Relationships Between Motor and Sensory Function in the Proximal Gut, Appetite, & Nutrients In Healthy Human Subjects

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SUMMARY

The motor and sensory interactions between nutrients and the proximal gut in humans are not well understood, despite the pivotal importance of these interactions on appetite, absorption and thus, nutrition. In part, this lack of knowledge results from technical difficulties in studying motor function in the human gut. In particular, the inability to continuously measure intraluminal flow with any degree of temporal resolution, has impeded progress in this field. The studies described in this thesis focus on nutrient-gut interactions; and the development of novel methodologies aimed at advancing our understanding and interpretation of the relationships between intraluminal pressures and flows.

Nutrient-Gut Interactions

To examine the roles of insulin and other gastrointestinal hormones in mediating appetite suppression in response to intraduodenal (ID) infusion of glucose, subjects received either ID glucose, ID saline or ID glucose with intravenous octreotide (somatostatin analogue) during euglycaemic hyperinsulinaemia. It was confirmed that ID glucose suppressed appetite and decreased intake compared to ID saline. Moreover, the suppression of appetite did not appear to be due to elevation of plasma insulin, and was abolished by octreotide implying a role for other gastrointestinal hormones in the production of satiety.

Different macronutrients may vary in their effects on gastrointestinal motor and sensory function. The relative potencies of ID lipid and glucose in suppressing appetite and stimulating pyloric motility was therefore compared. In young healthy subjects (18-40 yrs), lipid was found to be more potent at both suppressing appetite and stimulating pyloric motility.

As there is substantial evidence to suggest that regulation of appetite is impaired with ageing, this comparison was repeated in healthy older subjects (65-80 yrs). In the elderly, the two nutrients did not differ in their effects on appetite or intake. The older subjects were less hungry at baseline and had an enhanced phasic pyloric response to lipid, compared to the young. In addition, the elderly had both a higher fasting level of cholecystokinin (CCK) and a greater incremental CCK response to ID lipid. All of which is consistent with dysregulation of appetite with ageing.

Usual diet, and modification of intake are known to influence the gut's subsequent handling of meals containing the nutrient whose intake was altered. The motor mechanisms by which dietary changes influence proximal gut function are unknown. Whether regulation of appetite is likewise affected by changes in diet is also unknown. To determine whether motor modifications occur by "nutrient specific" mechanisms, and to determine whether appetite changes occur at all, pyloric motility and appetite (in response to separate ID infusions of glucose and lipid) were evaluated before and after dietary supplementation with glucose. The motor adaptation which occurred was nutrient specific; in that pyloric tone in response to glucose decreased after supplementation, whilst the motor response to lipid was not substantially altered. Dietary modification did influence appetite, but this change occurred across macronutrient class, with attenuation of the appetite suppressant effects of ID lipid seen after dietary glucose supplementation, whilst perception of appetite in response to ID glucose did not change.

Given the changes found in motor responses to nutrients after dietary manipulation, the effect of diet on fasting motility was then examined. Fasting small intestinal motility was evaluated in longstanding lacto-ovo vegetarian and omnivorous subjects, and also in omnivores who consumed a lacto-ovo vegetarian diet for a 14 day period. No differences in fasting motility were found between long-term vegetarian and control subjects; although when control subjects acutely adopted a vegetarian diet, their interdigestive motor cycle length decreased by approximately one third, due a shortening of phase II. This change was unrelated to total dietary fibre intake. Dietary change may therefore be capable of also modifying fasting motility.

Plasma glucose concentrations in the pathological range (such as seen in diabetes mellitus) are well documented to affect both motor and sensory function in the proximal gut. It is less clear whether plasma glucose within the physiological postprandial range has any effect, and whether physiological levels of hyperglycaemia interact with the presence of small intestinal nutrients. Gastric motor and sensory function were therefore studied at ~4-5 mmol/L (fasting level) and at ~8-9 mmol/L (physiological hyperglycaemia). Physiological hyperglycaemia increased the perception of fullness during fasting and decreased hunger during ID lipid infusion. It also suppressed antral pressure waves, and altered the temporal patterning of phasic pyloric pressures, but had no effect on proximal gastric compliance, or perception of distension. Thus, although physiological hyperglycaemia does affect some proximal gastrointestinal functions, others are spared.

Novel Methodology

To better define the spatiotemporal patterning of duodenal pressures, high resolution manometry along the length of the human duodenum was performed during fasting and 3 different rates of ID lipid infusion. The overwhelming majority of pressure wave (PW) sequences were short (1.5-4.5 cm). ID lipid was associated with a dose-related suppression of the number of PW sequences and regional variation along the duodenum in the patterning of PW sequences compared to fasting. Under all conditions, a greater proportion of sequences were antegrade than retrograde. Further interpretation of the mechanical significance of the temporospatial patterning of duodenal pressures, will require concurrent measurement of intraluminal flows. To date this has not been achievable in human subjects.

In order to concurrently measure intraluminal pressures and flows, a novel laser-Doppler velocimeter was developed. Fibre-optic technology was used to quantify particle speed within the gut lumen and this was implanted in a manometric assembly to enable concurrent pressure measurements. The initial human validation study of this instrument was performed in the oesophagus with concurrent assessment of flow by barium fluoroscopy. The onset of the flow signal from the velocimeter correlated well, particularly in the distal oesophagus, with the occurrence of flow documented fluoroscopically. In analysing data from this study, technical and timing problems with the instrument were discovered. Consideration of these matters has lead to further refinements of the instrument being proposed.

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or any other tertiary institution or material previously published or written by another person except where due reference has been made in the text.

I give consent for this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

Jane M Andrews
December 1999

DEDICATION

To my children, Oliver, Jessica and Tony, without whom this would not have been undertaken (but completed much faster) and to my husband, David without whom I could not have coped.

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Due to their complexity, a number of studies in this thesis involved input from, and collaboration with, a number of people, who each brought a different area of expertise. In Chapter 8A, Jacquie Lavin (visiting from Sheffield) introduced me to the area of "dietary" research, Caroline MacIntosh (Adelaide) was closely involved in Chapter 8C, Marc Verhagen (from Utretch) was a valuable collaborator in Chapters 9B and 12. Chris Rayner (Adelaide) particularly assisted in Chapters 9A and B and Rob Fraser (Adelaide) supervised (and funded) the study described in Chapter 10. In Chapter 12 Burt Nathan (Adelaide - Optical engineer) proved indispensable, as did Taher Omari (who built the combined manometric-velocimetric assemblies) and Shelley McDonald (biomedical engineering student).

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PUBLICATIONS ARISING FROM THE THESIS

The following publications (or manuscripts in preparation) have arisen from the work described in this thesis:

- JM Andrews, S Doran, GS Hebbard, G Rassias, WM Sun, M Horowitz. Effect of glucose supplementation on appetite and the pyloric motor response to intraduodenal glucose and lipid. Am J Physiol, 1998; 274 (Gastrointest. Liver Physiol. 37): G645-G652.

- JM Andrews, CK Rayner, S Doran, GS Hebbard, M Horowitz. Physiological changes in blood glucose affect appetite and pyloric motility during intraduodenal lipid infusion. Am J Physiol, 1998; 275 (Gastrointest. Liver Physiol. 38): G797-G804.

- JM Andrews, H Nathan, CH Malbert, MAMT Verhagen, M Gabb, GS Hebbard, D Kilpatrick, S MacDonald, CK Rayner, S Doran, T Omari, E O'Young, C Frisby, RJ Fraser, M Schoeman, M Horowitz, J Dent. Validation of a novel luminal flow velocimeter with video fluoroscopy and manometry in the human oesophagus. Am J Physiol., 1999; 276 (Gastrointest. Liver Physiol. 39): G886-G894.

- JM Andrews, SM Doran, GS Hebbard, CH Malbert, M Horowitz, J Dent. Nutrient-induced spatial patterning of human duodenal motor function. submitted to J. Physiol. (Lond) (Sept '99) - 1st revision with reviewers (Nov '99).

- JM Andrews, SM Doran, AC Di Matteo, CG Macintosh, Chiu, NW Read, RJ Fraser. Acute adoption of vegetarian diet alters fasting small intestinal motility: no effect of chronic lacto-ovo vegetarian diet. manuscript in preparation (Dec '99).

- JM Andrews & M Horowitz. Gastric function in the elderly. Clin Geriatr. 1996; 4: 21-43.

- CG Cook, JM Andrews, KL Jones, GA Wittert, I McP Chapman, Morley JE, M Horowitz. Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age. Am J Physiol, 1997; 273: (Regulatory Integrative Comp. Physiol. 42) R755-R761.

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- MA Verhagen, CK Rayner, JM Andrews, GS Hebbard, S Doran, M Samson, M Horowitz. Effect of physiological changes in blood glucose on proximal gastric compliance and the perception of gastric distension in normal subjects. Am J Physiol., 1999; 276 (Gastrointest. Liver Physiol. 39): G761-G766.

CHAPTER 1

The Role of the Gut in Appetite Regulation

1.1 INTRODUCTION

Appetite regulation is a complex process with many overlapping influences and is consequently difficult to study and poorly understood. Although, in the longer term, signals from body fat stores may regulate weight by altering both intake and metabolic

rate (Bennett 1995; Leibel et al 1995; Campfield et al 1996a & b; Jequier & Tappy 1999); in the short-term, signals arising from the gut, particularly in response to nutrients in the small intestine, appear to be important in the acute signalling of satiety and satiation and in decreasing food intake (Welch et al 1985; Lavin et al 1996). The terms satiety and satiation are used inconsistently in the literature, with some papers (and usual medical parlance - "early satiety") defining satiety as the process which terminates an acute episode of eating (Morley 1987; Rolls 1995a), whilst others define it as the feeling of satisfaction/sufficiency which delays the need to eat again, and use satiation to describe the process of meal termination (Blundell et al 1993a & 1994). In this thesis satiety will be used to refer to the acute process of becoming "full", and satiation as the intermeal feeling of satisfaction. The focus of this chapter is the role of the gut in human appetite regulation, although reference is made to the role of other organ systems and to relevant animal work in the field where appropriate.

In humans, eating is not only a physiological imperative, but also a social activity. Thus when examining appetite regulation in humans, it is essential to recognise the potential impact of social and psychological factors on appetite and eating behaviour, even in the absence of perceived stress or recognised psychological disorders. Accordingly, where possible, in both the review of the literature and in the performance of the studies presented in this thesis, these factors are identified and considered, if it has not been possible to eliminate them.

Food interacts with the gut along its length and it is possible that it is the combination of these interactions which regulates appetite. However, in order to dissect out the relative contribution of each region to appetite regulation and to examine possible mechanisms, each region of the proximal gut is considered separately, although interactions between regions will also be acknowledged where information is available. Different macronutrients appear to generate satiety signals by discrete pathways; it is possible that this may influence the magnitude of their effects on appetite. Usual dietary intake, and changes in it, alter motor function in the gut (Chapter 4), but again the influence on appetite is unclear. The evidence for nutrient-specific effects, and the possibility of adaptive changes in appetite in response to dietary change are therefore discussed.

1.2 PREABSORPTIVE MECHANISMS IN APPETITE REGULATION

1.2.1 Oral effects

While there is little direct work examining the effect of the oral presence of food on appetite, the sight and smell of food are known to generate a number of physiological responses, including salivation, and gastrointestinal hormone release, and food in the mouth further augments these responses (Lam et al 1993; Soffer & Adrian 1992). In general, for palatable foods, this oral phase of eating probably provides positive feedback to augment appetite (Smith et al 1990). Certainly in animals, oral exposure to food, which is then diverted from the gut, does not have any suppressant effect on intake (reviewed in Morley 1987). Although more recent work in humans suggests that when oral and intestinal exposure to nutrients are combined appetite is suppressed to a greater degree than with intestinal exposure alone (Cecil et al 1998a). Oral exposure to fat influences its subsequent metabolism in humans (Mattes 1996), suggesting that there is a specific oral ability to "sample" and signal the content of foods. Given that absorption and metabolism generate the release of several putative satiety factors, oral exposure to food may thus influence appetite.

Taste, which is influenced by individual preference and social factors, is thought to play an important role in the oral component of appetite regulation (Morley 1987; Read et al 1994). In animals specific oral abilities to "sense" fat (Rolls ET et al 1999) and sweet solutions (De Graaf et al 1993; Swithers & Hall 1994) have been described, and there is evidence that humans may also have macronutrient-specific oro-sensory abilities (Mattes 1996; Myers & Epstein 1997). In fact, in affluent societies, taste is now the primary determinant of food choice (Drewnowski 1988). The general principle is that pleasant tasting foods are consumed preferentially. Unfortunately, pleasant tasting foods tend to be energy dense, containing large amounts of fat and/or sugar, and are implicated in causing obesity in humans (Blundell et al 1993a & b; Rolls et al 1994a; Jequier & Tappy 1999) and animals (Hope et al 1999). It is also thought that the variety of tastes within one's diet affects the amount consumed, with Rolls et al (1981) proposing the concept of "sensory-specific satiety". They showed that consumption of a particular food was associated with a decrease in pleasantness rating, whilst the pleasantness of foods not eaten did not change; moreover, ratings of pleasantness then correlated with the subsequent amount of each food consumed. Thus, "sensory specific satiety" may be important in ensuring adequate calories and a balanced macronutrient intake are maintained. Animal studies have also supported this concept of sensory specific satiety. In one, a larger amount of novel-tasting food was

consumed, even in when rats were not food deprived (Holder & Di Battista 1994), and in the second, diminished oral responsiveness to a sweet solution, was demonstrated, which was specific for the sweet solution initially used and persisted for several hours (Swithers & Hall 1994).

Overall, oral food exposure alone does not appear to produce satiety or satiation, but influences food choice and interacts in potentially important ways with other factors to affect satiation and satiety. Oral exposure may affect appetite by influencing patterns of intake, modulating metabolism of nutrients and augmenting satiety signals arising from other regions in the proximal gut.

1.2.2 Gastric effects

The presence of food in the stomach is well documented to decrease hunger, increase fullness and reduce subsequent food intake in animals (reviewed in Houpt 1982) and humans (Sepple & Read 1989; Rolls 1995; Doran et al 1998). Gastric distension is proposed to be the primary mechanism by which suppression of appetite occurs, and is likely to be signalled by both vagal and humoral means (Morley 1987; Read et al 1994). Antral distension, in particular, has been found to correlate with postprandial satiation (Hveem et al 1996; Jones et al 1997b). It is not necessary for food to have been consumed orally to suppress appetite, as intragastric nutrient infusion also decreases appetite and food intake (Rolls 1995; Shide et al 1995; Stratton et al 1998), although, as discussed, not to the same degree as when consumed orally (Cecil et al 1998a).

In humans, it is difficult (and somewhat artificial) to separate out the purely "gastric" from the "small intestinal" component of appetite regulation, as in most cases, nutrients begin to empty from the stomach promptly after ingestion. However, an initial role for gastric distension in human satiety is supported by several pieces of data:- the rapid onset (~15 min) of decreased hunger and increased fullness after consumption (Doran et al 1998); the fact that a larger meal suppresses appetite more than a smaller meal (Doran et al 1998); and that greater gastric capacity is associated with larger intake from a test meal (Geliebter et al 1992). Not withstanding this, there are a number of doubts surrounding the magnitude of the role gastric distension plays in the day to day regulation of appetite. In particular, Sepple & Read (1989) note that satiation is present up to 4 hours after eating, when there is little left in the stomach. Morley (1987) has questioned whether gastric distension plays a role at all in the course of satiety during a

normal meal, instead signalling "over-distension" when large, celebratory meals are consumed. This contention is supported by the finding that intragastric pressure in spontaneously eating pigs is evenly maintained within a narrow range (Houpt 1994) only rising above this when given access to food and drink after a long (16-18 hour) period of deprivation. Moreover, in humans Feinle et al (1996) showed that gastric distension in the absence of small intestinal nutrients causes only a feeling of discomfort rather than "meal-like" satiety; and others have found that the initial appetite suppressant effects of an intragastric balloon (Geliebter 1988) do not persist (Pasquali et al 1990). On the other hand, impaired gastric relaxation in functional dyspepsia (which may give a greater "distension signal") has been associated with early satiety, and that this symptom decreased with improved fundal relaxation (Tack et al 1998).

The "gastric" mechanisms which sense nutrients seem to be relatively imprecise, as the accuracy of energy compensation seen in subjects when offered food subsequent to intragastric nutrients varies substantially. Shide et al (1995) showed very accurate compensation after rapid intragastric fat and carbohydrate (250 ml, 2092 kJ over 15 min), and slow fat (250 ml, 2092 kJ over 3.5 hr), but not after slow carbohydrate infusion. Whereas Stratton et al (1998) found that over 3 days, nasogastric feeding only suppressed usual oral intake by 17%. In large part these discrepancies are likely to reflect differences in study design, as each is likely to be assessing different gut sensing methods; with the earlier study design biased toward examining short-term, gastric effects, and the subsequent one, toward small intestinal, postabsorptive and metabolic mechanisms. It is also possible that sensing methods and their accuracy vary between nutrient classes, with some authors finding carbohydrate to be more accurately compensated for than fat (Blundell et al 1993a). As the mechanisms which sense nutrients and enable day to day balance in energy intake to be maintained are unlikely to be mediated by predominantly gastric means, they are considered in greater detail below (sections 1.3.1, 1.3.2 & 1.4)

The rate of gastric emptying also influences appetite regulation, as emptying rate is a significant factor in determining the duration over which small intestinal nutrient exposure occurs. As the rate of emptying is largely determined by nutrient-mediated small intestinal feedback, this issue is considered below (section 1.3.1).

1.3 ABSORPTIVE MECHANISMS IN APPETITE REGULATION

1.3.1 Role of the Small Intestine

Exposure of the small intestine to nutrients decreases hunger, increases fullness and decreases subsequent consumption in humans (Welch et al 1985; Sepple & Read 1989; Rolls 1995; Lavin et al 1996; Doran et al 1998). The presence of nutrients in the small intestine also modifies the perception of both gastric (Feinle et al 1996), and duodenal distension (Edelbroek et al 1994a). In humans, oral or gastric exposure to nutrients is not necessary for appetite suppression, as direct infusion of nutrients into the small intestine also suppresses appetite (Welch et al 1985; Lavin et al 1996). Although these effects may be mediated by either neural or humoral means, gastrointestinal hormones appear to play the larger role, as the appetite-suppressant effect of small intestinal glucose is largely abolished by the use of somatostatin (Lavin et al 1996) which prevents gastrointestinal hormone release, and gastric distension in the presence of intraduodenal lipid is perceived as less "meal-like" when cholecystokinin A (CCK-A) receptors are blocked (Feinle et al 1996). Also in support of the dominance of humoral factors over neural mechanisms, Meyer et al (1998c) have recently demonstrated persistence of small intestinal satiety in rats despite capsaicin blockade of chemosensory nerves. It is perhaps facile to consider neural and humoral means as completely separate however, as many factors recognised as gut hormones also act as neurotransmitters both peripherally and in the CNS (Wittert et al 1997).

Both the length and site of small intestine exposed to nutrients may affect the expression of satiety and satiation, and species differences are likely to exist. In rats, Meyer et al (1998b & c), have shown that degree of satiety correlated with length of intestine exposed to nutrients and that nutrients which suppressed intake were as effective whether infused into the duodenum or midgut (Meyer et al 1998a). In humans however, Welch et al (1985) found infusion of lipid into the distal small intestine immediately prior to a test-meal induced early satiety (and hence reduced the amount consumed), despite the subjects having no suppression of hunger prior to the test-meal. In contrast, more proximal nutrient infusions have resulted in both increased satiety and decreased hunger (Lavin et al 1996). Suggesting perhaps that the proximal and distal small intestine signal hunger and satiety by different means, and that hunger and fullness are not simple opposites, and may vary independently (Sepple & Read 1989).

The effect of nutrients on appetite may vary between macronutrient classes, with some authors proposing carbohydrates to be more potent appetite-suppressants than fats (reviewed in Blundell et al 1994; Cotton et al 1994). Other authors propose that fats exert a greater effect (see Foltin et al 1992); and still others reporting equivalent effects (De Graaf et al 1992; Cecil et al 1998b). Subject factors also appear to be important in nutrient specific effects, with Rolls et al (1994a) demonstrating accurate energy compensation following covert preloads of either carbohydrate or fat in normal weight men, whilst in females and men reporting restrained intake, the energy compensation after fat preloads was less accurate than that following the carbohydrate preload. Foltin et al (1990), also found that normal weight men were capable of compensating equally well for covert manipulations in either carbohydrate or fat intake.

The small intestine is the main site of intraluminal digestion and subsequent absorption of nutrients. Discrete pathways are responsible for digestion and absorption of different macronutrients, and the separateness of these pathways may account for some of the observed differences between macronutrients in terms of both appetite regulation and gut motor function (see section 4.2.5). In rats, Meyer et al (1998a, b & c) have reported that the chemical specificity of the nutrient determines the degree of satiety which occurs. Furthermore, for fat, they showed that the timing and degree of satiety correlate with the spread of lipolytic products along the small intestine, rather than with gastric distension (Meyer et al 1998c). This situation is analogous to the relationships between both the length of intestine exposed to nutrients and their chemical specificity and the degree to which nutrients slow gastric emptying in dogs (Lin et al 1989, 1990a & b); suggesting that a close relationship exists between the factors which determine satiety and those regulating gastric emptying - perhaps by controlling the timing and extent of small intestinal nutrient exposure.

In humans (Carney et al 1995), as in rats (Meyer et al 1998c), intraluminal digestion of fats is necessary for a reduction in hunger to occur. In rats the satiety demonstrated after fatty acids (Meyer et al 1998a) was shown to be directly related to chain length, with only those of 12 carbons or more being effective; and satiety was abolished when chylomicron transport and apolipoprotein A-IV (Apo A-IV) secretion were blocked (Meyer et al 1998c). Importantly, in a human study, only fatty acids with a chain length of 12 carbons or longer released CCK, and reduced antral contractile activity, although satiety was not measured (McLaughlin et al 1999); suggesting that a similar system is operant in man. Fatty acids of fewer than 12 carbons in length are sufficiently water soluble to be transported from the gut after absorption via the portal

blood, and do not generate chylomicrons or Apo A-IV, perhaps accounting for their lack of effect on satiety. In rats the production of chylomicrons also stimulates CCK release (Raybould et al 1999), which is also thought to be involved in the production of satiety in both humans and rodents (see 1.3.2.2). Apo A-IV is able to cross the blood brain barrier and hypothalamic Apo A-IV receptors which trigger satiety have been described in rodents (discussed in Meyer et al 1998c), providing a pathway by which it may mediate its effects.

In rats, for carbohydrates (after hydrolysis to monomeric sugars), the ability to stimulate the glucose transporter appears to be necessary to induce satiety (Meyer et al 1998a). Although this is not necessarily so in humans as fructose clearly suppresses intake compared to saline (Rayner et al 1999b). With respect to regulation of protein intake, studies have shown the amino acids L-phenylalanine and L-tryptophan to suppress intake (see Carney et al 1994; Meyer et al 1998a). This appetite suppression may be mediated via CCK release, at least for L-Phenylalanine (Ballinger & Clark 1994).

In both animals and humans, the length of time over which the small intestine is exposed to nutrients also affects the duration of appetite suppression. This is dependent on both the physico-chemical properties of the food and the rate of gastric emptying. For example, a high nutrient "meal" containing guar gum emptied from the stomach more slowly, and suppressed hunger for longer than a high nutrient meal without guar (French & Read 1994). However, the high nutrient meal with guar gum, emptied from the stomach only slightly slower than the high nutrient meal without guar (mean change in T1/2 ~15 min), but suppressed hunger for much longer (mean change ~65 mins), making it likely that the contact time of the nutrients with the small intestine rather than the gastric emptying rate, or duration of gastric distension, is the factor primarily responsible for the suppression of hunger. The physical state of meals is a major determinant of the rate of gastric emptying and absorption of nutrients from the meal (Lavin & Read 1995; Benini et al 1994 & 1995), with both the presence of fibre and frying of fats noted to reduce the glycaemic response and delay the return of hunger. Moreover, it is apparent that these effects are not wholly due to the slowing of gastric emptying (reviewed in Cherbut 1995), and that the physical state of the food alters the duration of small intestinal nutrient contact (Lavin & Read 1995).

In humans, usual diet (or an alteration of it) is capable of modifying the rate of gastric emptying (Corvilain et al 1995; Cunningham et al 1991a & b; Horowitz et al 1996;

Rigaud et al 1988; Robinson et al 1988), and thus changing the duration of small intestinal nutrient exposure. This alteration in the duration of small intestinal nutrient exposure is likely to also affect appetite, although this issue has not been clearly addressed. The existing studies have examined dietary modification and gastric emptying rates, but not appetite. Restriction of intake slows gastric emptying in both normal subjects (Corvilain et al 1995), and patients with anorexia nervosa (Rigaud et al 1988; Robinson et al 1988). Moreover, this slowing of gastric emptying appears to be due to small intestinal contact with nutrients, rather than signals from body fat stores, as gastric emptying in patients with anorexia normalises within days of resumption of normal intake, prior to attainment of normal weight (Rigaud et al 1988; Robinson et al 1988). This normalisation of gastric emptying rate is also seen in underweight patients with bulimia (where large amounts of food are consumed, but vomited before contacting the small intestine), upon adoption of normal intake. Supplementing the diet with either fat (Cunningham et al 1991a) or glucose (Cunningham et al 1991b; Horowitz et al 1996) accelerated the subsequent gastric emptying of the added (or closely related) substance. Shi et al (1997) have duplicated these observations in rats for dietary supplementation with protein, moreover they demonstrated the acceleration in gastric emptying to be specific for subsequent protein meals, and to possibly be mediated through a reduction in CCK release in the high protein group. Rats have also been shown to adapt both their pancreatic secretion (Spannagel et al 1996), and their small intestinal transit rate (Brown et al 1994) in response to alterations in small intestinal nutrient exposure, emphasising the plasticity in the relationship between intake and gut function in animals. Some similar plasticity is also likely to exist in humans, but it is not known whether the aforementioned effects of dietary supplementation on gastric emptying are macronutrient-specific, nor whether perception of appetite is modified. These issues are addressed further in chapter 8B.

1.3.2 Putative Satiety Factors/Mechanisms

Many of the gastrointestinal hormones released by nutrients in the small intestine are thought to play a role in appetite regulation. In particular, CCK, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 (GLP-1), insulin, somatostatin and bombesin have all been implicated. A number of other substances including opioids, neuropeptide Y (NPY), and corticotrophin releasing factor (CRF) are proposed to play a role centrally and possibly also peripherally; these are discussed in section 1.4.2.

Much of the evidence relating to the role of "satiety factors" comes from animal studies, given the relative ease with which such preparations can be manipulated and subsequently examined. Given the difficulty of discerning discrete effects in humans from the measurement of blood levels (despite the fact that the action may be local), or the use of exogenous administration (which might not reach the site of action), knowledge in humans is far more limited. Measurement of putative satiety factors in blood may mislead by detecting innocent bystanders, and administration of pharmacological doses may obscure subtle effects. Hence, except where specific antagonists are available for use in humans, the understanding of the roles of these factors in humans is extensively contributed to by extrapolation from animal work.

1.3.2.1 Cholecystokinin (CCK)

CCK is released from I cells in the small intestine in response to fat and protein in the gut. Its release appears to be partly dependent on the formation of chylomicrons subsequent to absorption of fatty acids of 12 or more carbons (McLaughlin et al 1999; Raybould et al 1999). It has well documented physiological roles in humans causing gallbladder contraction (hence its name), and slowing gastric emptying (Liddle et al 1986; Kleibeuker et al 1988).

Given the link between gastric emptying rate and duration of suppression of hunger after a meal (1.3.1), it is thought that CCK is likely to be involved in signalling satiety. Certainly, in monkeys, exogenous CCK slows gastric emptying and reduces intake, but only in the presence of gastric distension (Moran & McHugh 1982). In humans, as a group, a positive relationship exists between fullness and CCK, and an inverse one between hunger and CCK after a meal (French et al 1993), although in this study there was considerable variation between individual subjects in the presence and strength of these relationships. When CCK-33 was infused to result in physiological postprandial levels it suppressed hunger and decreased intake at a meal offered fifteen minutes after a preload (Lieverse et al 1994a); and there was no difference between lean and obese subjects. However no effect was seen in a similar study without the preload (Lieverse et al 1993a), so it would seem that other signals arising from the stomach or small intestine are necessary to act in concert with CCK to suppress appetite in humans.

Use of antagonists have shown at least partial reversal of CCK's ability to suppress food intake in animals (Silver et al 1989), and in humans (Lieverse et al 1994b), and under some conditions CCK antagonists have been found to increase intake compared

to basal (Silver et al 1989). Based on these studies, antagonists of CCK lead to ~25-30 % increase in intake (Lieverse et al 1994b). CCK-A receptors in humans are involved in signalling "meal like" feelings of satiety in response to gastric distension during intraduodenal lipid (Feinle et al 1996), and there is some evidence that CCK-A receptors may be involved in the production of nausea. This may cause a reduction of intake distinct from satiety, although probably not with the levels of CCK in the usual postprandial situation.

1.3.2.2 Glucose

Glucose is rapidly released by mucosal and intraluminal digestion of a number of commonly ingested carbohydrates. Absorption of glucose is rapid, and results in a postprandial rise in peripheral blood glucose. Given the temporal association between blood glucose elevation and satiation, it has been proposed to act as a centrally perceived satiety factor; with Mayer (1953) showing that hunger is perceived when the arterio-venous glucose increment falls below 10 mg per 100 ml. Moreover, elevations in blood glucose, although in diabetic subjects, have been associated with increased feelings of fullness, even prior to a meal (Jones et al 1997a), although this observation has not been consistent (Jones et al 1996a). Pathological levels of hyperglycaemia (~12 mmol/l) have been shown to alter both somatic (Thye-Ronn et al 1994) and visceral sensation (Hebbard et al 1996b), and to alter gastroduodenal motility (see Chapter 4)., Its effect on appetite however, has not been directly examined. Despite the evidence implying a role for blood glucose in appetite regulation, when peripheral blood glucose levels were matched, only intraduodenal (not intravenous) glucose suppressed appetite (Lavin et al 1996). Thus suggesting that it may be the interaction of glucose with the intestine, rather than its serum level which is more important.

1.3.2.3 Insulin

Insulin is released from the islet cells in the pancreas in response to hyperglycaemia, and the quantity of insulin secreted, at a given blood glucose level, is greater when the glucose load is given enterally than intravenously (see Fehmann et al 1995; Lavin et al 1996) emphasising the importance of the gut in mediating the insulin response. "Incretins" released from the gastrointestinal tract are believed to be responsible for this augmentation of insulin release following enteral glucose (Fehmann et al 1995).

There is extensive animal evidence that insulin has a role in appetite regulation (Woods et al 1984), with some studies showing insulin to enhance the satiating effect of a meal, whilst others find exogenous insulin may stimulate appetite (discussed in Lavin et al 1996). In humans the same controversy exists, with one group (Rodin et al 1985) finding insulin to increase hunger and food intake in humans, whilst others present evidence suggesting insulin either suppresses hunger (Lavin et al 1996), or has little or no role in appetite regulation (Lavin & Read 1995). In a state of insulin deficiency (Type 1 insulin dependent diabetes mellitus), hyperphagia occurs, which is thought by some authors to be due to a deficiency of insulin's appetite-suppressant effect in the CNS (Sipols et al 1994), although this has not been proven.

Suppression of appetite is seen when a glucose load is given intraduodenally, but not when the blood glucose is elevated to a similar level by intravenous glucose (Lavin et al 1996), suggesting that insulin (or the gut factor "incretin" which augment its secretion) is important in the suppression of appetite seen in response to small intestinal carbohydrates. However, other authors have found no relationship between physiological levels of insulin and appetite ratings or intake (Chapman et al 1998). The roles of the incretins are discussed below (1.3.2.4).

1.3.2.4 Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)

GIP and GLP-1 have recently been identified as "incretin" factors responsible for the augmented insulin response to intestinal as compared to intravenous glucose (see 1.3.2.3 above; and Fehmann et al 1995). GIP is found in the K cells of the intestine, and occurs in highest concentration in the duodenum. GLP-1 is present in the L cells, and is predominantly found in the distal bowel. They are both secreted in response to certain oral, but not intravenous nutrients (reviewed in Fehmann et al 1995). Glucose and mixed meals are the strongest secretagogues, although fatty acids and some amino acids are effective at higher doses (Wittert et al 1997), and fructose stimulates similar GLP-1 but less GIP than glucose (Rayner et al 1999b). Given their different sources, it is thought their release is likely to be triggered by different means, but it is proving difficult to find separate triggers in humans, as despite its predominant source in the distal small intestine, GLP-1 is released as promptly as GIP after oral nutrients (Fehmann et al 1995). Both GLP-1 and GIP stimulate insulin release in the presence of hyperglycaemia. In rats, both incretins have been shown to have effects on fat metabolism, the situation in humans is unclear.

In humans, for glucose, the amount of GIP and GLP-1 released is proportional to the enteral caloric load delivered (Schirra et al 1996), providing a mechanism whereby the quantity of nutrients ingested could be signalled. Gastric emptying was slowed in the presence of greater GLP-1 and GIP concentrations, however the caloric rate of duodenal glucose delivery was still higher as the gastric glucose load increased. GLP-1 required a threshold rate of delivery of >1.4 Kcal/min to the duodenum for secretion, whereas GIP had ongoing release at low rates of duodenal glucose (Schirra et al 1996). Exogenous GLP-1 inhibits the early phase of gastric emptying, causing a lower glucose, glucagon, and pancreatic polypeptide response and a greater insulin response (Schirra et al 1997 & 1998; Wishart et al 1998). These "normoglycaemic" properties have led to it being examined as a promising agent for the normalisation of blood glucose in diabetes mellitus (Holst et al 1998). GLP-1 could suppress appetite by the increased time over which small intestinal nutrient exposure occurs due to the slowing of gastric emptying; or via central actions, perhaps vagal afferents, as its effect on gastric motor function is known to be mediated via the vagi (Imeryuz et al 1997; Tolessa et al 1998; Wettergren et al 1998).

Some of the studies suggesting a role for insulin in appetite regulation (see 1.3.2.3), may have unwittingly been describing the appetite-suppressant effects of GLP-1 and/or GIP. Although there seems to be less evidence for an important effect for GIP (Fried et al 1989). In rats GLP-1 acts centrally to decrease appetite (Kalra et al 1999), possibly by blocking neuropeptide Y (NPY) in the hypothalamus (Tritos et al 1998). Central administration of GLP-1 also decreased intake in chicks via a NPY dependent pathway (Furuse et al 1997). Furthermore Turton et al (1996), have shown the appetite suppressant effects of GLP-1 to be abolished by the specific GLP-1 antagonist, exendin in rats. Exogenous GLP-1 has now been shown to increase satiety and decrease intake in lean humans (Flint et al 1998), however obese subjects maintained consumption despite increased feelings of satiety (Naslund et al 1998). Given the evidence discussed, GLP-1 in particular, appears to be a strong candidate to play a role in intestinally mediated satiety in humans.

1.3.2.5 Somatostatin

Somatostatin is present extensively in the proximal gut, and acts largely in a paracrine fashion to regulate (suppress) release of other hormones and modulate gastrointestinal motility (Wittert et al 1997). When somatostatin analogue is given intravenously the

measurable release of gastrointestinal hormones is prevented (Gyr & Meier 1993). In the fasting state, exogenous somatostatin may decrease appetite in humans (Lieverse et al 1995a). Whilst during nutrient infusion into the proximal small intestine, concurrent administration of somatostatin attenuates the development of feelings of satiety (Lieverse et al 1995a; Lavin et al 1996), presumably by preventing the release of other satiety factors from the gut.

1.3.2.6 Bombesin

Bombesin is a member of family of peptides including gastrin releasing peptide which is known to stimulate gastrin release and to contract the lower oesophageal sphincter (Wittert et al 1997). It is not widely studied as a satiety factor, however, in lean humans, exogenous bombesin has been shown to reduce hunger and slightly, but not significantly, decrease intake after a preload (Lieverse et al 1993b), whereas obese women do not appear to be sensitive to its appetite suppressant effects (Lieverse et al 1994c). It appears to act via a CCK independent route (Lieverse et al 1993b), but does not seem likely to play a major role in appetite regulation.

1.3.2.7 Neural pathways

The gut is richly innervated with sensory endings (see Chapter 3). In particular the mucosa contains sensory nerve endings from both the enteric nervous system (ENS) and the autonomic nervous system (ANS) which are capable of being stimulated by luminal substances, either directly or by second messengers during absorption (see 3.4.1). The signals perceived by these afferent fibres may act directly within the ENS to modulate motor function, such as slowing gastric emptying; or be transmitted centrally via vagal or spinal pathways, where they have the potential to interact with the central control of appetite regulation. CCK certainly exerts some of its effects via vagal fibres (McLaughlin et al 1999), as does GLP-1 (1.3.2.4). In addition to stimulating nerve endings in the periphery, satiety factors circulate and may cross the blood brain barrier, where they could directly stimulate hypothalamic and other regions involved in appetite regulation. Neural pathways, and interactions between nutrients and motor function are considered in greater detail in Chapters 3 & 4.

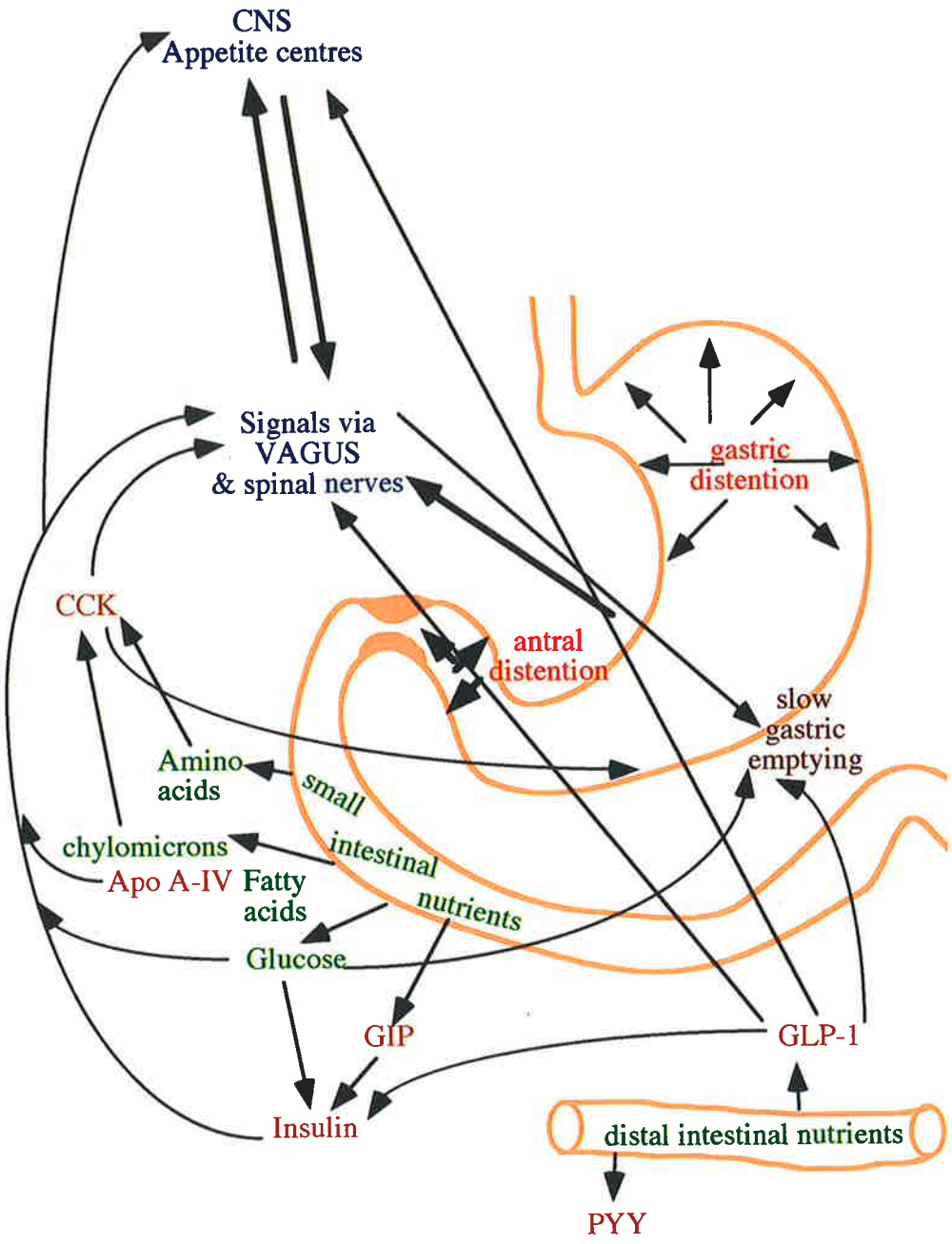


Figure 1.1
Some of the putative gastrointestinal satiety mechanisms. For further detail see text.

1.4 POSTABSORPTIVE MECHANISMS IN APPETITE REGULATION

1.4.1 Effects of portal and intravenous nutrients

Earlier theories of appetite regulation speculated that nutrients in the portal blood may be important in determining satiation and satiety (Russek 1971). However, animal experiments comparing intraduodenal with portal infusion of glucose (VanderWeele et al 1974) found that only intraduodenal glucose reduced intake, emphasising the importance of signals generated by small intestinal nutrients. Similarly, in humans, intraduodenal glucose suppressed appetite and intake, compared to intravenous glucose titrated to obtain similar blood glucose levels (Lavin et al 1996). Lipid has also been noted to suppress appetite to a greater degree when given intraluminally than intravenously (Welch et al 1985). While this observation does not preclude the possibility that absorbed lipid has a central appetite suppressant effect, it suggests this is of less importance than the role played by its preabsorptive/absorptive signals. Despite these clear short-term studies largely refuting a role for intravenous nutrients in appetite suppression, other investigators have found parenteral nutrition to decrease appetite in humans (Bursztein-De Myttenaere et al 1994), reduce intake in primates (Woods et al 1984), and to slow human gastric emptying (see MacGregor et al 1979; Bursztein-De Myttenaere et al 1994). These studies were performed over longer time courses, and unfortunately parenteral nutrition was not compared directly with enteral nutrition in terms of the degree of appetite suppression or delay in gastric emptying seen, making it difficult to assess the relative contribution of intestinal vs intravenous levels of nutrients to satiety. In addition, at least part of the effects observed are likely to be due to hyperglycaemia (MacGregor et al 1979; Woods et al 1984). The failure of intravenous nutrients to suppress appetite in short-term models is likely to be due to the bypass of gastrointestinal signalling (see 1.3.1 & 1.3.2), intravenous fat, for example, does not cause CCK release (DeBoer et al 1992b); in the longer term studies other factors (referred to in 1.1) are likely to be involved.

In contrast to the situation for increased blood levels of nutrients, decreased levels can certainly stimulate appetite, particularly for glucose. In particular, Campfield and Smith (1990) have shown small transient declines in blood glucose to precede each episode of feeding in rats, in addition this feeding response was blocked by infusion of glucose but not other nutrients. These dips in blood glucose concentration determined the timing of the onset of feeding episodes, but not the amount eaten. Moreover, Campfield et al (1996b) have now found evidence that transient declines in blood

glucose are also important in initiating intake in humans. Interestingly, this model is consistent with the glucostatic theory proposed some decades ago by Mayer (1953).

1.4.2 Central nervous system (CNS) appetite regulation

It is widely held that the regulation of appetite can be simplified into a central feeding drive and a peripheral satiation system (Morley & Silver 1988), although most of the work has been performed in rodents. The peripheral satiation system is predominantly based around the gut as discussed earlier (sections 1.2 & 1.3). The central feeding drive involves numerous substances within the central nervous system (CNS), with neuropeptide Y (NPY) (Kalra et al 1991), opioid peptides (such as dynorphin), serotonin (5HT) (Pijl et al 1993) and norepinephrine appearing to be of particular importance (see figure 1.2). In brief; opioid peptides increase intake, particularly of high fat and highly palatable foods; NPY particularly stimulates carbohydrate intake; and norepinephrine administered centrally also stimulates intake, particularly of carbohydrate, by increasing meal size (reviewed in Morley 1987 and Morley & Silver 1988). 5HT is implicated in inhibiting intake, although some 5HT receptor types stimulate intake. Norepinephrine probably promotes intake by inhibiting corticotrophin releasing factor (CRF) which is in turn an inhibitor of both opioids and NPY. A number of "gut" peptides such as GLP-1 (see 1.3.2.4) are also found in the CNS, and appear to be involved in modulating intake.

It is likely that a high degree of interplay exists between the gut and the CNS, and it is probably a more of useful paradigm than a true division to separate their role in appetite regulation. Nonetheless, it is beyond the scope of this thesis to consider the central regulation of appetite in more than a cursory fashion.

1.5 ASSESSMENT OF APPETITE

No ideal method for assessment of appetite in humans exists, both because of the constraints of study design on free living subjects, and the degree of introspection created, when subjects are aware of intake being quantified, with inaccuracy, under-reporting and alteration of normal intake being commonly recognised problems (Bingham & Day 1997). Most studies assessing human appetite and consumption have been performed in subjects with obesity or eating disorders and it is not known whether the problem of under-reporting is as prevalent when normal weight subjects are studied. Under-reporting can be minimised if consumption is observed. Likewise,

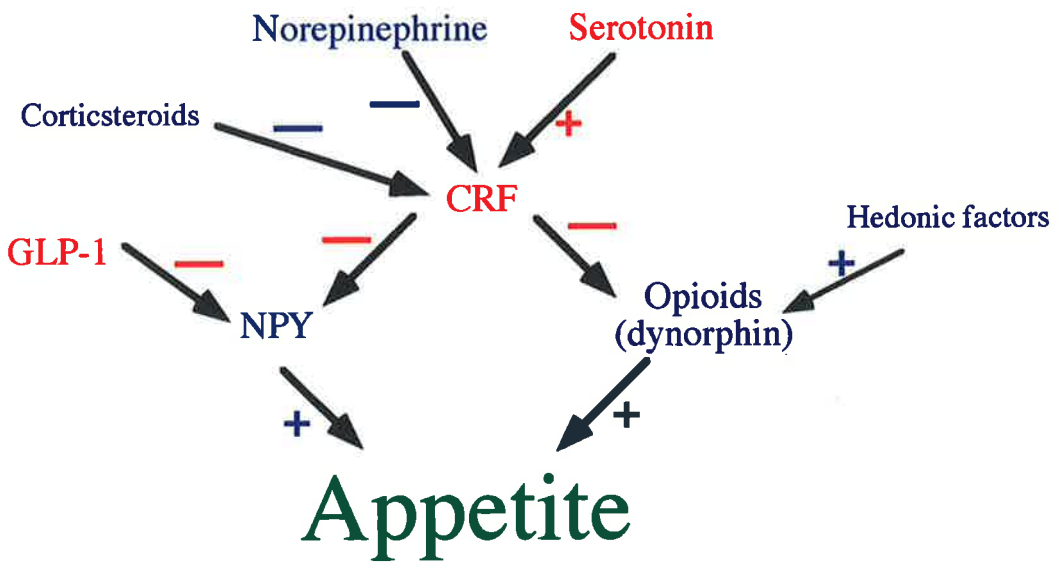


Figure 1.2.
Basic outline of proposed CNS mechanisms of appetite regulation. Factors which suppress appetite are shown in red, those which augment appetite, in blue.
(section 1.4.2)

the impact on results of under-reporting can be lessened by adopting a paired within-subject design. A brief overview of some commonly used methods for human appetite assessment are considered below; for a more detailed consideration of appetite assessment methods see Hill et al (1995).

In animals, appetite per se, cannot be accurately assessed, only its presumed effect, via observation of consumption, non-consumption, and volume and choice of foods consumed. Hence, when considering some of the animal data it is not possible to entirely exclude other factors at work such as boredom, fear, nausea, fatigue etc. As in humans, it is likely that eating has a social component for animals, and this is likely to be interfered with by study design. Paired within-subject study designs are best used where possible, as they control for these social confounding factors, the variation in eating behaviour between subjects, and between males and females, and the variation in intake by age (Chapter 5), and by activity level. In order to restrict observations to those based on normal physiology, subjects of normal weight, who are not "restrained eaters" (Stunkard & Messick 1985) are best selected.

