Gastric and Small Intestinal Determinants of

Postprandial Blood Pressure and Glycaemia

Thesis by

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Now the world is upside down,

I'm heading straight for the clouds…

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THESIS SUMMARY

This thesis presents clinical research studies relating to the roles of the stomach and small intestine in determining postprandial blood pressure (BP) and glycaemic responses in different patient groups. Postprandial hypotension (PPH), defined as a fall in systolic $BP \geq 20$ mmHg within two hours of a meal, is an important clinical problem in older individuals, which may lead to syncope and falls and is associated with increased mortality. PPH occurs when mechanisms responsible for the homeostatic maintenance of BP (including baroreceptor activation), are unable to compensate adequately for the shift in blood volume to the splanchnic circulation induced by meal ingestion. The gastrointestinal tract is also pivotal to the regulation of postprandial glycaemia. Postprandial glycaemia depends on the rate at which carbohydrate is delivered from the stomach to be absorbed from the small intestine, glucose disposal, and endogenous glucose production. The rate of gastric emptying (GE) is known to be a determinant of both the hypotensive and glycaemic responses to carbohydrate ingestion, so that when GE is relatively faster, the fall in BP and rise in blood glucose are more substantial. The broad focus of the work presented in this thesis, underlying the hypothesis of each chapter, is to achieve important insights into the physiology of the gastrointestinal tract with respect to the regulation of BP and glycaemia.

The prevalence of PPH is believed to be approximately 30% in individuals aged over 65 years, and even higher in patients with autonomic dysfunction,

hypertension or multiple co-morbidities. Estimates of the prevalence of PPH, however, are for the main part, derived from clinical studies in small, heterogenous cohorts and no study has been undertaken to determine the prevalence of PPH in otherwise healthy individuals. Furthermore, given the wide range of 'normal' GE, it is likely that relatively more rapid GE may be a risk factor for PPH. In the study reported in **Chapter 5**, we aimed to determine the prevalence of PPH, as well as the relationships between BP responses with GE, in a cohort of 88 healthy older volunteers, in response to a standardised 75 g oral glucose tolerance test (OGTT) 'meal'. In our cohort, the prevalence of PPH was ~13% and in patients with PPH, GE was faster. These findings are consistent with the concept that relatively more rapid GE may be a 'risk' factor for PPH, and that dietary and/or pharmacological strategies that slow GE may prove beneficial in the management of PPH.

The 75 g OGTT is regarded as the 'gold standard' for the diagnosis of impaired glucose tolerance and diabetes, but is subject to considerable variability which is likely to be accounted for, in part, by variations in GE. Furthermore, recent studies have questioned the diagnostic value of blood glucose at 60 min, used as an alternative to, or in conjunction with, the traditional 120 min value. We sought to determine the impact of GE on both 'early' and 'late' blood glucose responses to an OGTT in healthy older volunteers with and without impaired glucose tolerance. We obtained concurrent measurements of blood glucose, serum insulin and 'incretin' hormones (glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP)) in the cohort of older volunteers studied and reported in

Chapter 5. These results are presented in **Chapter 6**. Subjects were classified according to their glucose tolerance as either impaired glucose tolerance (IGT) or normal glucose tolerance (NGT). In both NGT and IGT, insulin sensitivity and GE were demonstrated to be independent, yet complimentary, determinants of the blood glucose response at both the 'early' and 'late' time points. These findings indicate that the inter-individual variability of the OGTT can be accounted for, in part, by differences in GE and insulin sensitivity. Furthermore, individuals with an overall faster rate of GE may potentially be at greater risk of developing IGT and type 2 diabetes.

Exposure of the gut lumen to nutrients results in the secretion of a number of hormones, including cholecystokinin (CCK), GIP and GLP-1. CCK and GIP are secreted from I- and K-cells, respectively, located in the proximal small intestine, whereas GLP-1 is released from L-cells located predominantly in the distal small intestine and colon. The mechanisms by which GLP-1 is released from the small intestine are incompletely understood; there appears to be a minimum threshold of nutrient delivery, above which GLP-1 is secreted, and the diversion of nutrients to the distal small intestine can potentiate GLP-1 secretion. We sought to determine region-specific effects of glucose exposure on gut hormone release by measuring the glycaemic, insulinaemic and incretin hormone responses to a duodenal glucose infusion in proximal (12 – 60 cm beyond the pylorus), distal (> 70 cm beyond the pylorus), and proximal and distal combined, small intestinal segments. This study is reported in **Chapter 7**. The findings from this study suggest the importance of the distal small intestine for GLP-1, and to a lesser extent GIP

and CCK, secretion. Therapies which target GLP-1 release in the distal small intestine may, therefore, potentially be more effective in blood glucose regulation than those that have a non-specific regional effect throughout the small intestine.

The pathophysiology of PPH is poorly defined. Although the gastrointestinal tract is important in the pathophysiology of PPH, a fall in BP must ultimately be regarded as an inadequate cardiovascular response to compensate for meal-related splanchnic blood pooling. Conversely, gastric distension, either nutrient or non-nutrient, stimulates sympathetic output and has the capacity to attenuate the fall in BP. The information relating to the effects of nutrients on cardiovascular function is limited and inconsistent, and no study of patients with PPH has employed echocardiography to assess postprandial cardiovascular changes. In the study reported in **Chapter 8**, we measured postprandial cardiovascular haemodynamics with echocardiography in response to drinks of water or glucose in individuals with and without PPH. We found that the fall in postprandial BP was greater in PPH but there were no differences in cardiac parameters. Interestingly, the hypotensive response to glucose and the hypertensive response to water were shown to be related. As the pressor response to water drinking is maintained and probably exaggerated in PPH, this represents a potential therapeutic target.

While delayed GE is a sequela of Parkinson's disease, the prevalence of delayed GE is uncertain, as is the impact of this phenomenon on postprandial BP and glycaemia. PPH is likely to occur frequently in Parkinson's disease,

particularly in cases with impairment of the autonomic nervous system. In healthy individuals, the postprandial fall in BP is related to the increase superior mesenteric artery (SMA) blood flow, but this has not been investigated in Parkinson's disease. In **Chapter 9**, we present the results of a cross-sectional study in which GE, BP, SMA blood flow and glycaemia were measured following an OGTT, in individuals with mild-to-moderate Parkinson's disease. Gastric emptying was delayed in 14% of these patients and 38% had PPH. Gastric emptying was related to autonomic dysfunction and a determinant of the glycaemic, but not apparently the hypotensive, responses to meal ingestion in this population. There was also no relationship between the rise in SMA blood flow and fall in BP.

GLP-1, by slowing GE and altering mesenteric blood flow, may potentially affect postprandial BP. The slowing of GE by GLP-1 is potent, so that it is the primary mechanism by which GLP-1 lowers postprandial glycaemia. GLP-1 and GLP-1 receptor agonists may affect BP, but clinical trials of GLP-1 receptor agonists have, for the main part, not discriminated between fasting and postprandial BP. We evaluated the effects of GLP-1 on postprandial BP in two studies, reported in **Chapters 10 and 11.** In **Chapter 10**, we report the effects of exogenous GLP-1 on the BP, heart rate, SMA blood flow and glycaemic responses to an intraduodenal glucose infusion in healthy older subjects. In **Chapter 11**, the effects of exogenous GLP-1 in response to an oral glucose load, in healthy older subjects and patients with type 2 diabetes, are reported. The findings of both of these studies suggest GLP-1 receptor agonists may be effective in the management of PPH; one potential

mechanism is through the slowing of GE, although GLP-1 was shown to attenuate the fall in BP during intraduodenal glucose infusion so other factors are also involved. It is clear that clinical trials that report the effects of GLP-1 and GLP-1 receptor agonists on BP should differentiate between the fasting and postprandial states.

When glucose is infused intraduodenally at rates spanning the normal range of GE, the increase in SMA blood flow and heart rate occurs in parallel with secretion of GIP, not GLP-1. A potential role for GIP in the regulation of the cardiovascular response to meal ingestion is suggested by the outcomes of the study reported in **Chapter 12**. We report the effects of sitagliptin (a dipeptidyl peptidase-4 inhibitor, which increases circulating levels of GLP-1 and GIP), on BP and heart rate during an intraduodenal glucose infusion at a rate of 2 kcal/min, where GIP is the major incretin in the circulation. Our findings suggest a potential role of GIP in the regulation of postprandial cardiovascular function but more studies are warranted.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signature: Date: February 2016

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PUBLICATIONS ARISING FROM THESIS

Trahair LG, Horowitz M, Jones KL. Postprandial Hypotension: A Systematic Review. *J Am Med Dir Assoc.* Mar 2014; **15**(6): 394-409.

Trahair LG, Horowitz M, Jones KL. Postprandial hypotension is associated with more rapid gastric emptying in healthy older subjects. *J Am Med Dir Assoc.* Jun 2015; **16**(6): 521-3.

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CHAPTER 1: BLOOD PRESSURE REGULATION

Introduction

Blood pressure (BP) is defined as the pressure or force exerted by blood on the wall of any blood vessel (1). It is dependent on the volume of blood contained within the vessel, as well as the compliance of the vessel walls (1). Systolic pressure is defined as the peak pressured exerted in arteries during ventricular contraction and diastolic pressure is the minimum arterial BP occurring during ventricular relaxation in between heartbeats (2). For a healthy individual these pressures are typically about 120 and 75 mmHg, respectively (2). In this chapter, current knowledge of the factors that are known to influence BP is discussed, with a focus on the definitions of and principal mechanisms underlying cardiac output, peripheral resistance and blood volume, as well as the homeostatic mechanisms by which these are regulated.

Maintenance of Blood Pressure

Blood pressure is physiologically determined by three principle variables: cardiac output, blood volume and peripheral resistance.

Cardiac output

Cardiac output is the amount of blood ejected by the left ventricle into the aorta every minute (1). Changes in cardiac output result either from changes

in cardiac function and/or venous return frequently as a consequence of changes in blood volume, increasing stroke volume (i.e. the volume of blood pumped out of each ventricle with each contraction of the heart) or heart rate (2). Accordingly, increasing cardiac output also increases BP (2). Conversely, a reduction in cardiac output is associated with a reduction in BP (2).

Blood volume

The total volume of the blood in the cardiovascular system is directly proportional to BP (2), such that any increase or decrease in blood volume from the normal level (approx 4 – 6 L in a healthy individual) results in an increase or decrease in BP.

Peripheral resistance

Peripheral resistance is the opposition to flow that the blood encounters in a vessel away from the heart, arising through the force of friction between the blood and its vessel walls (2). Peripheral resistance is dependent on the viscosity of blood, and the length and diameter of the vessel. An increase in viscosity (occurring with such conditions as dehydration or an unusually high number of red blood cells) or a decrease in viscosity (occurring with such conditions with a depletion of red blood cells) is associated with an increase, or decrease in BP (2). As the length of a vessel increases, the more cumulative friction it encounters, so that the pressure within a vessel decreases with distance. Since blood viscosity and vessel length do not change from moment to moment, changes in the diameter of the vessel are readily adjustable and

are the most significant mechanism that governs peripheral resistance (2). Vasoconstriction, the contraction of the smooth muscle and narrowing of a vessel, increases pressure, whereas vasodilation, the widening of a vessel through passive relaxation of the vessel wall, allows the BP to expand the vessel and decreases pressure (2).

Regulation of Blood Pressure

Blood distribution and pressure are controlled by neural, local and hormonal mechanisms. An overview of the relationships between the mechanisms that control BP is given in **Figure 1.1**.

Neural Control

Vasomotor centre

The vasomotor centre is a cluster of neurons located in the medulla oblongata which exerts sympathetic control over blood vessels (2). Increasing the number of sympathetic impulses sent from the vasomotor centre to the smooth muscle in the arteriole wall results in vasoconstriction in a majority of blood vessels, but dilation in the vessels of skeletal and cardiac muscle to meet increasing metabolic demands (2). Conversely, at times of reduced metabolic demand, vasodilation is achieved by decreasing sympathetic nerve stimulation.

Cardiovascular control centre

The cardiovascular control centre is located within the medulla in the brain stem and is responsible for activating parasympathetic nerves which slow the heart, decrease cardiac output and reduce BP (2).

Baroreceptor reflex

Baroreceptors are specialised 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 (1). Stimulation of baroreceptors occurs when there is an increase in BP, and results in an increase in signalling between the baroreceptors and cardiovascular control centre in the medulla. This input inhibits sympathetic stimulation and excites vagal fibres resulting in a decrease in heart rate and cardiac output, and consequently, BP (2). Conversely, a reduction in BP leads to decreased baroreceptor activity resulting in increased sympathetic, but decreased parasympathetic, outflow (2).

Chemoreflex

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 (1, 2). The chemoreflex primarily adjusts respiration to changes in blood chemistry, but when there is relative hypoxia (low blood oxygen), hypercapnia (excessive carbon dioxide) or acidosis (low blood pH), chemoreceptors are stimulated and send impulses to the vasomotor centre

which induces widespread vasoconstriction through sympathetic pathways, resulting in a rise in BP (2).

Medullary ischaemic reflex

When the blood supply to the medulla is inadequate, an autonomic response is triggered known as the medullary ischaemic reflex (2). Within seconds of detecting excessive carbon dioxide and insufficient oxygen in the blood, the cardiac and vasomotor centres of the medulla send sympathetic signals to the heart and blood vessels to induce an increase in heart rate and widespread vasoconstriction (2).

Higher brain centres

Cardiovascular responses associated with behaviours and emotions are mediated through the cerebral cortex – hypothalamic pathway (1). During states of stress, anger or arousal, the cardiac and vasomotor centres receive input from the cerebral cortex and increase or decrease sympathetic stimulation leading to changes in BP (1).

Local Control

Autoregulation

Autoregulation is the ability of tissues to regulate their own blood flow (2). If a tissue is inadequately perfused, it becomes hypoxic and this results in the presence of metabolites such as carbon dioxide, hydrogen and potassium ions,

lactic acid and adenosine accumulate, which stimulate vasodilation and increase perfusion (2). As the bloodstream delivers oxygen and removes the metabolites, the vessels reconstrict, achieving a homeostatic equilibrium which adjusts perfusion to the tissue's metabolic needs (2).

Hormonal Control

Vasoactive hormones

There are a number of hormones which affect BP by causing vasoconstriction or vasodilation of the arterioles. Epinephrine (adrenaline) and norepinephrine (noradrenaline) produced by the adrenal medulla bind to α-adrenergic receptors on the smooth muscle of most blood vessels to stimulates muscle contraction and vasoconstriction (2). In the coronary and skeletal blood vessels these chemicals bind to β-adrenergic receptors and cause vasodilation, thus increasing blood flow to the heart and muscles during exercise (2). Hormones such as nitric oxide, as well as certain prostaglandins, kinins and histamine, cause smooth muscle relaxation in arteries and promote vasodilation, allowing for local regulation of BP through hormonal mechanisms. Antidiuretic hormone (ADH) (also known as vasopressin) is produced by the hypothalamus and released from the neurohypophysis (the posterior lobe of the pituitary gland) causing vasoconstriction of the arterioles and raising BP (2).

Renal Control

The kidney has a major role in regulating BP by modulating water balance. Increased renal sympathetic activity, decreased BP at the juxtaglomerular cells, and/or decreased concentration of sodium and chloride ions at the macula densa stimulate the juxtaglomerular cells to secrete the enzyme renin. Renin readily hydrolyses circulating angiotensinogen into angiotensin I. Angiotensin I is then converted to angiotensin II by angiotensin converting enzyme (ACE) in the lungs. Angiotensin II raises BP by increasing sympathetic activity, increasing tubular sodium and chloride ion reabsorption, causing widespread vasoconstriction and promoting ADH secretion. Angiotensin II also stimulates the secretion of aldosterone from the adrenal cortex, which further acts to increase tubular sodium ion concentration and water reabsorption, thus promoting a higher blood volume and pressure (2). Atrial natriuretic peptide (ANP), secreted by atrial myocytes, has the opposite effect of aldosterone, increasing the excretion of sodium ions thus promoting vasodilation and reducing blood volume and pressure (2). Brain natriuretic peptide (BNP), produced mainly in the cardiac ventricles, has a similar effect to ANP.

Effects of Meal Ingestion

Meal ingestion is associated with splanchnic blood pooling and a systemic cardiovascular response (3). In healthy young individuals, splanchnic blood pooling is compensated for by an increase in cardiac output and heart rate, as well as vasoconstriction in skeletal muscle and peripheral vascular beds (4, 5), mediated by autonomic and baroreflex pathways. The cardiovascular response is affected by meal size and caloric load (6), and macronutrient composition

(7). If postprandial cardiovascular changes are inadequate, then BP will decrease. A modest postprandial fall in BP is common in healthy older (> 65 years) individuals (8), related to mild impairment of compensatory pathways. A sustained fall in BP following a meal, known as postprandial hypotension, will be discussed in detail in **Chapter 2**.

Conclusions

This chapter has presented a brief summary of the mechanisms involved in BP regulation relevant to this thesis. The changes in BP in response to the specific physical and metabolic demands of the body are required to provide an adequate blood supply to all tissues. It is determined by three principle variables: cardiac output, blood volume and peripheral resistance, with changes in peripheral resistance being the most physiologically modifiable. Homeostatic control of BP is achieved via a number of neural and hormonal mechanisms at both local and systemic levels.

Figures and Figure Legends
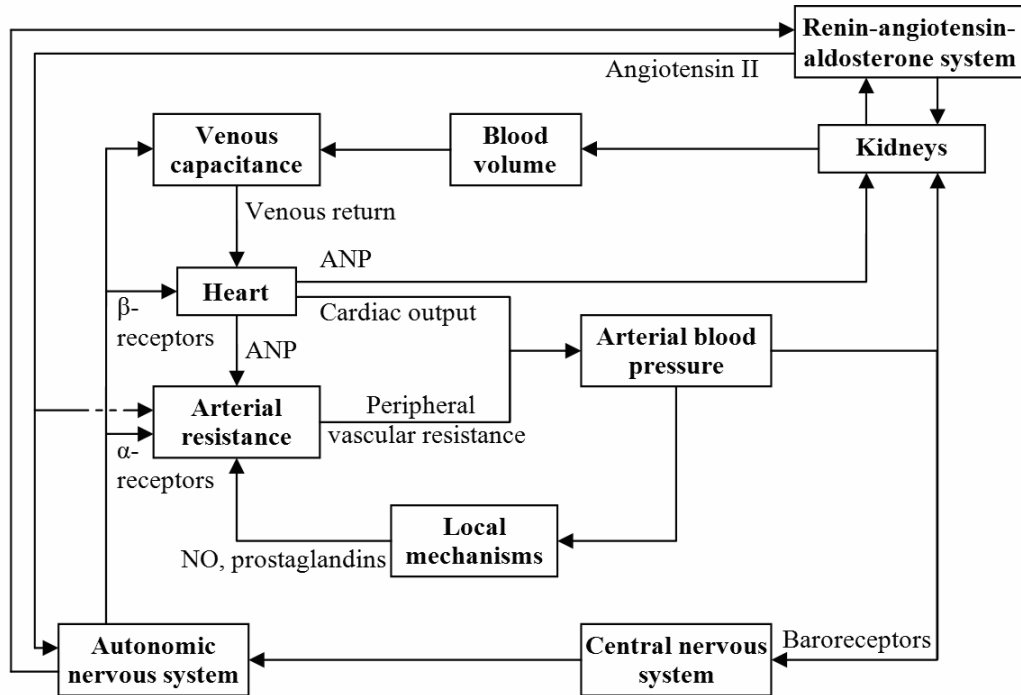
Figure 1.1: Mechanisms and pathways for maintaining blood pressure homeostasis.

CHAPTER 2: POSTPRANDIAL HYPOTENSION: A SYSTEMATIC REVIEW

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Principal Author

Candidate	Laurence G Trahair		
Contribution	Searching, identification and critical review of literature, drafting of the manuscript.		
Overall percentage	80%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-author	Michael Horowitz		
Contribution	Drafting of the manuscript.		
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Name of Co-author	Karen L Jones		
Contribution	Drafting of, and overall responsibility for, the manuscript.		
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Introduction

A significant, meal-related, postprandial decline in blood pressure (BP) was first reported in 1977, in a 65 year old male with Parkinson disease who experienced dizziness and visual disturbance following an oral glucose load, when his systolic BP was shown to decrease from 200 to 105 mmHg (9). Postprandial hypotension (PPH) is now recognised as an important clinical problem, being associated with increases in the risk of syncope, falls, stroke

and angina, as well as mortality (10). The primary aim of this review is to evaluate current knowledge relating to PPH, with particular focus on prevalence, pathophysiology and management, as well as future research priorities.

Methods

We performed a review of publications in the databases Pubmed, Embase, Cochrane Library and Web of Knowledge. The searches included all original, full-text, journal articles from database inception to the time of writing (January 2014); the keywords postprandial, hypotension and blood pressure were used to identify articles. Screening of studies was performed initially by assessment of the relevance of the abstract to PPH. Study inclusion was based on the guidelines for preferred reporting items for systematic reviews and meta-analyses (PRISMA) (11).

Results

A total of 1071 full-text articles were identified from the database search; abstracts and conference proceedings were excluded. **Figure 2.1** summarises the selection process. After the removal of duplicate papers, 417 remained, and following screening of the abstract, a further 169 articles were removed. Of the remaining 248 papers, reviews (n= 25), papers that were unavailable in the English language (n= 36), preliminary studies (n= 3), letters to the editor or comments (n= 6), and most case reports (n= 11 of 12) were removed, leaving a total of 167 papers which were reviewed.

Definition, prevalence and risk groups

PPH has been traditionally defined as a fall in systolic BP of > 20 mmHg, or a decrease to ≤ 90mmHg when the preprandial BP is ≥ 100 mmHg, within 2 hours of a meal (12). Postprandial changes in diastolic BP are, in general, less marked. This definition is empirical, rather than being based on evaluation of sensitivity/specificity, and derived from that used to define orthostatic hypotension (13). A fundamental difficulty in establishing a definition of PPH is that, as will be discussed, many, if not a majority of patients are asymptomatic (14). In otherwise healthy subjects with PPH, the nadir of the BP reduction is usually evident at about 45 min postprandially; but, in patients with autonomic failure, the maximum decrease usually occurs within the first 15 min, and is often greater (15). Studies relating to the prevalence of PPH are confounded by a lack of standardisation of a number of methodological issues, including the composition of the test meal, the timing of meal ingestion, the posture of the subject during the evaluation, the technique used to quantify BP, the duration of postprandial BP monitoring and the use of medications, particularly antihypertensives and diuretics. Arguably, the optimum 'meal' is that recommended by Jansen and Lipsitz (12), a drink with a total energy of 419 kcal, 77% from carbohydrate, 3% from fat and 20% from protein, however, there is little information about the precision of different meals. As will be discussed, PPH is intuitively less likely to occur with low calorie, higher volume meals and the macronutrient content may influence the timing of the fall in BP. If only one meal is to be assessed, 'breakfast' is arguably the best choice as the greatest fall in postprandial BP is usually seen at this time (16), although a case can be made for the increased use of ambulatory BP

monitoring (ABPM) for both diagnosis and determining the response to management. Home BP monitoring has also been used to diagnose PPH and has advantages over ABPM in terms of cost and patient tolerance (17). The intra-individual reproducibility of PPH appears to be high (18, 19), so that in most cases, a single test may be sufficient for diagnostic purposes (19). BP should ideally be measured every 10 min for 2 hours with an automatic sphygmomanometer. It has been suggested that when PPH is assessed with an ambulatory monitor, measurements should be performed every 15 min during the day (20-22) and every 60 min at night (20, 23). PPH and orthostatic hypotension (OH) frequently coexist (24-27) and prolonged (> 2 hours) supine rest may increase the propensity to PPH (28). Accordingly, posture should be standardised as part of the assessment of PPH and OH also assessed; the sitting position is recommended, although PPH occurs independent of posture (29). Medication should be standardised throughout the test period. It is arguable whether antihypertensive therapy should be withdrawn; although hypertensive subjects exhibit greater reductions in postprandial BP, when compared with age-matched normotensive subjects (17, 30-35). Diuretics, such as furosemide, may potentiate the postprandial fall in BP (36).

The prevalence of PPH has been reported in a number of distinct patient groups, including 'healthy', hospitalised and institutionalised 'older' subjects, patients with type 2 diabetes and those with Parkinson Disease (PD), as summarised in **Table 2.1 (Appendix 1)**. Whilst PPH has been identified in other populations, these studies did not specifically report 'prevalence.' In

addition to the issues discussed, interpretation of information is compromised by the modest size and heterogeneity of many of the cohorts evaluated, including the potential effects of illness(es). Despite these limitations it is clear that PPH occurs frequently and probably more often than OH (24, 26).

Twelve studies have evaluated the prevalence of PPH in, for the main part, unselected, populations of older subjects varying from 24 – 401 individuals (10, 14, 21, 37-45). There are no large population-based studies. In 2 studies data from the same cohort were reported twice (10, 38, 39, 42), 3 studies reported prevalence specifically in a population known to have hypertension (17, 20, 22), 2 in type 2 diabetes (23, 46), 4 in PD (18, 47-49) and 1 in Alzheimer's disease (50). Only 3 studies (37, 43, 51) reported race, and these studies were performed in Caucasians (51), Asians (43) and mixed-race (37) populations respectively, hence, it is not known whether the prevalence of PPH exhibits a racial variation. The prevalence of PPH in residential care cohorts has been evaluated in 5 studies (37, 39, 41, 43, 44), in hospitalised geriatric patients in 4 studies (14, 21, 40, 45) and 1 study evaluated prevalence in geriatric outpatients attending a falls clinic (38). The outcome of these studies suggests that the prevalence of PPH in 'healthy' older subjects and in inmates of residential care is 24 – 38% (37, 39), 20 – 91% (14, 21) in hospitalised geriatric patients, ~40% (23, 46) in type 2 diabetes and 40 – 100% (47, 52) in PD.

Of the 20 studies included in **Table 2.1 (Appendix 1)**, the test meal was controlled for in 11 (20, 23, 38, 40, 41, 44-48, 50), while in the other 9, BP

was measured following meals eaten ad libitum (14, 17, 21, 22, 37, 39, 43, 49, 53). Two studies, both relating to diabetes, employed 75 g or 50 g oral glucose loads (23, 46). Surprisingly, no studies used the test meal recommended by Jansen and Lipsitz (12). There was also a substantial variation in the time of day when PPH was assessed; 8 studies assessed PPH after breakfast (14, 23, 39, 41, 45-48), 6 at lunch, or in the early afternoon (17, 37, 40, 43, 44, 50), and 1 in either the morning or afternoon (38). In the 5 studies employing ABPM, there were no specific meal times (20-22, 49, 54). Posture was standardised in all but 4 studies (21, 22, 47, 55), all of which employed 24 hour ABPM, but the posture was inconsistent, i.e. 1 supine (23), 12 seated (14, 20, 37-41, 43, 45, 46, 48, 50) and in 1 study the position was standardised, but variable, in each patient (44). Only 4 studies assessed both OH and PPH (38, 45, 47, 48).

BP was measured in 3 ways in these studies; 8 with an automated, or manual sphygmomanometer (17, 37, 40, 43, 44, 46, 47, 50), 8 ABPM (6, 20-23, 39, 45, 49) (in 2 of these for less than 24 hours (39, 45)), 3 reported beat-to-beat BP monitoring (14, 38, 48), and in one study the method was not reported, but was presumably a sphygmomanometer (41). The duration of assessment was between 60 (21, 22) and 180 min (23), and excluding beat-to-beat methods, the frequency of measurements was between 10 (45, 50) and 30 (20) min. Disconcertingly, BP was monitored for at least 120 min in only 5 studies (23, 37, 46, 50, 56), particularly as this is a component of the definition of PPH. In only 2 studies (14, 41) was the use of medications potentially affecting BP excluded, in the others, patients continued their normal medications, including

anti-hypertensive medication, and, where applicable, withheld their dose immediately prior to the study.

Based on studies in small cohorts it is also clear that PPH occurs frequently in other patient groups, including paraplegia (39, 57, 58), PD (52, 59-61), primary autonomic failure (multiple system atrophy (MSA) or pure autonomic failure (PAF)) (15) and patients receiving haemodialysis (62, 63). PPH may occur more frequently in PAF compared to MSA (64).

Clinical manifestations

PPH is associated with a number of sequelae which impact adversely on quality of life, as well as increased mortality (10). However, it should be recognised that all studies relating to the clinical manifestations of PPH have substantial limitations, particularly in relation to the size of the cohorts studied, lack of appropriate control subjects, paucity of longitudinal assessments and potential confounders, including the diseases associated with PPH, in addition to other methodological issues, referred to previously. Large-scale, prospective studies are required; there is also a need for a validated instrument to assess symptoms of PPH.

The most common manifestations of PPH appear to be syncope, falls, angina, dizziness, nausea, light-headedness and/or visual disturbance (65-67) so that there is an overlap with symptoms of 'frailty' (12). Postprandial BP has been reported to be lower in older subjects who had recently experienced a fall when compared to those who had not (37, 39, 68) and an association of

syncope with PPH has been reported (65, 67, 69). For example, in a study of 16 older patients with unexplained syncope, 50% had PPH (65). However, in the majority of otherwise healthy individuals, a fall in BP of ~20 mmHg is insufficient to exceed the threshold for cerebral autoregulation (67); and it is, accordingly, likely that the majority of patients with PPH are 'asymptomatic (17).' While well designed studies to evaluate the relationship of symptoms with the magnitude and time course of the fall in BP are needed, there is anecdotal evidence that symptoms are more common, and severe, when the reduction is greater.

There is reasonable evidence that PPH is an independent predictor of mortality (10, 22, 70); what is much less certain is the strength of this association. PPH appears to increase both cardiovascular (22) as well as all-cause mortality (10) and to be a risk factor for cerebrovascular ischemia (71, 72) and arteriosclerosis (73). For example, in 401 older hypertensive patients, 73% whom had PPH, followed for 4 years, the presence of a decrease in systolic BP following breakfast was associated with an increase in cardiovascular mortality of ~20% (22). In a study of 179 low-level care residents (mean age 83 years) followed for 4.7 years, mortality was greater in individuals with PPH (145 vs. 99 per 1000 person-years) (10) and there was a linear relationship between mortality with the magnitude of the fall in BP so that mortality rates in patients who experienced BP falls of ≤ 10 mmHg, 11 – 19 mmHg, 20 – 39 mmHg and ≥ 40 mmHg being 89.1, 116.9, 144.4 and 156.1 per 1000 person-years, respectively (10). Mortality does not appear to

be predictable on the basis of symptoms; hence, even 'asymptomatic' individuals with PPH appear to be at higher risk.

Pathophysiology of PPH

The pathophysiology of PPH is incompletely understood, however, in the broadest sense, a significant postprandial fall in BP is indicative of inadequate cardiovascular compensation for meal-induced splanchnic blood pooling (74-76). In healthy young subjects, meal ingestion is not associated with a major change in BP, while in healthy older subjects any postprandial reduction in BP is modest (8, 77-80). Current evidence suggests that in most cases there is not a single, or dominant, aetiological factor (81) in PPH and that multiple factors are involved, including autonomic and neural dysfunction, changes in gastrointestinal hormones, meal composition, gastric distension and the rate of delivery of nutrients to the small intestine.

Autonomic and neural mechanisms

Definition of the neural mechanisms underlying PPH is of considerable interest, particularly because of the potential implications for management and represents a research priority. In healthy subjects, meal ingestion increases sympathetic nerve activity, triggered by gastric distension and the presence of nutrients in the small intestine. It has been estimated that following a meal, healthy older subjects require a ~200% increase in sympathetic nerve activity to maintain postprandial BP (82), and that this response is even greater in the healthy young (83). After a meal there is a modest increase in plasma

noradrenaline in healthy young and older subjects (69). There is also an increase in muscle sympathetic nerve activity (MSNA) in response to intraduodenal nutrient infusion, responses which are comparable in healthy young and older subjects, despite a modest reduction in BP in the latter group (56). However, the hypertensive and MSNA responses to gastric distension (the so-called 'gastrovascular reflex') are blunted in the elderly (84) and gastric distension also appears to have no effect on plasma noradrenaline in the healthy elderly (85). In patients with PPH, limited data indicate that there is no increase in MSNA (86), or plasma noradrenaline (87) following a meal, indicative of diminished sympathetic activation.

Spectral analysis of the heart rate (HR) response to a meal can be used to characterise the contribution of the baroreflex, a response mediated by the sympathetic nervous system, to HR variability. In healthy young and older subjects, meal ingestion affects HR variability (24, 88) indicative of greater baroreflex stimulation. In PPH (89-91), there is apparently less HR variability, suggestive of reduced baroreflex sensitivity (89). However, the latter does not per se appear to account for the hypotensive response to oral glucose (92).

Nitric oxide (NO), an important neurotransmitter with vasodilatory properties, is released endogenously in the gastrointestinal tract (93) and may regulate splanchnic blood flow (94). The role of NO pathways can be evaluated using inhibitors of its production, such as, NG-nitro-L-arginine-methyl-ester (L-NAME) (93), or NG-mono-methyl-L-arginine (L-NMMA) (94). In healthy older subjects, the fall in BP induced by an oral glucose load (50 g in 300 mL

water) is attenuated by L-NAME, without any change in gastric emptying (95).

The role of NO in the regulation of postprandial splanchnic blood flow in

humans has not been evaluated, nor has the relevance of NO mechanisms

been explored in patients with PPH. 5-hydroxytryptamine, an important

neurotransmitter in the sensing of small intestinal glucose, does not appear to

play a role in postprandial BP regulation (96).

Hormonal mechanisms

A number of gastrointestinal hormones, including insulin, glucagon-like

peptide-1 and 2 (GLP-1, GLP-2), calcitonin-gene-related peptide (CGRP),

neurotensin, vasoactive-intestinal peptide (VIP), bradykinin and substance P

have been implicated in PPH, Moreover, the postprandial hypotensive

response is attenuated by somatostatin (97) which inhibits the release of most

gastrointestinal hormones. However, the relevance of hormonal mechanisms

to PPH remains uncertain because of inconclusive evidence.

The concept that insulin is a factor in PPH has some supporting evidence (98-

100). There is a hypotensive response to oral glucose, but not fructose or

xylose, which do not stimulate insulin secretion substantially (66, 101, 102).

That intravenous administration of glucose stimulates insulin, but has no

effect on BP (100, 103), however, indicates that, at a minimum, other

mechanisms are likely to be important. The 'incretin' hormone, GLP-1, is

secreted from the intestinal L-cells in response to oral glucose (104), and acts

to stimulate glucose-induced insulin secretion, suppress glucagon (105), and

slow gastric emptying (106). Limited evidence suggests that GLP-1 may

attenuate the postprandial fall in BP, particularly in relation to the effects of acarbose which has the capacity to stimulate GLP-1, as well as slow gastric emptying (107). While GLP-1 agonists such as exenatide and liraglutide, which are now used widely in the management of type 2 diabetes, have been reported to lower BP modestly, effects of postprandial BP have, to our knowledge, not been specifically examined. Interestingly, in a recent case report, vildagliptin, an inhibitor of dipeptidyl peptidase-4 (and hence, GLP-1 degradation), was reported to be beneficial in the management of PPH (108). Additional studies are required to clarify the role of GLP-1. GLP-2, which is co-secreted with GLP-1 (109), has potent vasodilatory effects including in the mesenteric circulation (109, 110) and may also be important (105). Plasma CGRP, another vasodilatory peptide released in response to meal ingestion, was reported in one study to correlate with the magnitude of the fall in BP after an oral glucose load in 20 healthy older subjects (111). The potential role of neurotensin in PPH has been studied extensively (15, 103, 105, 112), with essentially negative outcomes. Vasoactive-intestinal peptide, bradykinin and substance P have powerful vasodilatory properties, but there is no evidence to suggest that either is important in PPH. For example, levels of plasma VIP, bradykinin and substance P are not affected by the ingestion of a meal and/or oral glucose in older subjects (113), hypertensive subjects (7) or patients with autonomic failure (30, 103, 114-116). Neither glucagon (112) nor pancreatic polypeptide (15, 112) appear to have significant cardiovascular effects.

Superior mesenteric artery blood flow

Following a meal, there is an approximate doubling of blood flow through the superior mesenteric artery (SMA), coupled with a decrease in vascular resistance and peripheral blood flow (117), particularly to skeletal muscle (118). SMA blood flow can be assessed readily with ultrasonography (119). The postprandial increase in splanchnic blood flow has been shown to be dependant on both the size of the meal (53) and the rate of small intestinal nutrient delivery (120). In healthy older subjects, intraduodenal infusion of glucose has a more profound effect on the increase in SMA blood flow, compared to fat or protein (121) **(Figure 2.2)**, and when these nutrients are administered orally, the onset of the increase in SMA flow in response to oral glucose is more rapid suggesting that the increase in SMA flow is triggered by the products of macronutrient digestion (122). There are limited data that the magnitude of the increase in SMA blood flow in response to oral (123) and intraduodenal (124) nutrients is comparable in patients with PPH, healthy young and older subjects (124). Octreotide attenuates the increase in SMA blood flow following a meal and reduces the fall in BP in chronic autonomic failure (125). Hence, it appears there are no fundamental differences in meal-induced SMA blood flow in PPH (126), implying that, in the broadest sense, the disorder reflects a lack of cardiovascular compensation for this increase.

Meal composition

All macronutrients have the capacity to decrease BP in older subjects significantly (19, 54, 55, 127-129) when administered orally or intraduodenally (121), whereas there is no fall in the healthy young (130). As

discussed, of the macronutrients, glucose appears to elicit the most rapid decrease in systolic BP in healthy older subjects (55) and patients with PPH (15). While a simple carbohydrate (78% glucose) induced a substantial fall in BP in healthy older subjects, this was not evident following complex carbohydrate (78% starch), probably reflecting a slower rate of small intestinal absorption (131). This may also account for the differential effects of glucose and xylose on BP (120), particularly as the effects of carbohydrate on glycaemia are not apparently related to the BP response (132). Similarly for the other macronutrients, it has been suggested that the hypotensive response is mediated by the products of their digestion and absorption, including the digestion of protein to amino acids (129), and triglyceride to fatty acids (55). Perhaps surprisingly, the lipase inhibitor, orlistat, increases the hypotensive response to a fatty meal in both healthy elderly and type 2 diabetic patients, which may be secondary to the acceleration of gastric emptying (133, 134). The effects of different nutrient combinations on PPH have not been formally evaluated, although, as discussed, numerous studies have employed mixed meals for diagnostic purposes.

Gastric distension

Distension of the stomach is associated with a number of systemic cardiovascular effects which appear to be important in mediating the postprandial haemodynamic response to a meal. The magnitude of the increase in MSNA induced by gastric distension, the 'gastrovascular reflex' (84) is dependant on the distension volume (135, 136) and the increase in sympathetic activity has been shown to be comparable to that induced by

traditional stimuli, such as caffeine (135). It is uncertain whether the pressor response to gastric distension with water reflects stimulation of the sympathetic nervous system (137), and/or changes in intravascular volume (138). In healthy older subjects, gastric distension with water, even at relatively low volumes (300 mL), attenuates the fall in BP induced by intraduodenal glucose (139). Moreover, the magnitude of the fall in BP is less following oral glucose, compared with an identical duodenal glucose load, probably due to the 'protective' effects of gastric distension (140). The region of gastric distension (proximal or distal) may also be important (141). Despite the fact that the 'gastrovascular reflex' is attenuated in healthy elderly (84) as well as other parameters of sympathetic activity (69), gastric distension with water is associated with a significant pressor response in patients with autonomic failure (137). As will be discussed, this has implications for the management of PPH.

Small intestinal nutrient delivery

There is a wide inter-individual variation in the normal rate of gastric emptying, which is between 1 – 4 kcal/min (142), regulated primarily by inhibitory feedback arising from interaction of nutrients with the small intestine (143). In contrast, the intra-individual variation is modest. Because gastric emptying is determined primarily by small intestinal feedback, both posture and meal volume have little effect on emptying rates (143). Abnormally delayed gastric emptying (gastroparesis) occurs frequently in a number of conditions associated with PPH, including type 2 diabetes (46) and

PD (61). Healthy ageing appears to be associated with a modest slowing of gastric emptying (144).

The hypotensive response to oral glucose has been shown to be related to the rate of gastric emptying in type 2 diabetes (46), i.e. when gastric emptying is relatively more rapid, there is a greater fall in BP. The outcome of subsequent studies employing direct intraduodenal infusion of glucose within the normal range for gastric emptying (i.e. 'bypassing' the potential effects of gastric distension) indicats that there is a non-linear relationship between nutrient delivery and the magnitude of the fall in BP in healthy older subjects (120) **(Figure 2.3)** so that there appears to be a threshold above which nutrient must be delivered to the small intestine in order to elicit a hypotensive response- probably between 1 – 2 kcal/min (120) an effect that is apparently independent of the concentration of glucose infused (145). Knowledge of the effects of direct small intestinal nutrient exposure in PPH is limited to a report of two cases- in one of these intraduodenal glucose at a rate of 3 kcal/min induced a substantial fall in systolic BP of 92 mmHg (146). It is intuitively likely that patients with PPH will be more 'sensitive' to the effects of small intestinal glucose, particularly when the protective effects of gastric distension are eliminated. The above observations support the concept that strategies which slow gastric emptying (dietary or pharmacological) may prove useful in the management of PPH.

Management

Management of PPH can be 'non-pharmacological' or 'pharmacological' and the outcomes have usually been assessed by quantifying the magnitude of the postprandial BP decline, particularly, systolic BP. Given that the association of symptoms with the postprandial decline in BP is weak, the effect of a treatment on symptoms can only be evaluated in studies of large cohorts- and even larger studies would be required to determine impacts of cardiovascular events/mortality. Previous studies are all acute and limited to small cohorts, often including individuals who had more modest postprandial falls in BP which did not meet the strict criteria for diagnosis of PPH. Accordingly, larger, chronic studies of the effects of treatment in PPH represent a priority. Despite these limitations, several management strategies for PPH appear effective.

Non-pharmacological

Numerous non-pharmacological interventions have been evaluated for the treatment of PPH (53, 115, 147-153); including the consumption of smaller meals more frequently, slowing gastric emptying, water drinking (to increase gastric distension), postprandial exercise and modifying meal temperature. These studies are difficult to classify systematically, as summarised in **Table 2.2 (Appendix 1)**. Two dominant strategies have been to delay the exposure of the small intestine to the products of nutrient digestion (53, 115, 148, 150, 152) and/or increase gastric distension (147, 154).

There is limited evidence that patients with PPH may benefit from consuming smaller meals, more frequently, instead of large meals (6, 53, 155, 156). In a

study of patients with autonomic failure and PPH, consumption of 6 smaller meals over a given day (including breakfast, lunch and dinner) was associated with less (11 – 20 mmHg) reduction in postprandial BP than consumption of the equivalent energy as 3 larger meals (53). Smaller meals may be associated with relatively less (magnitude and duration) splanchnic diversion of blood, requiring less cardiovascular compensation, but a reduction in meal size is also associated with less gastric distension which, as discussed, is protective (139). Further information is required before clinical recommendations can be made in relation to meal size in PPH. Lubart et al. reported that there was no difference in the prevalence of PPH (~40%) in inmates of residential care receiving nutrition by the oral, nasogastric or percutaneous gastrostomy routes when the same meal (600 kcal over 30 min) was given (40).

The effect of the addition of guar gum, a natural polysaccharide, to a meal has been assessed in 3 studies (148, 150, 152), with evidence that it is beneficial in healthy older subjects (148, 150) and type 2 patients (152) with an improvement, but not abolition of the postprandial BP reduction (148, 152) **(Figure 2.4)**. The mechanisms that mediate the effect of guar gum are likely to be both 'gastric' and 'small intestinal', by slowing both gastric emptying (148, 152) and small intestinal nutrient absorption (150). No studies have evaluated the effects of guar gum in PPH; moreover, its use is limited by unfavourable palatability and adverse gastrointestinal effects. The effects of other 'fibres' in PPH warrant evaluation.

