen et al 1990, Jansen and Lipsitz 1995). While oral ingestion of glucose (Mathias et al 1989a, Jansen et al 1990), sucrose (Visvanathan et al 2005) and to some extent starch (Heseltine et al 1991a), leads to a fall in blood pressure in normal elderly subjects and patients with chronic autonomic failure, intravenous infusion of glucose has little, if any, effect (Jansen and Lipsitz 1995), indicating that the response is mediated from the gastrointestinal tract. It has recently been demonstrated that the rate of nutrient delivery from the stomach to the small intestine is a major determinant of the hypotensive response to enteral glucose (O'Donovan et al 2002). For example, in healthy elderly subjects, when glucose is infused intraduodenally at a rate of ~3 kcal/min when compared to infusion at a rate of ~1 kcal/min (the normal physiological range for gastric emptying), the magnitude of the fall in systolic blood pressure and rise in heart rate, are substantially greater (O'Donovan et al 2002). Recent studies (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) indicate that ‘intragastric’ mechanisms related to gastric distension may reduce the postprandial fall in blood pressure.

Information relating to the effects of the other main nutrients, triglyceride and protein, on postprandial blood pressure is limited and inconsistent (Hoeldtke et al 1985, Potter et al 1989, Jansen et al 1990, Waaler and Eriksen 1992, Sidery et al 1993, Kearney et al
While it has been suggested that triglyceride (Potter et al 1989, Jansen et al 1990, Waaler and Eriksen 1992, Kearney et al 1996) and protein (Jansen et al 1990, Waaler and Eriksen 1992) have relatively little effect on blood pressure, other studies have reported a significant fall in blood pressure after high-triglyceride (Hoeldtke et al 1985, Sidery et al 1993) and protein meals (Potter et al 1989). Studies indicate that in healthy young subjects, the slowing of gastric emptying by triglyceride (Schwizer et al 1997) and protein (Burn-Murdoch et al 1978), and suppression of hunger after high-protein meals (Vandewater and Vickers 1996), are dependent on lipolysis of triglyceride to fatty acids (Feinle et al 2001) and proteolysis of protein to amino acids (Silk et al 1985). Accordingly, a triglyceride emulsion, which has been previously shown to slow gastric emptying in healthy young volunteers (Heddle et al 1989), and a whey (97%) protein concentrate with a low carbohydrate content, mainly lactose (~ 46 kcal of lactose apparently had no effect on the blood pressure response to a high-triglyceride drink (Visvanathan et al 2006)) were infused intraduodenally. The relative effects of triglyceride and protein on postprandial blood pressure in elderly individuals remain poorly defined and, therefore, have important implications for the dietary management of postprandial hypotension.

(Moneta et al 1988, Qamar and Read 1988, Waaler and Eriksen 1992, Sidery et al 1993, Sidery and Macdonald 1994) and protein (Moneta et al 1988, Qamar and Read 1988) loads, gastric emptying has not been quantified, therefore, differences in small intestinal nutrient delivery may have influenced the outcome of the studies.

The aim of this study was to determine the comparative effects of isocaloric and isovolaemic intraduodenal glucose, triglyceride and protein infusions on blood pressure, heart rate and superior mesenteric artery blood flow in healthy elderly subjects.

7.3 Research design and methods

Eight healthy elderly subjects, (four female and four male) with a median age of 74 years (range: 68 - 79 years) and body mass index (BMI) of 24.5 kg/m² (range: 21.2 - 28.2 kg/m²), recruited by advertisement, were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal, hepatic or cardiac disease, alcohol abuse or epilepsy, or was taking medication known to influence blood pressure or gastrointestinal function.

7.3.1 Experimental protocol

Each subject was studied on four days, separated by at least seven days, in single-blind, randomised order. On each study day, the subject attended the University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital, at ~ 08.30h following a fast (10.5h for solids; 8.5h for liquids) (Chapter 6). A silicone-rubber catheter (~ 4mm diameter)
which included an infusion channel with a port located ~ 10cm distal to the pylorus (ie in the duodenum) and two other channels, positioned in the antrum (2.5cm proximal to the pylorus) and duodenum (2.5cm distal to the pylorus), was introduced into the stomach via an anaesthetised nostril (O'Donovan et al 2002). The latter two channels were perfused with normal saline (0.9%). A saline-filled, reference electrode (20 gauge cannula) was inserted subcutaneously into the subject’s forearm to enable measurement of the antroduodenal transmucosal potential difference (TMPD) (O'Donovan et al 2002). The tip of the catheter was allowed to pass into the duodenum by peristalsis, which took between 30 and 90 minutes. Once the catheter was in position, the subject was placed in the recumbent position. An intravenous cannula was positioned in a left antecubital vein for blood sampling, and an automated blood pressure cuff placed around the left arm (O'Donovan et al 2002). Approximately 30 minutes after the catheter had been positioned correctly (ie at t = 0 minutes) an intraduodenal infusion of either glucose (64g), triglyceride (10% Intralipid®; Fresenius Kabi AB, Sweden), protein (72g, 97% whey protein concentrate containing 8.4% carbohydrate (mostly lactose); Perfect Protein, Aussie Bodies® Pty Ltd, Port Melbourne, Victoria, Australia) or saline (0.9%) in a total volume of 243ml, was commenced and continued at a rate of 2.7 ml/min for 90 minutes. Intraduodenal infusions were given using a volumetric infusion pump (Imed Gemini PC-1, San Diego, CA, USA) that was positioned out of the subject’s vision, to ensure that the subject was blinded to the study condition. The glucose, triglyceride and protein infusions all resulted in an energy delivery of ~ 3 kcal/min. On all days, between t = 90 - 120 minutes, saline (0.9%) was infused intraduodenally at the
same rate. At \( t = 120 \) minutes the catheter and intravenous cannula were removed, the subject was given a light meal and, soon after this, was allowed to leave the laboratory. On one day cardiovascular autonomic nerve function was evaluated soon after this (Ewing and Clarke 1982, Piha 1991).

### 7.3.2 Measurement of 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 minutes prior to commencement of the intraduodenal infusion and, subsequently, every three minutes between \( t = 0 - 120 \) minutes (O'Donovan et al 2002). ‘Baseline’ blood pressure and heart rate, ie \( t = 0 \) minutes, were calculated as the mean of measurements taken at \( t = -9, -6 \) and -3 minutes before commencement of the intraduodenal infusion. Postprandial hypotension was defined as a fall in systolic blood pressure ≥ 20mmHg that was sustained for at least 30 minutes (Jansen and Lipsitz 1995).

### 7.3.3 Measurement of superior mesenteric artery blood flow

Superior mesenteric artery blood flow was measured by Duplex ultrasonography (ie B-mode and Doppler imaging) using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, Australia) using the technique described in Chapter 4.7. The subject was scanned using a 3.5C broad spectrum 2.5 - 4 MHz convex transducer (Perko 2001) at \( t = -2 \) minutes, \( t = 5 \) minutes, \( t = 10 \) minutes and then at 15 minute intervals between \( t = 0 - 120 \) minutes. Recordings of peak systolic velocity, end-
diastolic velocity and time averaged mean velocity were acquired from Pulsed Doppler waveform complexes (Figure 7.1). The cross-sectional diameter of the superior mesenteric artery was measured in a longitudinal plane at $t = -2$ minutes using on-screen callipers operated manually (Sidery and Macdonald 1994, Sidery et al 1994, Perko 2001) and this value was utilised in subsequent calculations of blood flow.

7.3.4 Measurement of blood glucose concentrations
Venous blood samples (~ 5ml) were obtained immediately prior to commencement of the infusions ($t = -2$ minutes) and at 15 minute intervals from $t = 0$ - 120 minutes. Blood glucose concentrations were determined according to the method described in Chapter 4.8.

7.3.5 Assessment of cardiovascular autonomic nerve function
Autonomic nerve function was evaluated using standardised cardiovascular reflex tests (Ewing and Clarke 1982, Piha 1991). A description of this method is included in Chapter 4.10.

7.3.6 Statistical analysis
Data were evaluated using mixed model repeated measures 2 way Analysis of Covariance (ANCOVA), with ‘treatment’ and ‘time’ as within subject factors for superior mesenteric artery blood flow and blood glucose concentration and baseline was used as a covariate to analyse the change in systolic blood pressure, diastolic blood pressure and heart rate from baseline. Data were analysed from $t = -2$ - 90 minutes and t
= 90 - 120 minutes for superior mesenteric artery blood flow and blood glucose concentrations and from t = 0 - 90 minutes and t = 90 - 120 minutes for systolic blood pressure, diastolic blood pressure and heart rate to determine the comparative effects (‘treatment’ and ‘time’) of intraduodenal glucose, triglyceride, protein and saline. One-way ANOVA was used to analyse the effects of ‘time’ on systolic and diastolic blood pressure, heart rate, superior mesenteric artery blood flow and blood glucose. In all analyses, post-hoc comparisons of adjusted means were performed using Student’s t-test. The maximum fall in blood pressure was defined as the greatest mean change from baseline for a treatment at any given time point. Data are presented as ± standard error of the mean (SEM) and statistical significance was set at 5%. All analyses were performed by a professional statistician using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

7.4 Results

The studies were well tolerated. Four of the eight subjects experienced diarrhoea soon after completion of the triglyceride infusion and two subjects reported diarrhoea after the protein infusion. One subject reported nausea towards the end of the triglyceride and protein infusions. In all cases, these symptoms had resolved within three hours of the completion of each experiment. No subject had definite autonomic neuropathy; median score 0.75 (range: 0 - 2). Postprandial hypotension (ie a fall in systolic blood pressure ≥ 20 mmHg sustained for at least 30 minutes) was evident in one subject; this was during the triglyceride infusion. In one subject, acceptable superior mesenteric artery blood flow measurements could not be obtained on the saline study because the
vessel was obscured by abdominal gas and these data were, accordingly, not included. In the subject who experienced nausea, data collection on the triglyceride and protein days was terminated at t = 90 minutes. Blood glucose concentrations were not taken at t = 105 minutes and t = 120 minutes in one subject as the intravenous cannula was no longer patent.

7.4.1 **Blood pressure and heart rate**

There was no difference in baseline (ie t = 0 minutes) blood pressure or heart rate between the four days (saline vs glucose vs triglyceride vs protein): systolic blood pressure (125.5 ± 6.0 mmHg vs 126.1 ± 6.1 mmHg vs 127.9 ± 6.7 mmHg vs 124.8 ± 6.1 mmHg); diastolic blood pressure (65.6 ± 2.5 mmHg vs 67.1 ± 2.4 mmHg vs 69.3 ± 3.3 mmHg vs 67.5 ± 2.5 mmHg) and heart rate (61.2 ± 1.9 beats/minute (bpm) vs 58.9 ± 2.9 bpm vs 59.5 ± 3.9 bpm vs 60.0 ± 2.6 bpm).

7.4.1.1 **Systolic blood pressure**

Between t = 0 - 90 minutes there was a significant (P < 0.005) fall in systolic blood pressure during glucose, but no overall changes during triglyceride (P = 0.59) and protein (P = 0.44) infusions. In contrast, there was an overall modest rise during the saline (P < 0.05) infusion. The maximum fall in systolic blood pressure during the glucose (11.7 ± 2.8 mmHg), triglyceride (11.7 ± 4.8 mmHg) and protein (11.0 ± 1.5 mmHg), infusions were comparable (P = 0.97), however, the maximum fall occurred earlier during glucose (18 ± 3.0 minutes) when compared with triglyceride (46 ± 11.0 minutes, P = 0.02) and, protein (33 ± 7.0 minutes, P = 0.04), infusions; there was no
difference in the time of maximum fall between triglyceride and protein \((P = 0.12)\) infusions (Figure 7.2 (a)). There was a significant ‘treatment x time’ effect \((P < 0.01)\) for systolic blood pressure on all study days. Systolic blood pressure was lower between \(t = 0\) - 90 minutes during the glucose \((P < 0.05)\), triglyceride \((P < 0.05)\) and protein \((P < 0.01)\) infusions, when compared with saline and lower during glucose when compared with the triglyceride and protein \((P < 0.05 \text{ for both})\) infusions. After triglyceride, systolic blood pressure was initially greater (eg at \(t = 24\) minutes \((P = 0.03)\)) and subsequently lower (eg between \(t = 78\) - 90 minutes \((P < 0.05)\)) than protein. At \(t = 90\) minutes, systolic blood pressure was not significantly different from baseline after glucose \((P = 0.38)\), triglyceride \((P = 0.46)\), protein \((P = 0.56)\) or saline \((P = 0.90)\), infusions (Figure 7.2 (a)).

Between \(t = 90\) - 120 minutes there were no significant changes in systolic blood pressure during the glucose \((P = 0.72)\), triglyceride \((P = 0.18)\), protein \((P = 0.21)\) or saline \((P = 0.25)\) infusions (Figure 7.2 (a)). Systolic blood pressure was lower between \(t = 90\) - 120 minutes after the glucose and triglyceride \((P < 0.0001 \text{ for both})\) infusions than saline, however, there was no difference between protein and saline \((P = 0.34)\). Systolic blood pressure was lower after glucose when compared with protein \((P < 0.0001)\) without any difference \((P = 0.30)\) between glucose and triglyceride. After triglyceride, systolic blood pressure was lower \((P < 0.0001)\) than protein. At \(t = 120\) minutes, systolic blood pressure was significantly lower than baseline (ie \(t = 0\) minutes) after glucose \((P = 0.03)\), but not after triglyceride \((P = 0.43)\), protein \((P = 0.67)\) or saline \((P = 0.80)\) (Figure 7.2 (a)).
7.4.1.2 Diastolic blood pressure

Between $t = 0 - 90$ minutes there was a significant fall in diastolic blood pressure during glucose ($P < 0.05$) and protein ($P < 0.01$), but not during the triglyceride ($P = 0.83$) or saline ($P = 0.91$), infusions (Figure 7.2 (b)). There were comparable ($P = 0.44$) maximum falls in diastolic blood pressure during the glucose ($13.6 \pm 1.9$ mmHg), triglyceride ($11.6 \pm 2.6$ mmHg) and protein ($11.3 \pm 1.2$ mmHg), infusions, while there was no significant difference in the time of the maximal fall in diastolic blood pressure between the infusions (glucose: $64 \pm 7.0$ minutes vs triglyceride: $56 \pm 9.0$ minutes vs protein: $59 \pm 7.0$ minutes; $P = 0.17$). Between $t = 0 - 90$ minutes, diastolic blood pressure was less during glucose ($P < 0.01$) and protein ($P < 0.05$), when compared with saline infusion, and there was a trend ($P = 0.15$) for diastolic blood pressure to be less during triglyceride than the saline infusion. Diastolic blood pressure was lower during glucose when compared with the protein ($P < 0.05$) infusion. At $t = 90$ minutes, diastolic blood pressure was not significantly different from baseline after glucose ($P = 0.75$), triglyceride ($P = 0.68$), protein ($P = 0.67$) or saline ($P = 0.95$) (Figure 7.2 (b)).

Between $t = 90 - 120$ minutes there were no significant changes in diastolic blood pressure during the glucose ($P = 0.42$) or saline ($P = 0.46$), infusions, however, diastolic blood pressure fell during the triglyceride and protein ($P < 0.05$ for both), infusions (Figure 7.2 (b)). Diastolic blood pressure was lower between $t = 90 - 120$ minutes during glucose ($P < 0.001$), triglyceride ($P < 0.05$) and protein ($P < 0.05$) and when compared with saline infusion. Diastolic blood pressure was also lower after glucose than triglyceride and protein ($P < 0.01$ for both) but not after triglyceride when
compared with protein (P = 0.84) infusion. At t = 120 minutes, diastolic blood pressure was not significantly different from baseline after the glucose (P = 0.32), triglyceride (P = 0.76) protein (P = 0.46) or saline (P = 0.40), infusions (Figure 7.2 (b)).

7.4.1.3  **Heart rate**

Between t = 0 - 90 minutes there was a significant rise in heart rate during the glucose, triglyceride and protein (P < 0.0001 for all) infusions but no change (P = 0.61) during the saline infusion (Figure 7.2 (c)). The maximum rises in heart rate during the glucose (15.7 ± 2.3 bpm), triglyceride (17.2 ± 4.6 bpm) and protein (14.8 ± 2.7 bpm), infusions were comparable (P = 0.72), however, there was no difference in the time of the maximum heart rate between the infusions (glucose: 64 ± 9.0 minutes vs triglyceride: 54 ± 8.0 minutes vs protein: 64 ± 7.0 minutes, P = 0.65). There was a significant ‘treatment x time’ effect (P < 0.001) for heart rate on all study days. Heart rate was greater between t = 0 - 90 minutes during the glucose, triglyceride and protein (P < 0.05 for all) infusions when compared with saline. There was no significant difference in heart rate during glucose when compared with triglyceride or protein infusion. At t = 66 minutes, heart rate was greater with triglyceride when compared with glucose and protein (P = 0.02 for both) infusion. At t = 90 minutes, heart rate was significantly greater than baseline after the glucose (P = 0.02), triglyceride (P = 0.01) and protein (P = 0.0003), infusions but not significantly different from baseline after saline (P = 0.53) infusion (Figure 7.2 (c)).
Between t = 90 - 120 minutes there were significant falls in heart rate after the glucose (P < 0.01) and protein (P < 0.05) infusions but not after the triglyceride (P = 0.20) or saline (P = 0.37), infusion (Figure 7.2 (c)). Between t = 90 - 120 minutes, heart rate was greater during the glucose, triglyceride and protein (P < 0.001 for all) infusions when compared with saline. In contrast, there were no significant differences in heart rate between the three nutrient infusions. At t = 120 minutes, heart rate remained significantly greater than baseline after glucose (P = 0.04), triglyceride (P = 0.05) and protein (P = 0.05) and but not after saline (P = 0.43), infusion (Figure 7.2 (c)).

7.4.2 Superior mesenteric artery blood flow

There was no significant difference in baseline (ie t = -2 minutes) superior mesenteric artery blood flow between the four days (saline vs glucose vs triglyceride vs protein): 609.5 ± 61.5 ml/min vs 622.2 ± 88.2 ml/min vs 619.3 ± 104.6 ml/min vs 666.3 ± 102.0 ml/min (Figure 7.3). Between t = -2 - 90 minutes there was a significant rise in superior mesenteric artery blood flow during glucose (P < 0.0001), triglyceride (P < 0.001) and protein (P < 0.001) infusions and no change during the saline (P = 0.16) infusion (Figure 7.3). There was no significant difference in peak superior mesenteric artery blood flow between the three nutrient infusions, during the glucose (1927.1 ± 166.5 ml/min), triglyceride (1866.1 ± 309.7 ml/min) and protein (1442.8 ± 247.9 ml/min) infusions (P = 0.28). The time to peak superior mesenteric artery blood flow was not significantly different between the three nutrient infusions (glucose: 64 ± 7.0 minutes vs triglyceride: 56 ± 9.0 minutes vs protein: 45 ± 9.0 minutes; P = 0.23). There was a significant ‘treatment x time’ effect (P < 0.001) for superior mesenteric artery blood
flow on all study days. Superior mesenteric artery blood flow was greater during the glucose (P < 0.01), triglyceride (P < 0.001) and protein (P < 0.05) infusions than saline. Similarly, superior mesenteric artery blood flow was greater during both glucose (P < 0.05) and triglyceride (P < 0.01) than protein infusion and initially greater during glucose than triglyceride (P = 0.04) infusion. At t = 90 minutes superior mesenteric artery blood flow was significantly greater than baseline after the glucose (P = 0.0001), triglyceride (P = 0.004) and protein (P = 0.0001) infusions, but not after saline (P = 0.36) (Figure 7.3).

Between t = 90 - 120 minutes there were significant falls in superior mesenteric artery blood flow after the glucose (P < 0.01) and protein (P < 0.05) infusions but no change after the triglyceride (P = 0.18) or saline (P = 0.13) infusions (Figure 7.3). Superior mesenteric artery blood flow was greater between t = 90 - 120 minutes after the glucose, triglyceride and protein (P < 0.0001 for all) infusions when compared with saline, however, there was no difference in superior mesenteric artery blood flow during glucose when compared with triglyceride (P = 0.35) or protein (P = 0.16) or between triglyceride and protein (P = 0.64), infusions. At t = 120 minutes, superior mesenteric artery blood flow was significantly greater than baseline after glucose (P = 0.01), triglyceride (P = 0.008) and protein (P = 0.04) and but not after saline (P = 0.91) infusion (Figure 7.3).
7.4.3 **Blood glucose concentrations**

There was no significant difference in baseline (i.e., t = -2 minutes) blood glucose between the four days (saline vs glucose vs triglyceride vs protein): 5.5 ± 0.2 mmol/L vs 5.4 ± 0.1 mmol/L vs 5.4 ± 0.2 mmol/L vs 5.8 ± 0.2 mmol/L (Figure 7.4).