1.5.1 Questionnaire/Visual Analogue Scales (VAS)

Questionnaires or VAS are commonly used to assess human appetite. They may be administered by the researcher or completed by the subject, and may be used in either short-term or longer studies. Perceived problems with them include the lack of "objective" data, and the potential for bias. However, when administered carefully with a standard approach they appear to give consistent results (Sepple & Read 1989). By using study designs where subjects act as their own control for interventions, the impact of possible lack of objectivity is lessened. They give information on feelings of appetite, or intent to eat but do not actually measure consumption. However, they have been shown to correlate with subsequent consumption (Lavin et al 1996). Thus, they are suited to quantifying facets of appetite during an intervention, which may vary over time such as hunger, fullness, desire to eat, the amount one would eat if offered a meal etc; and can usefully measure other variables related to mood and state of arousal which may also affect appetite. Depending on the study design, and the hypothesis being addressed, results can either be presented as absolute values, or as change in ratings over time.

1.5.2 Spontaneous report

Spontaneous report for assessment of appetite relies on subjects volunteering subjective feelings of hunger, fullness, etc, and is likely to only yield reproducible results when subjects are at the extremes of these sensations. It is best used in combination with other measurement techniques, and may be useful in detecting unexpected events, such as nausea or drowsiness, that may occur during studies.

1.5.3 Observation

Observational techniques involve recording actions (food intake in this instance) of subjects during an intervention. Usually the subjects will be observed within set parameters, which will include location, activities permitted, and food and drink available. Apart from these provisos, and allowing for whatever restrictions the intervention necessitates, observation allows the subjects to be in a relatively "natural" state, although they will often have been removed from their home environment. Observational studies of greater than ~24 hours in humans are difficult to perform because of the cost and constraints involved. One must also consider that their results may not be generalisable if subjects have been kept in unusual circumstances, or when an unusual group of subjects (who are available for long periods of confinement) are studied. Intake from a buffet meal served at a time when subjects would usually consume a meal (after an intervention) is a commonly used technique (Blundell et al 1993a & 1994; Lavin et al 1996) which is well suited to use in addition to VAS. These techniques are used in Chapters 8 and 9.

Observational studies over long periods, of weeks months or years, are generally less fastidious in terms of control, but may be more reliable due to fewer constraints on the subjects. In studies of these time frames, other techniques such as monitoring weight, 24 hour dietary recall, and periodic diet diaries are often used in combination.

1.5.4 Diet diary

Diet diaries require that a subject keeps a written record of all foods consumed within a specified time period. If used correctly, they have the potential to give a very detailed picture of the consumption patterns of subjects in their natural surroundings (Bingham & Day 1997), without the limitations of observational studies; and they have an advantage over VAS of measuring actual intake, rather than subjective feelings. However, having to record food intake in detail has been thought to bias intake, and to

cause omissions from the diary, although this problem is greater in obese or restrained eating subjects (reviewed in Hill et al 1995). Their accuracy is also dependent on the instruction given to the subjects, their lifestyle, and their level of motivation. They are probably best used to assess proportions of macronutrients and patterns of intake, rather than precise energy consumption. The number of days over which a diet diary should be kept will depend on the reason for it. If one is merely wanting to assess a subject's usual diet, three weekdays and two weekend days is sufficient (Lavin et al 1996). To assess a subject's response to an intervention, the diary should be kept for a period of time appropriate to the proposed biological effector mechanism.

1.5.5 Dietary recall

Recall methods can usually be divided into three approaches, 24 hour recall, dietary history and food frequency (for detail see Hill et al 1995; Bingham & Day 1997). Twenty-four hour recall is usually an interview technique administered in a standard fashion, where an interviewer records responses from the subject. It is usually performed face to face or over the phone, although it can also be used in a "written" fashion by letter, fax or e-mail. It has the advantage of speed, low burden on the subjects, and can be used repeatedly over many occasions (weeks, months), and also has the ability to catch subjects by surprise which may improve veracity. It is however dependent on subjects being honest in their responses, and is reliant on the subjects' memory. Dietary history is similar to 24 hour recall, but attempts to gather information on intake and eating patterns over a longer period. It is best performed by a trained dietitian. Food frequency techniques assess only particular aspects of the diet, in a semi-quantifiable fashion, and are better suited to epidemiology work, and population studies because of their imprecision. These recall methods are useful for following long-term trends in intake, or checking compliance with diet, but have limited value in studies of the acute physiology of appetite regulation.

1.6 SUMMARY

Despite the undoubted role of longer term control mechanisms, the proximal gut plays a central role in short-term appetite regulation. Food given enterally causes the release of both neural and humoral factors which act to bring each episode of eating to a close, and to create an inter-meal feeling of satiation. Exposure of the small intestine to nutrients appears to be essential to this gut mediated response (1.3.1), although oral, and gastric exposure to food also exert an effect (1.2.1 &1.2.2), in the presence of

small intestinal nutrients. Appetite regulation is interrelated with gastroduodenal motor function, particularly gastric accommodation (via distension signals) when a meal is ingested, and the rate of gastric emptying which, in part, determines the duration of small intestinal nutrient exposure (1.2 & 1.3).

Because of the interactions between gastric motor function, nutrient delivery and appetite regulation, studies in this thesis have administered nutrients direct to the small intestine. Because of the interrelationships between usual diet and gut function (1.3.1, Chapter 4), subjects studied in this thesis were all of normal, stable weight, and their diet assessed (usually by diet diary) to ensure adequate caloric consumption and macronutrient intake approximating the community norm. VAS were used during infusions to assess subjective feelings of appetite, and where possible, these were validated by offering a test-meal from which intake was quantified following the study.

The particular role(s) of insulin, the incretins and blood glucose concentrations on appetite are examined in the studies described in Chapter 8A, and 9. As motor function has been documented to vary in response to dietary changes (1.3.1), perception of appetite may also exhibit dietary adaptation. The motor changes demonstrated appear to be macronutrient specific, although this is not certain in humans. These issues of macronutrient specificity and appetite adaptation in response to dietary change are examined in the study in Chapter 8B, and appetite regulation in the elderly (see Chapter 5), is the subject of study in Chapter 8C.

As pathological levels (≥12 mmol/L) of blood glucose have been associated with increased fullness (1.3.2.2), and alterations in gastroduodenal motor function (Chapter 4), the effect of lower, more physiological elevations of blood glucose (8-9 mmol/L) on appetite and gastric motor function is the subject of the studies in Chapter 9A & B.

CHAPTER 2

Human Gastroduodenal Motor Function and its Assessment

2.1 INTRODUCTION

It is somewhat artificial to consider gastroduodenal motor and sensory function separately, as without both the sensory (afferent) and motor (efferent) sides of the neural circuitry, no gut function would be possible. However, in the interests of organisation and clarity, motor function is considered in this chapter, and sensory function in the following chapter (Chapter 3).

This chapter reviews gastroduodenal function (2.2), structure (2.3 & 2.4), and electrical control activity of the smooth muscle (2.5), as they relate to observable motor events (2.6). Methods of assessment of motor function are also discussed, with particular regard to the strengths and limitations of each technique (2.7). The major issues are summarised in section 2.8.

Interactions between nutrients and gastroduodenal motor function are not discussed in this chapter, apart from brief consideration of some commonly observed motor patterns or outcomes, such as isolated pyloric pressure waves (IPPWs), the interdigestive motor cycle (IDMC), and gastric emptying (see 2.6.4 & 2.6.5). A fuller discussion of these nutrient/gut issues is contained in chapter 4, and specific consideration of the relationships between pressures and flow in chapter 6.

2.2 FUNCTIONS OF GASTRODUODENAL MOTOR ACTIVITY

The gastroduodenal region is responsible for initial reception of food (stomach), physically reducing the size of solids by "grinding" the intake (stomach), mixing of food with digestive enzymes and bile (stomach and duodenum), neutralising the acid pH of the chyme leaving the stomach (duodenum and pancreas) and regulating its orderly delivery to the small intestine where the majority of absorption occurs. In short, over the course of a day, the human gastroduodenal region converts 2 - 5 episodes of intermittent intake (meals), into a regulated small intestinal delivery of chyme at a rate and pH suited to optimising nutrient absorption, all of which occurs beneath consciousness (Read et al 1994).

2.3 GASTRODUODENAL ANATOMY

The stomach is the first part of the gastrointestinal tract within the abdominal cavity. It is a hollow viscus of an irregular "J" tubular outline, whose shape and capacity varies considerably dependent on its contents, and motor state. The oesophagus enters the

stomach at the cardia or gastro-oesophageal junction, which is situated immediately below the diaphragmatic hiatus. The fundus of the stomach lies laterally, adjacent to the cardia, under the dome of the diaphragm. The lesser curve of the stomach leads from the anteromedial aspect of the cardia, through the gastric body, down past the incisura, into the antrum and terminates at the pylorus. The greater curve leads down from the posterolateral aspect of the cardia, (below the fundus) through the gastric body, into the antrum and also terminates at the pylorus. The pylorus is a narrow zone which has specialised muscle and control systems and separates the stomach from the duodenal bulb; depending on conditions, it either facilitates or prevents gastric outflow.

The duodenum is a tubular "C" shaped organ which starts immediately distal to the pylorus and leads to the jejunum at the duodenojejunal flexure, formed by the ligament of Trietz. It is approximally 25-30 cm long, and by convention is divided into four parts, the bulb, the second part - into which the papilla of Vater opens, the third part and the fourth part. Bile and pancreatic digestive juices enter the duodenum through the papilla of Vater. The duodenum is largely a retroperitoneal organ, with the head of the pancreas within its curve. Like the stomach, its diameter is capable of variation in size. However, due to both its intrinsic anatomy and its fixed relations to the stomach, pancreas, liver and ligament of Trietz, its overall shape varies less; and allows it only limited mobility within the abdominal cavity (see figure 2.1).

2.4 STRUCTURE OF MUSCULAR LAYERS

The muscle of the gastroduodenal region is entirely smooth in type, with two muscle coats, the muscularis propria and the muscularis mucosae. In the stomach there are three layers within the main muscular coat (muscularis propria), the inner oblique, middle circular, and outer longitudinal layers. The longitudinal layer is mostly in continuity with the longitudinal muscle layer in the oesophagus proximally, and the duodenum distally. The circular layer is prominent in the antrum, and adjacent to the terminal antrum. The pylorus consists of thickened circular muscle with fibrous tissue partly separating it from the duodenum. In the duodenum there are only two layers in the muscularis propria, the inner circular and outer longitudinal layers. In both the stomach and duodenum, the muscularis mucosae is much thinner than the muscularis propria and lies between the lamina propria and the submucosa, with fingers extending into the villi of the mucosa. While the muscularis propria is responsible for the gross motility in the gastroduodenal region, the muscularis mucosae, by virtue of its

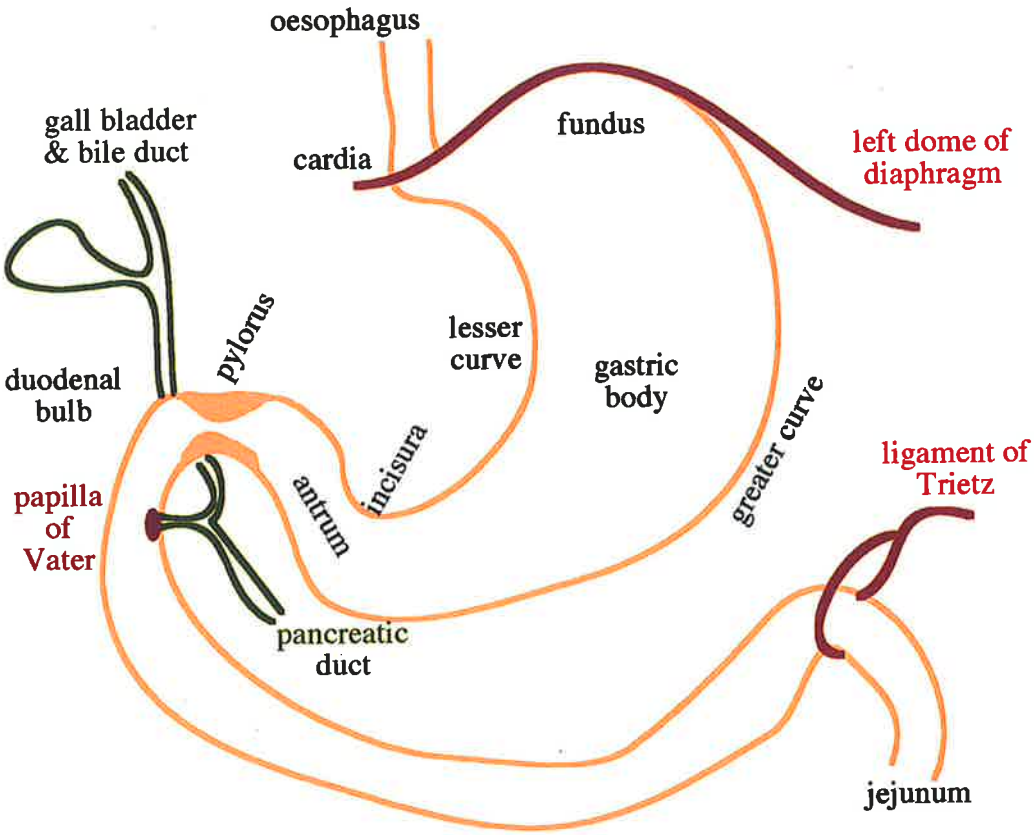


Figure 2.1
The basic anatomy of the gastoduodenal region is shown. See text for detail (section 2.3).

position, probably also has significant effects on the absorptive function of the mucosa by modulating mucosal contact with luminal nutrients (Angel et al 1982; Lee 1983).

2.5 ELECTRICAL CONTROL OF MUSCULAR LAYERS

The smooth muscle in the gastroduodenal region is richly innervated by both the enteric nervous system (ENS) and the autonomic nervous system (ANS), with the ANS forming the bridge between the ENS and the central nervous system (CNS). The gastroduodenal region receives its parasympathetic supply from the vagus nerves, and its sympathetic outflow from thoracic spinal nerves via the coeliac and superior mesenteric plexuses (Kumar 1993). The ENS is composed of the nerves and nerve-associated cells that lie within the walls of the gut. The nerve cell bodies lie within two ganglia located in the gut wall, one in the submucosa (submucous plexus), and the other between the two major muscle layers (myenteric plexus). Other, less complex nerve plexuses (not containing cell bodies) lie between circular and oblique muscle layers in the stomach, and deep within the circular muscle layer in the intestine. The ganglia of the myenteric plexus are extensively interconnected by interganglionic fascicles which form a sheet-like network between the muscle layers. Smaller branches from this network give rise to a secondary network and thence a tertiary network, which is fully developed only in the gastric antrum, small intestine and colon (reviewed in Christensen 1993).The structure of the submucous plexus is simpler, and consists only of a primary plexus.

The development and refinement of techniques such as electron microscopy, histo-immunofluorescence and functional nerve stimulation, has substantially advanced our understanding of the complex interactions between neural control elements in the gut. A full review of this field is beyond the scope of this chapter. The information presented here is limited to that necessary to appreciate the material which follows (section 2.6) and the studies described in chapters 8-12. Not all of the innervation of the gut is concerned purely with motor function, other important (and sometimes interrelated) functions include sensation (see chapter 3) and secretion (beyond the scope of this thesis).

The spatial patterning and rhythmicity of motor activity of the gastroduodenal region is largely determined by electrical control activity. Muscle cells in the proximal stomach (fundus) have a relatively steady, non-fluctuating membrane potential and, accordingly, this region of the stomach exhibits predominantly tonic muscle activity

(gastric tone). In the remainder of the stomach and small intestine, rhythmic partial depolarisations of membrane potential occur, referred to as slow waves or pacesetter potentials (Malagelada 1993). These pacesetter potentials determine the frequency at which phasic motor activity can occur. In general, phasic smooth muscle contraction occurs when an action potential (rapid membrane depolarisation) is superimposed on a slow wave. Thus not every pacesetter potential gives rise to a smooth muscle contraction, but a smooth muscle contraction cannot occur in the absence of slow wave activity. The frequency of the slow wave differs along the small intestine. In general more proximal regions have a faster slow wave frequency and "drive" distal areas. The stomach has a slower frequency than the small intestine, with the pylorus acting as a functional barrier between them; although antral slow waves have been shown to spread across the gastroduodenal junction into the proximal duodenum (Bortoff & Davis 1968). This provides a possible means whereby antral and proximal duodenal contractions may be coordinated (Bortoff & Davis 1968). The gastric pacemaker lies on the greater curve and cycles at ~3 per minute, with its slow wave propagating down the full length of the stomach. Whereas in the small intestine, several pacemakers exist, with each controlling a variable length of gut. The first intestinal pacemaker lies in the proximal duodenum and has a frequency of ~13 per minute. The slow wave frequency (and thus maximal contraction frequency) decreases along the small intestine to ~8 min-1 by the ileum. These rates are fairly consistent, although they have been found to vary with both distention and feeding (Lin et al 1996a).

The general pattern of innervation of the gut is such that a circumferential zone of relaxation travels in advance of muscle contraction, which is again followed by relaxation. In this fashion, boluses are propelled along the gut. In the majority of instances this occurs in an aboral direction, causing net distal transport of contents; however retrograde propagated contractions, and so retrograde propulsion, also occur. The advancing wave of relaxation is now believed to be largely mediated by nonadrenergic noncholinergic (NANC) pathways in which nitric oxide (NO) is implicated. The contraction which advances in the wake of this relaxation is thought to be mediated via withdrawal of NANC inhibition and subsequent cholinergic stimulation. In the intestine, intraluminal distension is known to be a reliable stimulus of this "peristaltic reflex" (Bayliss & Starling 1899). For the stomach, the relationship of pacesetter potentials and propagation patterns to mechanical outcomes is made substantially more complex by the different geometry of the organ, and the ability of the pylorus to stop outflow. Thus, though gastric contractions still occur in a ring-like fashion and travel distally, their functional outcome is less easily predicted. The

sequencing of lumen occlusion in the terminal antrum relative to the pylorus determines whether or not transpyloric flow occurs in response to gastric motor activity (Dent et al 1994). In order for contents to leave the stomach, the pylorus must be open, and the distal antrum must contain or receive contents. Lumen occlusion in the distal antrum or at the pylorus will prevent emptying in response to a given contractile event proximal to it, and will generate shearing forces which are thought to be important in triturating foods (Lin et al 1994b).

The factors determining whether or not a contraction occurs at a given site, and how far it travels from its site of onset are as yet not clearly defined. In the case of intact humans, we do not even have an accurate picture of the timing, site and extent of contractions at any more than a gross level, the available information being limited by short time base, poor resolution in time and space, or inexact techniques. Recent studies from intact guinea pigs have described the behaviour of calcium fluxes in the gut smooth muscle, which may help answer some of these questions about timing and extent of contractile activity (Stevens et al 1999; Hirst 1999). In these studies mixing movements in longitudinal muscle have been shown to result from spontaneous "waves" of elevated intracellular calcium which spread in all directions from discrete locations (which have been termed pacing sites), and to terminate by colliding with one another, or with refractory areas. Furthermore, activation of neural reflexes by mucosal stimulation of the gut was able to produce the typical peristaltic pattern of relaxation below, and contraction above, by modulating the number and activity of these pacing sites.

2.6 OBSERVED MOTOR PATTERNS

Smooth muscle contractions can be assessed in a number of ways (section 2.7). The method of assessment has a major impact on the nature of the information gained. Prior to consideration of observable gut motility, it is necessary to define the terminology used in this thesis, as there is no agreed standard usage of terminology in the study of gastrointestinal motility. Unfortunately, due to this lack of standard term usage, each study in the literature (and the measurement techniques used) must be carefully reviewed in order to discern the actual component of motility which is being measured. An additional difficulty when considering facets of motility from the cellular level across to the whole human, is that different techniques give vastly different sensitivities of measurement; for example, what may well cause a "contraction" (shortening or increased tension) in a muscle strip, may well not result in a change in

any measurable parameter (intraluminal pressure, wall motion, flow) in a whole subject.

In this thesis, the term "contraction" is used to refer to shortening or increased tension in the muscle in the gut wall. Thus it cannot be directly assessed in whole subjects (without the use of strain gauges, see section 2.7.1.1), and is usually said to have occurred from its consequences. The term "pressure" refers to intraluminal pressure (measured by manometric or barostatic techniques), and the term "pressure wave" refers to a phasic variation (section 2.6.1) of intraluminal pressure measured by manometry at a single point. "Pressure wave sequence" is used to describe a group of pressure waves related to each other in time and space. The precise temporospatial relationships are determined by the slow wave frequency (and other characteristics) of the region in which they occur, and therefore the characteristics of pressure wave sequences vary by region. "Wall motion" is used to describe visible (by imaging techniques), movement of the gut wall. To facilitate general description of non-controversial elements of motor function, the term "motor event" is used. It is purposefully an imprecise term used to cover any observable/measurable change in motility without prejudging its nature or causation, it thus includes contractions, pressures, pressure waves, pressure wave sequences and wall motion.

2.6.1 Tonic and phasic motor events

Motor events in the gastroduodenal region can be divided into two major types - tonic and phasic. Tonic and phasic events are distinguished on the basis of duration. Phasic events, have their duration determined by the slow wave frequency of the region in which they occur, whereas tonic (tone or wall tension) events last longer. Thus in the stomach, phasic events are shorter than ~20 s, and in the duodenum shorter than ~7 s. All sections of the gastroduodenal region exhibit both types of motor activity, tonic events being prominent in the proximal stomach and pylorus, and phasic events in the gastric corpus, antrum, pylorus and in the duodenum.

2.6.2 Lumen-occlusive vs non lumen-occlusive phasic motor events

Phasic events can be further subdivided into occlusive or non-occlusive, ie whether the opposing walls meet, obliterating the lumen. This distinction has been thought to be important, since these two types of phasic events have differing mechanical actions.

Unfortunately, not all techniques are capable of distinguishing between occlusive and non-occlusive events, although some studies correlating imaging techniques (wall motion) with pressure measurements have attempted to clarify this (Hveem et al 1995; Wright et al 1999). This distinction may be less important now than once thought, as on the basis of fluid transport principles, the timing of changes in intraluminal pressure (when the contraction starts to increase intraluminal pressure), and the pressure profile along the segment of gut (Clouse et al 1998) are primarily important in determining the outcome of a given motor event. From simultaneous oesophageal radiographic/manometric studies, occlusion is not necessary to alter intraluminal pressure and cause flow, although "near occlusion" is, and is usually quickly followed by occlusion (Li et al 1994). The importance of near occlusion is two-fold, in that it initially causes a local elevation of intraluminal pressure which would move of contents towards a region of lower pressure and subsequently causes compartmentalisation of the lumen, preventing flow across the area of near occlusion. In this fashion, the temporospatial sequencing of near lumen-occlusive events would determine the timing, extent and direction of luminal flows. This makes high temporospatial resolution during assessment of motor function desirable if the effects of individual contractile episodes along a length of gut are to be assessed (6.2).

2.6.3 Individual contractions

In humans, contractions are generally recognised by their consequences, i.e. when they cause either wall motion (endoscopy, ultrasonography, MRI), an increase in intraluminal pressure (barostat, manometry), or movement of an intraluminal substance (scintigraphy, radiology, plethysmography, impedancometry, ultrasonography) depending on the measurement technique applied (see 2.7). In animals, strain gauges (2.7.1.1) give direct measurement of muscle tension, but not lumen occlusion. Thus, in humans, as "contractions" are defined by varying parameters depending on the technique used, results of various studies may not be directly comparable.

2.6.4 Commonly recognised phasic motor patterns

Some motor patterns are relatively stereotyped, and occur consistently in certain situations; because of their ease of recognition with most recording techniques, these patterns mostly consist of phasic events. Some of the more commonly recognised phasic motor patterns include (i) "pylorospasm" or repeated isolated pyloric pressure waves (IPPWs) at the pylorus in response to a number of stimuli including small

intestinal nutrients and hyperglycaemia (sections 4.2.1.2 & 4.2.2), and (ii) propagated pressure wave sequences which travel along the small intestine. Many other motor patterns of grouped "contractions" have been described including "clustered contractions" (Kellow et al 1990), "giant migrating contractions" and "giant retrograde contractions" (Sarna & Otterson 1988). The mechanical/physiological significance of these patterns has not been fully elucidated, largely because the relative frequency, and/or their effect on transport of intestinal contents is unknown.

Four types of phasic motor patterns are discussed in detail in this thesis: IPPWs in Chapters 8 & 9; duodenal pressure wave sequences in Chapter 11; oesophageal peristalsis in Chapter 12; and the interdigestive motor cycle (IDMC) in Chapter 10. These are each dealt with in greater detail within the relevant chapter's discussion.

2.6.5 Global outcomes

Whilst the minute by minute control of gastroduodenal transport/flow is undefined, more is known about some of the commonly observed global outcomes of motor activity.

During fasting, the human gastroduodenal region exhibits a characteristic motor pattern consisting of a cycle of three periods of varying duration which differ in contraction rate (see figure 2.2). This pattern is interrupted by intraluminal nutrients (Sarna 1985). Mean cycle length is ~90-100 mins, although large inter and intra subject variation occurs. The three phases of the IDMC are, phase I (motor quiescence), phase II (irregular motor activity), phase III (regular contractions, at the maximal contraction rate). Phase III is also referred to as the migrating motor complex (MMC), as it sweeps down the small intestine. It is thought to be important in cleaning the bowel, preventing bezoar formation, and keeping the small bowel bacterial count low (Sarna 1985). During the fasting period, progression of phase III along the gut is responsible for about 50% of flow, whereas phase II accounts for 30-40% (Kerlin et al 1982). A phase III complex does not always occur in all sites within the gastroduodenal region during each cycle of the IDMC. Thus, only approximately a third of cycles have a gastric phase III, but in the distal duodenum, or proximal jejunum, the vast majority of cycles exhibit a phase III (Kellow et al 1986). Phases I and II are less studied and their functions largely unknown.

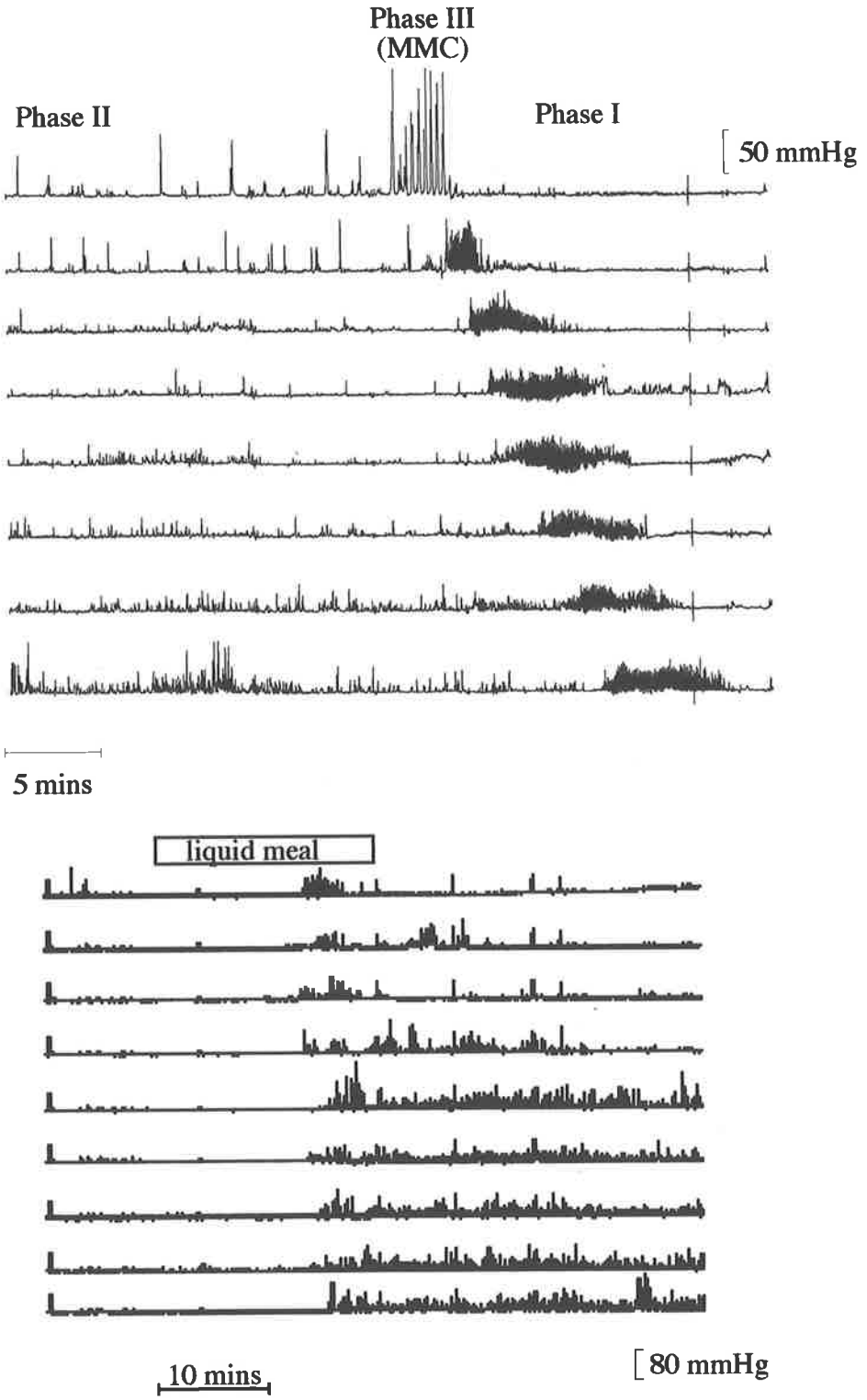


Figure 2.2
Fasting small intestinal motility, showing the three phases (unpublished data: JM Andrews), and the conversion to a fed motor pattern by the presence of small intestinal nutrients (Dr RJ Fraser) (section 2.6.5).

Consumption of food interrupts the IDMC and converts the motor pattern to one of irregular contractions in the stomach and duodenum, referred to as a "fed" motor pattern (Sarna 1985; Husebye 1999) (see figure 2.2). Although contractions appear to be irregular their timing is determined by the underlying frequency of the slow wave. The duration of the fed pattern depends on the amount of food consumed and the rate of gastric emptying (see Sarna 1985; Husebye 1999). When insufficient nutrient remains in the small intestine to stimulate the fed pattern, the fasting IDMC re-emerges. The disruption of the IDMC by food is dependent on both intraluminal nutrients and the ENS, as neither parenteral nutrition nor interruption of the ANS supply to the gut have an effect (Reviewed in Sarna 1985; Husebye 1999).

Food entering the stomach is held there for a variable period of time whilst it is mixed and ground into smaller pieces before being gradually expelled into the duodenum at a relatively steady caloric rate. The rate at which gastric emptying occurs is thus dependent on both the physical form of the food and its caloric density (Hunt et al 1985; Carbonnel et al 1994) (Figure 2.3). Gastric emptying, and gastric motor activity are also regulated by feedback mechanisms which are triggered by nutrients in the small intestine (Horowitz et al 1994; Read 1994; Hunt 1980), these mechanisms are discussed further in chapter 4. Gastric contractions are involved in both the trituration of food and its emptying (Figure 2.4). Although the precise relationship between contractions and emptying is still only partially understood, gastric emptying is now known to be mainly pulsatile (Malbert & Ruckebusch 1988) (Figure 2.5).

2.7 ASSESSMENT OF MOTOR FUNCTION

Ideally, techniques used to measure gastroduodenal motor function should be capable of directly measuring contractile activity, intraluminal pressure, and wall motion concurrently, without interfering with gut function, and be accurate, reliable, safe, well tolerated, non-invasive and cheap. Not surprisingly, such techniques do not exist. In order to recognise physiologically important aspects of individual contraction or pressure sequences, the technique used must have temporospatial resolution that is tailored to the region of the gut in which it is used. Temporospatial resolution of data, and its significance is further discussed in Chapter 6 (6.2). Techniques for measuring gastric emptying which cannot be adapted to yield temporal resolution sufficient to examine individual motor events, such as radio-isotopic breath tests and paracetamol absorption are thus not considered in this thesis. Likewise, measurement of electrical

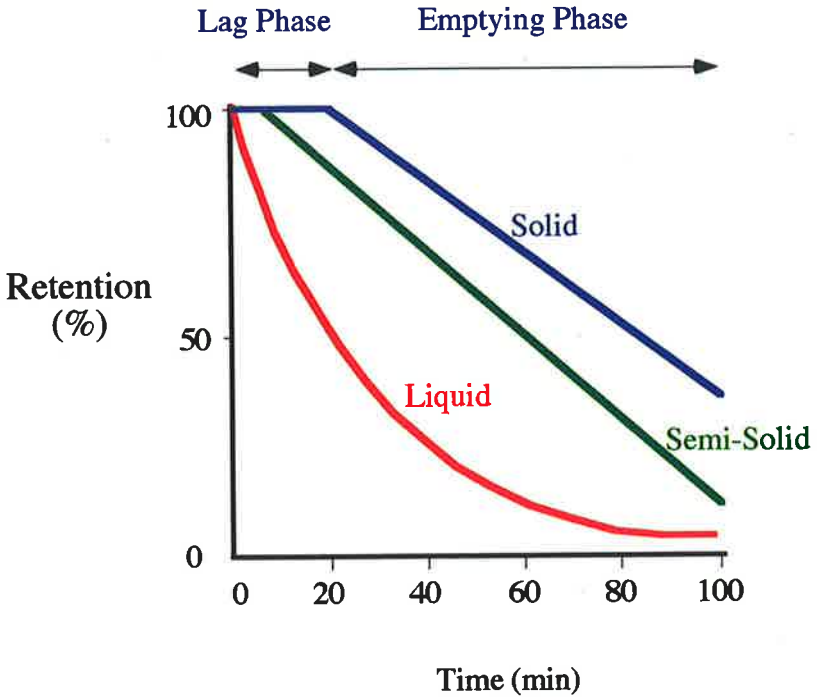


Figure 2.3
Gastric emptying curves for solid (pancake), semi-solid (porridge) and liquid (10% dextrose) show gastric retention over time. The overall pattern of gastric emptying of both solids and semi-solids is linear, following a lag phase which is longer for solids. In contrast, emptying of the low nutrient liquid is mono-exponential with minimal lag phase. (sections 2.6.5 & 2.7.4.1)

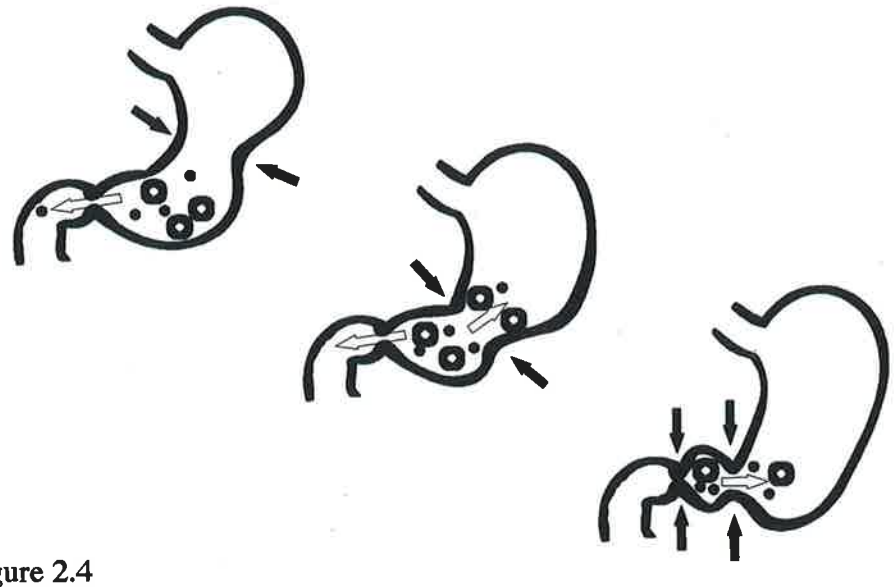


Figure 2.4
The outcome of a particular gastric contraction will depend on its site and the state of the pylorus. When the pylorus is open, pulsatile transpyloric flow occurs, when closed there is retropulsion of gastric contents, important in mixing and grinding. (section 2.6.5)

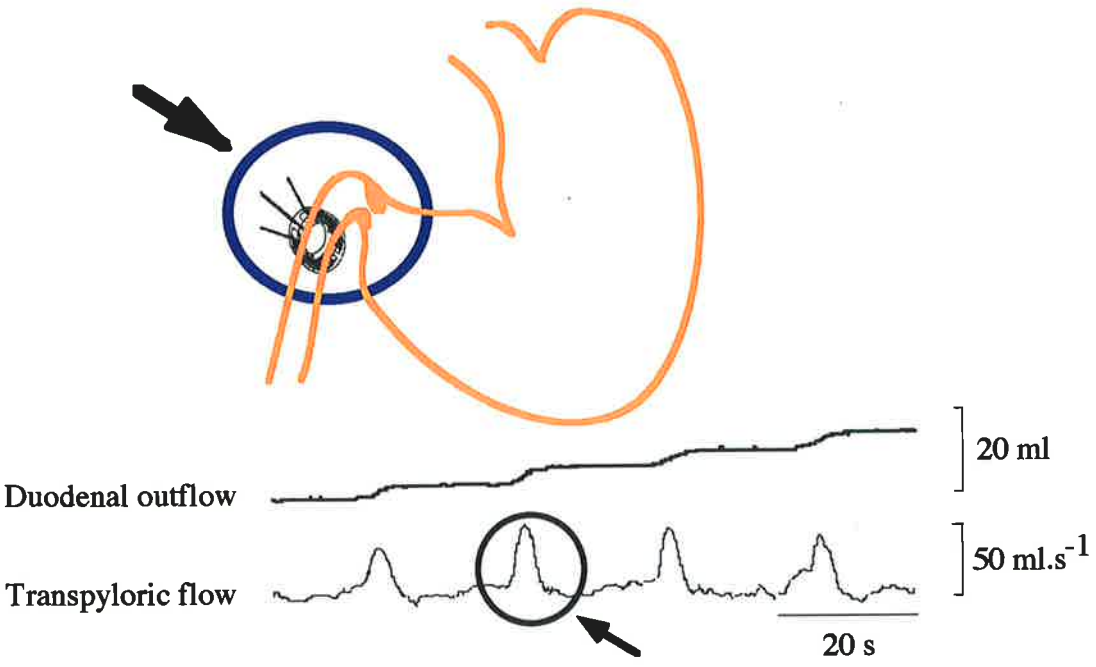


Figure 2.5
Evidence that transpyloric flow is infact pulsatile. In the pig an electromagnetic flow meter probe has been implanted in the proximal duodenum. (Diagram provided by Professor C-H Malbert) (section 2.6.5)

control activity, although important in determining the timing of contractile events, is not considered further. Some techniques described below, measure more than one aspect of motor function; these are classified by their predominant usage. As most modalities measure only limited aspects of motor function, they may be used in combination.

2.7.1 Techniques for detection of contraction(s)

2.7.1.1 Strain gauges

Strain gauges can be used in animal preparations for direct measurement of contraction(s) of the gut smooth muscle. They can be used for acute and chronic recording of contractile activity in muscle strip experiments, or when surgically implanted on the serosal surface of the gut, in whole animals. They are capable of excellent temporal resolution, and good spatial resolution over short lengths of gut, although due to their size, and the need to suture them to the gut, they cannot be closely spaced over long segments of gut, and in most studies only a few (2-10) are implanted in any one animal. They give the most direct measure of contractions available, but provide no information about lumen occlusion or movement of contents and cannot be used in humans.

2.7.2 Techniques for detection of wall motion

2.7.2.1 Endoscopy

Gastroduodenal motor activity can be directly visualised at upper gastrointestinal endoscopy. It provides an opportunity to see gross motor function, but objective measurements are not possible, the time over which observations can be made is short, and the setting is far from physiological. It is also difficult to estimate the mechanical consequences of contractions seen at endoscopy, or to assess tonic motor function. Therefore endoscopy is not recommended for evaluation of gastroduodenal motor function either clinically or in the research setting, although it is helpful in excluding strictures, obstruction, mass lesions and ulceration which may affect motility.

2.7.2.2 *Ultrasonography*

Ultrasonography is a readily available imaging technique which is routinely used in the upper abdomen for evaluation of the hepatobiliary region (Reece & Davies 1997). The quality of the image obtained however is known to be operator dependent, and is lessened by fat or gas. Despite these limitations, ultrasound has recently been shown to be useful for visualisation of the stomach, particularly the distal section, and given its temporal resolution and ability to provide real-time images, individual gastric contractions can be seen as episodes of gastric wall movement (King et al 1984). With the concurrent use of an intragastric manometric assembly, it is possible to correlate intraluminal pressures with wall movement (Hveem et al 1995). Other groups have visualised intragastric movement of contents, or flow, enabling the functional consequence of individual contractions to be assessed (King et al 1984 & 1988; Hausken et al 1992). Unfortunately the technique cannot be used in a substantial number of subjects - due to body habitus, or gas - and it cannot view the duodenum or the fundus with any degree of certainty. There is also a theoretical limit on the length of time over which it can be used continuously in the same site due to the risk of overheating the area. Ultrasound is not suitable for examining tonic motor function, except by visualising decreased luminal diameter, nor is it able to visualise the fasting (empty) stomach well.

2.7.2.3 *Magnetic Resonance Imaging (MRI)*

MRI is capable of directly visualising the gastrointestinal tract (Reece & Davies 1997) but its ability to image motor function with adequate spatial resolution, and in real-time is still limited even with the most advanced equipment. A number of studies have examined two dimensional antral motility and gastric emptying of liquids (Fraser et al 1994; Schwizer et al 1994 & 1996). However, in order to assess the consequences of individual phasic events, rather than global outcomes - such as gastric emptying, the instrument must be able to rapidly and repeatedly image slices of the region of interest in more than one plane and combine this information, to give real-time geometry (Evans et al 1993; Faas et al 1998; Indireshkumar et al 1998 a & b). Although the stomach can be viewed relatively easily, it is difficult to get more of the duodenum than the bulb in the same study, due to its angulation. As with ultrasound, MRI studies have been performed simultaneously with manometry, allowing limited correlation of pressures, flow and wall motion (Faas et al 1998; Indireshkumar et al 1998 a & b; Wright et al 1999). MRI cannot examine tonic motility directly, and because of the

very high costs involved, the technique is used for assessment of motility mainly for research purposes in only a small number of centers.

2.7.3 Techniques for detection of changes in intraluminal pressure

2.7.3.1 Manometry

Manometry can be performed using either solid state transducers, or low compliance water perfused systems (for a general review see Camilleri 1993). Only contractions which cause a rise in intraluminal pressure are registered by manometry, and the degree of wall motion necessary to increase intraluminal pressure will in turn depend on the cross sectional area of the gut in which it is employed. Manometry is thus most sensitive in detecting contractions where the lumen is relatively narrow, such as in the distal antrum and pylorus (Heddle et al 1988 a, b & c), small intestine (Russo et al 1999a) and colon (Bampton et al 1998 a & b). Previously, manometric assemblies were generally restricted to 8 or fewer pressure measuring sites (Camilleri 1993), and the use of chart recorders limited the temporal resolution possible. Newer assemblies are able to measure pressures at multiple (up to 21) sites simultaneously, and computer based recording systems now enable high temporal resolution (>10 Hz) to be routinely achieved (Hebbard 1997). The potential benefits of "high resolution" manometry are a focus of the study presented in Chapter 11. This ability to achieve high temporospatial resolution, over long periods of time, with modest cost and minimal subject discomfort sets modern manometric techniques apart from the other modalities considered here. High resolution manometry provides the opportunity for detailed analysis of the sequencing of pressures along a segment of gut. Correlation of these pressures with accurate observation of their outcomes (in terms of flow), will give a clearer understanding of the mechanics involved in the handling of gut contents.

The full potential of enhanced temporospatial resolution of manometric recordings is no use without high fidelity in the detection of pressures. With perfusion manometry, attention must be paid to the hydraulic characteristics of the system, in particular the pressure rise rate for each channel must exceed the highest rate of rise of pressures within the region from which recordings are being made. The amount of noise needs to be minimised by the use of high quality pressure amplification equipment, as these characteristics will affect the system's ability to resolve the onset time, variations of basal pressure among channels, and the amplitude of changes in intraluminal

pressures. Manometry is well suited to measuring phasic pressures; however, its quality, and the inferences which can be gained from it need to be carefully examined in the context in which it is performed. The addition of a sleeve sensor to a manometric assembly (Dent 1976), allows tonic pressures in sphincter regions to be assessed, with confidence that the pressures recorded are not at least in part caused by movement of point sensors relative to the narrow sphincter zones (Dent et al 1979; Heddle et al 1988b).

Although intraluminal pressures arise from contractions of gut smooth muscle (reviewed in Camilleri 1993), not every contraction causes an intraluminal pressure change, and some intraluminal pressures result from forces other than contractions; such as coughing, straining and movement. Thus, in this thesis, pressures will be referred to as pressures.

2.7.3.2 Barostatic measurements

Barostatic recording of gastrointestinal tone has been largely developed by Azpiroz and Malagelada (1985a). Barostats can be used in either of two modes; (i) to measure spontaneous gut motility, or (ii) to assess the gut's motor response (variation in wall tension) to a given distending pressure or volume. The luminal portion of a barostatic recording system consists of a non-distensible thin-walled bag which is inflated or deflated to maintain either a desired volume or pressure, which is operator selected. The bag itself offers negligible resistance or assistance to inflation/deflation, and thus functionally is highly compliant. It is able to measure both phasic and tonic motor activity, but due to time delays in inflation and deflation, mandated by the volume moved and the diameter of the tubing through which the air is moved (Hebbard 1997), is best suited to assessing tonic motility. Barostatic recordings have been made from the gastric fundus (Azpiroz & Malagelada 1985 a & b, 1986; Hebbard et al 1996 a & b) and antrum (Kreiss et al 1996), although in the antrum it is difficult to be sure the position of the bag is maintained. Some record of phasic gastric motor activity is also gathered in these positions, but is generally averaged out, to leave only the tonic component. Barostats can also be used to assess tonic motor function in the duodenum, providing the bag size is reduced appropriately (Azpiroz & Malagelada 1990). Due to the higher frequency of the slow wave in the duodenum, and the inflation/deflation dynamics of the barostat, phasic pressures cannot be recorded. The barostat then, is best suited to measure wall tension or compliance or to give graded distensions.

2.7.4 Techniques for detection of movement of contents

2.7.4.1 Scintigraphy

Solids and liquids can be labelled with a number of isotopes and their movement within the gut lumen followed by repeated imaging. The temporospatial resolution of scintigraphy depends on the amount of isotope used, the general principle being that with more isotope, greater resolution is obtained, in both space and time. As high radiation doses are not generally acceptable, this limits the information gained from scintigraphy. In routine usage, the temporal resolution of scintigraphic data is in blocks of between 30 seconds and three minutes (Jones et al 1996a), and spatial resolution is within approximately ± 3cm. This clearly limits its usage to studying the net effects of gastroduodenal motor function (Figure 2.3), rather than information about individual phasic events. With intake labelled with higher (2 mCi) radiation doses, a well defined gastric antrum allows observation of antral motility at a temporal resolution of less than a minute, but not less than a second (Urbain et al 1993). Scintigraphy cannot quantify tonic motor function, although some clues about the state of wall tension may be suggested by the diameter of the region under consideration. Some isotopes given intravenously are also able to directly label gastric mucosa (Sfakianakis & Conway 1981), enabling gastric wall motion to be visualised - although the radiation dose required is higher than generally acceptable.