Two studies have evaluated the effects of water drinking in PPH (137, 147). The onset of the pressor response to water is immediate, so water drinking should be performed immediately prior to a meal (157). In patients with autonomic failure, volumes of 480 mL (158) **(Figure 2.5)** and 350 mL (147) of water consumed prior to a meal reduced the magnitude of PPH by ~21 mmHg and ~13 mmHg, respectively, effects that were sustained for ≥ 60 min (147) and maintained for at least 7 days (147). The pressor effect of water drinking appears to be greater in this group than in 'healthy' older subjects (149). Water drinking does not abolish the hypotensive effects of a meal (147, 158), its effect on symptoms has not been formally evaluated, and further studies are indicated to determine the optimal volume and rate of ingestion of water. However, given its non-existent cost, safety and apparent efficacy, drinking a modest volume of water prior to a meal can be recommended as therapy for PPH.

Postprandial exercise has been evaluated as a treatment for PPH in 2 studies (149, 151); exercise is known to be associated with an increase in cardiac output and splanchnic vascular resistance, which could potentially increase postprandial BP. While 10 min of exercise (walking) performed 20 min following a meal in 14 frail elderly subjects with PPH (149) increased MAP by ~18 mmHg, these effects persisted only for the duration of the exercise (149). In contrast, in 12 patients with primary autonomic failure, peripheral vascular resistance and BP were less during light aerobic exercise (151). While PPH does not appear to reduce exercise capability in the frail elderly (159), it accordingly appears unlikely that exercise will be beneficial.

A single study examined the effects of meals served at different temperatures on the postprandial BP response (115). In healthy elderly subjects, a warm (50ºC) glucose drink (75 g) decreased MAP by 8.0 ± 1.1 mmHg, whereas when the drink was cold (5ºC) there was opposite effect, increasing MAP by 3.9 ± 1.3 mmHg (115). Colder meals are known to be associated with modest slowing of gastric emptying (160). Further studies are required.

Pharmacological

Studies which relate to the pharmacological management of PPH are summarised in **Table 2.3 (Appendix 1)**. The 26 studies describe a variety of approaches (86, 97, 103, 105, 107, 116, 125, 153, 157, 161-177), of which the most frequent have been to delay disaccharide absorption, using α-glucosidase inhibition (105, 107, 163, 172, 174, 177), blocking the release of/antagonizing the action of peptides potentially responsible for splanchnic vasodilation, using somatostatin analogues (97, 103, 125, 168-170) and vasopressin (86), lowering preprandial BP with antihypertensive agents (153, 171), and direct stimulation of the sympathetic nervous system (166) such as by caffeine (116, 161, 164, 165, 167, 173, 175).

Six studies relate to the use of α-glucosidase inhibitors (105, 107, 163, 172, 174, 177) (in 4, the cohorts comprised patients with PPH (105, 172, 174, 177), acarbose in 5 (in doses of 50 mg three times a day (172) or 100 mg once a day (105, 107, 163, 177)) and voligbose in 1 (174). No study was controlled, and the effect on symptoms potentially related to PPH was not formally evaluated (105, 172, 174, 177), however, the observations suggest that α-glucosidase

inhibition is effective in attenuating, but not eliminating, PPH (105, 172, 174, 177). Potential mechanisms to account for this effect include a reduction in splanchnic blood flow, consequent to slowing of gastric emptying and a delay in intestinal disaccharide absorption (107). As discussed, increases in GLP-1, and GLP-2, secretion secondary to the presence of carbohydrate in the more distal small intestine, may be relevant (107, 178). Acarbose does not delay the absorption of simple carbohydrates, such as glucose, and α-glucosidase inhibition is predictably associated with a high prevalence of gastrointestinal adverse effects (107, 163, 172), although these drugs are well tolerated in many patients with type 2 diabetes. While acarbose appears to be effective in a number of disorders associated with PPH; the elderly (172), PD (174), pure autonomic failure (177) and MSA (105, 174); hitherto, only acute studies have been performed and larger, controlled studies to determine the long-term efficacy and impact on symptoms are required before clear recommendations can be made. Despite this, acarbose may be currently the best pharmacological treatment for PPH.

The effects of the somatostatin analogue, octreotide, in PPH have been reported in 7 studies (97, 103, 125, 168-170, 179) (2 studies from the same cohort (97, 179)), in patients with PPH associated with autonomic failure (103, 125, 168, 169), hypertension (97, 170) and in the 'healthy' elderly (97) in doses ranging from ~0.4 µg/kg (103), to ~0.8 µg/kg (168, 169). Octreotide appears to be unique in that it is the only pharmacological treatment which may completely prevent PPH (97, 103, 125, 168-170), an effect that may relate to inhibition of the release of vasoactive gut peptides, with a consequent

reduction in splanchnic volume (97), and/or a direct effect on the splanchnic vasculature (125). Octreotide is expensive, requires daily injections and is associated with a high prevalence of adverse effects (103, 169). Longer-acting somatostatin analogues, such as lanreotide, are often tolerated better than octreotide (180), but there is hitherto no information about their use in PPH.

Eight studies have evaluated caffeine as a treatment for PPH (116, 157, 161, 164, 165, 167, 173, 175), and the evidence to support its efficacy is limited (161, 173). The doses evaluated have ranged from 50 mg (157) to 250 mg (116, 167, 173), as either a tablet (116, 161, 167, 173), coffee (164, 165, 175), or tea (157). Studies in older patients with PPH (157, 165), healthy older subjects (164, 165, 175) and in patients with autonomic failure (in whom PPH was not explicitly defined) (116, 167) suggest that caffeine has a modest effect to reduce the magnitude of PPH, presumably by direct stimulation of the sympathetic nervous system (176). While caffeine has been reported to be well tolerated, only 3 studies enrolled > 10 subjects, so this conclusion should be viewed circumspectly (157, 165, 175).

The effects of anti-hypertensive therapy on postprandial BP have been reported twice (153, 171), both studies evaluated elderly hypertensive populations without known PPH (153, 171). The drugs used were isosorbide dinitrate (20 mg) or nicardipine hydrochloride (20 mg) in a 3 week cross over study (153), and nitrendipine (20 mg) or hydrochlorothiazide (50 mg) in a 12 week parallel group study (171). Only 1 study was placebo controlled (153). Both studies indicated improvement, but not abolishment, of the modest

postprandial falls in BP that were evident in both populations (153, 171), which probably reflects lowering of pre-meal BP, as opposed to a direct effect of these anti-hypertensive medications on postprandial BP (153). The effects of withdrawal of anti-hypertensive therapy in symptomatic PPH have not been formally evaluated.

Four small studies have evaluated other potential therapies for PPH (86, 162, 166, 176). In patients with PPH, a combination of denopamine (10 mg) (a β_1-adrenergic agonist) and midodrine (4 mg) (α_1-adrenergic agonist) was reported to attenuate the postprandial fall in BP, while monotherapy with each agent was unsuccessful (166). It is likely that midodrine increased vascular resistance and denopamine increased cardiac output, targeting two pathways of haemodynamic dysregulation implicated in PPH (166). Intravenous vasopressin (0.3 U/min), a potent vasoconstrictor, administered continuously following oral glucose attenuated PPH in 5 patients with MSA (86). Neither study was placebo controlled, nor randomised, so the outcomes should at least be viewed as potential 'proof-of-concept' (86, 166). Oral vasopressin agents are available and may warrant evaluation in the treatment of PPH. The effects on the postprandial fall in BP of 4 doses of different drugs has been assessed in 6 subjects with autonomic failure, reporting that propranolol (40 mg) exacerbated, indomethacin (50 mg) attenuated and cimetidine (300 mg) and diphenhydramine (50 mg) had no effect on, the fall in BP following a mixed meal (181). There are 2 other reports with dihydroergotamine (50 mg), a vasoconstrictor without affect on the splanchnic vasculature (154), failing to prevent PPH in other patient groups (167, 168). 3,4-DL-threo-

dihydroxyphenylserine (DL-DOPS), a precursor of norepinephrine, apparently alleviated PPH in autonomic failure (162). Withdrawal of furosemide therapy may benefit patients with PPH (36). In 24 older subjects with heart failure, a single dose (40 mg) increased the hypotensive response to a mixed meal from 19.6 ± 2.1 mmHg to 28.5 ± 6.2 mmHg (182) and in 20 patients with heart failure (mean age 75 years), 55% of whom had PPH, withdrawal of furosemide therapy reduced the magnitude of PPH at a 3 month follow-up (183).

In summary, while a number of non-pharmacological and pharmacological treatments show promise, drinking water immediately prior to a meal is the only non-pharmacological, and acarbose (and possibly, octreotide) the only pharmacological therapies which can be recommended for the management of PPH. There is a rationale to manage PPH with a combination of therapies, e.g. water drinking and acarbose, however, this approach has not been assessed. Future studies should prioritise the inclusion of symptomatic patients with PPH, and include the effect on symptoms as an endpoint.

Conclusion

Postprandial hypotension is an important clinical problem, causing syncope, dizziness and falls, and is associated with an overall increased risk of mortality. Postprandial hypotension occurs frequently in several different disorders, including type 2 diabetes, PD and autonomic failure; but is also frequent in healthy older subjects. The pathophysiology of PPH is multifactorial; small intestinal nutrient delivery, splanchnic blood flow,

gastric distension and neural and hormonal mechanisms are all implicated. Current management strategies are both pharmacological, such as α-glucosidase inhibitors, and non-pharmacological, such as water drinking. Treatment is targeted at ameliorating the postprandial decline in BP, with the rationale that this will provide symptomatic relief. Studies focusing on the epidemiology and pathophysiology of this condition represent a priority.

Figures and Figure Legends
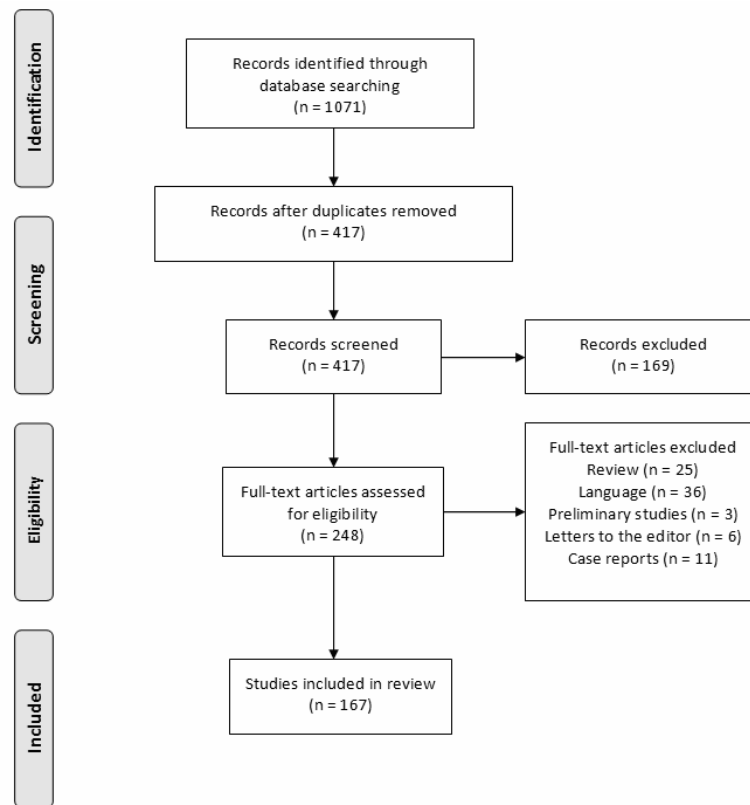
Figure 2.1: Flow diagram for the selection of studies for review based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2009 statement (11).
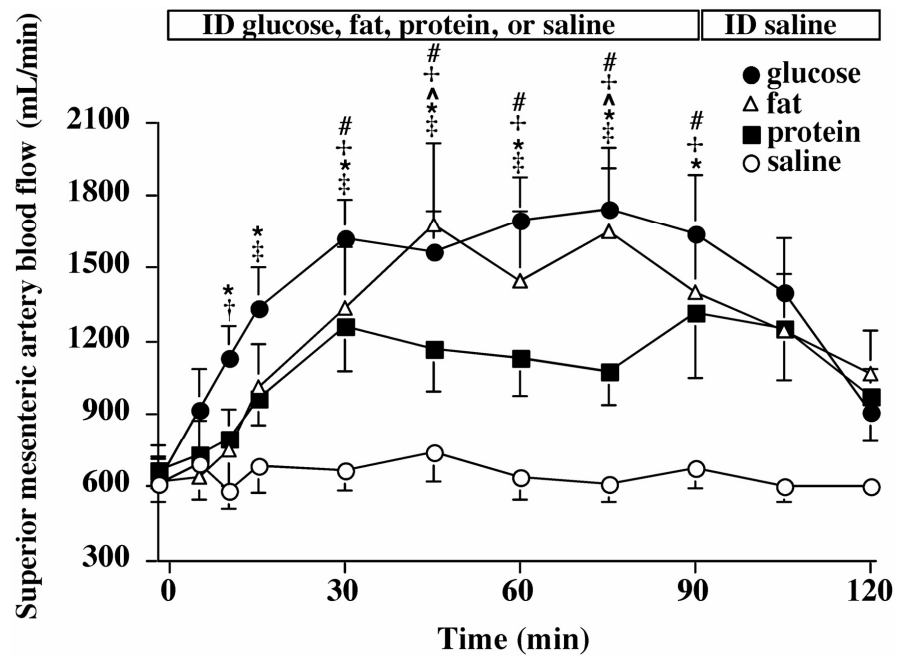
Figure 2.2: Effects of intraduodenal (ID) infusion of glucose, fat, protein and saline on superior mesenteric artery flow in 8 healthy older subjects. *P< 0.01 for glucose compared with saline; [†]P= 0.04 for glucose compared with fat; [‡]P< 0.05 for glucose compared with protein; P< 0.01 for fat compared with saline; [#]P< 0.05 for protein compared with saline; P< 0.01 for fat compared with protein (121).
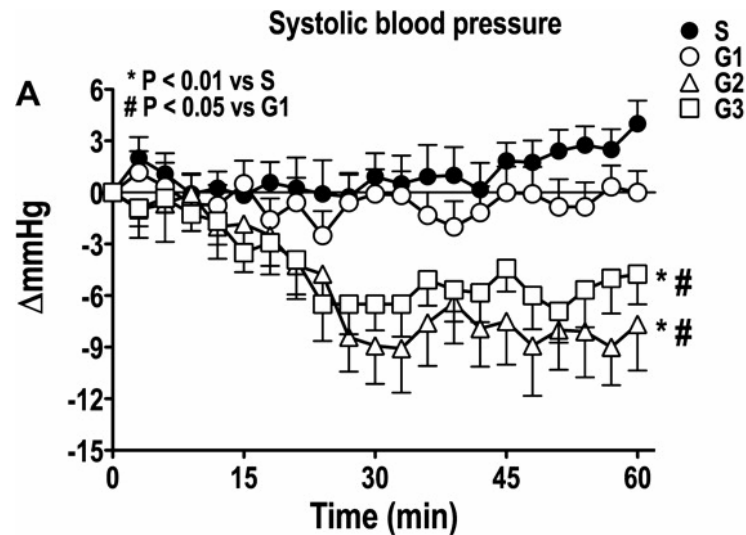
Figure 2.3: Effects of intraduodenal glucose at loads of 1 kcal/min (G1; ○), 2 kcal/min (G2; △) 3 kcal/min (G3; □), or saline (S; ●) on change in systolic blood pressure in 12 healthy older subjects. *P< 0.01 saline compared with G2 and saline compared with G3, #P< 0.05 G1 compared with G2 and G1 compared with G3 (120).
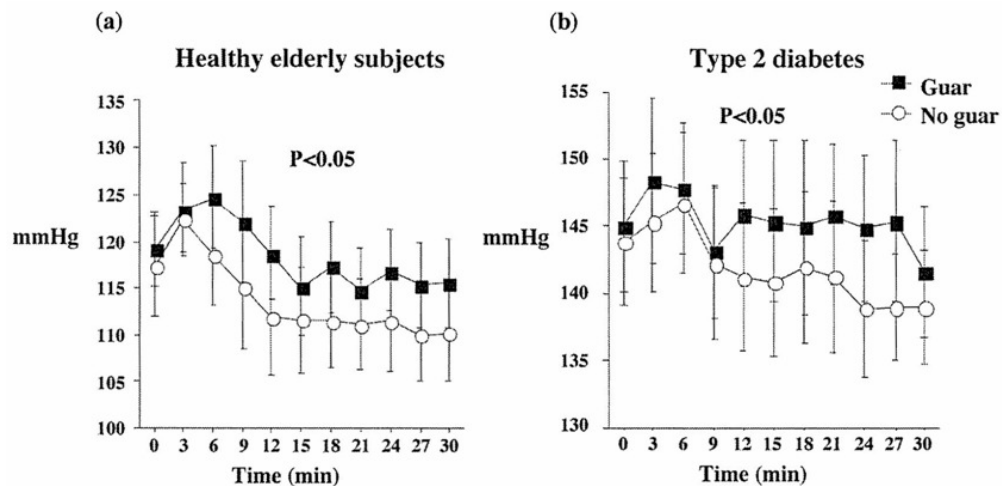
Figure 2.4: Change in systolic blood pressure after ingestion of 50 g glucose in 300 mL water with and without 9g guar gum in a) healthy older subjects (148) and b) type 2 diabetic patients. Differences between control and guar studies between t= 0 – 30 min by ANOVA are shown (152).
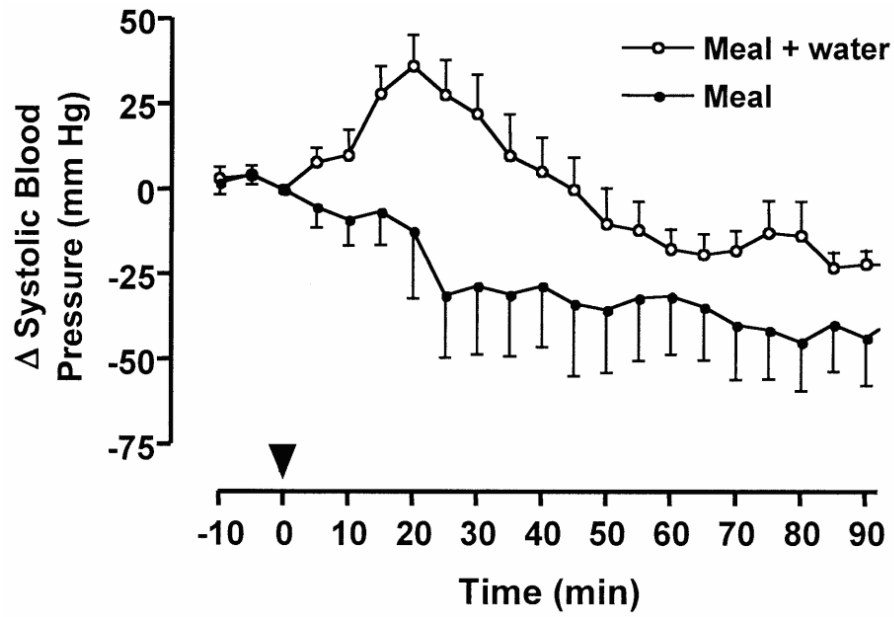
Figure 2.5: Effect of ingesting 480 mL water prior to a meal on systolic blood

pressure in 7 patients with primary autonomic failure. Treatment effect for

water drinking: $P < 0.001$ by ANOVA, compared with meal alone (158).

CHAPTER 3: GASTRIC EMPTYING AND GLYCAEMIA

Introduction

The stomach and small intestine play a pivotal role in blood glucose homeostasis. The rate at which the stomach delivers nutrients into the small intestine, known as gastric emptying (GE), is now recognised to be a major determinant of postprandial blood glucose concentrations. The relationships between GE and blood glucose are complicated by their interdependency; postprandial blood glucose concentrations are also a determinant of the rate of GE. This chapter provides an overview of the physiology of GE and the role of the gastrointestinal tract in glucose homeostasis.

Gastric Emptying

Physiology of gastric emptying

The primary function of the stomach is to receive and store ingested food, mix and grind digestible solid food with gastric secretions to small particles less than ~1 mm in size, and deliver this chyme into the small intestine at a tightly regulated rate that optimises absorption. Gastric emptying is predominantly a pulsatile process; most liquefied chyme enters the small intestine as a series of small pulses, with both antegrade and retrograde flow occurring (184, 185). The characteristics of these pulses, including their magnitude and duration, vary substantially and result from the integration of contractile activity of the proximal and distal stomach, pylorus and the upper small intestine, regulated

through extrinsic and intrinsic nervous, as well as neurohormonal, pathways (186). Current understanding of the relevant regulatory mechanisms is far from complete (187).

The migrating motor complex (MMC) is a cyclical pattern of electrical activity in the fasted state that generates gastric peristaltic contractions which remove residual food, secretions and cellular debris from the stomach and small intestine (188, 189). It is generally regarded as the 'housekeeper' of the gastrointestinal tract. There are 4 phases of the MMC with a total cycle time of between 80 to 120 min (190). Phase 1 is motor quiescence, lasting ~40 min, phase 2 is characterised by irregular contractions lasting ~50 min, phase 3 is regular, high amplitude contractions (lasting ~5 – 10 min) during which time indigestible solids are emptied and phase 4 is a period of reduction in motor activity, which may be very short or absent, before the resumption of phase 1 (186, 188, 191). The MMC is interrupted by food ingestion, which is associated with increases in the tonic activity of the proximal stomach and irregular contractile activity in the antrum (186).

Following food intake, the proximal stomach undergoes an initial state of 'receptive' relaxation, followed by a prolonged state of relaxation known as 'accommodation,' (186) mediated, at least in part, by the release of vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) from the nerves in the fundus (192). Gastric accommodation is triggered by gastric distension and allows intragastric pressure to remain relatively constant, irrespective of increasing volumes (193). The distal stomach receives solids from the

proximal stomach and grinds them into particles with a diameter of 1 – 2 mm by irregular antral, tonic and phasic pyloric contractions (186). 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 ~3 per min, although not every discharge results in a muscular contraction (186). The resultant particles are mixed with gastric juice and the chyme enters the duodenum predominantly in a pulsatile fashion against pyloric resistance (186).

Regulation of gastric emptying

Gastric emptying is regulated, in most cases, by inhibitory feedback from the small intestine, rather than 'intragastric' factors. The presence of nutrients in the small intestine generates neurohormonal feedback triggered by the interaction with receptors distributed throughout the small intestine (142). There are receptors for various nutrients (e.g. glucose, fatty acids and amino acids), with regional variations in receptor number and type (191). The magnitude of small intestinal feedback is dependent on both the length and region of small intestine exposed to nutrient (194-196) and is influenced by patterns of prior nutrient exposure (197). Hormones which slow GE include glucagon-like peptide-1 (GLP-1), cholecystokinin and peptide YY (186). Based on this feedback, the stomach empties nutrients at a rate of between 1 – 4 kcal/min (142, 194). This substantial inter-individual variation in small intestinal nutrient delivery is increased farther in type 2 diabetes due to the high prevalence of delayed (198), and occasionally rapid, GE (199). In

contrast, there is a relatively low intra-individual variation in the rate of GE (200).

Patterns of gastric emptying

Patterns of GE are dependent on the composition (solid and/or liquids) and macronutrient content (fat, protein, carbohydrate) of the meal ingested, and is largely unaffected by the volume of the meal (186). Solids empty in an overall linear fashion, following an initial lag phase, usually of 20 – 40 min in duration, during which solids move from the proximal to the distal stomach and are ground into small particles (201). Empting of liquids commences immediately after, and sometimes during their ingestion, with minimal, if any, lag phase. Emptying of high-nutrient liquids follows a linear pattern, similar to solids, whereas low-nutrient liquids follows a monoexponential pattern (142), i.e. as nutrient density increases, the rate of liquid emptying slows (186). If liquids and solids are ingested together, the liquids are preferentially emptied (~80% of the liquid component is emptied before the solid) and the presence of solids slows emptying of a simultaneously ingested liquid (202).

Gastrointestinal Determinants of Glycaemia

The gastrointestinal tract plays a pivotal role in blood glucose homeostasis. In the fasted state, blood glucose is determined by insulin and glucagon secretion and hepatic and peripheral glucose update, however, these factors only account for ~50% of the variance in blood glucose concentrations (203). Postprandial changes in blood glucose are determined by the rate of glucose

delivery and absorption from the stomach and small intestine, glucose disposal and endogenous glucose production (204). Theoretically, blood glucose will increase until the rate of glucose absorption matches that of glucose removal (204). Blood glucose concentrations increase from approximately 10 min after the start of a meal and, in health, peak concentrations are usually achieved within the first hour (205). In type 2 diabetes, peak blood glucose may occur up to 2 hours after a meal, reflecting the impairment in glucose disposal (205). The profile of the postprandial excursion in blood glucose is influenced, in varying degrees, by several factors including the preprandial glucose concentration, meal composition, the rate of GE, small intestinal glucose absorption and hormone secretion, insulin secretion and hepatic and peripheral glucose disposal (187, 189).

The incretin effect

The incretin effect refers to the increased insulin secretory response to oral/enteral glucose administration, when compared with an isoglycaemic intravenous infusion (206, 207), and accounts for 50 – 70% of the total insulin response to oral glucose in health (208). Glucagon-like peptide-1 (GLP-1) and glucose-dependant insulinotropic peptide (GIP) are the two known 'incretin hormones.' GLP-1 is secreted from the intestinal L-cells located predominantly in the distal ileum and colon, in response to fat, carbohydrate, protein and bile acids (209, 210) while GIP is secreted from the K-cells located predominantly in the proximal small intestine, primarily in response to glucose or fat ingestion (208). Both GLP-1 and GIP stimulate insulin secretion and suppress glucagon in a glucose-dependent manner; the blood

glucose needs to be > 8 mmol/L for these effects (208). GLP-1 also slows GE and increases satiation (208). The effects of exogenous GLP-1 on GE are substantial, so that the consequent reduction in postprandial glucose is associated with a reduction, rather than an increase, in plasma insulin (211). More recently, it has also been demonstrated that the slowing of GE by exogenous GLP-1 is subject to tachyphylaxis with sustained exposure (212).

The magnitude of the incretin effect is reduced in longstanding type 2 diabetes (213); in part this reflects a markedly reduced insulinotropic property of GIP- while the insulinotropic action of GLP-1 is relatively preserved (214). These observations have stimulated the development of GLP-1 based pharmacological therapies for the management of type 2 diabetes. Biologically secreted GLP-1 and GIP have a circulating half-life of 1 – 2 min and are rapidly inactivated by the ubiquitous enzyme dipeptidyl peptidase 4 (DPP-4). The two therapeutic classes currently available are DPP-4-resistant GLP-1 receptor agonists (such as exenatide, liraglutide and lixisenatide) (215) and DPP-4 inhibitors that inhibit the degradation of endogenously secreted GLP-1 and GIP (such as sitagliptin, vildagliptin and alogliptin) (216).

Effects of gastric emptying on glycaemia

The rate of GE has been shown to be a significant determinant of the initial (~15 – 60 min after a meal) glycaemic response to carbohydrate ingestion, accounting for up to ~35% of variance in the blood glucose response to oral glucose in health (217, 218) and type 2 diabetes (219, 220), so that when GE is faster, the rise in blood glucose is relatively greater. In contrast, the 'late'

blood glucose response (~120 – 180 min after a meal) is inversely related to the rate of GE (217, 218, 220), probably reflecting the latency in the insulinaemic response to the initial rise in blood glucose.

The relationship between glycaemia and small intestinal carbohydrate delivery appears to be non-linear (**Figure 3.1**). In health (221, 222), and type 2 diabetes (223), studies employing intraduodenal infusion of glucose spanning the normal range of GE demonstrate only a modest rise in blood glucose when the infusion rate is at the low end of the range (1 kcal/min), a substantially greater blood glucose response to 2 kcal/min, but only an incremental increase when the infusion rate is 4 kcal/min (**Figure 3.1**). These responses can be accounted for by the substantially greater serum insulin response following the 4 kcal/min, compared with the 2 kcal/min glucose infusion, probably mediated, in part, by the secretion of GLP-1 and GIP. As the rate of duodenal glucose infusion is increased from 1 – 4 kcal/min, there is a dose-dependent linear increase in the GIP response, whereas the GLP-1 response is non-linear. These observations, and others (210), suggest the release of GLP-1, unlike GIP, requires a carbohydrate load which exceeds the absorptive capacity of the proximal small intestine (224), in turn increasing the carbohydrate exposure to the more distal L-cells. More recent studies indicate that the magnitude of the incretin effect is greater when the rate of entry of glucose into the small intestine is greater in both health and type 2 diabetes (225). In health, it is likely that when the rate of GE is $\leq$ 2 kcal/min, the contribution of GIP to the incretin effect is greater than that of GLP-1, while GLP-1 is dominant at rates $\geq$ 3 kcal/min (205, 222).

In healthy subjects (226) and type 2 diabetic patients (227), when glucose is infused intraduodenally more rapidly initially, the resultant insulin and incretin responses are greater when compared to a constant infusion of glucose at an identical load; however, the overall glycaemic profile of both infusions remains unchanged (226, 227).

Effects of glycaemia on gastric emptying

Gastric emptying is a determinant of glycaemia; however, changes in glycaemia also influence the rate of GE. Marked acute hyperglycaemia (blood glucose ~15 mmol/L) delays GE of both solids and liquids (228, 229). Moreover, in both health and type 1 diabetes, changes in blood glucose within the physiological postprandial range, e.g. 8 mmol/L, compared with 4 mmol/L, slow GE (230). This slowing of GE is associated with suppression of antral pressure waves, a reduction in fundic tone and an increase in localised pyloric pressure (229), as well as an induction of gastric electrical dysrhythmias (231).

In contrast to the effect of hyperglycaemia, a substantial acceleration of GE occurs during insulin-induced hypoglycaemia (blood glucose level ~2.6 mmol/L) in both health and type 1 diabetes (232). These combined effects contribute towards blood glucose regulation; i.e. small intestinal nutrient delivery is slowed, or accelerated to mitigate hyperglycaemia or hypoglycaemia.

Conclusion

This chapter has provided insights into the physiology of GE and the role of the stomach in the regulation of blood glucose. Gastric emptying is a major determinant of, and is also determined by, the blood glucose concentration. Studies employing direct intraduodenal infusion of carbohydrates, to control the rate of GE, have provided valuable insights into the complex relationships between the rate of GE, glycaemia and the secretion of hormones pivotal to the regulation of blood glucose.

Figures and Figure Legends
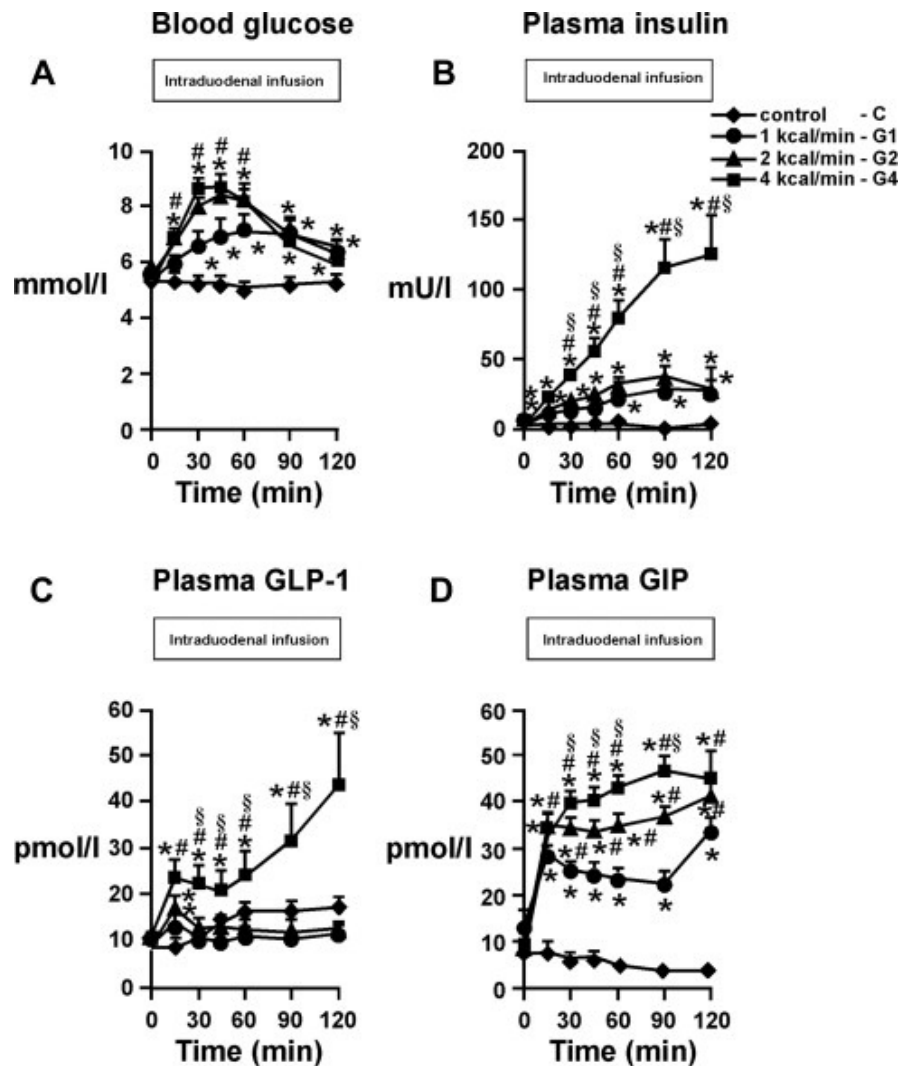
Figure 3.1: Blood glucose (A), plasma insulin (B), glucagon-like peptide-1 (GLP-1) (C), and glucose-dependant insulinotropic peptide (GIP) (D) in response to intraduodenal glucose (25%) infused over 120 min at rates of 1 (G1), 2 (G2), or 4 (G4) kcal/min, or saline (4.2%) control (C) in 10 healthy men. * Versus control: $P < 0.05$; #, versus G1: $P < 0.05$; §, versus G2: $P < 0.05$. Data are means ± SEM (221).

CHAPTER 4: METHODOLOGIES

Introduction

This chapter provides an overview and description of the common methodologies and techniques for the studies presented in this thesis. All of these techniques are well established, have been utilised extensively by our research group and are considered ethically acceptable.

Ethical Approval

All protocols were reviewed by either the Human Research Ethics Committee at the Royal Adelaide Hospital **(Chapters 5 – 7, 9 – 12)** or Central Adelaide Local Health Network (The Queen Elizabeth Hospital) **(Chapter 8)**. For studies involving pharmaceutical agents **(Chapters 10 – 12)**, the Investigational Drug Sub-Committee also reviewed the protocols. For studies involving the administration of unapproved therapeutic goods **(Chapters 10, 11)**, an appropriate notification was lodged with the Therapeutic Goods Administration, Department of Health, Australian Government, under the 'Clinical Trial Notification (CTN) Scheme'. Each subject provided written, informed consent prior to their inclusion of the study. All studies were carried out in accordance with the Declaration of Helsinki.

Subjects

Information relating to the subject groups included in the studies will be provided in each chapter, with specific reference to recruitment, inclusion and exclusion criteria and screening assessment.

Assessment and Definition of PPH

Subjects with PPH were identified and enrolled in the relevant studies. Healthy older subjects were screened with a 75 g oral glucose load **(Chapter 5)** and PPH was defined as a fall in blood pressure (BP) > 20 mmHg within 2 hours following the drink. Healthy older subjects enrolled in other studies who had PPH were included in this group, in subsequent studies.

Cardiovascular Parameters

Blood pressure and heart rate

Blood pressure (systolic and diastolic) and heart rate were measured in all studies using an automated, oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA). In all studies, there was a 'rest period' of no less than 15 min, during which BP was assessed, immediately prior to the commencement of the study. 'Baseline' BP and heart rate (t= 0 min) were defined as the mean of the measurements taken at t= -9, -6 and -3 min during this rest period.

Gastric Emptying

Several techniques can be used to assess gastric emptying (GE), each with their respective strengths and limitations. Both the study design and patient group were considered when selecting an appropriate technique. Scintigraphy is considered the 'gold standard,' but it is associated with a small radiation burden to the patient and requires access to specialised facilities. Stable isotope breath testing is inexpensive, safe and easily adapted to a laboratory setting, however, it only provides an indirect assessment of GE.

Scintigraphy

Studies employing scintigraphy **(Chapters 9, 11)** were performed in the Department of Nuclear Medicine, PET and Bone Densitometry, at the Royal Adelaide Hospital. Data were acquired with the subject seated with their back against a gamma camera (Genie, GE Healthcare Technologies, Milwaukee, WI, USA). Data were acquired immediately following the consumption of a glucose drink (75 g glucose in 300 mL water) labelled with 20 MBq of either ^{99m}Technetium (Tc) sulphur colloid **(Chapter 11)**, or ^{99m}Tc calcium phytate **(Chapter 9)**. Time zero (t= 0 min) was defined as the time at the completion of the drink. Radioisotopic data were acquired in 60 sec frames for the first 60 min, followed by 180 sec frames for the remainder of the study, i.e. until t= 120 min **(Chapter 11)**, or 180 min **(Chapter 9)**. Upon completion of the data acquisition, a 30 sec lateral image was taken with the subject seated with their left side against the gamma camera; based on the lateral images, correction factors for γ-ray tissue attenuation were derived (200). Data were also corrected for subject movement and radionuclide decay (200). Regions-of-

interest were drawn around the stomach and GE curves (expressed as % retention over time) derived (46). The time taken for 50% of the drink to empty (T50) was also determined where possible (200). The lag phase was defined visually as the time before the radioactivity had entered the proximal small intestine (200).

Stable isotope breath tests

The use of stable isotope breath tests to evaluate GE is well established in the research setting (233-235), and validated (236). Following ingestion, the emptying of the meal from the stomach into the duodenum is the 'rate-limiting' step. The subsequent absorption and metabolism gives rise to $^{13}CO_2$ that can be measured in the exhaled breath.

Subjects consumed a drink containing 75 g glucose and 150 mg ^{13}C-acetate **(Chapters 5, 6, 8)**, made up to 300 mL with water, within 3 min; t= 0 min was defined as the time of completion of the drink. Exhaled breath samples were collected in hermetically sealed 10 mL tubes (Exetainer, Buckinghamshire, England) immediately prior to the ingestion of the glucose drink (t= -3 min), every 5 min for the first hour, and then every 15 min for the subsequent 3 hours. The $^{13}CO_2$ concentration in breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK) with an online gas chromatographic purification system. The gastric 50% emptying time (T50) was calculated using the formula described by Ghoos et al (237), which has been validated against the scintigraphic measurement of GE (236).

Splanchnic Blood Flow

Ultrasound has been used widely for the assessment of superior mesenteric artery (SMA) blood flow (5, 6, 119, 122). SMA blood flow was measured by duplex ultrasonography using a Logiq^{TM} 9 (**Chapter 7**) or Logiq E (**Chapter 9 – 11**) ultrasound machine (GE Healthcare Technologies, Sydney, NSW, Australia). Scanning was conducted with a 3.5C broad spectrum 2.5 – 4 MHz convex transducer positioned immediately inferior to the xiphoid process, manoeuvred slightly to the left to visualise the abdominal aorta and then moved inferiorly so that the coeliac trunk and SMA were visualised (119). Peak systolic velocity, end-diastolic velocity and time-averaged mean velocity of the SMA were acquired from pulsed Doppler waveform complexes acquired 2 – 3 cm distal to its aortic origin. The cross-sectional diameter of the SMA was measured at this point, in the longitudinal plane, using manually operated on-screen callipers, and this value was utilised in subsequent calculations of blood flow. Blood flow (mL/min) was calculated automatically using the formula: $\pi \times r^2 \times TAMV \times 60$, where r= the radius of the SMA and TAMV is the time-averaged mean velocity (119).

Intraduodenal Infusion

Intraduodenal infusion of glucose was performed (**Chapters 11, 12**) using a modified 17-channel manometric silicone-rubber catheter (~4 mm diameter) (Dentsleeve International Ltd, Mui Scientific, Ontario, Canada), introduced into the stomach via an anaesthetised nostril. The catheter was allowed to pass through the stomach and into the duodenum by peristalsis. As depicted in **Figure 4.1**, the catheter consisted of 6 side holes, the first 3 of which were

positioned in the antrum, ~2.5 cm distal to the pylorus, one positioned across the pylorus (channel) and two in the duodenum (channel). The catheter incorporated a separate luminal channel for intraduodenal infusion ~10 cm distal to the pylorus. The correct positioning of the catheter was maintained by measurement of the antroduodenal transmucosal potential difference (TMPD) across the antrum (-40 mV) and duodenum (0 mV) (238, 239), using a saline-filled 20 gauge intravenous cannula inserted subcutaneously into the subject's forearm as a reference electrode.

Biochemical Measurements

Venous blood samples were obtained in all studies. Blood glucose, serum insulin, plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were analysed using appropriate techniques described in the relevant chapters. Serum and plasma were separated by centrifugation (3200 rpm, 15 min, 4°C) and stored at -70°C for subsequent analysis.

Blood glucose

Blood glucose (mmol/L) was determined immediately using a portable glucometer (Medisense Companion 2 meter, Medisense Inc. Waltham, USA) **(Chapters 6, 8 – 11)**.

Serum insulin

Serum insulin was measured by ELISA immunoassay (10-1113, Mercodia, Uppsala, Sweden) **(Chapters 6, 7, 10 – 12)**. The sensitivity of the assay was 1.0 mU/L and the coefficient of variation (CV) was 2.6% within, and 7.6% between, assays (120).

Plasma GLP-1

Total GLP-1 was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA) **(Chapters 6, 7)**. Minimum detectable limit was 3 pmol/L, intra- and inter-assay CVs were 7.7% and 9.4% respectively (120).

Plasma GIP

Plasma GIP was measured by radioimmunoassay **(Chapters 6, 7)**, with modifications to a published method (240) so that the standard curve was prepared in buffer, rather than extracted charcoal stripped serum and the radioiodinated label was supplied by Perkin Elmer (Boston, MA, USA). Minimum detectable limit was 2 pmol/L, inter-assay CV was 8.7% and intra-assay CV was 5.0% (240).

Cardiovascular Autonomic Nerve Function

Autonomic nerve function **(Chapters 7 – 11)** was evaluated using standardised cardiovascular reflex tests (241, 242). This involved the assessment of both parasympathetic function (the variation in R – R interval of the heart rate in response to deep breathing and the change in position from

lying to standing ("30:15" ratio) and sympathetic function (systolic BP fall in response to standing). Each of the test results was scored according to age-adjusted predefined criteria: 0= normal, 1= borderline and 2= abnormal for a total maximum score of 6 (242). A score of ≥ 3 was considered to indicate autonomic dysfunction (241).

Statistical Analysis

All analyses were performed using commercially available software (SPSS version 17.0, IBM software, New York, USA). In all studies, data are presented as mean values ± standard error of the mean (SEM). A P value < 0.05 was considered significant in all analyses.

Conclusion

This chapter has presented a brief overview of the techniques used in the studies included in this thesis. Additional detail is provided in the methodology section of individual chapters.