There was a rise in blood glucose between t = -2 - 90 minutes during the glucose (P < 0.0001) and protein (P < 0.01) infusions, a minor fall during saline (P < 0.05) and no change (P = 0.51) during the triglyceride infusion (Figure 7.4). There was a significant ‘treatment x time’ effect (P < 0.0001 for all) for blood glucose between t = -2 - 90 minutes. Blood glucose was greater during glucose than triglyceride (P < 0.01), protein (P < 0.0001) and saline (P < 0.05) infusion. Similarly blood glucose was greater during the protein infusion, than both triglyceride (P < 0.05) and saline (P = 0.05) infusions. At t = 90 minutes, the blood glucose concentration was significantly greater than baseline after glucose (P = 0.0001) and less than baseline after saline (P = 0.03) but there was no significant difference after the triglyceride (P = 0.92) or protein (P = 0.49) infusion (Figure 7.4).

Between t = 90 - 120 minutes there was a fall in blood glucose during glucose (P < 0.0001) infusion (Figure 7.4). There was a significant ‘treatment x time’ effect (P < 0.01) for blood glucose between t = 90 - 120 minutes. Blood glucose was greater during glucose when compared with triglyceride (P < 0.05), protein (P < 0.0001) and saline (P < 0.05) infusions. Similarly blood glucose was greater during the protein infusion, than both triglyceride (P < 0.05) and saline (P = 0.05), however, there was no difference in
blood glucose between triglyceride and saline infusions. At $t = 120$ minutes, there was a trend for the blood glucose concentration to be greater than baseline after glucose ($P = 0.09$) (Figure 7.4).

### 7.4.4 Relationships between blood pressure and heart rate with superior mesenteric artery blood flow

There were no significant relationships between blood pressure and heart rate with superior mesenteric artery blood flow (data not shown).

### 7.5 Discussion

This study establishes that in healthy elderly subjects, isocaloric and isovolaemic intraduodenal infusions of glucose, triglyceride and protein, induce a comparable fall in systolic blood pressure and rise in heart rate but that the stimulation of superior mesenteric artery blood flow may be less after protein.

It has been previously demonstrated that the hypotensive response to an intraduodenal glucose infusion in healthy elderly subjects is dependent on the rate of glucose delivery into the small intestine (O'Donovan et al 2002); intraduodenal glucose infusion at a rate of ~ 3 kcal/min resulted in falls in both systolic and diastolic blood pressures that were much greater when compared with a rate of ~ 1 kcal/min. Studies (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) indicate that ‘intragastric’ mechanisms related to
gastric distension may reduce the postprandial fall in blood pressure in healthy young and elderly subjects, hence, nutrients were infused directly into the small intestine.

It has been established that the magnitude of the postprandial fall in blood pressure is dependent on meal composition. Of the macronutrients, carbohydrate, particularly in the form of glucose appears to have the greatest effect on postprandial blood pressure (Mathias et al 1989b, Jansen et al 1990, Heseltine et al 1991a, Visvanathan et al 2005). While triglyceride has been reported to have relatively little effect on blood pressure (Potter et al 1989, Jansen et al 1990, Waaler and Eriksen 1992, Kearney et al 1996), it has recently been reported in healthy elderly subjects that ingestion of a high-triglyceride (88%) drink containing cream blended with milk (653 kcal), induced a comparable fall in systolic blood pressure (mean ~ 17 mmHg) when compared with an isocaloric glucose (75g and 93g Polyjoule in 300ml water) drink (mean ~ 13 mmHg), but that the onset of this fall was substantially later after the high-triglyceride (~ 26.5 minutes) compared with the glucose (~ 13 minutes) drink (Visvanathan et al 2006). However, gastric emptying was not measured and changes in intragastric distribution due to layering of the fat component (Horowitz et al 1993b) may have influenced the observations. In an early study of hypertensive elderly patients (> 70 years), ingestion of a protein (75g whey in 300ml water) drink had little effect on blood pressure (Jansen et al 1990) whereas in healthy elderly subjects, ingestion of a high-protein mixed meal resulted in a rise in postprandial blood pressure (Potter et al 1989). A limitation of these studies, was that gastric emptying was not measured and changes in intragastric distribution may have contributed to the outcome. In this current study, glucose,
triglyceride and protein were infused directly into the small intestine at a rate of \(~ 3\) kcal/min; the magnitude of the fall in blood pressure, and increase in heart rate, during the intraduodenal glucose infusion were comparable to those observed previously (O'Donovan et al 2002, O'Donovan et al 2005b). By infusing triglyceride and protein directly into the small intestine, it was confirmed that both nutrients induce a fall in systolic blood pressure and rise in heart rate that is comparable to intraduodenal infusion of glucose but that the maximum fall in systolic blood pressure after glucose occurs earlier (\(~ 18\) minutes) when compared with triglyceride (\(~ 46\) minutes) and protein (\(~ 33\) minutes). Furthermore, systolic blood pressure had returned to baseline values by 120 minutes after triglyceride, protein and saline infusions, however, the effect of glucose on blood pressure was still evident at this time.

Information about the effects of protein on postprandial blood pressure are inconsistent. Jansen et al (1990) found little fall in blood pressure after a protein drink (75g whey in 300ml water) in a group of hypertensive elderly subjects, while, in contrast, Potter et al (1989) demonstrated falls of up to \(~ 9\) mmHg in healthy elderly subjects after a high-protein (53%; chicken) mixed meal. The observations in this current study are consistent with those of Potter et al (1989), in that isocaloric protein and carbohydrate induced a comparable fall in postprandial blood pressure, suggesting that protein may mediate postprandial hypotension.

The slowing of gastric emptying, stimulation of gastrointestinal hormone release and suppression of energy intake by oral triglyceride are known to be mediated by fatty
acids, rather than triglycerides (Feinle et al 2001). Similarly, while the mechanisms responsible for the fall in blood pressure after protein remain unknown, protein has been shown to slow gastric emptying (Burn-Murdoch et al 1978) and increase satiety (Vandewater and Vickers 1996) in healthy young subjects. It would, accordingly, not be surprising if the hypotensive responses to triglyceride and protein are also dependent on lipolysis and proteolysis and this could account for the relative latency in the responses. Studies are now indicated to determine the effects of inhibition of triglyceride and protein digestion on postprandial blood pressure.

The pathophysiological mechanisms underlying postprandial hypotension remain poorly defined, however, impaired regulation of splanchnic blood flow is thought to play a role (Jansen and Lipsitz 1995). Following a meal, 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). The magnitude of the postprandial increase in mesenteric blood flow is comparable in healthy young and elderly individuals, despite the greater fall in blood pressure in the latter group, indicating that there is inadequate cardiovascular adjustment for the shift of blood volume into the splanchnic system (Lipsitz et al 1993, Sidery et al 1993). It has also been demonstrated, in healthy young and elderly subjects, that the magnitude of the postprandial increase in mesenteric blood flow is dependent on the type of nutrient ingested (Moneta et al 1988, Qamar and Read 1988, Sieber et al 1992, Waaler and Eriksen 1992, Sidery et al 1993, Sidery et al 1994), however, information relating
to the effects of carbohydrate, triglyceride and protein on blood flow is inconsistent. Sieber et al (1992) reported that the increase in superior mesenteric artery blood flow in healthy young subjects during isocaloric, isovolaemic and iso-osmolar intraduodenal infusions of triglyceride and protein were comparable, but the response to intraduodenal glucose was less when compared with intraduodenal triglyceride and protein infusions. In contrast, in this present study, the superior mesenteric artery blood flow responses to intraduodenal glucose and triglyceride infusions were greater when compared with protein infusion. The findings in this current study support those of Qamar and Read (1988), who reported that the maximal responses in superior mesenteric artery blood flow in healthy young subjects in response to ingestion of isocaloric and isovolaemic carbohydrate, triglyceride and protein liquid meals were not significantly different. However, while there was no difference in the time to the mean maximal responses, Qamar and Read (1988) reported that blood flow responses to oral triglyceride and protein loads were significantly slower than the response to ingestion of a carbohydrate liquid meal. Furthermore, in the healthy young volunteers, the time of peak superior mesenteric artery blood flow differed such that when individual data was analysed, there was not one nutrient that consistently induced the most rapid response (Qamar and Read 1988). In this study while the maximal responses in superior mesenteric artery blood flow after intraduodenal glucose, triglyceride and protein infusions occurred at ~64, 56 and 45 minutes, respectively, the maximum falls in systolic blood pressure were evident well before this time during glucose (~18 minutes), triglyceride (~46 minutes) and protein (~33 minutes) infusions. Furthermore, the rises in superior mesenteric artery blood flow after the nutrient infusions were gradual indicating that, to some
degree, the falls in blood pressure after intraduodenal glucose, triglyceride and protein are mediated by changes in superior mesenteric artery blood flow. The observation that intraduodenal saline infusion did not affect superior mesenteric artery blood flow is not surprising; ingestion of distilled water (~400ml) has been shown to have no effect on superior mesenteric artery blood flow in healthy young subjects (Qamar and Read 1988). There were no relationships between blood pressure and heart rate with superior mesenteric artery blood flow. This was not surprising as the number of subjects studied was relatively small and further studies in a larger population are now warranted.

The blood glucose responses to the three nutrient infusions were predictable (O'Donovan et al 2002, Erdmann et al 2004, O'Donovan et al 2005b, Visvanathan et al 2006). The modest rise in blood glucose that was observed after the protein infusion is likely to reflect the small amount of carbohydrate (mainly lactose) present in the infusion. Changes in blood glucose (O'Donovan et al 2002) and insulin (Jansen et al 1987, Jansen et al 1990) do not appear to play a major role in the hypotensive response to oral glucose; intravenous glucose does not affect blood pressure in the elderly (Jansen and Lipsitz 1995) and postprandial hypotension has been shown to occur in patients with type 1 diabetes, who are by definition insulin deficient (Jansen et al 1990).

In summary, isocaloric and isovolaemic intraduodenal infusion of glucose, triglyceride and protein appear to result in comparable falls in systolic blood pressure yet the response after glucose infusion occurs earlier when compared with triglyceride and protein infusion. All nutrient infusions were associated with a decrease in diastolic
blood pressure and increase in heart rate and superior mesenteric artery blood flow. Hence, glucose, triglyceride and protein all have the potential to contribute to postprandial hypotension. The mechanisms responsible for these changes remain unclear, however, the relatively slower blood pressure response to triglyceride and protein may potentially reflect the time taken for digestion of triglyceride to free fatty acids and protein to amino acids. Inhibition of both triglyceride and protein digestion may therefore represent an approach to the treatment of patients with postprandial hypotension treatment and warrants further evaluation.
Figure 7.1: Abdominal Duplex ultrasonography of the superior mesenteric artery demonstrating flow pulses.
Figure 7.2: Changes in systolic blood pressure (a), diastolic blood pressure (b) and heart rate (c), from baseline in eight healthy elderly subjects in response to intraduodenal infusion of glucose (●), triglyceride (▲), protein (■) and saline (○). Data are mean values ± SEM. P values indicate ‘treatment x time’ effect between t = 0 - 90 minutes.
**Figure 7.3:** Superior mesenteric artery blood flow in eight healthy elderly subjects in response to intraduodenal infusion of glucose (●), triglyceride (△), protein (■) and saline (○). Data are mean values ± SEM. P values indicate ‘treatment x time’ effect between t = 0 - 90 minutes.
Figure 7.4: Blood glucose concentrations in eight healthy elderly subjects in response to intraduodenal infusion of glucose (●), triglyceride (△), protein (■) and saline (○). Data are mean values ± SEM. P values indicate ‘treatment x time’ effect between t = 0 - 90 minutes and t = 90 - 120 minutes.

Blood glucose concentrations

![Graph showing blood glucose concentrations](image)

- t = 0 - 90 minutes
- P < 0.01 glucose vs triglyceride
- P < 0.0001 glucose vs protein
- P < 0.01 glucose vs saline
- P < 0.05 triglyceride vs protein
- P = 0.05 protein vs saline

- t = 90 - 120 minutes
- P < 0.05 glucose vs triglyceride
- P < 0.0001 glucose vs protein
- P < 0.05 glucose vs saline
Acarbose attenuates the hypotensive response to sucrose in the healthy elderly; associations between slowing of gastric emptying and stimulation of GLP-1
8.1 Summary

Current management of postprandial hypotension is less than optimal. Recent observations suggest that slowing of gastric emptying and small intestinal carbohydrate absorption may prove beneficial in the treatment of this condition. The aims of this study were to evaluate the effects of the α-glucosidase inhibitor, acarbose, on blood pressure and heart rate and the glycaemic, insulin, and glucagon-like peptide 1 (GLP-1) and glucose-dependant insulinotropic-polypeptide (GIP) responses to an oral sucrose load in healthy elderly subjects and to relate these effects to changes in gastric emptying. Eight healthy subjects (five male and three female, aged 65 - 79 years) were studied on two days. Blood pressure (systolic and diastolic), heart rate, gastric emptying, blood glucose, plasma insulin, GLP-1 and GIP were measured after ingestion of a 300ml drink containing 100g sucrose, labelled with $^{99m}$Technetium (Tc) sulphur colloid, with or without 100mg acarbose. The magnitude of the falls in systolic (P < 0.05) and diastolic (P = 0.0009) blood pressure and rise in heart rate (P = 0.03) were less after acarbose. Gastric emptying was delayed by acarbose (P < 0.05); this effect was evident after 90 minutes and associated with increased retention (P = 0.0008) of the drink in the distal stomach. The rises in glucose, insulin and GIP (P < 0.0001 for all) levels were attenuated by acarbose whereas, after 75 minutes, plasma GLP-1 was increased (P = 0.004). It is concluded that in healthy elderly subjects (i) acarbose attenuates the fall in systolic and diastolic blood pressure and increase in heart rate induced by oral sucrose, (ii) these effects are, at least in-part, independent of slowing of gastric emptying, (iii) the slowing of gastric emptying of sucrose by acarbose is evident some time after ingestion of a sucrose-containing drink and is associated with increased
retention in the distal stomach and (iv) stimulation of GLP-1 may contribute to the slowing of gastric emptying and suppression of postprandial glycaemia by acarbose.

8.2 Introduction

Postprandial hypotension is an important clinical problem (Mathias 1991, Jansen and Lipsitz 1995). Those most commonly affected include the elderly and patients with autonomic dysfunction, most often secondary to diabetes (Mathias 1991, Jansen and Lipsitz 1995). Postprandial hypotension is associated with significant complications, including syncope, falls and cerebrovascular accidents (Jansen and Lipsitz 1995, Aronow and Ahn 1997), and current approaches to management are sub-optimal (Jansen and Lipsitz 1995).

The mechanisms mediating postprandial hypotension are poorly defined (Jansen and Hoefnagels 1991, Puisieux et al 2000). The magnitude of the fall in blood pressure is dependent on meal composition (Jansen and Lipsitz 1995) with ingestion of carbohydrate, particularly glucose, reported to have the greatest effect (Jansen et al 1990). The effects of enteral glucose on blood pressure do not appear to be mediated by changes in blood glucose (O'Donovan et al 2002) or insulin (Jansen and Hoefnagels 1987b, Jansen et al 1990). Recent studies have demonstrated that the rate of nutrient delivery from the stomach to the small intestine is a major determinant of the hypotensive response to oral glucose (Jones et al 1998, Jones et al 2001, O'Donovan et al 2002, Russo et al 2003). For example, in healthy elderly subjects, when glucose is infused intraduodenally at a rate of ~ 3 kcal/min compared to infusion at a rate of ~ 1
kcal/min, the magnitude of the fall in systolic blood pressure and rise in heart rate, is substantially greater (O'Donovan et al 2002). The hypotensive responses to oral glucose in both healthy elderly subjects (Jones et al 2001) and type 2 diabetic patients (Russo et al 2003), and intraduodenal glucose in healthy elderly subjects (O'Donovan et al 2005b), are attenuated by concurrent administration of the viscous polysaccharide, guar, which slows gastric emptying and small intestinal glucose absorption (Meyer et al 1988).

Acarbose has been used in the treatment of type 2 diabetes for many years (Breuer 2003) and may prevent the development of type 2 diabetes in people with impaired glucose tolerance, as well as induce weight loss (Chiasson et al 2002). Acarbose reduces postprandial glycaemia without affecting fasting blood glucose (Chiasson et al 2002); the former is now recognised to be the major determinant of average glycaemic control, as assessed by glycated haemoglobin (El-Kebbi et al 2004), and may be an independent risk factor for cardiovascular disease (Chiasson et al 2002, Gerich 2003). The suppression of postprandial glycaemia by acarbose has been attributed to the slowing of small intestinal digestion and absorption of carbohydrate as a result of inhibition of α-glucosidase (Breuer 2003). While the determinants of postprandial glycaemia remain controversial (Gerich 2003), it is clear that even minor variations in gastric emptying of carbohydrate may have a major effect on postprandial glycaemic excursions in healthy subjects and type 2 patients (Jansen et al 1990, Schwartz et al 1994, Rayner et al 2001, O'Donovan et al 2004a). It is, therefore, of interest that two studies, both involving healthy young adult males, indicate that acarbose slows gastric
emptying (Ranganath et al 1998, Enç et al 2001). Ranganath et al (1998) employed the relatively insensitive, paracetamol technique to evaluate liquid gastric emptying and reported that acarbose (100mg), taken just before a drink containing sucrose and paracetamol, reduced plasma paracetamol levels after 60 minutes. The study was not placebo controlled. Subsequently, Enç et al (2001), reported that acarbose slowed gastric emptying of both carbohydrate-containing, and non-carbohydrate, solid meals; the latter was consumed after sucrose. While a precise radionuclide technique was used to measure gastric emptying of solids in this study, the acarbose was dissolved in 200ml of tea and gastric emptying of the tea was not quantified; gastric emptying of liquids is known to influence gastric emptying of a concurrently ingested solid meal (Collins et al 1983, Horowitz and Dent 1991). Hence, both studies (Ranganath et al 1998, Enç et al 2001) had methodological limitations which preclude a meaningful assessment of the magnitude of the effect of acarbose on gastric emptying; there is also no information about the effect of acarbose on intragastric meal distribution. Nevertheless, the evidence that acarbose slows both gastric emptying and small intestinal glucose absorption suggest a potential therapeutic role in the treatment of postprandial hypotension. This concept is supported by recent case studies in which acarbose apparently had a major beneficial effect to attenuate the postprandial fall in blood pressure in patients with type 2 diabetes (Sasaki et al 2001, Yamamoto et al 2006).

Oral ingestion of glucose elicits a greater rise in plasma insulin than a comparable amount of glucose given intravenously; this ‘incretin’ effect is mediated by the secretion of peptide hormones, including GIP and GLP-1 (Holst and Gromada 2004).
Acarbose and other α-glucosidase inhibitors, such as miglitol and voglibose, stimulate the release of GLP-1 after oral (non-glucose) carbohydrate, while suppressing GIP (Ranganath et al 1998, Seifarth et al 1998, Enç et al 2001, Lee et al 2002). The stimulation of GLP-1 may be relevant to the effects of acarbose on postprandial glycaemia (Nauck et al 1997b), gastric emptying (Nauck et al 1997a, Meier et al 2003), body weight (Chiasson et al 2002) and blood pressure (Edwards et al 1997).

The aims of this study were to evaluate the effects of acarbose, on gastric emptying, blood pressure, heart rate, blood glucose, insulin and GLP-1 following an oral sucrose load in healthy elderly subjects.

### 8.3 Research design and methods

Eight healthy elderly subjects, (five male and three female) with a median age of 71 years (range: 65 - 79 years) and body mass index (BMI) of 26.4 kg/m² (range: 22.2 - 30.8 kg/m²), recruited by advertisement, were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, or was taking medication known to influence blood pressure or gastrointestinal function.