2.7.4.2 Radiology:- barium studies, markers

The tubular gut is not well seen on radiology without use of radio-opaque intraluminal contrast, which can be in the form of a liquid, such as barium or gastrograffin alone, or incorporated into food stuffs, or as discrete non-absorbable solid markers. The gut is then repeatedly imaged in order to follow the movement of the contrast. The temporal resolution of radiology depends on the imaging technique adopted, and the limits of X-ray exposure. Fluoroscopy, with 30 frames a second, gives excellent temporal resolution of phasic motor events, but can only be used for a limited time period (~1-2 min per year in volunteers, slightly longer in patients) because of the high radiation burden, and is thus clearly unsuitable for repeated examinations, or long studies. Time-limited fluoroscopy has been used to good effect in some gut regions to correlate motor events with other measures such as manometry (Brasseur 1993; Li et al 1994; Rao et al 1996b). Barium meals, or small intestinal transit studies, are more

acceptable in terms of x-ray burden but like scintigraphy, give only gross information on transit. However, lumen calibre, mucosal pattern, wall thickness and presence of obstruction or deformation are well demonstrated by this technique, and this information is helpful in excluding possible physical causes of motility disorders. Radiology cannot measure tonic motor events, apart from revealing a reduction in luminal diameter.

2.7.4.3 Plethysmography

Plethysmography, or applied potential tomography, involves measuring the difference in electrical conductivity in the different tissues through the body. It is performed externally, and when applied over the stomach, is capable of measuring the gastric emptying of fluids (Brown et al 1985). It has low temporospatial resolution however, and apart from the absence of radiation exposure, gives no benefit over scintigraphy for assessing gastric emptying. It is unable to assess the duodenum or small intestine in any spatial detail, and cannot quantify tonic motor events.

2.7.4.4 Intraluminal impedancometry

This technique, largely developed by Silny (1991), relies on the measurement of differences in conductivity between electrode pairs on a multi-electrode catheter placed intraluminally. Intraluminal impedancometry does not actually measure phasic or tonic contractions, or pressure events, but their consequences in terms of bolus movement of intraluminal contents past each electrode pair. Impedancometry is capable of equal temporal and spatial resolution to that of manometry. Inferences are then made on the contractile activity which caused these "bolus transfer events" (BTEs) (Nguyen et al 1995 & 1997). Boluses can be gas or intraluminal contents. In theory, the signal generated will vary depending on intraluminal conductivity, which is affected by the chemical composition of the ingested food/liquid, bolus size (diameter), pH, air content of the gut and the tonic state of the gut, as changes in wall tension will alter diameter. The signal morphology itself is a further difficulty with interpretation of the data. For interpretation of the signal, lumen occlusion is defined in a somewhat circular fashion as the highest impedance recorded, and used as a comparative point for then recognising the transit of a bolus (lumen non-occluded). The signal is also variable in appearance from one BTE to the next, and does not have the stereotypy of pressure recordings. The signal is divided into five phases, the last of which should coincide with peak pressure measured manometrically, however on inspection on many of this

groups published BTEs it would be difficult to assign these five phases to each impedance trace in a reproducible fashion. Allowing for these difficulties with the data interpretation, it seems a useful technique in the oesophagus and small intestine, whereas its use in the stomach is made more problematic by the geometry of the organ, and its mixing and grinding activities. Thus, although impedancometry gives spatio-temporal resolution of conductance changes, it is less clear precisely how these changes relate to contractile activity of the gut. Rather than a method of assessment of motor function, it should perhaps be thought of as a technique for recognising lumen occlusion and non-occlusion, and transport of boluses.

2.8 SUMMARY

Current knowledge of gastroduodenal motor function in humans is limited to only a basic understanding of phenomenology, such as the fasting IDMC, patterns of gastric emptying and small intestinal transit. This is due to difficulty in studying motility relatively non-invasively in humans with sufficient temporospatial resolution to assess the functional outcomes (in terms of flow) of individual motor events. Intraluminal flow is also difficult to measure (section 6.8) which compounds the difficulty. In studies of motility, consideration of the strengths and limitation of the technique(s) used is essential

To move ahead with elucidating the specific outcomes of individual gastroduodenal motor events, greater temporospatial resolution of measurements is required. Intraluminal manometry, with its recent refinements, is an ideal technique for assessing the forces acting on gut contents with better resolution in both time and space. However, for a full appreciation of the consequence of individual pressures and/or pressure wave sequences, concurrent measurement of flow will be required. Hence, in this thesis both the technique of high resolution manometry (Chapter 11) and a novel intraluminal flow measurement instrument (Chapter 12) are further studied.

Motor function also interacts with food intake in important ways, and this is considered further in Chapter 4, and in the studies described in Chapters 8, 9 & 10.

CHAPTER 3

Human Gastroduodenal Sensory Function and its Assessment

3.1 INTRODUCTION

In lay terms, sensation is what we feel. However, more precisely, sensation is the information coming from the afferent limb of the neural circuits. Gastroduodenal sensation, like that in most areas of the gut, works on two levels, conscious and unconscious. The vast majority of the upper gut's sensory function is unconscious, which makes a realistic examination of normal physiological gastroduodenal sensory function in intact humans somewhat problematic. Complicating matters further,

conscious sensation commonly arises from noxious stimuli or pathological states, but can nonetheless contribute some insights into the physiology of sensory function. An additional problem with measurement of conscious sensation is that it is often described in subjective or emotive terms which clouds its assessment. These issues are considered in sections 3.2 & 3.6 - 3.8.

Sensation defined more narrowly, from a neurological perspective, as the information perceived by afferent fibres is easier to assess, albeit by invasive means, as these sensory signals can be recorded in intact animal preparations, or in excised tissues. Outcomes of direct nerve stimulation and physical and pharmacological interventions can also be recorded in such preparations. Many aspects of gut sensory function in animals are well described at this level, and are described in sections 3.4 & 5.

The techniques described in section 3.8 to assess gastroduodenal sensory function are confined to those suitable for human use, as a complete review of modern gut neurophysiological techniques is beyond the scope of this work. Broadly speaking, appetite and satiety are also gut sensations (at least in part), but due to their importance to the field of study addressed in this thesis, they are dealt with separately in Chapters 1 & 4.

3.2 PURPOSE OF GASTRODUODENAL SENSORY FUNCTION

Gastroduodenal sensation has two main purposes, the major one of which is to enable coordination on a continuous basis between gut contents, and the gut's response to those contents. This generally occurs without conscious perception. The second purpose is to provide a warning system to enable the organism to respond to noxious stimuli or events and, in order to serve this purpose, this type of sensation usually involves conscious perception. The unconscious, everyday business of ingestion and digestion is encompassed within the first sensory purpose, with afferent fibres continuously sensing gut wall tension and chemical composition of contents whilst food, secretions and motor activity are coordinated. The myriad of gut symptoms experienced by people everyday fall under the second purpose and include both physiological responses to pathological stimuli (such as pain with a peptic ulcer), and, at least in some cases, pathological perception (as noxious) of normal physiological events (such as pain in irritable bowel syndrome).

This is not to imply that these two sensory purposes are mediated by separate sensory systems, and indeed there is substantial overlap in both physiological and pathological

conditions. However, they are largely distinguished from each other by whether or not sensory information impinges on consciousness.

3.3 ANATOMY OF SENSORY FUNCTION

Both the motor and sensory components of the neural circuitry involved in the control of gut function can be regarded as existing within a hierarchy as described by Furness et al (1994). The simplest circuits exist within the gut wall and are activated locally to give local responses. The long reflex circuits which connect regions of the gut to each other are slightly more complex, but remain outside the central nervous system (CNS). At the next level there are reflexes which traverse the CNS, but which are not necessarily noted at a conscious level. The final level of complexity is the integration of information from the gut with control mechanisms of a central origin, which influence behaviour and control gastrointestinal activity.

There are three groups of sensory neurones which have endings within the gut; primary sensory neurones of the enteric nervous system (ENS), vagal (parasympathetic) neurones and spinal (sympathetic) neurones. In the past, sympathetic and parasympathetic routes were thought to subserve different functions, although it is now known there is not a precise correlation between the afferent pathway of projection and functional role (Cervero 1994). Controversy exists as to whether there is any overlap, or interconnection between these three sensory routes; and although they are generally thought to be independent of one another at the peripheral level (Grundy & Scratcherd 1989), central processing integrates the information from all sources.

Dogiel type II neurones, which have cell bodies in either the myenteric or submucous ganglia and numerous projections into the villi, are now thought to be the primary sensory neurone in the gut (Furness et al 1994). As testimony to the importance of sensation to gut function, these cells are estimated to make up ~25% of the nerve cells in the guinea pig intestine (Furness et al 1994). These sensory cells synapse monosynaptically with local motor neurones and also with both ascending and descending interneurones and thence onto further motor neurones to make up the circuitry necessary for local and remote reflexes to occur. The ENS does not have any direct central afferent projections

The vagal sensory endings in the gastroduodenal region, are present on neurones which have their cell bodies in the nodose ganglion (reviewed in Grundy & Scratcherd 1989; Cervero 1994). Two main types of vagal endings have been described, based on their response characteristics; tension receptors (within the muscle layers) and mucosal (or chemo-) receptors (Roman & Gonella 1987; Grundy & Scratcherd 1989). These afferents input centrally to the medulla, mostly via the main thoracic trunks of the vagus, although they may also travel with the splanchnic nerves, or to the spinal cord along with the intercostal nerves (Grundy & Scratcherd 1989). Some viscerotropic organisation has been suggested to exist in the nodose ganglion (Mei 1970), although not all authors agree. Whilst the vagus was once thought to be primarily a motor nerve, it is now known that at least 80% of its fibres are sensory (Agostini et al 1957), attesting to the importance of sensation in maintaining physiological function in the gut.

Sympathetic afferents from the gastroduodenal region travel via the coeliac plexus and/or superior mesenteric ganglia to the lower thoracic and upper lumbar dorsal root ganglia via the sympathetic chain (Cervero 1994). On entering the spinal cord, these afferents terminate in the dorsal horn. Second order neurones ascend within the cord and input to the dorsal motor vagal nucleus and other brainstem nuclei (Grundy & Scratcherd 1989). Unlike the extensive body of literature on vagal afferents, there is a paucity of work on the responses of sympathetic afferents in the gastroduodenal region. In animals, afferents which respond to gastric contractions, mechanical stimulation of the mesentery and certain chemicals have been demonstrated in splanchnic nerves (Cervero 1994). Visceral pain is generally thought to be transmitted via the spinal sympathetic system (Cervero 1994), and is thought to arise from stimulation of serosal and mesenteric sensory endings (Grundy & Scratcherd 1989).

3.4 GUT SENSING MECHANISMS

The gut responds to a number of stimuli including distension, mechanical distortion of the mucosa by compression or stroking and chemical stimuli from the lumen (reviewed in Furness et al 1994). Furthermore, different afferents respond to different stimuli. This ability of the afferent supply to code different stimuli implies that either several types of sensory neurones exist, or that sensory neurones can respond discretely to different stimuli, or possibly both. The answer is likely to be that the response from a given afferent nerve ending depends in large part on its location both along and within the gut to determine its specificity, as gastrointestinal sensory receptors do not appear

to show morphological specialisation (Grundy & Scratcherd 1989). Thus, anatomically, receptors can be divided into three major groups on the basis of their tissue level, mucosal, intramuscular and serosal, whilst the mechanisms which stimulate them can be divided into two - chemical and mechanical.

3.4.1 Chemical mechanisms

Mucosal nerve endings act as slowly adapting chemoreceptors (Christensen 1993), and react to a number of different chemical stimuli including pH, alkalis, and short-chain organic acids (Iggo 1986; Grundy & Scratcherd 1989). Mucosal nerve endings are also known as multimodal receptors as they also respond to mechanical stimulation of the mucosa. Intramuscular and serosal nerve endings do not react to luminal chemical stimuli.

In order to explain the gut's apparent ability to specifically sense intraluminal contents, specific classes of chemoreceptors (in addition to multimodal receptors) have been hypothesised to exist, although there has been no ultrastructural identification of receptor subtypes. In particular, glucoreceptors and amino acid receptors have been proposed (reviewed in Grundy & Scratcherd 1989) on the basis of the occurrence of increased discharge of vagal fibres following intraluminal perfusion of each particular substance in anaesthetised animals (Mei 1978; El-Ouazzani & Mei 1981; Perrin et al 1981). The possibility that these responses were caused by simultaneous mechanical sensitivity or stimulation of a postabsorptive "second messenger" has not been excluded; nor has the precise response mechanism of these proposed chemoreceptors been clarified. Given these deficiencies in methodology, the existence of specific chemoreceptors is still somewhat speculative.

Even if specific chemoreceptors do not exist, there appears to be a specific mechanism for sensing intraluminal glucose, and a number of other carbohydrates in the proximal gut of a number of species (reviewed in Grundy & Scratcherd 1989). Regardless of the exact means of signal transduction, the afferent neural response has been shown to travel centrally via the vagus, and to be proportional to the strength of solution infused (Mei 1978). These responses did not appear to be mediated by pH, mechanical or osmotic stimuli, although there were deficiencies in the methods employed to exclude these factors as stimuli (Grundy & Scratcherd 1989). Whilst the single unit recordings of Mei (1978) did not reveal much difference in responses between actively and passively absorbed sugars, nor between those requiring enzymatic breakdown or not,

carefully performed multi-unit recordings by Hardcastle et al (1978) found that only actively transported sugars evoked an afferent discharge. Species differences is the only readily apparent explanation for these discrepancies, aside from unrecognised confounding factors. In animals, vagal afferent fibres responsive to infusion of several amino acids have also been described (see Grundy & Scratcherd 1989), with some of these fibres also responding to 10% glucose. Given the inability to examine the response of single afferent fibres in humans, this issue of possible receptor or nutrient specificity will need to be untangled in animals, although there is certainly evidence in humans that an ability to encode specificity exists; via different profiles of second messengers (see below and Chapter 1).

Mucosal afferents may be stimulated directly by an intraluminal substance, or indirectly by a "second messenger". The second messenger may be distortion of the epithelium by water flux consequent on absorption of the substance, or an endogenous chemical (such as CCK) released in response to the intraluminal substance. Given the subepithelial location of the mucosal nerve endings, some absorption is likely to be necessary for any contact between substance and receptor to occur. It is thus possible that the apparent receptor specificity demonstrated derives from specificity of the second messenger. For lipids this now seems to be the case, as the formation of chylomicrons and the release of Apolipoprotein A-IV are necessary to signal the presence of intraluminal fat (Raybould et al 1999). For some amino acids, CCK is a strong candidate second messenger (Ballinger & Clark 1994).

3.4.2 Mechanical mechanisms

Mucosal nerve endings act as rapidly-adapting mechanoreceptors. They respond to stroking or deformation of the mucosa and may give "tactile" information on gut contents. Receptors in an intramuscular location mirror wall tension, and muscular contractions by acting as slowly-adapting mechanoreceptors which are arranged "in-series" with the both the longitudinal and circular muscle bundles (Christensen 1993; Grundy & Scratcherd 1989; Iggo 1986). These endings are referred to as "in-series tension receptors", and are able to monitor gut volumes as well as contractile force, dependent on their location in the gut. In the longitudinal muscle of the fundus and body of the stomach, for example, their discharge is largely dependent on wall tension, and is thought to be the means by which gastric volumes are tracked; whereas in the circular muscle of the antrum, their discharge is determined more by contraction patterns, and may possibly give feedback on the strength of contractions. Serosal

nerve endings are found along the gut and at junctions with the mesentery. They act as both rapidly and slowly adapting mechanoreceptors and respond to contractions, movement and distension of the gut. In contrast to the mucosal and intramuscular nerve endings which mostly relay sensory information to the brainstem via the vagi, serosal and mesenteric receptor fibres run predominantly via a spinal route in the splanchnic and pelvic nerves (Grundy & Scratcherd 1989; Cervero 1994).

3.5 LOCAL RESPONSES

Many of the common motor patterns in the tubular gut rely on reflex interaction between local sensory and motor function and are mediated within the ENS. These interactions are referred to as local responses, as the CNS is not necessary for their occurrence. The peristaltic reflex is the best recognised local response; whereby when the gut is distended, reflex muscle relaxation occurs distally and contraction proximally to the site of distension (Bayliss & Starling 1899). Other examples in the gastroduodenal region of proven local responses include (i) pyloric contractions in response to duodenal stimulation and pyloric relaxation in response to antral field stimulation (Allescher et al 1988); (ii) increased pyloric and duodenal pressures in response to intraluminal acid (Allescher et al 1989); and (iii) gastric relaxation in response to distension, (although vagal mechanisms also play a role), (Christensen 1993). Given that adequate gastroduodenal function re-establishes itself following vagotomy, or spinal cord injury, essential motor, sensory and secretory functions of the gut must be capable of implementation from wholly within the ENS, or with spinal modulation substituting for the brainstem and higher centres.

3.6 CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT

The CNS is linked to the ENS via the autonomic nervous system (ANS), and receives most of the information regarding gut status at brainstem level, without it impinging on consciousness. A number of vagovagal reflexes initiated from in-series tension receptors are good examples of this; such as the excitation of antral motility, stimulation of gastric secretion, and receptive relaxation of the stomach which each occur in response to gastric filling (Scratcherd & Grundy 1982; Roman & Gonella 1987); and the gastric relaxation following duodenal distension (De Ponti et al 1987).

Afferent vagal fibres terminate in the nucleus tractus solitarius (NTS) situated in the medulla. There does not appear to be an organ specific topographic arrangement of

incoming traffic. Vagal afferents also project to the dorsal motor nucleus of the vagus and to the area postrema, which is thought to be important in emesis. Projections from the NTS to other areas of the brain include reflex connections to other brainstem nuclei, and those which ascend through the brainstem to higher centres (for more detail see Grundy & Scratcherd 1989; Christensen 1993). Whilst the functional significance of many of these connections is unknown, specific areas within the CNS have been implicated in certain sensory functions, such as the hypothalamus in appetite regulation (Kalra et al 1999).

3.7 PERCEPTION

When a sensation impinges on consciousness, it is described as being perceived. Perception of a stimulus depends not only upon the type of stimulus, but also upon its strength, and other subject factors such as whether intraluminal nutrients are present (Feinle et al 1995a, b & c, 1996; Hebbard et al 1997), the level of sympathetic tone (Iovino et al 1992), or blood glucose (Morley et al 1984; Hebbard et al 1996a; Lingenfelser et al 1999), and is altered in certain disease states such as irritable bowel syndrome (Kellow et al 1990; Accarino et al 1995; Mayer 1995). Most work on upper gut sensation in humans concerns pain perception, nausea or appetite (see Chapter 1), and may not therefore provide a complete picture with regard to other sensations.

Measurement of perception threshold or intensity of sensation for various stimulus types is a commonly used technique for evaluation of sensation in humans. This approach is less reliable in animals, as responses to stimuli can only be observed, not described. This then involves making assumptions about the reasons for the animals' actions, for example decreased intake may represent satiety, nausea or even pain.

The fact that specialised nerve endings do not exist in the gut suggests that there are not separate receptors for conscious (perceived) and unconscious sensations. For many gut sensations a gradation of perception exists, with no awareness of an initial gentle stimulus, passing through perception, definite awareness, an unpleasantness and finally pain and/or nausea. In general, the intensity of discharge from a sensory nerve depends on the intensity of the stimulus applied to it, and for most stimuli, an increasing stimulus causes sensation to pass along this continuum. More intense stimuli also usually recruit a greater number of sensory nerves. Distension is a good example of this phenomenon. For instance, during gastric distension, subjects are initially unaware of increasing volumes and then become more conscious of discomfort

as volume increases further (Hebbard et al 1996a). The same pattern is seen during duodenal distension (Lingenfelser et al 1999). In both cases, not only does increased balloon volume cause a greater stimulus at each sensory ending, but the increased balloon volume encroaches on an increasing number of receptive fields.

Further consideration of the mechanisms whereby specific conscious sensations are generated can be found in detailed reviews such as Cervero (1994) and Grundy & Scratcherd (1989).

3.8 ASSESSMENT OF SENSORY FUNCTION IN HUMANS

The ideal technique for assessment of gastroduodenal sensation would be objective, cheap, non-invasive, easily used, yield internally and externally reproducible results and be able to assess both subconscious and conscious sensation. Such a technique does not exist. Measurement in humans is generally limited to conscious sensation, as it is not possible to use invasive neurophysiological techniques. With the exception of evoked potentials and perhaps functional MRI and positron emission tomography (PET) which measure direct physiological parameters (sections 3.8.5 & 3.8.6), the techniques described below address perception and the integrated conscious response to a stimulus, as much as actual gut sensation. Because of this limitation, if a high degree of precision is needed, it is desirable to use techniques which give complementary information simultaneously.

3.8.1 Barostat

Aside from the ability to measure wall tension (section 2.7.3.2), barostatic techniques can be used to give specified distension stimuli to the gastroduodenal region. Perception thresholds for volume and pressure can be measured. Subject blinding, and careful matching of other subject factors are important when using this technique, as substantial variability in response to distension is recognised, and anticipation of distension may decrease the sensory threshold for perception. The sensory response to barostat stimuli needs to be measured using other techniques such as visual analogue scales (VAS), (section 3.8.2), or spontaneous report (section 3.8.3).

3.8.2 Questionnaires/Visual analogue scales (VAS)

Questionnaires or VAS are used to measure sensory responses to given stimuli. Although they are subjective, this is a criticism which applies to any assessment of sensation in which central processing may play a role. These techniques must be used in literate subjects in appropriate language and be carefully explained prior to commencing a study in order to yield reproducible results. Given the potential variability inherent in assessing sensation, these instruments are best employed in experiments with paired within-subject observations. With these provisos, they have been shown to assess sensation reliably in a number of studies (Feinle et al 1996; Hebbard et al 1996a & b, & 1997; Lingenfelser et al 1999; Sepple & Read 1989).

3.8.3 Spontaneous report, descriptive

This technique relies on subjects volunteering their perceptions in response to stimuli and can be used alone, or in combination with questionnaires or VAS (section 3.8.2). Theoretically it has an advantage that, by not asking for a response at a particular moment, the investigator avoids placing suggestions in the subject's mind. It has substantial disadvantages, however, in that people can usually only be relied upon to spontaneously report intense sensations, and thus more subtle sensory information may be missed and, given the random occurrence of reported information, it is difficult to analyse data derived with this approach. Use of spontaneous reporting can however be useful in recognising unexpected events - such as change in character of sensation - which can be further tested in a future experiment.

3.8.4 Quantified stimuli, threshold/tolerance levels

A number of numerically quantifiable stimuli can be applied to the human gastrointestinal tract with safety. Stimuli include barostatic distensions (section 3.8.1), repeated small balloon inflations (Accarino et al 1995; Patel & Rao 1998), intraluminal electrical stimuli (Accarino et al 1995), and infusion of nutrients (Chapters 1 and 4). Sensory responses to these can be measured using questionnaires/VAS (section 3.8.2), spontaneous report (section 3.8.3), electrophysiological recordings over the spine or cranium (see section 3.8.5) or other techniques (section 3.8.6) to assess neurological parameters in response to the stimuli. Again, because of intersubject differences, other subject factors need to be carefully standardised, or a paired within-subject design adopted.

3.8.5 Evoked potentials, spinal & cerebral

Evoked potentials, which were initially used for testing auditory, optic and somatosensory pathways, have recently been measured over the scalp in response to oesophageal stimulation (De Vault et al 1993; Weusten et al 1994a & b), and over the both the scalp and spine in response to rectal stimulation (Chey et al 1995b; Rothstein et al 1996). Two main parameters are measured: latency, which is related both to the means by which the stimulus is sensed and to nerve conduction velocity; and amplitude, which has been shown to correlate with stimulus strength and perceived sensation (discussed in Russo et al 1999b). This technique has the theoretical advantage of measuring the electrical changes generated by afferent signals from the stimulated area, rather than the integrated conscious sensation arising from the stimulus. This is believed to render it an objective technique, however, it is not possible to entirely exclude the effect of central processing. It is able to give some anatomical data on the pathway taken by the afferent information and to localise the brain region in which the evoked potential occurs.

3.8.6 Techniques measuring blood flow and/or metabolism

Recently functional MRI and positron emission tomography (PET) have enabled cerebral blood flow or metabolic activity in various regions of the CNS to be visualised (Cohen et al 1996; Mega et al 1997; Hamdy et al 1999). This has the potential to enable the CNS response to various gut stimuli to be examined. Like evoked potentials, these techniques are more objective than the routine assessments of gastroduodenal sensation. Due to their novelty and cost, they are not yet widely used, and thus their utility is not yet clear.

3.9 SUMMARY

Gastroduodenal sensation is vital to the normal functioning of the gut. On a continuous basis, at a subconscious level, it provides the information on which motor and secretory function are modulated to optimise absorption and digestion and to empty the upper gut after meals. At a conscious level it makes us aware of potentially injurious situations and enables us to avoid noxious stimuli. The conscious perception of gastroduodenal sensation can be modified by a number of factors, and these must be considered when interpreting studies or symptoms. In particular, small intestinal

nutrients modulate both gastroduodenal sensation and motor function, and these interactions are further discussed in Chapter 4.

There is ongoing discussion as to whether the gastroduodenal region is able to sense certain nutrients specifically, and the means whereby this is achieved. Some of these issues of nutrient specificity are examined in Chapters 8B & C, and the specificity of the route of glucose administration is considered in 8A.

In humans, a number of techniques exist for assessing gastroduodenal sensation. As none are ideal, their limitations must be taken into account when considering results. In the studies in this thesis, we have used VAS and spontaneous report as they are minimally invasive, reliable in a paired within-subject design, and practical to apply when concurrent manometric studies are being performed.

CHAPTER 4

Effects of Small Intestinal Nutrients on Gastroduodenal Motor and Sensory Function

4.1 INTRODUCTION

This chapter attempts to integrate what is known about the effects of small intestinal nutrients on gastroduodenal motor and sensory function (including appetite regulation). Many of the issues have been mentioned in the preceding three chapters, particularly the effect of nutrients on appetite regulation, which is considered in some detail in Chapter 1. Therefore, in this chapter, the focus is predominantly directed at small intestinal nutrient effects with reference to gastroduodenal motor function (section 4.2), and conscious gut sensation (section 4.3). Issues concerning appetite

regulation are briefly revisited in section 4.4, and the major strands in the chapter are drawn together in section 4.5.

4.2 EFFECTS OF NUTRIENTS ON GASTRODUODENAL MOTOR FUNCTION

The presence of small intestinal nutrients causes a number of changes in fasting motility. These changes are specific for small intestinal nutrients, as mechanical stimuli such as equivalent intraluminal amounts of non-nutrient liquids (MacGregor et al 1976; Huge et al 1995), or gastric distension by balloons (Troncon et al 1995; Feinle et al 1996) do not reproduce the same effects. There is ongoing debate as to the means whereby intraluminal nutrients are detected (discussed in Grundy & Scratcherd 1989; Hunt 1980). These means may include small intestinal chemo- and osmoreceptors along with specific sensory nerve endings (glucoreceptors, amino acid receptors see section 3.4.1) and post absorptive signals (for fats CCK and Apo A-IV appear to be important, see section 1.3.1). In the following subsections, what is known about the gastroduodenal motor response to nutrients is considered. Initially the major outcomes (initiating the fed motor pattern - section 4.2.1.1 and slowing of gastric emptying - section 4.2.1.2) are discussed, followed by an examination of the motor effects resulting from both intraluminal and intravenous nutrient administration (section 4.2.2). Nutrient-specificity of motor effects is dealt with in section 4.2.3, and adaptation of motor responses resulting from dietary change in section 4.2.4.

4.2.1 Major outcomes

4.2.1.1 Conversion from fasting to fed motor pattern

The presence of small intestinal nutrients interrupts the interdigestive motor cycle and converts antropyloroduodenal motility to a pattern of irregular frequent contractions, referred to as the "fed" pattern (discussed in section 2.6.5). In the stomach, this pattern of contractions is believed to be responsible for mixing and trituration of food and, in the small intestine, for mixing chyme with pancreaticobiliary secretions, facilitating absorption by constantly bringing contents into close contact with the mucosa, and gradually moving contents along the gut. Of interest, in the stomach, the fed pattern varies depending on whether solids or only liquids are ingested. If there are no solids in the stomach, relative motor quiescence is seen in the antrum (Rees et al 1979b; Sun et al 1998), rather than the typical frequent, pressure waves seen when solids are

present (Fone et al 1990); and gastric emptying of liquids begins immediately following ingestion, without a lag phase (Horowitz & Dent 1991). The fed pattern in the small intestine has not been examined in as great detail, but may also vary depending on the nutrient infused (Schmid & Ehrlein 1993; Rao et al 1996a), or the viscosity of the gastric outflow, as this alters small intestinal transit time (Reppas et al 1991).

4.2.1.2 Slowing of gastric emptying

The presence of nutrients in the small intestine slows gastric emptying, and occurs in response to a variety of small intestinal nutrients including fats (Hunt & Knox 1968; Heddle et al 1989), carbohydrates (Brener et al 1983), protein (Shi et al 1997) and mixed meals (Hunt 1980). Hyperosmolar solutions in the small intestine are also associated with slowing of gastric emptying (Parr et al 1987), although their effects appear to be less potent than that of nutrients (Meeroff et al 1975; Lin et al 1993). The slowing of gastric emptying by small intestinal nutrients is achieved by decreasing the active emptying of the stomach, and increasing the post-gastric (duodenal or small intestinal) resistance to emptying. Collectively these alterations in motility are recognised as the gastroduodenal mechanisms which retard gastric empting.

Gastric motor patterns associated with nutrient-mediated slowing of gastric emptying include; fundal relaxation (Azpiroz & Malagelada 1985a & b, 1986; Hebbard et al 1996b), suppression of antral motility, and increased phasic and tonic pressures at the pylorus (Heddle et al 1989; Tougas et al 1992). Changes in duodenal motility associated with the slowing of gastric emptying vary according to the stimulus (Rao et al 1996a & b) and include dilatation and delayed clearance (Rao et al 1996b), high amplitude pressure waves and luminal constriction (Rao et al 1996b), and duodenal retroperistalsis of pressure waves which may cause duodenogastric reflux (Castedal et al 1997a & b). In this fashion, in the absence of other factors, the linear phase of gastric emptying is tightly regulated to deliver approximately 2 Kcal/min to the duodenum (Brener et al 1983; Carbonnel et al 1994), although increasing meal size and caloric density can accelerate this rate somewhat (Moore et al 1984; Hunt et al 1985; Wisen et al 1993; Doran et al 1998). The gastroduodenal mechanisms which retard gastric empting do not come into play immediately, and the early phase of gastric emptying (prior to full establishment of negative feedback) is more rapid than later (Schirra et al 1996), as the early phase appears to be more "load dependent",

accounting for the accelerant effect that increased meal size and energy density have on gastric emptying.

4.2.2 Effects of intraluminal as compared to intravenous nutrients

Oral nutrient exposure (section 1.2.1) appears to be sensed. At least for fats, this appears to have an effect on subsequent absorption and/or metabolism (Mattes 1996) and thus possibly on appetite regulation. Recently, it has been suggested that oral exposure to nutrients may further slow gastric emptying when compared to intragastric administration of the same nutrient load (Cecil et al 1998a), but it is not clear specifically how oral nutrients alter gastroduodenal motor function.

Intragastric nutrients affect gastroduodenal motility by their physical presence in the stomach. Intragastric contents cause an increase in gastric volume (gastric distension) which is proportional to the amount ingested and proximal gastric tone (Novitol et al 1995). In dogs, gastric distension is a sufficient stimulus to disrupt the fasting interdigestive motor pattern (Lee et al 1995); and increased gastric volume is thought to be a stimulus to antral contractions via a vasovagal reflex (Scratcherd & Grundy 1982); although only solids appear to give a strong contractile stimulus (section 4.2.1). Due to the stomach's ability to receptively relax (Azpiroz & Malagelada 1985a), gastric "distension" occurs (within the physiological range) without a significant increase in intragastric pressure. The volume change, however, is thought to be tracked by in-series tension receptors in the muscle of the body and fundus (see section 3.4.2). The initial phase of receptive relaxation is not mediated by nutrient-specific mechanisms, as gastric tone falls with the first swallow of a meal, before any small intestinal nutrient exposure or absorption could have occurred (Azpiroz & Malagelada 1985a). Receptive relaxation is prolonged by the presence of small intestinal nutrients (Azpiroz & Malagelada 1985a) and, indeed, can be induced in the absence of gastric contents, by nutrient infusion into the small intestine (Azpiroz & Malagelada 1985b).

Small intestinal nutrient exposure is more potent than oral or gastric nutrient exposure in stimulating the gastroduodenal mechanisms which retard gastric empting (see Hunt & Knox 1968). Small intestinal nutrient exposure slows gastric emptying (Hunt & Knox 1968; Heddle et al 1989; Lin et al 1989) and alters small intestinal motor patterns (Huge et al 1995), so that transit time is increased. Moreover, the degree of slowing of gastric emptying and intestinal transit is related to the length and site of intestine exposed to nutrient, the duration of contact of nutrients with the mucosa, and the

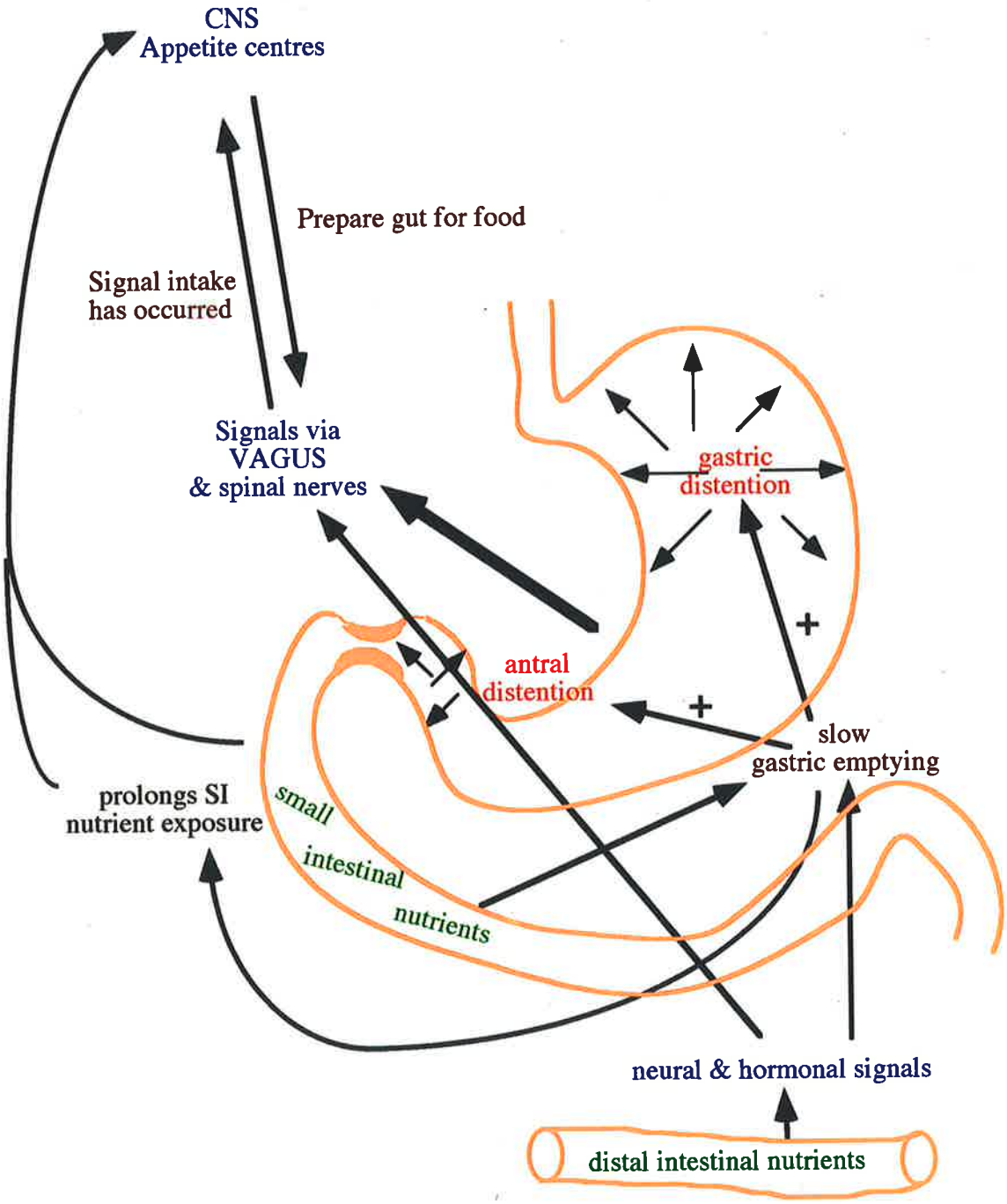


Figure 4.1
Schema illustrating the circular relationship between small intestinal nutrient exposure, slowing of gastric emptying, and prolongation of small intestinal nutrient exposure. This provides a means whereby motor and appetite regulation interact. This interaction will also affect the length of intestine exposed, which in turn, determines the degree of inhibition of gastric emptying. For further detail see text .

chemical specificity of the nutrients (Hunt & Knox 1968; Lin et al 1989, 1990b, 1992a & b, 1996b; French & Read 1994), with greater lengths of intestinal exposure leading to more potent slowing of gastric emptying. The motor mechanisms responsible for the slowing of gastric emptying, and prolongation of transit time are described in section 4.2.1.2 above.

Intravenous (IV) nutrients have been documented to alter gastroduodenal motor function (reviewed in Masclee et al 1996). For glucose these alterations are well studied in both animals and humans, whereas less information is available for other nutrients. The effect of IV nutrients on gastroduodenal motor and sensory function is important as it may be involved in the apparent role of intraluminal nutrients, as absorption occurs after enteral nutrient exposure. Comparison of IV and intraluminal nutrient effects is also useful in establishing whether some effects are specific (or more potent) for a specific route of administration, as this will help in pinpointing the mechanisms by which effects are mediated.

IV nutrients in humans have been shown to mimic some of the motor effects of enteral nutrients. In particular, IV fat slows gastric emptying and interrupts the interdigestive motor cycle, whereas IV amino acids in high doses stimulate gastric acid secretion, pancreatic secretion, gallbladder contraction and intestinal motility, whilst standard parenteral nutrition (PN) regimes are associated with gallbladder stasis (reviewed in Masclee et al 1996). IV amino acids do not interrupt the interdigestive motor cycle, but do shorten cycle length, slow gastric emptying, and prolong interdigestive duodenocaecal transit time (see Gielkins et al 1999). Mixed macronutrient PN also slows gastric emptying (MacGregor et al 1979; Bursztein De Myttenaere et al 1994). Although part of this effect may be due to hyperglycaemia (MacGregor et al 1979), other components of the PN exert a specific effect, as substitution of half the amino acids in standard PN with branched-chain amino acids markedly attenuates the slowing of gastric emptying seen during standard PN (Bursztein De Myttenaere et al 1994). Unfortunately these studies did not directly compare the effects of PN with a matched intraluminal load. It is therefore not possible to judge the relative potency of the IV as compared to the intraluminal effects, although it is clear that postabsorptive signals from nutrients (both fats and amino acids) do influence gastroduodenal motor function, in a similar fashion to intraluminal nutrients.

IV glucose, sufficient to acutely elevate blood glucose concentration, causes suppression of antral pressure waves, stimulation of phasic pyloric pressures, fundal

relaxation, slowing of gastric emptying, reduction in the number of duodenal and jejunal pressure waves, and stimulation of early onset of small intestinal phase III of the MMC (MacGregor et al 1976; Fraser et al 1990 & 1991; Hasler et al 1995; Hebbard et al 1996a & b; Russo et al 1996). Whilst some of these effects have only been demonstrated with pathological levels of hyperglycaemia (~12 mmol/L), suppression of antral pressure waves, and fundal relaxation, are seen even with minor elevations of blood glucose within the physiological range (MacGregor et al 1976; Hasler et al 1995; Schvarcz et al 1997). Conversely, hypoglycaemia has been shown to accelerate gastric emptying in both healthy volunteers and patients with insulin dependent diabetes (Schvarcz et al 1993 & 1995a). The effects of IV glucose have been demonstrated in both healthy subjects and in those with diabetes mellitus in short-term (single day) studies. Somewhat puzzlingly, the slowing of gastric emptying seen during elevated blood glucose has been reported by MacGregor et al (1976) to only occur if the gastric contents contain nutrients, whilst at least some of the motor mechanisms associated with delayed empting (see above and 4.2.1.2) are seen regardless of whether there are gastric contents or not (Hebbard et al 1996a). It may be that the magnitude of the effect is augmented by intragastric nutrients, or that the global measurement of gastric emptying rate by MacGregor et al (1976) was not sufficiently sensitive to detect the motor alterations demonstrated by Hebbard et al (1996a). Hyperglycaemia also alters gastrointestinal motor function at a number of other sites (De Boer et al 1992a & 1993b; Chey et al 1995a; Boeckxstaens et al 1997; Russo et al 1997), although this is some controversy as to its colonic effects (Maleki et al 1997). Since this is not directly relevant to the work in this thesis, it is not considered here in detail. As elevated blood glucose (up to ~12 mmol/L) usually follows a meal, "physiological hyperglycaemia" may be an important contributory mechanism to normal postprandial regulation of gastric emptying and small intestinal transit rates. As discussed in Chapter 1 (section 1.3.2.2), pathological hyperglycaemia has an effect on the perception of appetite, which may be partly mediated by the motor changes documented here, although the decrease in fasting appetite reported (Jones et al 1997a) could not be due to changes in gastric emptying and small intestinal transit rates.

4.2.3 Variation of motor effects between macronutrient classes

The nutrients commonly used to assess gastroduodenal motor function are simple carbohydrates, lipid emulsions, and proprietary mixed nutrient enteral liquid feeds. As the sensing mechanisms in the small intestine are capable of signalling some degree of nutrient specificity (sections 1.3.1, 1.3.2 & 3.4.1), it is possible that different

nutrients have different motor effects. Determining whether variations in specific motor responses between macronutrients occur in humans is difficult, as macronutrients have generally not been directly compared or, if so, making comparisons is complicated by different caloric content, volume and osmolality of nutrients, all of which may affect motility (Fone et al 1989; Fraser et al 1992a & b; Feinle et al 1995b & c). Anecdotally, fat has been thought to be the most potent macronutrient in modulating gastroduodenal motor function, although intraluminal carbohydrate and protein have also been shown to be capable of stimulating fundal relaxation, suppression of antral pressure waves, phasic and tonic pyloric pressures, and slowing of gastric emptying (Azpiroz & Malagelada 1985b; Heddle et al 1988c; Fone et al 1989; Feinle et al 1995b & c, & 1999). However, in dogs receiving proximal small intestinal isocaloric, isosmolar infusions of oleic acid, casein hydrolysate and maltose, fat has been shown to be the more potent stimulus, keeping the pylorus closed for longer, and slowing gastric emptying to a greater degree (Kumar et al 1987). Therefore in the studies described in Chapter 8B and C the relative potencies of small intestinal exposure to isocaloric amounts of lipid and glucose (in terms of pyloric stimulation and appetite suppression) are examined.

4.2.4 Adaptation of motor response to dietary changes.

As discussed in Chapter 1 (section 1.3.1) the motor response to small intestinal nutrients can be altered by varying (i) the amount of certain macronutrients consumed, (ii) the total number of calories consumed, and/or (iii) the physical form of the food.

In humans, dietary supplementation with fat or glucose is known to speed the gastric emptying of a subsequent meal of the same nutrient class (Cunningham et al 1991a & b; Horowitz et al 1996). In humans it is not known whether this adaptation is "macronutrient specific", although in animals it was, with those having a protein supplemented diet, only showing accelerated emptying of subsequent protein meals (Shi et al 1997). The adaptation in the rate of gastric emptying occurred relatively rapidly, with only ~3-7 days dietary glucose supplementation necessary in the human studies referred to above (Cunningham et al 1991b; Horowitz et al 1996) although 4 days was insufficient for the adaptation to fat to occur (Cunningham et al 1991a); and at least in animals the effect then persisted for several weeks after discontinuing the supplementation (Shi et al 1997).

There is evidence that "non nutrient specific" adaptation of gastroduodenal motility also occurs in response to dietary change, although this evidence is less direct. It appears to be seen in response to changes in the total number of calories consumed such that, after fasting (Robinson & Stephenson 1990; Corvilain et al 1995) or severely restricting intake such as is seen with eating disorders (Stacher et al 1986; Rigaud et al 1988; Robinson et al 1988; Kamal et al 1991), gastric emptying and small intestinal transit are slowed. When subjects with anorexia nervosa resume normal oral intake the rate of gastric emptying normalises (Rigaud et al 1988; Robinson et al 1988). Likewise in obese subjects who are eating a high calorie diet gastric emptying is more rapid (Wright et al 1983; discussed in Wisen & Hellstrom 1995), whereas eating a calorie restricted diet slows the rate of gastric emptying in obese subjects (Corvilain et al 1995).

Acutely changing physical attributes of food also alters gastroduodenal motility. In particular, adding fibre to foods slows gastric emptying (Read 1994; Vincent et al 1995), and removing fibre from foods accelerates it (Benini et al 1995). Variations in the mode of cooking also change the rate of gastric emptying (Benini et al 1994) as does the temperature of food (Sun et al 1995), changing particle size of solids (Vincent et al 1995), or altering the viscosity (Ehrlein & Prove 1982). There is no evidence in humans as to whether these acute effects persist but, given that adaptation to other dietary alterations occurs (see above), it is likely that the motor response to these physical modifications also adapts over time.

The more detailed motor mechanisms associated with these alterations of upper gastrointestinal motility are undocumented, as the studies discussed above employed techniques which assessed global outcomes (gastric emptying, transit time) rather than specific motor patterns. Therefore, motor consequences and alterations of appetite regulation in response to dietary manipulation are examined in more detail in the studies described in Chapters 8B and 10.

4.2.5 Mechanisms implicated in small intestinal nutrient exposure mediated regulation of gastroduodenal motor function

Both neural and hormonal mechanisms are implicated in producing the gastroduodenal mechanisms which retard gastric emptying in response to intraluminal nutrients (see specifically Hunt 1980; Moran & McHugh 1982; Azpiroz & Malagelada 1986; Eliasson et al 1995; Meyer et al 1998 a & c; Wishart et al 1998; Edwards et al 1999;

McLaughlin et al 1999; Raybould et al 1999; or Hasler 1995 for a general review). These motor responses are initiated consequent on nutrients being sensed by small intestinal "receptors" (sections 1.3.1, 2.6.5 & 3.4.1). Gastrointestinal hormones, particularly CCK and GLP-1, released in response to intraduodenal nutrients are known to slow gastric emptying (Anvari et al 1998; Raybould et al 1999; and section 1.3.2) and, as they are preferentially released by different macronutrients, they provide a potential mode by which specific nutrients may exert differing motor effects (as discussed in 4.2.3). The vagus is also known to be particularly important in regulating gastroduodenal motor function (Holle et al 1992); and gastrointestinal hormones, including CCK and GLP-1, at least partially exert their effect via vagal means (section 1.3.2). The ENS is also likely to be involved in sensing intraluminal nutrients via its primary sensory neurones (section 3.3) and modulating motor function via interneuronal relay to local motor neurones. Certainly, in rats, antral myenteric plexus denervation grossly interferes with gastric emptying of solids and liquids (Higham et al 1997). Spinal afferent pathways may also be involved in signal transduction, but this has not been studied in humans (see section 3.3).

Traditionally, signals from the gut released in response to intraluminal nutrients have been sought as the likely trigger for the gastroduodenal motor mechanisms seen in response to feeding. However, there is some evidence that signals from the CNS, in anticipation of feeding, also alter gastroduodenal motor function. Thompson et al (1993) have shown that the orexigenic peptide NPY (section 1.4.2), administered centrally in fasting dogs causes conversion of the fasting motor pattern to a pattern indistinguishable from the fed motor pattern. Moreover, others have shown in humans, that gastrointestinal hormone release (Witterman et al 1994) and gallbladder contraction (Masclee et al 1997) occur in response to sham feeding, perhaps by centrally mediated means.