Figures and Figure Legends
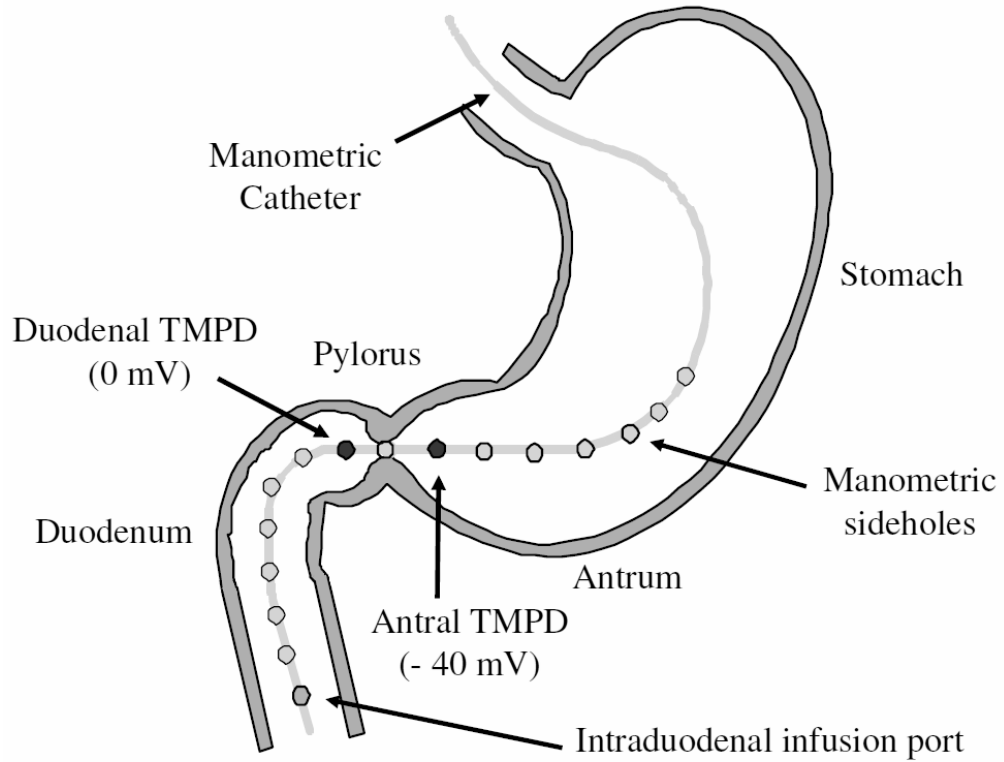
Figure 4.1: Diagram of the multi-lumen catheter used in intraduodenal

infusion (**Chapters 11, 12**). TMPD: transmucosal potential difference.

CHAPTER 5: POSTPRANDIAL HYPOTENSION IS ASSOCIATED WITH MORE RAPID GASTRIC EMPTYING IN HEALTHY OLDER SUBJECTS.

Statement of Authorship

Title of paper	Postprandial hypotension is associated with more rapid gastric emptying in healthy older subjects.
Publication Status	Published
Publication Details	Trahair LG, Horowitz M, Jones KL. Postprandial hypotension is associated with more rapid gastric emptying in healthy older subjects. *J Am Med Dir Assoc.* Jun 2015; **16**(6): 521-3.

Principal Author

Candidate	Laurence G Trahair
Contribution	Conception of the study, study design and coordination, subject recruitment, data collection and interpretation, statistical analysis and drafting of the manuscript.
Overall percentage	80%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary

| Signature | | Date | Feb 2016 |

author of this paper.

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-author	Michael Horowitz
Contribution	Conception and design of the study, data interpretation, statistical analysis and drafting of the manuscript.
Signature	Date Feb 2016

Name of Co-author	Karen L Jones
Contribution	Conception and design of the study, data interpretation, statistical analysis, drafting of the manuscript and had overall responsibility for the study.
Signature	Date Feb 2016

Introduction

Postprandial hypotension (PPH) is a clinically important disorder, occurring frequently, predisposing to syncope and falls (70), and associated with increased mortality (10). The prevalence of PPH in inmates of residential care is 25 – 40% (39, 70), and 20 – 91% in hospitalised geriatric patients (14, 21). No study has hitherto evaluated the prevalence of PPH in 'healthy' older subjects, with the exception of small cohorts of 'control' subjects (14, 118).

Although the pathophysiology of PPH is poorly understood, our recent studies indicate that the magnitude of the postprandial fall in blood pressure (BP) is dependent on the rate of nutrient entry to the small intestine. For example, in a small cohort of patients with type 2 diabetes, when gastric emptying (GE) of glucose was faster, the magnitude of the hypotensive response was greater (46) and, in healthy older subjects, when glucose was infused intraduodenally at rates within the 'physiological' range of GE of 1, 2 or 3 kcal/min, there was a substantial fall in systolic BP in response to the 2 and 3 kcal/min, but not the 1 kcal/min, load (120). Accordingly, given the wide range of normal gastric emptying (142), which is affected little by age (144) relatively more rapid GE may be a risk factor for PPH. This has hitherto not been evaluated and, if so, would have substantial implications for the management of PPH, which is suboptimal (243).

In this study we have characterised GE of, and the BP responses to, an oral glucose load in healthy older subjects to: (i) determine the prevalence of, and

(ii) evaluate the association of gastric emptying with, postprandial hypotension.

Materials and Methods

Subjects

Eighty eight healthy 'older' subjects (47 female and 41 male, age 71.0 ± 0.5 years (range: $65 - 90$ years), body mass index (BMI) 26.0 ± 0.3 kg/m^2 (range: $20.3 - 30.5$ kg/m^2)), were recruited by advertisements. Demographic information and a medical history were obtained. Subjects with a history of gastrointestinal disease or surgery, known diabetes, significant respiratory or cardiac disease, or alcohol intake (>20 g/day) were excluded. Any medication was withheld for 24 hours prior to the study.

Protocol

In each participant, BP and GE were measured concurrently on a single study day, which commenced at 08:30 after an overnight fast. Upon arrival, an intravenous cannula was inserted into an antecubital vein for blood sampling while the subject was supine. The participant was then seated and allowed to 'rest' for $15 - 30$ min before consuming a drink containing 75 g glucose and 150 mg C^{13}-acetate made up to 300 mL with water within 3 min; t= 0 min was the time of completion of the drink. BP was measured for 120 min and GE for 240 min, following the drink. Venous blood samples were obtained immediately prior to the glucose drink, and at regular intervals, for 240 min. Results of blood glucose and insulin in this cohort have been reported (218).

At t= 240 min, the cannula was removed and the participant given a meal, prior to leaving the laboratory.

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

Blood pressure and heart rate

Blood pressure and heart rate (HR) were measured using an automated oscillometric monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA), every 3 min during the 'rest' period, and every 5 min from t= 3 – 118 min. Baseline BP was calculated as an average of the measurements obtained at t= -9, t= 6 and t= -3 min) (120). PPH was defined as a sustained fall in systolic BP of $\geq$ 20 mmHg, occurring within the first 90 min following the drink (12).

Gastric emptying

Exhaled breath samples were collected in hermetically sealed 10 mL tubes (Exetainer, Buckinghamshire, England) prior to the ingestion of the drink (t= -3 min), every 5 min for the first hour, and then every 15 min for the subsequent 3 hours, for assessment of GE. The $^{13}CO_2$ concentration in breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK) with an online gas chromatographic

purification system. The gastric 50% emptying time (T50) was calculated using the formula described by Ghoos et al (237).

Statistical analysis

Blood pressure and HR were analysed as changes from baseline, and GE as absolute values. Maximum changes in BP and HR were calculated as the greatest change that occurred from baseline. Changes in BP and HR over time were assessed with one-way ANOVA. Differences between groups with and without PPH were assessed with Student's paired T-test. Pearson's correlation was used to evaluate relationships between variables. A P value < 0.05 was considered significant in all analyses. Data are presented as mean values $\pm$ SEM.

Results

The studies were well tolerated and there were no adverse events. Two subjects had diabetes (based on fasting and two hour blood glucose measurements) (244) and were excluded from the analysis. 5 subjects were taking antihypertensive medication. In 7 subjects, GE data were unavailable as an appropriate non-linear regression model fit to the measured $^{13}CO_2$ concentrations was not feasible. Accordingly, complete data were available in 79 subjects.

Blood pressure and heart rate

Baseline systolic BP was 122.3 ± 1.5 mmHg. Following the drink, there was a transient, modest rise, followed by a fall (P< 0.001) in systolic BP. The maximum fall was -14.2 ± 1.0 mmHg, occurring at 80.1 ± 3.5 min. Eleven subjects (12.8%) had PPH.

Baseline diastolic BP was 69.0 ± 0.8 mmHg. Following the drink, there was a transient, modest rise, followed by a fall (P< 0.001) in diastolic BP. The maximum fall was -11.9 ± 0.6 mmHg, occurring at 65.4 ± 3.6 min.

Baseline HR was 64.0 ± 0.9 BPM. Following the drink there was a prompt, and sustained, rise in HR (P< 0.05). The maximum increase in HR was 8.3 ± 0.5 BPM occurring at 43.8 ± 4.0 min.

Gastric emptying

The mean GE T50 was 138.9 ± 4.2 min (range: 55-256 min).

Comparison between non-PPH and PPH subjects

There was no difference in age (P= 0.14) or BMI (P= 0.24) between the groups. Baseline systolic BP was higher in subjects with PPH (non-PPH 121.0 ± 1.6 mmHg vs. PPH 131.6 ± 3.8 mmHg, P< 0.05), while there was no difference in diastolic BP (non-PPH 68.4 ± 0.9 mmHg vs. PPH 73.0 ± 2.7 mmHg, P= 0.14), or HR (non-PPH 63.4 ± 0.9 BPM vs. PPH 68.1 ± 3.5 BPM, P= 0.22) between the groups.

The GE T50 was shorter in subjects with PPH (non-PPH 142.3 ± 4.6 min vs.

PPH 118.0 ± 9.4 min, P< 0.05) **(Figure 5.1)**.

Relationships between variables

In the whole group, there was a trend for an inverse relationship between

maximum fall in systolic BP and maximum rise in HR (R= -0.20, P= 0.09), so

that when the fall in systolic BP was greater, there tended to be a greater rise

in HR. There was no significant relationship between age, BMI, or the

maximum fall in systolic and diastolic BP with the GE T50.

Discussion

We characterised the BP and GE responses to an oral glucose load in an

unselected cohort of 86 healthy older subjects aged > 65 years. 11 subjects

(12.8%) had PPH and GE was faster in this group.

Previous studies have evaluated the prevalence of PPH in cohorts in which

PPH is likely to be more common, including the frail elderly in residential

care facilities (39, 70), patients with dysautonomia (120), those with a history

of syncope or falls (21, 38), and patients with hypertension (17, 22). Given the

reported high prevalence in these groups, it is not surprising that 12.8% of our

healthy older subjects had PPH. We did not include a 'control' study for

logistical reasons, but in healthy young subjects ingestion of glucose is not

associated with a fall in BP (46). Studies to evaluate the association of

postprandial changes in BP with symptoms and mortality in healthy older subjects are indicated.

We hypothesized that PPH would be associated with relatively more rapid GE based on the outcome of our previous studies (46, 120) and this proved to be the case. GE in health exhibits a substantial inter-, but lower intra-individual variation (200), more rapid GE has been reported to predispose to postprandial hyperglycaemia and, possibly, the risk of type 2 diabetes (218). Hypertension has also been associated with more rapid GE (245) and is a risk factor for PPH (33), as confirmed in the current study. Although the breath test used to quantify GE has been validated against the 'gold standard' of scintigraphy (237), the resultant T50 should be regarded as notional. Accordingly, we cannot calculate a GE rate as kcal/min, although our previous studies suggest that the relationship of the postprandial fall in BP with the rate of small intestinal nutrient delivery is non-linear (120). Our novel observation of an association of PPH with more rapid GE has implications for the management of PPH, diseases which are frequently associated with PPH, such as diabetes (46) and Parkinson's disease (47) are also associated with disordered, albeit more frequently delayed, rather than more rapid, GE, while drugs which slow GE, such as the α-glucosidase inhibitor acarbose, may have efficacy in the management of PPH (107).

Figures and Figure Legends
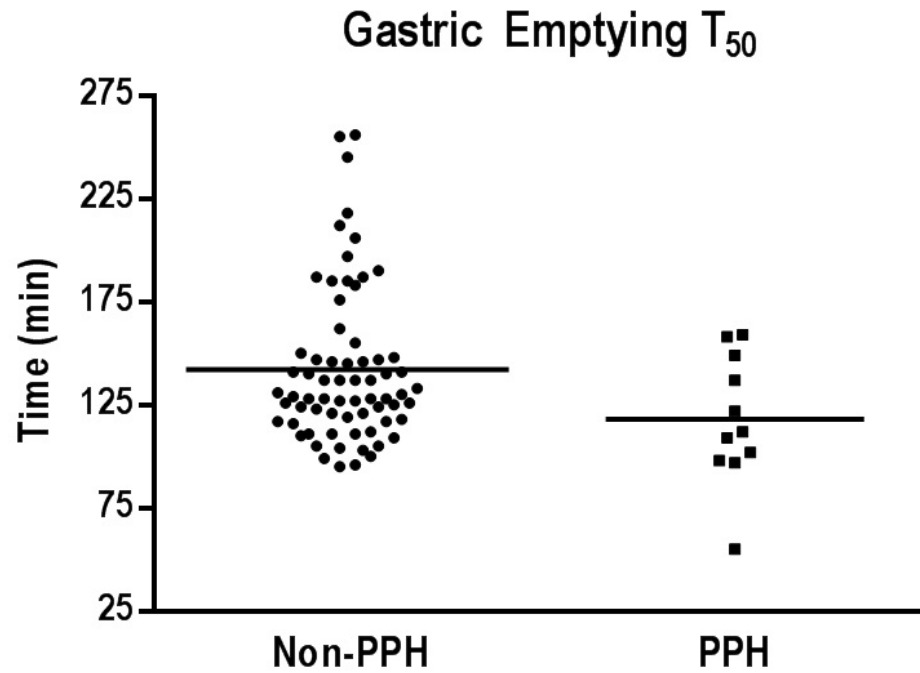
Figure 5.1: Gastric emptying half times (T50) in subjects without (n= 68, ●)

and with (n= 11, ■) postprandial hypotension (PPH) (P< 0.05).

CHAPTER 6: IMPACT OF GASTRIC EMPTYING TO THE GLYCAEMIC AND INSULINAEMIC RESPONSES TO A 75G ORAL GLUCOSE LOAD IN OLDER SUBJECTS WITH NORMAL AND IMPAIRED GLUCOSE TOLERANCE.

Statement of Authorship

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Principal Author

Candidate	Laurence G Trahair
Contribution	Conception of the study, study design and coordination, subject recruitment, data collection and interpretation, statistical analysis, and drafting of the manuscript.

Overall percentage	75%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Introduction

The World Health Organisation 75 g oral glucose tolerance test (OGTT) is regarded as the 'gold standard' for the diagnosis of impaired glucose tolerance (IGT) and diabetes (246) and is also predictive of the development of type 2 diabetes (247). The OGTT does, however, exhibit substantial variability (248) and there are uncertainties about the diagnostic value of the traditional 120 min glucose, as opposed to the 60 min value (249, 250). In particular, the 60 min plasma glucose may correlate better with insulin secretion and resistance (249, 250).

The variability of the OGTT is likely to be accounted for, in part, by gastric emptying (GE) which, in health, exhibits a wide inter-individual variation (200) so that nutrients, including glucose, usually enter the small intestine at an overall rate of 1 – 4 kcal/min, primarily as a result of inhibitory feedback arising from the small intestine (142). This inter-individual variation is increased in longstanding diabetes because of the high prevalence of delayed (191), and occasionally more rapid, GE (199). Studies in small cohorts have established that GE is a major determinant of the initial (from ~15 – 60 min) glycaemic response to oral glucose and carbohydrate-containing meals in healthy volunteers (197, 217, 251-253), type 2 patients with both normal and disordered GE (254, 255) and hypertensive patients (245). It has also been suggested that more rapid GE may predispose to the development of type 2 diabetes (199). The dependence of postprandial glycaemia on GE provides a rationale for the use of dietary and/or pharmacological (most recently, 'short-

acting' glucagon-like peptide-1 (GLP-1) agonists) interventions which slow GE to reduce postprandial glycaemic excursions (256).

In contrast to the above, there is much less information about the relationship of the blood glucose level at 120 min during an OGTT with GE (217, 251). Horowitz et al reported that, in health, this relationship is positive, rather than inverse, presumably reflecting the insulin levels achieved earlier (217). This also appears to apply to the blood glucose at 180 min (251). To our knowledge, there is no information about the impact of GE on the 120 min blood glucose in patients with IGT, or type 2 diabetes. Furthermore, studies which have evaluated the effect of GE on the glycaemic response to glucose have not assessed insulin secretion, or sensitivity. The incretin hormones, GLP-1 and gastric inhibitory polypeptide (GIP), modulate the glycaemic response to oral carbohydrate (213) and their secretion may be influenced by the rate of GE (221).

We hypothesized that GE would have a complementary effect to that of insulin sensitivity on the glycaemic and insulinaemic responses to a 75 g oral glucose load. This hypothesis could potentially be addressed by manipulating GE in isolation, which is problematic. Given the substantial inter-individual variation in GE, we have quantified GE as well as both 'early' and 'late' glycaemic responses, and insulin sensitivity in a cohort of older subjects with either normal glucose tolerance (NGT) or IGT.

Materials and Methods

Subjects

Eighty seven healthy 'older' subjects (47 female and 40 male, mean age 71.0 ± 0.5 years (range: 65 – 90 years), body mass index (BMI) 26.0 ± 0.3 kg/m^2 (range: 20.3 – 30.5 kg/m^2)), were recruited by advertisements placed in the local hospital campus and newspaper. Prior to their inclusion, demographic information and a basic medical history were obtained. Subjects with a history of gastrointestinal disease or surgery, known diabetes, significant respiratory or cardiac disease, alcohol abuse (consumption > 20 g/day) or epilepsy, were excluded. Any medication was withheld for 24 hours prior to the study.

Protocol

In each subject, concurrent measurements of GE, blood glucose, serum insulin and plasma GLP-1 and GIP were obtained on a single study day, which commenced at 08:30 after an overnight fast from solids for 14 hours and liquids for 12 hours. Upon arrival, an intravenous (IV) cannula was inserted into an antecubital vein for blood sampling while the subject was supine. The subject was then seated and allowed to 'rest' for 15 – 30 min before consuming a drink containing 75 g glucose and 150 mg C^{13}-acetate (Cambridge Isotope Laboratories, Massachusetts, USA), made up to 300 mL with water, within 3 min; t= 0 min was defined as the time of completion of the drink. Exhaled breath samples were collected in hermetically sealed 10 mL tubes (Exetainer, Buckinghamshire, England) prior to the ingestion of the drink (t= -3 min), every 5 min for the first hour, and then every 15 min for the subsequent 3 hours, for assessment of GE. Venous blood samples, for

measurement of blood glucose, serum insulin, plasma GLP-1 and plasma GIP, were obtained in tubes containing EDTA at t= -3, 15, 30, 45, 60, 90, 120, 180 and 240 min, centrifuged at 3200 rpm for 15 min and plasma, or serum, separated and stored at -70°C. At t= 240 min the IV cannula was removed and the subject offered a light lunch prior to them leaving the laboratory. The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written, informed consent. All experiments were carried out in accordance with the Declaration of Helsinki.

Gastric emptying

The $^{13}CO_2$ concentration in breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK) with an online gas chromatographic purification system. The gastric 50% emptying time (T50) was calculated (237).

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

Blood glucose (mmol/L) was determined using a portable glucometer (Medisense Companion 2 meter, Medisense Inc. Waltham, USA) and each subject classified, according to WHO (244) criteria, as having NGT (fasting blood glucose < 6.1 mmol/L, and 2 hour < 7.8 mmol/L), impaired fasting glucose (IFG) (fasting blood glucose < 7.0 mmol/L, but > 6.1 mmol/L), IGT (2 hour blood glucose < 11.1 mmol/L, but > 7.8 mmol/L), or diabetes (fasting blood glucose ≥ 7.0 mmol/L and/or 2 hour blood glucose ≥ 11.1 mmol/L) (244).

Serum insulin was measured by ELISA immunoassay (10-1113, Mercodia, Uppsala, Sweden). The sensitivity was 1.0 mU/L and the coefficient of variation (CV) was 2.6% within, and 7.6% between, assays (222). Total GLP-1 was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). Minimum detectable limit was 3 pmol/L, intra- and inter-assay CVs were 7.7% and 9.4% respectively (222). Plasma GIP was measured by radioimmunoassay. Minimum detectable limit was 2 pmol/L, inter-assay CV was 8.7% and intra-assay CV was 5.0% (240).

Insulin sensitivity and disposition index

The insulin sensitivity index (ISI) of Matsuda and DeFronzo (257) was calculated as:

$$\text{ISI} = \frac{10\,000}{\sqrt{(\text{Insulin}_{\text{fasting}} * \text{Glucose}_{\text{fasting}}) * (\text{Insulin}_{\text{Mean OGTT}} * \text{Glucose}_{\text{Mean OGTT}})}}$$

where insulin is in mU/L and glucose is in mg/dL. The ratio of the incremental changes from baseline in insulin and glucose at 30 min (ΔI30/ΔG30) was calculated as a measure of β-cell function (258). Insulin secretion, corrected for β-cell function (the oral disposition index (DI)), was calculated as the product of the Matsuda index and β-cell function (ΔI30/ΔG30*ISI) (259).

Statistical analysis

For blood glucose, serum insulin and plasma GLP-1 and GIP, changes from baseline and total areas under the curve (AUC) at t= 60, 120, 180 and 240 min

were calculated. Changes in each variable over time as were evaluated with a one-way repeated measures ANOVA. Data in subjects with NGT and IGT were compared (excluding subjects with IFG alone, or diabetes) using Student's T-test. Pearson's correlation was used to evaluate relationships between variables. A multiple regression model was used to assess the determinants of the blood glucose at t= 60, 120 and 180 min. In this model, covariates included the T50, ISI and DI. Results of the multiple regressions are reported as adjusted R^2 (R^2_{Adj}). Semipartial correlations are reported for the variables within each regression (R_{Part}). A P value < 0.05 was considered significant in all analyses. The statistical analysis was supervised and reviewed by a professional biostatistician. Data are presented as mean values ± SEM.

Results

The studies were well tolerated and there were no adverse events. Thirty-one subjects had NGT, 32 had IGT and 14 had both IFG and IGT; i.e. 46 had IGT. Eight had IFG alone and 2 had diabetes; these 10 subjects were excluded from the analysis, resulting in a cohort of 77 subjects. Demographic variables in the subjects are provided in **Table 6.1**. There were no differences in age or BMI between the groups with IGT and NGT. In 3 subjects (1 NGT, 2 IGT), GE data were unavailable due to degradation of the breath samples; in one the t= 180 and 240 min blood samples and in another the t= 240 min sample, were unavailable as the cannula was not patent. In two subjects, the insulin sample at t= 240 min was lost.

Gastric emptying

The GE T50 was 140.5 ± 4.3 min (n= 74, range: 95 – 256 min). There was no difference in the T50 between the groups with IGT and NGT (139.5 ± 5.7 min vs. 141.8 ± 6.5 min, P= 0.80).

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

Blood glucose increased following the drink (P< 0.001) and was less than baseline at both t= 180 and 240 min (P< 0.001 for both) **(Figure 6.1a)**. In the group with IGT when compared to those with NGT, blood glucose was greater at baseline, t= 15, 30, 45, 60, 90, 120 and 180 min (P< 0.05 for all), but not at t= 240 min (P= 0.34), and the AUC for blood glucose was greater at t= 60, 120, 180 and 240 min (P< 0.001 for all) **(Figure 6.1a)**.

Serum insulin increased following the drink (P< 0.001) and had returned to baseline by t= 240 min **(Figure 6.1b)**. In the group with IGT when compared to those with NGT, serum insulin was greater at t= 90, 120 and 180 min (P< 0.05 for all) **(Figure 6.1b)**.

There was an increase in plasma GLP-1 following the drink (P< 0.001), with a peak at ~t= 30 min and levels returning to baseline by t= 180 min **(Figure 6.1c)**. There was a sustained increase in plasma GIP (P< 0.001) until t= 120 min **(Figure 6.1d)**. There were no differences in absolute levels, or the AUC, for plasma GLP-1 or GIP between the groups with NGT or IGT **(Figure 6.1d)**.

Insulin sensitivity index, β-cell function and disposition index

In NGT, ISI (8.6 ± 0.9 vs. 6.0 ± 0.5, P< 0.01), $\Delta I30/\Delta G30$ (15.2 ± 1.8 vs. 9.4 ± 0.9, P< 0.05) and DI (110.1 ± 13.1 vs. 54.3 ± 9.2, P< 0.001) were greater when compared to those with IGT.

Relationships between the variables

(i) Glucose at 60 min

In the whole group (n= 77) the blood glucose at t= 60 min was related directly to the fasting blood glucose (R= 0.50, P< 0.001) and insulin (R= 0.28, P< 0.005), as well as the rise in insulin between t= 0 – 60 min (R= 0.33, P< 0.005) and inversely to the ISI (R= -0.48, P< 0.001) and DI (R= -0.68, P< 0.001). Similarly, the rise in blood glucose at t= 60 min was related inversely to the ISI (R= -0.45, P< 0.001) and DI (R= -0.68, P< 0.001).

In subjects with NGT (n= 31) the blood glucose at t= 60 min was related to fasting glucose (R= 0.38, P< 0.05), fasting insulin (R= 0.36, P< 0.05), and the rise in insulin between t= 0 – 60 min (R= 0.40, P< 0.05). In IGT (n= 46) the blood glucose at t= 60 min was also related to fasting glucose (R= 0.35, P< 0.05) and the rise in insulin between t= 0 – 60 min (R= 0.40, P< 0.01). In both groups, the blood glucose at t= 60 min was related inversely to both the ISI (NGT: R= -0.36, P< 0.05; IGT: R= -0.45, P< 0.005) and DI (NGT: R= -0.66, P< 0.001; IGT: R= -0.60, P< 0.001).

(ii) Glucose at 120 min

In the whole group, the blood glucose at t= 120 min was related to the fasting blood glucose (R= 0.45, P< 0.001), insulin (R= 0.29, P< 0.01), and insulin at t= 120 min (R= 0.43, P< 0.001) and inversely to the ISI (R= -0.43, P< 0.001) and DI (R= -0.53, P< 0.001). Similarly, the change in blood glucose at t= 120 was related inversely to both the ISI (R= -0.37, P< 0.005) and DI (R= -0.50, P< 0.001).

In NGT, the blood glucose at t= 120 min was not related to fasting serum insulin, but there was a relationship with serum insulin at t= 120 min (R= 0.38, P< 0.05); in IGT, blood glucose at t= 120 min was related to fasting serum insulin (R= 0.33, P< 0.05), but not the serum insulin at t= 120 min. In contrast, the blood glucose at 120 min was related inversely to both the ISI (NGT: R= -0.45, P< 0.05; IGT: R= -0.31, P< 0.05) and DI (NGT: R= -0.58, P< 0.001; IGT: R= -0.35, P< 0.05). In NGT, there was no relationship between the change in blood glucose at t= 120 min and the ISI (R= -0.20, P= 0.17), however, there was an inverse relationship with DI (R= -0.31, P< 0.05) and in IGT, the change in blood glucose at t= 120 min was related inversely to both the ISI (R= -0.38, P< 0.05) and DI (R= -0.53, P< 0.005).

(iii) GLP-1 and GIP

There were no significant relationships between either the absolute, or rises in plasma GLP-1 or GIP with blood glucose at t= 60 or 120 min in the whole group, or in NGT or IGT. In the whole group, there was a relationship between plasma GLP-1 at t= 60 min and insulin at t= 60 min (R= 0.35, P<

0.005), which was significant in IGT (R= 0.44, P< 0.005), but not NGT (R= 0.20, P= 0.29). There was no relationship between plasma GIP and insulin at t= 60 min.

Relationships with gastric emptying

(i) Glucose and insulin at 60 min

In the whole group (n= 74), there were inverse relationships between rises in both blood glucose (R= -0.30, P< 0.01, **Figure 6.2a**) and serum insulin (R= -0.23, P< 0.05) between t= 0 – 60 min and the T50. Similarly, there were inverse relationships between the absolute blood glucose (R= -0.27, P< 0.05) and serum insulin (R= -0.23, P< 0.05) at t= 60 min and the T50.

In the group with NGT (n= 30) neither the rises in, or absolute, blood glucose (R= -0.11, P= 0.54, **Figure 6.2b**) and serum insulin (R= -0.27, P= 0.14) between t= 0 – 60 min were related to the T50. In contrast, in IGT (n= 44) both the rise in blood glucose (R= -0.47, P< 0.001, **Figure 6.2c**), absolute blood glucose (R= -0.43, P< 0.005) and the AUC (R= -0.36, P< 0.05) from t= 0 – 60 min, but not serum insulin were related inversely to the T50

(ii) Glucose and insulin at 120 min

In the whole group, there was no relationship between the absolute, change in, or AUC for, blood glucose or serum insulin at t= 120 min and the T50 (**Figure 6.3a**), however, in subjects with NGT there was a trend for a relationship between both the change in blood glucose between t= 0 – 120 min (R= 0.34,

P= 0.06) and the absolute blood glucose at t= 120 min (R= 0.34, P= 0.06, **Figure 6.3b**), but not the AUC for blood glucose at t= 120 min (R= 0.03, P= 0.87) and the T50. In the group with IGT, the AUC for blood glucose (but not the absolute or change in blood glucose) at t= 120 min was related inversely to the T50 (R= -0.34, P< 0.05, **Figure 6.3c**).

(iii) Glucose and insulin at 180 min

In contrast to the rise, in the whole group there was a relationship between the change in blood glucose at t= 180 min (R= 0.55, P< 0.001, **Figure 6.4a)** and the T50. The absolute blood glucose at t= 180 min was also related to the T50 (R= 0.56, P< 0.001). Similarly, in the whole group both the change in (R= 0.54, P< 0.001), and absolute (R= 0.43, P< 0.001) serum insulin at t= 180 min were related to the T50.

In the NGT group, the absolute blood glucose (R= 0.74, P< 0.001), and serum insulin (R= 0.48, P< 0.01) at t= 180 min, and change in blood glucose (R= 0.73, P< 0.001, **Figure 6.4b)** and serum insulin (R= 0.55, P< 0.005) between t= 0 – 180 min, were related to the T50. In the IGT group, the absolute blood glucose (R= 0.53, P< 0.001) and serum insulin (IGT: R= 0.44, P< 0.001) at t= 180 min and the change in blood glucose (R= 0.50, P< 0.001, **Figure 6.4c**) and serum insulin (R= 0.57, P< 0.001) between t= 0 – 180 min, were related to the T50.

Determinants of the absolute and rises in blood glucose

(i) Glucose at 60 min

In the whole group (n= 74), a multivariable model incorporating the ISI, DI and T50, with the absolute blood glucose at t= 60 min as the dependent variable, was significant (P< 0.001), with individual significance for the ISI (P< 0.05), DI (P< 0.01) and the T50 (P< 0.05). In the group with NGT (n= 30) an identical model was significant (P< 0.001), however, the DI was the only significant variable in this model (P< 0.001). In the group with IGT (n= 44), this model was significant (P< 0.001) with significance for the T50 (P< 0.05), DI (P< 0.005), and a trend for the ISI (P= 0.08).

In the whole group, a model incorporating the ISI, DI and T50, with the rise in blood glucose at t= 60 min as the dependent variable, was significant (P< 0.001), with significance for the DI (P< 0.01) and T50 (P< 0.05), and a trend for the ISI (P= 0.06). In the group with NGT, this model was significant (P< 0.005), with the DI (P< 0.001) as the only significant variable. In the group with IGT this model was significant (P< 0.001), with significance for the DI (P< 0.005) and T50 (P< 0.01) only **(Table 6.2)**.

(ii) Glucose at 120 min

In the whole group, a multivariable model incorporating the ISI, DI and T50, with the absolute level of blood glucose at t= 120 min as the dependent variable, was significant (P< 0.001), with significance for the ISI (P< 0.05) and DI (P< 0.01), but not the T50. In the group with NGT this model was

significant (P< 0.001) with significance for the DI (P< 0.01), and a trend for ISI (P= 0.06) and T50 (P= 0.07). In the group with IGT, there was a trend for this model to be significant (P= 0.06).

In the whole group, a model incorporating the ISI, DI and T50, with the change in blood glucose at t= 120 min as the dependent variable, was significant (P< 0.001), with the DI (P< 0.001) as the only significant variable. In the group with NGT this model was significant (P< 0.005) with significance for the DI (P< 0.01) and a trend for the T50 (P= 0.08). In the group with IGT, this model was not significant **(Table 6.2)**.

(iii) Glucose at 180 min

In the whole group, a multivariable model incorporating the ISI, DI and T50, with the absolute level of blood glucose at t= 180 min as the dependent variable, was significant (P< 0.001), with significance for the T50 only (P< 0.001). In the group with NGT this model was significant (P< 0.001) with the T50 as the only significant variable (P< 0.001). In the group with IGT this model was significant (P< 0.001), with significance for the T50 (P< 0.001), and a trend for the DI (P= 0.06)

In the whole group, a model incorporating the ISI, DI and T50, with the change in blood glucose at t= 180 min as the dependent variable, was significant (P< 0.001), with significance for the T50 only (P< 0.001). In the group with NGT this model was significant (P< 0.001) with significance for the T50 only (P< 0.001). In the group with IGT this model was significant (P<

0.005) with a significance for the T50 (P< 0.001), and a trend for the DI (P= 0.08) **(Table 6.2)**.

Discussion

We have observed that the magnitude of the rise in blood glucose at 60 min is more closely related to GE in subjects with IGT than in those with NGT (i.e. when GE is relatively more rapid, the rise in glucose is proportionally greater), while the glycaemic response at 120 min tended to be related positively to GE (i.e. T50) in subjects with NGT, but was inversely related in those with IGT, probably reflecting the earlier insulinaemic response. We have also confirmed that the blood glucose levels at 60 and 120 min following an OGTT are related to insulin sensitivity in healthy older subjects.

Assessment of the relationship of GE with glycaemia is complicated by their interdependency. Acute elevations in glycaemia slow GE (228), while GE is a determinant of glycaemia (217). It has been assumed that the initial rise in blood glucose is modulated primarily by first-phase insulin secretion and hepatic insulin sensitivity (260). Given its potential diagnostic relevance (249, 250), we selected the blood glucose value at 60 min to reflect the 'early' glycaemic response. Our study demonstrates that the relationship of glycaemia and GE is time-dependent, likely reflecting changes in insulin sensitivity and secretion- while the blood glucose at 60 min was only significantly related to the rate of GE in the group with IGT. It should be appreciated that in NGT, the smaller variance in blood glucose at 60 min, as well as the earlier peak, may have contributed to the absence of a correlation.

Interestingly, at 180 min blood glucose levels were related to the rate of GE in both groups and in this case the relationship was inverse, i.e. when GE was relatively more rapid, the blood glucose at 180 min was less, presumably reflecting the proportionally greater insulin responses that occurred at earlier time points. Studies, utilizing intraduodenal infusions of glucose, have provided evidence that the relationships between small intestinal glucose delivery and initial glycaemic and insulinaemic responses are non-linear in both health (221) and type 2 diabetes (223). That such a relationship was not observed in the current study may reflect a lack of subjects with GE rates close to the upper limit of the normal range. With a breath test, the GE T50 should be regarded as notional, rather than precise, despite the demonstrated close correlation with scintigraphy (237), however, it is likely that in the majority of subjects GE was < 2 kcal/min, which is associated with only modest glycaemic and GLP-1 responses (221).

There were no differences in either the GIP, or GLP-1 responses between the NGT and the IGT groups, consistent with previous observations (104). However, in NGT the maximum glycaemic response only modestly exceeded the threshold (8 – 10 mmol/L) for insulinotropic effects of GIP and GLP-1 (261), unlike the case in subjects with IGT. Hence, the secretion of GIP and GLP-1 may be of greater relevance as a compensatory mechanism in the latter group and contribute to hyperinsulinaemia. We did not measure plasma glucagon, which is also modulated by both GIP and GLP-1 in a glucose-dependant manner (262). Insulin sensitivity and glucose disposition are recognised major determinants of the glycaemic response to oral glucose

(260). We calculated the ISI as described by Matsuda, and the DI adjusted for β-cell function and these were shown to be determinants of the rises in blood glucose at both 60 and 120 min, as would be predicted. It is well established that in cases of NGT, insulin sensitivity may be comparable to that in type 2 patients; however, it is only once the β-cell loses its capacity to compensate for the impaired insulin action that blood glucose concentrations increase.

Our observations are consistent with the concept that GE is a major determinant of the initial glycaemic response to carbohydrate-containing meals in type 2 diabetes and impacts on the overall glycaemic response (255). It is intuitively likely that GE will assume increased importance in type 2 patients as β-cell function declines; GLP-1 is of particular relevance given the diminished insulinotropic effects of GIP (214) and studies in type 2 patients employing mixed meals are indicated. While it should be recognised that blood glucose was quantified by glucometer, with its inherent limitations, our study supports the concept that the plasma glucose at 60 min during an OGTT provides clinically meaningful information (263); a cut-off of 8.6 mmol/L may represent a risk factor for type 2 diabetes (249, 250, 263). However, it may also represent a marker of relatively rapid GE per se. It should also be recognised that our cohort was exclusively > 65 years old and that ageing is characterised by diminished glucose tolerance, reflecting impairments in insulin sensitivity and β-cell function (264, 265). There is also a modest slowing of gastric emptying with age, but the rate of emptying usually falls within the normal range for the healthy young (144, 266).

We conclude that the rate of GE and insulin sensitivity appear to be independent, and complementary, determinants of both the 'early' and 'late' responses to an OGTT in healthy older subjects.

Figures and Figure Legends
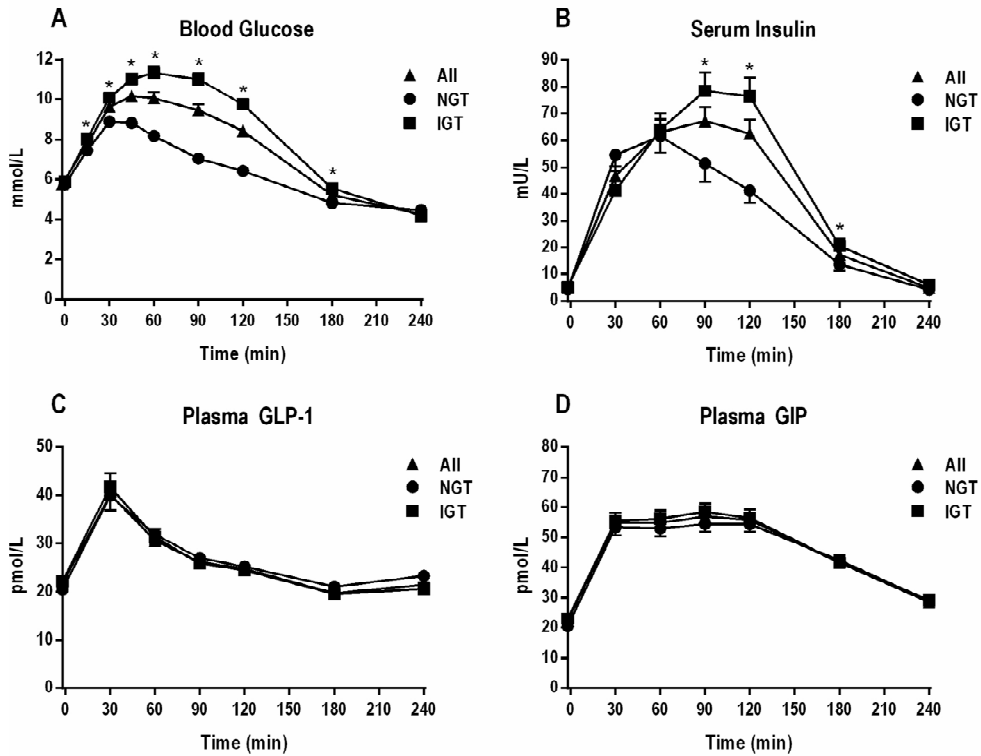
Figure 6.1: Blood glucose (A), serum insulin (B), plasma glucagon-like peptide-1 (GLP-1) (C) and plasma gastric inhibitory polypeptide (GIP) (D) immediately before and after a 75 g oral glucose load in all subjects (▲, n= 77), subjects with normal glucose tolerance (NGT, ●, n= 31) and those with impaired glucose tolerance (± impaired fasting glucose) (IGT, ■, n= 46) (* P< 0.05 NGT vs. IGT).
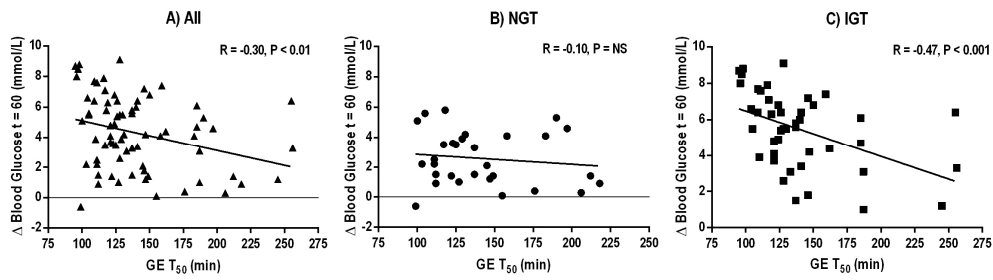
Figure 6.2: Relationships between the rise in blood glucose between t= 0 – 60 min and the T50 in (a) all subjects (n= 74, R= -0.26, P< 0.05), (b) normal glucose tolerance (NGT) (n= 30, R= 0.10, P= NS) and (c) impaired glucose tolerance (IGT) (n= 44, R= -0.47, P< 0.001).
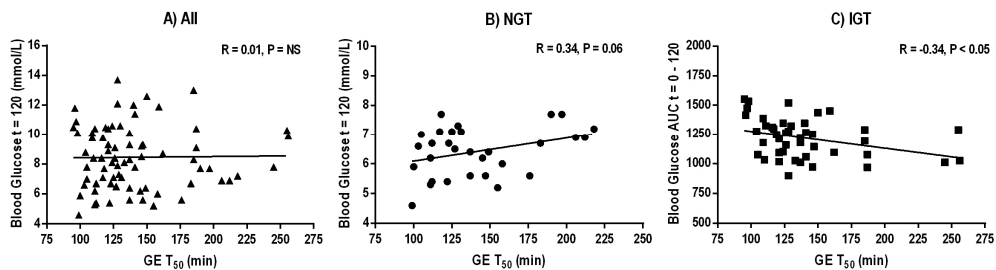
Figure 6.3: Relationships between absolute blood glucose at t= 120 min and the T50 in (a) all subjects (n= 74, R= 0.03, P= NS) and (b) normal glucose tolerance (NGT) (n= 30, R= 0.31, P= 0.06) and between blood glucose AUC 0-120 min and the T50 in (c) impaired glucose tolerance (IGT) (n= 44, R= -0.34, P< 0.05).
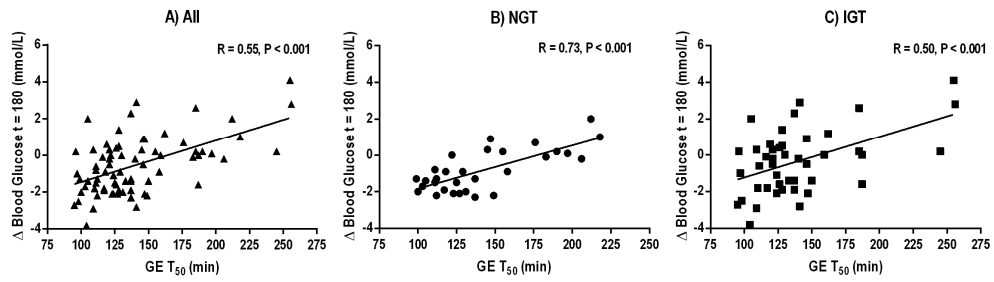
Figure 6.4: Relationships between the change from baseline for blood glucose between t= 0 – 180 min and the T50 in (a) all subjects (n= 74, R= 0.56, P< 0.001), (b) normal glucose tolerance (NGT) group (n= 30, R= 0.73, P< 0.001), and (c) the impaired glucose tolerance (IGT) group (n= 44, R= 0.50, P< 0.001).