#### 8.3.1 Experimental protocol

Each subject was studied on two separate days. On each day, subjects attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry, Royal Adelaide Hospital, at 08.30 hours following a fast (10.5 hours for
Acarbose attenuates the hypotensive response to oral sucrose solids; 8.5 hours for liquids) (Chapter 6). A cannula was placed in a right antecubital vein for blood sampling and subjects were seated with their back against a gamma camera with a blood pressure cuff around their left arm. Each subject rested comfortably in the sitting position for about 30 minutes. Subjects then consumed a drink comprising 100g sucrose and 30ml lemon juice dissolved in water with a total volume of 300ml and labelled with 20MBq $^{99m}$Tc sulphur colloid. On one of the days, 100mg acarbose (Bayer Australia Ltd, Pymble, NSW, Australia) 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 pressure (systolic and diastolic), heart rate, blood glucose, plasma insulin, GIP and GLP-1 were measured. At $t = 210$ minutes the intravenous cannula was removed, the subject was given a light lunch and, soon after this, 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).

### 8.3.2 Measurement of blood pressure and heart rate

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP, Johnson & Johnson, Tampa, FL, USA) prior to ingestion of the drink ($t = -30$, -15 and -2 minutes) at 3 minute intervals between $t = 0$ - 60 minutes and, subsequently, at 15 minute intervals until $t = 210$ minutes. Postprandial hypotension was defined as a fall in systolic blood pressure $\geq 20$mmHg after the sucrose drink that was sustained for at least 30 minutes.
(Jansen and Lipsitz 1995). ‘Baseline’ blood pressure, ie at t = 0 minutes, was calculated as the mean of measurements taken at t = -30, -15 and -2 minutes.

### 8.3.3 Measurement of gastric emptying and intragastric distribution

Subjects consumed the drink within two minutes. Time zero (t = 0 minutes) was defined as the time of completion of the drink. Radioisotopic data were acquired for 180 minutes using the method described in Chapter 4.4.1. 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) derived. The amounts of the drink remaining in the total, proximal and distal stomach at t = 0, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 minutes were calculated. The 50% emptying time (T50) was also determined (Collins et al 1983). The lag phase was defined visually as the time before any of the radioactivity had entered the proximal small intestine (Collins et al 1983).

### 8.3.4 Measurement of blood glucose, plasma insulin, GIP, and GLP-1 concentrations

Venous blood samples (~ 20ml) were obtained immediately prior to the drink (t = -2 minutes) and then at 15 minute intervals between t = 15 - 120 minutes, and at 30 minute intervals between t = 120 - 210 minutes. Blood glucose, plasma insulin, GIP and GLP-1 concentrations were measured using methods described in Chapters 4.8 and 4.9.
8.3.5 Assessment of cardiovascular autonomic nerve function

Autonomic nerve function was evaluated using standardised cardiovascular reflex tests (Ewing and Clarke 1982, Piha 1991). A description of this method is included in Chapter 4.10.

8.3.6 Statistical analysis

Data were evaluated using mixed model repeated measures 2 way Analysis of Covariance (ANCOVA), with ‘treatment’ and ‘time’ as within subject factors; baseline as covariate was used to analyse changes in systolic blood pressure, diastolic blood pressure and heart rate from baseline. Effects of ‘treatment’ and ‘time’ on gastric emptying (intragastric distribution), blood glucose, plasma insulin, plasma GIP and plasma GLP-1 concentrations were analysed using the same methodology. Differences in gastric emptying (total stomach) were analysed using mixed model repeated measures 2 way Analysis of Variance (ANOVA), with ‘treatment’ and ‘time’ as within subject factors. In all analyses, post-hoc comparisons of adjusted means were performed using Student’s t-test. The maximum fall in blood pressure was defined as the greatest mean change from baseline for a treatment at any given time point. Data are presented as mean values ± standard error of the mean (SEM), unless stated otherwise. All analyses were performed by a professional statistician using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). A P value < 0.05 was considered significant in all analyses.
8.4 Results

The studies were well tolerated. Flatulence and/or diarrhoea was reported by three of the eight subjects after acarbose - the onset of symptoms was approximately four hours after ingestion of the drink and in all cases symptoms had resolved within six hours. No subject had definite autonomic neuropathy; median score 1.0 (range: 0 - 2).

8.4.1 Blood pressure and heart rate

There was no difference in baseline blood pressure or heart rate between the two days (control vs acarbose): systolic blood pressure (128.3 ± 5.0 mmHg vs 128.0 ± 4.3 mmHg); diastolic blood pressure (66.9 ± 4.0 mmHg vs 68.2 ± 4.1 mmHg) and heart rate (58.3 ± 2.5 beats/minute (bpm) vs 61.8 ± 2.7 bpm).

Between t = 0 - 210 minutes, systolic blood pressure was less (P < 0.0001) on the control day compared with acarbose. There was a fall in systolic blood pressure on the control day, which was evident from t = 39 minutes; in contrast, there was an overall rise in systolic blood pressure after acarbose (P = 0.0003) (Figure 8.1 (a)). No subject experienced postprandial hypotension after either drink.

Between t = 0 - 210 minutes, diastolic blood pressure was less (P < 0.0001) on the control day compared with acarbose. There was a fall in diastolic blood pressure on the control day, which was evident from t = 9 minutes; in contrast, there was an overall rise in diastolic blood pressure after acarbose (P = 0.0002) (Figure 8.1 (b)).
There was a significant ‘treatment x time’ effect (P < 0.03) for heart rate for the two days. Between t = 0 - 210 minutes, heart rate was greater (P < 0.05) on the control day compared with acarbose. There was a rise in heart rate on both days, which was significant from t = 12 minutes on control and from t = 54 minutes after acarbose (Figure 8.1 (c)).

8.4.2  **Gastric emptying**

8.4.2.1  **Total stomach**

Gastric emptying approximated an overall linear pattern after a short lag phase (control: 2.6 ± 1.0 minutes vs acarbose: 3.9 ± 1.7 minutes; P = 0.13). There was a significant ‘treatment x time’ effect (P < 0.04) for gastric emptying for the two study days. Gastric emptying of the drink was faster (P < 0.05) between t = 90 - 180 minutes, on the control day, compared to acarbose. There was no difference before that time. There was no significant difference in the T50 between the two days (control: 100.1 ± 10.6 minutes vs acarbose: 123.5 ± 18.1 minutes; P = 0.10) (Figure 8.2 (a)).

8.4.2.2  **Intragastric distribution**

There was no difference in proximal stomach retention between the two study days (P = 0.99) (Figure 8.2 (b)). In contrast, retention of the drink in the distal stomach was less on the control day (P = 0.0008) (Figure 8.2 (c)).
8.4.3 **Blood glucose, plasma insulin, GIP, and GLP-1 concentrations**

There was no difference in baseline of (control vs acarbose) blood glucose (5.9 ± 0.2 mmol/L for both), plasma insulin (9.9 ± 3.1 mU/L vs 10.6 ± 3.5 mU/L), plasma GIP (2.8 ± 1.0 pmol/L vs 2.7 ± 1.2 pmol/L) or plasma GLP-1 (10.4 ± 3.6 pmol/L vs 8.3 ± 2.6 pmol/L) between the two days.

There was a significant ‘treatment x time’ effect (P < 0.0001) for blood glucose on both days. Between t = 30 - 75 minutes, blood glucose concentrations were greater (P < 0.001) on the control day compared with acarbose. Peak blood glucose concentrations were 9.4 ± 0.5 mmol/L at 51.0 ± 8.0 minutes and 7.9 ± 0.4 mmol/L at 83.0 ± 18.0 minutes (P = 0.0005) for control and acarbose days, respectively. Between t = 180 - 210 minutes, blood glucose was less on the control day (P < 0.0003) (Figure 8.3 (a)).

There was a rise in plasma insulin concentrations on both days (P < 0.05). There was a significant ‘treatment x time’ effect (P < 0.0001) for plasma insulin concentrations on both days. Between t = 15 - 150 minutes, plasma insulin was higher (P < 0.001) on control when compared with acarbose. Peak plasma insulin concentrations were 92.3 ± 17.0 mU/L at 75.0 ± 16.0 minutes and 33.1 ± 5.6 mU/L at 88.0 ± 17.0 minutes (P = 0.002) for control and acarbose days, respectively (Figure 8.3 (b)).

There was a rapid rise in plasma GIP concentrations on the control day from t = 15 minutes (P < 0.0001), but not on acarbose (P = 0.25). There was a significant ‘treatment x time’ effect (P < 0.0001) for plasma GIP concentrations on both days. Plasma GIP
concentrations were higher (P < 0.001) between t = 15 - 150 minutes, after control, compared with acarbose. Peak plasma GIP concentrations were (control vs acarbose): 61.7 ± 14.0 pmol/L at 86 ± 19.0 minutes and 8.7 ± 4.1 pmol/L at 22.0 ± 14.0 minutes (P < 0.001) (Figure 8.3 (c)).

There was a rise in plasma GLP-1 concentrations on both days from t = 15 minutes (P < 0.006). Similarly, there was a significant ‘treatment x time’ effect (P = 0.008) for plasma GLP-1 concentrations on the two days. However, plasma GLP-1 concentrations were less (P < 0.004) between t = 75 - 210 minutes, after control. Peak plasma GLP-1 concentrations were (control vs acarbose) 23.6 ± 6.0 pmol/L at 47.0 ± 13.0 minutes and 41.1 ± 7.7 pmol/L at 103.0 ± 16.0 minutes (P < 0.001) (Figure 8.3 (d)).

8.5 Discussion

This study indicates that in healthy elderly subjects: (i) acarbose attenuates the falls in systolic and diastolic blood pressure and increase in heart rate induced by oral sucrose; (ii) these effects appear to be, at least in-part, independent of the demonstrated slowing of gastric emptying by acarbose which is not evident until some time after ingestion of the sucrose drink and (iii) stimulation of GLP-1 may contribute to the slowing of gastric emptying and suppression of postprandial glycaemia by acarbose.

The observations that gastric emptying (Jones et al 1998, O'Donovan et al 2002) and the exposure of glucose to the small intestinal mucosa (Jones et al 2001, Russo et al 2003, O'Donovan et al 2005b), influence the hypotensive response to enteral glucose
suggest that acarbose may prove effective in the management of postprandial hypotension. The gradual fall in systolic and diastolic blood pressure and immediate rise in heart rate induced by consumption of a sucrose drink were attenuated by acarbose. With acarbose, both systolic and diastolic blood pressure rose, rather than fell, after the oral sucrose load and the rise in heart rate was modest. While no subject experienced postprandial hypotension, the magnitude of the effect was substantial.

A potential mechanism by which acarbose ameliorates postprandial hypotension is by slowing gastric emptying (Jones et al 1998, O'Donovan et al 2002) - as discussed, evidence for this effect is provided by two studies involving young subjects (Ranganath et al 1998, Enç et al 2001). By utilising a sophisticated radionuclide technique to measure gastric emptying we confirmed that acarbose slows gastric emptying of sucrose, but while this effect was major; it was only evident after ~ 90 minutes. In contrast, the fall in systolic blood pressure and rise in heart rate were evident well before this time. Hence, the initial effects of acarbose on blood pressure and heart rate were unrelated to gastric emptying and, presumably, reflect the slowing of small intestinal sucrose digestion and/or absorption. Gastric distension by liquid is known to attenuate the postprandial fall in blood pressure (Shannon et al 2002) and this is likely to account for the initial rise in blood pressure observed after the drink. The slowing of gastric emptying by acarbose may potentially contribute to the differences in blood pressure and heart rate observed after 90 minutes, and probably reflects increased small intestinal feedback by non-absorbed carbohydrate. In normal subjects gastric emptying of liquid glucose and other carbohydrates is known to be regulated at ~ 1 - 3 kcal/min
as a result of inhibitory feedback from receptors located throughout the small intestine (Brener et al 1983, Moran et al 2001). The extent of this feedback is dependent on the length of small intestine exposed (Lin et al 1989).

The slowing of gastric emptying by acarbose was shown to be associated with changes in intragastric distribution, so that relatively more of the drink was retained in the distal stomach. While the significance of this observation is uncertain, it is of interest that in both healthy young and elderly subjects the perception of postprandial fullness (Jones et al 1997, Santangelo et al 1998, Sturm et al 2004) and energy intake (Sturm et al 2004) are related to the content of the distal stomach and, presumably, antral distension (ie energy intake is less and fullness greater when antral content is greater) and that acarbose may decrease body weight (Chiasson et al 2002). The effects of acarbose on gastric emptying are also potentially relevant to patients with longstanding type 2 diabetes in whom there is a high prevalence of gastroparesis (Horowitz et al 1989a); the latter is attributable to gastric ‘pump failure’, as a result of autonomic neuropathy and hyperglycaemia (Horowitz et al 2002), rather than increased small intestinal feedback. In contrast, in ‘early’ type 2 diabetes gastric emptying of carbohydrate-containing liquids may be accelerated (Phillips et al 1992). There are, hitherto, no studies which have evaluated the effects of acarbose on gastric emptying in diabetes. While our study does not allow quantification of the relative contribution of delay in gastric emptying and impairment in carbohydrate absorption to the overall reduction in glycaemia induced by acarbose in type 2 diabetes, it is likely that the slowing of gastric emptying is important (Rayner et al 2001). It also remains to be determined whether upper
gastrointestinal symptoms associated with the use of acarbose (Chiasson et al 2002) may be attributable to a delay of gastric emptying and/or changes in intragastric meal distribution (Piessevaux et al 2003).

The study confirms that acarbose, as with other α-glucosidase inhibitors, stimulates the release of GLP-1, and suppresses GIP, after oral sucrose (Qualmann et al 1995, Ranganath et al 1998, Enç et al 2001). The stimulation of GLP-1 was substantial and likely to be physiologically relevant (Nauck et al 1997a, Meier et al 2003). GLP-1 is released from intestinal ‘L-cells’, which have their greatest density in the distal small intestine (Holst and Gromada 2004); the stimulation of GLP-1 after 60 minutes is likely to reflect the presence of unabsorbed sucrose (Ranganath et al 1998). GLP-1 has a number of properties - it increases glucose-dependent insulin release (Holst et al 1987), slows gastric emptying (Nauck et al 1997a, Meier et al 2003), suppresses appetite (Meier et al 2002), reduces glucagon secretion (Meier et al 2002) and may have trophic effects on pancreatic β cell function (Meier et al 2002). The incretin effect is reduced in type 2 diabetes (Nauck et al 1986); this reflects reduced GLP-1 (Vilsboll et al 2001), but preserved GIP (Toft-Nielsen et al 2001) secretion and a diminished insulinotropic effect of GIP (Nauck et al 1993), but not GLP-1 (Vilsboll et al 2002). The use of GLP-1 and its analogues, such as exenatide (Fineman et al 2003), in the treatment of type 2 diabetes is now being actively explored. Hence this property of acarbose is of considerable interest. Exogenous administration of GLP-1 is known to slow gastric emptying in both healthy subjects (Nauck et al 1997a) and type 2 patients (Meier et al 2003), moreover, Enç et al (2001), reported a relationship between the slowing of
gastric emptying induced by acarbose and stimulation of GLP-1, consistent with a causal association. The latter could be explored further using the GLP-1 antagonist, exendin-9-39 (Edwards et al 1999). There is evidence that GLP-1 may preserve pancreatic β cell mass in type 2 diabetes (Meier et al 2002) which could contribute to its demonstrated efficacy in the prevention of this condition (Chiasson et al 2002). There is little information about the effect of GLP-1 on blood pressure but in humans (Edwards et al 1998) and animals (Barragan et al 1996), exogenous administration of GLP-1 is known to increase blood pressure and heart rate; hence a role for GLP-1 in the observed effects of acarbose on blood pressure cannot be discounted.

Postprandial hypotension occurs in approximately 40% of nursing home residents (Aronow and Ahn 1997) and in 20% - 40% of patients with type 2 diabetes (Sasaki et al 1992, Jones et al 1998, Russo et al 2003) and is associated with an increased risk of sudden death, as well as myocardial and cerebral infarction (Sasaki et al 1992). Sasaki et al (2001) reported the case of a 58 year old male with insulin-treated type 2 diabetes in whom acarbose appeared to be effective in the management of postprandial hypotension; during acarbose therapy (300 mg/day) the fall in systolic blood pressure observed over a 24 hour period was reduced from ~ 45 mmHg to 18 mmHg. Sasaki et al (2001) attributed this effect, probably incorrectly, to the ‘suppression of the postprandial increase of blood glucose and resultant inhibition of excess postprandial secretion of insulin and gastrointestinal peptides’. Given the observations in healthy elderly subjects and the case report by Sasaki et al (2001), it is likely that acarbose
represents a therapeutic option for the treatment of patients with postprandial hypotension. Studies to formally evaluate this possibility are now indicated.
Acarbose attenuates the hypotensive response to oral sucrose

Figure 8.1: Effects of control (○) and acarbose (100mg) (■) on (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate. Data are mean values ± SEM.
Acarbose attenuates the hypotensive response to oral sucrose

Figure 8.2: Effects of control (○) and acarbose (100mg) (■) on (a) gastric emptying and (b) and (c) intragastric meal distribution. Data are mean values ± SEM.
Acarbose attenuates the hypotensive response to oral sucrose

Figure 8.3: Effects of control (○) and acarbose (100mg) (■) on (a) blood glucose, (b) plasma insulin, (c) plasma GIP and (d) plasma GLP-1 concentrations. Data are mean values ± SEM.
Gastric distension attenuates the hypotensive response to intraduodenal glucose in healthy elderly subjects
9.1 Summary

The pathophysiology of postprandial hypotension which is an important clinical problem, particularly in the elderly, is poorly defined. It has been established previously that the magnitude of the fall in blood pressure is related directly to the rate of gastric emptying. There is evidence that gastric distension may attenuate the postprandial fall in blood pressure. The aim of this study was to determine the effects of gastric distension with intragastric water on the blood pressure, heart rate and blood glucose responses to intraduodenal glucose infusion in healthy elderly subjects. Eight subjects (five male and three female, aged 65 - 76 years) were studied on three separate days, in single-blind, randomised order. Subjects received an intraduodenal (ID) infusion of either (i) 50g glucose in 300ml saline (‘ID glucose’) over 60 minutes (t = 0 - 60 minutes), (ii) 50g glucose in 300ml saline over 60 minutes and intragastric (IG) infusion of 500ml water between t = 7 - 10 minutes (‘IG water and ID glucose’) or (iii) intraduodenal (ID) saline infusion over 60 minutes and intragastric (IG) infusion of 500ml water (‘IG water and ID saline’) all followed by intraduodenal saline (0.9%) infusion for a further 60 minutes (t = 60 - 120 minutes). Blood pressure (systolic and diastolic) and heart rate (DINAMAP) and blood glucose (glucometer) were measured. Gastric emptying of the intragastric water was assessed by two-dimensional (2D) ultrasonography. Between t = 0 - 60 minutes, systolic and diastolic blood pressure were greater (P < 0.05 for both) with ‘IG water and ID saline’ compared with ‘IG water and ID glucose’, and less (P < 0.05 for both) with ‘ID glucose’ compared with ‘IG water and ID glucose’. During ‘ID glucose’ the maximum fall in systolic blood pressure was 14.3 ± 2.7 mmHg at t = 59 ± 14.0 minutes, however, there were no changes in blood
pressure during ‘IG water and ID glucose’ (P = 0.98). Between t = 0 - 60 minutes, heart rate was higher (P < 0.01) with ‘IG water and ID glucose’ compared with ‘IG water and ID saline’ but there was no difference between ‘ID glucose’ and ‘IG water and ID glucose’. Gastric emptying was faster with ‘IG water and ID saline’ when compared with ‘IG water and ID glucose’ (T50: ‘IG water and ID saline’: 41.0 ± 4.0 minutes vs ‘IG water and ID glucose’: 77.7 ± 8.0 minutes; P = 0.006). There was no difference in the blood glucose response to ‘ID glucose’ compared with ‘IG water and ID glucose’.

In healthy elderly subjects, intragastric administration of water markedly attenuates the hypotensive response to intraduodenal glucose, presumably as a result of gastric distension. The consumption of water before a meal, accordingly, may represent a simple approach to the management of postprandial hypotension.