Acute hyperglycaemia is thought to affect gastric motor function by a number of possible means, although the precise mechanisms are undefined. Autonomic neuropathy is associated with a higher incidence of delayed emptying in diabetics. Thus a vagal mechanism of action for hyperglycaemia has been proposed (Lin & Hasler 1995) but this, however, has not been proven. Concurrent hyperinsulinaemia is usually present, and does influence gastroduodenal motor function somewhat (Kong et al 1998; Eliasson et al 1995). However, it does not appear to be a major factor in altering gastroduodenal motor function (Chey et al 1995a; Hasler et al 1995), particularly as the effects of hyperglycaemia are also seen in type-1 diabetes mellitus,

where insulin is absent (Horowitz et al 1998). As hyperglycaemia has been reported to have both a stimulatory and inhibitory effect on smooth muscle, a direct smooth muscle effect of glucose is unlikely to account for the changes in motility seen (discussed in Horowitz et al 1998). The electrical control activity (slow wave) of the stomach is affected by hyperglycaemia (Jebbink et al 1994; Hebbard et al 1997), via a prostaglandin dependent pathway, albeit at a higher level than that at which it suppresses antral pressure waves (Hasler et al 1995), and this may be one of the means whereby hyperglycaemia slows emptying. Nitric oxide is known to be an important neurotransmitter in the gastroduodenal region (Allescher & Daniel 1994; Orihata & Sarna 1994; Schuurkes & Meulemans 1994; Meulemans & Schuurkes 1995), and in animal models of diabetes hyperglycaemia been shown to reduce nitric oxide synthase (NOS) activity in the gut (Wrzos et al 1997), and to increase NOS activity in the CNS (Serino et al 1998), thus providing a means whereby hyperglycaemia may change NO availability, and thus motor function, in the gut (Plourde et al 1994; Orihata & Sarna 1996; Ohta et al 1997).

The mechanism(s) by which IV nutrients, other than glucose, affect motor function is unclear, although it is possible that the serum levels of these substances may act directly on gut smooth muscle, or on vagal or CNS receptors involved in gastroduodenal motility or cause the release of other substances (eg CCK) which do so (Bursztein De Myttenaere et al 1994; Gielkins et al 1999).

The motor adaptation seen in response to dietary alterations (section 4.2.4) could arise by a number of means, none of which are proven. Adaptation mechanisms may include decreased receptor sensitivity, down-regulation of receptor number or signal, decreased length of intestine exposed due to increased (more efficient) absorption, or reduced responsiveness of the motor effector mechanisms to the negative feedback. Shi et al (1997) showed a reduced CCK response in rats to protein after protein supplementation, perhaps pointing to decreased hormone release as a factor in this situation. Given the interaction between length of intestine exposed to nutrients, absorption and the rate of gastric emptying (as discussed in Meyer 1994; Read 1994), measures which lead to more efficient absorption, and/or less gut exposed to nutrients are likely to be involved in adaptation to higher fat or carbohydrate diets.

4.3 EFFECTS OF NUTRIENTS ON CONSCIOUS GASTRODUODENAL SENSATION

In whole subjects, it is not feasible to separate out sensory afferent function from motor function. Therefore, as the effects of intraluminal nutrients on motor function considered above (section 4.2) all necessarily involve sensory afferent function, this aspect of sensory function is not reiterated. In this section, only conscious or perceived sensation, and how it is affected by nutrients is addressed. The information is somewhat limited, as only a few studies have examined perception (as opposed to appetite) during nutrient administration.

4.3.1 Modulation of sensations by intraluminal nutrients

Intraluminal nutrients are capable of generating conscious perception. In particular they are associated with feelings of fullness, meal-like feelings, and even nausea. Feinle et al (1995a) have demonstrated that a high-fat meal sensitises subjects to develop nausea when subjected to optokinetic rotation. Furthermore, small intestinal exposure to the fat is necessary, as when the optokinetic rotation was performed within minutes of eating nausea scores were no different between the zero-fat and high-fat meals, whereas when the rotation was delayed until part of the meal had emptied from the stomach, there was a clear increase in nausea after the high-fat meal. This group have also shown that concurrent intraduodenal lipid (Feinle et al 1996) and maltodextrin (Feinle et al 1995b) cause gastric distension to feel more meal-like, rather than discomforting and, for that lipid, this effect involves CCK-A receptors. Interestingly intraduodenal glucose did not elicit this change in sensory quality, but it was given at only half the caloric rate (1 Kcal/min) of the maltodextrin and lipid (2 Kcal/min) (Feinle et al 1995b; Feinle et al 1996). Of note, when maltodextrin and lipid were compared directly at 2 Kcal/min, they exerted differential sensory effects. Although they both caused distensions to be perceived as more meal-like, lipid was associated with a greater level of nausea during distensions, and maltodextrin (but not lipid) increased the sensory threshold for perception of distensions (Feinle et al 1995c). These studies are consistent with the clinical observation that subjects with functional dyspepsia have increased symptoms postprandially and that high fat meals appear to provoke symptoms preferentially (Barbera et al 1995a & b).

4.3.2 Modulation of sensations by intravenous nutrients

IV glucose sufficient to cause hyperglycaemia (~15 mmol/l) increases the intensity of a number of sensations, including fullness and nausea, in response to gastric distension during fasting or intraduodenal lipid infusion (Hebbard et al 1996a & b). In fact, hyperglycaemia appears to interact with signals from intraluminal nutrients to modulate sensation such that, during fasting, hyperglycaemia (~15 mmol/l) alone does not increase nausea, but, when combined with intraduodenal lipid, a significant increase in nausea occurs (Hebbard et al 1997). Hyperglycaemia has also been reported to both increase (Russo et al 1997), and decrease (Chey et al 1995a) rectal perception of stimuli in normals, whilst decreased perception has been reported in diabetes (Wald & Tunuguntla 1984; Caruana et al 1991). Unfortunately, gastroduodenal sensation in response to other IV nutrients has not been specifically evaluated.

4.4 EFFECTS OF NUTRIENTS ON APPETITE REGULATION

The effect of nutrients on appetite has been considered extensively in Chapter 1 and is therefore not repeated. In summary, the presence of small intestinal nutrients decreases appetite, and reduces consumption. There is discrepancy in the literature as to the magnitude of this effect, with some authors finding the subsequent reduction in intake to accurately compensate for the enteral nutrients given, whilst others finding a more limited effect (section 1.3.1). Other controversies in the literature include the relative strength of effects by nutrient class, and the effect of usual diet (discussed in 1.3.1).

Pathological hyperglycaemia affects appetite even prior to the presence of small intestinal nutrients (section 1.3.2.2). Hypoglycaemia is a positive stimulus to appetite, and some groups have hypothesised that physiological elevation of blood glucose acts as a satiety factor (Mayer 1953). In a recent study Lavin et al (1996) have reported that physiological changes in peripheral blood glucose do not appear to affect appetite in the absence of small intestinal nutrients. This is despite the effect of physiological hyperglycaemia on motor function (4.2.2) in slowing gastric emptying, which might have been expected to increase satiety and satiation (1.2.2 & 1.3.1). The mechanisms by which glucose may affect appetite may be related to the actual process of glucose absorption (perhaps via stimulation of insulin and GLP-1 release - see section 1.3.2), or the hyperglycaemia that results. It is unknown whether these effects of hyperglycaemia are specific for subsequent glucose loads or interact with the signalling of satiety by other nutrients.

Small intestinal nutrients suppress appetite directly (sections 1.3.1 & 1.3.2), and also indirectly by altering gastroduodenal motor function to slow gastric emptying (section 4.2.2), which prolongs both gastric distension (see 1.2.2) and the length of time over which there is nutrient driven stimulation of small intestinal satiety mechanisms. In the studies in this thesis, nutrients are presented directly to the duodenum to avoid this variable effect of gastric emptying on motor and appetite responses.

4.5 SUMMARY

Nutrients, both intraluminal and intravenous, interact with gastroduodenal motor patterns in such a way, that they prolong small intestinal nutrient exposure. Intraluminal effects are the more physiologically relevant as these occur after each meal in healthy subjects. Because of the prolongation of small intestinal nutrient exposure by these motor changes, further down-regulation of appetite (in addition to that signalled directly by the nutrients - as discussed in Chapter 1) is also likely to occur.

As the rate of gastric emptying varies within and between subjects and will affect the duration of gastric distension and small intestinal nutrient exposure, infusions of lipid and glucose are given intraduodenally in the studies reported here (Chapters 8, 9 & 11). Because of factors discussed earlier (section 1.5) all subjects were unrestrained eaters, who were of normal weight and healthy.

Both neural and hormonal mechanisms are involved in modulating intraluminal nutrient effects. The study in Chapter 8A examines the contribution of gastrointestinal hormones using octreotide (a somatostatin analogue) to block their release. Macronutrients may affect motor and sensory function in specific ways and the relative strength of these effects in humans is not well delineated, particularly for fats and carbohydrates, which form the caloric bulk of the diet. Therefore in Chapter 8B & C the relative potencies of isocaloric intraduodenal infusions of lipid and glucose are compared with respect to distal gastric motor function, appetite and sensation, in young (8B) and elderly (8C) subjects. Changes to diet are capable of causing adaptation in the gastroduodenal motor response to food. It is not known whether this effect is nutrient-specific in humans, nor whether adaptation in appetite also occurs. Thus, the specific gastroduodenal motor effects of dietary supplementation with glucose in response to subsequent glucose and lipid, and the effect on appetite regulation are addressed in Chapter 8B. Whereas changes in fed motility are known to

occur in response to dietary change (section 4.2.4), it is unknown whether fasting motor function is also altered. This issue is examined with respect to small intestinal motility in Chapter 10, where fasting small intestinal motility in response to acute and chronic vegetarian diets is presented.

Although pathological levels of hyperglycaemia are well recognised to cause similar motor changes to intraluminal nutrients, it now seems likely that physiological hyperglycaemia also modulates gastroduodenal motor and sensory function (section 4.2.2) and is thereby may be involved in the physiological regulation of appetite. This is the subject of study in Chapters 9A & B.

CHAPTER 5

Effects of Ageing on Appetite Regulation and Gastroduodenal Motor and Sensory Function in Humans

5.1 INTRODUCTION

Healthy ageing causes a decline in many physiological functions in humans. In particular it is known to cause a reduction in cardiorespiratory peak parameters, and in bone and muscle strength. The situation in the gut is less clear, as it has a large reserve capacity, and loss of function is generally only revealed by the co-presence of disease states.

Malnutrition and undernutrition are serious problems in the elderly with comorbidities (Keller 1993; Lovat 1996), and contribute to their poor general health. As the term

"malnutrition" also encompasses overnutrition, the more correct term to describe the problem which stems from reduced intake with ageing is "undernutrition". Some authors have proposed that even in the healthy elderly there is a physiological "anorexia of ageing", which predisposes to the development of severe anorexia when coupled with the presence of other illnesses or social stress (Morley & Silver 1988). The facts that during ageing weight generally increases until late middle age, and then plateaus and begins to fall (Rolls 1993), and that the elderly consistently eat less than young subjects (reviewed in De Castro 1993) both support this concept. Under-nutrition is associated with poor outcomes in a number of situations (reviewed in Keller 1993; Roberts et al 1994 and Morley 1996a), and places otherwise well elderly subjects at increased risk of morbidity and mortality. Given the increasing proportion of elderly people in the developed world, it is important to develop an understanding of this "anorexia of ageing" and to attempt to define the mechanisms by which it arises.

In the United States, between 15-20% of all subjects over 60 years of age consume fewer than 1000 calories a day; and it has been suggested that up to 58% of nursing home residents are affected by some degree of malnutrition (see Morley & Silver 1988). These rates of malnutrition in the elderly highlight the extent of the problem, and the comparison between different groups of elderly subjects emphasises the effect that additional social or medical factors have on determining whether significant weight loss results from the "anorexia of ageing". The literature on appetite regulation in the elderly generally refers to those over 60 or 65 years of age, and to avoid confounding factors, appetite regulation should initially be examined in the healthy elderly, ideally, those on no medications, who live in favourable financial and social settings.

In general, undernutrition results from insufficient intake for a given level of energy expenditure, although malabsorption may also contribute. Undernutrition in the elderly may also represent deficient intake of certain macronutrients, rather than simply insufficient calories, as discussed by Wurtman (1988). Insufficient intake of calories (or macronutrients) may be related to decreased appetite (section 5.2), taste disturbances (section 5.7.1), effects of intercurrent illnesses or medications (section 5.7.3), social and psychological factors (section 5.7.4), or to a combination of these. The amount of energy required for weight maintenance is dictated by activity level (section 5.7.2), and intercurrent illnesses (section 5.7.3). Absorptive function in the gut (section 5.5) is related to both motor (section 5.3) and sensory (section 5.4) function, and these in term are interrelated with appetite regulation (Chapters 1 - 4).

The effects of ageing on other areas of the gut are beyond the scope of this thesis, and are not considered here in any detail. For a general review see Pilotto (1999).

5.2 APPETITE

Caloric intake is decreased in both the institutionalised (Keller 1993) and the healthy elderly (De Castro 1993; Rolls 1993; Roberts et al 1994; Rolls et al 1995b). Although social factors related to ageing are thought to play a role in this decrease, when elderly and young subjects were studied under identical conditions in a residential laboratory, the elderly still ate fewer calories, carbohydrates and fats, despite the continuous availability of foods (Wurtman et al 1988). Confirming this, in a seven day diet diary study of over 300 healthy, free living subjects, De Castro (1993) found that increasing age was associated with lower caloric intake, smaller meal size, slower rate of consumption, eating earlier in the day, and less physical activity. He reported however, that in terms of intake, the elderly were as responsive as the young to other factors such as time of day, number of people sharing the meal and subjective state of hunger prior to meal. It is possible that decreased intake and appetite in the elderly does may, in a circular fashion, simply reflect the lesser amount the elderly subjects usually consumed (for other reasons), as usual diet affects gastric emptying and small intestinal transit rates (Chapter 4). Against this concept, however, when specifically asked about appetite elderly subjects had less desire to eat and less hunger postprandially than the young (Clarkson et al 1997). Of note, some authors have found older subjects to preferentially decrease the proportion of their diet taken as fat (De Castro 1993), this may be due to a differential sensitivity to the satiating effects of specific macronutrients (section 1.3.1), or simply be a result of a survival bias of those with a lower fat diet.

In addition to having less appetite, the elderly also appear to have a diminished ability to compensate for dietary alteration compared to younger subjects, implying a defect in the "set point" for appetite. In a study by Roberts et al (1994), when older and young subjects were overfed (or underfed) for 21 days, both age groups gained (lost) weight as expected. However, the elderly subjects continued to overeat (undereat) for some time after the dietary manipulation period, resulting in a persistently increased (decreased) weight. This has the potential to augment any anorexia, or decreased intake due to intercurrent medical or social factors. In a single day study Rolls et al (1995b) showed that elderly men compensated less well than young men when consuming a self-selected lunch after varying yogurt preloads. After the preload, young subjects

consumed within 10% of their baseline intake; whereas the elderly subjects overate by between 10-30% compared to baseline, confirming an inability to accurately adjust for dietary changes, compared to the young. Interestingly, the elderly react in an analogous fashion with respect to fluid intake, with an inability to accurately adjust for environmental conditions, which impairs their ability to maintain salt and water homeostasis (see Rolls 1993).

A number of disturbances in the central regulation of appetite have been proposed, however, most of the information is derived from rodents and may not be directly relevant to humans. Opioids are important in the central feeding drive (section 1.4.2); and in rodents there is substantial evidence that ageing reduces the strength of the feeding response to both endogenous and exogenous opioids (reviewed in Gosnell et al 1983; Morley & Silver 1988). The decline in the activity of the opioid feeding system may be related to zinc deficiency, due to inadequate zinc intake in the elderly (Morley & Silver 1988). Serotonin is another neurotransmitter involved in appetite regulation, and there is strong evidence that it produces satiety and decreased intake (Morley 1987). Blundell (1988) proposes that in humans, the serotoninergic satiety signal may be more potent (in terms of degree of appetite/intake suppression) than blockade of the opioid feeding drive; and although the relative roles of these two classes of neurotransmitters in appetite have not been directly compared, he goes on to suggest that a perturbation in serotonin balance may be the mechanism for the anorexia of ageing. Nitric oxide (NO) also appears to play a role in modulating intake. In elderly mice, levels of NO in the hypothalamus are higher and appear to play a more important role in stimulating feeding than in young mice (Morley et al 1996). Whilst in healthy young humans, inhibition of NO synthesis with L-NAME or L-NMMA has no effect on appetite (Vozzo et al 1999). With ageing, neuropeptide Y has been found to be less effective at stimulating food intake in some species of rodents, whereas the anorectic effects of corticotrophin-releasing hormone and amylin do not appear to alter with ageing (reviewed in Morley 1996).

5.3 MOTOR FUNCTION

The electrical control activity of the stomach appears to be well maintained, with no major differences described in healthy ageing compared to the young (Andrews & Horowitz 1996). Despite this, several authors have found gastric emptying of a mixed meal to be somewhat slower than in younger subjects (Horowitz et al 1984; Wegener et al 1988; Clarkson et al 1997), although whole gut transit time is not prolonged in the

healthy elderly (Wegener et al 1988; Clarkson et al 1997). Not all investigators agree however, with Madsen (1992) finding no influence of age on gastric emptying of radiopaque markers, but this relatively insensitive technique is unable to assess gastric emptying of liquids. Those studies showing slower gastric emptying of a meal may reflect the fact that the elderly subjects studied habitually consumed fewer calories (see 5.2 above) than the young subjects, as prior diet was not standardised. Regardless of the cause for the slowing of gastric emptying, this interaction between usual diet, gastric emptying and the subsequent duration of appetite suppression (via the presence of small intestinal nutrients) has the potential to create a "vicious circle" of decreasing intake in the elderly (Clarkson et al 1997).

Kupfer et al (1985) reported some evidence suggesting that the receptive relaxation of the fundus may be impaired in the elderly. Specifically, in the elderly they found gastric emptying of a liquid to be significantly faster in the first five minutes after drinking 500 ml of dilute cordial. After this period of initially more rapid emptying, the old and young subjects showed no difference in their gastric emptying rates. This is in contrast to Horowitz et al (1984) who found an initial delay in liquid gastric emptying in the elderly. Surprisingly, there is little other information regarding proximal gastric function in the elderly, although recent work from our laboratory (Rayner et al 1999a) also found an impaired accommodation response in healthy elderly subjects - with them taking 105 minutes to reach maximal gastric volume after a meal, compared to 36 minutes in the young. This may be due to decreased activity of nitric oxide synthase in the fundus, as some evidence for this has been found in rodents (Morley 1996).

Small intestinal pressure patterns in healthy elderly subjects have been studied using manometry, with only minor differences found (Husebye & Engedal 1992). Compared to the young, the elderly had a similar periodicity of MMC activity, duration of postprandial motility, amplitude and frequency of both fasting and postprandial pressure waves. They did, however, have a slower migration velocity of phase III and more frequent "propagated clustered contractions" than the young. These minor changes would not be expected to significantly affect transit time; and in fact, most authors report no difference in orocecal or whole gut transit time between age groups (Kupfer et al 1985; Wegener et al 1988; Clarkson et al 1997). Other studies of small intestinal motor patterns support the above finding of only minor age-related changes, despite the subjects studied having gastrointestinal symptoms, in the absence of diagnosable pathology (Fich et al 1989).

5.4 SENSORY FUNCTION

Gastroduodenal sensory function in the elderly has not been specifically evaluated in detail, however, autonomic dysfunction, which has been associated with disordered gastric emptying, is increasingly common with age (Clarkson et al 1997). Ageing is associated with atrophy in the CNS, it is not known whether this affects perception of gastroduodenal sensation in the absence of a diagnosable neurological disease. Of note, a greater proportion of chronic peptic ulcers present with bleeding or perforation in the elderly rather than pain, as in the young. Moreover, perception of other visceral pains is reduced in the elderly, with silent myocardial ischaemia and infarction, painless pneumonia, and occult biliary sepsis all being commonly recognised clinical phenomena.

Conscious visceral (oesophageal) sensation in elderly human subjects has been evaluated. Using graded intraoesophageal balloon distensions, Lasch et al (1997) found an age-related increase in the visceral pain threshold, with the older subjects' pain threshold being 27 ml compared to 17 ml in the young. As well as a decreased sensitivity to pain, these observations may also be explained by the elderly exhibiting a lesser inclination to complain. In support of a change in actual sensory signal, rather than complaint threshold, Weusten et al (1994b) found that several characteristics of the cerebral evoked potentials in response to rapid oesophageal balloon distension varied by age. They found that the amplitude of evoked potentials decreased and latencies increased with age. Somatic nerve conduction velocity decreases with ageing, and this is likely to hold true also for visceral nerves. It is likely to be the explanation for the prolonged cerebral evoked potential latencies described (Weusten et al 1994b). Whilst the exact significance of the decreased amplitudes is uncertain, it is clear that sensory function does alter with increasing age. The impact of this on gastroduodenal function is unknown.

In guinea pigs there is a loss of in the myenteric plexus with age, such that the older animals had only 40-60% of the number of myenteric neurones as the young (Gabella 1989). The appearance of the neurones was also markedly different, suggesting reorganisation with neuronal loss. It is not known whether a reduction of ENS neurones occurs in humans, or what its effect on motor or sensory function might be.

5.5 ABSORPTIVE FUNCTION

Gastric acid secretion is preserved with ageing, in the absence of atrophy (Katelaris et al 1993; Andrews & Horowitz 1996), however, as helicobacter pylori infection (which leads to atrophy) is more prevalent with increasing age, decreased acid secretion is commonly found. Hypochlorhydria has the potential to cause decreased absorption. However, when it has been specifically evaluated, absorption of most substances is unaffected (reviewed in Andrews & Horowitz 1996; Lovat 1996); although absorption of zinc (potentially important for appetite see section 5.2) and calcium is decreased; and theoretically, iron absorption may be reduced by achlorhydria. Certainly there is no gross malabsorption of calories to account for the decline in weight seen with ageing (Cook & Horowitz 1996). The main risk with marginal absorption of certain vitamins and minerals, is that with decreasing dietary variety with age (Rolls 1993), the elderly are more likely to develop occult micronutrient deficiencies.

5.6 POTENTIAL GASTROINTESTINAL MECHANISMS IN "ANOREXIA OF AGEING"

The gastrointestinal mechanisms involved in the anorexia of ageing, are at present ill-defined, likely to be multifactorial, and interrelate with alterations of the central regulation of appetite (discussed briefly in section 5.2).

Preliminary data in rodents suggests that the early satiety associated with ageing may be related to relative deficiency of nitric oxide in the fundus, impairing receptive relaxation (Morley 1996). In addition, slowing of gastric emptying (section 5.3) prolongs the duration over which the small intestine is exposed to nutrients. As small intestinal nutrient exposure is important in causing satiation and satiety (section 1.3.1), the longer duration over which this occurs in the elderly may play a causal role in the anorexia of ageing. Slower gastric emptying in the elderly may result from increased sensitivity to negative feedback generated by the presence of small intestinal nutrients with ageing. This increased sensitivity could result from the elderly having an enhanced sensory response to a given load of nutrients, an enhanced motor response to a given sensory signal, or a combination of these mechanisms; this is the subject of further study in Chapter 8C.

Habitual intake is likely to play a role in the anorexia of ageing. As discussed earlier (sections 1.3.1 & 4.2.6), usual diet (calories and macronutrient type) is known to influence both the rate of gastric emptying and small intestinal transit; and may well

influence appetite via determining the duration over which nutrients are in contact with the small intestine. Thus, in any investigation of appetite regulation with ageing, prior diet should be quantified.

Alteration of CCK mechanisms may also be involved in the changes seen with ageing. CCK is involved in the production of satiety (see 1.3.2.1), and elevated levels have been reported in elderly humans. In rodents, older animals are more sensitive to the satiating effects of CCK (Silver et al 1988; Morley & Silver 1988), providing a potential mechanism via which reduced appetite in the elderly may be explained. However, in one of these studies (Silver et al 1988), the elderly mice ate more than the younger ones under basal conditions, perhaps lessening the relevance of their findings to the situation in humans, where the elderly habitually consume less. This issue of potential change in the regulation of CCK release and the relative strength of its effect in elderly humans has not been fully evaluated. However, one group found no difference in humans in basal CCK levels, or the CCK response to a meal with healthy ageing (Berthelemy et al 1992). The relationship between CCK and intake in the healthy elderly is further examined in Chapter 8C.

Autonomic dysfunction is common with ageing (Clarkson et al 1997) and may impair transmission of afferent signals from gastroduodenal mucosal and tension receptors to the brainstem and other regions of the CNS (see Chapter 3). The loss of vagal integrity may partially explain the slowing of gastric emptying, minor changes in small intestinal manometric findings and impaired fundal relaxation seen with ageing (section 5.3). CCK is thought to possibly exert its satiety effects via stimulation of vagal afferent fibres (Silver et al 1989), thus enhanced sensitivity to CCK as a mechanism for anorexia (as suggested by Morley & Silver 1988), would appear to be restricted to those elderly without autonomic dysfunction.

5.7 CONFOUNDING FACTORS IN ASSESSING THE "ANOREXIA OF AGEING"

5.7.1 Taste/Oral function

Smell is an important component of taste, and decline in olfactory function with age is well documented. However, despite being poor discriminators of blended foods when blind-folded, elderly subjects rate taste as a major factor in their food choices (see Rolls 1993). Rolls (1993) suggests that the loss of taste may not be noticed as it occurs

gradually with ageing, and notes that other authors have found intake can be increased by adding flavour to foods. The loss of "sensory-specific satiety" (section 1.2.1; Rolls et al 1981) with age may also explain the shift to more monotonous diets by the elderly (Rolls 1993), placing them at risk of micronutrient deficiencies even if caloric intake is sufficient.

Decreased saliva may be partly responsible for loss of taste, and up to 40% of elderly subjects complain of dry mouth (Lovat 1996), which may be associated with a number of disease states common in the elderly. The elderly also have decreasing muscle strength, which affects their power when chewing, and are able to open their mouths less widely than young subjects (Lovat 1996). These factors combined with the high prevalence of poor dentition and problems with false dentures are likely to affect food choice and intake in the elderly.

5.7.2 Activity

In a seven day diet diary study, De Castro (1993) found the elderly to have reduced self-reported activity levels. Likewise, when evaluated by questionnaire, the average amount of strenuous activity was less in healthy elderly men than young men (Roberts et al 1994), as were the daily calories required for weight maintenance. Others studies are in accord with these findings and also report metabolic rate to be lower in the elderly (reviewed in De Castro 1993), although others assert that the decrease in metabolic rate is merely a reflection of declining metabolic mass, and that basal metabolic rate when considered as a function of metabolic mass, is stable (see Morley & Silver 1988). Some authors conclude that the reduction in intake in the elderly is thus appropriate to their needs (reviewed in De Castro 1993), but this does not explain the tendency to lose weight past ~65 years, when body weight is well defended (and, in fact, gradually increases) up to late middle age (Rolls 1993).

5.7.3 Intercurrent Illness/Medication

Intercurrent illness frequently has the effect of not only reducing appetite and intake, but also causing increased obligatory energy expenditure, particularly if there is an associated fever, or cardiac failure. Even if appetite is not reduced by intercurrent illness, the illness may make it more difficult for the individual to get access to food. In the well elderly, illness often creates problems in dealing with routine activities of daily living. This frequently leads to confusion and/or falls when even mildly unwell; and

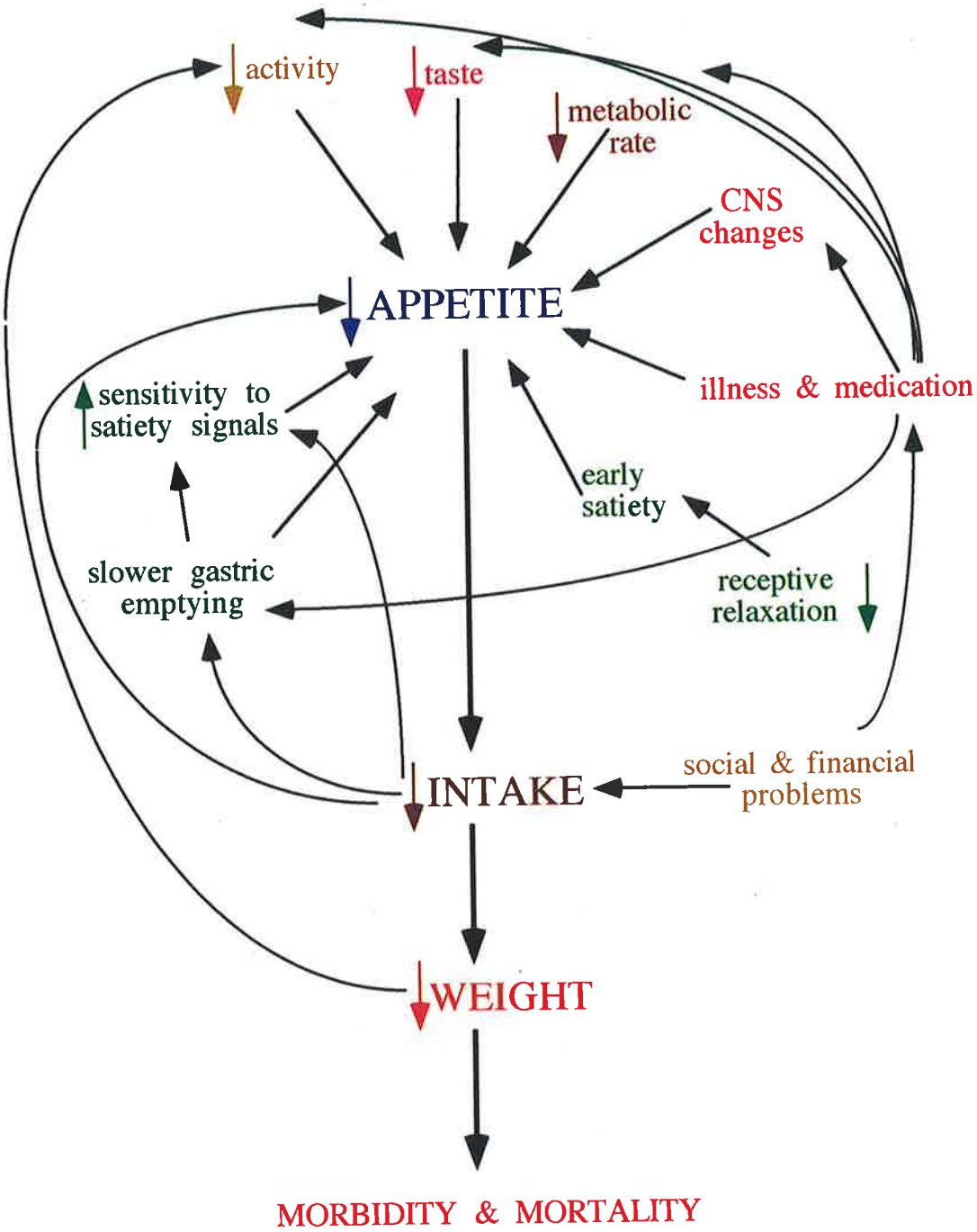


Figure 5.1
An attempt to illustrate the complex interplay between factors potentially affecting appetite regulation in the elderly. See text for detail.

unless food is readily available (or prepared for them) many elderly do not eat appropriately when unwell.

Many medications can affect appetite and taste, or cause nausea. The common culprits are antibiotics, non-steroidal anti-inflammatory agents and medications used for cardiovascular disorders. Given the widespread usage of these agents in the elderly, their potential impact on nutritional status must be anticipated. Other drugs commonly used in the elderly (such as antidepressants, calcium channel blockers) may affect gastrointestinal motility, and this in turn may reduce intake (see section 5.3). Medications may also reduce intake less directly by causing electrolyte imbalances, hypotension or confusion. Diuretics, antihypertensive and sedative/anxiolytic agents are the drug classes most commonly implicated in these disturbances, as they are all frequently prescribed in the elderly.

5.7.4 Social and psychological aspects

In young subjects, Shide & Rolls (1991) have shown that up to 50% more is consumed when a meal is eaten with friends, and this effect appears to hold true for the elderly also (De Castro 1993). The implications for the socially isolated elderly is obvious. Poverty, loneliness, boredom, vision impairment, mobility problems and difficulty shopping are all likely to have an impact on the nutritional status of elderly people in addition to any "physiological anorexia of ageing" they may have. Given the interaction between usual intake, motor function and appetite (discussed in 1.3.1 & Chapter 4) any factors, which decrease access to food are likely to augment anorexia, and further reduce intake leading to a poor nutritional outcome.

5.8 SUMMARY

There is strong evidence to support the concept of a "physiological anorexia of ageing" which is likely to be related to reduced energy requirements, reduced feeding drive, decreased enjoyment of foods and compounded by social and mobility factors. It also appears that the elderly have enhanced satiety and satiation following food; this may reflect, at least in part, slower gastric emptying, subtle changes in small intestinal motor function and absorption and possibly alterations in the perception of satiety signals, such as CCK. As usual diet influences gastric emptying and duration of small intestinal nutrient exposure, the habitually lower caloric intake of the elderly as a group predisposes them to further reductions in intake by enhancing satiety. The potential

interactions between these factors are shown in figure 5.1. Central transmitters involved in appetite regulation including opioids, serotonin and nitric oxide are also likely to be dysregulated with ageing.

In this thesis some of the potential gastrointestinal mechanisms associated with the anorexia of ageing are considered. In particular, in Chapter 8C, the possibility that the elderly have an enhanced sensitivity to the presence of small intestinal nutrients is considered. The relationships between appetite, antropyloroduodenal motility and gastrointestinal hormones including CCK, GLP-1 and GIP, in elderly and young humans are examined.

CHAPTER 6

The Relationships Between Gastrointestinal Pressures/Contractions and Flows

6.1 INTRODUCTION

As discussed in Chapter 2, one of the most important functions of gastroduodenal motor activity is the production of intraluminal flow. Despite quite detailed understanding of the modulation of motor activity at a gross level (section 2.6.5 & Chapter 4), there remains only limited understanding of the precise relationships between the spatial patterning of pressures or contractions and luminal flows on a second by second basis. This lack of insight means that it is not possible to accurately recognise clinically significant changes in motor patterns by the use of manometry. In part, this difficulty results from technical limitations, as there is no ideal method for assessing intraluminal flow in humans with adequate spatial and temporal resolution.

Also, measurement of pressures and/or contractions poses significant technical challenges, which are not always met (see 2.7). In this chapter, issues relevant to better understanding of the relationships of individual contractions and luminal flows are reviewed.

6.2 TEMPOROSPATIAL RESOLUTION AND ITS SIGNIFICANCE

The work of Ehrlein and colleagues in animals (section 6.7), demonstrates the demanding measurement requirements that must be met in humans in order to clarify the relationships between pressures (or contractions) and flows. Without an understanding of the function(s) of particular aspects of motility, its measurement becomes merely a study of phenomenology. As emphasised in Chapter 2, techniques used for assessing the relationships between individual motor events and flows must have detailed temporal resolution in order to define the flow correlates of individual motor events. For recording of both pressures and flow, temporal and spatial resolutions should be such that small fractions of the total time of the event at each location can be resolved. This approach has been demonstrated to be useful in the human oesophagus (Brasseur 1993), and in the small intestine of animals (section 6.7).

6.2.1 Temporal resolution

6.2.1.1 Pressures/Contractions

At any given site in the gastroduodenal region, the rhythmicity and maximum frequency of contractions (or pressure events) is determined by the slow wave (section 2.5). The duration of each contraction (at a single site) and the speed at which a contraction travels (pressure sequence) along the muscle wall of the gut is also tightly controlled. The relative pressures between adjacent areas along the gut is important in determining flow characteristics. Thus, the occurrence of a pressure change at a single site (during a given motor event) relative to the pressures in immediately adjacent areas is likely to be important in determining the movement of luminal contents. Consequently, temporal resolution of contraction and/or pressure data needs to be sufficiently high to resolve fractions of these events. Known values can be used to select an appropriate temporal resolution. In the body and antrum of the human stomach the slow wave frequency is ~3 cpm (Lin et al 1996a; Hebbard et al 1997), pressures at an individual site last ~5-25 s and migrate distally at ~ 0.15 - 1.5 cm/s

(Sun et al 1997b). In the human duodenum the slow wave frequency is ~12 cpm (section 2.5), pressures at an individual site last up to ~7 s (section 2.5), and migrate distally at ~0.5 - 4.5 cm/s (Ehrlein & Schemann 1992; Castedal et al 1998; Husebye 1999). Thus, a temporal resolution of at least 4 Hz is needed to examine gastroduodenal contractions/pressures in detail.

Computer polygraphs use digitisation to sample the data at fixed time intervals, and therefore may capture electronic spikes (caused by noise) when these co-coincide with the time of digitisation. The vulnerability of computer polygraph recordings to be thus affected by electrical "interference", is not well understood. The impact of this noise on measurement accuracy is greatly reduced by oversampling the data and applying a median filter. This then defines a single, more reliable value for each, still very brief, specified time period (Hebbard 1997, p169). Median filtering is now a cost-effective and very pragmatic approach to minimising the impact of electronic noise since it places only modest demands on the processing power of modern personal computers. The alternative solution of very low noise electronics, is costly and difficult to achieve in many recording environments.

6.2.1.2 Flows

Very little is known about the temporal characteristics of gastroduodenal intraluminal flows in humans. The temporal resolution used to study flows must be at least as high as that used to study pressures or contractions, otherwise one would be unable to correlate measured flows with the observed pressures. In the human pyloric region, flow velocities of up to 60 cm/s have been recorded with ultrasound (Hausken et al 1992); and in dogs, flows of ~9 ml/s have been measured using electromagnetic flow meters (Malbert & Ruckebusch 1989). In humans and animals, most gastroduodenal flow has been found to be pulsatile. In humans each flow pulse lasts ~2 - 10 s (King et al 1984; Hausken et al 1992). Based on these data, depending on the parameter one plans to measure, an intraluminal flow detector must be capable of resolution of velocities of ~10-80 cm/s, or volumes of 2-20 ml/s, with a temporal resolution of less than 1 s. These are demanding requirements

6.2.2 Spatial resolution

6.2.2.1 Pressures/Contractions

Many previous studies of gastroduodenal motor function have used motility indices, that sum the "amount" of pressure elevations over baseline within a defined period, rather than examining the spatial organisation of pressures. Not surprisingly, these studies have found that both slower (Malbert et al 1992), and more rapid (Schwizer et al 1996), transit are associated with higher motility indices (Merritt & Ruckebusch 1988). This supports the contention that the spatial patterning of pressures, rather than the "amount" of pressure, is important in determining flow. Except in sphincter regions, contractions or pressures in the gut rarely occur in isolation at a single site, and usually travel varying distances along the gut. This is consistent with what is seen in muscle strips in vitro (Stevens et al 1999). In humans, in the distal gastric antrum, pressure patterns are known to vary over short (~1.5 cm) distances (Sun et al 1997b), and this also appears to be the case in the proximal duodenum (Castedal et al 1997b & 1998); moreover, these changes in pressure patterns are associated with alterations in flow patterns (Castedal et al 1997a). Particularly in the distal antrum, detailed spatial resolution of pressures is vital, as their spatial organisation, and timing with respect to pyloric opening, is more important than the occurrence of pressures per se (Sun et al 1997a). As a broad generalisation, based on the studies discussed above, spatial resolution of at least 1.5 cm, is necessary in the gastroduodenal region. such detailed recordings impose significant technical demands, and have been rare in the past.

6.2.2.2 Flows

Compared to pressure, there is less information regarding flows and their spatial characteristics. Ideally, flows should be measured with the same spatial detail as contractions or pressures in order to determine the relationship of flow to motor activity. Unfortunately, even in animals, this has not been possible due to the size of flow measurement devices relative to the gut. Some studies have attempted to use fluoroscopy to measure flows (section 6.7), however fluoroscopy can only adjudge the presence or absence of contents within the lumen at a given site, and cannot give velocity, volume or direction of flows with precision. Measurement of flows with good spatial resolution is likely to yield interesting information, as changes in flow characteristics over a short distance have already been shown to occur in the proximal duodenum (Malbert & Ruckebusch 1989).

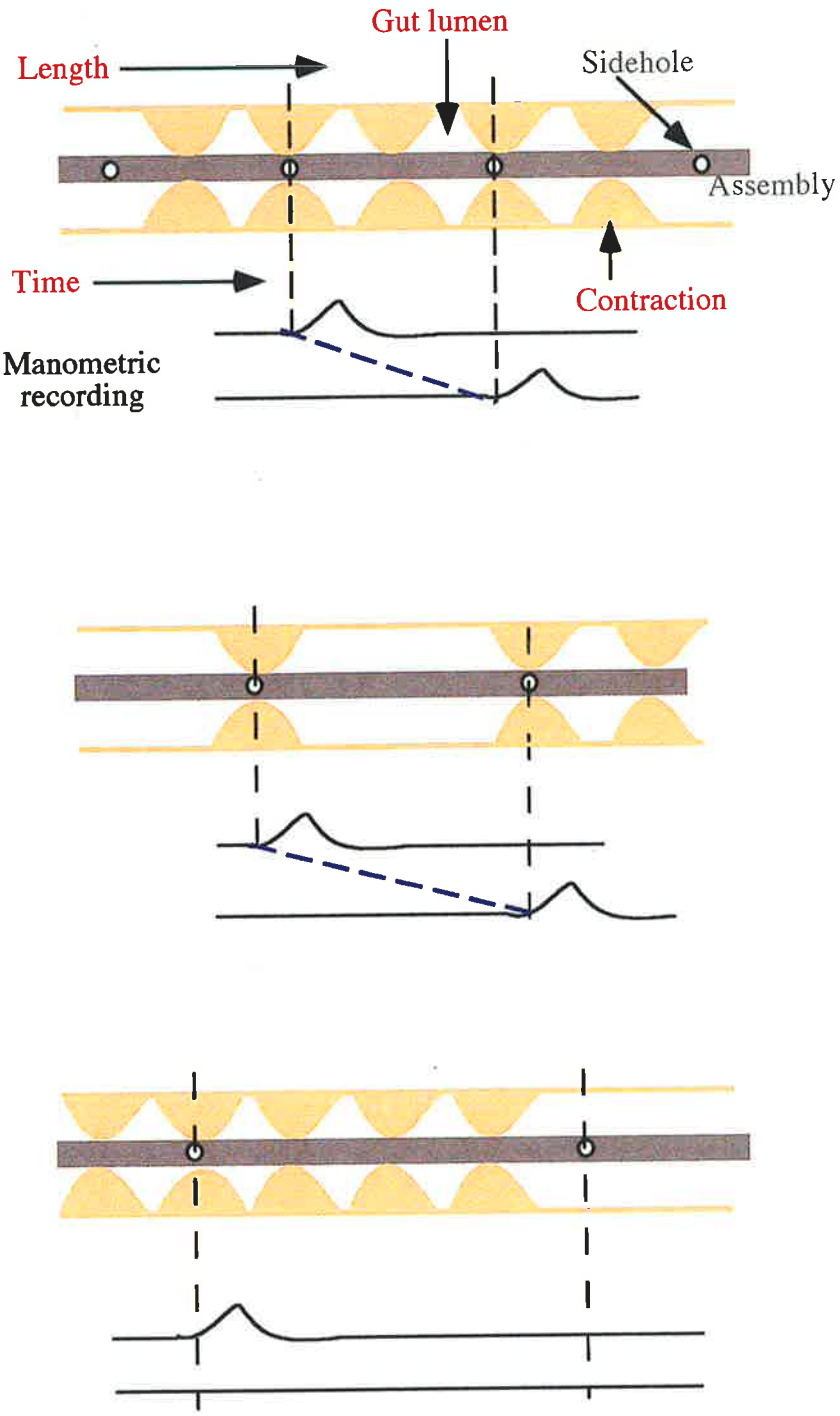


Figure 6.1
It can be appreciated that as a contraction (top) travels along a section of gut, it will only result in a measured pressure as it passes a sidehole. If the spacing of the sideholes is too wide this could lead to unrelated pressures being judged related (middle), or to a propagated pressure wave sequence (bottom) being judged as solitary (see 6.2.2.1).

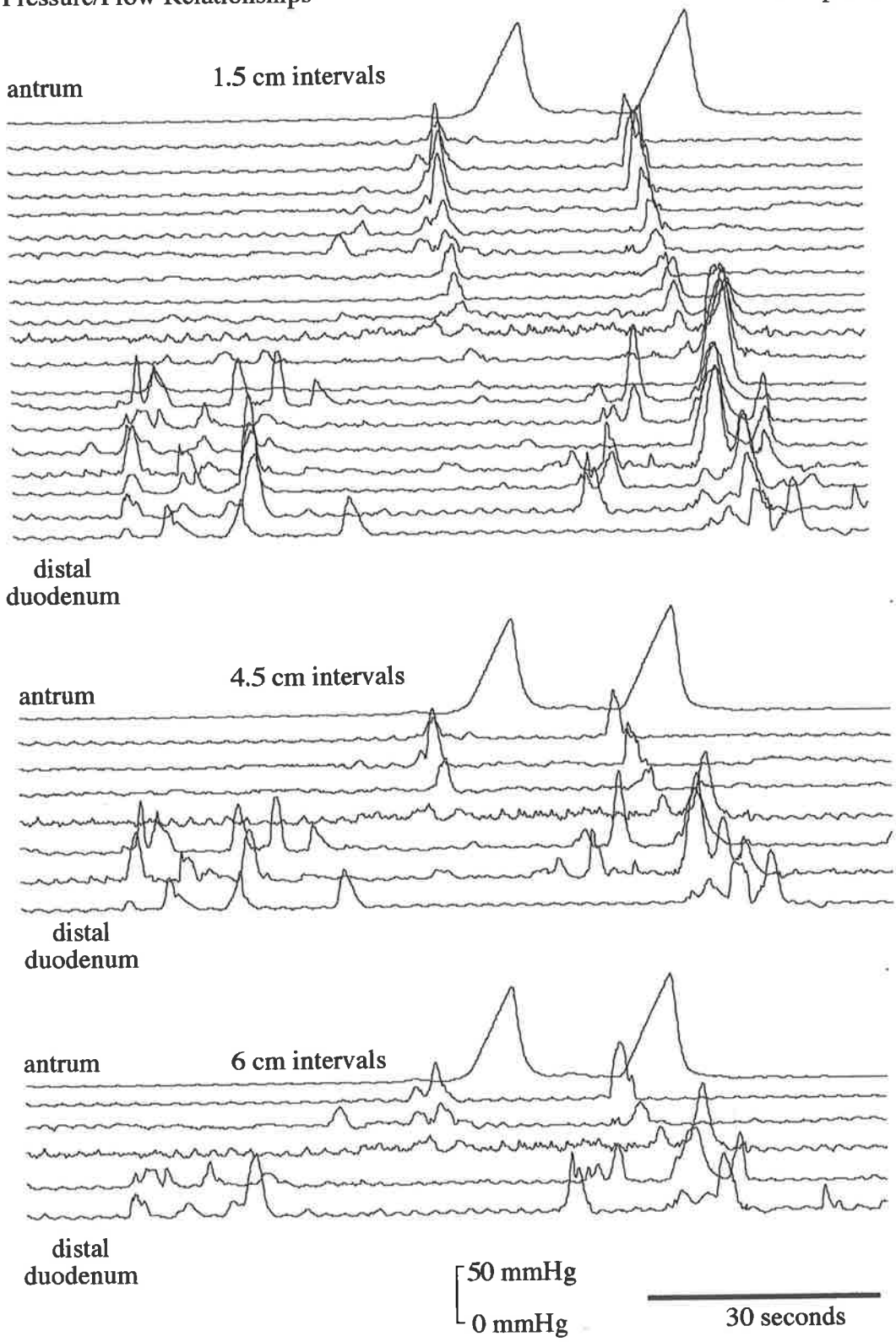


Figure 6.2
An example showing the loss of detail in spatial patterning of pressures as the interval between recording points is lengthened. Some short pressure wave sequences are no longer visible even at 4.5 cm intervals, and at 6 cm the spatial patterning of pressures is grossly simplified (see 6.2.2.1). Manometric pressures recorded from the length of the duodenum in humans (original data from the study described in Chapter 11).