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Tables

Table 6.1: Demographic variables.

	Whole Group	NGT	IGT
Age (years)	70.8 ± 0.5	69.8 ± 0.7	71.5 ± 0.7
Sex	39F, 38M	12F, 19M	27F, 19M
BMI (kg/m^2)	26.0 ± 0.3	25.6 ± 0.5	26.2 ± 0.4
GE T50 (min)	140.5 ± 4.3 (n= 74)	141.8 ± 6.5 (n= 30)	139.5 ± 5.7 (n= 44)

Demographic variables in the whole cohort (n= 77), subjects with NGT (n= 31) and IGT (n= 46). Data are mean ± SEM. BMI: body mass index, GE: gastric 50% emptying time (T50), IGT: impaired glucose tolerance, NGT: normal glucose tolerance.

Table 6.2: Relationships of glycaemia with gastric emptying, insulin sensitivity index and disposition index.

Blood Glucose Time	Variable	All Subjects (n= 74)	NGT (n= 30)	IGT (n= 44)
60 min	Overall Model	R^2_{Adj}= 0.52, P< 0.001	R^2_{Adj}= 0.38, P< 0.001	R^2_{Adj}= 0.45, P< 0.001
	T50	R_{Part}= -0.21, P< 0.05	R_{Part}= -0.20, P= 0.18	R_{Par}= -0.29, P< 0.05
	ISI	R_{Part}= -0.19, P< 0.05	R_{Part}= -0.14, P= 0.35	R_{Par}= -0.21, P= 0.08
	DI	R_{Part}= -0.49, P< 0.01	R_{Part}= -0.57, P< 0.001	R_{Par}= -0.35, P< 0.005
CFB 60 min	Overall Model	R^2_{Adj}= 0.52, P< 0.001	R^2_{Adj}= 0.38, P< 0.005	R^2_{Adj}= 0.44, P< 0.001
	T50	R_{Part}= -0.24, P< 0.05	R_{Part}= -0.22, P= 0.15	R_{Par}= -0.33, P< 0.01
	ISI	R_{Part}= -0.16, P= 0.06	R_{Part}= -0.12, P= 0.44	R_{Par}= -0.14, P= 0.22
	DI	R_{Part}= -0.51, P< 0.01	R_{Part}= -0.57, P< 0.001	R_{Par}= -0.37, P< 0.005
120 min	Overall Model	R^2_{Adj}= 0.32, P< 0.001	R^2_{Adj}= 0.41, P< 0.001	R^2_{Adj}= 0.12, P= 0.06
	T50	R_{Part}= 0.07, P= 0.48	R_{Part}= 0.27, P= 0.07	R_{Par}= 0.07, P= 0.64
	ISI	R_{Part}= -0.22, P< 0.05	R_{Part}= -0.28, P= 0.06	R_{Par}= -0.20, P= 0.17
	DI	R_{Part}= -0.38, P< 0.01	R_{Part}= -0.42, P< 0.01	R_{Par}= -0.23, P= 0.11
CFB 120 min	Overall Model	R^2_{Adj}= 0.27, P< 0.001	R^2_{Adj}= 0.34, P< 0.005	R^2_{Adj}= 0.04, P= 0.19
	T50	R_{Part}= 0.06, P= 0.56	R_{Part}= 0.27, P= 0.08	R_{Par}= 0.04, P= 0.80
	ISI	R_{Part}= -0.18, P= 0.08	R_{Part}= -0.23, P= 0.14	R_{Par}= -0.10, P= 0.52
	DI	R_{Part}= -0.38, P< 0.001	R_{Part}= -0.40, P< 0.01	R_{Par}= -0.23, P= 0.13
180 min	Overall Model	R^2_{Adj}= 0.35 P< 0.001	R^2_{Adj}= 0.53, P< 0.001	R^2_{Adj}= 0.31, P< 0.001
	T50	R_{Part}= 0.58, P< 0.001	R_{Part}= 0.76, P< 0.001	R_{Part}= 0.58, P< 0.001
	ISI	R_{Part}= -0.09, P= 0.37	R_{Part}= -0.12, P= 0.34	R_{Part}= 0.01, P= 0.97
	DI	R_{Part}= -0.16, P= 0.09	R_{Part}= 0.16, P= 0.22	R_{Part}= -0.25, P= 0.06
CFB 180 min	Overall Model	R^2_{Adj}= 0.29, P< 0.001	R^2_{Adj}= 0.52, P< 0.001	R^2_{Adj}= 0.26, P< 0.005
	T50	R_{Part}= 0.56, P< 0.001	R_{Part}= 0.75, P< 0.001	R_{Part}= 0.54, P< 0.001
	ISI	R_{Part}= -0.01, P= 0.96	R_{Part}= -0.08, P= 0.54	R_{Part}= 0.10, P= 0.47
	DI	R_{Part}= -0.13, P= 0.21	R_{Part}= 0.19, P= 0.19	R_{Part}= -0.24, P= 0.08

(Previous page) CFB: change from baseline, DI: disposition index, IGT: impaired glucose tolerance, ISI: insulin sensitivity index, NGT: normal glucose tolerance, R^2_{Adj}: adjusted R^2, R_{Part}: sempiartial correlation, T50: gastric 50% emptying time.

CHAPTER 7: REGIONAL SPECIFICITY OF THE GUT INCRETIN RESPONSE TO SMALL INTESTINAL GLUOCSE INFUSION IN HEALTHY OLDER SUBJECTS

Statement of Authorship

Title of paper	Regional specificity of the gut-incretin response to small intestinal glucose infusion in healthy older subjects
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Candidate Contribution

Candidate	Laurence G Trahair
Contribution	Data collection and interpretation, statistical analysis and drafting of the manuscript.
Overall percentage	50%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis.

Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	Feb 2016

Name of Co-author	Karen L Jones		
Contribution	Conception and design of the study, data interpretation, statistical analysis, drafting of the manuscript and had overall responsibility for the study.		
Signature		Date	Feb 2016

Introduction

Administration of macronutrients into the gut triggers the release of glucose-dependent insulinotropic polypeptide (GIP) from enteroendocrine K-cells situated most densely in the duodenum and jejunum, and glucagon-like peptide-1 (GLP-1) from L-cells which predominate in the distal small intestine and colon (208). GIP and GLP-1 are "incretin" hormones, accounting for the substantially greater insulin response to an enteral glucose load than an isoglycaemic intravenous glucose infusion (the "incretin" effect) (267). In type 2 diabetes, the insulinotropic effect of GIP is markedly diminished, whereas that of GLP-1 is relatively preserved (214). GLP-1 also slows gastric emptying and suppresses glucagon secretion and energy intake

(208). Diversion of nutrients from the proximal into the distal small intestine probably underlies the enhanced GLP-1 secretion after Roux-en-Y gastric bypass (RYGB) (268, 269).

The mechanisms by which GLP-1 is released from the intestine are incompletely understood. In humans, unlike rodents, exogenous GIP does not stimulate GLP-1 (270). Unlike GIP, the release of GLP-1 in response to intraduodenal glucose infusion displays a threshold, with evidence that only infusions at rates sufficient to exceed the absorptive capacity of the proximal small intestine stimulate substantial GLP-1 release (271). We reported in healthy humans that GLP-1 was stimulated when intraduodenally infused glucose was allowed to access the entire small intestine, but not when restricted to the proximal 60 cm (271). In contrast, the responses of GIP and cholecystokinin (CCK) were comparable between the two conditions, supporting the proximal gut as the origin of the latter two hormones (271). While there was a greater GLP-1 response to an additional load of glucose beyond the proximal 60 cm of the small intestine with access to the entire small intestine, the increases in blood glucose and plasma insulin were also greater (271) and the design of this study did not allow the potential effects of the region to be differentiated from those of the length of intestine exposed to glucose. In an analysis of unpaired observations on the effects of glucose infused at a rate of 2 kcal/min into either the duodenum or proximal jejunum (50 cm from the pylorus) (272), plasma GLP-1, GIP and insulin responses were greater following intrajejunal versus intraduodenal glucose administration, supporting the concept that diversion of nutrients to the more

distal small intestine enhances GLP-1 and insulin secretion (272). After RYGB very high GLP-1 and insulin responses are associated with extremely rapid emptying of glucose from the gastric pouch (~100 kcal/min). However, when the rate of intestinal glucose delivery is slowed to the upper end of the normal range for gastric emptying, both incretin hormone and blood glucose responses, are comparable to those in healthy controls following an identical rate of intraduodenal glucose infusion (273). These, and other, observations challenge the concept that direct exposure of distal L-cells to luminal content is the primary route for GLP-1 stimulation. Furthermore, CCK-signaling arising from the proximal gut potentiates GLP-1 release in response to fat (274).

The current study was designed to evaluate (i) the relative importance of the proximal (12 – 60 cm beyond the pylorus) versus distal (> 70 cm beyond the pylorus) exposure of the small intestine in incretin, CCK and insulin responses to luminal glucose, and (ii) the hypothesis that diversion of glucose from the proximal to the distal small intestine would increase GLP-1 secretion.

Materials and Methods

Subjects

Thirteen healthy older subjects (12 male and 1 female, aged 71.3 ± 1.3 years) were recruited through an existing database. All were non-smokers and none had a history of cardiovascular, hepatic, renal or gastrointestinal disease or

alcohol abuse. None had diabetes, or took medication known to influence gastrointestinal function.

Protocol

Subjects were studied on 3 occasions, separated by at least 7 days. On each study day, the subject attended our laboratory at the Royal Adelaide Hospital at 08:00 after an overnight fast (271). Upon arrival, a silicone-rubber, multilumen catheter (external diameter 4.2 mm) (Dentsleeve International, Mui Scientific, Mississauga, Canada) **(Figure 7.1)** was inserted into the stomach via an anesthetised nostril, and allowed to pass into the duodenum by peristalsis. The catheter included two infusion channels, positioned in the duodenum (~12 cm distal to the pylorus; channel 1) and the jejunum (~70 cm distal to the pylorus; channel 2), a ~10 cm long balloon (~60 cm distal to the pylorus to be inflated for isolating proximal and distal segments of the small intestine), and an aspiration channel (~3 cm proximal to the balloon to aspirate duodenal contents). The catheter also incorporated two 'air-return' channels, to equalise duodenal pressure during infusion and aspiration. Two other channels, located in the antrum (~2.5 cm proximal to the pylorus) and duodenum (~2.5 cm distal to the pylorus), were perfused continuously with saline (0.9%) and the correct positioning of the catheter maintained by continuous measurement of the transmucosal potential difference (TMPD) in the antral (-40 mV) and duodenal (0 mV) channels (124, 239). To measure TMPD, a 0.9% saline-filled cannula was inserted subcutaneously into the subject's forearm (239). After the catheter had been positioned correctly, the

subject was placed in a recumbent position and an intravenous cannula inserted into the left antecubital vein for blood sampling.

Commencing at t= -30 min, the balloon was inflated with air using a hand-held syringe until the subject reported a sensation of pressure, without discomfort (approximately 35 mL) (271, 275). The balloon was then deflated and the subject allowed to 'rest' for ~15 min. At t= 0 min, the balloon was re-inflated to the pre-determined volume, and intra-balloon pressure monitored throughout the study to ensure sustained inflation (271, 275).

Between t= 0 – 60 min, each subject received the following three infusions via infusion channel 1 and infusion channel 2: a) infusion channel 1: 25% glucose at 3 kcal/min + infusion channel 2: 0.9% saline (i.e. proximal small intestinal segment only exposed to glucose; "GP"), b) infusion channel 1: 25% glucose at 3 kcal/min + infusion channel 2: 25% glucose infused at an adjustable rate to allow the glucose recovered from the proximal segment to be infused concurrently (i.e. both the proximal and distal segments exposed to glucose; "GPD"), or c) infusion channel 1: 0.9% saline + infusion channel 2: 25% glucose at 3 kcal/min (i.e. distal segment only exposed to glucose; "GD").

All infusions were administered at 3.15 mL/min, with the exception of the variable rate glucose infusion during b). During the GPD infusion, for the first 10 min, glucose was infused into the proximal segment only, before the glucose concentration in the luminal aspirate was measured to determine the appropriate infusion rate for the distal small intestine, which was then re-

calculated and adjusted every 10 min. At the end of each 10 min epoch, 1 mL of the aspirate was diluted with 49 mL of saline (0.9%) and the glucose concentration measured with a 2300 Stat Plus glucose analyser (Yellow Springs Instruments (YSI), Ohio, USA). The glucose concentration in the aspirate was derived by multiplying this figure by 50, which was multiplied by the volume of aspirate to yield the total amount of glucose in the aspirate. This glucose load was replaced by infusion into the distal small intestine over the subsequent 10 min. At t= 60 min the infusion catheter and intravenous cannulae were removed and the subject offered a meal prior to leaving the laboratory.

The three study days were undertaken in single-blind, randomised fashion; the investigator delivering the infusions could not be blinded, but was independent of any other measurements. All infusions were performed using a volumetric infusion pump (Gemini PC-1, IMED, San Diego, CA, USA).

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

Blood glucose, serum insulin, plasma GLP-1, plasma GIP and plasma CCK

Venous blood samples (18 mL) were collected into ice-chilled EDTA-treated tubes prior to balloon inflation (t= -15 min) and at 15 min intervals between t=

0 – 60 min. Samples were separated by centrifugation at 3200 rpm for 15 min at 4˚C within 10 min of collection and stored at -70° for subsequent analysis.

Blood glucose concentrations (mmol/L) were determined with a 2300 Stat Plus glucose analyser.

Serum insulin (mU/L) was measured by ELISA immunoassay (Diagnostics 10-1113, Mercodia, Uppsala, Sweden). The sensitivity of the assay was 1.0 mU/L and intra- and inter-assay coefficients of variation (CV) were 2.1% and 5.3%, respectively (222). Serum insulin concentration was also expressed in relation to blood glucose at each time point, i.e. the insulin/glucose ratio (276).

Plasma total GLP-1 (pmol/L) was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). The minimum detectable limit was 3 pmol/L and intra- and inter-assay CV were 4.2% and 10.5%, respectively (222).

Plasma total GIP (pmol/L) was measured by radioimmunoassay with modifications of a published method (240). The minimum detectable limit was 2 pmol/L and intra- and inter-assay CV were 6.1% and 15.4%, respectively (222).

Plasma CCK (pmol/L) was measured following ethanol extraction using radioimmunoassay. The sensitivity of the assay was 2.5 pmol/L and intra- and inter-assay CV were 27% and 9%, respectively (271).

Statistical analysis

For all variables, incremental areas under the curve (iAUCs) were calculated using the trapezoidal rule. Maximum (peak) changes in each variable were calculated as the greatest change from baseline at any time point for each condition. Basal values, maximum changes and iAUCs were compared using one-factor repeated-measures ANOVA. Variables were also analysed using two-factor repeated measures ANOVA, with treatment and time as factors. Where significance was revealed by ANOVA, post hoc comparisons adjusted by Bonferroni-Holm's correction were performed. Based on our previous study, 8 subjects were required to detect, with 80% power, a difference in GLP-1 response between the GP and GPD conditions (271). All analyses were performed using SPSS 17.0.0 (SPSS Inc, Chicago, IL, USA). Data are presented as mean ± SEM. A P value < 0.05 was considered significant.

Results

One subject experienced nausea and vomited at ~45 min after the commencement of the glucose infusion on all 3 study days and was excluded from analysis. Two subjects withdrew due to the discomfort of the study procedure. In the remaining 10 subjects (9 male and 1 female, aged 70.3 ± 1.4 years (range 65 – 79 years), BMI 25.7 ± 0.8 kg/m^2 (range 22.3 – 30.1 kg/m^2)), who all tolerated the study well, there were no differences in baseline balloon pressure (GP: 128.6 ± 4.9 mmHg, GD: 132.7 ± 4.9 mmHg, GPD: 130.4 ± 4.6 mmHg; P= 0.58), balloon volume (GP: 35.3 ± 1.9 mL, GD: 35.5 ± 2.3 mL, GPD: 35.8 ± 1.7 mL; P= 0.85), glucose recovery in any 10 min epoch (**Figure 7.2)**, or % cumulative glucose recovery **(Figure 7.3)** between the study days.

Due to the glucose aspiration procedure, the total glucose load in the proximal small intestine was 101.9 ± 2.6 and 89.1 ± 2.5 kcal/h (P= 0.10) on the GP and GPD conditions respectively (n= 7 for both). In one subject, the insulin sample at the 45 min time point on the GD study day was lost. In 3 subjects, the glucose concentrations in the aspirate were not measured on the GP study day, due to technical problems; accordingly, the corresponding data on the GPD study day were excluded from analysis in these subjects.

Blood glucose

Fasting blood glucose concentrations did not differ between the study days. During glucose infusion, blood glucose increased progressively (time effect: P< 0.001 for each). There was a treatment × time interaction for blood glucose (P< 0.001), with concentrations being lower in response to both GP vs. GD, and GP vs. GPD at t= 60 min (P< 0.05 for both), without differences between GD and GPD. There were treatment effects on both the peak and iAUC for blood glucose (P< 0.01 and P< 0.05, respectively), such that the peak was higher with GPD vs. GP (P< 0.05), with a trend for a difference between GD and GP (P= 0.06) and no difference between GPD vs. GD; the iAUC was also higher in response to GPD vs. GP (P< 0.05), with no differences between GD vs. GP or GPD vs. GD **(Figure 7.4A)**.

Serum insulin

Fasting serum insulin concentrations did not differ between the study days. During glucose infusion, serum insulin increased (time effect: P< 0.001 for

each). There was a treatment × time interaction for serum insulin (P< 0.001), with concentrations being lower in response to GP vs. GD from ~t= 45 min (P< 0.05), and with GP when compared to both GD and GPD at t= 60 min (P< 0.05 for each), without differences between GD and GPD. There were treatment effects for both the peak and iAUC for serum insulin (P< 0.05 and P< 0.01, respectively); the peak was higher in response to both GD and GPD vs. GP (P< 0.05 for both), with no difference between GD and GPD; the iAUC was less for GP vs. GD (P< 0.05) **(Figure 7.4B)**.

Plasma GLP-1

Fasting plasma GLP-1 concentrations did not differ between the study days. During glucose infusion, plasma GLP-1 increased minimally in response to GP (time effect: P= 0.05), but substantially on both the GD and GPD days (time effect: P< 0.001 for each); the response occurred later in response to GPD compared to GD. There was a treatment × time interaction for plasma total GLP-1 (P< 0.001), with GLP-1 being higher in response to GD and GPD vs. GP from ~t= 45 min (P< 0.05 for each), and with GD vs. GPD at t= 45 min (P< 0.05). There were treatment effects for both the peak and iAUC for plasma GLP-1 (P< 0.001 for each), such that the peak was higher in response to both GPD and GD vs. GP (P< 0.001 for each) and with GD vs. GPD (P< 0.05), and the iAUC greater with GPD and GD vs. GP (P< 0.05 for each), and with GD vs. GPD (P< 0.05) **(Figure 7.4C)**.

Plasma GIP

Fasting plasma GIP concentrations did not differ between the study days. During glucose infusion, plasma GIP increased (time effect: P< 0.001 for each). There was a treatment × time interaction for plasma GIP (P< 0.001), with concentrations being higher for GPD vs. GP at t= 45 min (P< 0.05), and with GPD vs. both GP and GD at t= 60 min (P< 0.05 for each). There were treatment effects on both the peak and iAUC for plasma GIP (P< 0.001 and P< 0.05, respectively), such that the peak was higher for GPD vs. both GP and GD (P< 0.05 for both) and iAUC greater for GPD vs. GP (P< 0.05) **(Figure 7.4D)**.

Plasma CCK

Fasting plasma CCK did not differ between the study days. During glucose infusion, plasma CCK increased (time effect: P< 0.001 for each). Plasma CCK peaked at t= 15 min during GP infusion, while there was a marginal increase during GD infusion and a sustained elevation during GPD infusion. There was a treatment × time interaction for plasma CCK (P< 0.001), with concentrations being higher with GP vs. GD at t= 15 min (P< 0.01), and with GPD vs. both GP and GD at t= 60 min (P< 0.05 for each). There were treatment effects for the peak and iAUC for plasma CCK (P< 0.01 for each), such that the peak CCK was higher with both GP and GPD vs. GD (P< 0.05 for both), as well as GP vs. GPD (P< 0.05); the iAUC was greater with GPD vs. both GD and GP (P< 0.05 for both) with a trend for GPD vs. GP (P= 0.08) **(Figure 7.4E)**.

Insulin/glucose ratio

Fasting insulin/glucose ratio did not differ between the study days. During glucose infusion, the insulin/glucose ratio increased (time effect: $P < 0.001$ for each). There was a treatment × time interaction for the insulin/glucose ratio ($P < 0.001$), which was greater with both GD and GPD vs. GP at t= 45 min ($P < 0.05$ for both) and at t= 60 min ($P < 0.05$ for both), with a trend for it to be greater during GD vs. GPD at t= 45 min ($P = 0.07$) and t= 60 ($P = 0.10$). There were treatment effects for both the peak and iAUC for the insulin/glucose ratio ($P < 0.001$ and $P < 0.01$ respectively), such that the peak ratio was higher with GD vs. both GPD and GP ($P < 0.05$ for both), and the iAUC higher with GD vs. GP ($P < 0.01$), with a trend for GPD vs. GP ($P = 0.09$) **(Figure 7.4F)**.

Discussion

In this study of healthy older subjects (i) infusion of glucose into an isolated segment of proximal gut was associated with minimal GLP-1 secretion, but substantial release of GIP and CCK, (ii) combined infusions of glucose into proximal and distal segments induced greater GLP-1, GIP and CCK responses, associated with an increased insulin/glucose ratio, and (iii) there was a modestly increased GLP-1 and a substantial GIP, but diminished CCK, response with infusion into the distal segment alone, compared to proximal infusion, without differences in serum inulin or the insulin/glucose ratio. These observations attest to the importance of stimulation of the distal small intestine for GLP-1 and, to a lesser extent, GIP secretion.

The rate of intraduodenal glucose employed in the current study has been shown to induce substantial GLP-1 release in both health and type 2 diabetes (222, 223). It clearly exceeded the absorptive capacity of the proximal small intestinal segment; we used an intraluminal balloon to divide the small intestine into a proximal (12 – 60 cm beyond the pylorus) and a distal (> 70 cm beyond the pylorus) segment and showed that ~60% of intraduodenally administered glucose was absorbed. When glucose exposure was limited to the proximal segment, there was minimal GLP-1 release and when the unabsorbed glucose from the proximal segment was concurrently administered into the distal segment, there was a substantial, albeit delayed, response. It has been suggested that a threshold of small intestinal glucose delivery > 2 kcal/min is required to induce GLP-1 release (222, 223), but the observations in the current study indicate that this concept is overly simplistic. While proximal infusion alone had minimal effect on GLP-1, in the GPD condition, a lesser amount of glucose infused distally (~1.3 kcal/min) was associated with substantial GLP-1 release. Hence, the threshold load on GLP-1 release may well be lower with diversion of nutrients to the more distal small intestine, potentially reflecting the density of GLP-1-containing cells (208, 277). However, it also remains possible that exposure of the proximal segment to glucose potentiates GLP-1 secretion in response to enteral glucose available distally. In particular, despite more than double the rate of distal glucose infusion with GD when compared to GPD (3 vs. ~1.3 kcal/min), peak GLP-1 concentrations were comparable. GIP, which is released from the upper gut, induces GLP-1 secretion in rodents (278), but this is not the case in humans (270). In contrast, there is evidence that CCK may be an important

signal for GLP-1 release; antagonism of CCK receptors was reported to attenuate the GLP-1 response to intraduodenal fat infusion (274). Therefore, differences in plasma CCK may have influenced the GLP-1 responses to GPD and GD infusions. GLP-1 also has a physiologic action to slow upper gastrointestinal transit (208, 279), which might have attenuated the GLP-1 response to glucose during GD infusion.

Unlike GLP-1, plasma GIP and CCK increased on all study days, although GIP concentrations were higher after GPD than GD and GP, and CCK concentrations higher in response to GPD and GP than GD. The discrepancy in plasma GIP between the three study days, at least in part, is likely to reflect differences in the load of glucose (~102 kcal for GP vs. 180 kcal for GPD and GD); the response to GP would almost certainly have been greater with an equivalent glucose load (208, 280). It is, however, clear that exposure of the distal small intestine to glucose is associated with substantial secretion of GIP. In contrast to GIP, plasma CCK concentrations were substantially less after GD than GP suggesting that the proximal predominance of CCK-releasing I-cells is greater than of K-cells (281).

Serum insulin and the insulin/glucose ratio were lowest following GP infusion, mirroring responses of plasma GLP-1 and GIP. However, less glucose was absorbed during GP than GPD and GD, and blood glucose concentrations were also lowest for GP. That the insulinotropic effects of GIP and GLP-1 are comparable in health (262), unlike type 2 diabetes, probably accounts for why

neither serum insulin, blood glucose, nor the insulin/glucose ratio differed significantly between the two study days.

Potential limitations of our study should be recognised. The number of subjects studied was, of necessity, relatively small; however, the observed differences in the incretin hormone and CCK responses between the infusions were consistent. Because none of the parameters had returned to baseline by the end of the study, evaluation of the 'overall' responses to different glucose infusions was compromised. While we studied healthy older subjects, incretin release is preserved in this group (222). Given evidence to suggest alterations in the secretion and/or action of incretin hormones in obesity and type 2 diabetes, it would be of interest to evaluate patients with these conditions.

In summary, in healthy older subjects, exposure of the distal small intestine (> 70 cm beyond the pylorus) to glucose is necessary to induce substantial GLP-1 secretion. In contrast, the release of GIP from both the proximal and distal small intestine is substantial. These observations attest to the regional specificity of the gut-incretin axis.

Figures and Figure Legends
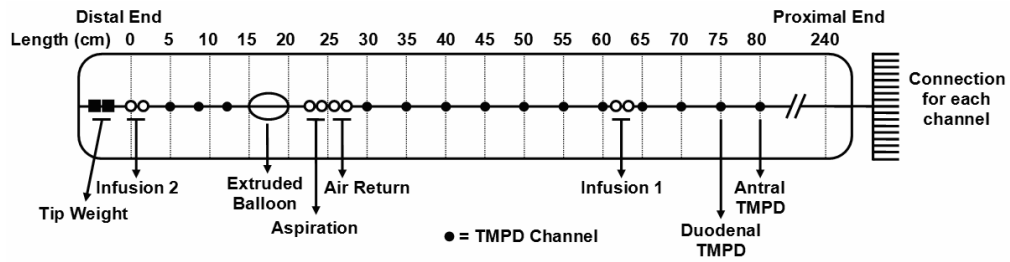
Figure 7.1: Schematic diagram of the multilumen catheter (external diameter 4.2 mm) used for intraduodenal infusion. TMPD: transmucosal potential difference.
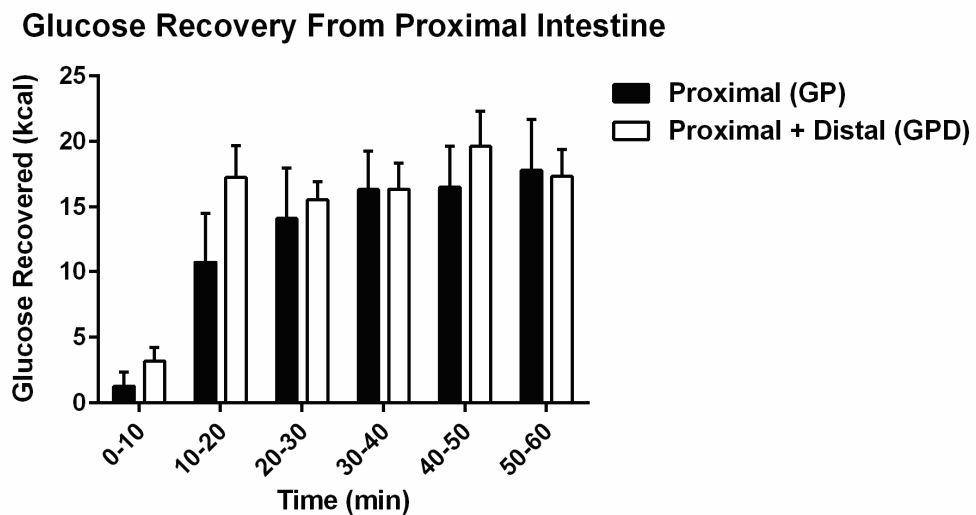
Figure 7.2: Glucose recovery (in kcal) in the aspirate collected from the proximal small intestine during intraduodenal glucose infusion into either (i) proximal (GP) or (ii) both proximal and distal segments (GPD) of small intestine in healthy older subjects (n= 7). Data are mean ± standard error, P= NS for GP vs. GPD at all time points.

Accumulation of % Glucose absorbed
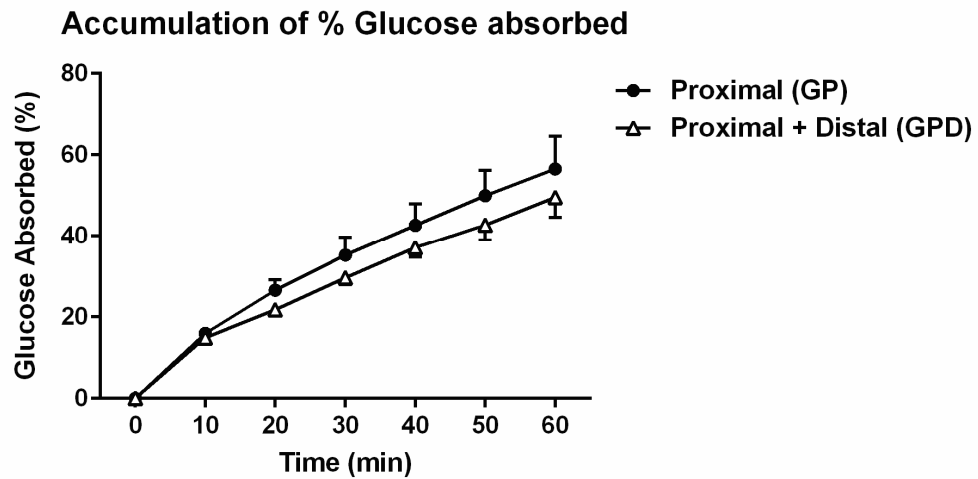
Figure 7.3: Cumulative glucose absorption (%) from proximal small intestine during intraduodenal glucose infusion into proximal (GP) and both proximal and distal segments (GPD) of small intestine in healthy older subjects (n= 7). Data are mean ± standard. P= NS for GP vs. GPD at all time points.
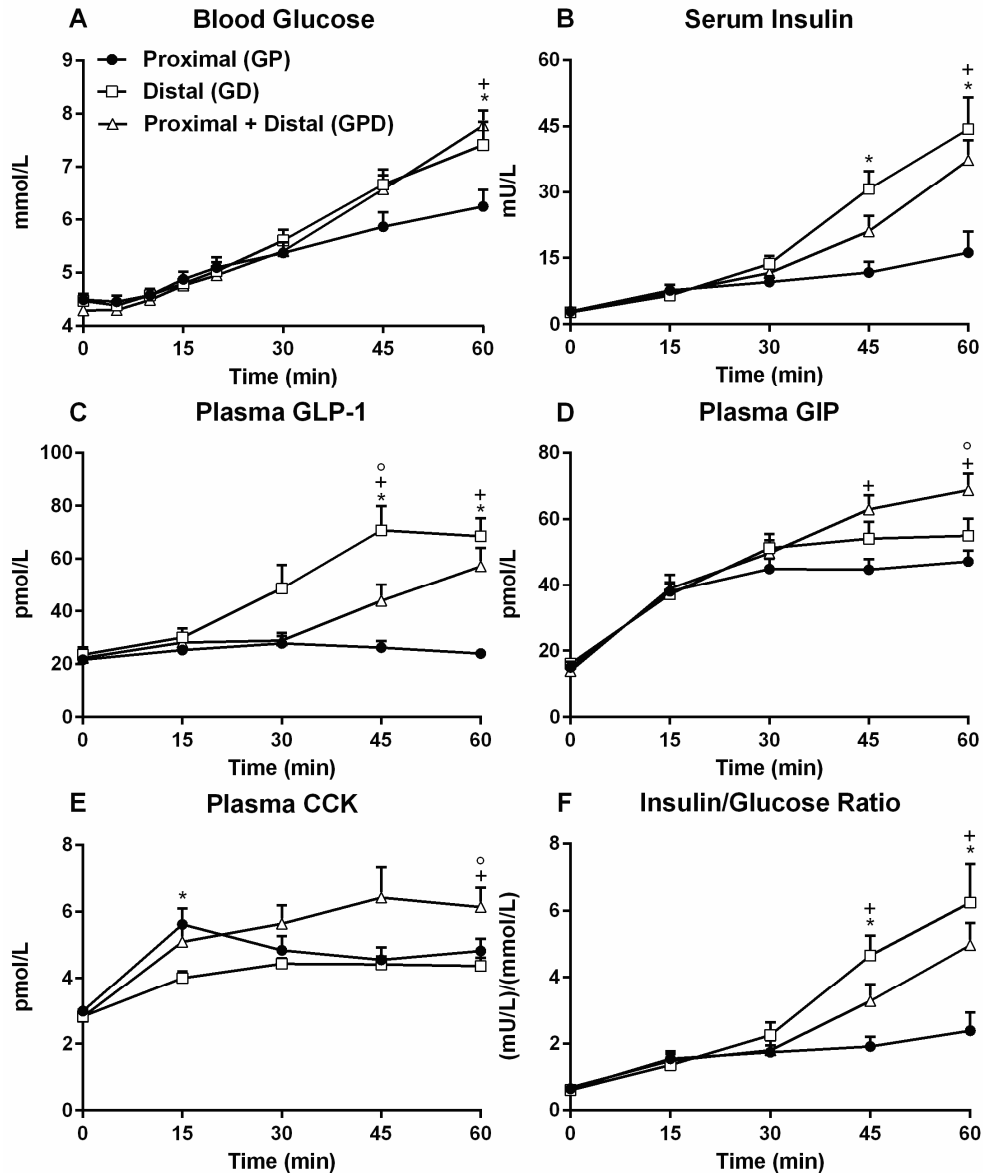
Figure 7.4: Effects of intraduodenal glucose infusion into proximal (GP, closed circle), distal (GD, open square), or both proximal and distal (GPD, open triangle) segments of small intestine on the (A) blood glucose, (B) serum insulin, (C) plasma glucagon-like peptide-1 (GLP-1), (D) glucose-dependent insulinotropic polypeptide (GIP), (E) cholecystokinin (CCK) and (F) insulin/glucose ratio in healthy older subjects (n= 10). Data are mean ± standard error, * $P < 0.05$ GP vs. GD, + $P < 0.05$ GP vs. GPD, ° $P < 0.05$ GD vs. GPD.

CHAPTER 8: COMPARATIVE EFFECTS OF GLUCOSE AND WATER DRINKS ON BLOOD PRESSURE AND CARDIAC FUNCTION IN OLDER SUBJECTS WITH AND WITHOUT POSTPRANDIAL HYPOTENSION

Statement of Authorship

Title of paper	Comparative effects of glucose and water drinks on blood pressure and cardiac function in older subjects with and without postprandial hypotension.
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Principal Author

Candidate	Laurence G Trahair
Contribution	Conception of the study, study design and coordination, subject recruitment, data collection and interpretation, statistical analysis and drafting of the manuscript.
Overall percentage	75%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research

	candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

  i) the candidate's stated contribution to the publication is accurate (as detailed above);

  ii) permission is granted for the candidate in include the publication in the thesis; and

  iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution	Conception and design of the study, data interpretation, statistical analysis, drafting of the manuscript and overall responsibility for the study.		
Signature		Date	Feb 2016

Introduction

Postprandial hypotension (PPH), defined as a fall in systolic blood pressure (BP) after a meal of > 20 mmHg (12), is an important clinical disorder, associated with an increased risk of syncope, falls and mortality (243). PPH occurs frequently- the prevalence in healthy older individuals is ~25% (243), in residents of aged care facilities 25 – 40% (243) and in patients with autonomic dysfunction 40 – 100% (243). Current management of symptomatic PPH is suboptimal.

The pathophysiology of PPH is poorly defined (243) but, in the broadest sense, a postprandial fall in BP implies that compensatory cardiovascular changes in response to splanchnic blood pooling following a meal are inadequate. In healthy young individuals, there are postprandial increases in cardiac output (CO) and heart rate (HR) (4, 5), as well as vasoconstriction in skeletal muscle and peripheral vascular beds (4), so that there is little, if any, change in BP (4, 5). These responses appear to be driven by unloading of arterial baroreceptors and a subsequent increase in sympathetic efferent outflow to the vasculature and heart; in healthy young subjects, meal ingestion is associated with increases in both cardiac and muscle sympathetic activity (3, 282) and a fall in vascular resistance which is accounted for by the rise in splanchnic blood flow (283). In contrast, in healthy older subjects, there is a variable postprandial reduction in BP (128) and the increases in CO, stroke volume (SV) and HR have been reported to be less than in healthy young (4), although the latter has not been a consistent observation (284), while increases in splanchnic blood flow appear comparable (124). There is also a reduction, rather than an

increase, in skeletal muscle vascular resistance (3). Similarly, in response to intraduodenal glucose infusion, systolic BP falls in healthy older subjects while there is little, if any, change in young subjects (124). That the increases in muscle sympathetic nerve activity induced by intraduodenal glucose infusion are comparable in healthy young and older subjects may be indicative of diminished sympathetic baroreflex sensitivity or, potentially, diminished responsiveness to sympathetic neural outflow at the neurovascular junction (56).

Surprisingly, the effects of meal ingestion on cardiac function in older people with PPH have hitherto not been evaluated directly. The postprandial increase in splanchnic blood flow appears to be comparable in patients with PPH than in healthy subjects (126), but the sympathetic response and reductions in blood flow to the postprandial vasculature may be diminished (112).

Although PPH may be considered to reflect inadequate cardiovascular compensation to meal induced splanchnic blood pooling, several gastrointestinal mechanisms are now recognised to be fundamental (243). In particular, gastric emptying (GE), which exhibits a wide inter-individual variation (285), is pivotal to the regulation of postprandial BP- in healthy older subjects and type 2 diabetes, when gastric emptying is relatively more rapid (46), or glucose is infused intraduodenally at a faster rate (124), the fall in systolic BP is greater. In 'healthy' older subjects PPH is associated with more rapid GE (286) and treatments such as acarbose, which delay both gastric emptying and small intestinal carbohydrate absorption, appear to be

effective in management (107). Contrary to the effects of small intestinal nutrient exposure, nutrient or non-nutrient gastric distension attenuates the postprandial fall in BP in healthy older subjects and patients with PPH and probably plays a protective role in the maintenance of postprandial BP (136, 139). Jordan et al (137) reported that ingestion of water (480 mL) is associated with a volume-dependant pressor response in healthy older subjects which is greater in patients with autonomic failure (137). Subsequently, Shannon et al (158) reported, in 7 patients with PPH associated with autonomic failure, that ingestion of 480 mL water markedly attenuated the fall in BP induced by a high carbohydrate meal for at least 60 min (158). That the fall in BP is less following oral, compared with an identical duodenal, glucose load, in healthy older subjects presumably reflects the 'protective' effects of gastric distension (140). In addition to the paucity of information about cardiac function in PPH, no studies have evaluated both the hypotensive response to oral carbohydrate and the pressor response to water drinking in patients with PPH, accordingly, it is not known whether these responses are related.

We sought to determine the comparative effects of drinks of water and glucose on BP, HR and cardiac haemodynamics in healthy older subjects and individuals with PPH. We hypothesized that the more substantial fall in BP after the glucose drink in subjects with PPH would be associated with inadequate cardiac compensation, while ingestion of the water drink will elicit comparable cardiac and BP responses between the groups.

Materials and Methods

Subjects

Eight healthy 'older' subjects (4 male and 4 female, age 71.0 ± 1.7 years (range: 66 – 79 years), body mass index (BMI) 26.7 ± 1.1 kg/m^2 (range: 20.5 – 30.3 kg/m^2)) and eight subjects with documented PPH (1 male and 7 female, age 75.5 ± 1.0 years (range: 70 – 79 years), BMI 26.8 ± 1.2 kg/m^2 (range: 19.8 – 31.6 kg/m^2)) were recruited through an existing database, or by advertisements placed in the local hospital campus. All subjects in the PPH cohort had previously demonstrated a fall in systolic BP >20 mmHg within 2 hours of a standardised test meal in recent research studies involving older subjects, did not have overt symptoms related to PPH, and were considered to have 'idiopathic' PPH (286). No subject with PPH was taking medication for the management of this condition, or had severe symptoms referrable to PPH. Five of the healthy subjects had participated in other research studies. Subjects with a history of gastrointestinal disease or surgery, diabetes, significant respiratory or cardiac disease, alcohol abuse or epilepsy, were excluded. Four subjects with PPH were taking an angiotensin II receptor antagonist and one of these was also taking a calcium channel blocker. All medication was withheld for ≥ 24 hours prior to each study day.

Protocol

Subjects were studied on two occasions, separated by a minimum of one week. On each study day, the subject attended the Echocardiography Suite in the Cardiology Unit at The Queen Elizabeth Hospital at 08:00 after an overnight

fast from solids (14 hours) and liquids (12 hours). Upon arrival, the subject was placed in a semi-recumbent position and an intravenous cannula inserted into an antecubital vein for blood sampling. An automated cuff was placed around the upper right arm to measure BP and HR. The subject was then allowed to 'rest' for 15 – 30 min before consuming a drink containing either 75 g glucose and 150 mg C^{13}-acetate (Cambridge Isotope Laboratories, Massachusetts, USA), made up to 300 mL with water; or 300 mL water, within 3 min; t= 0 min was considered as the time of completion of the drink. The order of treatments on the two study days was randomised (random number generator) prior to the enrolment of the first subject. BP, HR and transthoracic echocardiography measurements were obtained immediately prior to the consumption of the drink, and at regular intervals until t= 120 min. GE and blood samples for measurement of glucose were obtained until t= 180 min. Breath samples were only obtained after the glucose drink from t= 0 – 180 min. At t= 180 min the intravenous cannula was removed and the subject offered a light lunch prior to leaving the laboratory. On one of the two days, autonomic function was evaluated following lunch using standardised cardiovascular reflex tests (242).

The protocol was approved by the Human Research Ethics Committee of the Queen Elizabeth/Lyell McEwin/Modbury Hospitals, and each subject provided written, informed consent. All experiments were carried out in accordance with the Declaration of Helsinki.

Blood pressure and heart rate

BP and HR were measured using an automated oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA), every 3 min during the 'rest' period, and between t= 0 – 120 min. Baseline BP was calculated as an average of the three measurements obtained immediately prior to the consumption of the drink (i.e. t= -9, t= -6 and t= -3 min). Maximum changes in BP and HR were calculated as the greatest change from baseline. PPH was defined as a sustained fall in systolic BP of $\geq$ 20 mmHg (12).

Cardiac function and systemic vascular resistance

SV, CO, ejection fraction (EF), E/e' (a measure of diastolic filing pressure) and left ventricular systolic average global longitudinal strain (GLS) were determined by transthoracic echocardiography, using a Vivid 7 ultrasound system (GE Vingmed, Horten, Norway) and a 2.5 MHz phased array transducer, as described previously (287). Pulse-wave Doppler and tissue Doppler imaging was used for the measurement of left ventricular diastolic function. The methods of image acquisition and post-processing of GLS with speckle tracking have been described previously (288). Briefly, the apical 3-, 2-, and 4-chamber high frame rate grayscale acquisitions (40 to 80 frames/sec) were obtained. Echocardiography data were acquired at baseline, immediately prior to drink ingestion, and at 30 min intervals until t= 120 min. Data were analysed by a cardiologist, blinded to the subject group and treatment, with echoPAC BT-13 level software (GE Healthcare Technologies, Sydney, NSW, Australia). GLS data was analysed using the Q-analysis (2-D strain) feature

and all 18 myocardial segments were averaged to obtain the GLS %. In each subject, the sonographer who conducted the echocardiography was the same on the two visits. Systemic vascular resistance (SVR), in dys·s·cm^{-5}, was calculated as: 80 * mean arterial pressure (diastolic BP + 1/3[systolic BP – diastolic BP]), divided by CO (central venous pressure was considered negligible).