9.2 Introduction

Postprandial hypotension, defined as a decrease in systolic blood pressure ≥ 20mmHg, occurring within two hours of the end of a meal (Mathias et al 1989a, Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995), is recognised as an important clinical problem by leading to syncope and falls (Jansen and Lipsitz 1995) and in some cases, transient ischaemic attacks, stroke and angina (Vaitkevicius et al 1991, Jansen and Lipsitz 1995, Aronow and Ahn 1997). Those at greatest risk include the elderly and patients with autonomic dysfunction, the latter most often secondary to diabetes mellitus (Mathias et al 1989a, Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995). Current treatment options are suboptimal.
The mechanisms responsible for postprandial hypotension are unclear, but impaired regulation of splanchnic blood flow, the release of gastrointestinal hormones and sympathetic nerve activity appear important (Vaitkevicius et al 1991, Jansen and Lipsitz 1995, Aronow and Ahn 1997). The magnitude of the postprandial fall in blood pressure is known to be dependent on meal composition; ingestion of carbohydrate, particularly glucose, appears to have the greatest suppressive effect on blood pressure (Potter et al 1989, Aronow and Ahn 1997). In contrast to oral glucose, intravenous glucose has little, if any, effect on blood pressure (Jansen and Lipsitz 1995), indicating that postprandial hypotension is, at least in the broadest sense, triggered by gastrointestinal mechanisms. The onset of the postprandial fall in blood pressure after a meal is almost immediate, with a maximum response at 30 - 60 minutes (Jansen and Lipsitz 1995), suggesting a relationship to the rate of delivery of carbohydrate to the small intestine.

It has been recently established that the rate of nutrient delivery from the stomach to the small intestine is a major determinant of the hypotensive response to enterally administered glucose (Jones et al 1998, Jones et al 2001, O'Donovan et al 2002, Russo et al 2003). For example, in healthy elderly subjects, when glucose is infused intraduodenally at a rate of ~ 3 kcal/min, the magnitude of the fall in systolic blood pressure and rise in heart rate, are substantially greater (O'Donovan et al 2002) compared to infusion at a rate of ~ 1 kcal/min. In contrast to the effects of small intestinal nutrient exposure, studies (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) indicate that gastric distension may reduce the postprandial fall in blood pressure. For example, in young adult volunteers graded balloon distension of the proximal stomach using the so-called barostat device was shown to elevate systolic blood pressure and increase sympathetic neural tone progressively (Rossi et al 1998). Furthermore, while in healthy elderly subjects, stepwise (isobaric) barostat gastric distension, induces an increase in mean arterial blood pressure and muscle sympathetic nerve activity, in healthy young volunteers, the blood pressure response is attenuated and the increase in muscle sympathetic nerve activity is greater (van Orshoven et al 2004). Shannon et al (2002) reported in patients with autonomic failure that drinking water (480ml) immediately prior to a high carbohydrate meal, attenuated (mean ~ 21 mmHg) the fall in postprandial blood pressure. In these studies (Rossi et al 1998, Jordan et al 1999, Jordan et al 2000, Cariga and Mathias 2001, Shannon et al 2002), however, the effects on blood pressure were not related to changes in intragastric volume or pressure. Hence, the postprandial fall in blood pressure is related to ‘small intestinal’ nutrient exposure, while ‘intragastric’ mechanisms related to gastric distension, may attenuate the response. By definition, meal ingestion is associated with the stimulation of both ‘small intestinal’ and ‘intragastric’ mechanisms, for which in the majority of studies relating to postprandial hypotension have not attempted to discriminate (Chapter 1). This issue is of fundamental relevance to an understanding of the pathophysiology of postprandial hypotension and the development of effective therapy.
The aim of this study was to determine the effects of gastric distension with intragastric water on blood pressure, heart rate and the blood glucose response to intraduodenal glucose infusion in healthy elderly subjects. It is hypothesised that gastric distension would attenuate the fall in blood pressure induced by intraduodenal glucose infusion, and therefore, water ingestion before a meal may be considered a simple treatment strategy in patients with postprandial hypotension.

### 9.3 Research design and methods

Eight healthy elderly subjects, (five male and three female) with a median age of 71 years (range: 65 - 76 years) and body mass index (BMI) of 24.1 kg/m² (range: 21.1 - 28.2 kg/m²), recruited by advertisement, were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, or was taking medication known to influence blood pressure or gastrointestinal function.

#### 9.3.1 Experimental protocol

Each subject was studied on three occasions, each separated by a minimum of seven days, in single-blind, randomised order. On each day, the subject attended the University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital, at 08.30h following a fast (10.5h for solids; 8.5h for liquids) (Chapter 6). At that time, a silicone-rubber catheter (external diameter ~ 4mm) (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 \( \sim 1\text{mm} \) and was positioned so that the infusion port was located \( \sim 10\text{cm} \) distal to the pylorus (ie in the duodenum), as well as two other channels that were positioned in the antrum (2.5cm proximal to the pylorus) and duodenum (2.5cm distal to the pylorus), respectively. The latter two channels were perfused with normal (0.9\%) saline. A saline-filled, reference electrode (20 gauge intravenous cannula) was inserted subcutaneously into the subject’s forearm to enable measurement of the antroduodenal transmucosal potential difference (TMPD) (O'Donovan et al 2002). A smaller diameter (\( \sim 0.2\text{mm} \) internal) silicone tube was attached to the catheter with its outlet located \( \sim 10\text{cm} \) proximal to the pylorus, to allow intragastric infusion. The tip of the catheter was allowed to pass into the duodenum by peristalsis, which took between 40 and 205 minutes. An intravenous cannula was positioned in a left antecubital vein for blood sampling, and an automated blood pressure cuff placed around the left arm (O'Donovan et al 2002). Once intubated, the subject was allowed to rest in the recumbent position.

Approximately 30 minutes after the tube was positioned correctly (at \( t = 0 \) minutes) the subject was seated in a chair. At this time, the subject received either (i) an intraduodenal (ID) infusion of 50g of glucose dissolved in 300ml normal saline (‘ID glucose’) at a rate of 5 ml/min between \( t = 0 - 60 \) minutes, (ii) an identical intraduodenal (ID) infusion of 50g of glucose dissolved in 300ml normal saline between \( t = 0 - 60 \) minutes, and an intragastric (IG) infusion of 500ml water between \( t = 7 - 10 \) minutes (‘IG water and ID glucose’), or (iii) an intraduodenal (ID) saline (0.9\%) infusion at 5 ml/min between \( t = 0 - 60 \) minutes and an intragastric (IG) infusion of 500ml water between \( t = 7 - 10 \) minutes (‘IG water and ID saline’). On all three days, normal saline was infused intraduodenally at 5 ml/min between \( t = 60 - 120 \) minutes
Intraduodenal infusions were performed using a volumetric infusion pump (Imed Gemini PC-1, San Diego, CA, USA). For the intragastric infusions, water was pressurised (170 kPa) with oxygen and delivered at a rate of ~ 166 ml/min. Gastric emptying of the water was measured using ultrasound (Hveem et al 1996); in (i) sham ultrasonography measurements were performed to ensure that the subject remained blinded to the study conditions. At t = 120 minutes the catheter and intravenous cannula were removed. The subject was then given a light meal and was allowed to leave the laboratory soon after this. On one day cardiovascular autonomic nerve function was evaluated immediately after the completion of the study (Ewing and Clarke 1982, Piha 1991).

### 9.3.2 Measurement of 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 minutes prior to commencement of the intraduodenal infusions and then every three minutes between t = 0 - 120 minutes (O'Donovan et al 2002). ‘Baseline’ blood pressure and heart rate, ie ‘t = 0 minutes’, were calculated as the mean of measurements taken at t = -9, -6 and -3 minutes. 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 Measurement of gastric emptying

Antral area was measured by real-time ultrasonography using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, Australia) using the technique described in Chapter 4.4.2.1. The subject was scanned using a 3.5C broad spectrum 2.5 - 4 MHz convex transducer (Jones et al 1997) before (t = -2 minutes) the commencement of the intraduodenal infusion, at t = 15 minutes ie 5 minutes after the completion of the intragastric infusions and then every 15 minutes until t = 120 minutes. The circumference of the antrum was outlined and the area recorded during the fasting state (t = -2 minutes) was subtracted from subsequent measurements. The 50% emptying time (T50) was also determined, as previously described (Hausken and Berstad 1992).

9.3.4 Measurement of blood glucose concentrations

Venous blood samples (~ 5ml) were obtained prior to commencement of the intraduodenal infusions (t = -2 minutes) and at 15 minute intervals between t = 0 - 120 minutes. Blood glucose concentrations were determined according to the method described in Chapter 4.8.

9.3.5 Assessment of cardiovascular autonomic nerve function

Autonomic nerve function was evaluated using standardised cardiovascular reflex tests (Ewing and Clarke 1982, Piha 1991). A description of this method is included in Chapter 4.10.
9.3.6 **Statistical analysis**

Data were evaluated using mixed model repeated measures 2 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. Data were analysed separately from baseline - 60 minutes and from t = 60 - 120 minutes for systolic and diastolic blood pressure and heart rate and from t = -2 - 60 minutes and t = 60 - 120 minutes for blood glucose concentration to evaluate the effects (‘treatment’ and ‘time’) of intraduodenal glucose. Gastric emptying and blood glucose concentration were analysed as absolute values. One-way ANOVA was used to analyse the effects of ‘time’ on systolic and diastolic blood pressure, heart rate, gastric emptying and blood glucose. The maximum fall in blood pressure was defined as the greatest mean change from baseline for a treatment at any given time point. In all analyses, post-hoc comparisons of adjusted means were performed using Student’s t-tests. 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 shown as change from baseline and 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 and there were no untoward events. The median score for autonomic nerve dysfunction was 0.88 (range: 0 - 3); one of the eight subjects had definite autonomic dysfunction. While no subject experienced postprandial hypotension (ie a fall in systolic blood pressure > 20 mmHg sustained for at least 30 minutes), in
five of them, the magnitude of the fall in systolic blood pressure was > 20 mmHg at one or more time point. In one subject, gastric emptying data were not available because of technical difficulties.

9.4.1 Blood pressure and heart rate

There was no significant difference in baseline blood pressure or heart rate between the three days (‘IG water and ID saline’ vs ‘ID glucose’ vs ‘IG water and ID glucose’): systolic blood pressure (121.1 ± 4.7 mmHg vs 125.2 ± 4.5 mmHg vs 122.9 ± 4.5 mmHg); diastolic blood pressure (71.3 ± 2.4 mmHg vs 72.1 ± 2.6 mmHg vs 69.8 ± 2.3 mmHg) and heart rate (61.1 ± 2.7 beats/minute (bpm) vs 59.2 ± 3.2 bpm vs 58.5 ± 3.4 bpm).

Between baseline and 60 minutes, there was a rise (P < 0.01) in systolic blood pressure during ‘IG water and ID saline’, a fall (P < 0.05) during ‘ID glucose’ (maximum fall in systolic blood pressure: 14.3 ± 2.7 mmHg at t = 59 ± 14.0 minutes) and no change during ‘IG water and ID glucose’ (P = 0.98) ie intragastric water attenuated the fall in systolic blood pressure induced by intraduodenal glucose. There was a significant ‘treatment x time’ effect (P < 0.001) for systolic blood pressure for the three studies between baseline and 60 minutes so that systolic blood pressure was greater (P < 0.05) during ‘IG water and ID saline’ when compared with ‘IG water and ID glucose’, and less (P < 0.05) during ‘ID glucose’ when compared with ‘IG water and ID glucose’. At t = 60 minutes, systolic blood pressure was higher (P = 0.01) than baseline after ‘IG
water and ID saline’ but not with intraduodenal glucose, with (P = 0.84) and without (P = 0.44) IG water (Figure 9.1 (a)).

Between t = 60 - 120 minutes, systolic blood pressure was higher (P = 0.05) after ‘IG water and ID saline’ when compared with ‘IG water and ID glucose’. However, there was no difference (P = 0.70) in systolic blood pressure after ‘ID glucose’ compared with ‘IG water and ID glucose’. At t = 120 minutes, systolic blood pressure was higher (P = 0.03) than baseline after ‘IG water and ID saline’, while there was a trend for systolic blood pressure to be less (P = 0.08 for both) after intraduodenal glucose, with or without intragastric water (Figure 9.1 (a)).

Between baseline and 60 minutes, there was a trend (P = 0.09) for a rise in diastolic blood pressure during ‘IG water and ID saline’. In contrast, there was a fall (P < 0.0001) in diastolic blood pressure with ‘ID glucose’, but not after ‘IG water and ID glucose’ (P = 0.17). There was a significant ‘treatment x time’ effect (P < 0.0001) for diastolic blood pressure between baseline and 60 minutes. Diastolic blood pressure was greater (P < 0.05) with ‘IG water and ID saline’ when compared with ‘IG water and ID glucose’ and less (P < 0.05) during ‘ID glucose’ than ‘IG water and ID glucose’.

Between t = 60 - 120 minutes, there was a rise (P < 0.01) in diastolic blood pressure after ‘ID glucose’, but not following ‘IG water and ID glucose’ (P = 0.48) or ‘IG water and ID saline’ (P = 0.97). Between t = 60 - 120 minutes, there was a trend (P = 0.09) for a difference in diastolic blood pressure between the three study days. At t = 120
minutes, diastolic blood pressure was not significantly different from baseline following ‘IG water and ID saline’ (P = 0.88) or ‘IG water and ID glucose’ (P = 0.25) while there was a trend (P = 0.06) for diastolic blood pressure to be less after ‘ID glucose’ (Figure 9.1 (b)).

Between baseline and 60 minutes, there was no effect of ‘IG water and ID saline’ on heart rate (P = 0.23). In contrast, there was a progressive rise in heart rate (P < 0.0001 for all) during intraduodenal glucose with or without intragastric water. There was a significant ‘treatment x time’ effect (P < 0.0001) for heart rate between baseline and 60 minutes. Heart rate was higher (P < 0.01) with ‘IG water and ID glucose’ when compared with ‘IG water and ID saline’, with no difference between ‘ID glucose’ and ‘IG water and ID glucose’.

Between t = 60 - 120 minutes, there was no change (P = 0.23) in heart rate, during ‘IG water and ID saline’ and a progressive fall (P < 0.0001 for both) with intraduodenal glucose both with and without IG water. There was a significant ‘treatment x time’ effect (P < 0.01) for heart rate between t = 60 - 120 minutes so that heart rate was greater (P < 0.01) following ‘IG water and ID glucose’ when compared with ‘IG water and ID saline’. At t = 120 minutes, heart rate was not significantly differently from baseline (P = 0.11) following ‘IG water and ID saline’ but was higher than baseline following intraduodenal glucose with (P = 0.009) and without (P = 0.007) intragastric water (Figure 9.1 (c)).
9.4.2 Gastric emptying

Gastric emptying of water following ‘IG water and ID saline’ approximated a mono-exponential pattern. Following ‘IG water and ID glucose’, there was no significant emptying of water until \( t = 75 \) minutes (\( P = 0.04 \)). Gastric emptying was much faster (\( P < 0.01 \)) during ‘IG water and ID saline’ when compared with ‘IG water and ID glucose’ (Figure 9.2). Similarly, the \( T_{50} \) was less during ‘IG water and ID saline’ (41.0 \( \pm \) 4.0 minutes) compared with ‘IG water and ID glucose’ (77.7 \( \pm \) 8.3 minutes; \( P = 0.006 \)).

9.4.3 Blood glucose concentrations

There was no significant difference in baseline blood glucose between the three days (‘IG water and ID saline’ vs ‘ID glucose’ vs ‘IG water and ID glucose’): 6.0 \( \pm \) 0.2 mmol/L vs 6.0 \( \pm \) 0.2 mmol/L vs 5.9 \( \pm \) 0.1 mmol/L (Figure 9.3). There was a rise in blood glucose concentrations between \( t = -2 \) - 60 minutes during intraduodenal glucose both with (\( P < 0.0001 \)), and without (\( P < 0.0001 \)), intragastric water. In contrast, there was a minor, albeit significant (\( P < 0.05 \)), fall in blood glucose after ‘IG water and ID saline’. There was a significant ‘treatment x time’ effect (\( P < 0.0001 \)) for blood glucose between \( t = -2 \) - 60 minutes. Blood glucose was greater (\( P < 0.01 \)) with ‘IG water and ID glucose’ when compared with ‘IG water and ID saline’, with no difference between ‘ID glucose’ compared with ‘IG water and ID glucose’.

Between \( t = 60 \) - 120 minutes, there was no significant change (\( P = 0.10 \)) in blood glucose concentrations, during ‘IG water and ID saline’, whereas a fall (\( P < 0.0001 \) for
both) was evident with intraduodenal glucose both with and without intragastric water. There was a significant ‘treatment x time’ effect ($P < 0.0001$) for blood glucose between $t = 60 - 120$ minutes. Blood glucose was less ($P < 0.01$) after ‘IG water and ID saline’ when compared with ‘IG water and ID glucose’, while there was no difference between ‘ID glucose’ when compared with ‘IG water and ID glucose’. At $t = 120$ minutes, blood glucose concentrations were not significantly different than baseline after any of the infusions (‘IG water and ID saline’, $P = 0.30$; ‘ID glucose’, $P = 0.12$; ‘IG water and ID glucose’, $P = 0.26$) (Figure 9.3).

### 9.5 Discussion

The major novel observation of this study is that gastric distension induced by rapid intragastric instillation of 500ml water, prevents the falls in systolic and diastolic blood pressure induced by intraduodenal glucose infusion at a rate of ~ 3 kcal/min in healthy elderly subjects. There was a predictable increase in blood pressure in response to gastric distension by water in the absence of small intestinal nutrient exposure and a marked slowing of gastric emptying of water by concurrent small intestinal glucose, when compared with saline, infusion. While none of the subjects had postprandial hypotension, the magnitude of the fall in systolic blood pressure induced by intraduodenal glucose was substantial (maximum 14.3 ± 2.7 mmHg). Hence, observations from this study, which establish that the hypotensive response to small intestinal nutrients is attenuated by non-nutrient gastric distension, have implications for the management of postprandial hypotension.
Previous studies have established a relationship between the magnitude of the postprandial fall in systolic blood pressure and rise in heart rate with the rate of gastric emptying of glucose drinks (Jones et al 1998) and the exposure of glucose to the small intestinal mucosa (Jones et al 2001, Russo et al 2003, O'Donovan et al 2005b). In healthy subjects, gastric emptying of glucose solutions approximates an overall rate of ~ 2 - 3 kcal/min, after an initial emptying phase that may be somewhat faster (Horowitz et al 1996) as a result of inhibitory feedback arising from the small intestine (Lin et al 1989, Horowitz et al 2001). This formed the basis for the use of a ~ 3 kcal/min infusion in the current study - the magnitudes of the observed fall in blood pressure and rise in heart rate were comparable to those noted previously (O'Donovan et al 2002, O'Donovan et al 2005b). A previous study indicates that the response to small intestinal glucose is load, rather than concentration dependent (Chapter 6).