6.3 PROXIMAL GASTRIC PRESSURE/FLOW RELATIONSHIPS

The proximal stomach subserves a reservoir function after eating and drinking and is thought to be important in the regulation of emptying of liquids (Hasler 1995). Despite its potential importance to flow, little is known about the relationships between individual motor events and flows in this region in humans. The proximal stomach has been shown to be important in regulating the rate of gastric emptying, as in its absence, contents empty more rapidly (Rees et al 1979b). A pressure differential between the proximal stomach and the duodenum has been hypothesised to drive emptying of liquids in particular (Hasler 1995), but this is unproven, given that emptying still occurs when there is no gastroduodenal pressure gradient (Miller et al 1981). In these studies however, the temporal resolution was low, the measurement of basal pressure probably insufficiently accurate, and no information is available regarding the relationship between individual pressure events and individual transpyloric flow pulses. Anvari et al (1995) studied intragastric pressures in pigs at higher temporal resolution. They found that elevation of pressure by several mmHg throughout the gastric lumen by a non-occlusive gastric contraction - a gastric common cavity pressure wave - was important in initiating episodes of transpyloric flow when the pylorus was open

Proximal gastric pressures can be measured with either point sensors (manometric sideholes or micro-balloons) or with a large, distending, non-focal balloon technique (sections 2.7.3.1 & 2). Neither technique is ideal however for assessing fundal motility, as the majority of fundal contractions do not produce any intraluminal pressure change, and therefore would be undetected with either a sidehole or a micro-balloon. When a larger balloon, which contacts the gastric wall, is used, changes in pressure are registered, because of changes in wall tension around the balloon. As this technique occupies a significant amount of intraluminal space it is not suited for use when attempting to concurrently assess local intraluminal flows (section 2.7.3.2). Attempts to examine flow in the region have used scintigraphy and magnetic resonance imaging MRI (Schwizer et al 1996). The temporospatial resolution of scintigraphy (section 2.7.3.1) is too low for it to be used to look at flow in real time. MRI does not actually measure proximal gastric flow, but volume. Sequential measurements are made, and flow is inferred to have occurred, from the difference between volumes over time. Again temporal resolution is an issue, as the instrument must scan in two different modes to generate the three dimensional image required for these calculations. MRI is able to track volume changes, and wall motion concurrently, but cannot give information on intraluminal pressures. Currently MRI technology is evolving rapidly,

and it is likely that these current processor-dependent limitations on temporal resolution will be overcome within a few years. Doppler ultrasound, which has been helpful in measuring flow in the antropyloric area (King et al 1984; Hausken et al 1992), is unable to visualise the fundus because of its subcostal position and gas content.

6.4 ANTRAL PRESSURE/FLOW RELATIONSHIPS

Antral pressures have usually been assessed with regard to their effect on flow at the pylorus, as it is transpyloric flow which is the final common pathway for gastric emptying. Whilst a substantial body of literature exists which attempts to correlate overall antral motility (expressed as a "motility index", see 6.2.2.1) with the rate of gastric emptying (see Rees et al 1979a for example); there is far less information on the relationship between individual antral pressure events and transpyloric flow.

Valuable insights into pressure/flow relationships have been gained from animals fitted with implanted electromagnetic flowmeters. In sheep, Malbert & Ruckebusch (1988) found second-by-second variations in transpyloric flow velocity at the level of the duodenal bulb to be primarily related to the strength (amplitude) of antral contractions assessed by strain gauges. In a further study, in dogs, the same authors found the occurrence of flow pulses just distal to the pylorus to be temporally associated with antral contractile activity, but the volume of each flow pulse was not related to contractile strength (again assessed by strain gauges) (Malbert & Ruckebusch 1989). Of note, in this study, bi-directional flow was recorded in the juxtapyloric region. The amount of retrograde flow was inconsequential in the fasting state, but postprandially it comprised up to a third of the volume of each aboral flow pulse (Malbert & Ruckebusch 1989). Treacy et al (1996) showed that, in pigs, decreased gastric outflow was associated with decreased numbers of antropyloric pressure waves (APWs) and increased numbers of isolated pyloric pressure waves (IPPWs); and, in support of a discrete role for APWs in gastric emptying, the decrease in APWs still resulted in an inhibition of gastric outflow in the absence of IPPWs.

The importance of the spatiotemporal patterning of pressure wave sequences of the distal stomach in determining transpyloric flow patterns is emphasised by the findings of Anvari et al (1995). In pigs, they found the volume of each transpyloric flow pulse to be dependent on the time interval between the onsets of a gastric common cavity pressure wave and the next lumen occlusive pyloric pressure wave. This time interval appeared to be controlled by antral intramural nerves, as antral transection did not alter

the number of pressure wave sequences (or the number of flow pulses), but decreased the interval between the onsets of increased intragastric pressure and pyloric occlusion, and thus shortened the time over which flow occurred, thereby reducing its volume.

Concurrent manometry and fluoroscopy in humans has shown that lumen-occlusive distal antral pressure waves participated in the pumping of a non-nutrient liquid through an open pylorus (Tougas et al 1992). This is in agreement with human ultrasound data from Hausken et al (1992) who found pulses of transpyloric flow of a low nutrient liquid to occur with each episode of propagated wall motion visualised in the distal antrum. Antegrade flow commenced within ~5 seconds of the onset of the episode of propagated wall motion, lasted for ~8 seconds and was followed by retrograde flow for ~2 seconds, prior to the next episode of propagated wall motion. When lumen occlusion occurred in the distal antrum before contents were expelled through the pylorus, there was retropulsion within the stomach (Hausken et al 1992). King et al (1984; 1985 & 1988) have also used ultrasound to examine the relationship of transpyloric flow to antral and duodenal contractions. In their first study (King et al 1984), flow of dilute orange juice was intermittent and bi-directional. In their second study (King et al 1985), they also described episodes of propagated wall motion, which were generally associated with pyloric closure at their temporal midpoint, and 67% of these episodes were also associated with proximal duodenal contraction. The onset of duodenal contractions was from ~1 second before to ~2 seconds after pyloric closure. Unfortunately, in this study flow was not evaluated concurrently; but the description of repetitive episodes of propagated wall motion and their relationship to both pyloric and proximal duodenal contractions is entirely consistent with that of Hausken et al (1992) (above). Given these similarities, one is likely to be safe in assuming similar flow characteristics to those described by Hausken et al (1992). In a further study, King et al (1988) examined a range of liquids, and found that the duration of transpyloric flow was reduced for nutrient liquids. Although the mechanism for this was not evaluated, it is likely that this was a consequence of alteration in the sequencing of lumen occlusion via nutrient mediated feedback from the small intestine (section 1.3.1 & 4.2.1.2).

During direct observation of the human pylorus at endoscopy, the antrum and pylorus appeared to contract in sequence (Aste et al 1979). Antral contractions are thus ideally sited (in time and space) to either retard or enhance transpyloric flow; and other factors which modify gastric emptying may do so partly via their influence on the sequencing of distal antral contractions relative to pyloric opening/closure (see 6.5 below). The

motor activity in the fundus also interacts with that of the antropyloric region by causing common cavity pressure increases as a result of vigorous phasic contractions, and by acting as a reservoir that primes the antrum with gastric contents. It should be noted, that in the studies discussed above, the role of the proximal stomach was not evaluated. Unfortunately, little is known about how the motor function of these two regions inter-relate, as there are considerable technical demands in achieving detailed concurrent measurements of all the motor components of the stomach and pylorus.

6.5 PYLORIC PRESSURE/FLOW RELATIONSHIPS

Direct observation of the pylorus at endoscopy showed that pyloric closure occurred for only a few seconds at the end of ~ one third of distal antral contraction wave sequences. It was therefore suggested that the pylorus did not act as a true sphincter (Aste et al 1979). However these subjects were fasting. When nutrients are present in the small intestine, the pylorus can and does act as a sphincter separating the distal antrum from the duodenum (Tougas et al 1992; Heddle et al 1988b).

Transpyloric flow is pulsatile and bi-directional (Malbert & Ruckebusch 1989, Hausken et al 1992), and can occur as a result of a relatively small pressure gradient. Flow does not occur across the pylorus when it is closed continuously by pyloric tone and IPPWs (Tougas et al 1992) even in the face of a positive gastroduodenal pressure gradient (Treacy et al 1994), and does not necessarily occur when it is open. Thus, it has been easier to describe the pressure correlates of no flow at the level of the pylorus, ie. that when a non-propagating tonic and/or phasic pressure of greater than 4 mmHg is present at the pylorus, it is closed, and flow does not occur (Tougas et al 1992). Prior to the development of the manometric sleeve sensor it was difficult to demonstrate these pyloric pressures as they occur in a quite narrow (< 6 mm) zone of closure (Heddle et al 1988b), older studies using only sideholes and a "pull-through" technique were not routinely able to demonstrate this high pressure zone (discussed in Rees et al 1979a; Heddle et al 1988b & Tougas et al 1992).

Because the occurrence of flow at the pylorus is intimately related to pressure events in the distal antrum and proximal duodenum, its pressure-flow relationships are further considered in sections 6.4 and 6.6.

6.6 DUODENAL PRESSURE/FLOW RELATIONSHIPS

The practical restriction in the use of certain measurement techniques in humans means that much of the available information comes from animal studies. These sources are, at best, probably only indicative of patterns of function in the human duodenum. Like gastric outflow (see 6.4 & 6.5 above), duodenal flow is intermittent, however, flow pulses occur more frequently and are of smaller volume (Malbert & Ruckebusch 1989; Schulze-Delrieu 1992). Schulze-Delrieu (1992), observed isolated guinea pig duodenum directly, and found the motor pattern responded to load and flow variations in such a way that luminal clearance was maintained. Clearance was achieved by both antegrade and retrograde displacement of contents from the contraction site, thus the temporospatial sequencing of contractions along a length of gut determined net movement of contents. The contention that the temporospatial relationship between contractions/pressures is important in determining flow patterns, is further supported by Keinke and Ehrlein (1983) who observed flow fluoroscopically in dogs, and found that a higher incidence of "segmenting" contractions (measured by strain gauges) led to a slower rate of flow. Importantly, they were only able to determine which contractions were segmenting by their spatial relationship to other contractions by the use of closely spaced strain gauges.

Pressures or contractions in the proximal duodenum may directly influence transpyloric flow. Ultrasound studies in humans have shown that contractions in the duodenal bulb are associated with reflux across the pylorus, and raise the possibility that these duodenal bulb contractions start more distally and propagate in a retrograde direction (King et al 1984 & 1985; Hausken et al 1992). Shirazi et al (1988), have examined the effects of the duodenum on gastric emptying in cats. They calculated that the duodenum generates resistance to gastric emptying, beyond that of the pylorus, predominantly by reduction of stroke volume. It has, however, been difficult to define the motor correlates of this resistance on a second-by-second basis. Intraduodenal acids, hyperosmolar solutions and nutrient-containing solutions all slow gastric emptying, but as shown by Rao et al (1996a) these diverse solutions each cause a different pattern of duodenal pressures. In a related study, Rao et al (1996b) demonstrated that duodenal tone, as indicated by diameter, may also play a role in retardation of flow. In particular, they have shown that diverse solutions, which have a common effect in slowing gastric emptying, cause increased duodenal resistance by differing means; lipid produced fewer phasic pressures with a large luminal diameter and delayed clearance, whilst acid and 5% saline led to frequent, high amplitude phasic pressures and a closed lumen.

Apart from their influence on gastric emptying, duodenal pressures also determine flow characteristics within the duodenum itself, although this is less well studied. Some limited data exist in which duodenal flows and pressures, or movement of contents, have been found to interrelate. A combination of concurrent high resolution manometry and scintigraphy led Samsom et al (1999) to conclude that the transit of chyme through the duodenum and proximal small intestine in humans is related to the number of propagated pressure wave sequences. However, because of the limitations in temporospatial resolution of the scintigraphic technique, discrete episodes of flow cannot be related to individual pressure wave sequences, nor can the duodenum be distinguished reliably from the small intestine. Simultaneous manometric and videofluoroscopic studies in humans have categorised duodenal manometric pressure wave sequences as "stationary" (mixing), "antegrade" or "retrograde" (Borgstrom and Arborelius 1975a & 1978). The reliability of these manometric classifications must be considered relatively poor as these conclusions are based on only 4 recording points spaced at 3 cm intervals in the proximal half of the duodenum.

6.7 SMALL INTESTINAL PRESSURE/FLOW RELATIONSHIPS

A clear relationship between pressures propagated in a retrograde direction and orad intraluminal flow has been demonstrated in the small intestine of dogs. Studies have shown pressures/contractions travelling in an orad direction to precede emesis, with transport of intestinal contents in an orad direction (Ehrlein 1981; Furukawa & Hatano 1998). In somewhat more detailed studies, in which flow was observed fluoroscopically whilst motility was measured with closely spaced strain gauges in the jejunum of dogs, Schemann & Ehrlein (1986) found that net transit of contents slowed as the number of stationary (non-propagated) contractions increased. In addition, the number of contractions and length over which they propagated both decreased in response to nutrient-containing meals when compared to a non-caloric cellulose meal. Moreover they found that length of propagation of a contraction was the most important factor influencing transit. The same conclusions were reached for the canine ileum (Siegle and Ehrlein 1988). Using the same methodology in canine small intestine Ehrlein et al (1987) recognised several discrete patterns of contractions and their flow correlates. In each of these studies, the high temporospatial resolution used for measurement of both contractions and flows was essential for accurate recognition of individual contraction/flow events and their outcomes.

6.8 DESIRABLE CHARACTERISTICS FOR AN INSTRUMENT TO MEASURE INTRALUMINAL FLOWS

Ideally the instrument would be sensitive and specific for flow detection, have temporal resolution of <1 s, and be able to measure flow direction, velocity and volume. In order to be of use in humans it would also need to be safe, relatively small and adequately tolerated. Further desirable characteristics include the ability to measure flow at multiple points concurrently, and suitability for simultaneous use with other measures of motility - to enable precise correlations between pressures and flows. Chapter 12 describes the development, and initial validation studies, of a prototype instrument aimed at fulfilling the most important of these criteria.

6.9 SUMMARY

There is a paucity of information on the precise relationship between flows and contractions and/or pressures in the human gut. In order to accurately determine the relationship between pressures and flow along a segment of gut, temporospatial sequencing of pressures and flows must be recorded at closer intervals than has hitherto been standard in human studies. The detailed information gained from high resolution of pressures is likely to significantly add to our understanding of the hydraulic function of the duodenum and small intestine. A technique for measurement of intraluminal flow in humans is also necessary to define these relationships. Therefore, to pursue these aims, high resolution pressure recording from the human duodenum is the subject of study in Chapter 11; and a prototype laser-Doppler velocimeter designed for measurement of intraluminal flow in humans is described in Chapter 12.

CHAPTER 7

Methodologies Used in Multiple Studies.

7.1 INTRODUCTION

Several methodologies used in the studies contained in this thesis were already established in the motility laboratory at the Royal Adelaide Hospital. These are each described in section 7.2, and include; nasoenteric tube insertion, water perfused manometry, continuous measurement of transmucosal potential difference, use of a gastric barostat, measurement of blood glucose and insulin, intraduodenal (ID) nutrient infusions and assessment of appetite/sensation with visual analogue scales. Except where otherwise specified, standardised methods were used for recording, scoring and analysis of the intraluminal pressures in these studies; as described in section 7.3.

All human subjects gave written informed consent, and each protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital. The protocols for the animal studies contained in Chapter 12 were approved by the Animal Ethics Committee of the Institute for Medical and Veterinary Studies (IMVS), Adelaide.

Methodologies unique to a particular study are described within the chapter addressing the relevant study.

7.2 ESTABLISHED METHODS

7.2.1 Nasoenteric tube insertion

The manometric assembly is passed into the stomach through an anaesthetised nostril, the tip of the assembly being allowed to pass into the duodenum by peristalsis. The correct position of the sleeve is verified by measurement of the antroduodenal transmucosal potential difference (TMPD) gradient across the pylorus (Heddle et al 1988b) (see 7.2.3).

7.2.2 Water perfused manometry

All manometric assemblies were multilumen silicone rubber devices from *Dentsleeve* (Bowden, SA, Australia). In the studies concerned with antropyloric motility (chapter 8B, 8C, 9A), an 11 lumen sleeve/sidehole assembly was used. The 4.5 cm long sleeve (channel 7) was positioned across the pylorus using transmucosal potential difference (TMPD), as described below (7.2.3) (Heddle et al 1988b). Two sideholes located on the back of the sleeve 1.5 and 3 cm from its orad end (channels 8 and 9) were designated P1 and P2 respectively. Five antral channels (channels 1-5), were positioned proximal to the sleeve at 1.5 cm intervals. Channels 6 and 10 were positioned at either end of the sleeve, and perfused with saline for measurement of TMPD (7.2.3). ID nutrient infusions were administered through the remaining channel, 10 cm distal to the pylorus.

Assemblies were perfused with degassed, distilled water (or saline) at rates sufficient to yield pressure rise rates of greater than or equal to 150 mmHg, using pressure

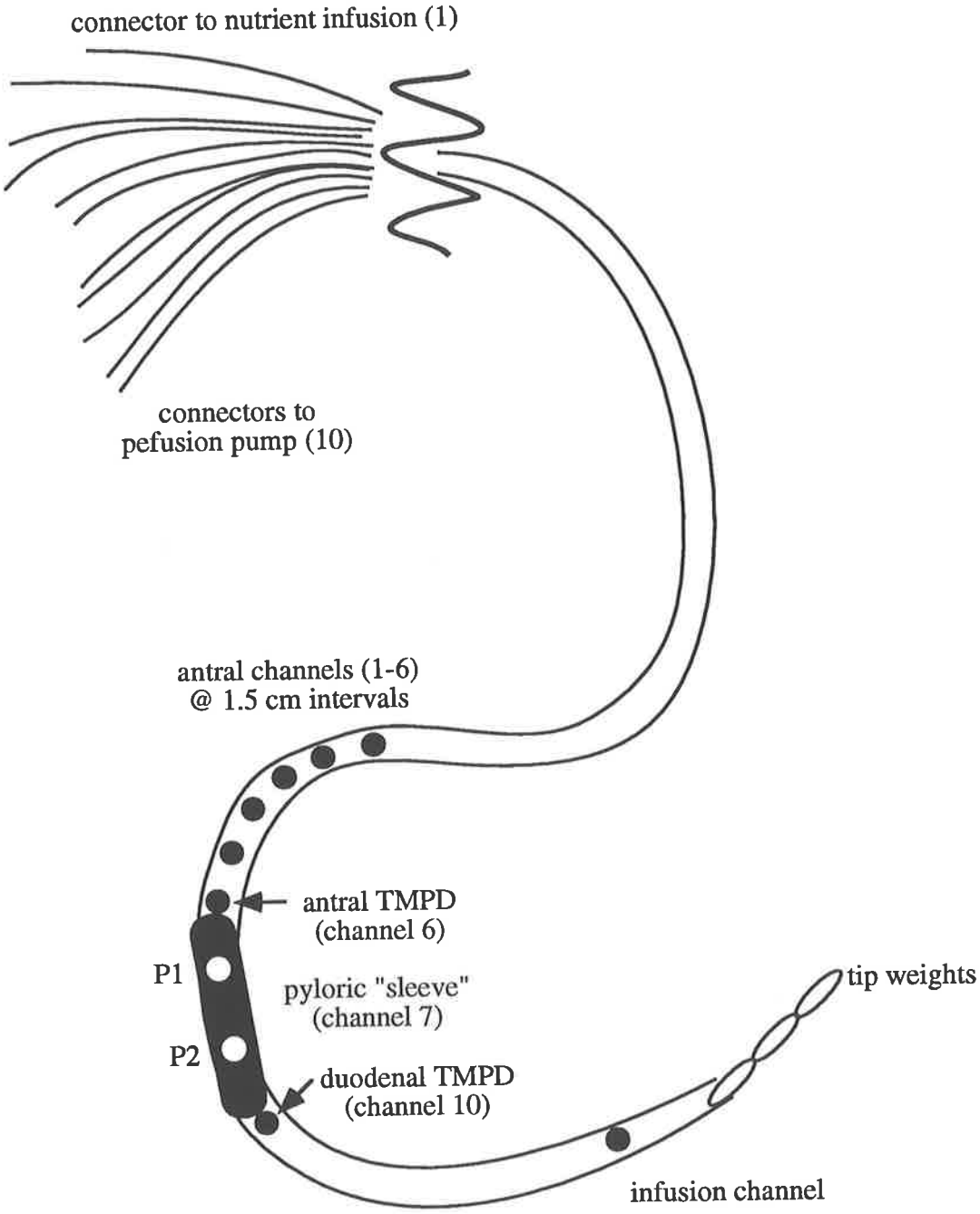


Figure 7.1
The manometric assembly referred to in 7.2.2 is shown, see text for detail.

driven fluid reservoirs and hydraulic resistors selected to give the rate of perfusion which delivered the desired rise rate.

7.2.3 Monitoring of transmucosal potential difference (TMPD)

Two lumina of the manometric assembly were perfused with saline from separate reservoirs, and connected via KCl-agar bridges to separate Calomel cells. A third Calomel cell acted as a reference. A small gauge saline-filled cannula was inserted into the subject's forearm and connected via an KCl-agar bridge to the reference cell. The transmucosal potential difference (TMPD) could thus be measured at two sites along the assembly. It was continuously measured in millivolts and logged to computer concurrently with the manometric pressures. In this fashion the characteristic gastroduodenal gradient in TMPD could be observed and used to maintain the correct assembly position continuously. When positioning each assembly, initially the two most distal sideholes of the assembly were perfused with saline and used to record TMPD. When these were both noted to be in the duodenum according to established criteria (Heddle et al 1988b), a more proximal sidehole was used to record TMPD so that the progressive passage of the assembly across the gastroduodenal junction could be monitored. TMPD is thus used to position the assembly precisely and maintain its position during each study.

7.2.4 Gastric barostat

An electronic barostat (Distender Series II™, G & J Electronics, Ontario, Canada), containing a rigid cylinder with an internal moveable piston of 1200 ml maximum displacement, was used to perform the gastric distensions. The barostat was connected via a polyvinyl chloride catheter assembly (750 mm long, internal diameter 2.5 mm) to a sealed polyethylene bag. The volume of the bag was changed at a rate of 33 ml/sec. The intrabag pressure, measured by a pressure sensor in the electronic barostat, was monitored via a separate channel (internal diameter 0.97 mm). At maximal inflation, the shape of the bag was approximately spherical and its volume ~1000 ml. At the volume range used (0 -800 ml) in the study (Chapter 9B) the bag had an extremely high compliance (> 4000 ml/mmHg) (Hebbard et al 1996b).

The catheter assembly also contained two manometric sideholes (internal diameter 0.5 mm), positioned 25 and 75 mm above the barostat bag, which were connected to a low-compliance manometric perfusion pump. These pressure recordings were used to

the position the intragastric bag so that the point of respiratory reversal at the diaphragm was located between these two manometric sideholes.

Data from the barostat (sampled at 1 Hz) were recorded on a Powermac 7100 computer (Apple Computer Inc., Cupertino, CA, USA), using custom-written data-acquisition software (Labview, National Instruments Corporation, Austin, TX, USA). This software also programmed the barostat to perform distensions in stepwise volume (isovolumetric distensions) or pressure (isobaric distensions) increments.

Data were imported into a display and analysis program (Acqknowledge, Biopac Systems, Goleta, CA, USA). The mean volume and pressure were determined during the last 2 minutes of each distension step, after at least 1 minute for equilibration.

7.2.5 Blood glucose & insulin measurement

In studies requiring glucose and insulin measurement, venous blood was taken via an intravenous cannula in the forearm or cubital fossa concurrently with the visual analogue scales (VAS) (see 7.2.7). Arterialisation of blood was achieved by wrapping the forearm in a heated pad. Plasma glucose was assessed with a glucometer (MediSense 2, Balwyn, Vic, Australia) at the bedside and the accuracy of this was later verified by comparison with laboratory measurements using the hexokinase enzymatic reagent (Trace Scientific Pty. Ltd., Baulkham Hills, New South Wales, Australia). Plasma insulin was measured by radioimmunoassay (Phadesph Insulin RIA, Pharmacia Diagnostics, Sweden).

7.2.6 Intraduodenal (ID) nutrient infusion

In the studies addressing appetite and pyloric motility (chapters 8A, 8B, 8C, 9A), intraduodenal (ID) nutrient infusions (glucose or lipid) were administered through a channel 10 cm distal to the pylorus. In the study addressing high resolution measurement of duodenal motility (Chapter 11), the ID lipid infusion was given into the first 4.5 cm of the duodenum.

7.2.7 Visual analogue scales (VAS's)

Appetite was assessed using previously described 100 mm linear VAS's (Sepple & Read 1989). Fullness, hunger, projected quantity of consumption and desire to eat were quantified. Subjects were familiarised with these scales at the commencement of each study day and instructed to make a single mark on the VAS corresponding to their own assessment of their current feelings. The -5 min and 0 min values were averaged to provide a baseline and the change in ratings from baseline during the study measured. Where two conditions were studied on the one day, a second baseline was calculated in the five minutes prior to each condition examined.

7.3 SHARED MANOMETRIC RECORDING AND ANALYSIS TECHNIQUES

7.3.1 Recordings

Except where otherwise specified, a computer based recording system was used (Powermac 7100/75, Apple Computer, Cupertino, CA), running software (MAD) written by Prof C Malbert (Rennes, France) in Labview 3.0.1 (National Instruments). Manometric pressures were digitised with an NBM1016H data acquisition board and then recorded direct to disk at a frequency of 10 Hz for later analysis.

7.3.2 Scoring of Pressure Events

7.3.2.1 Isolated Pyloric Pressure Waves (IPPWs)

An IPPW was defined as a pressure wave of greater than 10 mmHg recorded by the sleeve sensor, with or without a concurrent pressure wave in either P1 or P2 (see 7.2.2), but not in both, in the absence of an antral or duodenal pressure wave with an onset within 5 seconds of the IPPW onset. The frequency (in ten minute segments) and amplitude of isolated pyloric pressure waves (IPPW's) were noted. IPPWs were manually identified and scored.

7.3.2.2 Antral Pressure Waves (APWs)

An antral pressure wave was defined as a pressure increase over baseline of 10 mmHg or greater in any antral channel (channels 1 - 6, for the assemblies described in 7.2.2). These were scored manually.

7.3.2.3 Pyloric Tone

Pyloric tone was calculated each minute by in-house software (MAD, Dr C Malbert, Rennes, France). The mean pressure (excluding phasic pressure waves) in the antral channel 1.5 cm proximal to the sleeve sensor was subtracted from the mean pressure in the sleeve channel. Tone was then averaged over five minute blocks. Five minute blocks were only included in the analysis if the sleeve was correctly positioned for three or more minutes of the five. The mean pyloric tone in the five minutes prior to commencing the test intervention was taken as the basal pyloric tone and, in experiments with more than one intervention examined, tone during the five minutes prior to commencing each subsequent intervention was taken as the basal pyloric tone for that segment of the study. Pyloric tone is presented as change from baseline.

7.3.3 Statistical Analysis

Except where otherwise noted, where different conditions were compared, each subject acted as their own control. Data analysis was performed under the guidance of a biostatistician. Differences over time and between conditions were examined by tests for fixed effects, with mixed model, repeated measures analysis of variance (SAS Institute Incorporated, Carey, Nth Carolina). The total numbers of APWs and IPPWs, were compared using a two-tailed paired Students' t-test. Data are shown as mean ± SEM. In all analyses, a P value of < 0.05 was regarded as significant.

CHAPTER 8

Effects of Intraduodenal Nutrients on Appetite and Antropyloric Motility: Role of Gastrointestinal Hormones, Nutrient Class, Dietary Adaptation and Ageing

Appetite Suppression By Intraduodenal Nutrients

Dietary Glucose Supplementation

Appetite Regulation In The Elderly

8.1 INTRODUCTION

The presence of nutrients in the gastrointestinal tract suppresses appetite and alters gastroduodenal motor and sensory function (Chapters 1 & 4). In this chapter three closely related studies which examine the effect of small intestinal nutrients on gastroduodenal motor and sensory function and appetite are presented. To facilitate interpretation, each study is presented separately and hence there is some overlap in the discussion of each study, although this has been minimised.

The first study (8A) was performed to evaluate the means by which intraduodenal (ID) glucose suppresses appetite. In an attempt to exclude insulin, or changes in blood glucose concentration as satiety factors (sections 1.3.2.2 & 1.3.2.3), subjects were studied using a hyperinsulinaemic, euglycaemic clamp technique. To determine whether gastrointestinal peptides other than insulin (section 1.3.2) are likely mediators of appetite suppression by ID glucose, their release in response to ID glucose infusion was prevented by co-administration of octreotide (a somatostatin analogue) on one study day.

The second study (8B) addressed nutrient-specificity of the effects of small intestinal nutrients on appetite (section 1.3.1) and antropyloric motility (section 4.2.3) by comparing the effects of isocaloric ID infusions of fat (lipid emulsion) and carbohydrate (glucose). The impact of dietary modification on appetite and antropyloric motility (section 1.3.1 & 4.2.4) was then evaluated by repeating the ID infusions after a week of dietary glucose supplementation.

Study 8C examined the hypothesis that ageing modifies the effects of intraduodenal nutrients on appetite and antropyloric motility. Specifically, appetite and antropyloric

motility in response to the same ID infusions were evaluated in healthy elderly and young subjects.

CHAPTER 8A

The Interaction of Insulin, GLP-1, GIP and Appetite in Response to Intraduodenal Carbohydrate

8A.1 INTRODUCTION

As discussed in Chapter 1, small intestinal nutrients suppress appetite and decrease intake (1.3.1). Several different mechanisms for these effects have been proposed (1.3 & 1.4), but the role of several of these factors is unclear due to the cascade of potential mediators which are released consequent on small intestinal nutrient exposure. In particular, since insulin concentrations rise after eating (in a time frame corresponding to the reduction in appetite), it has been implicated as a satiety factor (1.3.2.3), as have other gut hormones such as GLP-1 and GIP, released in response to food (1.3.2.4).

This study was performed to examine the relationships among hyperinsulinaemia, other putative gastrointestinal satiety hormones, and short-term satiety caused by small intestinal carbohydrate. Eight fasted, healthy male volunteers received intraduodenal (ID) infusions of either glucose (two days) or saline (one day) under hyperinsulinaemic and euglycaemic conditions in random order. Insulin, glucose, GIP, GLP-1 and appetite ratings were measured during, and food intake after the nutrient infusions. On one of the ID glucose days, intravenous octreotide was administered to prevent the release of endogenous gastrointestinal hormones.

8A.2 METHODS

8A.2.1 Subjects

Eight healthy male volunteers (aged 19 to 33 years), each with normal body mass index (BMI) (range 20 to 25) were recruited by advertisement. Each scored less than 8 (2.5 ± 0.7; mean $\pm$ SEM) on the restraint factor of the Eating Inventory Questionnaire (Stunkard & Messick 1985), indicating that they were not restrained eaters. All were non smokers and none was taking medication.

8A.2.2 Experimental design

Three studies were carried out in the subjects in random order and single-blind fashion. Each experiment was separated by at least 5 days (range 5 to 36 days, median = 8 days). Volunteers were instructed to consume a weight-maintaining diet containing at least 200g/day of carbohydrate for 3 days before each experiment. They were also instructed not to consume alcohol or indulge in strenuous exercise for 24 hours prior to each experiment. Subjects fasted from 8.00 PM on the night before each study and attended the Department of Medicine at 8.00 am.

All three studies were carried out under hyperinsulinaemic conditions designed to yield plasma insulin concentrations of ~ 360 pmol/L using a primed, continuous infusion of insulin (De Fronzo et al 1979). Plasma glucose was maintained in the normal postprandial range 5.0-9.5 mmol/L, using IV infusion of glucose where necessary. In one study, glucose was infused into the duodenum and saline IV (ID Gluc). In the second, saline was infused intraduodenally and glucose IV (ID Sal) and, in the third, ID glucose was given whilst IV octreotide was infused (Gluc/Oct). The third protocol (Gluc/Oct) was conducted in 6 of the 8 subjects, who were selected at random.

The study protocol is summarised in figure 8A.1. After placement of the nasoenteric tube (section 7.2.1), two IV cannulae were inserted into forearm veins for infusion of insulin, potassium chloride (KCl) and glucose. A further IV cannula was inserted into the opposite hand for obtaining blood samples (t = -75 min). Subjects then rested quietly for 30 minutes. They were then (t = -45 min) asked to complete a visual analogue scale (VAS) (section 7.2.7) assessing hunger and fullness. VAS's were administered every 15 minutes thereafter throughout the study. After 5 min (t=-40), a 12 ml venous blood sample was obtained. Subsequently, 2 ml samples were taken every 5 min throughout the experiment for immediate measurement of blood glucose and 10 ml samples every 10 min for subsequent measurement of plasma glucose, insulin, C-peptide, GIP and GLP-1.

At t = -30 min, an IV infusion of insulin (Actrapid, NovoNordisk, Australia) was given at a rate to raise and maintain plasma insulin concentrations at ~360 pmol/L (Figure 8A.3). An IV infusion of 25% glucose was also commenced at a rate (between 25-130 ml/h) designed to maintain blood glucose in the euglycaemic range, and KCl was administered at 5 mmol/h (in 0.9% saline) to offset the intracellular transport of potassium induced by the high levels of insulin and glucose. During Gluc/Oct an IV infusion of octreotide (Sandostatin, Sandoz Pharma Ltd., Basle, Switzerland) (in

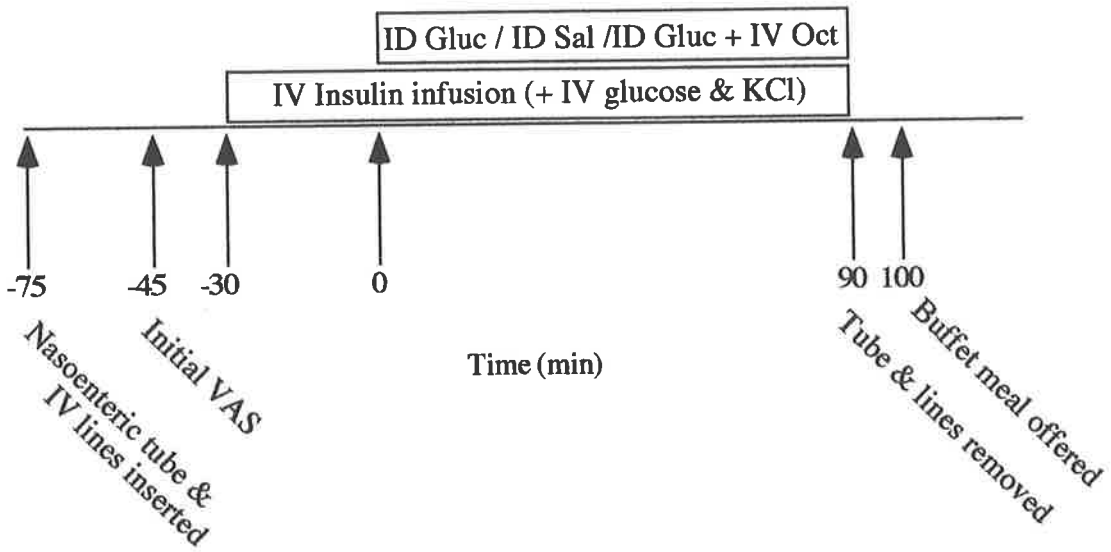


Figure 8A.1
Protocol showing experimental design for each of the three study days.
For further detail see text.

0.9% saline) was also commenced at a rate of 50 ug/h. At t = 0 min (30 min after hyperinsulinaemia was induced), an ID infusion of either 20% glucose (ID Gluc, Gluc/Oct) or 0.9% saline (ID Sal) was given at 4 ml/min (3.2 Kcal/min for glucose) and continued for 90 min. During ID Gluc, the IV infusion of glucose was stopped when blood glucose concentrations began to rise due to absorption of glucose from the small intestine. At t = 90 min all infusions were discontinued and the nasoenteric tube and IV lines removed.

Subjects were presented (t=100 min) with a cold, buffet style, meal (Table 1) prepared in excess of what they would normally be expected to eat and invited to eat as much as they wished. The meal consisted of a number of food and drink items, varying in macronutrient content, from which the subjects were free to select what they wished. The rate of ingestion and amount of food consumed was recorded. Energy and macronutrient intake was subsequently calculated using COMP-EAT diet analysis software (COMP-EAT, Lifeline Nutritional Services Ltd, London, UK). Subjects were allowed 30 minutes to consume their meal and, at this time, another venous blood sample was taken and a VAS administered. They remained in the laboratory for a further 30 minutes when a final blood sample was taken and VAS completed.

8A.2.3 Blood measurements

Blood glucose was measured immediately on the 2 ml blood samples using a portable blood glucose meter (MediSense Companion 2 Blood Glucose System, MediSense, Inc. Waltham, MA 02154). Plasma from the samples taken at 10 minute intervals was separated within 30 minutes of collection and stored at -70°C for later analysis. Plasma glucose was determined with the hexokinase enzymatic reagent (Trace Scientific Proprietary Ltd., Baulkham Hills, New South Wales, Australia) and plasma insulin by radioimmunoassay (Phadeseph Insulin RIA, Pharmacia Diagnostics, Uppsala, Sweden). The intra-assay coefficient of variation for the insulin assay was < 5%.

Blood samples for the measurement of GLP-1 and GIP were collected into chilled EDTA tubes containing 400 x 10^3 kallikrein inhibitory units of aprotinin/L of blood. GIP was measured using a previously described radioimmunoassay (Wishart et al 1992). GLP-1 7-36 was measured using an antibody provided by Professor SR Bloom (Hammersmith Hospital, London) which did not cross react with glucagon, GIP or other gut or pancreatic peptides (Kreymann et al 1987) The radioimmunoassay method used was an adaptation of that used by Orskov et al (1994). The interassay

Table 1: Energy and macronutrient content of test foods served as a lunch buffet[1]

Food	Weight	Energy	Fat	CHO[2]	Protein
	g	kJ	g	g	g
Sliced ham	100	502	5.1	0.0	18.4
Sliced chicken	100	665	9.0	4.0	15.0
Sliced cheese	100	1693	34.0	0.1	24.7
Whole-meal bread	228	2048	5.7	94.9	21.0
Tomatoes	100	71	0.3	3.1	0.7
Cucumber	100	42	0.1	1.5	0.7
Lettuce	100	59	0.5	1.7	0.8
Potato salad	250	1463	20.5	40.1	3.9
Bean salad	150	539	0.0	21.0	10.5
Mayonnaise	50	1513	39.7	0.1	1.0
Margarine	50	1526	40.5	0.1	0.0
Tomato sauce	50	205	0.0	1.1	12.0
Canned peaches	280	1020	0.0	64.1	1.1
Canned pears	280	903	0.0	56.0	1.1
Ice cream	200	1622	19.6	48.8	7.2
Choc. custard dessert	400	1585	12.0	61.6	10.4
Whole milk	300	828	11.7	14.4	9.6
Fresh orange juice	330	456	1.3	28.1	1.3
Total	-	16737	198.7	451.5	128.4

[1] Amounts given as served. [2] Carbohydrate

The total percentages of energy from fat, carbohydrate and protein were 44.7 %, 42.3 % and 12.8 % respectively.

coefficient of variation was 18%. ^{125}I-labelled GLP 7-36 was prepared by the lactoperoxidase method and purified by reverse phase high performance liquid chromatography on a 5 mm Techsil C_{18} column using a 120 minute 30-50% gradient of acetonitrile in water containing 0.1% TFA (Orskov et al 1994).

Prior to radioimmunoassay, plasma samples and standards (made up in serum treated by charcoal stripping) were extracted in 70% ethanol, dried down under nitrogen and resuspended to the original volume in assay buffer (0.1 M Phosphate buffer, pH 7.4, 1g/L human serum albumin, 0.6 mM Thiomerusal, 3.9 g/L, EDTA, 1.3g/L α-amino caproic acid). Aliquots of 100 µl of the reconstituted samples and standards were incubated for 48 hours with 100 µl diluted antiserum and 100 µl labelled peptide (~ 10,000 cpm). The bound fraction was separated from the free by the addition of dextran-coated charcoal containing gelatin (0.015g gelatin, 0.09g dextran, 0.15g charcoal in 30 ml assay buffer) and the radioactivity determined in the supernatant.

8A.2.4 Statistical analysis

Statistical analysis was performed using SPSS for Windows v.6.0 (SPSS Inc., USA). To determine whether the hyperinsulinaemic clamp technique itself had any effect on appetite ratings, one-way repeated-measures analysis of variance (ANOVA), with time as a main factor, was applied to ratings of hunger and fullness measured in the 45 min before ID infusions commenced. Because all 3 experimental conditions were the same up to this point, the data from the three studies were grouped for this analysis. Changes in hunger and fullness from baseline, during the ID infusions were evaluated using two-way repeated-measures ANOVA, with infusion type and time as main factors. The mean of two measurements taken for each condition, when insulin concentrations had been raised and stabilised (t = -15 & 0), were used as baselines. Differences in plasma glucose, insulin, GIP and GLP-1 levels during the ID infusions were evaluated using two-way repeated-measures analysis of covariance (ANCOVA). Plasma measurements taken immediately prior to the ID infusions were used as covariates. Following repeated-measures ANOVA, post hoc tests were carried out using Student's t-tests to compare data between two studies (ID Gluc vs ID Sal) and Tukey's procedure for comparisons involving 3 conditions (ID Gluc vs ID Sal vs Gluc/Oct). Differences in energy and macronutrient intake were tested using Student's t-test (ID Gluc vs ID Sal) or ANOVA followed by Tukey's procedure (ID Gluc vs ID Sal vs Gluc/Oct). As study C was conducted in 6 of the 8 subjects, the effects of ID

glucose during infusion of octreotide were only evaluated on the data for the 6 subjects who completed all three studies. Data are shown as mean ± SEM. A P value of < 0.05 was considered significant.

8A.3 RESULTS

8A.3.1 ID Glucose vs. ID Saline

8A.3.1.1 Plasma glucose, insulin, GLP-1 and GIP levels

Infusion of insulin alone had no effect on plasma GIP or GLP-1 (Fig 8A.2). Plasma glucose concentrations increased from 5.3 ± 0.1 mmol/L during the baseline period to 7.7 ± 0.4 mmol/L (ID Gluc) and 6.8 ± 0.3 mmol/L (ID Sal) during the ID infusions. Plasma glucose concentrations were higher during ID glucose compared with ID saline ($F_{[1,7]} = 9.00$, $P < 0.05$; Figure 8A.3). Plasma insulin concentrations were raised to 356.4 ± 4.8 pmol/L due to the IV infusion of insulin and further increased to 779.4 ± 114.0 pmol/L during ID glucose, so that insulin levels were higher during ID glucose than during ID saline ($F_{[1,7]} = 14.08$, $P < 0.01$; Figure 8A.3). Post hoc tests revealed that this increase occurred within 20 minutes of commencing the ID infusions.

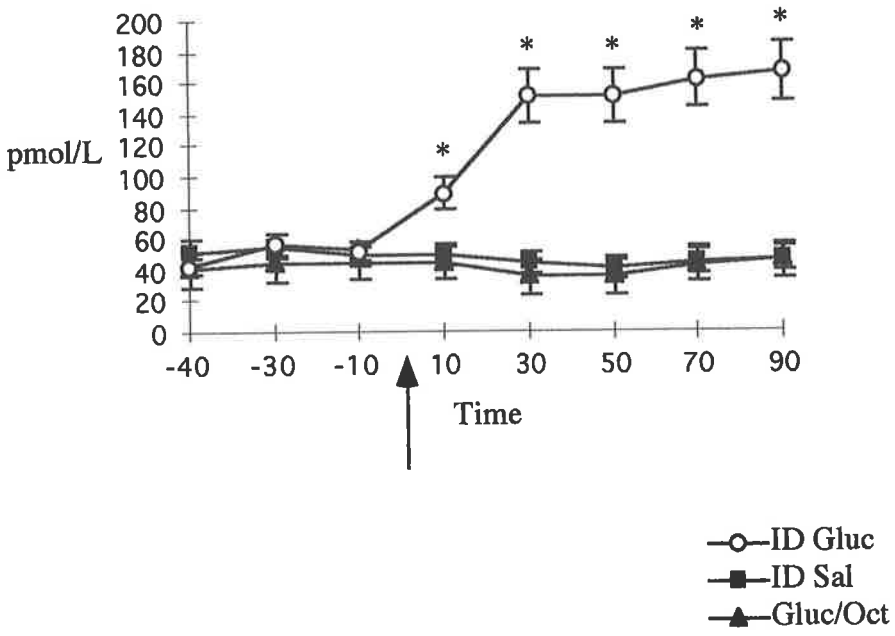
Plasma GLP-1 levels also increased during ID glucose infusion, but not during ID saline ($F_{[1,7]} = 24.10$, $P < 0.01$). This increase did not occur until 30 minutes after starting the ID infusions and levels continued to rise progressively throughout the 90 min infusion period. Plasma GIP levels also increased in response to ID glucose, but not ID saline infusion ($F_{[1,6]} = 31.03$, $P < 0.01$) and post hoc tests indicated that this increase was evident within the first 10 minutes of ID glucose, when there was a rapid rise before concentrations plateaued at ~30 minutes. (One subject had GIP levels > 2 standard deviations from the mean of the group at all times and his data was therefore excluded from this part of the analysis).

The total amount of energy received from ID and IV infusions of glucose was greater during ID glucose compared with ID saline (376 ± 14 vs. 262 ± 18 Kcal; $P < 0.001$).

8A.3.1.2 Appetite ratings

Raising plasma insulin levels with IV insulin had no effect on hunger ($F_{[3,63]} = 0.48$) or fullness ($F_{[3,63]} = 1.21$) before the ID infusions began (Figure 8A.4). Moreover, there

Plasma GIP



Plasma GLP-1

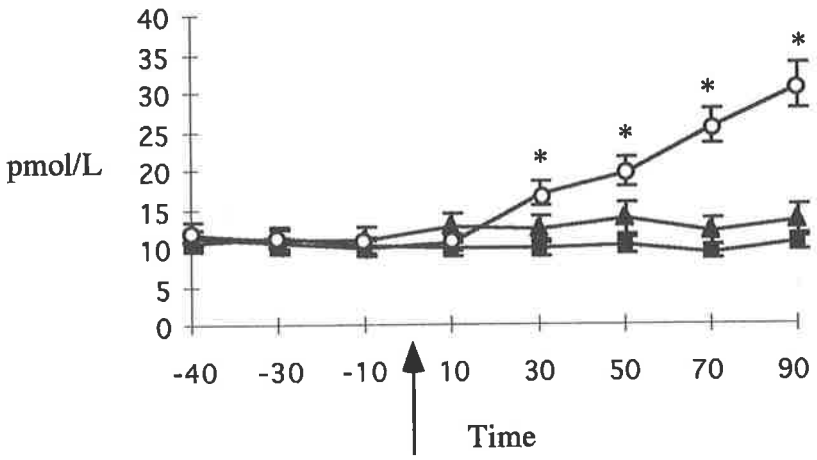


Figure 8A.2
Plasma GIP & GLP-1 (mean +/- SEM). The arrows (at t = 0) indicate the onset of ID glucose or saline infusions . Hyperinsulinaemia is present from t = -40. * significantly higher during ID Gluc than ID Sal and ID Gluc/Oct; $P < 0.05$ (Tukey's procedure). During ID Gluc and ID Sal n=8 for GLP-1, and n=7 for GIP; During ID Gluc/Oct n=6 for GLP-1, and n= 5 for GIP.