Gastric emptying

On the study day with the glucose drink, exhaled breath samples were collected in hermetically sealed 10 mL tubes (Exetainer, Buckinghamshire, England) prior to the ingestion of the drink (t= -3 min), every 5 min for the first hour, and then every 15 min for the subsequent 2 hours, for assessment of GE. The $^{13}CO_2$ concentration in the breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK) with an online gas chromatographic purification system. The gastric 50% emptying time (T50), and gastric emptying coefficient (GEC) were calculated according to the formula by Ghoos et al (237).

Blood glucose

Blood glucose (mmol/L) was determined immediately using a portable glucometer (Medisense Companion 2 meter, Medisense Inc. Waltham, USA) on venous blood samples obtained at t= -3, 30, 60, 90, 120 and 180 min.

Autonomic nerve function

Autonomic nerve function was assessed using standardised cardiovascular reflex tests (242). 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 BP in response to standing. Each test result was scored according to age-adjusted predefined criteria as 0= normal, 1= borderline and 2= abnormal for a total maximum score of 6. A score $\geq$ 3 was considered to indicate autonomic dysfunction (242). Orthostatic hypotension (OH) was defined as a reduction in systolic BP of > 20 mmHg within 3 min of standing.

Statistical analysis

BP, HR, SV, CO, EF, E/e', GLS and SVR were assessed as changes from baseline, whereas GE and blood glucose were analysed as absolute values. The maximum change from baseline (rise or fall) for each variable was calculated. Areas under the curve (AUC) between t= 0 – 120 min were calculated, using the trapezoidal rule. Initial rises (t= 0 – 15 min) and changes in each variable over time (t= 0 – 120 min), during each condition, were assessed with one-way repeated measures ANOVA. Differences between the conditions (i.e. treatment × time effect) and between subject groups (i.e. treatment × group effect) were assessed with two-way repeated measures ANOVA. Differences between the treatments and subject groups were assessed with two-way repeated measures ANOVA of AUC. Maximum changes from baseline, GE parameters and AUC were compared with Student's paired t-test. Relationships between the variables were assessed

with Pearson's correlation. A P value < 0.05 was considered significant in all analysis. Data are presented as mean ± SEM.

Results

The studies were well tolerated and there were no adverse events. Baseline variables are summarised in **Table 8.1.** Subjects with PPH were slightly older than the control subjects (P< 0.05) with no difference in BMI between the groups. No subject had autonomic neuropathy (control: score 1.1 ± 0.2, PPH: 0.88 ± 0.2, P= 0.45). Two subjects with PPH, but no healthy subject, had OH. In 1 healthy, and 1 PPH subject, GE data were unavailable due to degradation of the breath samples. In one subject with PPH, capillary, rather than venous, blood glucose was collected due to restricted venous access. In one subject with PPH, analysis of GLS was not feasible, as the data could not be retrieved by the echoPAC software.

Systolic blood pressure

Healthy older subjects

Baseline systolic BP was slightly higher (P< 0.05) on the study day with water ingestion **(Table 8.1)**. Following the glucose, but not the water drink, there was a transient rise (time effect: P< 0.05) in systolic BP, with a return to baseline at t= ~15 min **(Figure 8.1A)**. Between t= 0 – 120 min there was a modest decrease following the glucose drink (time effect: P< 0.001), and no overall change in systolic BP following the water drink (time effect: P= 0.68) with no difference in the AUC for each treatment (P= 0.47).

PPH subjects

There was no difference in baseline systolic BP between the two days, although mean values were higher prior to the water drink (P= 0.10) **(Table 8.1)**. Following ingestion of both drinks, there was a transient rise (time effect: P< 0.01 for both) in systolic BP, with a return to baseline at t= ~15 min **(Figure 8.1A)**. After the glucose drink, there was a decrease in systolic BP (time effect: P< 0.001); with a maximum fall of -19.8 ± 2.0 mmHg. The AUC for systolic BP was less following the glucose drink (P< 0.05). In 6 of the 8 PPH subjects the maximum fall in systolic BP was > 20 mmHg and in the other 2 the fall was > 10 mmHg. In contrast, following water there was a further rise in mean systolic BP after t= ~15 min which was sustained until t= 120 min and systolic BP between t= 0 – 60 min was higher following the water drink (treatment × time: P< 0.001).

Comparison between groups

Baseline systolic BP was higher (treatment × group: P< 0.05), and the maximum fall in systolic BP following glucose greater (treatment × group: P< 0.05), in the subjects with PPH, compared with the controls. There was a trend for the rise in systolic BP following water between t= 0 – 120 min to be greater (treatment × group: P= 0.07) in the PPH group.

Diastolic blood pressure

Healthy older subjects

There was no difference in baseline diastolic BP between the two study days (P= 0.11) **(Table 8.1)**. Following the glucose, but not the water drink, there was a transient rise (time effect: P< 0.05) in diastolic BP **(Figure 8.1B)**. Between t= 0 – 120 min there was a modest decrease following the glucose drink (time effect: P< 0.001) and no overall change in diastolic BP following the water (time effect: P= 0.24), with no difference in the AUC for each treatment (P= 0.87).

PPH subjects

There was no difference in baseline diastolic BP between the days (P= 0.10) **(Table 8.1)**. Following the glucose, but not the water drink, there was a transient rise (time effect: P< 0.05) in diastolic BP **(Figure 8.1B)**. Between t= 0 – 120 min, there was a decrease (time effect: P< 0.001) after the glucose drink, with a maximum fall of -15.4 ± 1.9 mmHg, no overall change in diastolic BP following the water drink (time effect: P= 0.13) and no difference in the AUC between the two treatments (P= 0.11)

Comparison between groups

There was no difference in baseline diastolic BP on the two study days, between the two groups (treatment × group: P= 0.94). There was a trend for the maximum fall in diastolic BP following the glucose drink to be greater (treatment × group: P= 0.09) in subjects with PPH.

Heart rate

Healthy older subjects

There was no difference in baseline HR between the study days (P= 0.72) **(Table 8.1)**. Following the glucose drink, there was a modest, particularly initial, rise (time effect: P< 0.001) in HR, while after the water drink there was a modest decrease (time effect: P< 0.001) in HR **(Figure 8.1C).** There was a difference in the AUC between the two treatments, so that the AUC for HR was greater following the glucose drink (P< 0.05).

PPH subjects

There was no difference in baseline HR between the two study days (P= 0.45) **(Table 8.1)**. Following the glucose drink there was a modest, particularly initial, rise in HR (time effect: P< 0.05), while following the water drink, there was no significant (time effect: P= 0.27) change in HR **(Figure 8.1C)**. There was a significant difference in the AUC between the two treatments, so that the AUC for HR was greater following the glucose drink (P< 0.01).

Comparison between groups

There was no difference in baseline HR on the two study days between the groups (P= 0.97). While there was no difference in the AUC for each treatment between the groups (treatment × group: P= 0.64), the maximum increase in HR after the glucose drink was greater in the healthy older subjects (treatment × group: P< 0.05).

Cardiac function

There were no differences in baseline parameters on the two study days between the groups **(Table 8.1)**.

Healthy older subjects

Following the glucose drink, there was an increase in SV (time effect: $P< 0.05$), sustained until t= 120 min with a maximum rise of 15.8 ± 3.4 mL, while following the water drink, there was a trend for a small increase in SV (time effect: $P= 0.08$), with a difference in the AUC between the treatments ($P< 0.05$) **(Figure 8.2A)**.

There was a substantial, and sustained, increase in CO following the glucose drink (time effect: $P< 0.05$) while ingestion of water had no effect on CO (time effect: $P= 0.34$), with a difference in the AUC for CO between the treatments ($P< 0.05$) **(Figure 8.2B)**.

Neither drink affected EF (water time effect: $P= 0.10$, glucose time effect: $P= 0.65$) **(Figure 8.2C)** or the E/e' (water time effect: $P= 0.76$, glucose time effect: $P= 0.15$) **(Figure 8.2D)**.

Following the glucose drink, there was a trend for a decrease (time effect: $P= 0.08$) in global longitudinal strain (GLS), while there was a no change in GLS following the water drink (time effect: $P= 0.14$). There was no difference in the AUC between the treatments ($P= 0.42$) **(Figure 8.2E)**.

PPH subjects

Following the glucose drink, there was an increase in SV that was sustained until t= 120 min (time effect: P< 0.001), while ingestion of the water drink had no effect on SV (time effect: P= 0.56). There was a trend (P= 0.06) for a difference in the AUC between the treatments **(Figure 8.2A)**.

Following the glucose drink, there was an increase in CO that was sustained until t= 120 min (time effect: P< 0.05); and a maximum rise of 0.8 ± 0.2 L, while ingestion of water had no effect on CO (time effect: P= 0.38). There was a trend for a difference in the AUC between the treatments (P= 0.09) **(Figure 8.2B)**.

Following the glucose drink there was a trend for an increase in EF (time effect: P= 0.10), while the water drink did not affect the EF (time effect: P= 0.74). There was a trend for the AUC following the glucose drink to be greater (P= 0.07) **(Figure 8.2C)**. Neither drink affected the E/e' **(Figure 8.2D)**.

Following the glucose drink, there was a decrease in GLS (time effect: P< 0.01), while there was no change following the water drink (time effect: P= 0.84). There was a significant difference in the AUC between the treatments (P< 0.05) **(Figure 8.2E)**.

Comparison between groups

There were no differences between the groups in either EF, SV, CO or E/e' after either drink. For GLS, there was a significant treatment effect between

the groups (treatment effect: P< 0.05), so that GLS was lower following the glucose drink, irrespective of subject group, but there was no significant group (P= 0.20) or treatment × group effect (P= 0.38).

Systemic vascular resistance

Healthy older subjects

There was no difference in baseline SVR between the days (P= 0.55) **(Table 8.1)**. Mean SVR initially fell following the glucose drink, but this change was not significant (time effect: P= 0.17) and values had returned to baseline by t= 120 min, and there was no change following the water drink (time effect: P= 0.48). There was a difference in the AUC between the treatments (P< 0.05) **(Figure 8.2F)**.

PPH subjects

There was no difference in baseline SVR between the study days (P= 0.11) **(Table 8.1)**. Mean SVR exhibited a sustained fall following the glucose drink, which did not achieve significance (time effect: P= 0.06), and there was no change following the water drink (time effect: P= 0.74). There was also no difference in the AUC between the treatments (P= 0.16) **(Figure 8.2F)**.

Comparison between groups

There were no differences in baseline SVR on two study days between the groups (P= 0.66). There was a significant treatment effect between the groups

(P< 0.05), so that SVR was less following the glucose drink, irrespective of subject group, but there was no significant group (P= 0.63) or treatment × group effects (P= 0.96).

Blood glucose

Healthy older subjects

There was no difference in baseline blood glucose between the study days (P= 0.36) **(Table 8.1)**. There was a sustained increase in blood glucose following glucose (time effect: P< 0.001), and no change following water (time effect: P= 0.42), with a difference in the AUC between the treatments (P< 0.001).

PPH subjects

There was no difference in baseline blood glucose on the two study days between the groups (P= 0.20) **(Table 8.1)**. There was a sustained increase in blood glucose following glucose (time effect: P< 0.001) and no change in blood glucose following water (time effect: P= 0.75), with a difference in the AUC between the treatments (P< 0.005).

Comparison between groups

There were no differences in baseline blood glucose on the two days between the groups, nor any difference in AUC for each treatment, between the two groups (P= 0.62).

Gastric emptying

There was no difference in gastric emptying of glucose between the groups (Control T50 199 ± 32 min, PPH T50 282 ± 37 min, P= 0.68; Control GEC 3.47 ± 0.12, PPH GEC 3.50 ± 0.11, P= 0.88).

Relationships between responses to water and glucose

In the PPH group, but not the controls, the initial (t= 15 – 20 min) response to ingestion of water and glucose were related, e.g. at t= 18 min, (R= 0.83, P< 0.05). There was also an inverse relationship between the fall in systolic BP with glucose and the rise during water at t= 45 min (R= -0.75, P< 0.05) **(Figure 8.3)**.

Discussion

This study evaluated the comparative effects of 300 mL drinks containing 75 g glucose, or water, on BP, HR and cardiac haemodynamics in healthy older subjects and patients with PPH. In both groups, oral glucose was associated with a fall in BP, increases in HR, SV, CO and improvement in GLS, while ingestion of water was associated with an increase in BP and a modest reduction in HR, without changes in either SV, CO, EF or GLS. In patients with PPH, the pressor response to water tended to be increased and more sustained. The falls in systolic and diastolic BP in response to glucose were, predictably, substantially greater in the PPH group, but the compensatory increase in HR was comparable in both groups. Interestingly, in the PPH group, there was an inverse relationship between the hypotensive response to

glucose and the hypertensive response to water. These observations accordingly suggest that, in PPH, the hypotensive response to oral glucose is associated with inadequate compensatory increases in HR and CO, while the pressor response to water ingestion is maintained and, possibly, exaggerated.

Our study is the first to evaluate cardiac function directly (i.e. SV, CO, EF and GLS) in older subjects with and without PPH. The groups were reasonably well matched demographically- that systolic BP at baseline was higher in the PPH group is not surprising given that hypertension is known to predispose to PPH (243). A 75 g glucose load has been used widely in the diagnosis of PPH (243). That the fall in BP in response to oral glucose was more marked for systolic than diastolic BP is to be expected, given that the former is primarily dependent on preload and contractility, both of which would be reduced if the increase in sympathetic output is inadequate, and the latter is highly dependent on vascular resistance (289). The rate of GE, which is a determinant (46), as well as a risk factor (286), for PPH was comparable in the two groups; this lack of difference is likely to be attributable to the modest size of the two groups (237). Our PPH subjects were relatively 'asymptomatic' and otherwise 'healthy' with no conditions, per se, that might have affected autonomic function. Not surprisingly, none had abnormal cardiovascular autonomic function. In healthy older subjects, following ingestion of a high carbohydrate meal, the postprandial rise in superior mesenteric artery blood flow is not accompanied by a lesser increase in CO compared to the young (128), nor is there a postprandial increase in skeletal muscle vascular resistance (128). While we did not study young subjects,

there were consistent cardiac hemodynamic responses to glucose with increases in SV and CO and an improvement in GLS- the latter is a robust marker of global left ventricular function and a predictor of adverse cardiac events (290). These responses were not influenced by PPH. In healthy young and older subjects intraduodenal glucose infusion is associated with comparable increases in mesenteric blood flow and vascular conductance (124), and we anticipated that oral glucose would be associated with a reduction in SVR, but there was no difference between the two groups. Taken together, our observations, accordingly, indicate that in PPH the hypotensive response to oral glucose is associated with inadequate compensatory increases in both baroreceptor and myocardial function greater than that which usually occurs with normal ageing (291) and suggest that PPH represents a continuum of ageing.

Subjects with PPH exhibited a substantial pressor response to the water drink which tended to be greater, and more sustained, than in health. The response also appeared biphasic, with a nadir at ~15 min followed by a subsequent, sustained rise. This elevation in blood pressure was not associated with changes in SV, CO, EF, GLS or SVR, but a modest reduction in HR, as has been reported in patients with autonomic neuropathy (137, 138). It has been suggested that impairment of baroreflex function accounts for the pressor response to water in healthy older, but not young, subjects, as attested to by the modest fall in HR in comparison to the substantial rise in BP (137). Gastric distension induced by a balloon increases muscle sympathetic nerve activity, the so-called 'gastrovascular reflex,' a response known to be

attenuated in the healthy elderly (84). Water drinking also increases peripheral resistance (138) and may attenuate orthostatic tachycardia in patients with idiopathic orthostatic intolerance (158). Jordan et al (137) reported that the pressor response to water was exaggerated in patients with autonomic failure, some of whom had PPH (consistent with our observations) despite an apparently reduced release of noradrenaline, and postulated that this may reflect upregulation of the vascular α_1-adrenoreceptors and/or impaired baroreflex buffering (137). While direct stimulation of sympathetic activity triggered by visceral stretch is likely to be important, as evidenced by the response to distension of the stomach with a balloon (84), changes in intravascular volume may also be relevant (138). More recently, there is evidence that hypo-osmotic signalling via hepatic afferent fibres may influence sympathetic outflow directly via a local spinal reflex (292). Girona et al (293) reported that the effect of water ingestion on cardiac function in healthy young individuals is also dependent on its temperature- ingestion of water at 30°C and 22°C increased SV, while water at 37°C did not. Recently, Grobety et al (284) studied healthy older adults who drank either 100 mL or 500 mL of water before breakfast and reported that the postprandial fall in systolic BP was less in response to 500 mL, which tended to increase SV and CO more (124). Our observations add to the recommendation for the use of water drinking in the management of PPH (243).

In interpreting our observations, some limitations should be recognised. In particular, the number of subjects with PPH was small, none had severe PPH or PPH associated with autonomic dysfunction (as assessed by standardised

cardiovascular reflex tests) and PPH subjects were older than the healthy controls. It would be of interest to study these groups, e.g. PPH in Parkinson's disease (243). Baseline systolic BP was higher in the PPH group, which may impact on autonomic function and, as discussed, would favour a greater fall in BP (289). Cardiac parameters, BP and HR were evaluated intermittently, rather than on a continuous basis, albeit, relatively frequently and using 2-D, rather than 3-D imaging. Because of the technical demands of the study, we did not measure splanchnic blood flow, or vascular responses in different beds (e.g. skeletal muscle).

In conclusion, in PPH, the hypotensive response to oral glucose is associated with inadequate cardiac compensation, the acute pressor response to water ingestion sustained and possibly more pronounced and hypotensive and pressor responses are inversely related.

Figures and Figure Legends
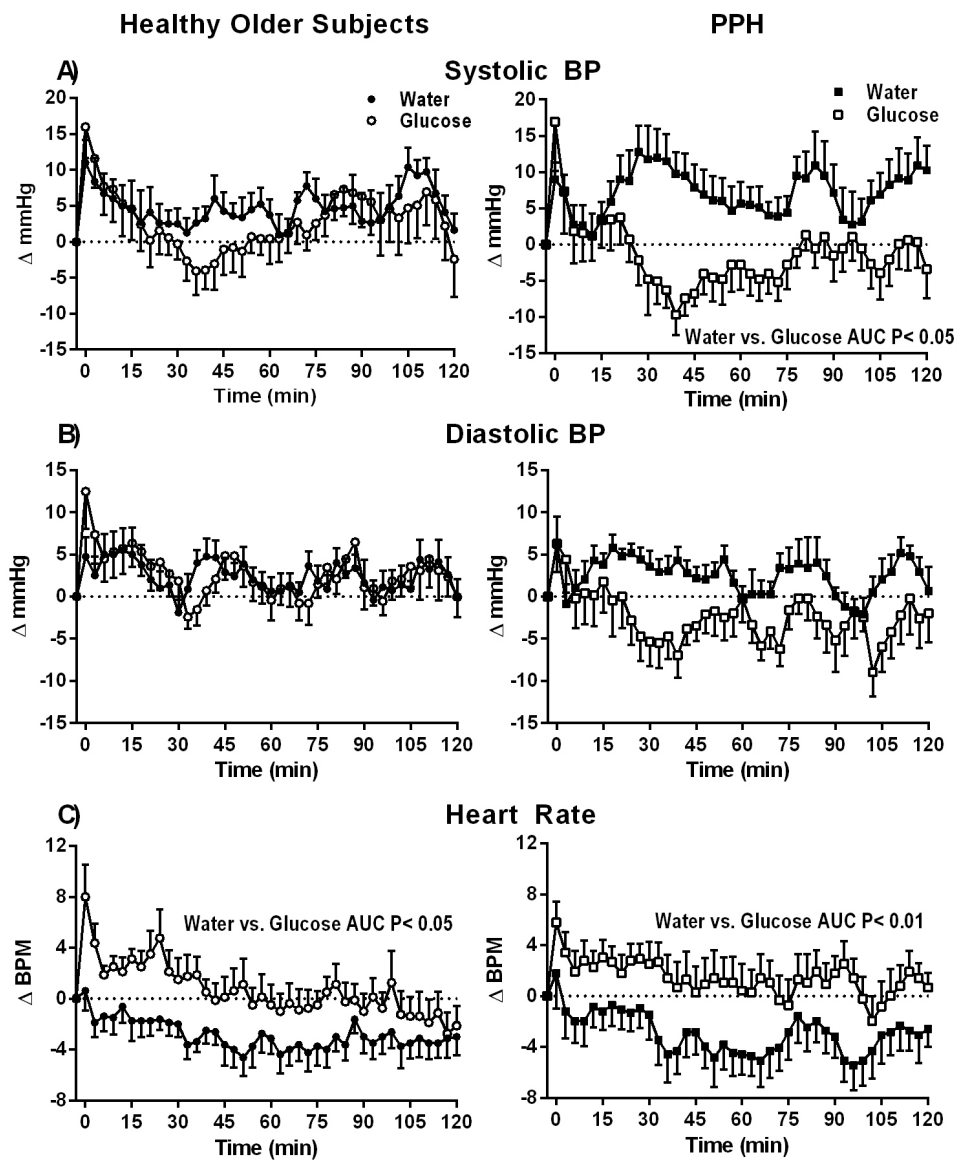
Figure 8.1: (A) Systolic blood pressure (BP), (B) diastolic BP and (C) heart rate before and after 300 mL drinks of 75 g glucose (open symbols) and water (closed symbols) in healthy older subjects (circle) and subjects with postprandial hypotension (PPH) (square).

Healthy Older Subjects   **PPH**

A) **Stroke Volume**

B) **Cardiac Output**

C) **Ejection Fraction**

D) **E/e'**

E) **Global Longitudinal Strain**

F) **Systemic Vascular Resistance**
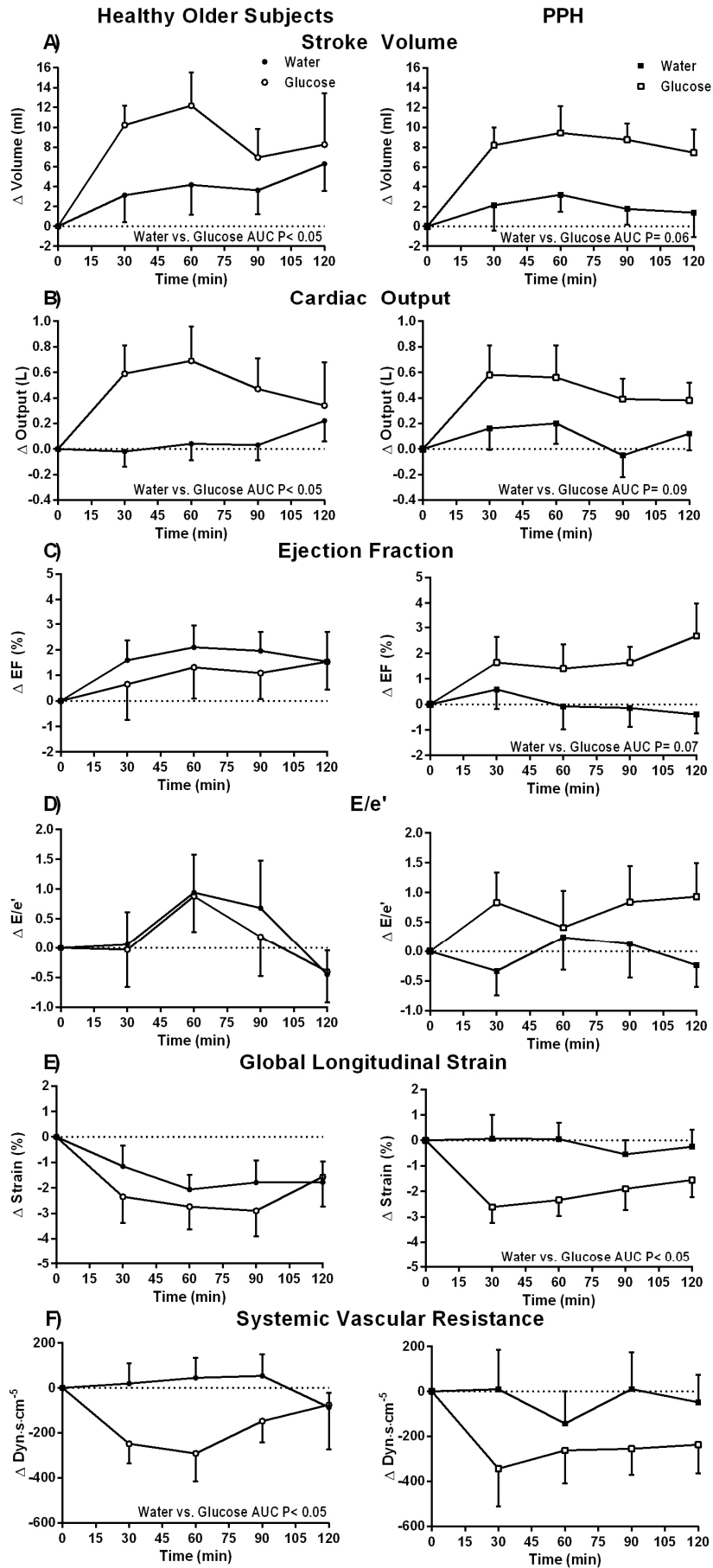
Figure 8.2 (Previous Page): (A) Stroke volume, (B) cardiac output, (C) ejection fraction, (D) E/e', (E) global longitudinal strain, and (F) systemic vascular resistance before and after 300 mL drinks of 75 g glucose (open symbols) and water (closed symbols) in healthy older subjects (circle) and subjects with postprandial hypotension (PPH) (square).

Change in systolic BP at t= 45 min
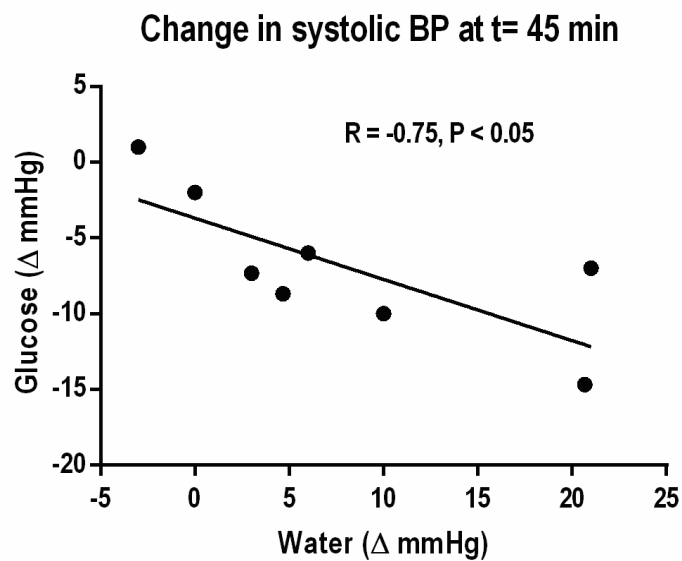
R = -0.75, P < 0.05

Figure 8.3: Relationships between the rise in blood pressure (BP) during water and fall in BP during glucose at t= 45 min (R= -0.75, P< 0.05) in subjects with postprandial hypotension (PPH).

Tables

Table 8.1: Baseline variables.

	Healthy older subjects			PPH			P value (healthy older subjects vs. PPH)
	Water	Glucose	P value (water vs. glucose)	Water	Glucose	P value (water vs. glucose)	
Systolic BP (mmHg)	129.8 ± 4.8	123.1 ± 4.3	< 0.05	147.8 ± 3.3	137.5 ± 6.1	0.10	< 0.05
Diastolic BP (mmHg)	72.6 ± 2.5	69.3 ± 2.7	0.11	73.4 ± 1.6	69.0 ± 2.8	0.10	0.94
Heart Rate (BPM)	62.6 ± 2.8	63.4 ± 3.3	0.72	63.4 ± 2.8	62.4 ± 2.1	0.45	0.97
Stroke Volume (mL)	60.9 ± 3.7	57.3 ± 2.6	0.06	64.8 ± 2.7	63.2 ± 4.0	0.47	0.29
Cardiac Output (L)	3.8 ± 0.2	3.7 ± 0.2	0.20	3.9 ± 0.4	4.0 ± 0.4	0.44	0.59
Ejection Fraction (mL)	59.9 ± 0.9	60.4 ± 1.7	0.72	59.8 ± 1.2	59.1 ± 1.7	0.37	0.72
E/e'	9.0 ± 1.2	8.6 ± 0.8	0.49	10.7 ± 0.6	9.8 ± 0.4	0.07	0.16
Global Longitudinal Strain (%)	-15.5 ± 0.5	-16.2 ± 0.6	0.23	-17.7 ± 1.1	-17.1 ± 1.2	0.34	0.12
Systemic Vascular Resistance ($Dyn{\cdot}s{\cdot}cm^{-5}$)	1949.9 ± 88.1	1918.3 ± 100.3	0.55	2144.6 ± 242.5	1931.2 ± 192.8	0.11	0.66
Blood Glucose (mmol/L)	5.3 ± 0.2	5.4 ± 0.2	0.36	5.3 ± 0.1	5.5 ± 0.1	0.20	0.77

Baseline variables in both groups prior to each treatment in healthy older subjects and subjects with PPH. BP: blood pressure, BPM: beats per minute, PPH: postprandial hypotension. All values are mean ± SEM.

CHAPTER 9: GASTRIC EMPTYING, POSTPRANDIAL BLOOD PRESSURE, GLYCAEMIA AND SPLANCHNIC BLOOD FLOW IN PARKINSON'S DISEASE.

Statement of Authorship

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Principal Author

Candidate	Laurence G Trahair
Contribution	Conception of the study, study design and coordination, subject recruitment, data collection and interpretation, statistical analysis and drafting of the manuscript.
Overall percentage	75%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or

	contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

      i) the candidate's stated contribution to the publication is accurate (as detailed above);

      ii) permission is granted for the candidate in include the publication in the thesis; and

      iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	Feb 2016

Introduction

While gastrointestinal dysfunction occurs frequently in Parkinson's disease (PD) (294, 295), the prevalence of abnormally delayed gastric emptying (GE) remains uncertain because of substantial variations in both the cohorts studied and the methodology used to quantify GE. Delayed GE has been associated with upper gastrointestinal and motor symptoms, as well as impaired absorption of dopaminergic therapy (296, 297).

There is little or no information about the potential impact of GE in two other areas: postprandial blood pressure (BP) and glycaemia. Postprandial

hypotension (PPH), a fall in systolic BP of $\geq$ 20mmHg within 2 hours of a meal (12), was reported for the first time in 1977 in a patient with PD (9) and is a clinically important disorder, predisposing to syncope and falls and being associated with increased mortality (243). PPH may occur frequently in PD, but information is limited (47). It has also been suggested that PPH represents an 'early' marker of autonomic dysfunction in PD (47, 298). Our studies have established that GE is pivotal to the regulation of postprandial BP- in healthy older subjects and patients with type 2 diabetes, the magnitude of the hypotensive response is greater when GE is relatively faster (46). When glucose is infused intraduodenally in healthy older subjects at 1, 2 or 3 kcal/min, there is a substantial fall in systolic BP in response to the 2 and 3 kcal/min, but not the 1 kcal/min, load (120). In contrast to the effect of GE, gastric distension attenuates the fall in BP (136), and consumption of water has been advocated as a treatment for PPH (243). Only one study has evaluated the impact of GE on BP in PD and found no relationship in a cohort of 12 patients with mild to moderate disease (61); BP was not a primary outcome in this study. The hypotensive response to a meal may relate to splanchnic blood pooling, as assessed by measurement of superior mesenteric artery (SMA) blood flow using Doppler ultrasound (119).

GE is an important determinant of postprandial glycaemia, which is a major contributor to 'overall' glycaemic control in diabetes, as assessed by glycated hemoglobin (299). Accordingly, in health (218), subjects with impaired glucose tolerance (218, 220) and type 2 diabetes (220), when GE is faster, there is a greater initial glycaemic response. While diabetes per se does not

appear to increase the propensity to PD (300), type 2 diabetes may be associated with greater impairments in postural stability and gait (301). PD is associated with impaired insulin signalling in the brain (302) and drugs developed for the management of diabetes, particularly glucagon-like peptide-1 agonists, may have efficacy in treatment (303). There is no information about the impact of GE on postprandial glycaemia in PD.

The primary aims of this study were to quantify the GE, BP, SMA flow and blood glucose responses to oral glucose in mild to moderate PD and evaluate the relationships of changes in BP and glycaemia with the rate of GE. We hypothesized that there would be a high prevalence of delayed GE, that consumption of glucose would result in a fall in BP and rises in both SMA flow and blood glucose, and that these responses would be related to GE.

Methods

Subjects

Twenty one subjects with mild to moderate PD were recruited through advertisements placed in a local Parkinson's newsletter, and outpatient referral by a neurologist (TK). Mild to moderate PD was defined as a score $\leq$ 2.5 on the modified Hoehn and Yahr scale (304). Subjects who were unable to move independently, or who had a history of falls, gastrointestinal disease (unrelated to Parkinson's), diabetes, significant respiratory or cardiac disease, alcohol abuse or epilepsy, were excluded. Thirteen males and 8 females, age 64.2 ± 1.6 years (range: 51 – 77 years), body mass index (BMI) 25.2 ± 0.8

kg/m^2 (range: 20.3 – 34.5 kg/m^2) and known duration of PD 6.3 ± 0.9 years (range: 1 – 16 years), were studied. Two patients were receiving antihypertensive drugs, which were withdrawn for 24 hours before the study day. Details of anti-Parkinsonian medication are summarised in **Table 9.1**. Four subjects had received deep brain stimulation for the management of their PD.

Protocol

At an initial screening visit 6 – 65 days before the study day, a medical history and staging of Parkinson's symptoms on the modified Hoehn and Yahr scale were performed by a neurologist (TK) (304) and a questionnaire to asses symptoms referrable to delayed GE completed (219, 305).

On the study day, subjects attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry at the Royal Adelaide Hospital at 08:30 after an overnight fast from solids (14 hours) and liquids (12 hours). Where possible, subjects were asked to withhold the morning dose of anti-Parkinsonian medication. On arrival, the subject was seated in front of a gamma camera and an intravenous cannula inserted into the left antecubital vein for blood sampling. An automated cuff was placed around the upper right arm to measure BP and HR. The subject was then allowed to 'rest' for approximately 15 min (120). At t= -3 min, the subject consumed a drink comprising 75 g glucose and 5 g 3-O-Methyl-D-gluco-pyranose (3-OMG) (Carbosynth, Berkshire, UK) dissolved in water (total drink volume 300 mL), labelled with 20 MBq ^{99m}Tc-calcium phytate (Radpharm Scientific,

Belconnen, ACT, Australia) within 3 min. GE, BP, SMA blood flow and blood glucose were measured for 180 min following the drink. At t= 180 min, the intravenous cannula was removed and the subject given a meal. Evaluation of autonomic function, using standardised cardiovascular reflex tests (242), was then performed, prior to the subject leaving the laboratory.

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

Gastric emptying

Radioisotopic data was acquired for 180 min following consumption of the drink (60 sec frames between t= 0 – 60 min, then 180 sec frames from t= 60 – 180 min), where t= 0 was the time of completion of the drink. Data were corrected for subject movement, radionuclide decay and γ-ray attenuation (107). A region-of-interest was drawn around the total stomach and gastric emptying curves (expressed as percentage retention over time) derived. The amount of the drink remaining in the total stomach at 15 min intervals between t= 0 – 180 min, as well as the 50% gastric emptying time (T50) (107), were calculated. The normal range for the T50 of this drink is 43 – 157 min, based on data in 21 healthy subjects (age 64.8 ± 1.8 years), matched for age (i.e. within 2 years) to each subject with PD (220). GE was considered to be abnormally fast or slow when the T50 was above, or below, this normal range.

Blood pressure and heart rate

BP and HR were measured using an automated BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA), every 3 min during the 'rest' period, and from t= 0 – 180 min. Baseline BP was calculated as an average of the three measurements obtained immediately prior to the consumption of the drink (i.e. t= -9, t= 6 and t= -3 min) (120). Maximum changes in BP and HR were calculated as the greatest change that occurred from baseline. Subjects were categorised according to the maximum fall in systolic BP following the drink, i.e. those in which the fall was ≤ 10 mmHg, > 10 mmHg but < 20 mmHg and ≥ 20 mmHg. PPH was defined as a sustained (> 10 min) fall in systolic BP of ≥ 20 mmHg (12).

Superior mesenteric artery blood flow

SMA flow was measured using a Logiq^{TM} e ultrasound system (GE Healthcare Technologies, Sydney, NSW, Australia) and a 3.5C broad spectrum 2.5 – 4 MHz convex linear array transducer. Measurements were obtained immediately prior to the consumption of the drink (t= -3 min), every 15 min between t= 0 – 60 min, and then at t= 90 min, 120 min and 180 min. Blood flow (mL/min) was calculated automatically using the formula: $\pi \times r^2 \times$ TAMV x 60, where r= the radius of the SMA and TAMV is the time-averaged mean velocity (119). In all subjects two measurements were acquired by the same, experienced investigator (LT) at each time point.

Blood glucose

Venous blood was sampled immediately prior to the consumption of the drink (t= -3 min), every 15 min between t= 0 – 60 min and then at t= 90 min, 120 min and 180 min. Blood glucose (mmol/L) was determined immediately using a portable glucometer (Medisense Companion 2 meter, Medisense Inc. Waltham, USA). Results were classified, according to World Health Organisation criteria, as normal glucose tolerance (NGT) (fasting blood glucose < 6.1 mmol/L, and 2 hour < 7.8 mmol/L), impaired fasting glucose (IFG) (fasting blood glucose < 7.0 mmol/L, but > 6.1 mmol/L), impaired glucose tolerance (IGT) (2 hour blood glucose < 11.1 mmol/L, but > 7.8 mmol/L), or diabetes (fasting blood glucose ≥ 7.0 mmol/L and/or 2 hour blood glucose ≥ 11.1 mmol/L) (244).

Upper gastrointestinal symptoms

Upper gastrointestinal symptoms assessed at the screening visit by questionnaire (219), included anorexia, nausea, early satiety, bloating, vomiting, abdominal pain, dysphagia, heart burn and acid regurgitation. Each was scored as: 0= none, 1= mild, 2= moderate or 3= severe, for a maximum score of 27 (219).

Cardiovascular autonomic nerve function

Autonomic nerve function (ANF) was assessed using standardised cardiovascular reflex tests (242). 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 BP in response to standing. Each of the results was scored according to age-adjusted predefined criteria as 0= normal, 1= borderline and 2= abnormal for a total maximum score of 6. A score $\geq$ 3 was considered to indicate definite autonomic dysfunction (241, 242). Orthostatic hypotension (OH) was defined as a sustained reduction in systolic BP of >20 mmHg within 3 min of standing (13).

Statistical analysis

BP and HR were assessed as changes from baseline, whereas GE, SMA flow and blood glucose were analysed as absolute values. The maximum changes from baseline in BP, HR and blood glucose were also calculated. Areas under the curve (AUC) were calculated for BP, HR, SMA flow and blood glucose using the trapezoidal rule. Changes in each variable over time were evaluated with ANOVA. Pearson's correlation was used to evaluate relationships between variables. Relationships of BP, upper gastrointestinal symptoms and glycaemia with GE were assessed using the GE T50, given the observed overall linear pattern. A P value < 0.05 was considered significant in all analyses. The number of subjects included was based on power calculations derived from our previous study (46). The statistical analysis was supervised and reviewed by a professional biostatistician. Data are presented as mean ± SEM.

Results

The studies were well tolerated and no adverse events were reported. The mean Hoehn and Yahr score was 1.4 ± 0.1 (range: $1 - 2.5$) and duration of known PD 6.3 ± 0.9 years (range: $1 - 16$ years). Three subjects were unwilling, or unable to withhold their morning anti-Parkinson medications because of the risk of significant motor dysfunction. Three subjects had definite autonomic neuropathy, in 10 subjects, the score was ≥ 2; the mean ANF score was 1.8 ± 0.3 (range: $0 - 5$); 5 subjects had OH. Eight subjects had PPH. In another 8, the maximum fall was >10 mmHg but < 20 mmHg and in 5 subjects the fall was < 10 mmHg. Four of the 5 subjects with OH also had PPH. The mean score for upper gastrointestinal symptoms was 1.5 ± 0.4 (range: $0 - 5$).

Gastric emptying

Gastric emptying of the drink approximated an overall linear pattern. The T50 was 106 ± 0.5 min. In three subjects, GE (T50) was abnormally slow; no subject had abnormally rapid GE.

Blood pressure and heart rate

Baseline systolic BP was 116.9 ± 2.4 mmHg. Following the drink, there was a transient modest rise, followed by a fall, in systolic BP (P< 0.001, **Figure 9.1A**), which was sustained until the end of the study. The maximum fall was -18.6 ± 2.0 mmHg, occurring at t= 76.5 ± 12.8 min.

Baseline diastolic BP was 69.1 ± 1.6 mmHg. Following the drink, there was a transient initial rise, and then a fall, in diastolic BP (P< 0.001, **Figure 9.1B**), with a nadir between t= 30 – 45 min, which was sustained until the end of the study. The maximum fall in diastolic BP was -15.6 ± 0.9 mmHg, occurring at t= 85.9 ± 11.6 min.

Baseline heart rate was 69.5 ± 2.1 BPM. Following the drink, there was an increase in heart rate (P< 0.001, **Figure 9.1C**), which had returned to baseline by ~t= 60 min. The maximum increase in HR was 9.5 ± 0.7 BPM occurring at 75.6 ± 13.3 min.

Superior mesenteric artery blood flow

Baseline SMA flow was 565.0 ± 62.5 mL/min. Following the drink, there was a prompt increase in SMA flow (P< 0.001, **Figure 9.2**), which had returned to baseline by t= 180 min. The maximum SMA flow was 1208.8 ± 123.0 mL/min, occurring at t= 54.8 ± 8.1 min.

Blood glucose

Baseline blood glucose was 5.6 mmol/L. Following the drink, there was an increase in blood glucose (P< 0.001, **Figure 9.3**), which had returned to baseline by t= 180 min. The maximum blood glucose was 10.2 ± 0.5 mmol/L, occurring at 48.9 ± 4.0 min. 5 subjects had IGT, 2 had both IFG and IGT and 1 had 'marginal' diabetes (fasting and 2 hour blood glucose of 7.1 mmol/L and 11.1 mmol/L, respectively).

Relationships between variables

There were no significant relationships between the changes in systolic BP, diastolic BP, HR or SMA flow at any time point (absolute values and AUCs). The T50 was related directly to the ANF score (R= 0.55, P< 0.01, **Figure 9.4**). Upper gastrointestinal symptoms were also related to the score for ANF (R= 0.45, P< 0.05), but not GE.

There was an inverse relationship between the blood glucose at t= 30 min (R= -0.52, P< 0.05, **Figure 9.5**), while the blood glucose at t= 180 min (but not 120 min) was related directly (R= 0.49, P< 0.05) to the T50.

There were no significant relationships between T50, Hoehn and Yahr score, duration of disease, or age.

Discussion

Our study has quantified the GE, BP, SMA and glycaemic responses to oral glucose in mild to moderate PD. In the majority of patients, oral glucose induced a significant fall in systolic BP; i.e. in 16 of 21 patients (76%), this fall was > 10 mmHg and 8 (38%) had PPH. GE of glucose was abnormally delayed in 3 patients (14%), a prevalence lower than we anticipated and slower in those patients with cardiovascular autonomic neuropathy, and gastric emptying was not accelerated in any subject. There was, however, no relationship between the magnitude of the fall in BP with GE. A relationship between the initial glycaemic response to glucose with GE, comparable to that observed in subjects without PD, was demonstrated.