Recent studies have suggested that gastric distension influences 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, Young and Mathias 2004a, Young and Mathias 2004b, Jones et al 2005). In an initial study in healthy young subjects, proximal gastric distension with a barostat device was shown to increase blood pressure (mean ~ 8 mmHg) and muscle sympathetic nerve activity, the so-called ‘gastrovascular reflex’ (Rossi et al 1998); these effects were also evident in healthy elderly subjects where the magnitude of the increase in blood pressure (mean ~ 12 mmHg) was substantial (van Orshoven et al 2004). While there was an increase in blood pressure following gastric distension and intraduodenal glucose, intragastric water did not induce a rise in heart
rate following intraduodenal infusion of saline. The effects of gastric distension on heart rate remain unclear. It is also unknown why the attenuation in blood pressure by gastric distension had no affect on the rise in heart rate after intraduodenal glucose. Consumption of water (~ 240ml - 480ml) before a meal has been shown to increase blood pressure in healthy adult subjects (Jordan et al 1999, Jordan et al 2000), as well as in patients with multiple system atrophy and pure autonomic failure pressure (Jordan et al 1999, Jordan et al 2000, Cariga and Mathias 2001, Young and Mathias 2004a, Young and Mathias 2004b). More recently, in patients with autonomic failure, drinking 480ml of water immediately prior to a high carbohydrate meal, was shown to markedly attenuate (mean ~ 21 mmHg) the postprandial fall in blood pressure (Shannon et al 2002). Ingestion of water (~ 480ml) has also been shown to have no effect on (Imai et al 1998), or result in a decrease (Senard et al 1999) or increase (Routledge et al 2002) in blood pressure in other groups. Furthermore, in a recent study in healthy elderly subjects (Jones et al 2005), the fall in blood pressure was less when glucose drinks of the same concentration (12.5%) were ingested at a higher volume (600ml vs 200ml). There is also evidence that rapid (ie < 5 minutes) water ingestion is required to attenuate postprandial falls in blood pressure (Jordan et al 1999, Jordan et al 2000, Cariga and Mathias 2001, Young and Mathias 2004a, Young and Mathias 2004b). In healthy adult subjects (Jordan et al 1999, Jordan et al 2000) and patients with autonomic failure (ie multiple system atrophy and pure autonomic failure) (Jordan et al 1999, Jordan et al 2000, Cariga and Mathias 2001, Young and Mathias 2004a, Young and Mathias 2004b), the onset of the pressor response is first evident between ~ 5 and 15 minutes following water ingestion, reaches a peak at ~ 30 - 35 minutes, although
maximum peaks in blood pressure have also been demonstrated at ~ 14 - 20 minutes (Cariga and Mathias 2001, Shannon et al 2002), and then blood pressure returns to baseline levels between ~ 60 - 90 minutes (Young and Mathias 2004b).

The mechanisms mediating the pressor response to acute ingestion of water remain unclear. It has been suggested that the increase in blood pressure may be a result of resetting of the baroreflex (Rossi et al 1998), reduced compliance of the splanchnic arteries (van Orshoven et al 2004), reflex sympathetic activation (Jordan et al 2000), the hypo-osmotic properties of water (Brown et al 2005), the release of vasoactive substances and the overall fluid status of individuals (Young and Mathias 2004b).

Gastric emptying of water was predictably much slower during intraduodenal glucose infusion so that emptying was only evident after ~ 75 minutes, whereas emptying of intragastric water commenced immediately during intraduodenal saline infusion, as a result of small intestinal feedback stimulated by the presence of glucose.

While an increase in gastric distension appears to attenuate the postprandial fall in blood pressure, its role in the management of postprandial hypotension, may be influenced by changes in intragastric volume and the site of gastric distension (Jones et al 2005). The volume of water ingested has been shown to influence the pressor response. While rapid (ie < 5 minutes) ingestion of water in volumes as low as 120ml may be associated with a substantial pressor effect (Shannon et al 2002), when the volume of water is increased to 480ml, the magnitude of the elevation in systolic blood
pressure is substantially greater (Shannon et al 2002). Furthermore, the site of gastric distension is likely to be important in the observed effects of gastric distension on postprandial blood pressure (Jones et al 2005). While proximal stomach distension (Rossi et al 1998) increases blood pressure in healthy young subjects, an attenuation of the fall in blood pressure after a glucose drink, has been shown to be related to an increase in proximal gastric content in healthy elderly subjects (Jones et al 2005). However, the site of gastric distension, as determined by the distribution of a meal within the stomach, may be influenced by the meal volume and composition and body posture (Horowitz et al 1993b).

It has been established (Jones et al 1998, Jones et al 2001, O'Donovan et al 2002, O'Donovan et al 2004b, Jones et al 2005, O'Donovan et al 2005b, Visvanathan et al 2006) that the hypotensive response to meals is triggered by the interaction of nutrients with the small intestine and support the concept that ‘gastric distension’, whether non-nutrient or nutrient-induced, attenuates the postprandial fall in blood pressure (Jones et al 2005). Given that small intestinal nutrient-mediated feedback is known to play a major, and probably dominant, role in the regulation of gastric emptying (Brener et al 1983), this finding is not surprising.

In this current study, there was no effect on glycaemia following intraduodenal glucose infusion with or without intragastric water infusion, consistent with previous findings (Jones et al 2005). This is not surprising given that, as discussed, there was relatively little water emptied during the first 60 minutes.
Current treatment options for postprandial hypotension are suboptimal. Octreotide, attenuates the postprandial fall in blood pressure but is expensive, requires multiple subcutaneous injections and is associated with a high prevalence of adverse effects (Jansen et al 1989b). Caffeine has been suggested as a simple approach to therapy, however, information relating to its efficacy is inconsistent (Lipsitz et al 1994). Guar gum slows gastric emptying, oral glucose absorption and decreases postprandial blood pressure reductions, but it is unpalatable (Jones et al 2001, Russo et al 2003). Based on the present observations and those of others, drinking water before or immediately after a meal may represent a relatively simple and non-invasive approach to the treatment of postprandial hypotension. Accordingly, while further studies are required, including those to evaluate the effect of chronic gastric distension, drinking ~ 500ml of water before a meal could be regarded as a simple adjunctive treatment strategy in patients with postprandial hypotension, although this may not be always feasible in the elderly because of the substantial volume, as well as the potential risk of water intoxication (Shannon et al 2002).

In conclusion, water-induced gastric distension attenuates the falls in systolic and diastolic blood pressure induced by intraduodenal infusion of glucose. Enteral glucose induced a rise in heart rate, with and without gastric distension, and gastric emptying of intragastric water was slower during intraduodenal glucose infusion. Hence, gastric distension may represent a simple approach to the management of patients with postprandial hypotension treatment although further studies in patients with postprandial hypotension are now warranted.
Figure 9.1: Change in systolic blood pressure (a), diastolic blood pressure (b) and heart rate (c), from baseline in eight healthy elderly subjects after intraduodenal infusion of glucose alone (‘ID glucose’) (□), intraduodenal glucose infusion followed by intragastric infusion of water (‘IG water & ID glucose’) (●) and intraduodenal saline infusion followed by intragastric infusion of water (‘IG water & ID saline’) (○). Data are mean values ± S.E.M.
Figure 9.2: Gastric emptying of 500ml water in eight healthy elderly subjects after intraduodenal glucose (‘IG water and ID glucose’) (●) and intraduodenal saline (‘IG water and ID saline’) (○). Data are mean values ± SEM.
Figure 9.3: Blood glucose concentrations in eight healthy elderly subjects after intraduodenal infusion of glucose alone (‘ID glucose’) (□), intraduodenal glucose infusion followed by intragastric infusion of water (‘IG water and ID glucose’) (●) and intraduodenal saline infusion followed by intragastric infusion of water (‘IG water and ID saline’) (○). Data are mean values ± SEM.
The role of nitric oxide mechanisms in gastric emptying of, and the blood pressure and glycaemic responses to, oral glucose in healthy elderly subjects
10.1 Summary

Postprandial hypotension is an important clinical problem, particularly in the elderly, and is associated with an increased incidence of falls, stroke and angina. Recent studies have established that the magnitude of the fall in blood pressure is related to the rate of gastric emptying. Animal studies indicate that nitric oxide mechanisms are important in the regulation of postprandial splanchnic blood flow. The primary aims of this study were to evaluate the effects of the nitric oxide synthase inhibitor, NG-nitro-L-arginine-methyl-ester (L-NAME), on gastric emptying of, and the blood pressure, glycaemic, insulin and incretin responses to, oral glucose in elderly subjects. Eight healthy subjects (four male and four female, aged 66 - 76 years) were studied on two separate days, in double-blind, randomised order. Subjects received an intravenous infusion of either L-NAME (180 µg/kg/hr) or saline (0.9%) at a rate of 3 ml/min for 150 minutes. Thirty minutes after the commencement of the infusion (t = 0 minutes), subjects consumed a 300ml drink containing 50g glucose labelled with 20 MBq 99mTc sulphur colloid, while sitting in front of a gamma camera. Gastric emptying, blood pressure (systolic and diastolic), heart rate, blood glucose, plasma insulin, plasma glucose-dependant insulino-tropic-polypeptide (GIP) and plasma glucagon-like peptide 1 (GLP-1), were measured. L-NAME had no effect on gastric emptying or plasma GIP and GLP-1. Between t = -30 - 0 minutes L-NAME had no effect on blood pressure or heart rate. After the drink (t = 0 - 60 minutes) systolic and diastolic blood pressure fell (P < 0.05 for both) and heart rate increased (P < 0.01) during saline; these effects were attenuated (P < 0.001 for all) by L-NAME. Blood glucose levels were higher (P = 0.007), and plasma insulin less (P < 0.0001), after L-NAME. In conclusion, the fall in
blood pressure, increase in heart rate and stimulation of insulin secretion by oral glucose in elderly subjects are mediated by nitric oxide mechanisms by an effect unrelated to gastric emptying or changes in incretin hormones.

10.2 Introduction

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20mmHg, occurring within two hours of a meal (Mathias et al 1989a, Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995), is an important clinical problem by predisposing to a number of disorders including syncope, transient ischaemic attacks, stroke and angina (Vaitkevicius et al 1991, Jansen and Lipsitz 1995, Aronow and Ahn 1997). Those most at risk include the elderly and patients with autonomic nerve dysfunction; the latter is often secondary to diabetes mellitus (Mathias et al 1989a, Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995). Current therapies are sub-optimal (Jansen and Lipsitz 1995).

The potential mechanisms mediating postprandial hypotension are poorly defined, and include impaired regulation of splanchnic blood flow (Puisieux et al 2000), the release of gastrointestinal peptides (Jansen and Hoefnagels 1991) and disordered sympathetic nerve activity (Masuo et al 1996). The magnitude of the fall in blood pressure is known to be dependent on meal composition; ingestion of carbohydrate, particularly glucose, is thought to have the greatest effect on blood pressure, while it has been suggested that the effects of fat, protein or water are substantially less (Potter et al 1989, Jansen et al 1990). It has recently been established that the rate of nutrient delivery to the small
intestine is an important determinant of the hypotensive response to enterally administered glucose (Jones et al 2001, O'Donovan et al 2002, Russo et al 2003). For example, in healthy elderly subjects the magnitude of the fall in systolic blood pressure and rise in heart rate are substantially greater when glucose is infused intraduodenally at a rate of ~ 3 kcal/min when compared to ~ 1 kcal/min (O'Donovan et al 2002).

Nitric oxide is an important neurotransmitter in the gastrointestinal tract (Andrawis et al 2000). The role of nitric oxide mechanisms is optimally addressed by the use of specific inhibitors of its production, such as NG-monomethyl-L-arginine (L-NMMA) (Chiodera et al 1994), L-NAME (Chiodera et al 1996) or glyceryl trinitrate (GTN) (Andrawis et al 2000). Studies employing nitric oxide synthase blockers have established that nitric oxide mechanisms are important in the regulation of splanchnic blood flow in animals (Alemany et al 1997, Matheson et al 1997). The effects of nitric oxide synthase inhibition on gastric emptying in humans have been assessed in two studies with inconsistent observations (Konturek et al 1999, Hirsch et al 2000).

The role of nitric oxide mechanisms in glucose-induced insulin secretion is controversial (Jansen et al 1990, Kearney et al 1998). In particular, the outcome of animal studies and in-vitro studies relating to the role of nitric oxide mechanisms on insulin secretion are inconsistent (Coiro et al 1997, Smukler et al 2002). To date, no human studies have evaluated the role of nitric oxide in the insulin response to oral glucose; the latter is known to be dependent on the secretion of the so-called ‘incretin hormones’, GIP and GLP-1 (Schirra et al 1996). The major aims of this study were to
evaluate the role of nitric oxide mechanisms in mediating the hypotensive response to oral glucose in healthy elderly subjects and to determine whether any effect was related to changes in gastric emptying. The study design also allowed evaluation of the effects of nitric oxide blockade on the glycaemic, insulin and incretin hormone responses to oral glucose.

10.3 Research design and methods

Eight healthy elderly subjects, recruited by advertisement, (four male and four female) with a median age of 71 years (range: 66 - 76 years) and body mass index (BMI) of 26.5 kg/m² (range: 22.2 - 29.2 kg/m²), were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal or cardiac disease, chronic alcohol abuse or epilepsy, or was taking medication known to influence blood pressure or gastrointestinal function. In all subjects resting (sitting) systolic and diastolic blood pressure were less than 160 mmHg and 90 mmHg respectively and the electrocardiogram (ECG) was normal.

10.3.1 Experimental protocol

Each subject underwent two studies in double-blind, randomised order, separated by at least seven days. On one day, subjects received an intravenous infusion of L-NAME, and on the other an intravenous infusion of saline, for 150 minutes. Subjects attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry, Royal Adelaide Hospital, at 08.30 hours following a fast (10.5 hours for solids; 8.5 hours for liquids) (Chapter 8). A cannula was placed in a right forearm vein
for the infusion and in a left antecubital vein for blood sampling. Subjects were seated with their back against a gamma camera, and a blood pressure cuff around their left arm.

Each subject rested comfortably in the sitting position for approximately 30 minutes. At t = -30 minutes, an intravenous infusion of either L-NAME (180 µg/kg/hr) (Clinalfa AG, Switzerland) (Su et al 2001) or saline (0.9%) was commenced and continued at a rate of 3 ml/min for 150 minutes ie until t = 120 minutes. The dose of L-NAME was based on previous studies (Vozzo et al 1999, Su et al 2001). At t = 0 minutes, subjects consumed a drink which was at room temperature and comprised 50g glucose, 30ml lemon juice and 20 MBq $^{99m}$Tc sulphur colloid, made up to a total volume of 300ml with water. At t = 150 minutes the intravenous cannulae were removed; the subject was then given a light meal and allowed to leave the laboratory. Cardiovascular autonomic nerve function was evaluated on one of the study days (Ewing and Clarke 1982, Piha 1991).

10.3.2 Measurement of blood pressure and heart rate

Blood pressure (systolic and diastolic) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP, Johnson & Johnson, Tampa, FL, USA) immediately prior to the infusion (t = -30 minutes) at three minute intervals between t = -30 - 60 minutes and then at 15 minute intervals between t = 60 - 150 minutes (Jones et al 1998). Postprandial hypotension was defined as a fall in systolic blood pressure $\geq$ 20 mmHg after the glucose drink that was sustained for at
least 30 minutes (Jansen and Lipsitz 1995). ‘Baseline’ blood pressure (ie \( t = 0 \) minutes) was calculated as the mean of measurements taken at \( t = -3, -6 \) and -9 minutes immediately before consumption of the drink.

### 10.3.3 Measurement of gastric emptying

Subjects consumed the drink within two minutes. Radioisotopic data were acquired for 120 minutes according to the method described in Chapter 4.4.1. Regions-of-interest were drawn around the total stomach and 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 minutes. The 50% emptying time (T50) was also determined (Collins et al 1983).

### 10.3.4 Measurement of blood glucose, plasma insulin, plasma GIP, and plasma GLP-1 concentrations

Venous blood samples (~20ml) were obtained immediately prior to the infusion (\( t = -30 \) minutes), at \( t = -15 \) minutes, \( t = -2 \) minutes, and at 15 minute intervals between \( t = -30 - 60 \) minutes, and 30 minute intervals between \( t = 60 - 150 \) minutes. Blood glucose, plasma insulin, GIP and GLP-1 concentrations were measured using methods described in Chapters 4.8 and 4.9.
10.3.5 Assessment of cardiovascular autonomic nerve function

Autonomic nerve function was evaluated using standardised cardiovascular reflex tests (Ewing and Clarke 1982, Piha 1991). A description of this method is included in Chapter 4.10.

10.3.6 Statistical analysis

Repeated measures 2 way Analysis of Variance (ANOVA) was used to examine the overall effects of ‘treatment’ and ‘time’ on the change in systolic and diastolic blood pressure, heart rate, blood glucose, plasma insulin, plasma GIP and plasma GLP-1 concentrations ie for the 30 minute period immediately preceding the drink (ie t = -30 - 0 minutes) and from t = 0 - 60 minutes and t = 0 - 120 minutes for systolic and diastolic blood pressure (as the maximum postprandial fall in blood pressure is known to occur during this time) (Jansen and Lipsitz 1995), heart rate and gastric emptying and from t = 0 - 150 minutes for blood glucose, plasma insulin, plasma GIP and plasma GLP-1 concentrations. One-way repeated measures ANOVA were conducted to evaluate the effects of ‘time’ on systolic and diastolic blood pressure, heart rate, gastric emptying, blood glucose, plasma insulin, plasma GIP and plasma GLP-1 concentrations. 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 ± standard error of the mean (SEM), unless stated otherwise. A P value < 0.05 was considered significant in all analyses.
10.4 Results

All studies were well tolerated and there were no untoward events. No subject had definite autonomic neuropathy; the median score for autonomic nerve dysfunction was 1.0 (range: 0 - 2).

10.4.1 Blood pressure and heart rate

There was no difference in systolic or diastolic blood pressure or heart rate at t = -30 minutes between the two groups. Furthermore, there was no significant change in any of these parameters between t = -30 - 0 minutes; nor any difference between the saline and L-NAME infusions (Figure 10.1).

Between t = 0 - 60 minutes, systolic blood pressure fell (P < 0.005) during the saline, but did not change (P = 0.17) during the L-NAME, infusion (Figure 10.1 (a)). During saline infusion, the maximum fall in systolic blood pressure was 9.5 ± 3.0 mmHg at 46.5 ± 4.0 minutes; in one subject, the magnitude of the fall in systolic blood pressure was > 20mmHg. Systolic blood pressure had returned to baseline by ~ 75 minutes on the saline day. Systolic blood pressure was less during the saline, when compared to the L-NAME, infusion between t = 0 - 60 minutes (P < 0.001) and between t = 0 - 120 minutes (P < 0.01).

Between t = 0 - 60 minutes there was a fall in diastolic blood pressure during both saline (P < 0.05), and L-NAME (P < 0.02) infusions. Diastolic blood pressure was less
during the saline, than the L-NAME, infusion between t = 0 - 60 minutes (P < 0.001) and t = 0 - 120 minutes (P < 0.05) (Figure 10.1 (b)).

Between t = 0 - 60 minutes there was a rise in heart rate during the saline (P < 0.01) and a fall in heart rate (P < 0.005) during the L-NAME, infusion (Figure 10.1 (c)); between t = 0 - 60 minutes and t = 0 - 120 minutes heart rate was higher (P < 0.001 for both) during the saline, than the L-NAME, infusion.

10.4.2 Gastric emptying

Gastric emptying approximated an overall linear pattern on both days. There was no significant difference in gastric emptying (P = 0.40) (Figure 10.2) or T50 between the two days (saline: 55.8 ± 3.8 minutes vs. L-NAME: 61.8 ± 3.8 minutes; P = 0.28).

10.4.3 Blood glucose, plasma insulin, plasma GIP, and plasma GLP-1 concentrations

There were no initial differences in blood glucose concentrations between the two days, however, between t = 90 - 150 minutes, blood glucose levels were higher (P = 0.007) during the L-NAME infusion (Figure 10.3 (a)). Plasma insulin levels were lower after L-NAME (P < 0.0001) (Figure 10.3 (b)). There was no difference in plasma GIP (P = 0.39) or plasma GLP-1 (P = 0.79) concentrations between the two days (Figure 10.3 (c) and (d)). There was a significant rise in plasma GIP (P < 0.001 for both) on both days (Figure 10.3 (c)). Between -2 - 150 minutes, there was a significant rise in plasma GLP-
1 (P = 0.02) over time, during the saline infusion, but not after L-NAME (P = 0.81) (Figure 10.3 (d)).

10.5 Discussion

The major observations in this study are that the nitric oxide synthase blocker, L-NAME, when administered acutely to healthy elderly subjects in a dose of 180 µg/kg/hr: (i) had no effect on either blood pressure or heart rate in the 30 minute period before a glucose drink, (ii) attenuated the fall in systolic and diastolic blood pressure and increase in heart rate after oral glucose, (iii) had no effect on gastric emptying of glucose; (iv) attenuated the glucose-induced rise in plasma insulin, and (v) had no effect on the incretin hormone (ie GIP and GLP-1) response to oral glucose. The study, accordingly, indicates that the magnitude of the fall in blood pressure and increase in heart rate and stimulation of insulin secretion induced by oral glucose in healthy elderly subjects are mediated by nitric oxide mechanisms by an effect unrelated to changes in gastric emptying, or the secretion of GIP and GLP-1.