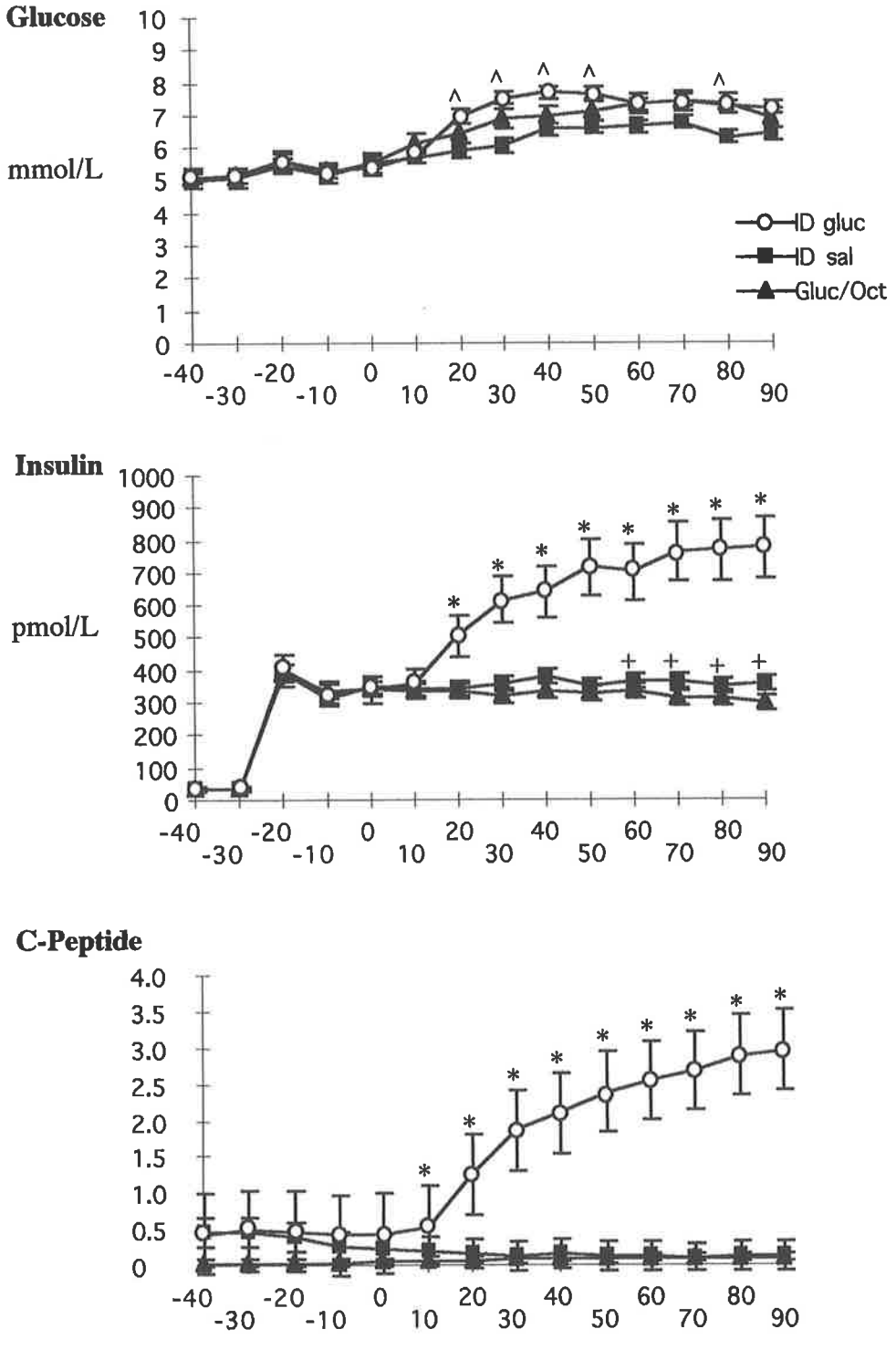


Figure 8A.3
Plasma glucose, insulin and C-peptide for the three study conditions, mean +/- SEM. For detail see text. ^ significantly higher during ID gluc vs ID sal; $P < 0.05$ (student's T-test). * significantly higher during ID gluc than during ID sal, and ID sal/Oct; $P < 0.05$ (Tukey's procedure). + significantly higher during ID sal vs ID gluc/Oct; $P < 0.05$ (Tukey's procedure). In ID gluc & ID sal, n=8; and during ID gluc/Oct, n=6.

were no changes from baseline in ratings of hunger ($F_{[9,63]}$ =0.98) or fullness ($F_{[9,63]}$ = 0.92) during ID saline studies.

Compared with ID saline, ID glucose infusion decreased hunger (condition x time interaction, $F_{[5,35]} = 4.33$, $P < 0.01$) and increased fullness (condition x time interaction, $F_{[5,35]} = 4.74$, $P < 0.01$). These effects became evident during the last 30 minutes of the ID infusions when there was a further decrease in hunger and increase in fullness during ID glucose, whereas during ID saline ratings began to return to baseline (Figure 8A.5). These changes resulted in a significant difference in fullness by 90 minutes ($P< 0.02$) and a trend for a difference in hunger at this time ($P = 0.08$).

8A.3.1.3 Energy and macronutrient intake

Energy intake from the test meal was less following infusion of ID glucose compared with ID saline ($t_{[7]}$ =3.58, $P < 0.01$). This was associated with a reduction of fat and protein intakes following ID glucose ($t_{[7]}$ =3.64, $P < 0.01$), and a trend towards a decreased intake of carbohydrate ($t_{[7]}$ =2.18, $P = 0.07$). There was no difference in the percentage of energy provided by the macronutrients between the two studies and no difference in the time spent eating (Table 2).

8A.3.2 Effect of octreotide on responses to ID glucose

8A.3.2.1 Plasma glucose, insulin, GLP-1 and GIP

Plasma glucose concentrations increased from 5.3 ± 0.1 mmol/L during the baseline period to 7.4 ± 0.2 mmol/L during ID glucose infusion with octreotide (Gluc/Oct), this was not significantly different from the plasma glucose response to ID glucose alone. (Figure 8A.3). Plasma insulin levels during ID infusions, however, did differ between the three studies ($F_{[2,10]} = 14.07$, $P < 0.001$). Post hoc tests indicated that the rise in plasma insulin during ID glucose was abolished by concurrent octreotide ($P < 0.05$ for all timepoints after $t = 20$; Figure 8A.3) and, in fact, plasma insulin concentrations were slightly lower during Gluc/Oct compared with ID saline ($P < 0.01$, from $t = 60$).

There were also significant differences in GIP and GLP-1 concentrations between the three studies (condition x time interactions, $F_{[10,40]} = 9.00$, $P < 0.001$ and $F_{[10,50]}$ =5.68, $P < 0.001$; for GIP and GLP-1 respectively; Figure 8A.2). Post hoc tests confirmed that IV octreotide prevented the increase in both GIP and GLP-1 which had

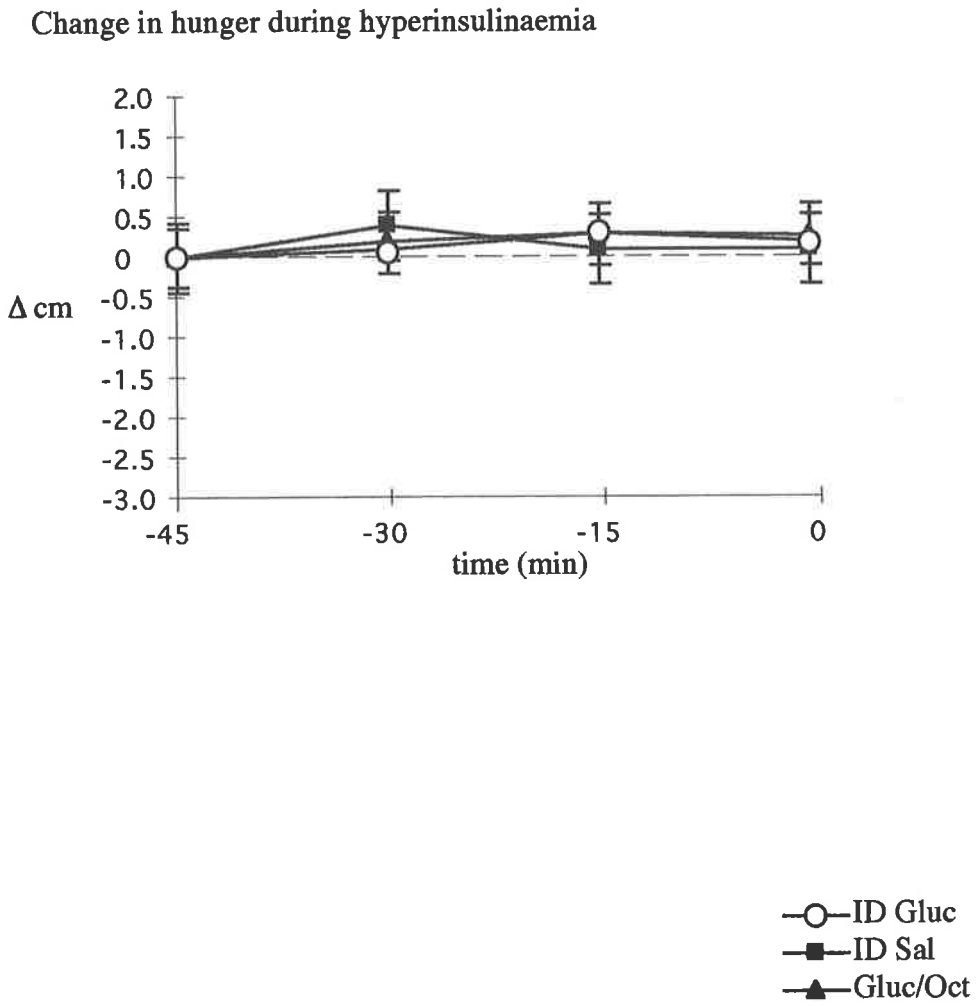


Figure 8A.4
Hunger ratings (mean +/- SEM) during the 45 minute basal period of induced hyperinsulinaemia, before commencing the ID infusions, are shown. There is no change in ratings attributable to hyperinsulinaemia.

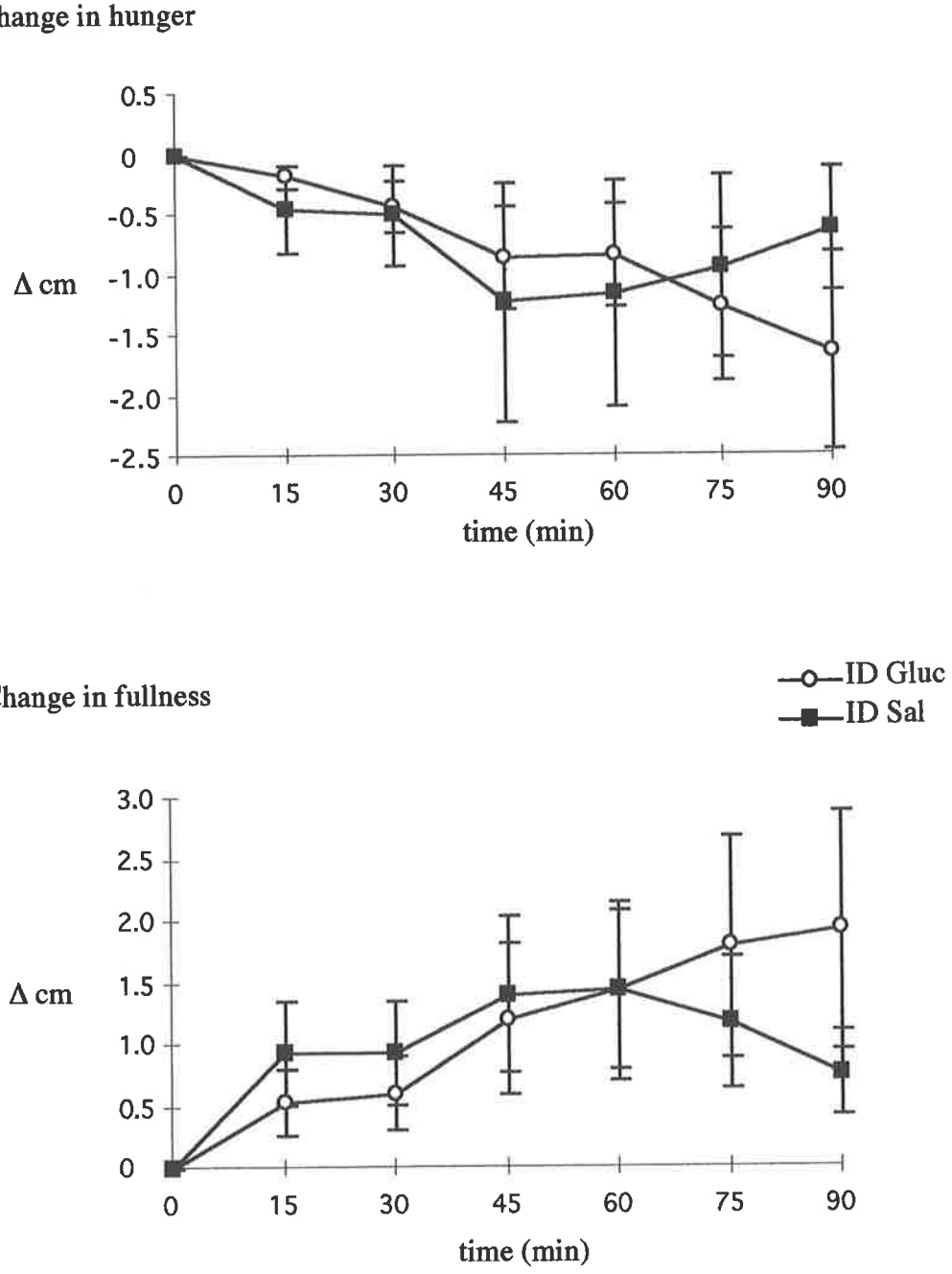


Figure 8A.5
Change in hunger and fullness (mean +/- SEM) during ID glucose and saline. Intraduodenal infusions commenced at t = 0. There was a decrease in hunger ($P < 0.01$), and an increase in fullness ($P < 0.01$) during ID Gluc vs ID Sal (condition x time interaction). n=8 in each study.

occurred during ID glucose ($P < 0.05$ for all time points from t =10 and $P < 0.05$ for all time points from t =30; for GIP and GLP-1 respectively), such that concentrations were no different than those during ID saline. IV Octreotide had no effect on basal (non-stimulated) levels of GIP or GLP-1. The total amount of energy provided from the ID and IV infusions of glucose was greater during Gluc/Oct (469 ± 27 Kcal) compared with ID Gluc (380 ± 18; $P < 0.05$) and ID Sal (259 ± 24; $P < 0.001$).

8A.3.2.2 Appetite ratings

There was a significant effect of the ID infusions on ratings of hunger (condition x time interaction, $F_{[10,50]} = 1.96$, $P < 0.05$) and fullness (condition x time interaction, $F_{[10,50]} = 2.33$, $P < 0.02$), which was apparent 90 min after the infusions began. Post hoc tests indicated that IV octreotide reversed the trend in hunger and fullness ratings observed in response to ID glucose alone; octreotide abolished the decrease in hunger and increase in fullness, resulting in ratings no different than those observed during ID saline (Figure 8A.6).

8A.3.2.3 Energy and macronutrient intake

There was a significant difference in energy intake from the test meal following the three studies ($F_{[2,10]} = 5.79$, $P < 0.05$). Post hoc tests showed that the reduction in energy intake following ID glucose compared with ID saline ($P < 0.05$) was no longer observed when IV octreotide was administered. Similarly, ANOVA revealed significant differences in fat ($F_{[2,10]} = 6.07$, $P < 0.02$) and protein ($F_{[2,10]} = 4.72$, $P < 0.05$) intakes from the test meal between the three studies and post hoc tests showed that the decreases in fat and protein intakes following ID glucose ($P < 0.05$) were no longer seen with IV administration of octreotide. There were no differences in carbohydrate intake between the 3 studies. The percentage of energy provided by the macronutrients after ID glucose was not affected by infusion of octreotide and there was also no difference in rate of eating (Table 3).

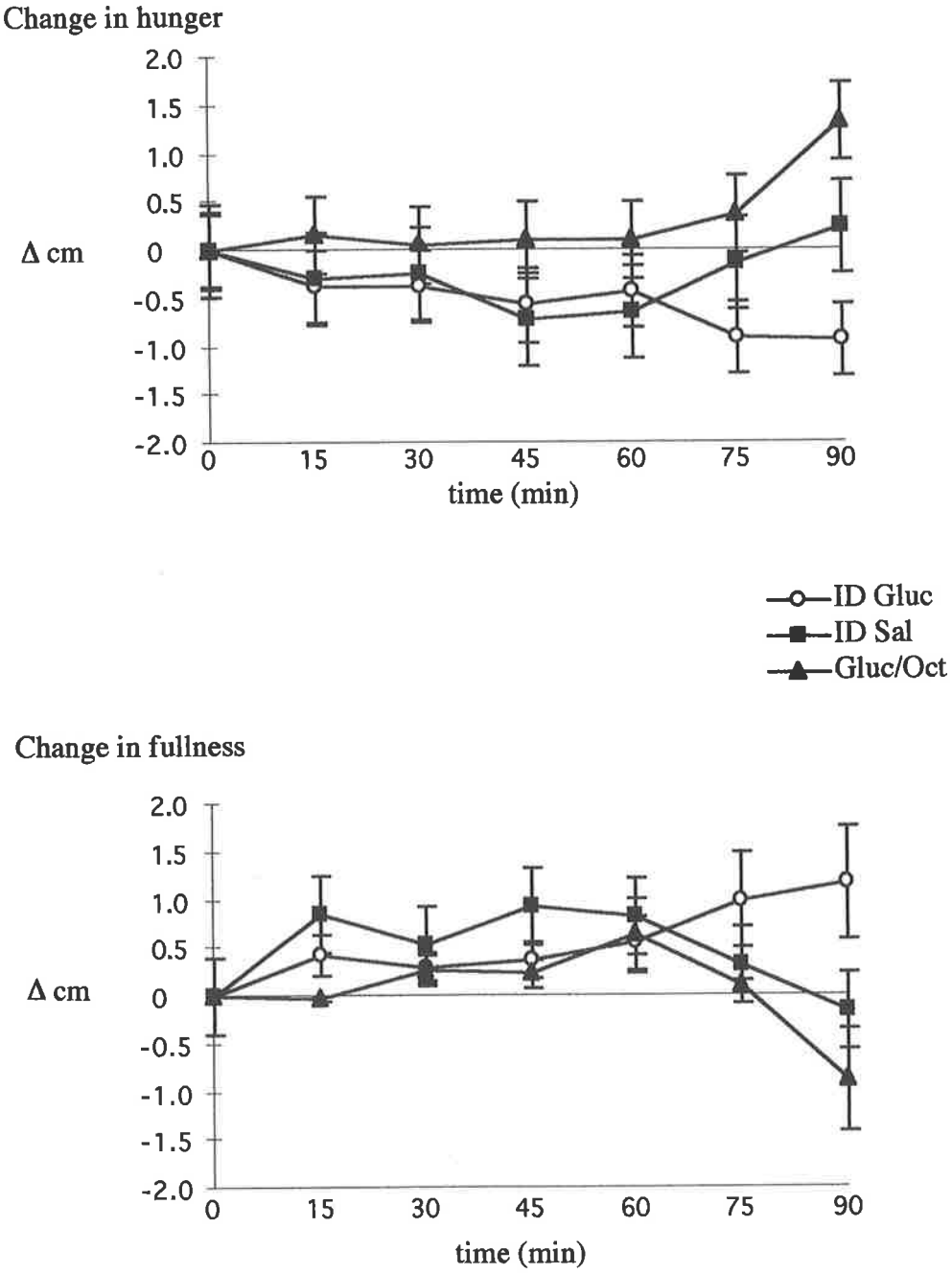


Figure 8A.6
Change in hunger and fullness (mean +/- SEM) during each of the three studies are shown. Intraduodenal infusions commenced at t = 0. With octreotide, there was a reversal of the decrease in hunger ($P < 0.05$) and increase in fullness ($P < 0.05$) seen during ID glucose (condition x time interactions). There was no difference in change in hunger or fullness between ID saline and ID glucose + octreotide. n = 6 in each study.

Table 2: Energy and macronutrient intakes from test meal following ID infusion of glucose (ID Gluc) and saline (ID Sal).

	ID Gluc	ID Sal
Energy (Kcal)	1138 ± 150 *	1539 ± 152
Fat (g)	47.1 ± 8.0 *	69.8 ± 7.1
Carbohydrate (g)	139.5 ± 17.3	177.4 ± 18.1
Protein (g)	47.4 ± 8.4 *	60.6 ± 7.4
Fat (% energy)	36.1 ± 2.8	40.9 ± 1.5
Carbohydrate (% energy)	47.4 ± 3.9	43.3 ± 2.1
Protein (% energy)	16.3 ± 1.6	15.6 ± 0.8
Rate of eating (mins)	24.8 ± 1.5	25.0 ±1.3

n = 8. * Significantly different from ID saline, $p < 0.01$.

Table 3: Energy and macronutrient intakes from test meal following ID infusions

	ID Gluc	ID Sal	Gluc/Oct
Energy (Kcal)	1201 ± 197 *	1617 ± 179	1360 ± 182 †
Fat (g)	50.8 ± 10.3 *	73.8 ± 8.0	62.1 ± 9.7 †
Carbohydrate (g)	139.0 ± 22.9	181.4 ± 23.0	150.1 ± 21.3
Protein (g)	54.9 ± 9.3 *	67.2 ± 7.7	59.1 ± 8.1
Fat (% energy)	36.7 ± 3.4	41.4 ± 2.0	40.7 ± 3.2
Carbohydrate (% energy)	44.9 ± 4.5	41.8 ± 2.5	41.9 ± 3.9
Protein (% energy)	18.3 ± 1.3	16.6 ± 0.6	17.3 ± 0.8
Rate of eating (mins)	25.5 ± 1.9	26.3 ± 0.8	23.8 ± 2.1

n = 6 * Significantly different from ID saline, $p < 0.05$

† Significantly different from ID glucose without octreotide, $p < 0.02$

8A.4 DISCUSSION

This study shows that insulin, when infused to levels above the normal postprandial range, did not decrease appetite and did not inhibit its own release in response to an ID infusion of glucose. In response to ID glucose, plasma GIP levels increased rapidly, whilst GLP-1 levels responded more slowly and were associated with an increase in insulin and decrease in appetite and subsequent food intake.

This study has confirmed previous observations that when postprandial levels of glycaemia are maintained by IV glucose infusion, ID infusion of glucose decreases appetite in humans compared with an ID infusion of saline (Lavin et al 1996). This was observed as a reduction in ratings of hunger, an increase in ratings of fullness and a decrease in energy intake from a test meal, with no change in macronutrient selection. The administration of 72 g of glucose in 90 min may be slightly supraphysiologic, albeit within the normal range for gastric emptying (Hunt et al 1985). Although plasma glucose levels in the current experiment were slightly higher during ID glucose compared with ID saline, this is unlikely to have affected our observations as the difference was only small (<1 mmol/L) and a previous study showed that the effects of intestinal glucose on appetite are not mediated via an increase in blood glucose per se (Lavin et al 1996). The observation that changes in appetite only occurred during the last 30 minutes of the ID infusion is also in accordance with this previous study (Lavin et al 1996). This delay might suggest either a postabsorptive mechanism influencing satiety, or simply reflect the time taken to recruit sufficient intestinal receptors (Welch et al 1988a & b). Lin et al (1989 & 1990b) showed that inhibition of gastric emptying is influenced more by the length of small intestine exposed to nutrients than the concentration of nutrients. Thus, it is possible that stimulation of a critical number of receptors was required to release sufficient peptides to induce the satiating effect of ID glucose in the present study.

Although the IV insulin infusion elevated plasma insulin concentrations to slightly above the normal postprandial range (Tasaka et al 1975), it was not possible to match the plasma insulin concentrations obtained when saline was infused into the duodenum with those obtained during ID glucose infusion because, in contrast to published data (Marchetti et al 1995; Piatti et al 1994), raising plasma insulin artificially did not suppress endogenous insulin release. Marchetti et al (1995) found that that 200 or 400 mU/ml (1200 or 2400 pmol/L) insulin decreased first- and second-phase secretion of insulin from perfused human islets. In a study on human subjects, Piatti et al (1994) found that second-phase, but not first phase, arginine-induced insulin release was

suppressed by both high dose (1.20 $mU.kg^{-1}.min^{-1}$ or 7.2 $pmol.kg^{-1}.min^{-1}$) and low dose (0.33 $mU.kg^{-1}.min^{-1}$ or 1.98 $pmol.kg^{-1}.min^{-1}$) insulin infusions. The high dose infusion resulted in mean plasma insulin levels of 455 pmol/L, similar to those obtained in the current study.

Despite being unable to match plasma insulin levels between the experiments, two observations suggest that, by itself, an increase in plasma insulin level did not influence appetite in the present study. Firstly, raising insulin concentrations with IV infusion had no effect on appetite ratings before the ID infusions began. Secondly, there was no significant effect of IV insulin on hunger or fullness during the control, ID saline, infusion. These results support the observations of Woo et al (1984) that elevating plasma glucose and insulin to normal postprandial concentrations with IV infusions does not affect food intake from a test meal. The decrease in appetite seen during ID glucose infusion did, however, occur whilst insulin concentrations were further raised by endogenous insulin secretion. It is not possible, therefore, to entirely exclude a role for this increase in plasma insulin in the suppression of appetite. However, Rodin et al (1985) reported that raising plasma insulin to much higher levels, via endogenous secretion, actually increased hunger in normal subjects after a delay of ~60 min. Moreover, Chapman et al (1998) have recently reported that the infusion of insulin either at or higher than postprandial concentrations (to approximate postprandial concentrations in the portal vein) under euglycaemic conditions had no effect on sensations of appetite or subsequent food intake. Therefore, on the basis of current evidence, insulin does not appear to be a physiological mediator of satiation.

In contrast, these data support the concept that gastrointestinal incretin peptides, in particular GLP-1, play a role in the suppression of appetite by ID glucose. Hunger and food intake were suppressed and GLP-1 and GIP levels were increased in response to ID glucose but not ID saline. GLP-1, when given as an intracerebroventricular injection, inhibits feeding in both rats (Turton et al 1996) and chickens (Furuse et al 1997). Peripheral administration of GLP-1 did not decrease food intake in rats (Turton et al 1996), however peripherally released GLP-1 has been shown to cross the blood brain barrier (Drewe et al 1997), and a recent study in healthy humans demonstrated that exogenous administration of GLP-1, which resulted in physiological plasma levels, decreased hunger and food intake (Gutzwiller et al 1997), and in obese subjects GLP-1 is lower postprandially than in normals (Naslund et al 1997a).

In contrast to GLP-1, there is no good evidence to suggest that GIP is involved in the regulation of appetite. In this study, there was a much closer temporal relationship between the suppression of appetite by ID glucose and GLP-1 release, than GIP release. GLP-1 levels were above baseline at about 30 minutes and continued to rise while appetite decreased, supporting the concept that GLP-1 has a substantial role in the stimulation of satiation by carbohydrate. By contrast, GIP concentrations were elevated by 10 minutes, peaked by ~ 30 minutes and levels plateaued after this time.

Schirra et al (1996) have evaluated the effects of ID glucose (1.1 and 2.2 Kcal/min, or 4.6 and 9.2 kJ/min) on plasma GLP-1 and GIP in normal subjects. They reported that plasma GIP increased after 15 minutes of ID glucose at both caloric rates in a dose-dependant manner, and plateaued by 30 minutes. Whereas, plasma GLP-1 levels increased at about 15 minutes at 2.2 Kcal/min and remained constant after this time while ID glucose at 1.1 Kcal/min did not result in elevation of GLP-1. The plasma GIP levels reported here, in response to ID glucose, are consistent with those reported by Schirra et al (1996). However, the GLP-1 response (a later rise and progressive increase) contrasts with that observed by Schirra et al (1996). The reason for this discrepancy, particularly as there was little difference in the infusion rates, is uncertain. GLP-1 is released from L cells which are localised mainly in the ileum, also occur in the jejunum but are absent in the duodenum (Eissele et al 1992). Therefore, the delay in the increase of GLP-1 relative to GIP may relate to the time taken to activate the more distally located L- cells. In this study, a close temporal association between GLP-1 and insulin was observed, which is consistent with other data suggesting a major role for GLP-1 in the regulation of insulin release and postprandial glycaemia in humans (Holst 1994).

That octreotide prevented the rise in GIP and GLP-1 levels during ID glucose is consistent with a study showing octreotide prevented the rise in insulin in response to incretin peptides (Lavin et al 1996). Although octreotide abolished the decrease in hunger and food intake, separate actions of insulin and/or other gastrointestinal hormones (such as GIP and GLP-1) in appetite suppression cannot be discriminated because octreotide also prevented the further increase in plasma insulin induced by ID glucose. It is clear, however, that stimulation of intestinal receptors by glucose per se is not involved in the production of satiety, at least not without the involvement of gastrointestinal hormones. Moreover, the observation that greater amounts of IV glucose were received during octreotide (than during ID gluc alone) also contradicts the notion that changes in appetite were solely related to increased glucose metabolism.

CHAPTER 8B

Glucose and Lipid Differentially Affect Appetite and Antropyloroduodenal Motility; Glucose Supplementation Alters These Responses

8B.1 INTRODUCTION

As different nutrient classes have the potential to stimulate small intestinally mediated satiety by discrete pathways (1.3.1; 1.3.2; 3.4.1; 4.2.3), it is possible that the effect of different nutrients on appetite and antropyloric motor function will differ. Although some work has examined these motility differences in animals (4.2.3), there is only anecdotal information about nutrient specificity of motor effects in humans.

Alterations in usual diet are known to effect both the rate of small intestinal transit and gastric emptying of a subsequent meal containing the nutrient whose intake was manipulated (1.3.1; 4.2.4). The specific motor mechanisms whereby this occurs are unknown, nor is it known whether the documented change in motor function in response to dietary alteration also affects perception of appetite.

Therefore, in this study, the effects of two different macronutrients on appetite and antropyloric motility, and the impact of short-term dietary glucose supplementation on these responses were evaluated. Specifically, ten healthy young males received isocaloric intraduodenal (ID) infusions of glucose and lipid whilst antropyloroduodenal motility and appetite were assessed by manometry and visual analogue scales (VAS). Effects of each ID nutrient on appetite and motility were evaluated prior to, and following 7 days of dietary supplementation with glucose.

8B.2 METHODS

8B.2.1 Subjects

10 healthy male volunteers were studied; mean age 26 years (range 19-38) and body mass index (BMI) 25 kg/m^2 (range 21.7-26.9). No subject had any history of eating

disorder, chronic gastrointestinal disease, gastrointestinal surgery, nor was on any medication. Subjects were recruited by advertisement and were predominantly university students. Prior to entering the study, all volunteers completed a five-day diet diary to ensure their usual diet approximated the "standard" Australian diet (34% fat, 21% protein and 44% carbohydrate), but no attempt was made to standardise the subjects' diets.

8B.2.2 Experimental design

The experimental design is illustrated in Figure 8B.1. In brief, after satisfying entry criteria (section 8.2.1), subjects were booked for the 2 study days on which isocaloric intraduodenal (ID) nutrient infusions of glucose and lipid were given, henceforth referred to as *day 1* (pre-supplementation) and *day 2* (post-supplementation). At the completion of the *day 1* study, subjects were instructed in the consumption of a preweighed glucose supplement (*Poly-Joule*, glucose polymer, Sharp Laboratories, Ermington, NSW, Australia) and asked to take five sachets (80 g each) daily (total 400 g daily) for 7 days immediately preceding study *day 2*. Subjects were provided with 35 sachets and compliance was assessed by weighing of unused sachets on the return visit. Subjects were weighed on enrolment and on study *days 1 and 2*.

In 9 of the 10 subjects, the 2 study days were completed within 10 days. In one subject the interval was 35 days. This delay resulted from a minor back injury sustained after *day 1*, and the second experiment was only performed when he could lie still for a prolonged period of time without experiencing any discomfort.

On the 2 study days, each subject attended the laboratory at 9 AM after an overnight fast. The manometric assembly, (section 7.2.2 & Figure 7.1) was positioned as described in sections 7.2.1 and 7.2.3. Fasting motility was observed until the occurrence of phase III of the interdigestive migrating motor complex (MMC). Immediately after cessation of phase III MMC activity, an intravenous cannula was placed in the left antecubital vein for blood sampling. The subject then rested quietly for a further 10-15 minutes before the baseline blood sampling and visual analogue scales (VAS) to evaluate appetite were administered (Sepple & Read 1989).

At t = 0 min an isocaloric ID infusion of either 10% lipid (Intralipid, Kabi Pharmacia AB, Sweden) or 25% glucose (Baxter Healthcare, Old Toongabbie, NSW, Australia) was commenced at 2.9 Kcal/min for 90 minutes (2.6 ml/min lipid; 3 ml/min glucose).

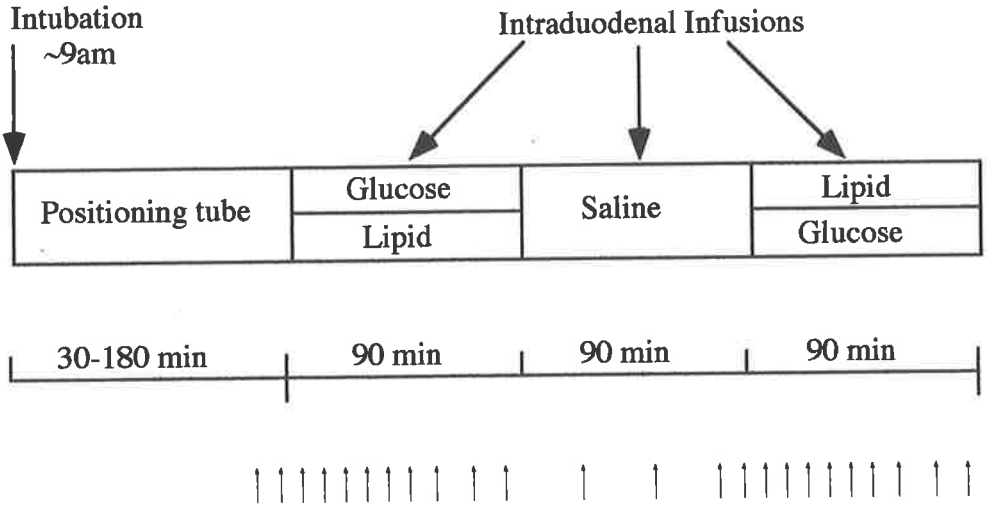


Figure 8B.1
Diagramatic representation of the protocol for the 2 study days on which ID infusions of glucose and lipid were administered. Dietary supplementation with glucose was administered for 7 days immediately preceeding the second study day. For detail see section 8B.2.2.

Blood samples were taken and VAS administered throughout the ID infusions at t =-5, 0, 5, 10, 20, 30, 40, 50, 60, 75 and 90 min. After 90 min the first ID nutrient infusion was ceased, and ID saline (0.9%, 3 ml/min) was given for 90 minutes ("washout"). After this, each subject received the alternate nutrient for a final 90 min period. ID nutrient infusions were administered through a channel in the manometric assembly 10 cm distal to the pylorus. Antropyloroduodenal (APD) motility was monitored during each of these three 90 min periods. The order of administration of ID nutrient infusions was randomised and single-blind (so that 5 subjects received ID lipid first and 5 ID glucose). Each subject, however, received the ID nutrients in the same order on the 2 study days (ie *day 2* was not randomised) to enable a direct comparison of the effects of ID nutrients on *days 1 and 2*.

8B.2.3 Outcome measures

8B.2.3.1 Plasma glucose and insulin

Venous blood was taken concurrently with the VAS. Plasma glucose and insulin were measured as described in section 7.2.5.

8B.2.3.2 Appetite

Appetite was assessed with VAS as described in section 7.2.7. Subjects were familiarised with the VAS at the commencement of each study day and instructed to make a single mark on it corresponding to their own assessment of their current feelings.

8B.2.3.3 Antropyloroduodenal (APD) pressures

APD pressures were assessed with manometry, using an 11 lumen water-perfused silicone rubber sleeve/sidehole assembly as described in section 7.2.2 and Figure 7.1. Pressures were recorded and analysed as described in sections 7.3.1 and 7.3.2.

APD pressures were only analysed when the sleeve sensor was positioned correctly across the pylorus (Heddle et al 1988b) and variables quantified included:

(i) the number of antral pressure waves (as defined in section 7.3.2.2).

(ii) the total number, frequency and amplitude of isolated pyloric pressure waves (IPPW) (as defined in section 7.3.2.1) during ID nutrient infusions. The frequency and amplitude of IPPW were assessed in two ways: (a) in ten minute

segments during the ID nutrient infusions and (b) comparing the "early" response (IPPW/10 min) defined as that between 20-50 min of the ID nutrient infusion with the "late" response – between 50-90 min.

(iii) pyloric tone (as defined in section 7.3.2.3) during ID nutrient infusions.

8B.2.4 Statistical analyses

Differences between nutrients between the 2 study days and between the early and late responses were evaluated using Mixed Model ANOVA, a model with a mixture of fixed and random effects (SAS Institute Incorporated, Carey, Nth Carolina). Paired comparisons were done using tests of simple effects (slices of interactions) (Winer 1971). Missing data points were left as blanks in the analysis. Data are presented as means ± SEM; and a P value <0.05 was considered significant unless otherwise stated.

8B.3 RESULTS

The study was well tolerated with all volunteers completing the protocol. As assessed by the five day diet diary, usual macronutrient intake was 16.7% protein (range 14-20%), 36% fat (31-47%) and 45% carbohydrate (35-52%). Compliance with the glucose supplement was excellent, with volunteers consuming 84-96% of the dispensed supplement. There was no significant weight gain between studies (81.02 ± 3.29 kg vs 81.48 ± 3.25 kg). Two subjects vomited during the ID lipid infusion on both study days; both had already completed both the ID glucose and ID saline infusions. On *day 1* they vomited 30 and 40 minutes after commencement of ID lipid. On *day 2* both tolerated the infusion for a longer period, vomiting at 60 and 50 min respectively. The onset of nausea was only 3-5 min prior to emesis. In these subjects, VAS from the first time point at which nausea was reported were not included in the analysis. No other subjects reported nausea.

8B.3.1 Plasma glucose and insulin concentrations

During ID glucose plasma glucose increased on both days, whereas there was no change during ID lipid. There was no significant difference in the magnitude, nor the time course, of the rise in plasma glucose during ID glucose between *day 1* and *day 2* (*day 1* baseline 4.6 ± 0.4 mmol/L - peak 8.9 ± 0.4 mmol/L; *day 2* baseline 4.8 ± 0.4 mmol/L - peak 8.6 ± 0.4 mmol/L). Plasma insulin also increased during ID glucose on both days and did not change during ID lipid. While there was no significant difference

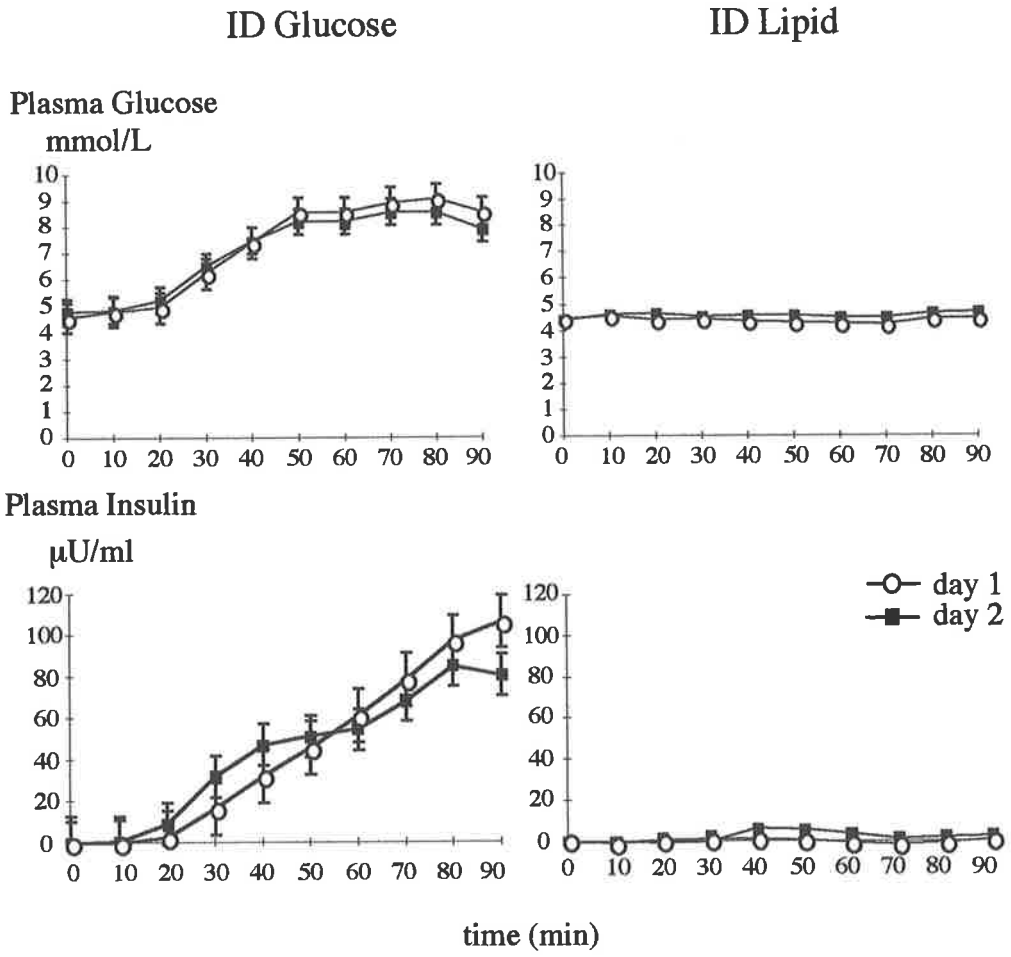


Figure 8B.2
Plasma glucose and insulin (mean +/- SEM) during ID glucose and lipid infusions, both pre- (*day 1*) and post-supplementation (*day 2*). See section 8B.3.1.

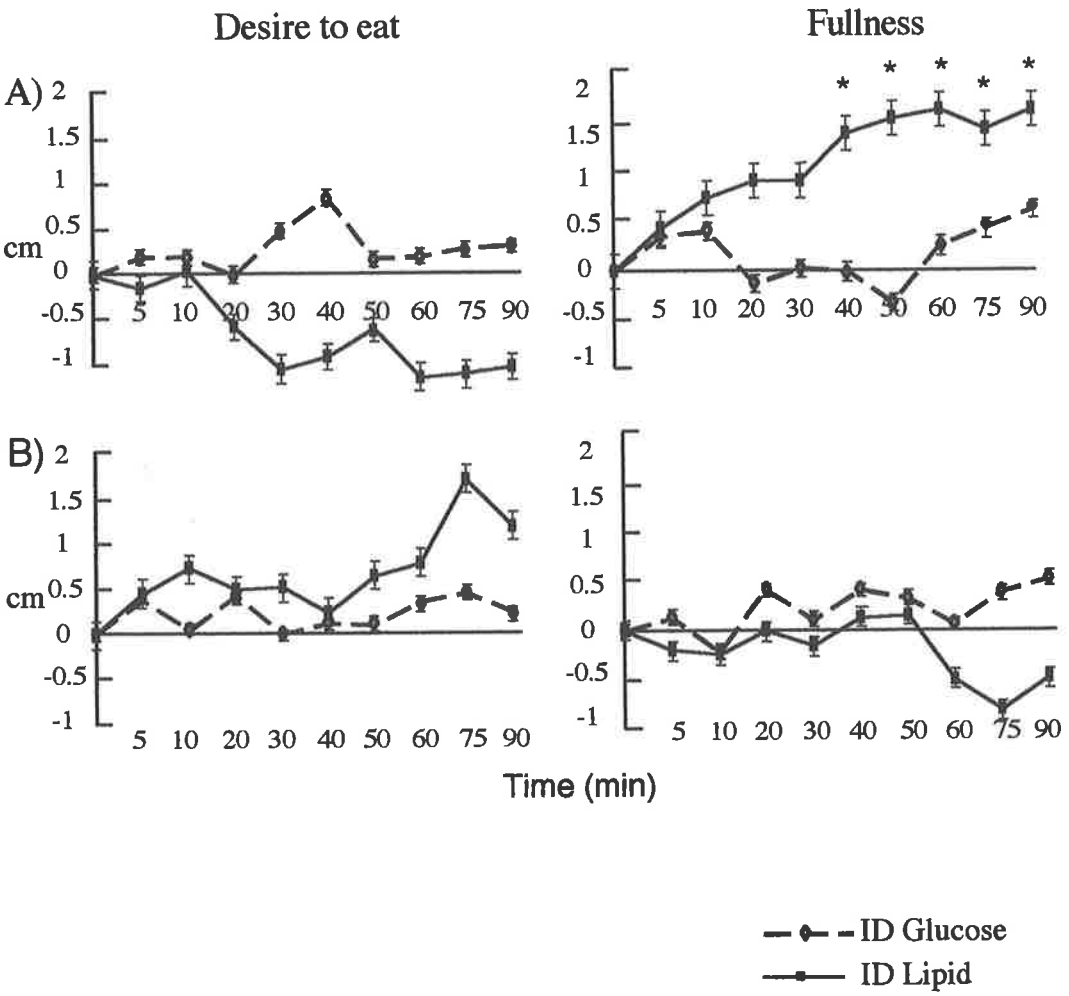


Figure 8B.3
Change from baseline in desire to eat and fullness during ID lipid and glucose, (mean +/- SEM), before (A) and after (B) dietary glucose supplementation. On *day 1* ID lipid reduced desire to eat ($P < 0.05$) and increased fullness ($P < 0.05$), whereas there was no change in appetite ratings during ID glucose cf. baseline. Desire to eat was less during ID lipid than during ID glucose ($P = 0.013$ by ANOVA for whole curves), and fullness was greater from 40 mins on during ID lipid (*$P < 0.05$ cf baseline). After dietary glucose supplementation neither ID nutrient had a significant effect on appetite ratings compared with baseline and there was no difference between nutrients. (see 8B.3.2).

in the magnitude nor the time course of the rise in plasma insulin between the two study days (*day 1* baseline 6.8 ± 9.5, peak 112.6 ± 9.6 μU/ml; *day 2* baseline 6.8 ± 9.5, peak 90.8 ± 9.6 μU/ml), there was a trend for plasma insulin at 90 minutes to be higher on *day 1* when compared to *day 2* (*day 1* vs *day 2*, 112.6 vs 85.7 μU/ml, P = 0.066 by ANOVA). (see Figure 8B.2).

8B.3.2 Appetite ratings

On *day 1*, ID lipid was associated with a reduction in desire to eat ($P < 0.05$) and increase in fullness ($P < 0.05$). In contrast, on both *days 1 and 2*, appetite ratings did not change from baseline during ID glucose. On *day 1*, ID lipid was more potent at suppressing appetite than ID glucose, as evidenced by an overall reduction in desire to eat during ID lipid when compared to ID glucose ($P = 0.016$), and increase in fullness during ID lipid after 40 minutes ($P < 0.05$). The greater satiating effect of ID lipid compared to ID glucose on *day 1* was not evident on *day 2* (desire to eat on *day 2* lipid vs glucose, $P > 0.05$; fullness on *day 2* lipid vs glucose, $P > 0.05$). The difference between the responses on *days 1 and 2* reflected a decrease in the suppression of appetite by ID lipid (desire to eat lipid *day 1* vs *day 2*, $P = 0.006$), as there was no difference in the response to ID glucose between *days 1 and 2*, $P = 0.9$ (Figure 8B.3).