The outcome of studies relating to the prevalence of disordered GE in PD is inconsistent. We measured GE using the 'gold-standard' technique of scintigraphy and, while a liquid, rather than a solid, 'meal' was used, the precision of solid and high-nutrient liquid meals in the diagnosis of delayed GE appears comparable (306). It should, however, be recognised that our definition of delayed GE- a T50 that was greater than the range observed in healthy subjects, was deliberately stringent, so that more modest gastric motor function cannot be excluded. The observations of a relationship between GE and the severity of autonomic dysfunction and the high prevalence of autonomic dysfunction are not surprising. The pathophysiology of disordered GE in PD is heterogeneous- alpha-synuclein aggregation, abnormalities in the dorsal motor nucleus of the vagus and enteric nervous system, and drugs such as L-dopa may all be important (294). As with previous studies, there was no significant relationship between GE and the duration of PD (307). Patients had mild upper gastrointestinal symptoms, possibly in part because the majority were studied off dopaminergic therapy, although symptoms were more common in patients with impaired ANF.

The high prevalence of PPH is comparable to that reported previously- 8% had PPH and the fall in systolic BP was $\geq$ 10 mmHg in 16 of 21 (76%) subjects (47). That the latter may have adverse consequences, even in apparently 'asymptomatic' patients (71), dictates the need for greater recognition. We did not observe a relationship between the magnitude of the fall in BP and GE, for which there are a number of potential explanations. Baseline systolic BP was in most cases 'normal,' which is predictive of a

smaller postprandial fall (33). We have demonstrated in healthy older subjects that the relationship between the fall in BP and the rate of duodenal glucose delivery is non-linear, so that a 'threshold' between 1 – 2 kcal/min must be exceeded to elicit a hypotensive response (120). In the current study, based on the T50, GE was $\geq$ 2 kcal/min in only 4 subjects. Hence, it would be appropriate to re-evaluate this hypothesis further in a larger group of patients. The current study certainly does not exclude the possibility that PD patients with relatively more rapid GE are at increased risk for PPH.

There was an approximate doubling in SMA flow following the glucose drink, as anticipated. In healthy subjects and patients with autonomic failure (123), comparable increases in SMA flow have been observed, but a reduction in BP was only evident in patients with autonomic failure, probably reflecting inadequate sympathetic compensation (123). The absence of a relationship between BP and SMA flow, may reflect the relatively narrow distribution of the rises in SMA flow, and modest size of the cohort. OH is a frequent manifestation of autonomic involvement in PD and a concordance of PPH and OH in PD has been reported (47), and supported by our study.

The relationship between the initial glycaemic response to the drink and the rate of GE in PD is consistent with observations in health (218), impaired glucose tolerance (218, 220), and type 2 diabetes (220) as well as the effect of delayed GE on the absorption of L-dopa in PD (294). It is now recognised that postprandial glycaemic excursions are a major determinant of overall glycaemic control in type 2 diabetes, assuming increasing importance as

glycated hemoglobin normalises (299). 8 of our 21 subjects (38%) had impaired glucose tolerance (7 subjects) or 'marginal' diabetes (1 subject); that the blood glucose level at 180 min, but not 120 min, was inversely, rather than directly, related to GE, presumably reflecting higher insulin levels achieved earlier, associated with insulin resistance (218). In healthy subjects an inverse relationship is evidence at 120 min after a 75 g oral glucose load (220). The recognition that GE is a determinant of glycaemia in PD is not surprising, but potentially important- slower GE, including that induced by dopaminergic therapy, would potentially be advantageous in optimising glycaemic control in type 2 patients with PD. Interestingly, GLP-1 agonists, such as exenatide BD which are undergoing evaluation of their efficacy in the management of PD (303), diminish postprandial glycaemic excursions primarily by slowing GE (211).

In interpreting our observations, it should be recognised that 3 subjects did not withdraw their medication, which may represent a cofounder. We also did not include a control (water) drink because of potential ethical concerns. A normal range for GE allowed the prevalence of disordered GE in PD to be determined- a formal control group was not included because the focus of the present study was on relationships between variables within the Parkinson's group. As discussed, one subject had diabetes, based on fasting and 2 hour blood glucose, but these levels were only marginally above the diagnostic cut-offs and this subject was not excluded.

In conclusion, in this unselected population of patients with mild to moderate PD, GE was delayed in only a minority, oral glucose induced a substantial reduction in BP, as well as rises in SMA flow and blood glucose, and GE was an important determinant of the glycaemic, but not the BP, response.

Figures and Figure Legends
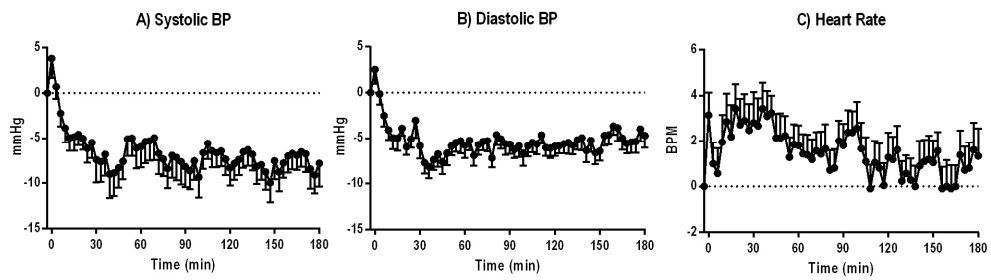
Figure 9.1: Systolic blood pressure (BP) (A), diastolic BP (B) and heart rate (C) immediately before and after 75 g oral glucose load in 21 patients with Parkinson's disease.
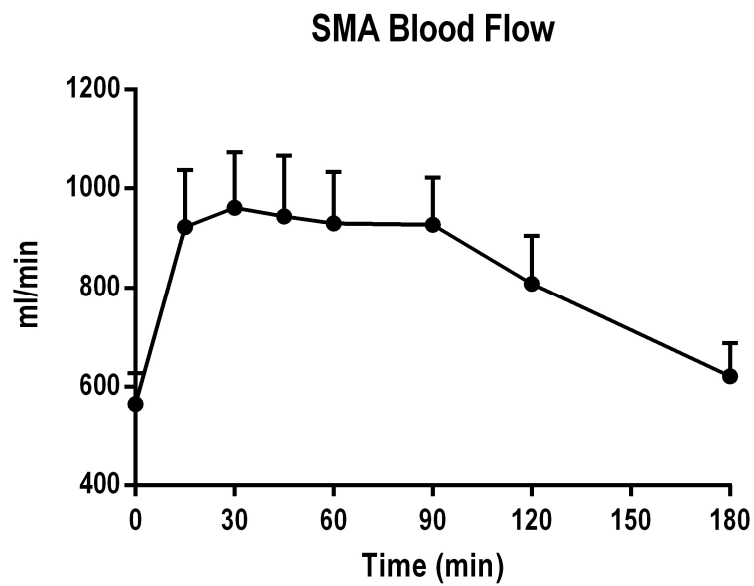
Figure 9.2: Superior mesenteric artery (SMA) blood flow immediately before and after 75 g oral glucose load in 21 patients with Parkinson's disease.
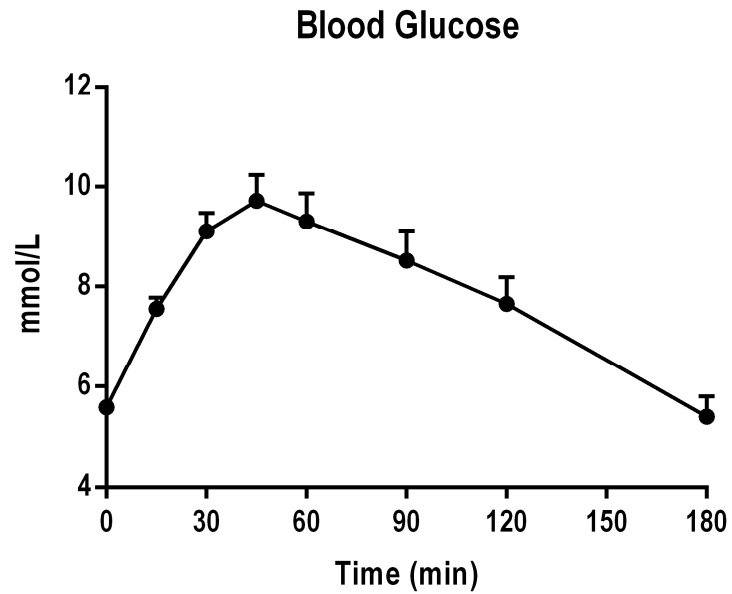
Blood Glucose

Figure 9.3: Blood glucose immediately before and after 75 g oral glucose load in 21 patients with Parkinson's disease.

Relationship between T_{50} and ANF score
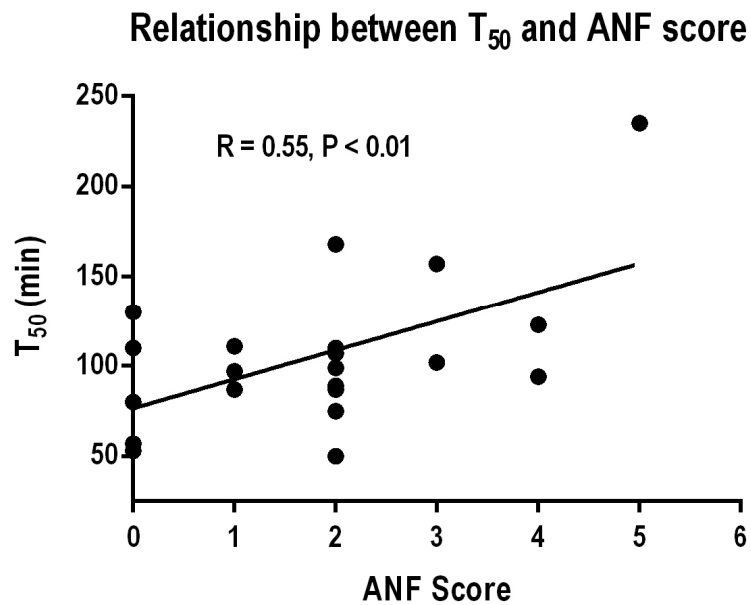
Figure 9.4: Relationship between gastric half emptying time (GE T50) and autonomic nerve function (ANF) score (R= 0.55, P< 0.01).

Relationship between blood glucose (t = 30 min) and T$_{50}$
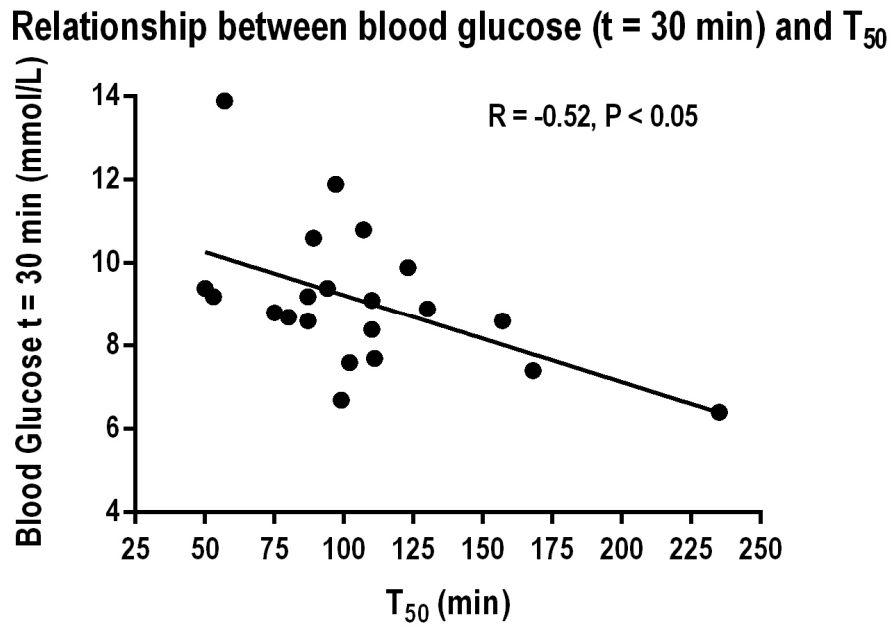
Figure 9.5: Relationship between the absolute blood glucose at t= 30 min with the gastric half emptying time (GE T50) (R= -0.52, P< 0.05).

Tables

Table 9.1: List of anti-Parkinsonian medications in 21 patients with

Parkinson's disease.

Drug	Number of patients	%
Pramipexole	11	52
Levodopa	9	43
Levodopa & Carbidopa	8	38
Levodopa, Carbidopa & Entacapone	4	19
Rasagiline	3	14
Amantadine	1	5
Apomorphone	1	5
Pregabalin	1	5
Selegiline	1	5

CHAPTER 10: EFFECTS OF EXOGENOUS GLUCAGON-LIKE PEPTIDE-1 ON THE BLOOD PRESSURE, HEART RATE, MESNETERIC BLOOD FLOW AND GLYCAEMIC RESPONSES TO INTRADUODENAL GLUCOSE IN HEALTHY OLDER SUBJECTS.

Statement of Authorship

Title of paper	Effects of exogenous glucagon-like peptide-1 on the blood pressure, heart rate, mesenteric blood flow, and glycemic responses to intraduodenal glucose in healthy older subjects.
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Principal Author

Candidate	Laurence G Trahair
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	manuscript.		
Overall percentage	75%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution	Conception and design of the study, data interpretation, statistical analysis, drafting of the manuscript and overall responsibility for the study.		
Signature		Date	Feb 2016

Introduction

Postprandial hypotension (PPH), defined as a fall in systolic blood pressure (BP) of > 20mmHg, within 2 hours of a meal (12), is now recognised as an important clinical problem, occurring frequently in the elderly and patients with autonomic dysfunction (15, 37) and being associated with a number of adverse sequelae, particularly syncope and falls (70), as well as increased mortality (10). Current management is suboptimal (243).

Our studies have established that the hypotensive response to a meal is triggered by the interaction of nutrients, as determined by meal composition, with the small intestine and that gastric distension, whether non-nutrient or nutrient-induced, attenuates this fall (136, 140). Accordingly, when healthy young and older subjects received intraduodenal (ID) glucose infusions at rates, spanning the physiological range for gastric emptying (GE) (142) of 1, 2 or 3 kcal/min, there was no change in BP in 'young' subjects, whereas in 'older' subjects systolic BP fell substantially in response to the 2 and 3 kcal/min loads (124). This hypotensive response was related to the marked increase in splanchnic blood flow, as assessed by measurement of superior mesenteric artery (SMA) blood flow with Doppler ultrasound (124). Conversely, gastric distension with a balloon, at volumes as low as 100 mL, abolished the hypotensive response to a 3 kcal/min ID glucose infusion in healthy older subjects (85).

There is considerable interest in the pharmacological effects of glucagon-like peptide-1 (GLP-1), particularly with the advent of GLP-1 agonists, which are

now used widely in the management of type 2 diabetes (308, 309). Amongst its properties, pharmacological doses of GLP-1 slow GE, stimulate insulin and suppress glucagon- the latter two effects in a dose-dependent manner (262). The acute slowing of GE by exogenous GLP-1 is substantial, so that the consequent reduction in postprandial glucose is associated with a reduction, rather than an increase, in plasma insulin (211, 310). The slowing of GE by exogenous GLP-1 is subject to tachyphylaxis with sustained exposure (311). Short-acting GLP-1 agonists, such as twice daily exenatide and once-daily lixisenatide, have a more robust effect to reduce postprandial glucose, whereas long-acting GLP-1 agonists, such as once-daily liraglutide and once-weekly exenatide, have a more pronounced effect on fasting/preprandial plasma glucose (312), differences which are likely to reflect the pharmacokinetic profile of GLP-1 receptor activation as a result of the impact on the slowing of GE (312). We have reported that the α-glucosidase inhibitor, acarbose, used extensively in type 2 patients, attenuates the hypotensive response to oral (107), and ID (163), sucrose in healthy older subjects, an effect which is associated temporally with marked stimulation of GLP-1, presumably reflecting the presence of unabsorbed carbohydrate in the distal small intestine, where the GLP-1 producing L-cells are predominantly located. We have, accordingly, reasoned that exogenous GLP-1 (and 'short-acting' GLP-1 agonists) have potential application to the management of postprandial hypotension by slowing GE. However, GLP-1 and GLP-1 agonists have other cardiovascular effects, which are receiving increased attention (313). In animals, the effects of GLP-1 on BP and heart rate (HR) are species-specific, but in most cases increases in both have been observed after acute

administration (314), which may relate to changes in sympathetic nerve activity and vasopressin (315). In humans, exogenous GLP-1 has been reported to increase BP in two studies (316, 317), but not another (318). GLP-1 may also affect blood flow and endothelial function (319), effects which may not be mediated by the known GLP-1 receptor (320). The majority of clinical trials relating to the effects of GLP-1 agonists in type 2 diabetes or obesity have reported a reduction in systolic BP of 2 – 6 mmHg and a rise in HR of 2 – 4 BPM, changes which appear to be independent of weight loss (313). Importantly, to our knowledge, none of these studies has discriminated between potential effects on fasting and postprandial BP and HR.

Given that GLP-1 has the potential to modulate splanchnic blood flow, we sought in this study to determine whether exogenous GLP-1 modulates the effects of an ID glucose infusion (bypassing the potential effects of gastric distension) on BP, HR and splanchnic blood flow in healthy older subjects.

Materials and Methods

Subjects

Ten healthy 'older' subjects (9 male and 1 female, mean age 73.2 ± 1.5 years (range: 69 – 79 years), body mass index (BMI) 26.2 ± 0.8 kg/m2 (range: 20.3 – 30.5 kg/m2)), were recruited through an existing database, or by advertisements placed in the local hospital campus. Subjects with a history of gastrointestinal disease or surgery, known diabetes, significant respiratory or cardiac disease, alcohol abuse or epilepsy, were excluded. All medication was

withheld for 24 hours prior to the study. Diabetes had been excluded by an oral glucose tolerance test in 4 subjects and measurement of glycated hemoglobin in 3 others. All subjects had normal fasting blood glucose. 7 subjects had participated previously in research studies involving nasogastric intubation.

Protocol

Subjects were studied on two occasions, separated by a minimum of one week. On each study day, the subject attended the laboratory at 08:30 after an overnight fast from solids (14 hours) and liquids (12 hours). There was no formal restriction on the type of fluid consumed before each study. Upon arrival, the subject was seated and a silicone rubber multilumen nasoduodenal catheter (external diameter ~4 mm) (Dentsleeve International, Mui Scientific, Mississauga, Canada) was inserted into the stomach via an anesthetised nostril and allowed to pass into the duodenum by peristalsis. The catheter incorporated an infusion channel (internal diameter ~1 mm) opening 10 cm distal to the pylorus. Two other channels, located in the antrum (2.5 cm proximal to the pylorus) and duodenum (2.5 cm distal to the pylorus), were perfused continuously with 0.9% saline and the correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference (TMPD) in the antral (-40 mV) and duodenal (0 mV) channels (124, 239). For the purpose of measuring TMPD, a 0.9% saline-filled cannula was inserted subcutaneously into the subject's forearm (239). After the catheter had been positioned correctly, the subject was placed in a recumbent position and intravenous (IV) cannulae were inserted into the left

antecubital vein for blood sampling (blood glucose and serum insulin) and into the right antecubital vein for infusion of GLP-1. An automated cuff was placed around the upper left arm to measure BP and HR. The subject was then allowed to 'rest' for approximately 15 min (124). Superior mesenteric artery (SMA) blood flow was quantified using duplex ultrasonography (124)

Commencing at t= -30 min, each subject received an IV infusion of GLP-1 (Bachem, Bubendorf, Switzerland) at a rate of 0.9 pmol.kg^{-1}.min^{-1}, or control (0.9% saline) for 90 min (i.e. t= -30 – 60 min). 30 min after the commencement of this infusion, an ID infusion of glucose at 3 kcal/min was commenced and continued for 60 min (i.e. t= 0 – 60 min). All infusions were performed using an automated volumetric infusion pump (IMED Gemini PC-1: IMED Corporation, San Diego, CA, USA). The order of treatments on the two study days was randomised and double-blind, being determined by an independent investigator prior to the enrolment of the first subject; this investigator also prepared the GLP-1 infusions, to ensure allocation concealment. At t= 60 min the nasoduodenal catheter and IV cannulae were removed and the subject offered a meal prior to leaving the laboratory. On one of the two days autonomic function was evaluated using standardised cardiovascular reflex tests (242).

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

Blood pressure and heart rate

BP and HR were measured using an automated oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA), every 3 min for 15 min prior to the IV infusion, i.e. t= -45 to -30 min, and then every 3 min between t= -30 – 60 min. Pre-IV baseline BP and HR were calculated as an average of the three measurements obtained immediately prior to the commencement of the IV infusion (GLP-1 or saline) i.e. t= -39, t= -36 and t= -33 min. Pre-ID baseline BP and HR were obtained immediately prior to the commencement of the ID glucose infusion i.e. t= -3 min. The maximum changes in BP and HR were calculated as the greatest change that occurred from baseline. PPH was defined as a fall in systolic BP $\geq$ 20 mmHg sustained for at least 20 min.

Superior mesenteric artery blood flow

SMA flow was measured using a LogiqTM e ultrasound system (GE Healthcare Technologies, Sydney, NSW, Australia) and a 3.5C broad spectrum 2.5 – 4 MHz convex linear array transducer. Measurements were obtained immediately prior to the commencement of the IV and ID infusions, i.e. t= -33 and -3 min, and then every 15 min between t= 0 – 60 min. Blood flow (mL/min) was calculated automatically using the formula: π x r2 x TAMV x 60, where r= the radius of the SMA and TAMV is the time-averaged mean velocity [119]. In all subjects at each time point, two measurements were acquired by the same experienced investigator (LT), and these measurements were reviewed and confirmed by a second, independent investigator (TH). Both investigators were blinded to the intervention.

Blood glucose and serum insulin

Venous blood was sampled immediately prior to the commencement of the IV and ID infusions, i.e. t= -33 and -3 min, and then every 15 min from t= 0 – 60 min. Blood glucose (mmol/L) was determined immediately using a portable glucometer (Medisense Companion 2 meter, Medisense Inc. Waltham, USA). Serum insulin was measured by ELISA (10-1113, Mercodia, Uppsala, Sweden). The sensitivity of the assay was 1.0 mU/L and the coefficient of variation was 2.6% within, and 7.6% between, assays (222).

Cardiovascular autonomic nerve function

Autonomic nerve function was assessed on one of the two days using standardised cardiovascular reflex tests (242). Parasympathetic function was evaluated by the variation (R - R interval) of the heart rate during deep breathing and the response to standing ("30:15" ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0= normal, 1= borderline and 2= abnormal for a total maximum score of 6. A score > 3 was considered to indicate autonomic dysfunction (241, 242).

Statistical analysis

Blood pressure, HR and SMA flow were assessed as changes from the pre-IV or pre-ID baseline, whereas blood glucose and serum insulin were analysed as absolute values. The maximum change from baseline for BP, HR and SMA

flow were also calculated. Areas under the curve (AUC) were calculated for BP and HR between t= -30 and 0 min, and for all variables between t= 0 – 60 min. Baseline measurements (i.e. pre-IV and pre-ID), and changes in each variable over time, were evaluated with repeated measures ANOVA, and post-hoc pairwise analysis were conducted if an effect was present. Maximum change from baseline and AUC were compared with Student's paired t-test. A P value < 0.05 was considered significant in all analyses. The number of subjects included was based on power calculations derived from our previous studies in which glucose has been administered intraduodenally at a rate of 3 kcal/min to healthy older subjects, with 80% power to detect a 7 mmHg difference in the maximum fall in systolic BP between the study days (120). Data are presented as mean ± SEM.

Results

The studies were well tolerated and there were no adverse events. No subject had autonomic neuropathy (mean score 1.0 ± 0.3, range: 0 – 2) or postprandial hypotension.

Blood pressure and heart rate

There was no difference in baseline systolic BP before the IV infusion (**Table 10.1**) and no change in systolic BP between t= -30 – 0 min, nor any difference in AUC for systolic BP. Similarly, there was no difference in systolic BP immediately prior to the commencement of the ID infusion (**Table 10.1**). Between t= 0 – 60 min, there was a fall in systolic BP (Control: P< 0.001,

GLP-1: P< 0.001), and the magnitude of this decrease was greater with saline than GLP-1 (AUC: Control: -369 ± 156 mmHg vs. GLP-1: -30 ± 137 mmHg, P< 0.005). Similarly, the maximum decrease in systolic BP was greater with saline (Control: -13.6 ± 3.1 mmHg vs. GLP-1: -8.7 ± 2.3 mmHg, P< 0.05) **(Figure 10.1a)**.

There was no difference in baseline diastolic BP before the IV infusion **(Table 10.1)** and no change between t= -30 – 0 min, nor any difference in the AUC for diastolic BP. Similarly, there was no difference in diastolic BP immediately prior to the commencement of the ID infusion **(Table 10.1)**. Between t= 0 – 60 min, there was a modest fall in diastolic BP (Control: P< 0.001, GLP-1: P< 0.001), without any differences in the magnitude of this decrease (AUC: Control: -150 ± 113 mmHg vs. GLP-1: -210 ± 76 mmHg). Similarly, there was no difference in the magnitude of the maximum fall in diastolic BP (Control: -9.3 ± 2.2 mmHg vs. GLP-1: -11.8 ± 1.5 mmHg) **(Figure 10.1b)**.

There was no difference in baseline HR before the IV infusion **(Table 10.1)** and no change between t= -30 – 0 min, there was no change in HR with either condition, nor were there any differences in AUC for HR **(Table 10.1)**. There was no difference in HR immediately prior to the commencement of the ID infusion. Between t= 0 – 60 min there was an increase in HR during both conditions (Control: P< 0.001, GLP-1: P< 0.001), without any difference overall (AUC: Control: 429 ± 125 BPM vs. GLP-1: 451 ± 93 BPM), however,

the maximum HR tended to be greater with GLP-1 (Control: 12.9 ± 2.7 BPM vs. GLP-1: 14.9 ± 2.6 BPM, P= 0.09) **(Figure 10.1c)**.

Superior mesenteric artery blood flow

There were no differences in baseline SMA flow before the IV infusion **(Table 10.1)**. Between t= -30 and 0 min there was a modest decrease in SMA flow during GLP-1, and rise during saline (Control: P< 0.05, GLP-1: P< 0.05), without any difference between them. Between t= 0 – 60 min, there was a prompt increase in SMA flow during both conditions (Control: P< 0.001, GLP-1: P< 0.001) and the magnitude of the increase was greater during GLP-1 (AUC: Control: 16248 ± 2687 mL/min vs. GLP-1: 31256 ± 4461 mL/min, P< 0.05) **(Figure 10.2a)**.

Blood glucose and serum insulin

There was no difference in baseline blood glucose prior to the IV infusion, **(Table 10.1)**. Between t= -30 and 0 min, blood glucose decreased slightly during GLP-1 (P< 0.05), but not saline, and immediately prior to the ID infusion, there was a trend for blood glucose to be lower with GLP-1 (P= 0.08). Between t= 0 – 60 min, there was an increase in blood glucose (Control: P< 0.001, GLP-1: P< 0.001) and the magnitude of this increase was greater with saline (AUC: Control: 463 ± 13 mmol/L vs. GLP-1: 413 ± 12, P< 0.005) **(Figure 10.3a)**.

There was no difference in baseline serum insulin prior to the IV infusion **(Table 10.1)**. Between t= -30 and 0 min, serum insulin increased slightly during GLP-1 ($P< 0.05$) but was unchanged during saline. Immediately prior to the ID infusion, serum insulin was higher during GLP-1 than saline ($P< 0.05$). Between t= 0 – 60 min, there was an increase in serum insulin (Control: $P< 0.001$, GLP-1: $P< 0.001$) without a significant difference between the conditions (AUC: Control: 1643 ± 334 vs. GLP-1: 2016 ± 379) **(Figure 10.3b)**.

Discussion

This study establishes that acute administration of GLP-1, in a dose of 0.9 $pmol.kg^{-1}.min^{-1}$, markedly attenuates the hypotensive response to ID glucose infusion in healthy older subjects while potentiating the stimulation of SMA blood flow. GLP-1, predictably, diminished the glycaemic response to ID glucose, at least in part, as a result of its insulinotropic effects. While the observed effect on SMA flow was, from our perspective, surprising, the effects of GLP-1 on BP should encourage further evaluation of this hormone, and its agonists, for the management of PPH. Given that exogenous GLP-1, and short-acting GLP-1 agonists, also slow GE substantially (256), a major effect of these agents to attenuate the hypotensive response is, intuitively, likely.

The ID glucose load (3 kcal/min) employed is representative of the upper end of the normal physiological range for GE of glucose, i.e. 1 – 4 kcal/min (142) and the observed changes in BP, HR, SMA flow and glycaemia, in the

absence of exogenous GLP-1 are comparable to those seen previously (120). The dose of GLP-1 used results in modestly pharmacological plasma levels and is well tolerated (321). The attenuation of the fall in BP induced by ID glucose by exogenous GLP-1 was not apparently attributable to an increase in HR, although it should be appreciated that the increase was slightly greater with GLP-1, and is, presumably, unrelated to the observed increase in SMA blood flow, which would favour a fall in BP. Interestingly, GLP-1 had no effect on BP, HR or SMA flow in the fasted state. We did not measure cardiac output, sympathetic activity and/or atrial natriuretic peptide (ANP), which may be of relevance (315, 322). The hypotensive properties of GLP-1 receptor agonists are attenuated by ANP agonists in mice, suggesting a role for ANP (323) and GLP-1 has been reported to increase left ventricular contractility in dogs (324). The mechanisms (320) by which GLP-1 modulates mesenteric flow remain uncertain, including whether this effect is mediated by the GLP-1 receptor, or not and whether similar effects will be evident with GLP-1 agonists that are resistant to degradation in vivo by dipeptidyl peptidase-4 (DPP-4). It would be of interest to evaluate the effects of blockade of the GLP-1 receptor using exendin-9-39 (325) on SMA flow. Degradation-resistant GLP-1 receptor agonists have hitherto not been shown to have vasodilatory properties (320) and GLP-19-36, the primary degradation product of native GLP-1, has vasodilatory effects independent of the GLP-1 receptor (320). Recent studies indicate that the expression of the GLP-1 receptor within the cardiovascular system is predominantly in the atrial cardiomyocytes (323), potentially accounting for discrepant effects on BP and HR.

The reduction in glycaemia by GLP-1 was anticipated, although the dominant mechanism by which acute administration of GLP-1 diminishes postprandial glycaemic excursions is by slowing of GE (211), which was deliberately bypassed in the current experimental paradigm. The relationship between the effects of exogenous GLP-1 on gastric emptying and mesenteric flow warrants evaluation. The magnitude of the elevation in blood glucose was above the threshold required for insulinotropic and glucagonostatic effects of GLP-1 (320); interestingly, GLP-1 also had a very modest insulinotropic effect before blood glucose concentration increased in response to ID glucose We would anticipate, that the magnitude of glucose-lowering by exogenous GLP-1 would be greater after oral glucose because of the impact of slowing of GE (211).

In interpreting our observations, some limitations of the study should be recognised. In particular, we evaluated the effects of acute administration of a single dose of GLP-1 in a relatively small cohort. However, the observations appeared consistent between subjects and it is unlikely that substantially different outcomes would be observed with a larger study.

In summary, acute administration of GLP-1, in a dose of 0.9 $pmol.kg^{-1}.min^{-1}$, attenuates the hypotensive effect of an ID glucose load and potentiates the increase in SMA flow. While the underlying mechanisms remain uncertain it would be appropriate for studies relating to the potential effects of GLP-1 and GLP-1 agonists on BP to make a distinction between measurements obtained in the fasted and postprandial states.

Figures and Figure legends
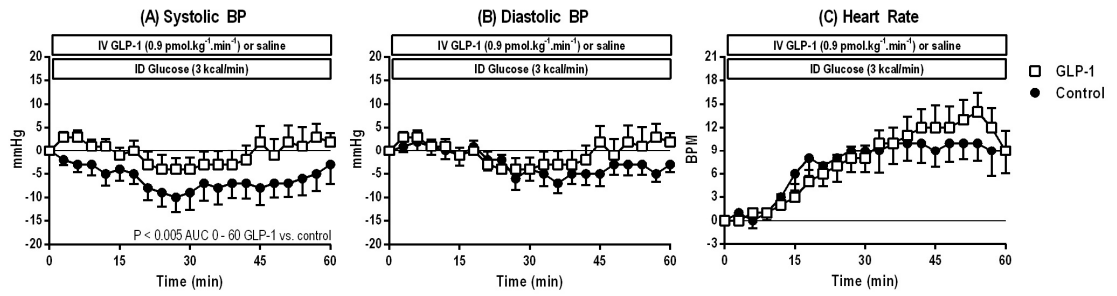
Figure 10.1: Effects of intravenous GLP-1 (0.9 pmol.kg^{-1}.min^{-1}) on the (A) systolic blood pressure (BP), (B) diastolic blood pressure and (C) heart rate (HR) responses to intraduodenal (ID) glucose infusion at 3 kcal/min in healthy older subjects (n= 10), P< 0.005 systolic BP area under the curve (AUC) t= 0 – 60 min GLP-1 vs. control.
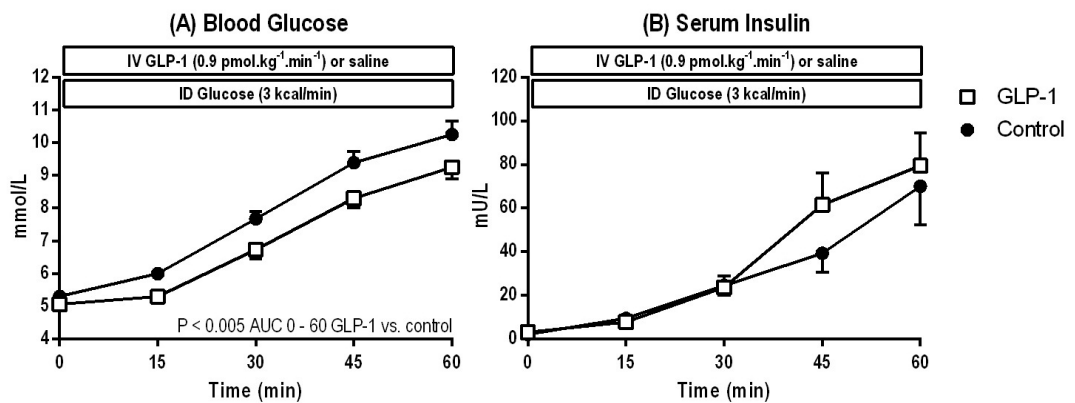
Figure 10.2: Effects of intravenous GLP-1 (0.9 pmol.kg^{-1}.min^{-1}) on the superior mesentery artery blood flow response to ID glucose infusion at 3 kcal/min in healthy older subjects (n= 10). P< 0.05 area under the curve (AUC) t= 0 – 60 min GLP-1 vs. control.

SMA Blood Flow
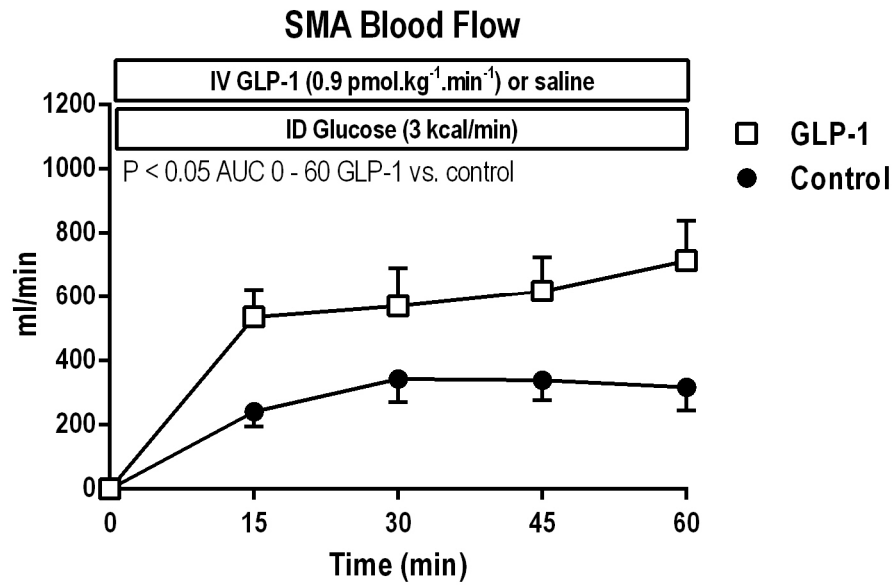
Figure 10.3: Effects of intravenous GLP-1 (0.9 pmol.kg^{-1}.min^{-1}) on the (A) blood glucose and (B) serum insulin responses during ID glucose infusion at 3 kcal/min in healthy older subjects (n= 10). P< 0.005 blood glucose area under the curve (AUC) t= 0 – 60 min GLP-1 vs. control, P< 0.05.

Tables

Table 10.1: Baseline variables.

Variable		Pre-IV infusion	Pre ID-infusion	Difference	Treatment	Time	Treatment × time
Systolic BP (mmHg)	Control	127.7 ± 4.3	133.0 ± 4.7		P= 0.12	P= 0.58	P= 0.08
	GLP-1	128.1 ± 3.1	129.6 ± 4.6				
Diastolic BP (mmHg)	Control	71.5 ± 2.6	70.0 ± 3.6		P= 0.62	P= 0.78	P= 0.08
	GLP-1	69.9 ± 2.8	72.1 ± 2.4				
HR (BPM)	Control	56.8 ± 2.3	55.6 ± 2.3		P= 0.61	P< 0.05	P= 0.24
	GLP-1	52.7 ± 1.9	53.3 ± 2.1				
SMA flow (mL/min)	Control	611 ± 66	720 ± 91	P< 0.05	P= 0.76	P= 0.83	P< 0.005
	GLP-1	694 ± 56	604 ± 67	P< 0.05			
	Difference	P= 0.25	P= 0.22				
Blood Glucose (mmol/L)	Control	5.2 ± 0.1	5.3 ± 0.1	P= 0.20	P= 0.52	P= 0.27	P< 0.05
	GLP-1	5.2 ± 0.1	5.1 ± 0.1	P< 0.05			
	Difference	P= 0.86	P= 0.08				
Insulin (mU/L)	Control	2.5 ± 0.6	2.3 ± 0.5	P= 0.29	P= 0.14	P= 0.25	P< 0.05
	GLP-1	2.2 ± 0.4	3.1 ± 0.7	P< 0.05			
	Difference	P= 0.33	P< 0.05				

Baseline variables prior to intravenous (IV) infusion of glucagon-like peptide-1 (GLP-1) (0.9 pmol.kg^{-1}.min^{-1}) or saline (0.9%) control, and intraduodenal (ID) glucose infusion at 3 kcal/min in healthy older subjects (n= 10). Data are mean ± SEM. BP: blood pressure, HR: heart rate, SMA: superior mesenteric artery.

CHAPTER 11: EFFECTS OF EXOGENOUS GLUCAGON-LIKE PEPTIDE-1 ON THE BLOOD PRESSURE, HEART RATE, GASTRIC EMPTYING, MESENTERIC BLOOD FLOW AND GLYCAEMIC RESPONSES TO ORAL GLUCOSE IN OLDER INDIVIDUALS WITH NORMAL GLUCOSE TOLERANCE AND TYPE 2 DIABETES

Statement of Authorship

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Principal Author

Candidate	Laurence G Trahair
Contribution	Conception and design of the study, coordination, participant recruitment, data collection and interpretation, statistical analysis and drafting of the manuscript.
Overall percentage	75%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature		Date	Feb 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution	Conception and design of the study, data interpretation and statistical analysis and drafting of the manuscript.		
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Introduction

There is considerable interest in the cardiovascular effects of glucagon-like peptide-1 (GLP-1) and its agonists (313, 326, 327). Phase 3 studies focusing on the blood-glucose-lowering effects of GLP-1 agonists indicate durable, modest reductions in systolic, and, less consistently, diastolic blood pressure (BP), and a slight rise in heart rate (HR) (328-331). Postprandial hypotension (PPH), defined as a fall in systolic BP $\geq$ 20 mmHg within 2 hours of a meal (12), is a common disorder, associated with substantial morbidity and increased mortality (10), for which current management is suboptimal (243). Groups most affected are older individuals, particularly those in residential care, and patients with autonomic dysfunction, including those with diabetes (243).

In healthy older individuals and patients with type 2 diabetes, the postprandial fall in BP and increase in superior mesenteric artery (SMA) blood flow are greater when the rate of gastric emptying (GE), or intraduodenal glucose infusion, is relatively faster (46, 120), while gastric distension attenuates the fall in BP (136). GE in healthy individuals exhibits a wide inter-individual variation of ~1 – 4 kcal/min (200); this is increased in diabetes because of the high prevalence of delayed (198), and occasionally rapid, GE (199). The reduction in postprandial glucose following acute administration of GLP-1 (211, 310, 332) or 'short-acting' GLP-1 agonists (333, 334) relates primarily to slowing of GE but clinical studies relating to the effects of GLP-1 and its agonists on BP have not discriminated between effects on fasting versus postprandial BP.

We recently reported that exogenous GLP-1 attenuates the hypotensive response to intraduodenal infusion of glucose at 3 kcal/min (335). That GLP-1 has a pressor effect when the 'protective' effects of gastric distension are bypassed, supporting the concept that it may have efficacy in the management of PPH.

We hypothesized that intravenous infusion of GLP-1 would slow the GE of orally administered glucose in healthy older individuals and patients with type 2 diabetes, and that this effect would be associated with attenuation of the fall in BP and increases in SMA flow and glycaemia.

Materials and Methods

Participants

Fourteen healthy older individuals (6 male, 8 female, age 72.1 ± 1.1 years, BMI 26.0 ± 0.7 kg/m^2) and 10 patients with type 2 diabetes (6 male, 4 female, age 68.7 ± 3.4 years, BMI 28.0 ± 1.2 kg/m^2, duration of known diabetes 9.4 ± 1.8 years and glycated haemoglobin (HbA1c) 6.6 ± 0.2% (48.5 ± 2.0 mmol/mol)) were recruited via a database, or advertisement. Nine of the patients with type 2 diabetes were taking oral hypoglycaemic medication (nine taking metformin, two a sulfonylurea and one sitagliptin); none was using insulin. Antihypertensive medication was being taken by 4 of the healthy individuals (all angiotensin-converting enzyme (ACE) inhibitors), and 5 patients with type 2 diabetes (four ACE inhibitors and one calcium-channel blocker). None of the patients with type 2 diabetes had microvascular

complications and plasma creatinine was normal. Individuals with previous gastrointestinal disease or surgery, significant respiratory or cardiac disease or alcohol abuse, were excluded. All medication was withheld for 24 hours before each study day.

Protocol

Participants were studied on two occasions, separated by at least 1 week when they attended the Department of Nuclear Medicine, Positron Emission Tomography and Bone Densitometry at the Royal Adelaide Hospital at 0830h after an overnight fast. On arrival, the participant was seated in front of a gamma camera and one intravenous (IV) cannula was inserted into the left antecubital vein for blood sampling and another into the right antecubital vein for infusion of GLP-1. A cuff was placed around the upper left arm to measure BP and HR. Commencing at t= -30 min, each participant received in randomised, double-blind fashion an IV infusion of GLP-1 (Bachem, Bubendorf, Switzerland) at a rate of 0.9 pmol.kg^{-1}.min^{-1}, or control (0.9% wt/vol saline, 154 mmol/L NaCl) for 150 min (i.e. from t= -30 – 120 min) (335). A t= -3 min, each subject consumed a drink comprising 75 g glucose and 5 g 3-O-Methyl-D-gluco-pyranose (3-OMG) (Carbosynth, Compton, UK) dissolved in water (volume 300 mL), labelled with 20 MBq ^{99m}Tc-calcium phytate (Radpharm Scientific, Belconnen, ACT, Australia) within 3 min. At t= 120 min, the IV cannulae were removed. On one of the two days, autonomic function was then evaluated using standardised cardiovascular reflex tests (242).