Recent studies (Jones et al 1998, Jones et al 2001, Russo et al 2003) have established that gastric emptying is a determinant of the hypotensive response to oral glucose in both healthy elderly subjects and type 2 diabetes. For example, the slowing of gastric emptying and glucose absorption induced by guar is associated with a reduction in the magnitude of the fall in blood pressure after a glucose drink (Jones et al 2001, Russo et al 2003). Hence, in interpreting the present observations it was important to determine the effects of nitric oxide blockade on gastric emptying, particularly as the outcome of
two previous studies is conflicting (Konturek et al 1999, Hirsch et al 2000). While Konturek et al (1999), reported that the nitric oxide synthase inhibitor, L-NMMA, administered in a dose of ~ 1 mg/kg/hr, accelerated gastric emptying of a nutrient-liquid as assessed by a carbon breath test, Hirsch et al (2000) found that L-NMMA, given in a dose of 3.75 mg/kg for 5 minutes and 7.1 mg/kg/hr thereafter, had no effect on gastric emptying of a pancake meal as assessed by the more sensitive scintigraphic method. Both studies involved healthy, young adult males. The current findings concur with those of Hirsch et al (2000) in that a dose of L-NAME, which has biological effects, did not affect gastric emptying, and discounts a role for gastric emptying in the observed effects of L-NAME on the blood pressure and heart rate responses to oral glucose. Hence, these effects must be mediated by other mechanism(s).

The relatively low dose of L-NAME used in this study has been shown to affect cardiovascular function in young, healthy subjects (Vozzo et al 1999, Su et al 2001); much higher doses have been used by others (Sander et al 1999, Frandsen et al 2001, Chavoshan et al 2002, van den Meiracker et al 2002). Given that highly significant effects on blood pressure and heart rate were observed, it appears that the degree of nitric oxide inhibition was adequate. Moreover, effects have also been observed in humans using lower doses of L-NAME (Coiro et al 1997). Hence, although ageing may be associated with defective nitric oxide release (Lyons et al 1997, Kamper et al 2004), and it would certainly be of interest to evaluate the effects of a dose of L-NAME higher than utilised in this current study, this may pose ethical issues. It is also appropriate to note that it cannot be determined whether the cardiovascular effects induced by the
infusion of L-NAME reflect, the accumulation of the long-acting metabolite, NG-nitro-L-arginine (L-NNA), which may play a role in blood pressure regulation (Avontuur et al 1998, Frandsen et al 2001). This may have relevance to the observation that L-NAME had no effect on blood pressure or heart rate in the 30 minute period before consumption of the drink. Changes in sympathetic activity may also contribute to the cardiovascular effects of L-NAME (Owlya et al 1997, Sander et al 1999). Although only one of the subjects had postprandial hypotension, the mean fall in systolic blood pressure during the saline infusion was substantial at 9.5 mmHg; this fall was abolished by L-NAME. These findings may, accordingly, have implications for the treatment of postprandial hypotension and the effects of nitric oxide synthase inhibition warrant evaluation in this group.

There are a number of potential pathways by which nitric oxide mechanisms could influence the cardiovascular response to oral glucose. Changes in splanchnic blood flow are likely to be important. Following a meal, there is an increase in splanchnic blood flow (Mathias 1991), associated with reductions in total systemic vascular resistance and skeletal muscle blood flow (Lyons et al 1997, O'Mara and Lyons 2002). The magnitude of the increase in mesenteric blood flow is comparable in normal young and elderly individuals, despite the greater fall in blood pressure in the elderly (Lipsitz et al 1993). 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, 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). Further studies are also indicated to determine whether the effects of L-NAME on blood pressure reflect changes in systemic and/or splanchnic blood flow. The effects of L-NAME on systolic blood pressure and heart rate were sustained until at least 120 minutes (the time of completion of the L-NAME infusion); at this time gastric emptying was not completed and we are, accordingly, unable to determine whether the effects were dependent on ongoing nutrient entry into the small intestine, or not.

Various peptides released by food, including insulin, vasoactive intestinal polypeptide, substance P, neurotensin and calcitonin gene-related peptide have been implicated in the aetiology of postprandial hypotension (Jansen and Hoefnagels 1991, Mathias 1991, Jansen and Lipsitz 1995). While insulin has vasodilatory properties (Scherrer and Sartori 1997), the observations that postprandial hypotension occurs in patients with type 1 diabetes, who are by definition insulin deficient (Jansen et al 1990) and that intravenous glucose does not affect blood pressure in the elderly (Jansen and Hoefnagels 1987b), argue against a significant role.

The observation that blood glucose levels between 90 - 150 minutes after the drink were higher, and plasma insulin between 15 - 150 minutes less, after L-NAME indicates that nitric oxide mechanisms play a role in mediating insulin secretion induced by oral glucose. The involvement of nitric oxide mechanisms in insulin secretion remains controversial (Jones et al 1992, Schmidt et al 1992, Coiro et al 1997, Gross et al 1997, Akesson et al 1999, Smukler et al 2002, Schneid et al 2003). The
Current findings concur with recent in-vitro studies (Smukler et al 2002, Kaneko et al 2003), which support the concept that endogenous nitric oxide is involved in the regulation of insulin release; the effect of nitric oxide on insulin secretion may be concentration-dependent (Kaneko et al 2003). There is little information about the role of nitric oxide in insulin release in humans. Coiro et al (1997) reported that L-NAME inhibited the stimulation of insulin secretion induced by intravenous L-arginine (the substrate for nitric oxide) but not intravenous glucose in healthy young subjects. It should however, be noted that in this study (Coiro et al 1997) after intravenous glucose, plasma insulin levels were less with L-NAME. Furthermore, the dose of L-NAME used (ie 90 µg/kg/hr) was much less than in our study (Coiro et al 1997) and the negative observations may, accordingly, reflect a lesser degree of nitric oxide blockade. It is also possible that the effects of nitric oxide blockade on glucose-induced insulin release are dependent on the route of glucose administration (ie enteral or parenteral), sympathetic nerve activity and/or blood flow. The relative increase in plasma insulin in response to oral, when compared to intravenous, glucose is accounted for by the secretion of the incretin hormones, GIP and GLP-1 (Herrmann et al 1995, Schirra et al 1996). L-NAME had no significant effect on plasma GIP or GLP-1, indicating that the reduction in insulin secretion is not attributable to a diminished incretin response.
Figure 10.1: Effects of intravenous administration of saline (0.9%) (○) and L-NAME (180 µg/kg/hr) (■) on (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate. The intravenous infusions were given between t = -30 and 120 minutes. Data are mean values ± SEM. P values indicate ‘treatment’ effect between t = 0 - 120 minutes.
Figure 10.2: Effects of intravenous administration of saline (0.9%) (○) and L-NAME (180 µg/kg/hr) (■) on gastric emptying. The intravenous infusions were given between t = -30 and 120 minutes. Data are mean values ± SEM. P value indicates ‘treatment’ effect.
Figure 10.3: Effects of intravenous administration of saline (0.9%) (○) and L-NAME (180 µg/kg/hr) (■) on (a) blood glucose, (b) plasma insulin, (c) plasma GIP and (d) plasma GLP-1 concentrations. The intravenous infusions were given between t = -30 and 120 minutes. Data are mean values ± SEM. P values indicate ‘treatment’ effect.
Effects of the 5-HT3 antagonist, granisetron, on blood pressure, heart rate, and antropyloroduodenal motility in response to intraduodenal glucose infusion in healthy elderly subjects
11.1 Summary

Postprandial hypotension is an important clinical problem, particularly in the elderly - the pathogenesis is poorly defined and current treatment approaches are less than optimal. Studies indicate that 5-hydroxytryptamine 3 (5-HT3) mechanisms may be important in the regulation of postprandial splanchnic blood flow and blood pressure, and may also play a role in mediating the effects of small intestinal nutrients on small intestinal and gastric motility. The aims of this study were to evaluate the effects of the 5-HT3 antagonist, granisetron, on the blood pressure, heart rate, antropyloroduodenal motility and glycaemic responses to intraduodenal glucose in healthy elderly subjects.

Ten subjects (five male and five female, aged 65 - 76 years) were studied on two occasions, in double-blind, randomised order. Each subject received an intraduodenal glucose infusion (~ 3 kcal/min) for 60 minutes (t = 0 - 60 minutes), followed by intraduodenal saline for a further 60 minutes (t = 60 - 120 minutes). At t = -25 minutes, subjects were given either granisetron (10 µg/kg) or control over 5 minutes. Blood pressure (systolic and diastolic), heart rate, antropyloroduodenal pressures (manometry) and blood glucose were measured between t = 0 - 120 minutes. Granisetron had no effect on either systolic (P = 0.46) or diastolic (P = 0.48) blood pressure or heart rate (P = 0.68) in the 25 minute period before intraduodenal glucose infusion. During intraduodenal glucose, there were falls in systolic and diastolic blood pressure and a rise in heart rate (P < 0.0001 for all); granisetron had no effect on these responses.

Between t = 0 - 60 minutes, there was a decrease in the antral (P < 0.01) and duodenal (P < 0.05) pressures and stimulation (P = 0.07) of pyloric pressures on control, but the reduction in the number and amplitude (P < 0.05 for both) of duodenal pressures from
baseline after granisetron was greater. Granisetron had no effect on the glycaemic response to intraduodenal glucose. In conclusion, in healthy elderly subjects 5-HT3 mechanisms modulate the ‘local’ motor effects of small intestinal glucose, but not the cardiovascular response.

11.2 Introduction

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥ 20 mmHg sustained for at least 30 minutes, within 2 hours of a meal, is now recognised, albeit relatively recently, as a major cause of morbidity and mortality (Jansen and Lipsitz 1995, Fisher et al 2005) by predisposing to a number of disorders including syncope, transient ischaemic attacks, stroke and angina (Vaitkevicius et al 1991, Jansen and Lipsitz 1995, Aronow and Ahn 1997). Postprandial hypotension occurs more frequently than orthostatic hypotension (Jansen and Lipsitz 1995) and those at greatest risk include the elderly and patients with autonomic dysfunction; the latter usually secondary to diabetes (Jansen et al 1995, Jansen and Lipsitz 1995). Current treatment options are suboptimal.

The mechanisms mediating postprandial hypotension are poorly defined, although several factors, including impaired regulation of splanchnic blood flow, the release of gastrointestinal hormones and duodenal sympathetic nerve activity have been identified as possible pathophysiological mechanisms (Jansen et al 1995, Jansen and Lipsitz 1995). The magnitude of the fall in blood pressure is known to be dependent on meal composition; ingestion of carbohydrate, particularly glucose, has the greatest
suppressive effect on blood pressure (Jansen et al 1990). The onset of the fall in blood pressure after a meal is almost immediate, with a maximum response at 30 - 60 minutes (Jansen and Lipsitz 1995), suggesting a relationship to the rate of delivery of carbohydrate to the small intestine. Studies have shown that, in patients with recently diagnosed type 2 diabetes, there is a significant relationship between the magnitude of the fall in mean arterial blood pressure and the rate of gastric emptying of glucose (Jones et al 1998). Subsequently, it was demonstrated that the hypotensive responses to oral (Jones et al 2001, Russo et al 2003) and intraduodenal glucose (O'Donovan et al 2005b), are attenuated by concurrent administration of the viscous polysaccharide, guar, which slows gastric emptying and small intestinal glucose absorption (Meyer et al 1988). More recently, it was established that in healthy elderly subjects, when glucose is administered intraduodenally at a rate of ~ 1 kcal/min or ~ 3 kcal/min respectively (O'Donovan et al 2002), a greater fall in blood pressure and increase in heart rate is evident during the ~ 3 kcal/min infusion, indicating that the fall in blood pressure is dependent on the rate of nutrient delivery into the small intestine. Furthermore, the response to small intestinal glucose appears to be load, rather than concentration dependent (Chapter 6).

Previous studies have established that the presence of glucose in the small intestine inhibits antral and proximal duodenal pressures and stimulates both pyloric tone and phasic pyloric activity (Heddle et al 1988, Verhagen et al 1998). In healthy subjects, gastric emptying of glucose is known to be regulated at ~ 2 - 3 kcal/min, after an initial emptying phase that may be somewhat faster (Horowitz et al 1996), as a result of
inhibitory feedback arising from receptors located throughout the small intestine (Lin et al 1989, Horowitz et al 2001). Hence, in order to discriminate between ‘intragastric’ and ‘small intestinal’ mechanisms, glucose was infused directly into the small intestine.

5-hydroxytryptamine (5-HT) is an important neurotransmitter in the gastrointestinal tract, formed predominantly in enterochromaffin cells of the intestinal mucosa (Sanger 1996). Recent studies have demonstrated that 5-HT is released from BON cells, derived from human enterochromaffin cells (Kim et al 2001a, Kim et al 2001b) that act as ‘glucose sensors’ in the small intestine, activating signal transduction pathways, in the presence of D-glucose, galactose and α-D-glucopyranoside (αMG) (Kim et al 2001a, Kim et al 2001b). The release of 5-HT by D-glucose is also known to inhibit gastric emptying in rodents by activation of nervous pathways containing 5-hydroxytryptamine 3 (5-HT3) receptors (Raybould and Zittel 1995, Li et al 2000, Zhu et al 2001, Kim et al 2001a, Raybould et al 2003). Following a meal, 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). The magnitude of the postprandial increase in mesenteric blood flow is comparable in healthy young and elderly individuals, despite the greater fall in blood pressure in the latter group, indicating that there is inadequate cardiovascular adjustment for the shift of blood volume into the splanchnic system (Lipsitz et al 1993, Sidery et al 1993). There is evidence that 5-HT mechanisms play a role in the regulation of splanchnic blood flow (Zinner et al 1983, O'Donovan et al 2005b). A study in the canine model has
demonstrated an increase in gastrointestinal blood flow in response to low (4 μg/kg/min) and high (10 μg/kg/min) 5-HT infusions, suggesting a role for 5-HT in postprandial haemodynamic responses (Zinner et al 1983).

The role of 5-HT3 mechanisms on antropyloroduodenal motility responses to intraduodenal glucose in humans has been assessed in one study (Müller et al 2003), which demonstrated that the 5-HT3 antagonist, granisetron (10 μg/kg/5min), attenuated the suppression of antral waves after intraduodenal lipid, but not intraduodenal glucose (5ml), in healthy young subjects. A limitation of this study was that the amount of glucose infused was small (5ml of 25% dextrose) and given over a short time interval (60 seconds). Furthermore, the study design did not allow a distinction between the ‘local’ response and that of antropyloroduodenal motility, to intraduodenal glucose. The role of 5-HT mechanisms in the regulation of postprandial blood pressure, heart rate and antropyloroduodenal motility has not yet been evaluated in healthy elderly subjects, or any other group susceptible to postprandial hypotension.

The major aims of this study were to evaluate the potential role of 5-HT3 mechanisms in mediating the effects of intraduodenal glucose on blood pressure and antropyloroduodenal motility in healthy elderly subjects.

11.3 Research design and methods

Ten healthy elderly subjects, (five female and five male) with a median age of 70 years (range: 65 - 76 years) and body mass index (BMI) of 24.5 kg/m² (range: 21.1 - 29.1
kg/m$^2$), recruited by advertisement, were studied. All subjects were non-smokers. None had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, or was taking medication known to influence blood pressure or gastrointestinal function.

### 11.3.1 Experimental protocol

Each subject was studied on two separate occasions, separated by at least seven days, in double-blind, randomised order. On each day, the subject attended the University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital, at 08.30h following an overnight fast (10.5h for solids; 8.5h for liquids) (Chapter 6). At that time, a 17-channel manometric catheter (Dentsleeve International Ltd, Mui Scientific, Ontario, Canada) was introduced into the stomach via an anaesthetised nostril (Cook et al 1997). The catheter was allowed to pass through the stomach and into the duodenum by peristalsis (Cook et al 1997) (which took between 20 and 165 minutes) and included 16 side holes (spaced 1.5cm apart) and an infusion channel with a port located ~ 10cm distal to the pylorus (ie in the duodenum, 1.5cm distal to channel 16). Six side-holes (channels 1 - 6) were positioned in the antrum, a 4.5cm sleeve sensor (channel 7), with 2 channels (channels 8 and 9) on the back of the sleeve, was positioned across the pylorus, and 7 channels (channels 10 - 16) were positioned in the duodenum (Little et al 2005). The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by measurement of the antroduodenal transmucosal potential difference (TMPD) using a saline-filled, reference electrode (20 gauge intravenous cannula) inserted subcutaneously into the subject’s forearm as a reference (O'Donovan et al
2002). The manometric channels were perfused with degassed, distilled water, except the TMPD channels which were perfused with degassed saline (0.9%), at 0.15 ml/min (Heddle et al 1989). An intravenous cannula was positioned in a right antecubital vein for blood sampling, and an automated blood pressure cuff placed around the left arm (O'Donovan et al 2002). Once intubated, the subjects were studied in the recumbent position. Approximately 25 minutes after the tube was in position (t = -25 minutes), an intravenous dose of granisetron (Kytril', F. Hoffmann-La Roche, Basel, Switzerland) (10 µg/kg) (ie 0.58 - 0.87ml) in a volume of 25ml or placebo (25ml normal saline) was given as a bolus over a 5 minute period. Twenty minutes later (ie at t = 0 minutes), an intraduodenal infusion of 50g glucose dissolved in saline (0.9%) in a total volume of 300ml was commenced and maintained at a rate of 5 ml/min for 60 minutes. This infusion resulted in an energy delivery of ~ 3 kcal/min (O'Donovan et al 2002). Between t = 60 - 120 minutes, saline (0.9%) was infused intraduodenally at the same rate. At t = 120 minutes the catheter and intravenous cannula were removed and the subject was given a light meal. On one day cardiovascular autonomic nerve function was evaluated immediately following the study (Ewing and Clarke 1982, Piha 1991).

11.3.2 Measurement of 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) immediately prior to intravenous administration of granisetron (ie t = -25 minutes), at the commencement of intraduodenal infusion (ie ‘baseline’ at t = 0 minutes), and then every 3 minutes until t = 120 minutes (O'Donovan
et al 2002). Postprandial hypotension was defined as a fall in systolic blood pressure ≥ 20 mmHg after glucose that was sustained for at least 30 minutes (Jansen and Lipsitz 1995). Blood pressure and heart rate before the administration of granisetron, was calculated as the mean of measurements taken at t = -34, -31 and -28 minutes.

11.3.3 Antropyloroduodenal pressures

Manometric pressures were digitised and recorded on a computer-based system running commercially available software (Flexisoft®, Version 3, Associate Professor GS Hebbard, Royal Melbourne Hospital, Melbourne, Australia, written in Labview 3.1.1 (National Instruments)), and stored for subsequent analysis. Antropyloroduodenal pressures pertaining to channels 1 - 16 were analysed for: (i) number and amplitude of antral pressure waves (PWs), (ii) basal pyloric pressure (tone), (iii) number and amplitude of isolated pyloric pressure waves (IPPWs), (iv) number and amplitude of duodenal PWs, and (v) number and length of pressure wave sequences involving the antrum, pylorus and duodenum, using tailored, custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, The Netherlands (Samsom et al 1998)). In addition, pressures recorded by the closest channel to the infusion port (ie 16) were analysed for the number and amplitude of duodenal PWs to evaluate the ‘local’ effects of intraduodenal glucose. Basal pyloric pressure (tone) was determined by subtracting the mean basal pressure recorded at the most distal antral side-hole from the mean basal pressure recorded at the sleeve, using custom-written software (MAD, Professor Charles Malbert, Institut National de la Recherche Agronomique (INRA), Rennes, France) (Heddle et al 1988c). Phasic PWs in the antrum
and pylorus were defined by pressure increases which lasted 1 - 20 seconds and that had
an amplitude of $> 10$ mmHg, with a minimum interval of 15 seconds between peaks.
Phasic PWs in the duodenum were defined as those having an amplitude of $> 10$
mMg, with a minimal interval of 3 seconds between peaks. Antropyloroduodenal
pressure wave sequences (PWSs) were defined as two or more temporally related PWs
with onsets within $\pm 5$ seconds (in the antrum), or $\pm 3$ seconds (in the duodenum) of
each other (Samsom et al 1998).