8B.3.3 Antropyloric pressures

No antral pressure waves were seen during the ID nutrient infusions. The total number of IPPWs during the 90 min ID infusions was greater with ID lipid than ID glucose; (*day 1* lipid vs glucose 86.6 ± 16.0 vs 59.6 ± 14.6, $P = 0.048$; *day 2* lipid vs glucose 95.5 ± 21.3 vs 62.2 ± 11.7, $P = 0.06$). There was no significant difference between the total number of IPPWs induced by each nutrient on *days 1 and 2* (*day 1* lipid vs *day 2* lipid, $P = 0.74$; *day 1* glucose vs *day 2* glucose, $P = 0.89$). The temporal patterning of IPPWs varied between nutrients (Figure 8B.4), so that the maximum rate of IPPWs was greater during ID lipid than ID glucose on both *days 1 and 2* (*day 1*, $P = 0.05$; *day 2*, $P = 0.059$ by ANOVA for whole curves). Comparison of early (20-50 min) and late (60-90 min) responses demonstrated attenuation of the IPPW response during both ID nutrient infusions on *day 1*; (*day 1* lipid early vs late response, 15.78 ± 2.12 vs 8.40 ± 2.28 IPPW/10 min, $P < 0.002$; and *day 1* glucose early vs late response, 9.8 ± 2.12 vs 4.67 ± 2.12 IPPW/10 min, $P < 0.02$). After supplementation, attenuation of the frequency of the IPPW response was still evident for ID glucose (*day 2* glucose early vs late response, 9.40 ± 2.12 vs 4.70 ± 2.12, $P < 0.02$), but not

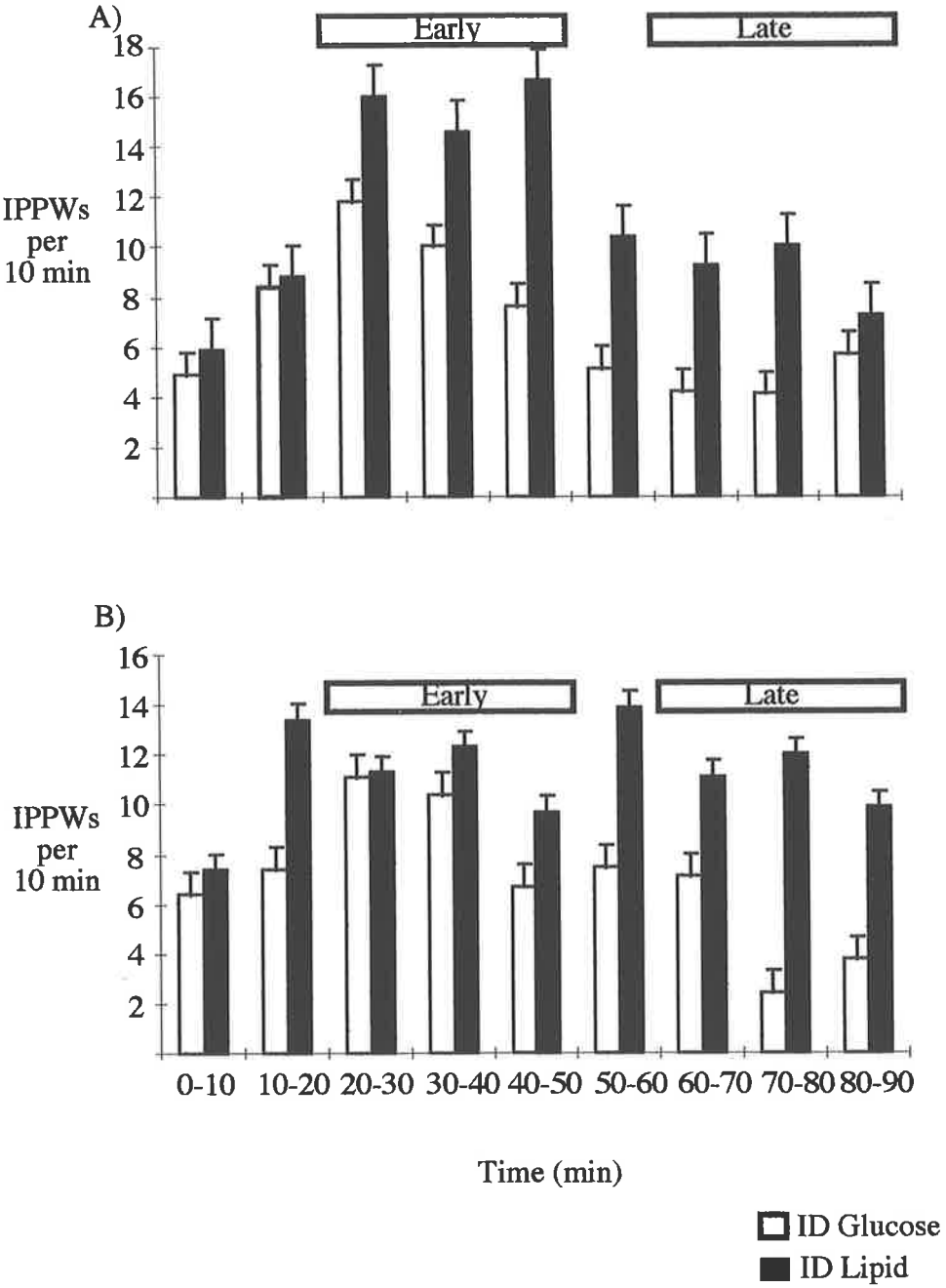


Figure 8B.4
Frequency of isolated pyloric pressure waves (IPPW) during ID lipid and ID glucose, (mean +/- SEM) in 10 minute segments before (A) and after (B) dietary glucose supplementation. On both days the maximum rate of IPPWs was higher during ID lipid than glucose, although this failed to reach statistical significance postsupplementation (*day 1*, P = 0.05, *day 2*, P = 0.06 by ANOVA for whole curves). Comparing the early (20-50 min) and late (60-90 min) IPPW response, attenuation of the frequency of IPPWs was evident during all ID nutrient infusions except ID lipid on *day 2*. (see 8B.3.3).

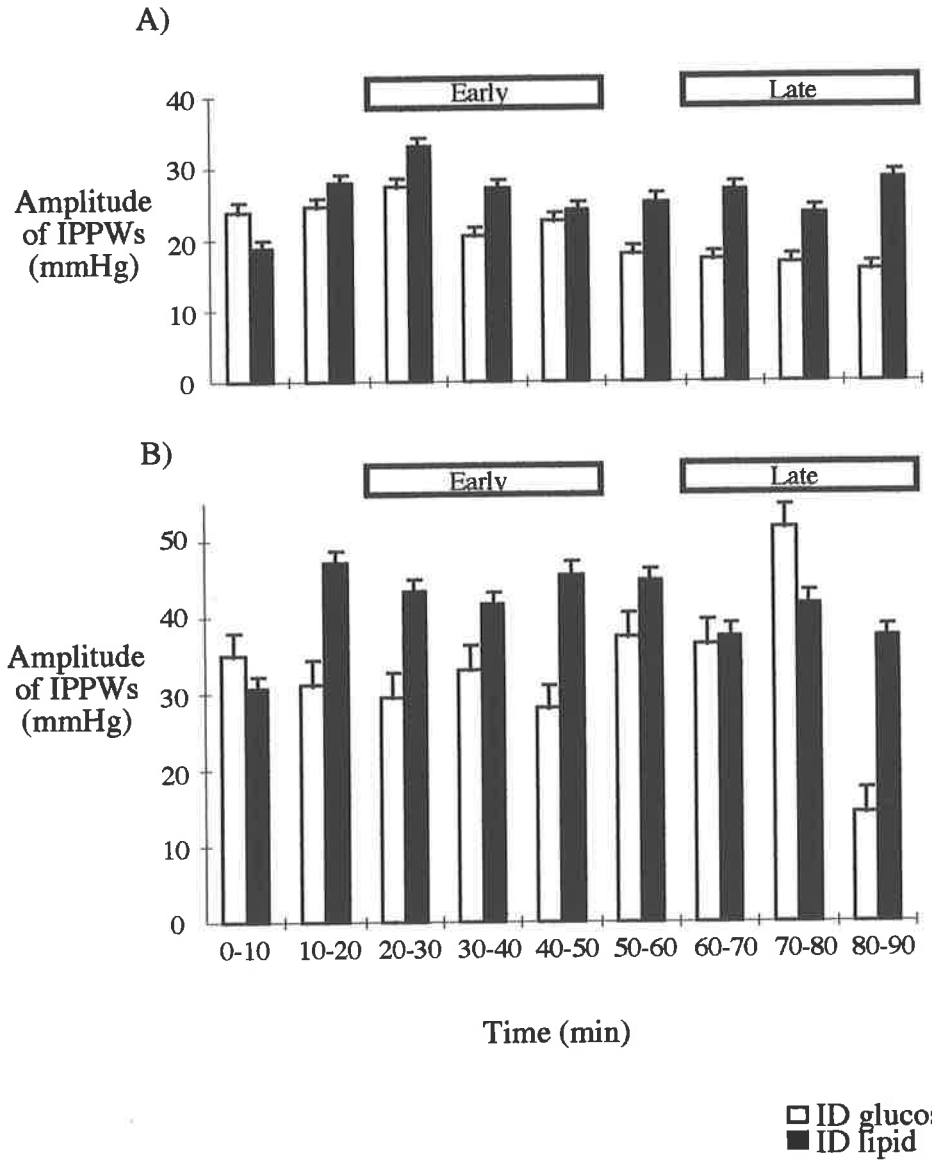


Figure 8B.5
Amplitude of IPPW during ID lipid and ID glucose, (mean +/- SEM) in 10 min segments before (A) and after (B) dietary glucose supplementation. On *day 1* comparison of the early and late response demonstrated attenuation during ID glucose ($P = 0.038$), but not lipid, although there was no overall difference between the two nutrients. On *day 2* the amplitude of IPPWs was greater during ID lipid than ID glucose ($P = 0.016$) by ANOVA for whole curves). The attenuation of the mean amplitude of IPPWs during ID glucose on *day 1* was not evident on *day 2*. For both nutrients the mean amplitude of IPPW was greater ($P < 0.001$) on *day 2* than *day 1*. (see 8B.3.3).

for ID lipid (*day 2* lipid early vs late response, 11.10 ± 2.12 vs 11.02 ± 3.57, P = 0.98).

Before dietary glucose supplementation, there was no difference in the temporal patterning of the amplitude of IPPWs (Figure 8B.5) between the two nutrients (P = 0.25). In contrast, on *day 2*, the mean amplitude of IPPWs was greater during ID lipid (*day 2* lipid vs glucose, P = 0.016 by ANOVA for whole curves). Attenuation of the amplitude of IPPWs was evident during ID glucose on *day 1* (*day 1* glucose early vs late response, 25.73 ± 3.67 vs 17.66 ± 3.93 mmHg, P = 0.038). There was no significant difference in the amplitude of IPPWs between 20-50 min compared to 60-90 min during ID lipid on *day 1* (P = 0.23), nor for either nutrient on *day 2* (P > 0.36 for both). For both ID nutrients, the amplitude of IPPWs was greater on *day 2* compared to *day 1* (*day 1* lipid vs *day 2* lipid and *day 1* glucose vs *day 2* glucose, P < 0.001 by ANOVA for whole curves).

On *day 1*, the increase in pyloric tone in response to both ID lipid and ID glucose was not significantly different; both being higher than baseline (P < 0.05) after 10 min of infusion, and remaining elevated out to 90 min (P < 0.05) for both. While there was no overall difference in the tonic responses to the two ID nutrients on *day 2*, the curves were clearly divergent from 10 to 40 min, with tone slower to rise, and reaching a lower peak value in response to ID glucose when compared with ID lipid (Figure 8B.6).

8B.4 DISCUSSION

This study established that there are substantial differences in the effects of ID infusion of two nutrients of different macronutrient class on appetite and pyloric motility in healthy male volunteers. The major novel observations are that ID lipid when compared to an isocaloric glucose load (i) suppressed appetite more, (ii) stimulated a higher frequency, but similar amplitude, of phasic pyloric pressure waves (IPPW), and (iii) stimulated a similar increase in pyloric tone. Moreover, we have shown that short-term alteration in diet has the capacity to modify the effects of ID nutrients on both appetite and pyloric motility. In particular, after dietary supplementation with glucose for one week, (i) ID lipid no longer had a differential effect on appetite compared to an isocaloric ID glucose load, and (ii) pyloric tone in response to ID glucose was attenuated.

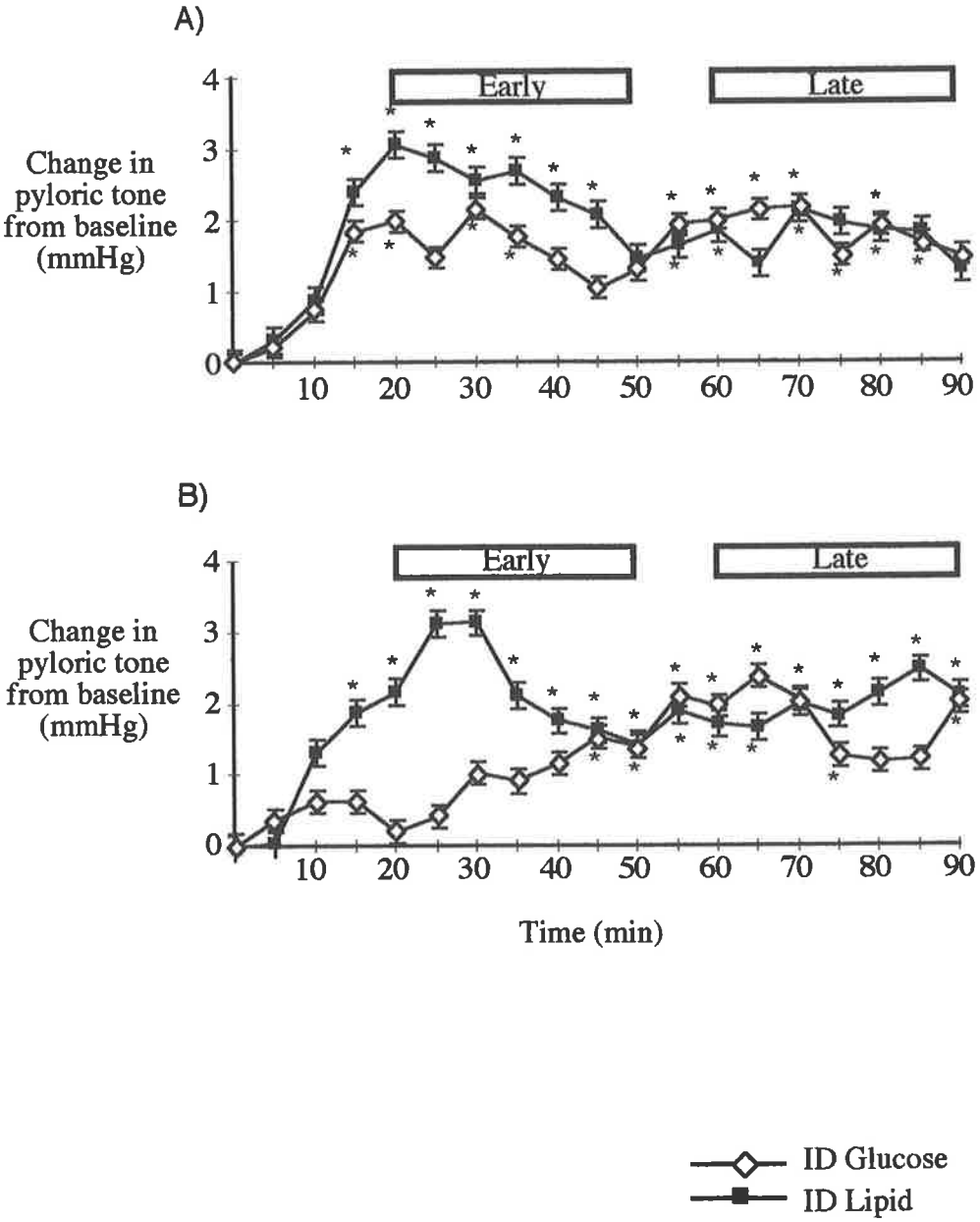


Figure 8B.6
Change in pyloric tone during ID lipid and ID glucose, (mean +/- SEM) in 5 min blocks both before (A) and after (B) dietary glucose supplementation. On *day 1* both ID nutrients stimulated a similar rise in pyloric tone, which began within 15 min of commencing the nutrient infusion. On *day 2*, pyloric tone in response to ID glucose failed to rise above baseline until 45 min. The response to ID lipid is unchanged. (*P < 0.05 cf. baseline). (see 8B.3.3)

In interpreting these observations the potential limitations of the study should be considered. The studies were not randomised (and hence no sham supplementation given) because of the substantial logistical difficulties that this would have presented, associated with an increased likelihood of alterations in diet and weight. To minimise the possibility of a carryover effect, half the subjects received ID glucose first and half ID lipid on *day 1*, and the order of infusions on *day 2* was identical. The diet of all subjects was not standardised as this would have limited the applicability of the observations. It is unlikely that these issues influenced our observations, as responses to each nutrient on *day 2* varied, with some being increased and others decreased. Moreover, it has been shown previously that both ID lipid and ID glucose stimulate pyloric motility (Fone et al 1989; Fraser et al 1992a; Heddle et al 1988a & 1989), that ID glucose suppresses appetite in healthy males (Lavin et al 1996) and that dietary glucose supplementation accelerates gastric emptying of glucose (Cunningham et al 1991a; Horowitz et al 1996) in doses comparable to those used in this study. The possibility that differences in osmolality may have influenced the observed responses to the two ID nutrients on *day 1* cannot be excluded. However, as 25% glucose has a much greater osmolality than 10% Intralipid, it may have been anticipated that, if osmolality was a major factor, the stimulation of pyloric motility and suppression of appetite by ID lipid would have been less than that observed with ID glucose, not greater as was seen. While both ID nutrients were infused at a rate of 2.9 Kcal/min, there was a minor difference in the volume infused of 0.4 ml/min. However, if volume was a significant factor contributing to the effects of nutrients on appetite and pyloric motility, it may have been expected that ID glucose (given as the larger volume) would have had a greater effect than ID lipid, but the converse was seen. Some data points were missing due to incorrect positioning of the manometric assembly and vomiting in two subjects. The robustness of these results is strengthened by the stringency of the analysis, as these missing data points were included as blanks rather than creating "dummy" values or collapsing time points which can obscure time-dependent phenomena.

8B.4.1 Appetite

This study has established for the first time that adaptation of appetite in response to dietary manipulation occurs across macronutrient classes. The ID delivery of nutrients at 2.9 Kcal/min approximates the overall rate of gastric emptying of nutrients (Hunt et al 1985), and, for glucose, has been shown to decrease appetite and stimulate release of putative satiety hormones including insulin, gastric inhibitory polypeptide (GIP) and

glucagon-like peptide-1 (GLP-1) (Katchinski et al 1995; Lavin et al 1996; Chapter 8A) in normal subjects. Therefore, although the protocol did not include a control (no nutrient) condition, it is justified to conclude that ID lipid really decreased appetite as its effects were greater than those of ID glucose, which has been previously shown to suppress appetite ratings and decrease subsequent food intake (Lavin et al 1996; Chapter 8A). By delivering nutrients directly into the small intestine, the potential contributions of gastric distension and variability in gastric emptying rates to appetite regulation were eliminated (section 1.2.2 & Chapter 4). Although previously hypothesised to be an important satiety mechanism, gastric distension is now thought to play a lesser role in modulating intake than stimulation of small intestinal nutrient receptors (Sepple & Read 1989 & 1990; section 1.2.2). The observation that intravenous nutrient loads have a lesser effect on appetite (Lavin et al 1996; Welch et al 1985; section 1.4.1), suggests that post-absorptive signals play a minor role in the short term regulation of appetite.

It is controversial whether fat and carbohydrate exert different effects on appetite (section 1.3.1). For example, Blundell et al (1993a) have reported a greater degree of appetite suppression by fat in one study, and by carbohydrate in another, although the subjects studied appear similar. In young, unrestrained eaters of normal weight acutely administered, isocaloric, enteral (oral and intragastric) loads of fat and carbohydrate appear to suppress hunger to a similar degree (reviewed in Rolls 1995). It may be that any differences between fat and carbohydrate are time dependent, so that the time interval between intake and evaluation of appetite is critical. In the obese, there is evidence, based on acute clinical studies, longitudinal studies and diet surveys, that fat is less satiating than carbohydrate (reviewed in Blundell et al 1993a and Rolls 1995). Although it should be recognised that convenience, taste and social situations are likely to confound some of these results. In this study it has been clearly demonstrated that ID lipid (a triglyceride emulsion) has a greater immediate satiating effect than ID carbohydrate prior to dietary alteration.

After dietary glucose supplementation, the effects of ID fat on appetite were diminished, so that there was no difference from ID glucose. This observation is important as current hypotheses of the mechanisms of satiation tend to invoke relatively nutrient-specific signals as discussed in 1.3.1, 1.3.2 & 4.2.5. In studies which have examined the effects of dietary supplementation on gastric emptying (Brown et al 1994; Cunningham et al 1991a & b; Horowitz et al 1996), gastric emptying adapted (accelerated) in response to the macronutrient which had been given

as a supplement. The nutrient-specificity of this adaptation was not adequately examined, nor was appetite evaluated. In patients with anorexia nervosa, gastric emptying is generally delayed whilst they have inadequate caloric intake, but improves once refeeding commences and before normal body weight is obtained (Rigaud et al 1988; Robinson et al 1988; Stacher et al 1986). This suggests that the caloric load presented to the small intestine, rather than its specific nutrient composition, is the major factor modulating gastric emptying. Although appetite was not formally evaluated in these latter studies, there is anecdotal evidence that symptoms of bloating and early satiety diminish in patients with anorexia nervosa once an adequate enteral intake is maintained, supporting the concept that the rate of nutrient entry into the small intestine has the capacity to alter appetite signals, as well as motor function. These effects may be mediated by changes in receptor sensitivity, receptor number, length of intestine exposed to nutrients, or the central response to a given satiety signal. Taking the above observations and the results of the current study together, it is likely that in the regulation of appetite, "nutrient-general" (rather than specific) small intestinal mechanisms are involved.

8B.4.2 Pyloric motility

The presence of nutrients within the small intestine retards gastric emptying (Heddle et al 1989; Lin et al 1989, 1990b &1992a; Welch et al 1985 &1988a). This is associated with suppression of antral motility and elevation of tonic and phasic pressures in the pylorus (Fone et al 1989; Heddle et al 1988a & 1989; Tougas et al '92; section 4.2.1.2). This study demonstrates, for the first time, that ID lipid is a more potent stimulus of phasic pyloric activity (IPPW) than ID glucose, both pre- and post- dietary glucose supplementation, with a higher frequency and amplitude of IPPW and less attenuation in this response over time. This was not completely unexpected given that intestinal lipid has also been shown to cause greater proximal gastric relaxation than carbohydrate in dogs (Azpiroz & Malagelada 1985b). Although there was no statistically significant difference between the two nutrients, the stimulation of pyloric tone by ID lipid appeared to be greater than with ID glucose prior to supplementation. Furthermore, on *day 2*, the initial effect of ID glucose on pyloric tone was diminished, despite preservation of the frequency and an increase in the amplitude of IPPWs. The major significance of these findings lies in the notion that pyloric tone and IPPWs are not all or none phenomena, and that different nutrient receptors may act via discrete pathways to influence pyloric motor patterns, or generate quantitatively differing signals. A discrepancy between the tonic and phasic pyloric motility has been

previously reported in humans (Edelbroek et al 1994b; Fraser et al 1991, 1992b & 1993), for example in response to ID infusions of D- and L-tryptophan (Edelbroek et al 1994b), acute hyperglycaemia (Fraser et al 1991) and intravenous cholecystokinin octapeptide (Fraser et al 1993). In this study this discrepancy may reflect that phasic and tonic pyloric pressures are mediated via different neural or humoral signals, have different sensitivities to stimuli, or possibly because a "ceiling" had been reached for stimulating pyloric tone (but not IPPW) by delivering nutrients at a relatively high caloric rate. Previous studies of pyloric motility in humans with ID lipid infusions used caloric rates one or two thirds of those used here (Fraser et al 1992a; Heddle et al 1988a); however, others have given ID dextrose at higher caloric rates (Fone et al 1989). Because the two nutrients were not directly compared in these previous studies, and because ID infusions were performed at only one caloric rate in the current study, the question regarding dose cannot be answered. Both the tonic and phasic pyloric responses to both ID dextrose and ID lipid have been shown to be sensitive to atropine, perhaps reducing the likelihood that they are mediated by entirely separate mechanisms (Fone et al 1989; Fraser et al 1992a).

Prior to dietary glucose supplementation, both the tonic and the phasic pyloric pressures remained above baseline during both ID lipid and ID glucose throughout the infusions; although there was attenuation of the phasic response (both frequency and amplitude) to ID glucose over the last 30 minutes as reported previously (Edelbroek et al 1992). A previous study reported attenuation in the tonic pyloric motor response to ID lipid infusion (Fraser et al 1992a). Late attenuation in pyloric tone was not seen during any ID nutrient infusion in the current study, however, the low caloric rate of delivery of lipid in the previous study (1.1 Kcal/min compared to 2.9 Kcal/min here) may account for this difference.

IPPWs are associated with the slowing of gastric emptying caused by nutrient in the small intestine (Heddle et al 1988a & 1989; Tougas et al 1992), however, it is unlikely that they are primarily responsible for retarding transpyloric flow. Even at their peak frequency of about 3/min, IPPWs occupy only a fraction (less than a third) of the time course during which flow is stopped (Tougas et al 1992). A sustained rise in pressure is likely to contribute to the retardation of gastric emptying, as increases in pyloric tone of as little as 3-4 mmHg are likely to be mechanically significant (Tougas et al 1992; section 6.4).

The observation of altered pyloric motor responses following dietary supplementation with glucose extends earlier observations that modification of intake of a nutrient affects gastric emptying; the usual response being that increasing the intake of a substance accelerates its subsequent gastric emptying, and decreasing intake retards it (Brown et al 1994; Cunningham et al 1991a & b; Horowitz et al 1996). Gastric emptying was not evaluated in this study, however, it should be noted that the glucose supplement used was similar to that previously documented to accelerate gastric emptying of a subsequent load of both glucose and fructose (Cunningham et al 1991b; Horowitz et al 1996). Therefore, the fact that there was attenuation of pyloric tone in response to glucose after dietary glucose supplementation (with the phasic pyloric response to glucose being unaltered), leads one to hypothesise that this may be responsible for the acceleration of gastric emptying reported in these studies (Cunningham et al 1991b; Horowitz et al 1996). Proximal gastric motor mechanisms were not evaluated and, therefore, one cannot exclude the possibility that changes in fundal tone contributed to acceleration in gastric emptying (Azpiroz & Malagelada 1985b). However, pyloric tone is likely to be the final arbiter in gastric emptying of a liquid, as emptying cannot occur against a closed sphincter (Tougas et al 1992; 6.5). Unlike the effect on appetite, this adaptation of pyloric tone in response to ID nutrients was "nutrient-specific", with no modification in the response to ID lipid, suggesting that motor and sensory adaptation to dietary manipulation may be mediated by different mechanisms.

CHAPTER 8C

The Effects of Small Intestinal Nutrients on Appetite, Pyloric Motility and Gastrointestinal Hormones are Altered by Healthy Ageing.

8C.1 INTRODUCTION

As discussed in Chapter 5, there is now strong evidence that a physiological anorexia of ageing exists (5.1). There is also some evidence to suggest that the elderly consume a lesser amount of fat than young subjects (5.2), which may be due to a greater satiating effect. Moreover, in rodents, ageing is associated with increased sensitivity to the appetite suppressant effects of CCK (5.6), which is released in response to fat (1.3.2.1). Gastric emptying - which in part determines the duration over which small intestinal nutrient exposure occurs (1.2.2 & 4.2.1.2) - is also known to be slightly, but significantly, slowed by ageing (5.3). As small intestinal nutrient exposure is important in mediating short-term satiety and satiation (1.3.1), it is possible that ageing alters antropyloric motility and perception of appetite in response to small intestinal nutrients.

This study was performed to determine whether elderly subjects were more sensitive to the appetite suppressant effects of intraduodenal (ID) nutrients than young subjects, and whether ID lipid exerted a greater effect than ID glucose in the elderly as it did in the young (Chapter 8B). The gastrointestinal hormones CCK, GLP-1 and PYY were measured to determine whether reduced appetite in the elderly is related to greater release of these putative satiety factors. Antropyloric motility in response to ID lipid was measured to determine the means by which gastric emptying is slowed in the older compared to young subjects.

8C.2 METHODS

8C.2.1 Subjects

Eight healthy older males, mean age 70 years (range 65-75) and mean body mass index (BMI) 25.8 kg/m^2 (range 18.2-30) and 7 healthy "young" males, mean age 27 years (range 20-34) and mean BMI 26.8 kg/m^2 (range 24.4-31.8), were recruited by advertisement. All subjects were assessed as unrestrained eaters with a caloric intake >1500 Kcal/day, and were non-smokers. All subjects had plasma albumin measured at baseline. None had a history of gastrointestinal disease, gastrointestinal surgery, and none was taking medication known to influence gastrointestinal motility. One older subject was taking allopurinol for treatment of gout and another enalapril for hypertension, but otherwise no subject was taking regular medication at the time of the study.

8C.2.2 Experimental design

Each subject underwent paired studies, on separate days, in random order. The two study days were separated by a mean interval of 10 days (range 5-14). Before the first study day, each subject was required to keep a food diary for five successive days (three weekdays, Saturday and Sunday) to confirm that their dietary intake conformed to a "standard" Australian diet (44% carbohydrate, 34% fat and 21% protein), with a total energy requirement that was appropriate for weight (Lavin et al 1996).

The experimental protocol is summarised in Figure 8C.1. On each of the two study days, subjects attended the laboratory at 9:00 AM after a 12 hour overnight fast. Upon arrival, a silicone rubber manometric assembly (7.2.2; Figure 7.1) was inserted and positioned (7.2.1 & 7.2.3). This took ~ 20-180 min. Once the assembly was positioned correctly across the pylorus an intravenous cannula was placed in a left antecubital vein for blood sampling. At time = 0 an isocaloric (2.9 Kcal/min) ID infusion of either 25% glucose (Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) at 3 ml/min, or lipid (10% Intralipid, Kabi Pharmacia AB, Sweden) at 2.6 ml/min was commenced and continued for 120 min. The ID infusions (7.2.6) were commenced ~ 30 min after the assembly was correctly positioned. Subjects were asked to record their feelings of hunger, desire to eat and fullness on visual analogue scales (VAS) at t = -15, -5, 0, 10, 20, 30, 45, 60, 75, 90, 105, and 120 min (7.2.7).

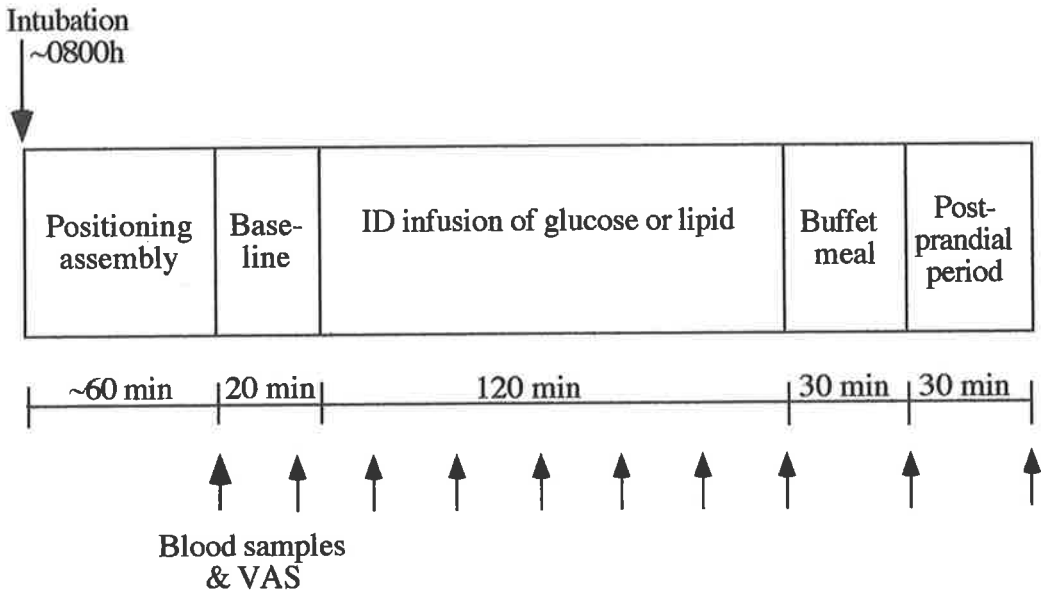


Figure 8C.1
Schema showing the experimental design for each of the two study days (section 8C.2.2). Each subject received isocaloric ID infusions of glucose and lipid for 120 min on separate days. After completion of the ID infusions, a buffet meal was offered. The timing of venous blood samples and administration of visual analogue scales (VAS) to assess desire to eat, hunger and fullness are indicated by arrows.

Antropyloric motility was recorded during the ID lipid infusion but not during ID glucose (for logistical reasons relating to the availability of recording equipment). Lipid was chosen as it had been shown to be a more potent stimulus of pyloric motility (Chapter 8B). At t = 120 min the ID infusion was ceased and the manometric catheter removed. Subjects were then presented with a cold buffet-style meal, prepared in excess of what they would normally eat, and invited to eat as much as they wished. The rate of ingestion and total amount consumed were quantified (Lavin et al 1996).

8C.2.3 Assessment of appetite

During ID infusions, appetite was assessed using VAS (7.2.7). After ID infusions, appetite was assessed from the total amount (g) of food consumed at the buffet meal (300 ml full cream milk; 300 ml unsweetened orange juice; 4 slices wholemeal bread; 4 slices white bread; 4 slices ham; 4 slices processed chicken; 4 slices cheddar cheese; 4 slices of tomato, cucumber and lettuce; 2 sachets each of mayonnaise, margarine, butter and tomato sauce; 1 pear; 1 orange; 1 banana; 1 apple; 200 g chocolate custard; 200 g strawberry yoghurt; 50 g ice cream), and the rate of food intake (Kcal/min) (Lavin et al 1996). Food intake from the meal was analysed using the DIET/1 Nutrient Calculation software (Xyris Software, Australia, Pty Ltd) to determine both the energy intake and macronutrient composition (% protein, fat and carbohydrate) (Lavin et al 1996).

8C.2.4 Measurement of plasma CCK, GLP-1 and PYY

Venous blood was collected in ice-chilled dipotassium EDTA tubes containing 400 x 10^6 IU aprotinin (Trasylol; Bayer Australia Ltd, Pymble, NSW) per litre of blood for measurement of CCK, GLP-1 and PYY. Plasma was separated by centrifugation within 30 minutes of collection and stored at -70°C until assay. Plasma CCK was measured by a radioimmunoassay technique (Jansen & Lamers 1983) using antibody T_{204}, which binds to all biologically active CCK peptides containing the sulfated tyrosine region with almost equal affinity. The detection limit of the assay was between 0.5 and 1 pmol/L in plasma. The intra-assay precision ranged from 4.6 to 11.5% in the steep part of the standard curve. All samples were assayed in the same run. Plasma GLP-1 7-36 was measured, as described in 8A.3.3. Plasma PYY was measured by a radioimmunoassay technique (Chen & Rogers 1997) using antiserum raised in rabbits immunised to the purified porcine or bovine PYY. The antiserum showed minimal to no cross reactivity to bovine or porcine pancreatic polypeptide, NPY or neurotensin.

The minimal detectable limit for the PYY radioimmunoassay was 0.3 pmol/L with intra-assay coefficients of variation of 6-11% in the working range of the assay.

8C.2.5 Measurement of antropyloric pressures

Antropyloric pressures were recorded during ID infusion of lipid as described previously (7.2.2; 7.2.3; 7.3.1). The 11 lumen sleeve/sidehole manometric assembly was as illustrated in Figure 7.1. Manometric data were only analysed when the sleeve sensor was positioned correctly across the pylorus, as defined by TMPD criteria (7.2.3), and variables analysed included:

(i) The number and amplitude of isolated pyloric pressure waves (IPPW) (7.3.2.1);

(ii) The number of antral pressure waves (7.3.2.2); and

(iii) pyloric tone (7.3.2.3).

For temporal analysis of IPPWs, recordings were divided into 10 min segments, while for analysis of pyloric tone, recordings were divided into 5 min segments. "Early" (0 - 40 min) and "late" (40 - 120 min) IPPW and pyloric tone responses, were also compared.

8C.2.6 Statistical analyses

Before ID nutrient infusions, comparisons between the young and older groups in the macronutrient content of the previous diet, scores for hunger, desire to eat and fullness, fasting plasma albumin, basal hormone (CCK, GLP-1, PYY) concentrations and BMI were performed using Student's unpaired *t*-test, as these data were normally distributed. The effects of the ID nutrient infusions on VAS, IPPWs and pyloric tone, and energy and macronutrient intake from the buffet meal in the two groups were compared using repeated measures mixed model analysis of variance (ANOVA). The effects of nutrient infusions on absolute plasma hormone concentrations were analysed using a repeated measures three-way ANOVA with time, age (young vs older) and nutrient (glucose vs lipid) as the factors, (plasma GLP-1 and PYY concentrations were log transformed before this analysis as this data was not normally distributed).

To test the hypothesis that ID lipid produced a greater CCK response in the older than young subjects, CCK data during the ID lipid infusion were expressed as the change in CCK concentrations from baseline and analysed using a repeated measures two-way ANOVA, with time and age as the factors. This ANOVA was performed using SigmaStat Statistical Software for Windows Version 1 (Jandel Corporation, San

Rafael, CA). Relationships between the changes in plasma hormone concentrations and changes in both appetite ratings and pyloric pressures were evaluated by linear regression with robust variance estimation via Mixed Model analysis to allow for repeated values in each subject (White 1980). A generalised R-square for each relationship was determined in this analysis using 'ordinary' maximum likelihood (Cox & Snell 1989). Relationships between basal and changes in plasma hormone concentrations and body weight (kg); BMI (kg/m^2); and previous energy intake (kJ) were analysed using linear regression. Differences between means of regression lines were analysed by Student's *t*-test for unpaired observations. Differences between slopes of regression lines were analysed by F-test. Data are presented as mean values ± SEM. A P value < 0.05 was considered significant in all analyses.

8C.3 RESULTS

The study protocol was generally well tolerated. One of the elderly subjects experienced severe nausea, which had a rapid onset 65 min after commencing ID lipid infusion, and on this day only data up to 60 min were included in the analysis. The manometric catheter was positioned correctly across the pylorus 90.8% of the time (90.3% in the young vs 91.4% in the older subjects). As assessed by the five-day diet diary, caloric intake was less in the older than the young subjects (1,764 ± 227 vs 2,439 ±149 Kcal; $P < 0.05$). This was predominantly due to the older men eating less during main meals than young, as there was no significant difference in the number of snacks eaten between meals in young compared with older men (1.7 ± 0.29 vs 1.4 ± 0.32, $P = 0.5$). The proportion of intake as carbohydrate was less in older than young subjects (43.3 ± 1.4 vs 48.3 ± 1.8%; $P < 0.05$), while there was no difference in the proportion of fat (37.0 ± 1.2 vs 34.3 ± 2.5%) or protein (17.1 ± 0.7 vs 15.7 ± 0.8%) intake between age groups. In all subjects plasma albumin levels were within the normal range for healthy adults (34-48 g/L), with no difference between age groups.

8C.3.1 Appetite

Before commencing the ID nutrient infusions, mean scores for both hunger (1.9 ± 0.6 vs 5.2 ± 0.8 cm; $P < 0.01$) and desire to eat (2.4 ± 0.5 vs 5.1 ± 0.7 cm; $P < 0.05$) were less in older compared to young subjects, whilst there was no difference in the baseline score for fullness (0.6 ± 0.3 vs 0.7 ± 0.4 cm) between age groups.

In young subjects there was a significant reduction from baseline in the scores for desire to eat ($P < 0.05$) and hunger ($P < 0.01$) during ID lipid infusion but not during ID glucose. In young subjects hunger also decreased during ID lipid, compared to ID glucose ($P < 0.05$). During both ID nutrient infusions, the score for fullness increased ($P < 0.05$) with no significant difference between ID glucose and lipid (Figure 8C.2).

In older subjects neither ID nutrient affected scores for hunger or desire to eat compared to baseline. In contrast, both ID glucose and lipid infusions increased fullness ($P < 0.01$ for both), with no significant difference in the magnitude of the response between nutrients (Figure 8C.2).

During ID glucose the decrease in ratings of desire to eat and hunger was greater in the young than the older subjects ($P < 0.05$ for both), whereas there was no difference in fullness ratings between age groups. During ID lipid, the decrease in desire to eat ($P < 0.05$) and hunger ($P < 0.01$) was greater in the young than the older subjects, while there was no difference in fullness ratings between age groups (Figure 8C.2).

Older and young subjects ate a similar amount (weight and calories) after the ID nutrient infusions. In both age groups, intake from the buffet meal was less following ID lipid than glucose, although this difference was only significant ($P < 0.05$) for the young (Figure 8C.3). The macronutrient content of their intake (% protein, fat and carbohydrate) was not significantly different between young and older subjects, nor between ID lipid and glucose (data not shown). There was also no effect of either age or ID nutrient type on the rate of eating (after ID glucose and lipid respectively: young 47.8 ± 7.1 and 51.7 ± 7.1; older 47.9 ± 6.6 and 43.5 ± 6.6 Kcal/min).

8C.3.2 Gastrointestinal hormones

8C.3.2.1 Plasma CCK

Before ID nutrient infusions, fasting plasma CCK concentrations were higher in the older than the young subjects (mean of both study days; 4.7 ± 0.3 vs 3.2 ± 0.2 pmol/L, $P < 0.001$), (Figure 8C.4). Three-way ANOVA revealed a significant effect of age, with higher CCK concentrations in the older than young subjects throughout the nutrient infusions (mean 0-120 min 7.8 ± 0.5 vs 5.7 ± 0.4, $F_{1,13} = 11.9$; $P < 0.01$). There was a significant effect of treatment ($F_{1,240} = 186.4$; $P < 0.001$), with a greater CCK response to lipid than glucose, and of time ($F_{9,240} = 17.7$; $P < 0.001$). The age ×

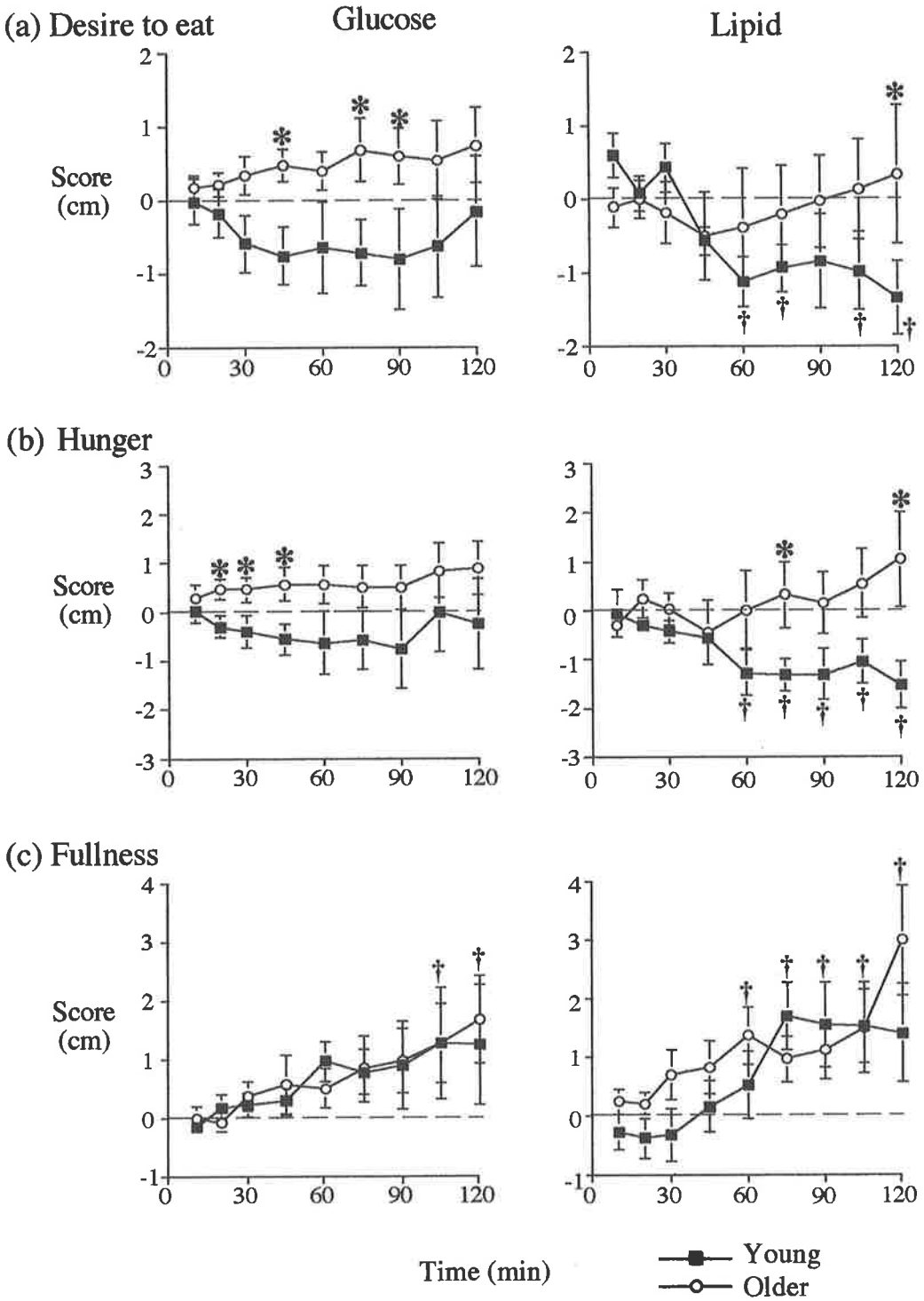


Figure 8C.2
Appetite ratings (mean +/- SEM), in young and older subjects during ID infusions of glucose (left) and lipid (right). † $P < 0.05$ cf baseline; *$P < 0.05$ young vs elderly at individual time points. (section 8C.3.1).

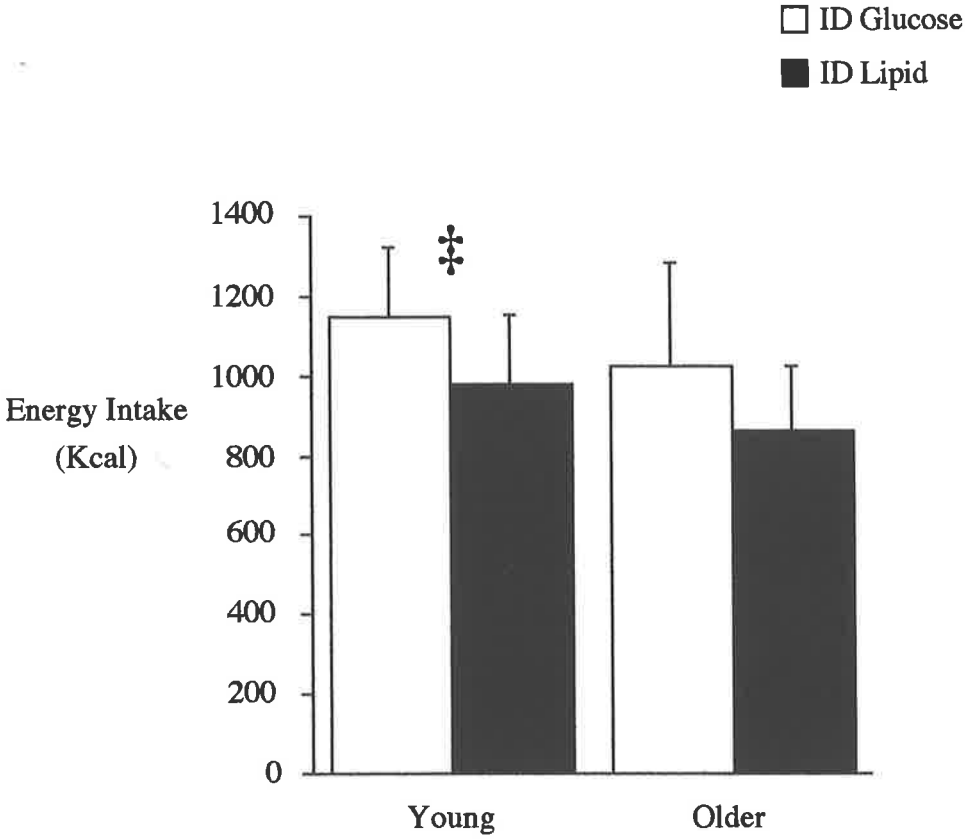


Figure 8C.3
Energy intake at buffet lunch (mean +/- SEM) after ID infusion of glucose or lipid in young and older subjects. ‡ $P < 0.05$ glucose vs lipid. (section 8C.3.1).

time interaction was not significant ($F_{9,240}$ = 1.3; P = 0.27), however there was a significant treatment × time effect ($F_{9,240}$ = 21.8; P < 0.001). There was no significant age × treatment × time interaction ($F_{9,240}$ = 1.13; P = 0.35), indicating that the timing of the increase in CCK concentrations was not significantly different between older and young subjects. The age × treatment interaction was not significant ($F_{1,240}$ = 3.13; P = 0.078). However, analysis of the CCK response to ID lipid infusion alone, by two-way ANOVA, indicated a significant effect of age as a result of a greater increase in CCK concentrations in older than young subjects (mean increase from baseline, 10-120 min. 7.1 ± 0.5 vs 5.3 ± 0.6 pmol/L; P = 0.048, Figure 8C.5). Neither basal CCK concentrations, nor response to ID nutrients, were related to body weight, BMI or previous energy intake in either age group.