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

Blood pressure and heart rate

BP and HR were measured using an automated BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA), every 3 min for 15 min prior to the IV infusion (t= -45 to -30 min) and every 3 min between t= -30 and 120 min. Pre-IV 'baseline' BP and HR were calculated as an average of the measurements made at t= -39, -36 and -33 min. Pre-drink 'baseline' BP and HR was the measurement made at t= -3 min. PPH was defined as a fall in systolic BP $\geq$ 20 mmHg that was sustained for at least 20 min.

Gastric emptying

Radioisotopic data were acquired for 120 min following the drink (60 sec frames between t= 0 and 60 min, then 180 sec frames from t= 60 and 120 min). Data were corrected for movement, radionuclide decay and γ-ray attenuation (107). The amount of the drink remaining in the stomach at 15 min intervals between t= 0 and 120 min and, where possible, the 50% GE time (T50), were calculated (107).

Superior mesenteric artery blood flow

SMA flow was measured using a Logiq^{TM} e ultrasound system (GE Healthcare Technologies, Sydney, NSW, Australia) and a 3.5C broad spectrum 2.5 – 4 MHz convex linear array transducer. Measurements were made prior to the commencement of the IV infusion and consumption of the drink (i.e. at t= -33 and t= -3 min, respectively), and every 15 min between t= 0 and 120 min. Blood flow (mL/min) was calculated as: π x r^2 x TAMV x 60, where r is the radius of the SMA and TAMV is the time-averaged mean velocity (119). Two measurements were made at each time point by the same investigator (LT).

Blood glucose and serum insulin

Venous blood was collected at t= -33 min and t= -3 min, every 15 min from t= 0 – 60 min, and at t= 90 and t= 120 min. Blood glucose (mmol/L) was determined, using a glucometer (Medisense Companion 2 meter, Medisense Inc. Waltham, MA, USA). Serum insulin was measured by ELISA (10-1113: Mercodia, Uppsala, Sweden); the sensitivity was 6.0 pmol/L and the coefficient of variation was 2.6% within, and 7.6% between, assays (222).

Cardiovascular autonomic nerve function

Autonomic nerve function was assessed using cardiovascular reflex tests (242). Parasympathetic function was evaluated by the variation (R-R interval) of the HR during deep breathing and the response to standing ('30:15' ratio) and sympathetic function by the fall in systolic BP in response to standing.

Each result was scored as 0= normal, 1= borderline and 2= abnormal for a total maximum score of 6 (242). A score $\geq$ 3 was considered to indicate autonomic dysfunction (242).

Statistical analysis

BP and HR and were assessed as changes from the pre-IV or pre-drink baselines, whereas GE, SMA flow, blood glucose and serum insulin were analysed as absolute values. Areas under the curve (AUC) between t= -30 and t= 0 min and between t= 0 and 60 min were calculated using the trapezoidal rule. Changes in each variable over time (from t= -30 – 0 min and from t= 0 – 60 min) were evaluated with repeated-measures ANOVA. Differences between treatments and groups (i.e. treatment × group interaction) were evaluated with repeated-measures ANOVA. Baseline measurements and AUC within groups were compared using Student's paired t-test. The number of participants was based on a power calculation, derived from our study in which GLP-1 in identical dosage was administered to healthy older individuals (335), so that there was 80% power to detect an 8 mmHg difference in the AUC for systolic BP between the study days. A P value < 0.05 was considered significant.

Results

The studies were all well tolerated. There was no difference in age (P= 0.36), BMI (P= 0.17) or the score for autonomic neuropathy (P= 0.61), when comparing the healthy individuals with the patients with type 2 diabetes. No

participant had autonomic neuropathy. In three healthy individuals and three diabetic patients, SMA measurements were not feasible due to intra-abdominal gas.

Blood pressure and heart rate

Healthy individuals

Between t= -30 min and t= 0 min there was no change in systolic BP on either study day **(Table 11.1)**. Following the glucose drink there was a rise, followed by a sustained fall, in systolic BP ($P < 0.001$ for both infusions), with no difference between the study days (treatment, P= 0.47, time, P< 0.001; treatment × time, P= 0.12), although at t= ~15 – 60 min mean systolic BP was lower on the control day **(Figure 11.1a)**.

Between t= -30 min and t= 0 min there was no change in diastolic BP on either study day **(Table 11.1)**. Following the glucose drink there was a fall in diastolic BP (P< 0.001 for both infusions), which was greater during the control infusion (treatment, P< 0.001; time, P< 0.001; treatment × time, P< 0.001). The AUC for diastolic BP was smaller during the control than during the GLP-1 infusion (P< 0.001) **(Figure 11.1c)**.

Between t= -30 min and t= 0 min there was no change in HR on either study day **(Table 11.1)**. Following the glucose drink there was no significant change in HR on either study day, although mean values were higher during the control than during the GLP-1 infusion **(Figure 11.1e)**.

Type 2 patients

Between t= -30 min and t= 0 min there was no change in systolic BP on either study day **(Table 11.1)**. Following the glucose drink there was a rise on both study days, followed by a sustained fall in systolic BP during the control infusion (P< 0.05) but not during the GLP-1 infusion (P= 0.32). Systolic BP was lower in patients receiving the control infusion (treatment, P= 0.06; time, P= 0.22; treatment × time, P< 0.05) and the AUC for the control infusion tended to be smaller (P= 0.06) **(Figure 11.1b)**.

Between t= -30 min and t= 0 min there was no change in diastolic BP on either study day **(Table 11.1)**. Following the drink there was no change in diastolic BP on either study day, but diastolic BP was lower in those receiving the control infusion than in those receiving GLP-1 (treatment, P= 0.14; time, P= 0.13; treatment × time, P< 0.05) **(Figure 11.1d)**.

Between t= -30 min and t= 0 min there was no change in HR **(Table 11.1)**. Following the drink there was no change in HR on either study day (P= 0.18). HR increased during GLP-1 infusion (P< 0.01), although there was no difference between the study days (treatment, P= 0.21; time, P= 0.94; treatment × time, P= 0.45) **(Figure 11.1f)**.

Comparison between the groups

There was no difference between the two groups in the AUC t= 0 – 60 min for systolic BP, diastolic BP or HR for either study day.

Gastric emptying

Healthy individuals

The T50 on the control study day was 121.7 ± 11.7 min. GE was slowed by GLP-1 (e.g. retention at t= 120 min: control, 47.7 ± 4.6%; GLP-1, 71.4 ± 4.8%, P< 0.001) **(Figure 11.2a)**.

Type 2 patients

The T50 on the control study day was 87.7 ± 10.4 min. GE was slowed by GLP-1 (e.g. retention at t= 120 min: control, 32.1 ± 5.3%; GLP-1, 63.5 ± 7.9%, P< 0.005) **(Figure 11.2b)**.

Comparison between the groups

The T50 on the control study day was shorter (P< 0.05) and the percentage of drink retained in the stomach at t= 120 min was lower (P< 0.05) in the patients with type 2 diabetes than in the healthy individuals. There was no difference between the groups in the retention at t= 120 min for the GLP-1 study day (P= 0.40).

SMA blood flow

Healthy individuals

Between t= -30 min and t= -3 min there was a trend for SMA flow to be lower on the control study day (P= 0.06), without any change during GLP-1 (P= 0.50) **(Table 11.1)**. Following the glucose drink there was an increase in SMA

flow (P< 0.001 for both infusions); this increase was less during GLP-1 infusion (treatment, P< 0.001; time, P< 0.001; treatment × time, P< 0.01). The AUC for SMA blood flow was smaller during the GLP-1 than the control infusion (P< 0.001) **(Figure 11.3a)**.

Type 2 patients

At the pre-drink baseline measurement, SMA flow was slightly less on the control study day than on the GLP-1 study day (P< 0.05) **(Table 11.1)**. Between t= -30 min and t= -3 min there was no change in SMA flow on either study day. Following the glucose drink, there was an increase in SMA flow during the control infusion (P< 0.05) and no change during the GLP-1 infusion (P= 0.16). While there was no difference between the study days (treatment, P= 0.07; time, P< 0.01; treatment × time, P= 0.18), the AUC for SMA flow was smaller during the GLP-1 than the control infusion (P< 0.05) **(Figure 11.3b)**.

Comparison between the groups

There were no differences between the healthy individuals and the patients with type 2 diabetes in SMA blood flow at either the pre-IV or pre-drink baseline **(Table 11.1)** and there was no difference in the AUC t= 0 – 60 min for either study day.

Blood glucose, serum insulin

Healthy individuals

Blood glucose at pre-IV baseline was slightly less on the control day than on the GLP-1 day (P< 0.05) **(Table 11.1)**. Between t= -30 min and t= -3 min, there was a modest decrease in blood glucose during the GLP-1 infusion (P< 0.05) but no change during the control infusion (P= 0.40). Following the glucose drink, there was an increase in blood glucose on both study days (P< 0.001 for both) which was less during GLP-1 infusion (treatment, P< 0.001; time, P< 0.001; treatment × time, P< 0.001). The AUC for blood glucose was smaller during the GLP-1 than the control infusion (P< 0.001) **(Figure 11.4b)**.

At the pre-drink baseline serum insulin was higher on the GLP-1 study day (P< 0.01) **(Table 11.1)**. Between t= -30 min and t= -3 min there was an increase in insulin during the GLP-1 infusion (P< 0.005) but no change during the control infusion (P= 0.26). Following the glucose drink there were comparable increases (P< 0.001 for both) in serum insulin on both study days (treatment, P< 0.05; time, P< 0.001; treatment × time, P= 0.37), although the AUC for serum insulin was smaller during the GLP-1 than the control infusion (P< 0.05) **(Figure 11.4c)**.

Type 2 patients

Between t= -30 min and t= -3 min there was a slight decrease in blood glucose during the GLP-1 infusion (P< 0.05) but no change during the control infusion (P= 0.48) **(Table 11.1)**. Following the glucose drink, there was an increase in

blood glucose (P< 0.001 for both infusions) which was less during the GLP-1 infusion (treatment, P< 0.005; time, P< 0.001; treatment × time, P< 0.001). The AUC for blood glucose was smaller during the GLP-1 than the control infusion (P< 0.005) **(Figure 11.4b)**.

At the pre-drink baseline serum insulin was higher on the GLP-1 study day (P< 0.05) **(Table 11.1)**. Between t= -30 min and t= -3 min there was an increase in serum insulin during the GLP-1 infusion (P< 0.05) but no change during the control infusion (P= 0.71). Following the glucose drink, there was an increase in serum insulin on both study days (P< 0.001 for both). Serum insulin levels were higher during the GLP-1 infusion (treatment, P= 0.38; time, P< 0.001; treatment × time, P< 0.05), although there was no difference in AUC (P= 0.68) between the two infusions **(Figure 11.4d)**.

Comparison between the groups

Pre-IV and pre-drink baseline blood glucose levels (P< 0.001 for both study days) **(Table 11.1)** and the blood glucose AUC t= 0 – 60 min were greater in patients with type 2 diabetes than in healthy individuals (P< 0.001 for both infusions).

Pre-IV and pre-drink baseline serum insulin levels were greater in patients with type 2 diabetes than in healthy individuals (P< 0.005 for both study days) **(Table 11.1)** but there was no difference in the AUC t= 0 – 60 min for either study day.

Relationships between the variables

In patients with type 2 diabetes the difference in AUC for blood glucose was inversely related to the difference in intragastric retention at t= 120 min between the control and GLP-1 infusions (R= -0.65, P< 0.05) indicating that the slower the GE the greater the reduction in glycaemia. There were no significant relationships between changes in BP or SMA flow and GE.

Discussion

This study establishes that acute, intravenous, administration of GLP-1 (0.9 pmol.kg^{-1}.min^{-1}) to healthy older individuals and patients with type 2 diabetes attenuates the fall in BP and rise in SMA flow induced by orally administered glucose and confirms that GLP-1 slows GE and diminishes glycaemia. The effect on BP is consistent with our hypothesis and supports the concept that GLP-1 and GLP-1 agonists may prove effective in the management of PPH.

GLP-1 and GLP-1 receptor (GLP-1R) agonists induce a sustained elevation of BP in rodents (313, 327, 336), attributable to central and peripheral mechanisms, but not in larger-animal models (337) or apparently humans (338, 339). However, in a recent study a modest (~5 mmHg) increase in systolic (but not diastolic) BP, associated with an increase in cardiac output attributable to rises in stroke volume as well as HR, was evident in healthy middle-aged men in response to an intravenous infusion of GLP-1 (1.5 pmol.kg^{-1}.min^{-1}) (340). It was suggested that the increase in cardiac output may be a compensatory response to vasodilatation elsewhere. The effectiveness of GLP-1R agonists in reducing BP in clinical trials of type 2

diabetes and obesity is most evident in individuals with higher baseline BP (326, 329, 330) and may be attributable to reductions in tubular sodium reabsorption (341) or central sympathetic output (315) as well as peripheral vasodilatory effects (320). In contrast to findings in rodents (323) GLP-1 and GLP-1R agonists do not appear to promote the secretion of atrial natriuretic peptide in humans (339, 340). In our study GLP-1 had no effect on BP or HR in the 30 min preceding ingestion of a glucose drink which reduced BP in both healthy older individuals and patients with type 2 diabetes. GLP-1 attenuated the glucose-drink-induced fall in diastolic, but not systolic, BP in healthy older individuals. In the patients with type 2 diabetes both systolic and diastolic BP post-glucose drink were greater following GLP-1, compared with the control infusion. The magnitude of the effect of GLP-1 on BP was substantial (~10 mmHg difference) and may relate to the slowing of GE, but other mechanisms are likely to be important, particularly as GLP-1 reduces the hypotensive response to intraduodenal glucose (335). In healthy older individuals, orally (148) and intraduodenally (150) administered guar reduces the hypotensive response to glucose administered via the same routes, and the α-glucosidase inhibitor acarbose has comparable effects on the response to orally (107) and intraduodenally administered (163) sucrose. These observations suggest that the slowing of nutrient absorption from the small intestine is a critical factor. In view of recent observations made using a higher dose of GLP-1 (340), a direct pressor effect of GLP-1 represents an alterative explanation and it would be interesting to evaluate the effect of GLP-1 infusion without an oral glucose load.

Cross-sectional studies have reported there to be a 30 – 50% prevalence of gastroparesis in longstanding type 1 or 2 diabetes (198, 306), although GE may be accelerated (199), perhaps particularly in 'early' type 2 diabetes (306). Our patients had type 2 diabetes of relatively short duration, good glycaemic control and no microvascular complications. The observed acceleration in GE may be an underestimate given that acute hyperglycaemia slows GE (228). The acute slowing of GE by exogenous GLP-1 is substantial (211, 310, 332), as we confirmed, so that the consequent reduction in glucose may be associated with a reduction, rather than an increase, in insulin (211, 310, 332). While blood glucose levels were lower during GLP-1 than the control infusion, the overall glycaemic profile was predictably greater in patients with type 2 diabetes. That GLP-1 had a mild insulinotropic effect prior to the glucose drink is consistent with previous observations (335). In healthy individuals, blood glucose exceeded the threshold for an insulinotropic effect of GLP-1 (320) only during the control day, whereas in patients with type 2 diabetes, levels were above this threshold during both study days. This may account for why serum insulin was higher following GLP-1, compared with control, in patients with type 2 diabetes, whereas in the healthy individuals, the opposite was observed. In healthy individuals, the reduction in blood glucose caused by GLP-1 was greater than we observed in response to an intraduodenal infusion of glucose at 3 kcal/min (335), which is likely to reflect the slowing of GE. Glucose-lowering per se also represents a potential confounder in interpreting the effects of GLP-1 on BP. Ideally; we would have included a positive control for the anti-hyperglycaemic effect of GLP-1.

The glucose-stimulated increase in SMA flow was reduced in both healthy individuals and patients with type 2 diabetes when GLP-1 was administered. This effect is probably related to the slowing of GE rather than a direct effect of GLP-1, particularly as GLP-1 in identical dosage potentiated the increase in SMA flow induced by intraduodenal glucose (335).

In interpreting these results other potential limitations should be recognised. While most volunteers were normotensive, some were taking antihypertensive medication- although medication was withdrawn for a minimum of 24 hours. As the postprandial fall in BP is greater when the preprandial BP is higher (243), it is likely that the effects of GLP-1 will be greater in patients with hypertension. Although blood glucose was quantified with a glucometer, the observed changes appeared consistent between individual participants. All the patients with type 2 diabetes had relatively well-controlled, uncomplicated diabetes and studies of the effects of GLP-1 on postprandial BP in diabetes associated with autonomic neuropathy are warranted, given that the response to GLP-1 may be influenced by autonomic function (342). There is also potential for a discordance between the effects of intravenous and subcutaneous administration of GLP-1 on BP, as appears to be the case for glucose-lowering in patients with type 2 diabetes and, possibly, the induction of upper gastrointestinal symptoms (343).

Despite its importance, information relating to the dietary and pharmacological management of PPH is limited (243). The current study should be regarded as 'proof of principle' as we did not study patients with

PPH. However, in the elderly, PPH probably represents a continuum (i.e. in healthy older individuals there is predicably a postprandial fall in BP which is not the case in healthy young (46)). Given our observations, evaluation of the effect of GLP-1 agonists on BP in PPH is indicated. It will be important to determine whether the acute effects that we observed are maintained with chronic administration. We assessed the acute responses to GLP-1, and it is now recognised that the slowing of GE by exogenous GLP-1 is subject to tachyphylaxis with sustained exposure (212, 311). Intuitively, once- or twice-daily administration of 'short-acting' preparations of GLP-1 agonists, such as exenatide and lixisenatide, which predominantly diminish postprandial glucose by slowing GE (186, 312), may have greater efficacy than once-weekly administration of longer-acting drugs, such as liraglutide and modified-release exenatide, where the slowing of GE appears to be modest with chronic use (186, 312).

In summary, in healthy older individuals and patients with type 2 diabetes, acute intravenous administration of GLP-1, in a dose of 0.9 $pmol.kg^{-1}.min^{-1}$, attenuates the hypotensive response to orally administered glucose. This effect is associated with slowing of GE and reductions in SMA blood flow and glycaemia. These observations suggest the following: (i) GLP-1 agonists may be effective in the management of PPH and (ii) studies relating to the effects of GLP-1 and its agonists on BP should discriminate between the fasted and postprandial states.

Figures and Figure Legends
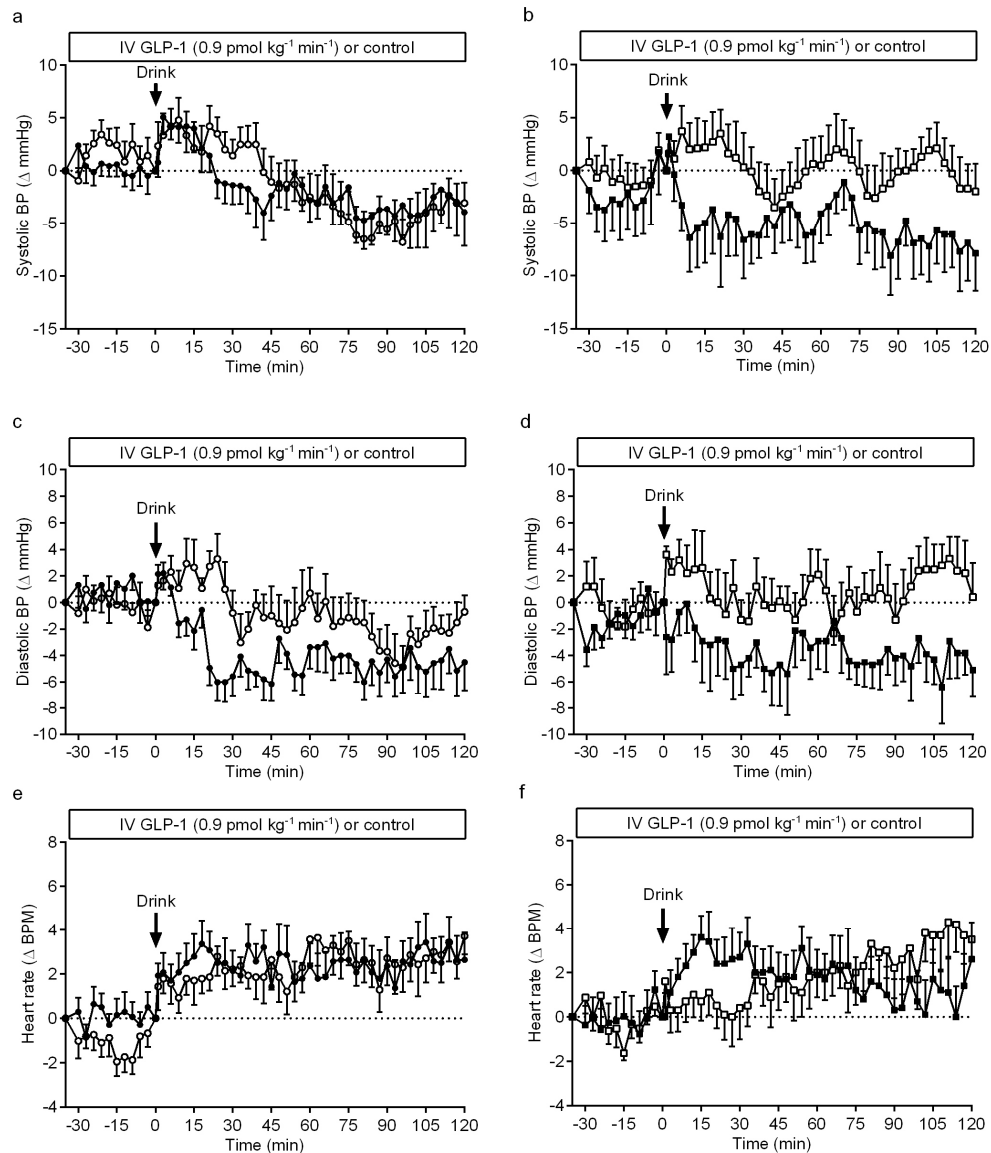
Figure 11.1: Effects of intravenous infusion of GLP-1 (0.9 $pmol.kg^{-1}.min^{-1}$, white symbols) or control (0.9% saline, black symbols) on systolic BP (a, b), diastolic BP (c, d) and HR (e, f) before and after 75 g oral glucose in healthy older individuals (n= 14; a, c, e) and patients with type 2 diabetes (n= 10; b, d, f). Systolic BP: type 2 patients, $P< 0.05$. Diastolic BP: healthy older individuals, $P< 0.001$; type 2 patients, $P< 0.05$; p values are for GLP-1 vs. control ANOVA t= 0 – 60 min.
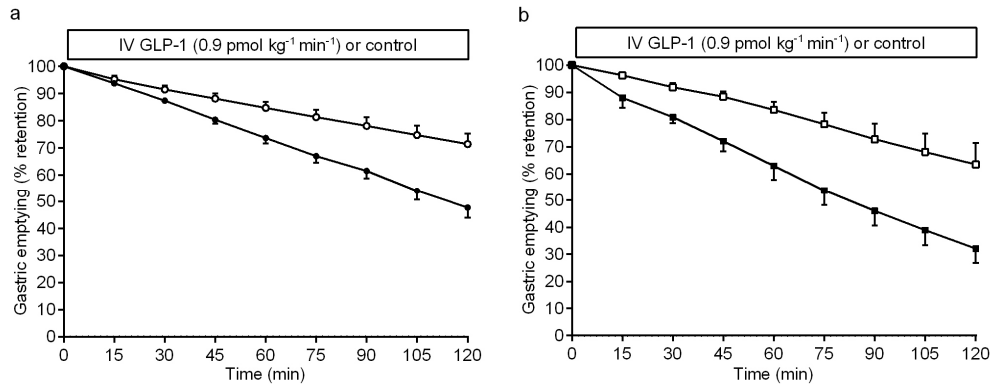
Figure 11.2: Effects of intravenous infusion of GLP-1 (0.9 pmol.kg^{-1}.min^{-1}, white symbols) or control (0.9% saline, black symbols) on gastric emptying of a 75 g oral glucose load in healthy older individuals (n= 14; a) and patients with type 2 diabetes (n= 10; b). Gastric retention at t= 120 min, GLP-1 vs. control: healthy older individuals, P< 0.001; type 2 patients, P< 0.005.
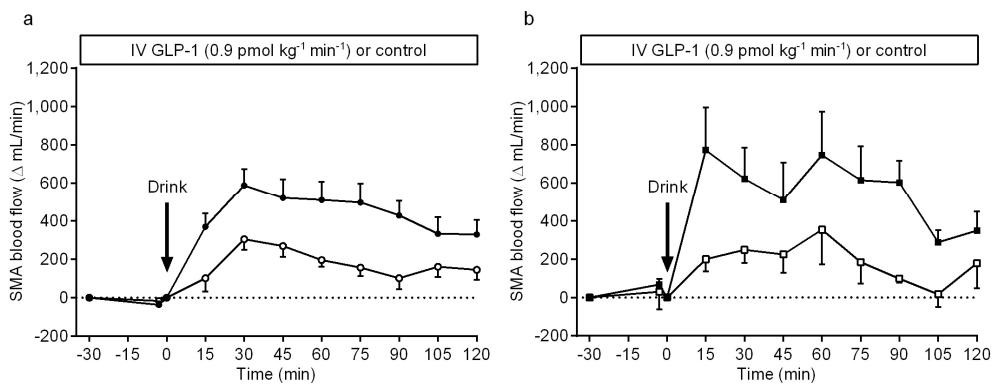
Figure 11.3: Effects of intravenous infusion of GLP-1 (0.9 pmol.kg^{-1}.min^{-1}, white symbols) or control (0.9% saline, black symbols) on the SMA blood flow response to oral administration of 75 g glucose in healthy older individuals (n= 11; a) and patients with type 2 diabetes (n = 7; b). AUC t= 0 – 60 min, GLP-1 vs. control: healthy older individuals, P< 0.001; type 2 patients, P< 0.05.
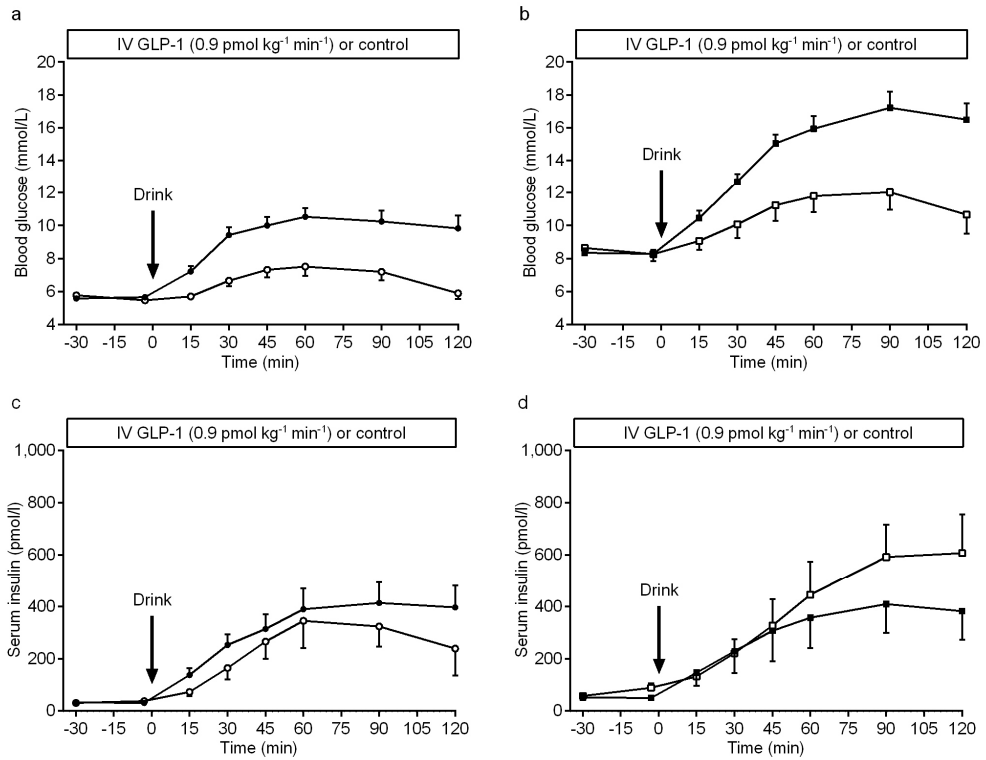
Figure 11.4: Effects of intravenous infusion of GLP-1 (0.9 pmol.kg^{-1}.min^{-1}, white symbols) or control (0.9% saline, black symbols) on blood glucose (a, b) and serum insulin (c, d) before and after 75 g oral glucose in healthy older individuals (n= 14; a, c) and patients with type 2 diabetes (n= 10; b, d). Blood glucose: healthy older individuals, P< 0.001; type 2 patients, P< 0.001. Serum insulin: type 2 patients, P< 0.05; p values are for GLP-1 vs. control ANOVA t= 0 – 60 min.

Tables

Table 11.1: Baseline variables.

		Healthy Subjects (n= 14)		Type 2 Diabetes (n= 10)	
Variable	Condition	Pre-IV infusion	Pre Drink	Pre-IV infusion	Pre Drink
Systolic BP (mmHg)	Control	119.0 ± 4.5	118.5 ± 5.1	125.0 ± 7.0	126.7 ± 8.0
	GLP-1	122.6 ± 5.2	124.0 ± 4.5	120.1 ± 5.1	122.0 ± 5.2
Diastolic BP (mmHg)	Control	65.1 ± 2.5	65.3 ± 2.4	69.0 ± 2.8	68.3 ± 3.3
	GLP-1	66.6 ± 3.2	64.8 ± 2.9	66.6 ± 2.1	65.9 ± 2.7
HR (BPM)	Control	60.9 ± 2.4	61.3 ± 2.4	66.8 ± 3.3	67.9 ± 3.2
	GLP-1	60.6 ± 2.5	59.9 ± 2.6	66.5 ± 3.1	67.0 ± 3.1
SMA flow (mL/min) (n= 11 and 7)	Control	542 ± 34	504 ± 39	587 ± 91	654 ± 113
	GLP-1	551 ± 54	534 ± 63	741 ± 139	771 ± 119
Blood Glucose (mmol/L)	Control	5.6 ± 0.1	5.6 ± 0.2	8.4 ± 0.3	8.3 ± 0.3
	GLP-1	5.8 ± 0.1	5.5 ± 0.1	8.6 ± 0.4	8.3 ± 0.4
Insulin (pmol/L)	Control	32.6 ± 3.0	30.6 ± 3.7	51.2 ± 3.7	50.0 ± 5.4
	GLP-1	31.0 ± 3.2	37.7 ± 3.7	57.5 ± 8.0	53.1 ± 16.8

Baseline variables prior to intravenous (IV) infusion of GLP-1 (0.9 pmol.kg⁻¹.min⁻¹) or control (0.9% saline), and consumption of a 75 g glucose drink in healthy older individuals (n= 14) and type 2 patients (n= 10). Data are mean ± SEM. GLP-1: glucagon-like peptide-1, BP: blood pressure, HR: heart rate, SMA: superior mesenteric artery.

CHAPTER 12: EFFECTS OF SITAGLIPTIN ON BLOOD PRESSURE AND HEART RATE IN RESPONSE TO INTRADUODENAL GLUCOSE INFUSION IN TYPE 2 DIABETES: A POTENTIAL ROLE FOR GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE.

Statement of Authorship

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Candidate Contribution

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Contribution	Data interpretation, statistical analysis and drafting of the manuscript.
Overall percentage	50%

Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i) the candidate's stated contribution to the publication is accurate (as detailed above);

ii) permission is granted for the candidate in include the publication in the thesis; and

iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Introduction

Meal ingestion is associated with splanchnic blood pooling and frequently inadequate compensatory increases in heart rate (HR), cardiac output and peripheral vascular resistance in older individuals and patients with type 2 diabetes, leading to postprandial hypotension, which may be associated with syncope, falls, and even coronary and cerebrovascular ischemic events (344). These responses are evident after oral, rather than intravenous glucose, indicating a gastrointestinal influence on cardiovascular function, i.e. a "gut-heart" axis (344).

Postprandially, the release of "incretins", i.e. glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), may participate in the control of postprandial cardiovascular function, although rapidly inactivated by dipeptidyl peptidase 4 (DPP-4) (313). DPP-4 inhibitors have been reported to attenuate inflammation and oxidative stress, improve endothelial function and blood pressure (BP) (313, 345), via the pleiotropic actions of GLP-1 (313, 345), while little is known regarding the effects of GIP. However, when intraduodenal (ID) glucose was infused at rates spanning the normal range for gastric emptying (i.e. 1 – 4 kcal/min) (142), variations in superior mesenteric artery blood flow (a marker of splanchnic blood pooling) and HR occur in parallel with the secretion of GIP, but not GLP-1 (124, 222, 223), warranting further clarification of the cardiovascular effects of GIP postprandially.

In the present study, we evaluated the effects of sitagliptin on HR and systolic and diastolic BP in type 2 patients, during ID glucose infusion at a rate of 2 kcal/min, where GIP is the major incretin in the circulation (222, 223).

Methods

Subjects

We retrospectively evaluated HR and BP from 10 Caucasian males (mean age 66.2 ± 1.4 years, BMI 30.0 ± 1.3 kg/m^2, HbA1c 6.6 ± 0.1% (48.5 ± 1.5 mmol/mmol), duration of known diabetes 3.6 ± 1.1 years) enrolled in a study evaluating the effects of sitagliptin in patients with type 2 diabetes. Blood glucose and hormone data from these subjects have been reported previously (346). All subjects provided written, informed consent. None had significant comorbidities, a history of gastrointestinal surgery or autonomic dysfunction (assessed by standardised cardiovascular reflex tests (241)), was a smoker, or was taking any medication known to affect gastrointestinal function. Three patients with hypertension and treated with perindopril arginine and indapamide hemihydrate (Coversyl Plus, 5/1.25 mg per day) were instructed to withhold the dose 24 hours prior to the study. The protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee.

Protocol

Each subject was studied twice, separated by 3 days, in a randomised, double-blind fashion. After an overnight fast from the standardised beef lasagne meal (McCain Foods, Victoria, Australia) at 19:00, subjects attended the laboratory

at 08:00 the following day, when a multilumen silicone rubber catheter (Dentsleeve International, Ontario, Canada) was inserted transnasally and allowed to pass into the duodenum by peristalsis (222, 223). The catheter was positioned with an infusion port opening 12 cm beyond the pylorus. A cannula was inserted into a forearm vein for blood sampling. 100 mg sitagliptin or matching placebo was administered orally with 30 mL water (t= -30 min). Subjects were then allowed to "rest" for 30 min (t= -30 – 0 min), followed by an ID glucose infusion (60 g glucose dissolved in water to a volume of 240 m; 2 kcal/min) over 120 min (t= 0 – 120 min; 2 kcal/min). HR, systolic and diastolic BP were measured every 15 min during glucose infusion, using an automatic sphygmomanometer (DINAMAP ProCare 100, Milwaukee, USA). Blood was sampled frequently into ice-chilled EDTA tubes for plasma glucose and hormone measurements, with DPP-4 inhibitor (DPP4-010; Linco Research, MO, USA; 10 μL/mL) added to tubes for intact incretin measurements. Plasma was separated and stored at – 70°C for subsequent analysis.

Measurements

Plasma glucose concentrations were measured by the glucose oxidase technique (Yellow Springs Instruments 2300 STAT Plus, OH, USA). GLP-1, GIP and glucagon analyses were performed as previously described (347, 348). Intact GLP-1 was measured using a two-site ELISA (C-terminally directed GLP-1F5 catching antibody; N-terminally directed Mab26.1 detecting antibody) (347). Total GLP-1 was assayed using C-terminally directed assay, detecting intact GLP-1 and the primary (N-terminally

truncated) metabolite (347). Intact and total GIP were analysed with the N-terminally and C-terminally directed antisera, 98171 (347) and 80867 (348), respectively. Glucagon immunoreactivity was determined using the C-terminally directed antiserum 4305, which measures glucagon of pancreatic origin (347). Insulin was measured by ELISA (10-1113, Mercodia, Uppsala, Sweden).

Statistical analysis

Outcomes were analysed using repeated measures ANOVA, with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed, if ANOVAs revealed significant effects. Within-subject correlation analyses were used to assess the relationships between the differences in HR response to ID glucose and the rises in insulin and intact GLP-1 and GIP after sitagliptin (349), for which areas under the curves (AUC) were calculated using the trapezoidal rule. Means and AUC were compared by paired t-test. All analyses were performed using SPSS 19 (IBM Corporation). Data are means ± standard error; $P < 0.05$ was considered statistically significant.

Results

All subjects tolerated the study well.

Plasma GLP-1 and GIP, glucose, glucagon, and serum insulin

During ID glucose infusion, total GLP-1 **(Figure 12.1a)** did not change, whereas total GIP **(Figure 12.1c)** increased markedly (P< 0.001), without any differences between the two days. As expected, intact GLP-1 **(Figure 12.1b)** was marginally higher (treatment × time interaction: P= 0.044; AUC: P= 0.087), and intact GIP **(Figure 12.1d)** was substantially higher (treatment × time interaction: P= 0.003; AUC: P= 0.009), after sitagliptin than placebo.

Neither plasma glucose nor glucagon differed between placebo and sitagliptin during ID glucose infusion, although insulin was modestly greater after sitagliptin than placebo (treatment × time interaction: P= 0.041; AUC: P= 0.097).

Blood pressure and heart rate

Prior to ID glucose infusion, neither systolic **(Figure 12.1e)** or diastolic BP **(Figure 12.1f)** differed between study days. During glucose infusion, systolic and diastolic BP slightly reduced within the first 60 min, and slowly recovered to baseline subsequently on both days (P< 0.001 for each), without significant differences between placebo and sitagliptin.

Prior to ID glucose infusion, HR **(Figure 12.1g)** did not differ between study days. During glucose infusion, HR increased (P< 0.001), and this increase was greater after sitagliptin than placebo (treatment effect and AUC: P= 0.001). The differences in HR between sitagliptin than control were directly

associated with changes in both intact GLP-1 (R= 0.67, P= 0.024) and intact GIP (R= 0.75, P= 0.008), but not insulin.

Discussion

In our experimental model, sitagliptin resulted in a large increase in plasma intact GIP, but minimal rise in intact GLP-1 (346), in which sitagliptin acutely increased HR, without affecting BP, in type 2 patients who had relatively good glycaemic control and no evidence of autonomic dysfunction. Furthermore, we demonstrated a strong relationship between the increments in HR and plasma intact GIP concentrations. These observations are in line with findings that functional GIP receptor signalling is present in the myocardium and vasculature (313), and that intravenous GIP can increase vagal activity (evident by increased plasma pancreatic polypeptide) (350) and cause splanchnic blood pooling (351, 352), elevation in HR and reduction in BP (353), thus warranting further clarification of a potential role of GIP in the regulation of the gut-heart axis in humans.

The strengths of the present study are that the selective elevation of plasma GIP concentrations was achieved via a physiological route, rather than intravenous infusion of exogenous peptide, and that confounding effects due to inter-individual variations in the rate of gastric emptying were excluded by administering glucose at a controlled rate (142). The observed increase in plasma intact GLP-1 was minimal; although we cannot exclude a local effect of endogenous GLP-1 on insulin secretion, neither plasma glucose nor glucagon was reduced. While GIP may be more important for the modest

increase in insulin in this study, its insulinotropic and glucose-lowering properties are largely impaired in type 2 diabetes (346).

It remains to be established whether sitagliptin increased HR predominantly via increasing plasma intact GIP in the present study, which, however, can be validated by using GIP antagonist. Although insulin was reported to increase peripheral sympathetic nerve activity (354), elevation of peripheral insulin within physiological levels does not alter HR, systolic or diastolic BP in humans (355). Several other "cardiovaso-active" peptides, including stromal cell-derived factor-1α, substance P and B-type natriuretic peptide, are DPP-4 substrates, but are not influenced by nutrient intake (356), so are unlikely to be physiologically important after meals. Although DPP-4 is localised to the endothelial membrane (345), the effects of direct inhibition of the enzymatic activity of DPP-4 on the cardiovascular system have not been reported. HR has not generally been reported to be increased during treatment with sitagliptin in clinical trials (357); the increase in HR could be related to incretin involvement in the cardiovascular response to food ingestion rather than an effect of sitagliptin in general.

Both systolic and diastolic BP appeared to be lower on sitagliptin, but none reached statistical significance, probably because the type 2 patients studied were "normotensive" and without autonomic dysfunction, so the rise in HR could compensate for any hypotensive response to ID glucose. Alternatively, the failure to show statistical significance may represent a type II error. In this

case, we cannot rule out a compensatory increase in HR as a result of fall in BP

In conclusion, sitagliptin increases the effect of ID glucose on HR when infused at 2 kcal/min, associated with augmentation of intact GIP concentrations. Further clarification of endogenous GIP in the physiological control of the gut-heart axis is warranted.

Figures and Figure Legends
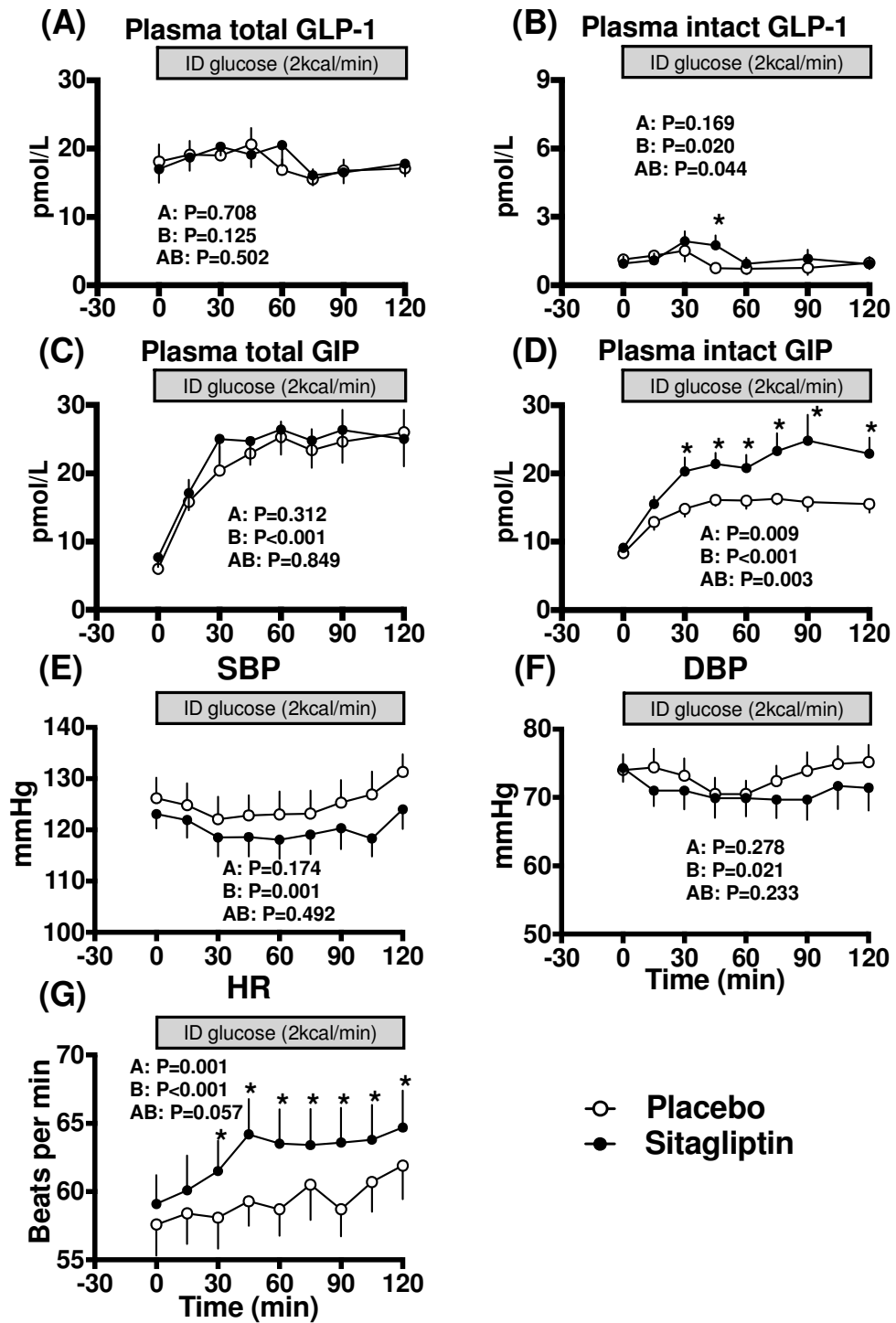
Figure 12.1 (previous page): Plasma total and intact glucagon-like peptide-1 (GLP-1) (A and B) and glucose-dependent insulinotropic polypeptide (GIP) concentrations (C and D), systolic blood pressure (SBP) (E), diastolic blood pressure (DBP) (F) and heart rate (HR) (G) during intraduodenal (ID) glucose infusion at 2 kcal/min (t= 0 – 120 min) after placebo and sitagliptin (at t= -30 min) in patients with type 2 diabetes (n= 10). Repeated measures ANOVA was also used to determine the statistical significance, with treatment and time as factors. Post hoc comparisons, corrected by Bonferroni's correction, were performed, if ANOVAs were significant. Results of ANOVA are reported as P-values for (A): differences by experiment, (B) differences over time and (AB): differences due to the interaction of experiment and time. Post hoc comparisons, adjusted by Bonferroni's correction, were performed, if ANOVAs were significant. *P< 0.05 for each. Data are means ± SEM.