11.3.4 Measurement of blood glucose concentrations
Venous blood samples (~ 5ml) were obtained both prior to the intravenous dose of
granisetron ($t = -27$ minutes) and commencement of the intraduodenal infusion (ie $t = -2$
minutes), and then at 15 minute intervals from $t = 0 - 120$ minutes. Blood glucose
concentrations were determined according to the method described in Chapter 4.8.

11.3.5 Assessment of cardiovascular autonomic nerve function
Autonomic nerve function was evaluated using standardised cardiovascular reflex tests
(Ewing and Clarke 1982, Piha 1991). A description of this method is included in
Chapter 4.10.

11.3.6 Statistical analysis
Data were evaluated using repeated measures 2 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. Differences in blood
glucose were analysed as absolute values. The maximum fall in blood pressure was defined as the greatest mean change from baseline for a treatment at any given time point. All antropyloroduodenal pressure data, with the exception of the number of antropyloroduodenal PWSs, are expressed as change from baseline. For the number and amplitude of antral PWs, duodenal PWs and IPPWs, basal pyloric pressures and number of antropyloroduodenal PWSs, baseline values were calculated as the mean of values obtained between $t = -15 - 0$ minutes (Little et al 2005). The number and amplitude of antral PWs, duodenal PWs and IPPWs and basal pyloric pressures were expressed as mean values over 15 minute periods during the 120 minute duodenal infusion period (ie 0 - 15 minutes, 15 - 30, ..., 105 - 120 minutes) (Little et al 2005). The number and amplitude of antral PWs, duodenal PWs and IPPWs and basal pyloric pressures were analysed with ‘time’ ($t = -15 - 0$, 0 - 15, 15 - 30, ..., 105 - 120 minutes) and ‘treatment’ as factors (Little et al 2005). Antropyloroduodenal PWSs were expressed as the total number of PWs travelling over 2 (ie 1.5cm), 3 (ie 3cm), ..., 15 (ie 21cm) channels during the 120 minute infusion period (Little et al 2005). The number of antropyloroduodenal PWSs was analysed with length of propagation (1.5, 3, 4.5, ..., 21cm) and ‘treatment’ as factors (Little et al 2005). Phase III activity was excluded from the data analysis. Systolic and diastolic blood pressure, heart rate, blood glucose concentration, the number and amplitude of antral PWs, duodenal PWs and IPPWs and basal pyloric pressures were analysed separately from baseline - 60 minutes and from $t = 60 - 120$ minutes. One-way ANOVAs were used to analyse the effects of ‘time’ from baseline - 60 minutes and from $t = 60 - 120$ minutes on systolic blood pressure, diastolic blood pressure, heart rate, blood glucose concentrations, the number and
amplitude of antral PWs, duodenal PWs and IPPWs, basal pyloric pressures and the effects of ‘length of propagation’ of the number of antropyloroduodenal PWSs. In all analyses, post-hoc comparisons of adjusted means were performed using Student’s t-tests. 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), and a P value < 0.05 was considered significant in all analyses.

11.4 Results

The studies were well tolerated. Three of the ten subjects reported constipation after granisetron - in all cases symptoms had resolved within 48 hours of completion of each experiment. One subject reported feeling depressed for approximately 3 hours after granisetron. The median score for autonomic nerve dysfunction was 1.2 (range: 0 - 3); one of the ten subjects had definite autonomic dysfunction. Two had postprandial hypotension (ie a fall in systolic blood pressure ≥ 20 mmHg sustained for at least 30 minutes) on both study days. The one subject with autonomic neuropathy did not have postprandial hypotension on either day. In one subject, basal pyloric pressure (tone) data were not available because of technical difficulties.

11.4.1 Blood pressure and heart rate

There was no difference in blood pressure or heart rate at t = -25 minutes (ie immediately prior to intravenous granisetron) between the two study days: systolic blood pressure (control: 133.0 ± 5.6 mmHg vs granisetron: 131.9 ± 4.5 mmHg, P =
0.64); diastolic blood pressure (control: 69.9 ± 1.9 mmHg vs granisetron: 70.5 ± 1.8 mmHg, P = 0.57) and heart rate (control: 57.1 ± 2.4 beats/minute (bpm) vs granisetron: 58.3 ± 2.7 bpm, P = 0.43). Nor was there any difference in baseline (ie t = 0 minutes) blood pressure or heart rate between the two days: systolic blood pressure (control: 133.2 ± 5.3 mmHg vs granisetron: 130.8 ± 4.3 mmHg, P = 0.37); diastolic blood pressure (control: 69.3 ± 2.8 mmHg vs granisetron: 66.6 ± 2.2 mmHg, P = 0.27) and heart rate (control: 57.3 ± 2.3 bpm vs granisetron: 57.1 ± 2.7 bpm, P = 0.88). There was no significant effect of granisetron on systolic blood pressure (P = 0.46), diastolic blood pressure (P = 0.48) or heart rate (P = 0.68) prior to the intraduodenal glucose infusion (ie between t = -25 minutes and t = 0 minutes).

Between t = 0 - 60 minutes there was a fall in systolic blood pressure on both the control day and after granisetron (P < 0.0001 for both) with no difference (P = 0.65) between the two days. There were comparable maximum falls in systolic blood pressure (control: 17.3 ± 4.7 mmHg vs granisetron: 17.5 ± 3.3 mmHg; P = 0.95) and no difference in the time of maximal fall in systolic blood pressure on the control day (37 ± 5.0 minutes) when compared with granisetron (51 ± 9.0 minutes; P = 0.19). Between t = 60 - 120 minutes, the rise in systolic blood pressure was not statistically significant on the control day (P = 0.37) or after granisetron (P = 0.99) and there was no difference in systolic blood pressure between the two days (P = 0.69). At t = 120 minutes, systolic blood pressure was not significantly different (P = 0.26) from baseline (ie t = 0 minutes) on the control day but there was a trend (P = 0.08) for systolic blood pressure to be lower than baseline after granisetron (Figure 11.1 (a)).
There was a fall in diastolic blood pressure between $t = 0 - 60$ minutes on both the control day and after granisetron ($P < 0.0001$ for both) but no difference ($P = 0.16$) between the two days. There was a rise in diastolic blood pressure between $t = 60 - 120$ minutes both on the control day and after granisetron ($P < 0.0001$ for both) but no difference ($P = 0.87$) between the study days. At $t = 120$ minutes, diastolic blood pressure was not different ($P = 0.57$) from baseline (ie $t = 0$ minutes) on the control day or after granisetron ($P = 0.50$) (Figure 11.1 (b)).

Between $t = 0 - 60$ minutes there was a rise in heart rate both on the control day and after granisetron ($P < 0.0001$ for both) with no difference ($P = 0.24$) between the two days. The maximum heart rate on the control day ($16.6 \pm 1.9$ bpm) and after granisetron ($15.2 \pm 1.9$ bpm; $P = 0.45$) was comparable and there was no difference in the time of maximum heart rate on the control day ($61 \pm 2.0$ minutes) when compared with granisetron ($68 \pm 10.0$ minutes; $P = 0.48$). There was a fall in heart rate between $t = 60 - 120$ minutes both on the control day and after granisetron ($P < 0.0001$ for both) but no difference ($P = 0.53$) between the two days. At $t = 120$ minutes, heart rate was greater than baseline on both the control day ($P = 0.001$) and after granisetron ($P = 0.02$) (Figure 11.1 (c)).
11.4.2 Antropyloroduodenal pressures

11.4.2.1 Antral pressures

There was no difference in the baseline (ie t = -15 - 0 minutes) number of antral PWs between the two days (control: 31.7 ± 9.0 vs granisetron: 45.9 ± 11.5; P = 0.34). Between baseline and t = 0 - 15 minutes, there was no change in the number of antral PWs on the control day (P = 0.22), however, there was a significant fall (P = 0.02) in the number of antral PWs after granisetron, and no difference between the two days (P = 0.26). Between t = 0 - 60 minutes, there was a decrease in the number of antral PWs from baseline on both the control day (P < 0.01) and after granisetron (P < 0.001), but no difference between the two days (P = 0.70). There was a significant, albeit modest, increase (P < 0.05) in the number of antral PWs between t = 60 - 120 minutes on the control day, but not after granisetron (P = 0.76), however, the difference between the two days was not significant (P = 0.40). Between t = 105 - 120 minutes there was a trend for the number of antral PWs to be less than baseline on the control day (P = 0.06), and significantly less, after granisetron (P = 0.02) (Table 11.1).

There was no difference in the baseline (ie t = -15 - 0 minutes) amplitude of antral PWs between the two days (control: 57.1 ± 16.1 mmHg vs granisetron: 58.0 ± 15.3 mmHg; P = 0.95). There was no change in the amplitude of antral PWs between baseline and t = 0 - 15 minutes on the control day (P = 0.21), however, the amplitude of antral PWs after granisetron was lower (P = 0.04) and there was no difference between the two days (P = 0.99). Between t = 0 - 60 minutes, there was a decrease in the amplitude of antral PWs
on the control day \(P < 0.01\), but not after granisetron \(P = 0.36\), and no difference between the two days \(P = 0.40\). There was an increase in the amplitude of antral PWs between \(t = 60 - 120\) minutes on the control day \(P < 0.05\) but not after granisetron \(P = 0.20\) and no difference between the two study days \(P = 0.83\). Between \(t = 105 - 120\) minutes the amplitude of antral PWs was not significantly different from baseline on the control day \(P = 0.29\) or after granisetron \(P = 0.24\) (Table 11.1).

### 11.4.2.2 Pyloric pressures

(i) Basal pyloric pressure (tone)

Between baseline and \(t = 0 - 15\) minutes, there was no change in basal pyloric pressure on the control day \(P = 0.23\) or after granisetron \(P = 0.79\), and no difference between the two days \(P = 0.86\). Similarly, there were no significant changes in basal pyloric pressure on the control day \(P = 0.89\) or after granisetron \(P = 0.74\) between \(t = 0 - 60\) minutes or between the two days \(P = 0.60\). There was a trend for a decrease in basal pyloric pressure between \(t = 60 - 120\) minutes on the control day \(P = 0.06\), but not after granisetron \(P = 0.14\), nor between the two days \(P = 0.46\). Between \(t = 105 - 120\) minutes basal pyloric pressure was not significantly different from baseline on either the control day \(P = 0.21\) or after granisetron \(P = 0.32\) (Table 11.2).

(ii) Phasic pressures

There was no difference in the baseline (ie \(t = -15 - 0\) minutes) number of IPPWs between the two days (control: \(4.1 \pm 1.3\) vs granisetron: \(5.5 \pm 2.7\); \(P = 0.61\)). There was an increase in the number of IPPWs between baseline and \(t = 0 - 15\) minutes on the
control day (P = 0.03) and a trend for an increase after granisetron (P = 0.06), but there was no difference between the two days (P = 0.76). There was also a trend for a decrease in the number of IPPWs between t = 0 - 60 minutes on the control day (P = 0.07), but not after granisetron (P = 0.25) and there was no difference between the two days (P = 0.62). There was no significant change in the number of IPPWs between t = 60 - 120 minutes on the control day or after granisetron (P = 0.58), nor was there any difference in the number of IPPWs between the two study days (P = 0.39). Between t = 105 - 120 minutes, the number of IPPWs was not significantly different from baseline on the control day (P = 0.26) or after granisetron (P = 0.39) (Table 11.2).

There was no difference in the baseline (ie t = -15 - 0 minutes) amplitude of IPPWs between the two days (control: 24.4 ± 8.1 mmHg vs granisetron: 24.5 ± 9.0 mmHg; P = 0.10). Between baseline and t = 0 - 15 minutes, there was no change in the amplitude of IPPWs on either the control day (P = 0.31) or after granisetron (P = 0.18), and no difference between the two days (P = 0.50). Similarly, there was no change in the amplitude of IPPWs between t = 0 - 60 minutes on the control day (P = 0.49), or after granisetron (P = 0.25), or between the two days (P = 0.98). Between t = 60 - 120 minutes, there was also no overall change in the amplitude of IPPWs on the control day (P = 0.50) or after granisetron (P = 0.51) and there was no difference between the two days (P = 0.69). Between t = 105 - 120 minutes the amplitude of IPPWs was not significantly different from baseline on the control day (P = 0.82) or after granisetron (P = 0.76) (Table 11.2).
11.4.2.3 **Duodenal pressures**

(i) Channels 10 - 16

There was no difference in the baseline (ie t = -15 - 0 minutes) number of duodenal PWs between the two days (control: 129.9 ± 33.0 vs granisetron: 144.9 ± 34.0; P = 0.75). There was no change in the number of duodenal PWs between baseline and t = 0 - 15 minutes on either the control day (P = 0.44) or after granisetron (P = 0.87), and no difference between the two days (P = 0.96). Between t = 0 - 60 minutes, there was a trend for a reduction in the number of duodenal PWs from baseline on the control day (P = 0.12) and a significant decrease (P < 0.01) after granisetron; without any difference between the study days (P = 0.40). There was no change in the number of duodenal PWs between t = 60 - 120 minutes on the control day (P = 0.31) and a trend for an increase after granisetron (P = 0.11), with no difference between the two study days (P = 0.54). Between t = 105 - 120 minutes the number of duodenal PWs was not significantly different from baseline on the control day (P = 0.72) or after granisetron (P = 0.62) (Table 11.3).

There was no difference in the baseline (ie t = -15 - 0 minutes) amplitude of duodenal PWs between the two days (control: 28.1 ± 3.8 mmHg vs granisetron: 22.9 ± 2.0 mmHg; P = 0.30). Between baseline and t = 0 - 15 minutes, there was no change in the amplitude of duodenal PWs on either the control day (P = 0.44) or after granisetron (P = 0.61), and no difference between the two days (P = 0.27). Between t = 0 - 60 minutes, there was no overall change in the amplitude of duodenal PWs from baseline on either the control day (P = 0.45) or after granisetron (P = 0.36) and no difference between the
two days (P = 0.90). There was also no change in the amplitude of duodenal PWs between t = 60 - 120 minutes on the control day (P = 0.32) or after granisetron (P = 0.17) or between the two study days (P = 0.50). Between t = 105 - 120 minutes the amplitude of duodenal PWs was not significantly different from baseline on the control day (P = 0.65) or after granisetron (P = 0.67) (Table 11.3).

(ii) Channel 16

There was no difference in the baseline (ie t = -15 - 0 minutes) number of duodenal PWs between the two days (control: 20.1 ± 4.7 vs granisetron: 36.7± 11.4; P = 0.19).

There was an increase in the number of duodenal PWs between baseline and t = 0 - 15 minutes on the control day (P = 0.03) but no change in the number of IPPWs after granisetron (P = 0.19), and no difference between the two days (P = 0.89). Between t = 0 - 60 minutes, there was a significant decrease in the number of duodenal PWs from baseline on both the control day (P < 0.05) and after granisetron (P < 0.001) and the reduction in the number of duodenal PWs from baseline was greater (P < 0.05) after granisetron when compared with the control day. There was no overall change in the number of duodenal PWs between t = 60 - 120 minutes on the control day (P = 0.48) or after granisetron (P = 0.25); and no difference between the two study days (P = 0.12). Between t = 105 - 120 minutes the number of duodenal PWs was not significantly different from baseline on the control day (P = 0.96), however, there was a trend for the number of duodenal PWs to be lower after granisetron (P = 0.09) (Table 11.4).
There was no difference in the baseline (ie t = -15 - 0 minutes) amplitude of duodenal PWs between the two days (control: 23.5 ± 1.8 mmHg vs granisetron: 22.7 ± 1.5 mmHg; P = 0.68). Between baseline and t = 0 - 15 minutes, there was no change in the amplitude of duodenal PWs on either the control day (P = 0.18) or after granisetron (P = 0.27), however, there was a trend (P = 0.08) for a reduction in the amplitude of duodenal PWs from baseline after granisetron when compared with control. Between t = 0 - 60 minutes, there was no overall change in the amplitude of duodenal PWs on the control day (P = 0.11), however, the amplitude of duodenal PWs was decreased after granisetron (P < 0.001); the reduction in the amplitude of duodenal PWs from baseline between t = 0 - 60 minutes was greater (P < 0.05) after granisetron when compared with control. While there was also no change in the amplitude of duodenal PWs between t = 60 - 120 minutes on the control day (P = 0.35), there was a trend for an increase in the amplitude of duodenal PWs after granisetron (P = 0.10); there was no difference (P = 0.37) in the amplitude of duodenal PWs between the two days. Between t = 105 - 120 minutes the amplitude of duodenal PWs was not significantly different from baseline on the control day (P = 0.10) or after granisetron (P = 0.38) (Table 11.4).

11.4.2.4 Antropyloroduodenal sequences

The number of PWs sequences decreased with increasing length of propagation on both the control day and after granisetron (P < 0.0001 for both) without any significant difference (P = 0.17) between the two study days (data not shown).
11.4.3 Blood glucose concentrations

There was no significant difference at \( t = -25 \) minutes in blood glucose between the two days (control: 6.3 ± 0.2 mmol/L vs granisetron: 6.5 ± 0.2 mmol/L; \( P = 0.47 \)), or at baseline (control: 6.2 ± 0.2 mmol/L vs granisetron: 6.3 ± 0.1 mmol/L; \( P = 0.72 \)).

There was a rise between \( t = -2 - 60 \) minutes in blood glucose concentrations on both days (\( P < 0.0001 \) for both) with a subsequent fall between \( t = 60 - 120 \) minutes (\( P < 0.0001 \) for both). Between \( t = 0 - 60 \) minutes (\( P = 0.27 \)) and \( t = 60 - 120 \) minutes (\( P = 0.94 \)), there was no difference in blood glucose concentrations between the two study days. At \( t = 120 \) minutes, blood glucose concentrations were not significantly different from baseline on control (\( P = 0.66 \)) or granisetron (\( P = 0.48 \)) (Figure 11.2).

11.5 Discussion

The major observations in this study are that the 5-HT3 receptor antagonist, granisetron, when administered acutely to healthy elderly subjects in a dose of 10 µg/kg has no effect on blood pressure, heart rate or antral and pyloric motor responses but modulates the duodenal motor response, to intraduodenal glucose. This study indicates that the cardiovascular and antropyloric motor responses to enteral glucose do not appear to be influenced by the stimulation of 5-HT3 receptors, but may be involved in the modulation of the duodenal motor response to intraduodenal glucose in healthy elderly subjects.
Using oral glucose loads (Jones et al 1998, Jones et al 2001, Russo et al 2003) and direct intraduodenal glucose infusions (O'Donovan et al 2002, O'Donovan et al 2005b), it has been established that in healthy elderly subjects there is a relationship between the magnitude of the postprandial fall in blood pressure and rise in heart rate with the rate of nutrient delivery from the stomach to the small intestine; these effects were evident within 60 minutes of ingestion and infusion of glucose. The latter studies excluded the potential effects of gastric emptying, hence, in this present study, glucose was infused directly into the small intestine for a period of 60 minutes and at a rate (ie ~ 3 kcal/min) that has previously resulted in falls, in healthy elderly subjects, in both systolic and diastolic blood pressures that were much greater when compared with a rate of ~ 1 kcal/min (O'Donovan et al 2002). The dose of granisetron (ie 10 µg/kg/5min) employed was comparable to that given intravenously in previous studies (Boike et al 1997, Ladabaum et al 2001, Müller et al 2003), and is the recommended effective dose for clinical use (Boike et al 1997). Enteral glucose induced a gradual fall in systolic (control: 17.3 ± 4.7 mmHg vs granisetron: 17.5 ± 3.3 mmHg) and diastolic blood pressure (control: 11.6 ± 2.2 mmHg vs granisetron: 10.7 ± 1.5 mmHg), and rapid increase in heart rate (control: 16.6 ± 1.9 bpm vs granisetron: 15.2 ± 1.9 bpm), that were comparable to those observed previously in the healthy elderly (O'Donovan et al 2002, O'Donovan et al 2005b). Furthermore, in accordance with previous studies, on the control day the presence of glucose in the small intestine reduced the number of antral contractions and proximal duodenal pressure waves and increased the frequency of isolated pyloric pressure waves (Heddle et al 1988a, Heddle et al 1988b, Edelbroek et al 1992).
The effect of 5-HT on the cardiovascular system is complicated, however, the blood pressure response usually consists of a short lasting bradycardia and relative hypotension (via the Bezold - Jarisch reflex involving the stimulation of 5-HT3 receptors), followed by a middle pressor phase (due to vasoconstriction by the activation of 5-HT2 receptors) and a longer-lasting hypotension (mediated by 5-HT1-like receptors) (Saxena and Villalon 1990). In a recent study in healthy adult volunteers (Boike et al 1997), a clinical dose of granisetron (ie 10 µg/kg) when delivered over a 5 minute period, did not exert any effects on systolic blood pressure or heart rate. It is unclear, however, whether granisetron was administered to the subjects in the postprandial or fasting state. In the presence of enteral glucose, there was no effect of granisetron on systolic and diastolic blood pressure or heart rate suggesting that the stimulation of 5-HT3 receptors are not involved in the observed effects of glucose on blood pressure and heart rate in healthy elderly subjects. It has been demonstrated that 5-HT is released with increasing intraluminal pressures in the small intestine (Bulbring and Crema 1959, Burks and Long 1966). Hence, there is a potential for 5-HT3 receptors to modulate distension-induced postprandial changes in blood pressure. In a study in rodents, decreases in diastolic blood pressure were observed during duodenal distension resulting in intraluminal pressures of 10 - 75 cmH₂O (Moss and Sanger 1990). While, intravenous granisetron in doses of 1 - 100 µg/kg, reduced the blood pressure response in this animal model (Moss and Sanger 1990), granisetron had no effect on the falls in systolic and diastolic blood pressure after intraduodenal glucose in this current study. It is possible that greater distension of the small intestinal wall is
required before attenuation of the postprandial hypotensive response to intraduodenal glucose becomes evident.