CHAPTER 13: CONCLUSIONS

This thesis has presented studies that provide important and novel insights into the role of the gastrointestinal tract in the regulation of blood pressure (BP) and glycaemia.

In **Chapter 5**, postprandial BP responses to an oral glucose tolerance test (OGTT) were reported in a cohort of healthy older individuals. It has previously been established that the magnitude of the fall in BP following a meal is related to the rate of gastric emptying (GE), so that when GE is relatively faster, the fall in BP is greater. In our cohort, the prevalence of postprandial hypotension (PPH) was ~13%. Our study did not report symptoms of PPH and it would be of interest to evaluate the association of postprandial changes in BP with symptoms and mortality in older subjects. We confirmed the hypothesis that PPH is associated with relatively more rapid GE; in our study, GE was faster in subjects with PPH, consistent with the concept that relatively more rapid GE may be a 'risk' factor for PPH. Pharmaceutical and non- pharmaceutical strategies which slow GE may represent an effective management strategy in PPH.

In **Chapter 6**, we measured the GE and glycaemic responses to an OGTT in a relatively large cohort of older individuals. These subjects were then classified according to their glucose tolerance. In individuals with impaired glucose tolerance (IGT), there was a direct relationship between GE and glycaemia at t= 60 min (so that when GE was faster, the rise in blood glucose was

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relatively greater), whereas this was not the case in individuals with normal glucose tolerance (NGT), potentially due to the smaller variance in blood glucose at this time. In IGT, the 'late' blood glucose response, at t= 120 min, was inversely related to GE which is likely to reflect the greater insulinaemic response to the earlier blood glucose levels. In both NGT and IGT, insulin sensitivity and GE were demonstrated to be independent, yet complimentary, determinants of the blood glucose response at both the 'early' and 'late' time points. Our observations are consistent with our underlying hypothesis that GE is a major determinant of the initial glycaemic response to carbohydrate-containing meals in health and type 2 diabetes. As it is intuitively likely that GE will assume increased importance in type 2 patients as β-cell function declines, studies evaluating glycaemic, insulinaemic and incretin responses to mixed meals would be of interest. Individuals with an overall faster rate of GE may potentially be at greater risk of developing IGT and type 2 diabetes and strategies which slow GE may reduce the progression to type 2 diabetes.

In **Chapter 7**, we measured gut hormone responses to an intraduodenal infusion of glucose into either proximal, distal, or proximal and distal combined small intestinal segments, in healthy older subjects. Infusion of glucose proximally resulted in minimal glucagon-like peptide-1 (GLP-1) secretion, but substantial gastric inhibitory polypeptide (GIP) and cholecystokinin (CCK) responses, whereas infusion into both proximal and distal segments induced greater GLP-1, GIP and CCK responses, compared with proximal alone, and this was associated with a reduction in the insulin/glucose ratio. The secretion of GLP-1 was greatest following the distal

infusion, but the GIP and CCK responses were less than during the proximal and distal segment infusion, with no difference in insulin/glucose ratio. These observations attest to the importance of the distal small intestine for GLP-1, and to a lesser extent GIP and CCK, secretion. We confirmed the hypothesis that the diversion of glucose from the proximal to the distal small intestine potentiates GLP-1 secretion and therefore, therapies which target GLP-1 release in the distal small intestine may potentially be more effective in blood glucose regulation than those that have a non-specific regional effect throughout the small intestine. Studies evaluating such responses in conditions where the secretion and/or action of incretin hormones are known to be altered, such as obesity and type 2 diabetes, would be of interest.

In **Chapter 8**, we report the BP and cardiovascular responses to drinks of glucose and water in healthy older subjects and patients with PPH. In both groups, ingestion of glucose resulted in a fall in BP and increases in cardiac function, particularly cardiac output and stroke volume, whereas the water drink was associated with an increase in BP and a modest reduction in heart rate, with no change in cardiac function. The fall in BP following glucose was greater in the PPH group, whereas there were comparable increases in heart rate and cardiac output, i.e. the increase in cardiac output is less than that required to compensate for the postprandial fall in BP in these patients. The latter is likely to reflect age-related impairments in both baroreceptor and myocardial compensatory pathways. In the group with PPH, we also observed relationships between the hypotensive response to glucose and the hypertensive response to water. As the pressor response to water drinking is

maintained in PPH, this represents a potential therapeutic target and studies to evaluate the efficacy in the management of symptomatic PPH are warranted.

Chapter 9 presents the results of a cross-sectional study of patients with Parkinson's disease, in which we measured GE using the 'gold-standard' technique of scintigraphy, as well as BP, superior mesenteric artery (SMA) blood flow and glycaemia, in response to a 75 g oral glucose load. We studied 21 patients with mild-to-moderate Parkinson's disease. GE was delayed in 14% of these patients and 38% had PPH. GE was related to autonomic dysfunction and a relationship between the initial glycaemic response to glucose with GE, comparable to that previously observed in subjects without Parkinson's disease, was demonstrated. We did not observe a relationship between GE and the fall in BP, or rise in SMA blood flow, in response to the glucose drink, perhaps due to the small number of subjects with GE times towards the upper end of the normal range. There was also no relationship between the rise in SMA blood flow and fall in BP. This study provides novel insights into the prevalence and implications of delayed GE and PPH in Parkinson's disease, as well as the relationships between these variables. Studies in patients with more advanced Parkinson's disease, or who are known to have symptomatic PPH would be of interest.

In **Chapter 10 and Chapter 11**, the effects of exogenous GLP-1 on postprandial BP are reported. In **Chapter 10**, we show that when glucose is infused intraduodenally, bypassing the stomach and the 'protective' effects of gastric distension, exogenous GLP-1 attenuates the fall in BP and rise in blood

glucose in healthy older individuals. Interestingly, GLP-1 potentiated the increase in SMA blood flow in response to the intraduodenal infusion, via an uncertain mechanism. In **Chapter 11**, it is shown that exogenous GLP-1 attenuates the fall in BP, and rise in SMA blood flow and blood glucose induced by an OGTT, in both healthy older subjects and patients with type 2 diabetes, effects that are likely to be secondary to the slowing of GE. Together the outcomes of these two studies suggest that GLP-1 receptor agonists may be effective in the management of PPH, through slowing of GE, and certainly warrant further evaluation. It is clear that clinical trials that report the effects of GLP-1 and GLP-1 receptor agonists on BP should differentiate between the fasting and postprandial states.

The outcomes of the study reported in **Chapter 12** suggest a potential role for GIP in the regulation of cardiovascular function. Administration of sitagliptin, a dipeptidyl peptidase-4 inhibitor, was associated with an increase in the heart rate response to intraduodenal glucose in type 2 diabetes. The rate of intraduodenal glucose infusion employed in this study stimulated the secretion of GIP, but not GLP-1. These findings suggest a potential role of GIP in the regulation of postprandial cardiovascular function, and further studies of this role are warranted.

GLOSSARY

+ve: Positive

-ve: Negative

ABPM: Ambulatory blood pressure monitoring

ACE: Angiotensin converting enzyme

AD: Alzheimer's disease

ADH: Antidiuretic hormone

AN: Autonomic neuropathy

ANF: Autonomic nerve function

ANOVA: Analysis of variance

ANP: Atrial natriuretic peptide

AUC: Area under the curve

BP: Blood pressure

BMI: Body mass index

BNP: Brain natriuretic peptide

BPM: Beats per minute

CCK: Cholecystokinin

CGRP: Calcitonin-gene-related peptide

CHO: Carbohydrate

CO: Cardiac output

CTN: Clinical trial notification

CV: Coefficents of variation

DI: Disposition index

DL-DOPS: 3,4-DL-threo-dihydroxyphenylserine

DM: Diabetes mellitus

DPP-4: Dipeptidyl peptidase 4

EF: Ejection fraction

GD: Glucose distal

GE: Gastric emptying

GEC: Gastric emptying coefficient

GIP: Gastric inhibitory polypeptide

GLP-1: Glucagon-like peptide-1

GLP-2: Glucagon-like peptide-2

GLS: Global longitudinal strain

GP: Glucose proximal

GPD: Glucose proximal and distal

HR: Heart rate

ID: Intraduodenal

IQR: Inter-quartile range

ISI: Insulin sensitivity index

IV: Intravenous

L-NAME: NG-nitro-L-arginine-methyl-ester

L-NMMA: NG-mono-methyl-L-arginine

MAP: Mean arterial pressure

MMC: Migrating motor complex

MSA: Multiple system atrophy

MSNA: muscle sympathetic nerve activity

NGT: Nasogastric tube

NO: Nitric oxide

OGTT: Oral glucose tolerance test

OH: Orthostatic hypotension

PAF: Pure autonomic failure

PD: Parkinson's disease

PEG: Percutaneous endoscopic gastrostomy

PPH: Postprandial hypotension

PRISMA: Preferred reporting items for systematic reviews and meta-analyses

RYGB: Roux-en-Y gastric bypass

SD: Standard deviation

SEM: Standard error of the mean

SMA: Superior mesenteric artery

SV: Stroke volume

SVR: Systemic vascular resistance

T50: Gastric 50% emptying time

TAMV: Time-averaged mean velocity

TMPD: Transmucosal potential difference

VIP: Vasoactive-intestinal peptide

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APPENDIX 1

Table 2.1: Studies which have reported the prevalence of postprandial hypotension in various patient groups.

Study	Pt Group and Setting	Test Meal	Number	Patient Characteristics	Design	Systolic BP	Prevalence of PPH and symptoms	Assessment
Aronow and Ahn (1994) (37)	Older, long-term health care facility residents	Not controlled, normal lunch	499 subjects	80 ± 9 years 145M;354F	Prospective, cohort study	Mean max fall 15 ± 6 mmHg	118 (24%) of subjects	Mercury sphygmomanometer, every 15 min for 75 min, then at 120 min
Barochiner et al (2013) (17)	Hypertensive older subjects, home setting	Not controlled, normal lunch	230 hypertensive subjects	74 (IQR 17) years 80M;150F	Cross-sectional, observational study	PPH +ve preprandial 139 ± 17 mmHg, postprandial 124 ± 15 mmHg; PPH -ve preprandial 127 ± 14 mmHg, postprandial 125 ± 14 mmHg	63 (27%) of subjects	Subject-assessed BP with home automatic sphygmomanometer, 1 hour before and after lunch.
Ejaz et al (2006) (51)	Parkinson Disease (PD) patients	Not controlled	13 idiopathic PD patients	IPD 77 ± 6 years 9M;4F	Retrospective, cross-sectional study	Not reported	100% had at least one episode of PPH	24 hour ABPM 15 min daytime, 30 min night

Table 2.1 (continued)

Study	Pt Group and Setting	Test Meal	Number	Patient Characteristics	Design	Systolic BP	Prevalence of PPH and symptoms	Assessment
Idiaquez et al (1997) (50)	Alzheimer's disease (AD) patients, healthy older control subjects	Liquid meal, 500 kcal, 54.5% CHO, 14% protein, 31.5% fat	10 patients with probable AD, 23 controls	AD 74 ± 6 years, 3M;7F, control subjects 72 ± 6 years, 12M;11F	Prospective, case-controlled study	Mean max fall 20 mmHg in AD	7 (70%) AD patients, 6 (26%) control subjects	Automated sphygmomanometer, every 10 min for 120 min
Jones et al (1998) (46)	Type 2 diabetic patients, older subjects, young control subjects	OGTT (75 g glucose in 350 mL water)	16 recently diagnosed type 2 (3 – 12 months), 9 older, 10 young controls	Type 2 57 years 11M;5F, Older 48 years 6M;3F, Young 23 years 9M;1F	Prospective, case-controlled study	Mean max fall type 2 patients 12 mmHg, older subjects 5 mmHg, young subjects 7 mmHg	7 (44%) type 2 patients, 3 (33%) older subjects	Automated sphygmomanometer, every 15 min for 180 min
Kohara et al (1998) (20)	Older, hospitalised patients with essential hypertension	Standardised meals, 30 kcal/kg ideal wt/day, 66% CHO, 16% protein, 18% fat	121 hypertensive patients	PPH +ve 68 ± 8 years 7M;11F; PPH -ve 60 ± 9 years 14M;10F; remainder 54 ± 14 years 44M;35F	Prospective, uncontrolled study	In all subjects, mean max fall 3 ± 17 mmHg. Individual groups not presented	14% PPH +ve (> 10mmHg), 19% PPH -ve (< 10mmHg, >5 mmHg)	24 hour ABPM 30 min daytime, 60 min night.

Table 2.1 (continued)

Study	Pt Group and Setting	Test Meal	Number	Patient Characteristics	Design	Systolic BP	Prevalence of PPH and symptoms	Assessment
Lagro et al (2012) (38)	Geriatric clinic patients with a history of falls	Mixed liquid meal, 292 kcal, 65 g CHO, 4 g protein, 2 g fat	302 patients	PPH +ve 80 ± 8 years 67M;108F, PPH -ve 77 ± 8 years 44M;83F	Retrospective, cohort study	Specific BP data not reported	175 (58%) of patients	Finapress beat-to-beat finger BP monitoring for 75 min
Le Couteur et al (2003) (39)	Older subjects in residential care	Not controlled, normal breakfast	179 semi-independent older subjects	83 ± 7 years, 36M;143F	Cross-sectional, observational study	At breakfast, preprandial 160 ± 27 mmHg, postprandial 144 ± 24 mmHg, mean fall 16 ± 21 mmHg	68 (38%) of subjects	ABPM, single measurement at 60 min
Loew et al (1995) (47)	Parkinson Disease patients, healthy older control subjects	Mixed lunch, 2500 kJ, 50% CHO, 10% protein, 35% fat	10 PD patients, 10 controls	IPD 82 ± 9 years 5M;5F, controls 85 ± 5 years 3M;7F	Observational, case-controlled study	Max fall from 154 ± 26 mmHg to 128 ± 18 mmHg in PD, no fall in controls	4 (40%) of patients	Semi-automated sphygmomanometer, every 30 min for 10 hours
Lubart et al (2006) (40)	Older, orally and tube fed hospitalised patients	Mixed meal, 600 kcal, 65% CHO, 15% protein, 20% fat	50 orally (OF), 50 nasogastric (NGT), 50 gastrostomically (PEG) fed patients	OF 78 ± 8; NGT 74 ± 12; PEG 81 ± 9. 49M,101F	Prospective, case-controlled study	Mean fall 34 ± 13 mmHg, occurring before 75 min in 68% of population	64 (43%) of total patients, no differences between groups; one OF patient symptomatic	Automated sphygmomanometer, every 15 min for 90 min

Table 2.1 (continued)

Study	Pt Group and Setting	Test Meal	Number	Patient Characteristics	Design	Systolic BP	Prevalence of PPH and symptoms	Assessment
Mehagnoul-Schipper et al (2001) (48)	PD patients, healthy control subjects	Mixed liquid meal, 292 kcal, 100 mL milk, 65 g CHO, 4 g protein, 2 g fat	17 PD patients, 17 controls	IPD 75 ± 2 years 8M;9F, controls 74 ± 1 years 8M;9F	Double-blind, randomised, cross-over trial	Max fall for PD 34 ± 5 mmHg, controls 16 ± 3 mmHg	14 (82%) PD patients; 9 symptomatic; 7 (41%) controls	Finapress beat-to-beat finger BP monitoring 90 min
Peitzman and Berger (1989) (41)	Older subjects, in retirement and regular communities	Mixed meal, 1848 kJ, 30% CHO, 20% protein, 50% fat. Water control.	16 subjects, 8 controls	82 years 6M;10F	Randomised, controlled, observational study	Preprandial 136 ± 18 mmHg, postprandial 120 ± 18 mmHg. Control preprandial 133 ± 23 mmHg, postprandial 136 ± 24 mmHg	4 (25%) of subjects	Method not reported, every 15 min for 60 min
Puisieux et al (2000) (21)	Geriatric ward patients with history of syncope or falls	Not controlled, normal meals	45 patients with falls, 75 with syncope, 34 controls	Falls 81 ± 9 years 12M;33F, syncope 81 ± 8 years 22M;53F, controls 79 ± 7 years 11M;23F	Descriptive, cross-sectional study	Max fall: falls 12 ± 11 mmHg, syncope 13 ± 9 mmHg, controls 9 ± 9 mmHg	31 (20%) of subjects; 8 (18%) in falls, 20 (27%) in syncope, 3 (8.5%) in control group	24 hour ABPM 15 min day time 30 min night
Saski et al (1992) (23)	Type 2 diabetic patients, healthy control subjects	Not controlled during ABPM, and OGTT (75 g in 300 mL water)	35 type 2 diabetic patients (mean duration 11 years), 15 healthy control	Type 2 52 years (range 28 – 60 years), control 41 years (range 25 – 63 years)	Observational, case-controlled study	Only PPH data reported	ABPM, 13 (37%); OGTT, 7 (20%) type 2 patients, 100% symptomatic	24 hour ABPM 30 min daytime, 60 min night. OGTT, automated sphygmomanometer, every 10 min for 180 min

Table 2.1 (continued)

Study	Pt Group and Setting	Test Meal	Number	Patient Characteristics	Design	Systolic BP	Prevalence of PPH and symptoms	Assessment
Son and Lee (2012) (43)	Older, nursing home residents	Not controlled, mixed meal, rice and vegetable soup	121 subjects	82 ± 7 years 25M;96F	Descriptive, cross-sectional study	91% experienced fall in BP, mean fall 13 ± 10 mmHg at 45 min	39 (32%) of subjects	Mercury sphygmomanometer, every 15 min for 90 min
Tsukamoto et al (2013) (49)	Parkinson disease patients, older hospitalised patients as controls	Not controlled	37 patients with PD, 44 hospitalised patients	PD 75 years (range 46 – 91 years), 18M;19F, patients 73 years (range 39 – 89 years), 20M;24F	Observational, cross-sectional study	Only prevalence reported	71% PD patients, 51% other patients (BP > 90mmHg for at least 2 out of 3 meals)	24 hour ABPM, every 30 min for 24 hours
Vaitkevicius et al (1991) (44)	Older, nursing home residents	Mixed meal, 650 kcal, 65% CHO, 15% protein, 20% fat	113 subjects, 7 controls who did consume meal	78 ± 9 years 30M;83F	Descriptive, cohort study	109 (96%) experienced fall in BP, mean fall 18 ± 16 mmHg within 75 min	41 (36%) of patients, 2 symptomatic	Automated sphygmomanometer, every 15 min for 90 min
Van Orshoven et al (2010) (14)	Geriatric ward patients, healthy older control subjects	Not controlled, normal breakfast	22 patients, 20 controls	Patients, 84 ± 5 years 7M;15F, controls 82 ± 4 years 2M;18F	Descriptive, case-controlled study	Mean fall 24 ± 20 mmHg in patients compared to 10 ± 16 mmHg in controls	20 (91%) of patients, 8 (40%) of controls, 18 symptomatic	Portapres, beat-to-beat finger BP monitoring for 135 min

Table 2.1 (continued)

Study	Pt Group and Setting	Test Meal	Number	Patient Characteristics	Design	Systolic BP	Prevalence of PPH and symptoms	Assessment
Vloet et al (2005) (45)	Geriatric ward patients	Mixed meal, 100 mL liquid CHO, 100 mL milk, 65 g solid CHO, 2 g fat, 4 g protein	80 patients	80 ± 1 years 44M;41F	Descriptive, cross-sectional study	Mean fall at 50 min in patients with PPH 23 ± 16 mmHg, non-PPH 0 ± 9 mmHg	57 (67%) of patients, 42 (79%) symptomatic	ABPM every 10 min for 90 min
Zanasi et al (2012) (22)	Hypertensive older patients at cardiology clinic	Not controlled, normal meals	401 patients with essential hypertension	78 ± 11 years, 187M;214F	Prospective, cohort study	PPH preprandial 139 ± 17 mmHg, postprandial 113 ± 15 mmHg, non-PPH, preprandial 130 ± 15 mmHg, postprandial 121 ± 16 mmHg	292 (73%) PPH at least once, 160 (55%) symptomatic, 106 (36%) had 2 episodes of PPH	24 hour ABPM every 15 min

+ve: positive, -ve: negative, ABPM: ambulatory blood pressure monitoring, AD: Alzheimer's disease, BP: blood pressure, CHO: carbohydrate, IQR: inter-quartile range, NGT: nasogastric tube, OGTT: oral glucose tolerance test, PEG: percutaneous endoscopic gastrostomy, PD: Parkinson's disease, PPH: postprandial hypotension. All values are mean ± SD, PPH is defined as a fall in systolic BP > 20mmHg, unless otherwise stated.

Table 2.2: Studies relating to the non-pharmacological management of postprandial hypotension.

Study	Patient characteristics	Study design	Test meal	Intervention	Efficacy	Assessment
Deguchi et al (2007) (147)	5 subjects with MSA, aged 63 ± 6 years, 3M;2F, 100% with PPH, medications not withheld	Unblinded, non-randomised, intervention study	Standardised breakfast, 380 cal, 52 g CHO, 15 g protein, 9 g fat	Water drinking, 350 mL, immediately prior to standardised breakfast, then every morning for 7 days	Effective: fall in BP at baseline (no water) 32 mmHg, reduced to 19 mmHg (day 1) then further to 16 mmHg (on day 7)	Automatic sphygmomanometer, every 5 min for 90 min
Jones et al (2001) (148)	10 healthy older subjects, aged 67 – 78 years, 5M;5F, 3 demonstrated PPH during study, not on medication	Unblinded, randomised study	OGTT, 50 g glucose, 30 mL lemon juice ± 9 g guar gum made to 300 mL with water	Slowing of GE by the addition of guar gum	Effective: drink containing guar gum caused significantly less fall in BP in first 30 min of study	Automatic sphygmomanometer, every 3 min for 60 min, every 15 min between 60 – 120 min
Kuipers et al (1991) (115)	15 healthy older subjects, aged 74 ± 3 years, 8M;7F, no PPH, not on medication	Unblinded, randomised study	OGTT, 75 g in 300 mL water	Test drink served either cold (5ºC) or warm (50ºC)	Effective: cold glucose increased MAP from 107 ± 3 mmHg to 111 ± 4 mmHg at 15 min; warm glucose decreased MAP 108 ± 4 mmHg to 100 ± 3 mmHg at 30 min	Arteriosonde every 5 min for 120 min
Oberman et al (1999) (149)	14 frail elderly patients, aged 88 ± 7 years, 5M;9F, PPH not reported, medications withheld	Unblinded, randomised study	Not standardised, breakfast, mean consumption 444 ± 110 kcal	10 min postprandial walking, commencing 20 min after breakfast	Temporarily effective: MAP fell both days, exercise increased MAP by 18 ± 4 mmHg, however, returned to pre-exercise levels 10 min after exercise ceased	Automatic sphygmomanometer, every 5 min for 60 min

Table 2.2 (continued)

Study	Patient characteristics	Study design	Test meal	Intervention	Efficacy	Assessment
O'Donovan et al (2005) (150)	8 healthy older subjects, aged 70 ± 3 years, 4M;4F, no PPH, not on medication	Single blind, randomised study	Intraduodenal infusion of 50 g glucose in 300 mL water ± 4 g guar gum (rate of delivery 3 kcal/min)	Slowing of small intestinal glucose absorption by the addition of guar gum	Effective: fall in BP evident from 15 – 60 min, significantly less with addition of guar gum	Automatic sphygmomanometer, every 3 min for 120 min
Puvi-Rajasingham et al (1998) (151)	12 older subjects with primary autonomic failure, mean age 56 years, 5M;7F, PPH present, but not reported, medication withheld	Unblinded, randomised study	Liquid meal, 2300kJ, 66 g CHO, 30 g protein, 15 g fat	Supine exercise for 9 min in 3 min blocks of increasing workload	Not effective: BP fell following meal with no exercise from 159 ± 8 mmHg to 129 ± 6 mmHg; during exercise, further fall to 123 ± 6 mmHg	Automatic sphygmomanometer, every 5 min for 60 min
Puvi-Rajasingham and Mathias (1995) (53)	7 subjects with primary autonomic failure, aged 45 – 69 years, 4M;3F, PPH present, but not reported, medication withheld	Unblinded, randomised study	Breakfast, cornflakes, toast, tea, just; lunch, ham salad, yoghurt, bread; dinner, chicken, potatoes, cabbage and rice pudding; total energy 2.5 MJ	Adjusting meal size-6 smaller meals vs. 3 larger meals, eaten over a 12 hour period	Effective: in 3 positions, lying, sitting, standing Systolic BP lower by 20 mmHg, 11 mmHg and 14 mmHg respectively following the 3 larger meals	ABPM every 30 min between 0630 and 2100h on each test day

Table 2.2 (continued)

Study	Patient characteristics	Study design	Test meal	Intervention	Efficacy	Assessment
Russo et al (2002) (152)	11 type 2 diabetic patients, aged 62 ± 1 years, 8M;3F, no PPH, not on medication	Unblinded, randomised study	OGTT, 50 g glucose, 30 mL lemon juice ± 9 g guar gum made to 300 mL with water	Slowing of GE by the addition of guar gum	Effective: dink containing guar gum reduced fall in MAP in first 30 min of study	Automatic sphygmomanometer, every 3 min for 60 min, every 15 min between 60 – 120 min
Shannon et al (2002) (158)	18 older subjects with autonomic failure (either MSA or PAF), aged 70 ± 2 years, 12M;6F, PPH present, but not reported, medication withheld	Unblinded, randomised study	Breakfast, 414 cal, 52 g CHO, 14 g protein, 17 g fat; with or without water prior	Water drinking, 480 mL, immediately prior to standardised breakfast (in 7 patients only)	Effective: max fall following meal alone, 43 ± 36 mmHg, following water consumed before meal, max fall 22 ± 10 mmHg; at 90 min	Automatic sphygmomanometer, every 5 min for 90 min

BP: blood pressure, MSA: multiple system atrophy, OGTT: oral glucose tolerance test, PAF: pure autonomic failure, PPH: postprandial hypotension, CHO: carbohydrate, GE: gastric emptying. All values are mean ± SD, unless otherwise stated.

Table 2.3: Studies relating to the pharmacological management of postprandial hypotension.

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Barakat et al (1993) (161)	10 elderly subjects with renal failure receiving dialysis, 58 ± 5 years, no history of PPH during dialysis	Double blind (to treatment), randomised, cross over study	Mixed meal, 2 pieces of toast, 2 eggs, 5 mL marmalade, butter and 50 mL juice, given 1 hour into dialysis treatment	Caffeine 200 mg 1 hour pre-dialysis	Not effective: % fall in MAP during dialysis with food ingestion 12 ± 2 mmHg vs. dialysis with no food 2 ± 4 mmHg; caffeine had no effect when given prior to meal	Automatic sphygmomanometer, every 15 min for 240 min
Connelly et al (1994) (153)	17 hypertensive elderly subjects; 76 ± 1 years, 11M;6F	Double blind, randomised, crossover study	Liquid meal, 390 to 420 kcal; prior to treatment, and after each 3 week treatment block	Isosorbide dinitrate or nicardipine hydrochloride 20 mg 3/day orally	Effective: significant fall in BP reported after no treatment, ameliorated after both isosorbide dinitrate and nicardipine hydrochloride	Automatic sphygmomanometer, every 5 min for 60 min
Freeman et al (1996) (162)	11 subjects with autonomic failure, aged 54 ± 13 years, 7M;4F (6 with MSA, 5 with PAF); medications withheld	Double blind, randomised, placebo controlled, cross over study	Standardised mixed liquid meal, 400 kcal, 40% CHO, 45% fat, 15% protein	3,4-dl-threo-dihydroxyphenyserine (DL-DOPS) 1000 mg given 3 hours prior to meal	Effective: systolic BP at baseline vs. 30 min, placebo 169 ± 9 mmHg vs. 127 ± 11 mmHg; DL-DOPS 184 ± 10 vs. 148 ± 13 mmHg	Automatic sphygmomanometer, every 15 min for 90 min
Fukushima et al (2012) (105)	24 subjects with MSA, 10 had PPH, aged 64 ± 5, 15M;9F, medication withheld	Single blind, non-randomised, crossover study	250 mL liquid test meal; 375 kcal, 51.5 g CHO, 13.2 g protein, 13.2 g fat	Acarbose 100 mg orally, immediately prior to test	Effective: acarbose reduced AUC of postprandial BP significantly, compared to no treatment	Automatic sphygmomanometer, every 3 min for 60 min
Gentilcore et al (2005) (107)	8 healthy elderly subjects, aged 71 years, 5M;6F, not on medication	Double blind, randomised, crossover	100 g sucrose in 300 mL water	Acarbose 100 mg orally, dissolved in drink on one test day	Effective: acarbose increased BP from 0 – 210 min, no fall detected, compared to sustained fall in BP with no treatment	Automatic sphygmomanometer, every 3 min for 60 min, every 15 min 60 – 210 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Gentilcore et al (2011) (163)	8 healthy elderly subjects, aged 66 – 77 years, 4M;4F, not on medication	Double blind, randomised, crossover	Intraduodenal sucrose, 100 mg in 300 mL for 60 min (5 mL/min)	Acarbose 100 mg dissolved in infusion on one test day	Effective: max fall during control 11 ± 2 mmHg, no fall with acarbose	Automatic sphygmomanometer, every 3 min for 60 min
Hakusui et al (1991) (86)	4 patients with MSA, aged 56 ± 7, 2M;2F, 100% with PPH, medication withheld	Single test, additional study visit following initial test	OGTT, 75 g glucose in 225 mL water	Vasopressin 40U/400 mL saline, infused IV at 0.3 U/min	Effective: PPH was prevented with IV vasopressin administration	Automatic arterial sphygmomanometer, every 1 min for 60 min
Heseltine et al (1991) (164)	7 healthy elderly subjects, aged 67 years, 2M;7F; not on medication	Double blind, randomised, cross over study	Standardised mixed lunch, 585 kcal, 71% CHO, 21% protein, 8% fat	Caffeine, 200 mg given as coffee, or decaffeinated coffee as placebo, with meal	Effective: mean max fall following placebo 14 mmHg, following caffeine, increase 9 mmHg occurring at 60 min	Automatic sphygmomanometer, every 15 min for 60 min, then at 90 min
Heseltine et al (1991) (165)	20 frail elderly subjects, aged 84 ± 5 years; 10M;10F; 2 symptomatic with PPH, medication withheld	Double blind, randomised, cross over study	Standardised 400 kcal glucose drink	Caffeine, 100 mg given as coffee, or decaffeinated coffee as placebo, given following glucose	Effective: mean fall following placebo 8 mmHg, following caffeine, increase 2 mmHg	Automatic sphygmomanometer, every 15 min for 60 min
Hirayama et al (1993) (166)	8 patients with autonomic failure (5 with MSA, 61 ± 4years, 3M;2F; 2 with PAF, 66 ± 12 years 2M; 1 with PD, 71 years, F), 100% had PPH, medication withheld	Single test, additional study visit following initial test for PPH	OGTT, 75 g glucose in 225 mL water	Denopamine (selective β1-adrenergic agonist), 10 mg and 4 mg midodrine-HCl (selective α1-adrenergic agonist), administered 30 min prior to meal	Effective: PPH was prevented when treated with both drugs, compared with no treatment	Automatic sphygmomanometer, every 2 min for 60 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Hoeldtke et al (1986) (103)	8 subjects with AN, aged 56 ± 8 years; 4M;4F, 100% with PPH, medication withheld	Single blind, placebo controlled, randomised, crossover	Mixed breakfast, 2.31 MJ, 1 egg, toast, cereal, orange juice	Octreotide 0.4 µg/kg, 0.2 µg/kg, placebo given immediately prior to breakfast	Effective: mean fall 35 ± 10 mmHg at 60 min after breakfast, prevented by 0.4 µg/kg octreotide	Automatic sphygmomanometer, every 15 min for 180 min sitting
Hoeldtke et al (1986) (167)	8 elderly patients with orthostatic hypotension, 1 with PPH, age 54 ± 7 years, 5M;4F, only 5 treated with dihydroergotamine and caffeine	Single blinded, randomised, placebo controlled cross over study	Breakfast, 550 cal, one egg, toast, cereal, orange juice; lunch, 600 cal, meat, potatoes, milk, fruit	Either dihydroergotamine 10 µg/kg IV 1hr prior to breakfast, and caffeine 250 mg 30 min prior to breakfast orally, or each treatment alone, NaCl placebo	Partially effective: dihydroergotamine prevented PPH in 2 patients, caffeine in 1 patient; combination therapy had synergistic effect	Automatic sphygmomanometer every 15 min after breakfast, every 30 min after lunch
Hoeldtke et al (1989) (169)	11 older subjects (6 with MSA, aged 53 – 73 years, 4M;2F, 5 PAF, aged 41 – 84 years, 3M;2F), 100% with PPH; 14 healthy controls, aged 36 – 89, 9M;5F, medication withheld	Single blind, placebo controlled, randomised, crossover	OGTT, 50 g glucose in 300 mL water	Octreotide 0.8 µg/kg or NaCl placebo, immediately prior to meal	Effective: MSA group, mean fall in BP, placebo 86 mmHg to 66 mmHg; octreotide 92 mmHg to 102 mmHg at 45 min; PAF group placebo 84 mmHg to 55 mmHg; octreotide 74 mmHg to 87 mmHg at 60 min	Automatic sphygmomanometer, every 15 min for 180 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Hoeldtke et al (1991) (168)	13 hospitalised patients with autonomic failure (3 with MSA, aged 68 –77 years, 2M;1F, 5 PAF, aged 42 –86 years, 3M;2F, 5 with Diabetic AN aged 17 – 62 years, 2M;3F), 100% with PPH; 6 healthy controls, aged 59 – 78, 2M;4F	Single blind, placebo controlled, randomised, crossover	Standardised liquid breakfast, 400 kcal; 41% CHO, 39% protein, 20% fat	Octreotide 0.8 µg/kg; dihydroergotamine 7.0 µg/kg (in 9 patients only); placebo control immediately prior to meal	Only octreotide effective: fall during control 98 ± 6 mmHg to 70 ± 6 mmHg; following octreotide 97 ± 6 mmHg to 115 ± 3 mmHg; following dihydroergotamine, modest improvement (values not reported)	Automatic sphygmomanometer, every 8 min for 64 min
Jansen et al (1988) (171)	22 hypertensive elderly subjects; 9 in nitrendipine group, 73 ± 3 years, 4M;5F, 13 in hydrochlorothiazide group, 76 ± 4 years, 3M;10F	Double blind, randomised, parallel-group trial	OGTT, 75 g glucose in 300 mL water prior to treatment, then at 12 weeks post-treatment	Nitrendipine 20 mg once daily or hydrochlorothiazide 50 mg once daily for 12 weeks	Effective: nitrendipine before, 174 ± 4 mmHg to 160 ± 5 mmHg at 60 min, after 12 weeks treatment, 161 ± 4 mmHg to 154 ± 5 mmHg at 60 min; hydrochlorothiazide before, 175 ± 6 mmHg to 166 ± 5 mmHg at 60 min, after 12 weeks treatment, 157 ± 7 mmHg to 155 ± 6 mmHg at 60 min, both medications had significant effects on baseline BP	Arteriosonde every 5 min for 120 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Jansen et al (1989) (170)	10 hypertensive subjects, aged 73 ± 3, 7M;3F, medication withheld	Single blind, randomised, crossover	OGTT, 75 g glucose in 300 mL water	Octreotide 50 μg SC and Insulin 0.3 U/kg at 30 min and 10 min prior to meal respectively	Effective: max fall during control day 12 ± 3 mmHg, minor increase during octreotide	Automatic sphygmomanometer, every 5 min for 120 min
Jansen et al (1989) (97)	10 hypertensive subjects, aged 74 ± 4 years, 4M;6F, 10 normotensive subjects, aged 74 ± 4 years, 3M;7F	Single blind, placebo controlled, randomised, crossover	OGTT, 75 g glucose in 300 mL water	Octreotide 50 μg SC, or NaCl placebo, 30 min prior to meal	Effective: normotensive; mean fall in MAP, placebo baseline 94 ± 2 mmHg, 60 min 87 ± 2 mmHg; octreotide baseline 95 ± 2 mmHg, 60 min 95 ± 2 mmHg; hypertensive placebo baseline 123 ± 3 mmHg, 60 min 109 ± 3 mmHg; Octreotide baseline 122 ± 3 mmHg, 60 min 120 ± 3 mmHg	Automatic sphygmomanometer, every 5 min for 120 min
Jian and Zhou (2008) (172)	43 healthy elderly subjects in nursing home, 100% had PPH, aged 80 ± 6 years, 33M;10F, medication not reported	Single blind, non-randomised, crossover study	Semi-liquid meal, 104.6-125.5 kj·kg^{-1}·day^{-1}; allocated between 3 meals. 50 – 60% CHO, 10 – 15% protein, 20 – 30% fat	Acarbose 50 mg with each meal (breakfast, lunch and dinner)	Effective: acarbose reduced max postprandial fall from 26 ± 14 mmHg to 9 ± 16 mmHg, effective in 63% of patients, 15 still demonstrated PPH	ABPM, every 15 min for 120 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Lipzitz et al (1994) (173)	9 elderly subjects, aged 76 ± 9 years, 2M;7F, 100% with symptomatic PPH, medication withheld	Double blind, randomised, placebo controlled study	Standardised liquid meal, 400 kcal, 40% CHO, 45% fat, 15% protein	Caffeine 250 mg or placebo given orally with meal	Not effective: max fall following placebo 19 ± 6 mmHg, following caffeine 31 ± 7 mmHg	Automatic sphygmomanometer, every 5 min for 90 min
Maruta et al (2006) (174)	11 elderly subjects (4 with PD, 5 with MSA, 1 with DM), 100% had PPH, medication withheld	Single test, additional study visit following initial test for PPH	OGTT, 75 g glucose in 300 mL water	Voglibose 200 µg 10 min prior to meal	Effective: voglibose reduced PPH in magnitude (42 ± 13 mmHg vs. 21 ± 13 mmHg) and duration (52 ± 28 min vs. 17 ± 23 min)	Automatic sphygmomanometer, every 5 min for 120 min
Onrot et al (1985) (116)	6 patients with autonomic failure, aged 63.8 ± 2.9 years, 3M;3F	Randomised, controlled, cross over study	Standardised breakfast, one egg, 2 pieces of bacon, 1 slice of toast, 1 teaspoon jelly, 5g margarine, 1 cup decaffeinated coffee	Caffeine 250 mg given 30 min prior to meal, then every morning for 7 days	Effective: max fall following placebo 28 mmHg, following caffeine, 11 mmHg at 60 min. 7 day treatment continued to ameliorate PPH	Automatic sphygmomanometer, every 5 min for 120 min
Raimbach et al (1989) (125)	7 subjects with primary autonomic failure, aged 40 – 55 years, medications withheld	Single blind, placebo controlled, randomised, crossover	Glucose ingestion at 1 g/kg, water control	Octreotide 50 µg SC, or placebo 30 min prior to meal	Effective: significant fall 150 ± 12 mmHg to 121 ± 12 mmHg at 30 min during control, octreotide 150 ± 10 mmHg to ~160 mmHg at 30 min	Automatic sphygmomanometer, every 5 min for 90 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Rakic et al (1996) (175)	171 elderly subjects; 62 normotensive, aged 72 ± 1 years; 46 untreated hypertensives, aged 77 ± 1 years; 63 treated hypertensives aged 77 ± 1 years	Randomised, controlled, parallel group study	Standardised high CHO meal, 2420 kJ, 72% CHO, 21% protein, 7% fat	2 week treatment with 300 mg caffeine/day from coffee, or no caffeine, or 300 mg caffeine/day from tea (in treated hypertensive subjects only)	Effective: reduction in postprandial BP fall only in normotensive coffee drinkers, and treated hypertensive with tea drinking; BP values not reported	Automatic sphygmomanometer, at baseline and again at 60 min
Robertson et al (1981) (176)	10 patients with autonomic neuropathy, aged 54 – 74 years, 8M;2F, medication withheld	Single blind, randomised, cross over study	Breakfast, 456 cal, 46% CHO, 12% protein, 42% fat	In 6 subjects only, cimetidine 300 mg and 50 mg diphenhydramine, 50 mg indomethacin or 40 mg propranolol; 6 hour intervals 24 hours prior to meal. Placebo used between treatments	Not effective: no drug prevented PPH following the breakfast meal, propranolol potentiated the depressor effect of meal ingestion	Automatic sphygmomanometer, every 3 min for 180 min

Table 2.3 (continued)

Study	Patient characteristics	Study design	Test meal	Treatment	Efficacy	Assessment
Shibao et al (2007) (177)	13 patients with autonomic failure (12 PAF and 1 PD), 100% had PPH, aged 65 ± 3, 5M;8F, medication withheld	Single blind, non-randomised, crossover in 4 patients (pilot); double blind, randomised, crossover in 9 patients	Standardised breakfast; 423 kcal; 42.3 g CHO, 19.5 g protein, 19.9 g fat	Acarbose 100 mg orally, prior to meal	Effective: acarbose reduced mean postprandial fall by 17 mmHg, compared with no treatment	Automatic sphygmomanometer, every 5 min for 90 min
Son and Lee (2012) (157)	29 elderly subjects, aged 82 ± 7 years, 2M;27F, 100% with PPH, medication permitted	Randomised, controlled, cross over study	Not standardised, mean rice eaten 163 ± 24 g, mean soup intake 174 ± 50 mL	Caffeine, 50 mg given as green tea, 20 min prior to breakfast meal	Effective: fall following meal only 134 ± 13 mmHg to 120 ± 12 mmHg, following caffeine 132 ± 14 mmHg to 135 ± 12 mmHg at 45 min	Automatic sphygmomanometer, every 15 min for 90 min

AN: autonomic neuropathy, BP: blood pressure, CHO: carbohydrate, DM: diabetes mellitus, MSA: multiple system atrophy, OGTT: oral glucose tolerance test, PAF: pure autonomic failure, PD: Parkinson's disease, PPH: postprandial hypotension. All values are mean ± SD, unless otherwise stated.