The mechanisms influencing the cardiovascular response to glucose remain unclear. In health, ingestion of nutrients, promotes an increase in splanchnic blood flow (Mathias 1991), that is associated with reductions in total systemic vascular resistance and skeletal muscle blood flow (O'Mara and Lyons 2002). In the elderly, the magnitude of the increase in mesenteric blood flow is associated with a fall in blood pressure that is not evident in young volunteers (Lipsitz et al 1993). A previous study has suggested that 5-HT mechanisms may play a role in the regulation of splanchnic blood flow (Zinner et al 1983). In a canine model, low (4 µg/kg/min) and high (10 µg/kg/min) exogenous infusions of serotonin have been reported to increase gastrointestinal blood flow by acting as a vasodilator; this effect was not associated with any changes in either cardiac output or mean arterial pressure (Zinner et al 1983).

In humans, approximately 90% of the total body 5-HT is found in the gastrointestinal tract (Read and Gwee 1994) with the majority located in the enterochromaffin cells of the mucosa (Farthing 1991). The other 10% is contained within enteric neurons situated in the myenteric plexus (Read and Gwee 1994). In animals, the release of 5-HT is dependent on vagal stimulation (Gronstad et al 1985), the application of pressure to the mucosa (Bulbring and Crema 1959), pathophysiological stimulus, such as toxins (Beubler and Horina 1990, Read and Gwee 1994), and the ingestion of a meal (Farthing 1991). 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, activating signal transduction pathways, in the presence of D-glucose, galactose and 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 in rodents by activation of extrinsic vagal afferent pathways containing 5-HT3 receptors (Raybould and Zittel 1995, Li et al 2000, Zhu et al 2001, Kim et al 2001a, Raybould et al 2003). In humans, the reported effects of 5-HT3 antagonists on gastric emptying are unclear. 5-HT3 antagonists have been shown to have no effect on (Talley et al 1989, Gore et al 1990, Nielsen et al 1990, Stacher et al 1991, Netzer et al 2002), accelerate (Akkermans et al 1988) or delay (Stacher et al 1990) gastric emptying. A recent study (Müller et al 2003) suggests that the suppression of antral waves by intraduodenal lipid infusion is mediated via a 5-HT pathway; intravenous granisetron (10 µg/kg) attenuated the suppression of antral waves after intraduodenal lipid, but not intraduodenal glucose, in healthy young subjects. Glucose also failed to induce a significant duodenal motor response (Müller et al 2003). However, the amount of glucose infused was small (5ml of 25% dextrose) and given over a short time interval (60 seconds) (Müller et al 2003). In this current study, glucose infused at a rate of ~ 3 kcal/min over 60 minutes failed to exert any effects on the number or amplitude of antral or pyloric pressure waves. In contrast, while there were no significant effects of granisetron in the motor response across all duodenal channels (ie channels 10 - 16), a greater reduction in the number and amplitude of duodenal pressure waves from baseline after granisetron was observed in the channel most proximal to the infusion port (ie channel 16). Furthermore, there was a short increase in duodenal motility within the first 15 minutes of the
commencement of the glucose infusion on the control day consistent with previous observations (Fone et al 1989, Verhagen et al 1998). The findings in this current study suggest that the attenuation of the duodenal motor response to glucose is mediated via 5-HT3 receptors and that enterochromaffin cells may potentially play a role by releasing 5-HT in response to the presence of glucose.

Changes in blood glucose concentrations have been shown to have an impact on antropyloroduodenal motility (Fraser et al 1991). In healthy young volunteers, hyperglycaemia is associated with the stimulation of localised pyloric contractions and inhibition of antral contractions (Fraser et al 1991). In this study, granisetron had no effect on the glycaemic response to intraduodenal glucose (Lin et al 1989, Erdmann et al 2004) discounting a role for glycaemia in the observed effects on antropyloroduodenal motility. Furthermore, in contrast to oral glucose, intravenous glucose, which is a substantial stimulus to insulin secretion, does not appear to affect blood pressure in the elderly (Jansen and Hoefnagels 1987a, Jansen and Hoefnagels 1991) and postprandial hypotension has been shown to occur in patients with type 1 diabetes, who are by definition insulin deficient (Jansen et al 1990).

In conclusion, the falls in systolic and diastolic blood pressure and rise in heart rate induced by intraduodenal infusion of glucose is unaffected by granisetron suggesting that the cardiovascular response to glucose is mediated by mechanisms unrelated to the stimulation of 5-HT3 receptors. Granisetron was associated with a greater reduction in
the duodenal motor response to intraduodenal glucose indicating that 5-HT3 receptors may be involved in the modulation of small intestinal motility.
Figure 11.1: Effects of control (○) and granisetron (10 µg/kg) (●) on the change in (a) systolic blood pressure, (b) diastolic blood pressure and (c) heart rate from baseline during intraduodenal infusion of glucose (~ 3 kcal/min). Data are mean values ± SEM. P values indicate ‘treatment’ effects between t = 0 - 60 minutes and t = 60 - 120 minutes.
Figure 11.2: Effects of control (○) and granisetron (10 µg/kg) (●) on blood glucose concentrations during intraduodenal infusion of glucose (~ 3 kcal/min). Data are mean values ± SEM. P values indicate ‘treatment’ effect between t = 0 - 60 minutes and t = 60 - 120 minutes.
Table 11.1: Effects of control and granisetron (10 µg/kg) on the change in the number and amplitude of antral pressure waves from baseline during intraduodenal glucose at a rate of ~ 3 kcal/min. Data are presented as control vs granisetron and are mean values ± SEM.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Antral pressure waves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>0 - 15 min</td>
<td>-15.5 ± 11.9 vs -22.5 ± 8.3</td>
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<td>15 - 30 min</td>
<td>-19.5 ± 10.5 vs -28.2 ± 7.0</td>
</tr>
<tr>
<td>30 - 45 min</td>
<td>-25.6 ± 10.4 vs -29.9 ± 8.3</td>
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<tr>
<td>45 - 60 min</td>
<td>-24.4 ± 10.2 vs -28.2 ± 9.3</td>
</tr>
<tr>
<td>60 - 75 min</td>
<td>-20.1 ± 9.4 vs -29.2 ± 8.1</td>
</tr>
<tr>
<td>75 - 90 min</td>
<td>-23.8 ± 9.7 vs -29.2 ± 8.2</td>
</tr>
<tr>
<td>90 - 105 min</td>
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</tr>
<tr>
<td>105 - 120 min</td>
<td>-3.5 ± 10.6 vs -25.6 ± 8.8</td>
</tr>
<tr>
<td>Time (min)</td>
<td>Basal pyloric pressure (tone)</td>
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<td>------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td></td>
<td>Amplitude (mmHg)</td>
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<tr>
<td>0</td>
<td>1.4 ± 1.1 vs 1.3 ± 4.7</td>
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<td>45</td>
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<tr>
<td>60 - 75 min</td>
<td>1.8 ± 1.4 vs -3.3 ± 5.0</td>
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<td>75 - 90 min</td>
<td>1.2 ± 1.5 vs -2.8 ± 5.8</td>
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<td>90</td>
<td>0.3 ± 0.8 vs 5.2 ± 5.3</td>
</tr>
<tr>
<td>105 - 120 min</td>
<td>-4.7 ± 3.5 vs -5.7 ± 5.4</td>
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</table>

**Table 11.2:** Effects of control and granisetron (10 µg/kg) on the change in the amplitude of basal pyloric pressure waves and the number and amplitude of pyloric phasic pressure waves from baseline during intraduodenal glucose at a rate of ~ 3 kcal/min. Data are presented as control vs granisetron and are mean values ± SEM.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Duodenal pressure waves</th>
</tr>
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<tbody>
<tr>
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</tr>
<tr>
<td></td>
<td>Number</td>
</tr>
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<tr>
<td>105 - 120 min</td>
<td>13.8 ± 37.2 vs -15.4 ± 30.4</td>
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Table 11.3: Effects of control and granisetron (10 µg/kg) on the change in the number and amplitude of duodenal pressure waves (channels 10 -16) from baseline during intraduodenal glucose at a rate of ~ 3 kcal/min. Data are presented as control vs granisetron and are mean values ± SEM.
<table>
<thead>
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<th>Time (min)</th>
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<th>Amplitude (mmHg)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 15 min</td>
<td>25.7 ± 10.2 vs -11.0 ± 7.8</td>
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<td>4.0 ± 2.7 vs -3.2 ± 2.7</td>
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<td>15 - 30 min</td>
<td>0.5 ± 4.3 vs -31.1 ± 11.5</td>
<td>&lt; 0.05</td>
<td>-2.8 ± 2.9 vs -14.2 ± 3.1</td>
<td>&lt; 0.05</td>
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<td>45 - 60 min</td>
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<td>-4.7 ± 3.8 vs -11.0 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>60 - 75 min</td>
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<tr>
<td>75 - 90 min</td>
<td>-13.1 ± 4.4 vs -25.5 ± 12.0</td>
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<td>-7.6 ± 2.2 vs -8.8 ± 4.4</td>
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<tr>
<td>90 - 105 min</td>
<td>-10.0 ± 3.9 vs -27.2 ± 10.9</td>
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<td>-3.5 ± 1.5 vs -6.2 ± 2.3</td>
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<tr>
<td>105 - 120 min</td>
<td>-0.4 ± 7.4 vs -21.9 ± 11.7</td>
<td></td>
<td>-5.3 ± 2.9 vs -3.9 ± 4.2</td>
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</tr>
</tbody>
</table>

Table 11.4: Effects of control and granisetron (10 µg/kg) on the change in the number and amplitude of duodenal pressure waves (channel 16) from baseline during intraduodenal glucose at a rate of ~ 3 kcal/min. Data are presented as control vs granisetron and are mean values ± SEM.
Conclusions
The studies presented in this thesis have provided new insights into the role of the gastrointestinal tract in postprandial blood pressure regulation. Methodological approaches to the evaluation of gastric emptying in healthy young subjects and the pathophysiological mechanisms underlying postprandial hypotension, an important clinical problem that is a major cause of morbidity and mortality, particularly in the elderly and in patients with type 2 diabetes, with a particular focus on gastric and small intestinal mechanisms and their potential therapeutic relevance have been examined in healthy elderly volunteers.

In Chapter 5, two techniques to measure gastric emptying (scintigraphy and three-dimensional (3D) ultrasonography) of low- and high-nutrient liquids in healthy young volunteers were compared. Ultrasonography has potential advantages over scintigraphy as it is non-invasive, associated with a low cost, does not entail a radiation burden, and is widely available (Hveem et al 1996). In particular, 3D ultrasonography may allow a precise measure of gastric emptying, by utilising a direct method of volume determination. Simultaneous measurements of gastric emptying of both low- and high-nutrient drinks using 3D ultrasonography techniques and the ‘gold standard’ scintigraphy demonstrated that there was a good correlation and agreement between the two techniques. While the results of this study indicated that 3D ultrasonography is a promising technique for measurement of gastric emptying, it does have disadvantages. Intragastric air, particularly in the fundus has the potential to limit the visualisation of the gastric outline (Gilja et al 1997), the technique is user dependent (De Schepper et al 2004), magnetic interference can cause spatial distortion of images (Gilja et al 1997).
and it cannot discriminate between the solid and liquid components of a meal (Holt et al 1986, Hveem et al 1996). Despite these issues, 3D ultrasonography allows visualisation of anatomical detail (Molin et al 1999) and offers great accuracy and precision in determining gastric emptying of liquids (Gilja et al 2005). The concordance between the two techniques is yet to be confirmed in patients with disordered gastric emptying. Furthermore, gender differences and an acceptable normal range for measurement of gastric emptying with 3D ultrasonography are required to determine the clinical applicability of the technique. These issues represent priorities for future studies.

The study reported in Chapter 6 demonstrated that in healthy elderly subjects, small intestinal glucose load, rather than glucose concentration, contributes to postprandial hypotension. While it was found that varying glucose concentrations from 4.1 - 16.7% (233 - 928 mM) do not alter the hypotensive response to ~ 3 kcal/min of glucose, it is of interest to determine whether the observed effects on blood pressure would be evident at lower glucose concentrations and at different glucose loads. Furthermore, while it is likely that the effects of glucose concentration and load on postprandial blood pressure would be exacerbated in patients with postprandial hypotension, this issue remains to be formally assessed.

Meal composition has been reported to influence the hypotensive response to a meal, however, current information remains inconsistent. In Chapter 7, the comparative effects of isocaloric and isovolaemic intraduodenal infusion of glucose, triglyceride and protein on the magnitude of the postprandial fall in blood pressure and rise in heart rate
and superior mesenteric artery blood flow were evaluated in healthy elderly subjects. It was established that isocaloric and isovolaemic intraduodenal infusions of glucose, triglyceride and protein, induced a comparable fall in systolic blood pressure and rises in heart rate but that the maximum fall in systolic blood pressure occurred later after triglyceride and protein. Furthermore, the stimulation of superior mesenteric artery blood flow was less after protein. While the mechanisms mediating the effects of nutrients on postprandial blood pressure are uncertain, the relatively slower systolic blood pressure response after triglyceride and protein may potentially reflect the time taken for digestion (ie triglyceride to free fatty acids and protein to amino acids). Inhibition of triglyceride and protein digestion may therefore represent an effective approach to the treatment of patients with postprandial hypotension. Further evaluation is now warranted in healthy elderly volunteers and in patients with postprandial hypotension.

The effects of the anti-diabetic drug acarbose, on blood pressure, heart rate, gastric emptying of, and the glycaemic, insulin, glucagon-like peptide 1 (GLP-1) and glucose-dependant insulinotropic-polypeptide (GIP) responses to, an oral sucrose load in healthy elderly subjects were evaluated in the study reported in Chapter 8, and established that acarbose, attenuated the postprandial fall in blood pressure and increase in heart rate induced by oral sucrose. While the initial effects of acarbose on blood pressure and heart rate presumably reflect the slowing of small intestinal sucrose digestion and/or absorption, the latter effects were potentially related to slowing of gastric emptying and in particular, increased small intestinal feedback by non-absorbed
carbohydrate. Acarbose was also associated with increased retention in the distal stomach. Studies to determine whether upper gastrointestinal symptoms associated with the use of acarbose may be attributable to a delay in gastric emptying and/or changes in intragastric meal distribution are warranted. Furthermore, the effects of acarbose on gastric emptying are also potentially relevant to patients with longstanding type 2 diabetes in whom there is a high prevalence of gastroparesis and should be evaluated. Acarbose stimulated the release of GLP-1 and suppressed GIP, after oral sucrose, consistent with previous observations (Qualmann et al 1995, Ranganath et al 1998, Enç et al 2001) and may have contributed to the slowing of gastric emptying and suppression of postprandial glycaemia by acarbose. While exogenous GLP-1 has been reported to increase blood pressure and heart rate in healthy young subjects (Edwards et al 1998) and animals (Barragan et al 1996), no studies have evaluated the effects of exogenous GLP-1 on blood pressure in the elderly or patients with postprandial hypotension.

In Chapter 9, the effects of gastric distension on blood pressure and heart rate during intraduodenal infusion of glucose at a constant load and concentration were evaluated in healthy elderly subjects. Intragastric administration of water markedly attenuated the falls in systolic and diastolic blood pressure induced by intraduodenal glucose. Heart rate increased, with and without gastric distension, in response to intraduodenal glucose infusion but not after intraduodenal saline infusion. As a result of small intestinal feedback, gastric emptying of intragastric water was markedly slower during intraduodenal glucose infusion when compared with intraduodenal saline infusion. The
mechanisms mediating the pressor response to water remain unclear. It is of interest to evaluate the effects of chronic gastric distension on postprandial blood pressure and heart rate in patients with postprandial hypotension since the consumption of water before a meal may represent a simple approach to the management of postprandial hypotension. In the majority of previous studies, the effects of gastric distension on postprandial blood pressure have been evaluated after oral loads. A comparison of the effects on postprandial blood pressure of oral glucose and intraduodenal glucose infusion and the impact of ‘intragastric’ mechanisms warrant further evaluation in healthy elderly subjects and patients with postprandial hypotension.

Nitric oxide is an important neurotransmitter in the gastrointestinal tract and the role of nitric oxide mechanisms is optimally addressed by the use of specific inhibitors of its production such as NG-nitro-L-arginine-methyl-ester (L-NAME) (Chiodera et al 1996). The role of nitric oxide mechanisms in gastric emptying of, and the blood pressure and glycaemic responses to, oral glucose were evaluated in healthy elderly subjects and reported in Chapter 10. L-NAME attenuated the postprandial fall in blood pressure and increase in heart rate but had no effect on gastric emptying of glucose. Furthermore, L-NAME attenuated the glucose-induced rise in plasma insulin but had no effect on the GIP and GLP-1 hormone responses to oral glucose indicating that the observed effects on blood pressure, heart rate and insulin are mediated by nitric oxide mechanisms by an effect unrelated to changes in gastric emptying, or the secretion of GIP and GLP-1. While nitric oxide mechanisms have been reported to increase splanchnic blood flow in response to a meal in the animal model (Alemany et al 1997), the role of nitric oxide
mechanisms in the regulation of splanchnic blood flow in humans, in particular healthy elderly volunteers, has not yet been established.

5-hydroxytryptamine (5-HT) is an important neurotransmitter in the gastrointestinal tract, that is predominantly formed in enterochromaffin cells of the intestinal mucosa (Sanger 1996). The release of 5-HT by D-glucose is also known to slow gastric emptying in rodents by activation of extrinsic vagal afferent pathways containing 5-HT3 receptors (Raybould and Zittel 1995, Li et al 2000, Zhu et al 2001, Raybould et al 2003). A study in the canine model has demonstrated an increase in gastrointestinal blood flow in response to low (4 µg/kg/min) and high (10 µg/kg/min) 5-HT infusions, suggesting a role for 5-HT in postprandial haemodynamic responses (Zinner et al 1983). The effects of the 5-hydroxytryptamine 3 (5-HT3) antagonist, granisetron, on the blood pressure, heart rate, antropyloroduodenal motility and glycaemic responses to intraduodenal glucose infusion were assessed in healthy elderly subjects in Chapter 11. It was established that granisetron, when administered acutely to healthy elderly subjects in a dose of 10 µg/kg, had no effect on blood pressure, heart rate or antral and pyloric motor responses but modulates the duodenal motor response, to intraduodenal glucose indicating that the cardiovascular and antropyloric motor responses to enteral glucose do not appear to be influenced by the stimulation of 5-HT3 receptors, but may be involved in the modulation of the duodenal motor response to intraduodenal glucose in healthy elderly subjects.
While the studies presented in this thesis have provided fundamental insights into the role of the gastrointestinal tract in the healthy elderly, future studies focussing on the role of both ‘gastric’ and small intestinal’ mechanisms are now warranted in patients with postprandial hypotension.


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