8C.3.2.2 Plasma GLP-1

Baseline plasma GLP-1 concentrations were not significantly different between older and younger subjects (9.2 ± 1.3 vs 6.9 ± 0.9 pmol/L; P = 0.17, Figure 8C.4). By three-way ANOVA, there was no significant effect of age ($F_{1,13}$ = 0.79; P = 0.39), however there was a significant effect of treatment ($F_{1,240}$ = 14.1; P < 0.001), with higher GLP-1 concentrations during ID lipid than glucose infusion; and of time ($F_{9,240}$ = 93.5; P < 0.001). Age × treatment and age × time interactions were not significant ($F_{1,240}$ = 0.16; P = 0.69; $F_{9,240}$ = 0.59; P = 0.80, respectively). However, there was a significant treatment × time effect ($F_{9,240}$ = 2.47; P < 0.05), with plasma GLP-1 concentrations peaking at 60 min during lipid infusion but rising throughout ID glucose. There was no significant age × treatment × time interaction ($F_{9,240}$ = 0.60; P = 0.79), indicating that the timing of the increase in GLP-1 was not significantly different between age groups. Basal GLP-1 concentrations were not related to body weight, BMI or previous energy intake in either group. However, the mean change in GLP-1 concentration during ID glucose infusion was inversely related to BMI (kg/m^2) (F = 7.0; P = 0.046) and weight (kg) (F = 13.8; P = 0.014) in younger subjects.

8C.3.2.3 Plasma PYY

Before ID glucose and lipid infusions, there was no significant difference in fasting plasma PYY concentrations between older and younger subjects (11.3 ± 0.6 vs 10.5 ± 0.6 pmol/L, P = 0.13, Figure 8C.4). There was no significant effect of age ($F_{1,13}$ = 0.01; P = 0.94), however there was a significant effect of treatment ($F_{1,240}$ = 491.5; P < 0.001), with greater plasma PYY concentrations during ID lipid than glucose, and of

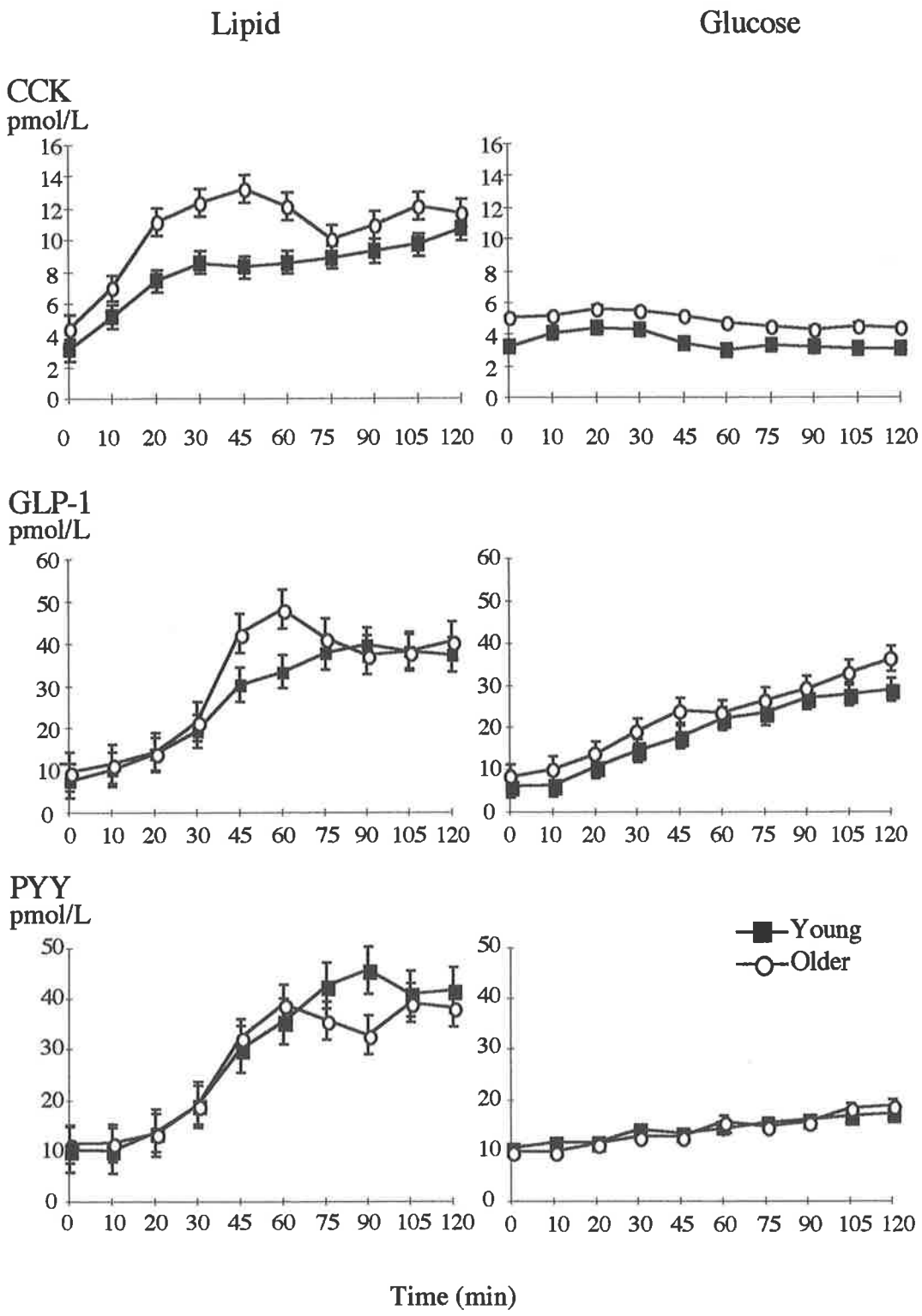


Figure 8C.4
CCK, GLP-1 and PYY (mean +/- SEM) response to ID lipid and glucose infusion, in young and older subjects. (section 8C.3.2).

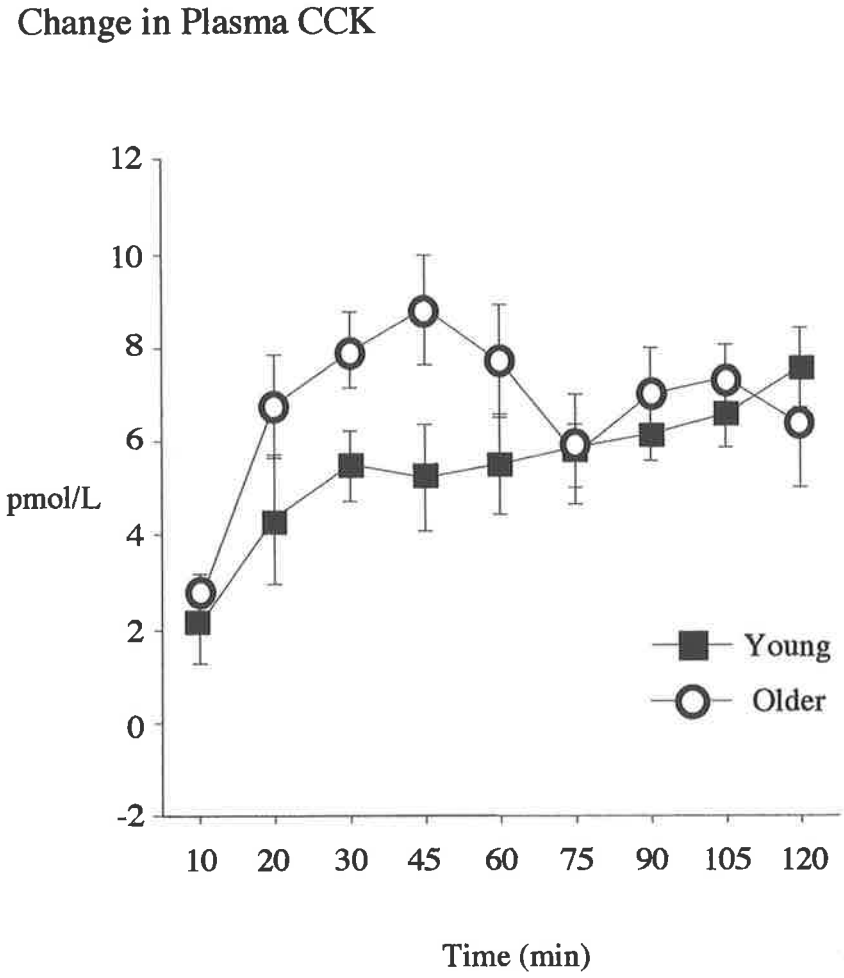


Figure 8C.5
Change in plasma CCK (mean +/- SEM) during ID lipid infusion in young and older subjects. The mean increase in older subjects was greater than in young subjects (P = 0.048). (section 8C.3.2).

time ($F_{9,240}$ = 78.9; P < 0.001). Age × treatment and age × time interactions were not significant ($F_{1,240}$ = 0.11; P = 0.74 and $F_{9,240}$ = 1.03; P = 0.41, respectively). But there was a significant treatment × time effect ($F_{9,240}$ = 23.6; P < 0.001), with the plasma PYY concentrations peaking earlier during ID lipid than glucose. There was no significant age × treatment × time interaction ($F_{9,240}$ = 0.60; P = 0.793), indicating that the timing of the increase in PYY was not significantly different between age groups. Neither the basal PYY concentrations, nor the response to ID nutrients, were related to body weight, BMI or previous energy intake in either young or older subjects.

8C.3.3 Antropyloric motility

8C.3.3.1 Antral pressure waves

There were no antral pressure waves after the onset of ID lipid in either age group.

8C.3.3.2 Isolated pyloric pressure waves (IPPW)

In both groups, ID lipid infusion stimulated an increase in the frequency (P < 0.01) and amplitude (P < 0.03) of IPPWs. The frequency of IPPWs was greater (P < 0.05) in the older than young subjects by ANOVA on the whole curves (Figure 8C.6). Whilst the amplitude of IPPWs was greater at every time point in the older than young subjects (mean 55.5 ± 10.3 vs 37.1 ± 3.3 mmHg), this difference was not significant when the whole curves were analysed by ANOVA. In both the young and older subjects, both the frequency and amplitude of IPPWs decreased over time (P < 0.05 for both), after a peak response at ~ 50 min.

8C.3.3.3 Pyloric tone

Before ID lipid infusion there was no difference in pyloric tone between the young and older subjects. In both young and older subjects there was an initial increase (P < 0.01 for both) in pyloric tone during ID lipid, and the overall response was not significantly different between age groups (Figure 8C.7). In both groups there was attenuation (P < 0.05 for both) of pyloric tone over time from a peak at ~ 30-40 min. Although the "late" response was less in the elderly than the young, this difference was not statistically significant (P = 0.16).

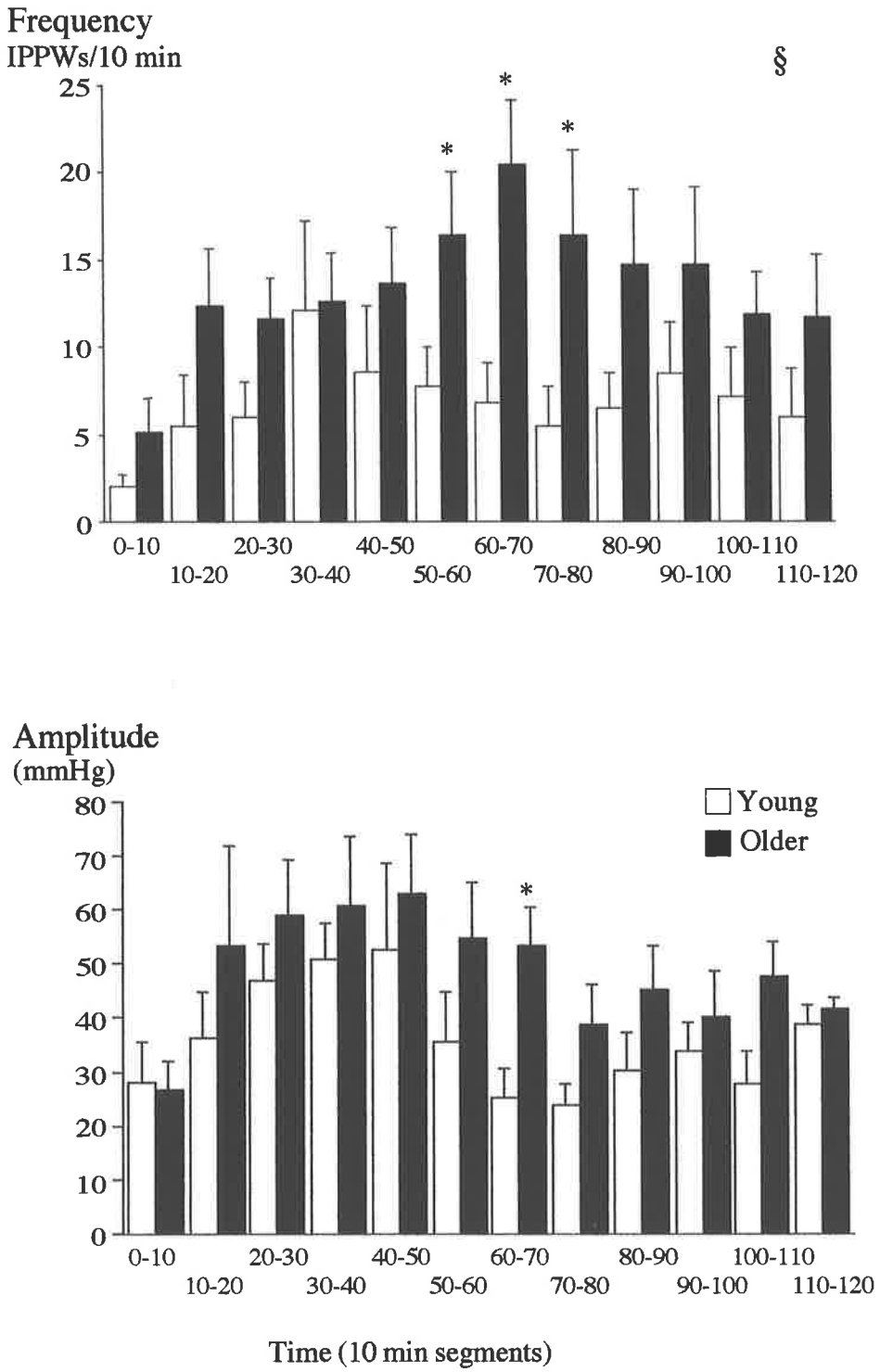


Figure 8C.6
Frequency (above) and amplitude (below) of phasic pyloric pressure waves (IPPW) during ID lipid infusion in young and elderly subjects. § $P < 0.05$ young vs older for whole curve; * $P < 0.05$ young vs older at individual time points. Data are mean ± SEM. (section 8C.3.3.2).

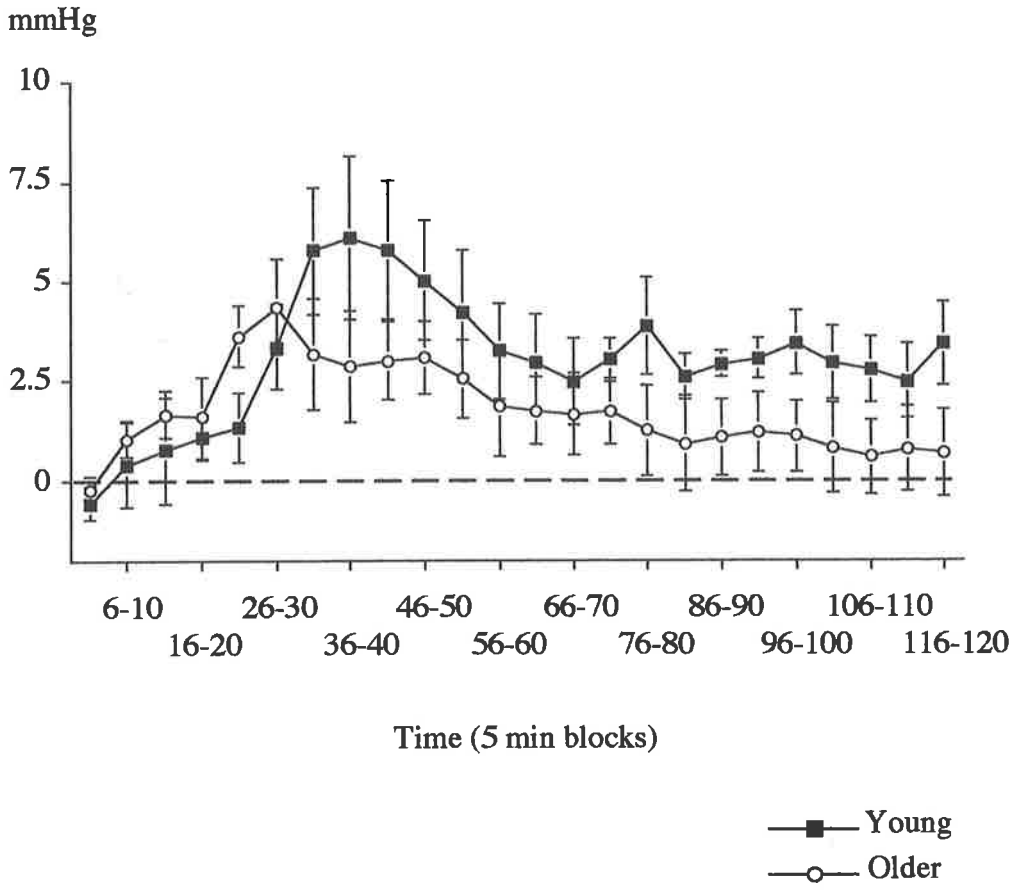


Figure 8C.7
Pyloric tone (tonic pyloric pressure) expressed as change from baseline during ID lipid infusion in young and elderly subjects. By ANOVA, $P < 0.05$ for each curve vs baseline. Data are mean ± SEM. (section 8C.3.3.3).

8C.3.4 Relationships between gastrointestinal hormones and appetite

8C.3.4.1 CCK

During ID lipid infusion the change in hunger ratings was inversely related to the change in plasma CCK concentrations in the young ($R^2 = 0.064$; P= 0.030), but not the older (P = 0.71), subjects. There was no significant relationship between the change in fullness and the change in plasma CCK concentrations in either younger (P = 0.32) or older (P = 0.095) subjects. During ID glucose infusion there was no significant relationship between the change in ratings of either hunger (young P = 0.73, older P = 0.60) or fullness (young P = 0.90, older P = 0.51) and the change in plasma CCK.

8C.3.4.2 GLP-1

During ID lipid infusion the change in hunger ratings was inversely related to the change in plasma GLP-1 in the young (R^2= 0.16; P = 0.0004), but not the older (P = 0.58) subjects. There was also a significant relationship between the increases in fullness and plasma GLP-1 concentrations in both the young (R^2= 0.22; P = 0.0001) and older (R^2= 0.21; P = 0.0001) subjects. During ID glucose, there was no significant relationship between the change in hunger and the change in plasma GLP-1 in either age group (young P = 0.15; older P = 0.19) and the change in fullness was related to the change in plasma GLP-1 in the older (R^2= 0.17; P = 0.0004), but not the young (P = 0.23) subjects.

8C.3.4.3 PYY

During ID lipid the change in hunger ratings was inversely related to the change in plasma PYY in the young (R^2= 0.27; P = 0.0001), but not the older subjects (P = 0.81). There was also a significant relationship between the change in fullness and the change in plasma PYY in both age groups (young R^2= 0.29, P = 0.0001; older R^2= 0.29, P = 0.0001). During ID glucose there was a significant inverse relationship between the change in hunger and the change in plasma PYY in the young (R^2= 0.12; P = 0.003) but not the older subjects (P = 0.84). There was a significant relationship between the change in fullness and the change in plasma PYY in both the young (R^2= 0.15; P = 0.0005) and older (R^2= 0.24; P = 0.0001) subjects.

8C.3.5 Relationships between gastrointestinal hormones and pyloric pressures

8C.3.5.1 CCK

There was no significant relationship between the increase in the frequency of IPPWs and the increase in plasma CCK during ID lipid infusion in either the young (P = 0.99) or the older subjects (P = 0.31). There was, however, a positive relationship between the increase in the amplitude of IPPWs and the increase in plasma CCK concentrations in the older subjects, which did not quite reach significance (R^2= 0.04; P = 0.052). In contrast, this tended to be a (non-significant) 'inverse' relationship in the young (P = 0.15). There was a significant inverse relationship between the increase in pyloric tone and the increase in plasma CCK concentrations during ID lipid infusion in the young (R^2= 0.076; P = 0.020), but not the older (P = 0.63) subjects.

8C.3.5.2 GLP-1

There was a positive relationship between the increase in the frequency of IPPWs and the increase in plasma GLP-1 during ID lipid in the older (R^2= 0.087; P = 0.009), but not the young (P = 0.60) subjects. In contrast, there was an inverse relationship between the increase in amplitude of IPPWs and the increase in plasma GLP-1 in the young (R^2= 0.14; P = 0.0001), but not the older (P = 0.28) subjects. There was an inverse relationship between the increase in pyloric tone and the increase in plasma GLP-1 concentrations in young (R^2= 0.044; P = 0.023), but not the older subjects (P = 0.093).

8C.3.5.3 PYY

There was a positive relationship between the increase in the frequency of IPPWs and the increase in plasma PYY during ID lipid infusion in the older (R^2= 0.090; P = 0.009), but not the young (P = 0.25) subjects. There was an inverse relationship between the increase in amplitude of IPPWs and the increase in plasma PYY in the young (R^2= 0.10; P = 0.001), but not the older (P = 0.88), subjects. Similarly, there was an inverse correlation between the increase in pyloric tone and increase in plasma PYY in the young (R^2= 0.070; P = 0.020), but not the older (P = 0.51) subjects.

None of the intercepts or slopes of the lines for these regression calculations of plasma CCK, GLP-1 and PYY vs appetite or pyloric motility differed between age groups.

8C.4 DISCUSSION

This study has demonstrated that the effects of isocaloric ID infusions of lipid and glucose on appetite and pyloric motility are different in healthy older compared to young men. The major findings are that:
(i) older men are less hungry and have less desire to eat after an overnight fast and eat less compared with young men in their usual environment. But consume a similar amount to young men when presented with a prepared meal;
(ii) In contrast to the response in younger men, ID lipid does not have a greater subjective appetite suppressant effect than glucose in healthy older men;
(iii) ID lipid causes greater increases than ID glucose of CCK, GLP-1 and PYY in both age groups;
(iv) older men have higher basal and stimulated CCK levels than young men;
(v) the stimulation of phasic, but not tonic, pyloric pressures by ID lipid is greater in older than young men.

These results show that (a) subjective perception of appetite, in response to small intestinal nutrients, is impaired in older persons; (b) the greater appetite suppressant effect of ID lipid in the young may result from its greater release of gastrointestinal hormones; (c) the slower gastric emptying reported in older persons may result from increased nutrient-mediated small intestinal feedback on phasic pyloric motility, and (d) despite decreased subjective feelings of hunger, actual intake in older males is no different to young males when presented with a pre-prepared meal. Taken together, these findings suggest that the physiological anorexia of ageing (section 5.2) does not primarily result from enhanced sensitivity to the appetite suppressant effects of small intestinal nutrients, although small intestinal nutrients may contribute, by slowing gastric emptying (1.2.2 & 4.2.1.2).

That both energy intake in their usual diet and hunger before a meal were less in healthy elderly compared to young subjects is consistent with previous observations (Clarkson et al 1997; Rolls et al 1995; Wurtman et al 1988a & b) and compatible with the concept of a physiological anorexia of ageing (Chapter 5). The diets of older subjects are also reported to be more monotonous than those of younger adults (Morley 1996), which is consistent with age-related impairment in taste (5.7.1) and reduction in so-called "sensory-specific satiety" in healthy older persons (1.2.1 & 5.7.1).

The rate of ID delivery of nutrients used in this study (2.9 Kcal/min) although slightly greater than the usually quoted rate of gastric emptying (~2-2.5 Kcal/min) of glucose (Horowitz & Dent 1991) and lipid (Horowitz et al 1993), was selected as discussed in 8B.4.1. The inclusion of a control non-nutrient (saline) ID infusion would have allowed the effects of each nutrient to be evaluated separately but would have required three study days, which was considered not to be feasible in the older subjects. Moreover (as discussed in 8B.4.1) this caloric rate has previously been shown to reduce appetite in healthy young volunteers (Lavin et al 1996; Read et al 1994; Welch et al 1985), with a progressive increase in scores for hunger and desire to eat evident during ID saline infusion (Lavin et al 1996). Therefore, it is reasonable to assume that both nutrient infusions would cause a decrease in hunger in the young subjects. Only males were included in this study because they appear to have the greatest capacity to regulate intake in response to energy manipulation (Rolls et al 1995) and these observations may not necessarily apply to females. The small number of subjects raises the possibility of Type 2 statistical errors; eg older subjects ate less after ID lipid than ID glucose, and pyloric tone was lower during ID lipid in the older subjects but these differences did not reach significance, although they appear likely to be real effects. That the effect of ID lipid infusion on hunger was substantially less in the elderly than the young may potentially result from the lower baseline scores for both hunger and desire to eat in the elderly before commencement of the ID infusions. However, this seems unlikely since there was no suggestion of a reduction in hunger ratings during either ID nutrient infusion in the elderly. In both age groups there was an increase in ratings of fullness during the ID nutrient infusions, supporting the idea that perceptions of hunger and fullness are mediated by different means (Read et al 1994; section 1.3.1).

8C.4.1 Appetite

There is a lack of consensus as to whether fat and carbohydrate exert different effects on appetite (section 1.3.1). In this study, we confirm that ID lipid has a greater suppressive effect on hunger and subsequent food intake than an isocaloric ID glucose load in young males (Chapter 8B). In contrast, a differential effect of the two nutrients on appetite was not evident in older persons, although intake at the buffet meal was no different in the older compared to the young subjects and tended to be less after ID lipid. These results are compatible with the concepts that the homeostatic mechanisms

which regulate appetite and body weight are impaired in older persons (Roberts et al 1994; Rolls et al 1995) and that subjective feelings of appetite do not correlate closely with subsequent food consumption (Sepple & Read 1989). Our findings suggest that the presence of small intestinal nutrients alone does not play a major role in the subjective perception of the anorexia of ageing.

8C.4.2 Gastrointestinal hormones

In both young and older men, both ID lipid and glucose increased CCK, GLP-1 and PYY concentrations, and lipid increased CCK, GLP-1 and PYY concentrations more than glucose. Older men had higher fasting CCK and greater increases in CCK during lipid infusions than younger men, whilst there was no effect of ageing on plasma GLP-1 or PYY concentrations, either during fasting or ID nutrient infusions. ID nutrient administration bypasses a number of gastric mechanisms (especially variations in the rate of gastric emptying) which potentially affect secretion of these hormones. Therefore, it cannot be assumed that plasma CCK concentrations would be higher in older than young subjects after oral or intragastric nutrients.

Previous studies of the effect of ageing on plasma CCK concentrations (5.6) have produced conflicting results. In rodents, the satiating effects of CCK are greater with ageing (Silver et al 1988). In humans, Masclee et al (1988) found CCK concentrations to be higher in older subjects following ID fat infusion, but no difference in fasting CCK concentrations between the two age groups. Berthelemy et al (1992) reported no difference in CCK concentrations, either fasting or following a liquid meal, between well nourished young and older people, but that postprandial CCK concentrations were higher in malnourished older subjects. This is consistent with evidence that suboptimal energy intake and/or reduced body weight may be associated with elevated CCK (Tamai et al 1993; Pirke et al 1994; Naslund et al 1997b) . In the current study, differences in weight were not responsible, however, as the BMIs of the two age groups did not differ. Qualitative and quantitative differences in previous diet are also unlikely to have contributed to the increased CCK concentrations in the elderly as (i) the diet of the older subjects had only a slightly lower carbohydrate composition, while the percentage fat and protein were similar across age groups; (ii) no subjects were malnourished according to the criteria of Berthelemy et al (1992) [ie energy intake less than 4184 kJ/day and body weight more than 20% below ideal and/or plasma albumin

concentration less than 20 g/L] and (iii) there was no relationship between plasma CCK and daily energy intake.

There is circumstantial evidence that CCK is a physiological satiety factor (1.3.2.1). Administration of exogenous CCK to healthy adults increases sensations of fullness and reduces sensations of hunger and subsequent food intake (Kissileff et al 1981; Stacher et al 1982; Shaw et al 1985; Muurahainen et al 1988). Many reports of CCK-induced satiety involve studies with plasma CCK concentrations above physiological post-prandial levels. Nevertheless, Lieverse et al (1995b) have reported suppression of food intake by exogenous CCK at physiological post-prandial plasma CCK concentrations. Additionally, food intake is suppressed in both rats (McLaughlin et al 1983; Garlicki et al 1990) and humans (Hill et al 1990) by administration of an inhibitor of trypsin, a compound which inhibits release of endogenous CCK. Whilst the finding here of elevated CCK in the elderly suggests increased endogenous CCK activity to be involved in the anorexia of aging, the fact that hunger ratings were not suppressed and no correlation existed between CCK and hunger ratings in older men (cf the situation in young men) (consistent with French et al 1993), during ID lipid suggests otherwise. These results, in fact, provide indirect evidence that human ageing may be associated with reduced sensitivity to CCK's satiating effects.

GLP-1 may also function as a satiety factor (1.3.2.4 & Chapter 8A). As ID glucose infusion is not a significant stimulus for CCK release, the observation that suppression of hunger by ID glucose is abolished by administration of octreotide (Chapter 8A), implies that gastrointestinal hormones other than CCK are important in mediating the effects of small intestinal glucose on appetite (Lavin et al 1996). In the current study, GLP-1 concentrations increased more during ID lipid than glucose in both age groups. The lack of a significant difference between plasma GLP-1 concentrations in young and older subjects under any study condition makes it unlikely that changes in circulating GLP-1 contribute to the reduced appetite accompanying normal ageing.

Fat or unabsorbed nutrients in the intestine stimulate PYY release by luminal cells (Pironi et al 1993; Chen & Rogers 1997). In animals, feeding is stimulated by central PYY administration (Corp et al 1990), but unaffected by intraperitoneal administration (Garlicki et al 1990). The role, if any, of PYY in the control of human feeding is unknown. The effects of ageing on plasma PYY concentrations have, hitherto, not been reported. Plasma PYY concentrations, did not differ between older and young

subjects in the present study, indicating that changes in circulating PYY concentrations are also unlikely to contribute to the anorexia of aging.

8C.4.3 Pyloric pressures

There is considerable controversy as to the effects of healthy aging on gastrointestinal function (see Chapter 5), although it is generally accepted that healthy aging is associated with a modest slowing of the rate of gastric emptying (Horowitz et al 1984; Wegener et al 1988; Clarkson et al 1997). The effects of small-intestinal nutrients on gastroduodenal motility are described in Chapter 4 and, consistent with this, ID lipid stimulated IPPWs and abolished all antral contractions. The increase in frequency and amplitude of IPPWs during lipid infusion was greater in older than young subjects. Whilst it is unknown whether the tonic or phasic pyloric response is more important in mediating the slowing of gastric emptying (Tougas et al 1992), in the young, pyloric tone appears to be of substantial importance (Chapter 8B). However, the greater phasic pyloric response to ID lipid in older men found here provides a possible mechanism for the slower gastric emptying observed with ageing (see above). Pyloric tone in response to ID lipid was not initially different between age groups and may, in fact, be less in the elderly. A discordance between the tonic and phasic response of the pylorus has previously been reported in humans (8B.4.2). The increased stimulation of IPPWs by ID lipid in the elderly may reflect a reduced activity of inhibitory neural pathways (Lovat 1996). There was a non-significant trend for the attenuation of the tonic pyloric response to be more marked in the elderly and, although the number of subjects studied was small, attenuation of both the phasic and tonic pyloric motor responses during prolonged ID nutrient infusion has been demonstrated previously in young, normal volunteers, albeit at lower rates of ID nutrients (8B.4.2). Evaluation of the effects of ageing on other motor mechanisms which play a role in the regulation of gastric emptying, such as fundic motility, would also be of interest. Further studies evaluating fundal behaviour would be of substantial interest, particularly in view of the possibility of fundal impairment with ageing (5.3 & 5.6).

8C.4.4 Relationships among CCK, GLP-1, PYY and appetite and pyloric pressures

Previous studies have demonstrated that CCK (Scarpignato et al 1981; Muurahainen et al 1988), GLP-1 (Young et al 1996; Schirra et al 1997) and PYY (Allen et al 1984; Savage et al 1987) are each associated with the slowing of gastric emptying. CCK

(Fraser et al 1993) and GLP-1 (Schirra et al 1997) appear to stimulate IPPWs and inhibit distal antral contractions, while PYY inhibits interdigestive migrating contractions in the small intestine (Lin et al 1996c). The finding that all three hormones increased to a greater degree during ID lipid than during ID glucose is consistent with previous results for CCK (Raybould & Lloyd 1994), but contrasts with previous reports that plasma GLP-1 (Layer et al 1995) and PYY (Pedersen-Bjergaard et al 1998) increase equally in healthy men after ingestion of high-carbohydrate and high-fat meals. For PYY, this discrepancy could reflect oro-sensory or gastric mechanisms augmenting its release.

Interpreting the relationships found among gastrointestinal hormones, appetite and pyloric pressures is somewhat problematic and it is likely that several of them result from coincident occurrence of small-intestinal nutrient-mediated effects, rather than representing causal relationships. For example, it would seem unlikely that CCK causes a decrease in pyloric tone in the young, despite an inverse relationship found between Δ tone and Δ CCK during ID lipid. Findings of potential importance include (i) the increases in plasma CCK were positively related to the increases in IPPW amplitude in the older subjects, although this relationship did not quite achieve statistical significance; (ii) a positive relationship between lipid-induced plasma GLP-1 and PYY concentrations and frequency of IPPWs in older subjects; and (iii) the lack of a relationship between CCK or GLP-1 and hunger in the elderly, whilst inverse relationships existed in the young subjects. The small subject number of subjects in this study makes it difficult to draw strong conclusions from these relationships. Nevertheless, they suggest that the enhanced stimulation of pyloric motility in the older subjects by ID lipid may be due to increased CCK and/or increased sensitivity to the stimulating effects of CCK, GLP-1 and PYY on phasic pyloric motility, whilst the elderly seem less sensitive to the appetite suppressant effects of CCK and GLP-1 seen in the young.

8.2 CONCLUSION

This group of studies examining interactions between small intestinal nutrients, appetite and motility have provided many novel observations, and confirmed some results from other investigators. In particular, the studies described in Chapter 8A confirm that ID glucose suppresses appetite and that the effect is dependent on the release of gut peptides. They demonstrate that insulin, at levels slightly above the upper postprandial range, does not inhibit its own release and also support the concept that GLP-1, rather than insulin or GIP, is likely to be involved in mediating the suppression of appetite caused by ID glucose.

In Chapter 8B, it was demonstrated that, in humans, nutrients of different macronutrient classes yield a differential response in terms of appetite regulation and pyloric motor function. It was also established that both the appetite signals generated by, and motor responses to, these nutrients are altered by dietary manipulation. The adaptation in perception of appetite occurred across macronutrient class, whilst the adaptation of the motor response was more "nutrient-specific".

In Chapter 8C, substantial differences in perception of appetite, gastrointestinal hormones and pyloric motility between young and elderly subjects were demonstrated. It had been expected that these motor and sensory changes might be related, in view of the evidence linking the rate of gastric emptying to satiation. Therefore, the observation that ID nutrients had a lesser effect on perception of appetite, but a greater effect on the stimulation of phasic pyloric motility in the elderly was somewhat unexpected. On this basis, it now seems unlikely that the physiological anorexia of ageing primarily reflects increased subjective sensitivity to small intestinal nutrients, although, increased motor sensitivity to small intestinal nutrients does appear to be present, as evidenced by the increased phasic pyloric activity. Whilst healthy ageing was associated with increased fasting and lipid-induced plasma CCK concentrations, this did not coincide with decreased appetite. The results actually suggest that resistance to the appetite-suppressant effects of CCK may occur with ageing. These data suggest that the slowing of gastric emptying (perhaps via increased pyloric activity), rather than small intestinal nutrient exposure, may be the more important determinant of the physiological anorexia of aging.

CHAPTER 9

Effects of Physiological Hyperglycaemia on Gastric Motility, Compliance and Perception

Physiological Hyperglycaemia, Antropyloric Motility & Appetite

Physiological Hyperglyceamia, Proximal Gastric Compliance & Perception Of Distension

9.1 INTRODUCTION

As discussed in Chapter 4 (4.2.2), studies show that acute changes in blood glucose concentration affect proximal gastrointestinal motor function. In particular, elevation of blood glucose to pathological levels (12-16 mmol/L) has been shown to decrease peristaltic velocity in the oesophagus (De Boer et al 1992a), slow gastric emptying (Fraser et al 1990), inhibit antral motility (Barnett & Owyang 1988; Fraser et al 1991; Hasler et al 1995; Samsom et al 1997), stimulate pyloric motility (Fraser et al 1991), increase proximal gastric compliance (Hebbard et al 1996a) and prolong small intestinal transit time (Russo et al 1996). Moreover, "physiological" changes in blood glucose (in the normal postprandial range: 8-11 mmol/L) have also been shown to affect some gastrointestinal motor functions, specifically to accelerate peristaltic velocity in the oesophagus (Boeckxstaens et al 1997), slow gastric emptying (MacGregor et al 1976; Schvarcz et al 1997), and alter fasting gastric motility (Barnett & Owyang 1988).

Pathological hyperglycaemia modulates the perception of distension- and nutrient-induced sensations arising from the gut (4.3.2). While oesophageal sensation varies with modest elevation of blood glucose ~8 mmol/L (Boeckxstaens et al 1997), it is unknown whether physiological hyperglycaemia affects gastric sensation. Controversy also exists as to whether physiological hyperglycaemia affects appetite (4.4). Small intestinal nutrients modify a number of gut functions such that gastrointestinal motility, and perception of appetite (Chapter 8C; Lavin et al 1996) and distension (Feinle et al 1996) are all substantially different in the fasted as compared to the fed states.

The following studies (9A & 9B), were performed to evaluate more fully the effects of physiological hyperglycaemia on gastric motor and sensory function, including appetite.

CHAPTER 9A

Physiological Changes in Blood Glucose Concentrations Affect Appetite and Antropyloric Motility in the Fasted State and During Intraduodenal Lipid Infusion.

9A.1 INTRODUCTION

By virtue of its position at the gastric outlet, the pylorus plays a crucial role in determining the rate of gastric emptying (6.4). During fasting, marked hyperglycaemia increases phasic pyloric motility (Fraser et al 1991). The effect of more modest "physiological" elevation of blood glucose on pyloric motility is unknown. Small intestinal nutrients may modulate the pyloric motor response to physiological hyperglycaemia as gastric emptying of saline, as compared to nutrients, is unaffected by hyperglycaemia (MacGregor et al 1976). As discussed in 9.1, the effect of physiological hyperglycaemia on appetite is unknown. Therefore, this study was performed to determine whether physiological hyperglycaemia affects antropyloric motility, or perception of appetite; and whether small intestinal nutrients (of a different macronutrient class) modify these effects

9A.2 MATERIALS AND METHODS

9A.2.1 Subjects

Ten healthy males, aged 19 - 40 years (mean 24 years), body mass index 22.5 - 29.6 kg/m^2 (mean 26.8 kg/m^2), were recruited by advertisement. None had any history of gastrointestinal disease or was taking any medication.

9A.2.2 Experimental design

Subjects attended the laboratory on two separate days at 9 AM after an overnight fast. On one day the blood glucose was maintained at 5 mmol/L, and on the other 8 mmol/L. The two studies were performed in random order, in single blind fashion. The protocol is summarised in figure 9A.1. On arrival, subjects were intubated, as described in 7.2.1, with an 11 lumen silicone rubber manometric assembly as in 7.2.2

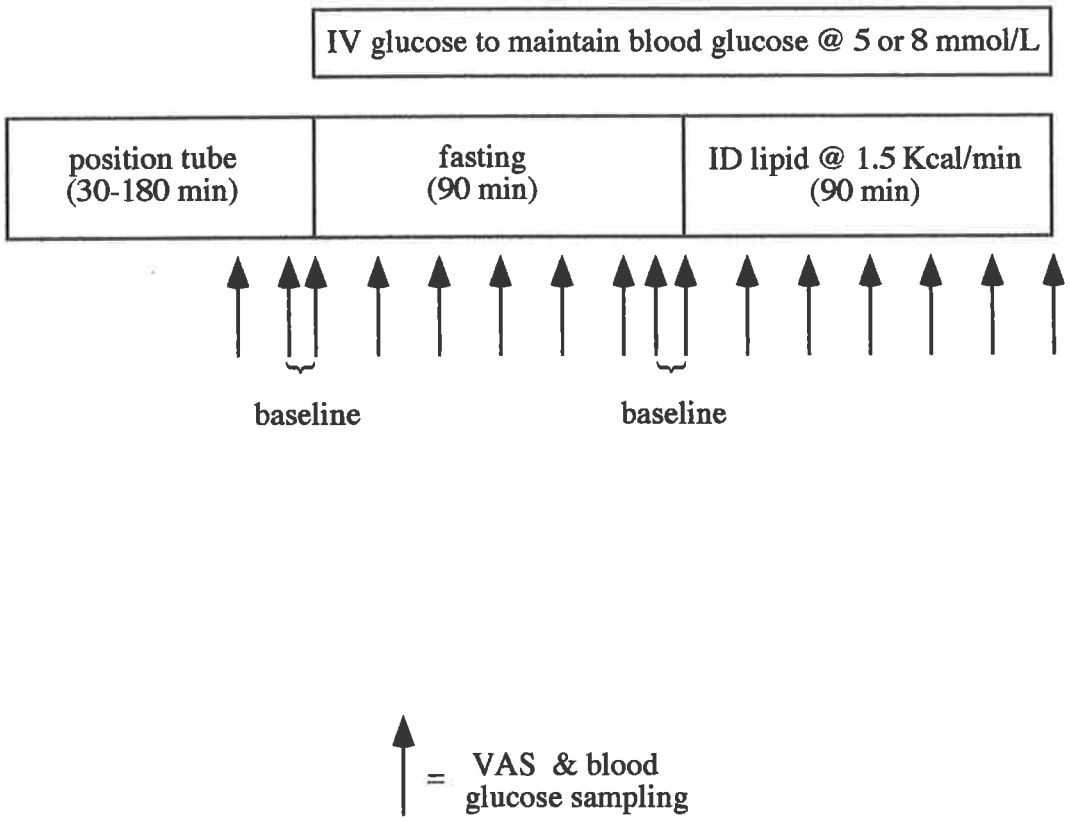


Figure 9A.1
Protocol for each of the two study days, see text for detail (9A.2.2).

(Figure 7.1). The assembly was positioned and its position verified (7.2.3). Intraluminal pressures were recorded as described earlier (7.3.1).

Once the manometric assembly was correctly positioned, an intravenous (IV) cannula was inserted in each arm; in the right forearm for administration of 25% glucose or normal saline (Baxter Healthcare, Old Toongabbie, NSW, Australia); and in the left cubital fossa for sampling of venous blood. At time (t) = -15 min each subject was familiarised with the visual analogue scales (VAS) (7.2.7) which were used to assess appetite. At t = 0 min, an IV infusion of either 25% glucose or normal saline was commenced, in a single-blind fashion, and continued at a rate to maintain the blood glucose at either ~5 mmol/L or ~8 mmol/L. Venous blood glucose was measured every 5 - 15 min with a portable glucometer (MediSense 2, Balwyn, Vic, Australia) and the infusion rate of IV glucose adjusted accordingly. At t = 90 min an ID infusion of lipid emulsion (10% lipid, Intralipid, Kabi Pharmacia AB, Sweden) was commenced at 1.5 Kcal/min (82 mls/hr) and continued for 90 min.

9A.2.3 Outcome measures

9A.2.3.1 Appetite

Appetite was assessed using VAS as described in 7.2.7. VAS were administered at t = -5, 0, 15, 30, 45, 60, 75 and 90 min during both the fasted state and the ID lipid infusion.

9A.2.3.2 Antropyloric Pressures

Antropyloric pressures were only analysed (7.3.2) when the sleeve sensor was correctly positioned according to established TMPD criteria (Heddle et al 1988b). For clarity, pressures occurring in the two 90 min periods (during fasting, and ID lipid infusion) are reported separately. Variables analysed include:

(i) the number of antral pressure waves (7.3.2.2);

(ii) the number, frequency (in ten minute segments) and amplitude of isolated pyloric pressure waves (IPPWs) (7.3.2.1); and

(iii) pyloric tone (7.3.2.3). Mean pyloric tone in the 5 min prior to commencing the blood glucose clamp was taken as the basal pyloric tone for the fasting state and tone during the 5 min prior to commencing ID lipid infusion was taken as the basal pyloric tone for this segment of the study. Tone is then presented as change from each of these baselines.

9A.2.4 Statistical analysis

Statistical analysis was performed as described earlier (7.3.3).

9A.3 RESULTS

Of the 10 subjects recruited, 2 declined to return for the second study day, their data are not included in the analysis. The other 8 subjects completed the protocol, with both study days being completed within a mean of 12 (range 7 - 19) days. No subject had significant nausea, nor vomited on the day on which the blood glucose was 5 mmol/L. In contrast 3 subjects had troublesome nausea on the 8 mmol/L day, with 2 withdrawing 61 mins into the ID lipid infusion, and a third vomiting after 90 min at extubation. In all 3 subjects the onset of nausea was sudden and data from time points when significant nausea was present are not included (apart from in ratings of nausea). Blood glucose concentrations closely approximated the desired levels (Figure 9A.2).

9A.3.1 Appetite

During the 90 min fasting period (Figure 9A.3), there were no significant changes in hunger, desire to eat or projected consumption when compared to baseline. Neither were there any differences between a blood glucose of 5 and 8 mmol/L (hunger $P = 0.15$, desire to eat $P = 0.96$, projected consumption $P = 0.92$). In contrast, fullness was greater at a blood glucose of 8 mmol/L than at 5 mmol/L ($P = 0.01$), although at neither blood glucose level was fullness significantly different from baseline. No nausea was reported during fasting.

During the 90 min ID lipid infusion, (Figure 9A.4), positive symptoms of appetite decreased from baseline at a blood glucose of 8 mmol/L, (hunger $P = 0.034$, desire to eat $P = 0.0001$, projected consumption $P = 0.0002$), whereas, at a blood glucose of 4 mmol/L they were no different from baseline. Consequently, during ID lipid, positive symptoms of appetite were less at a blood glucose of 8 than 5 mmol/L, (hunger $P = 0.02$, desire to eat $P = 0.04$, projected consumption $P < 0.001$). Fullness, however, increased at a blood glucose of 5 mmol/L when compared to baseline ($P = 0.003$), whilst remaining unchanged at a blood glucose of 8 mmol/L, so that the change in fullness during ID lipid was greater at a blood glucose of 5 compared to 8 mmol/L ($P < 0.001$). This latter result is partly accounted for by the fact that fullness did not change between fasting and the period of ID lipid infusion at a blood glucose of 8 mmol/L (P

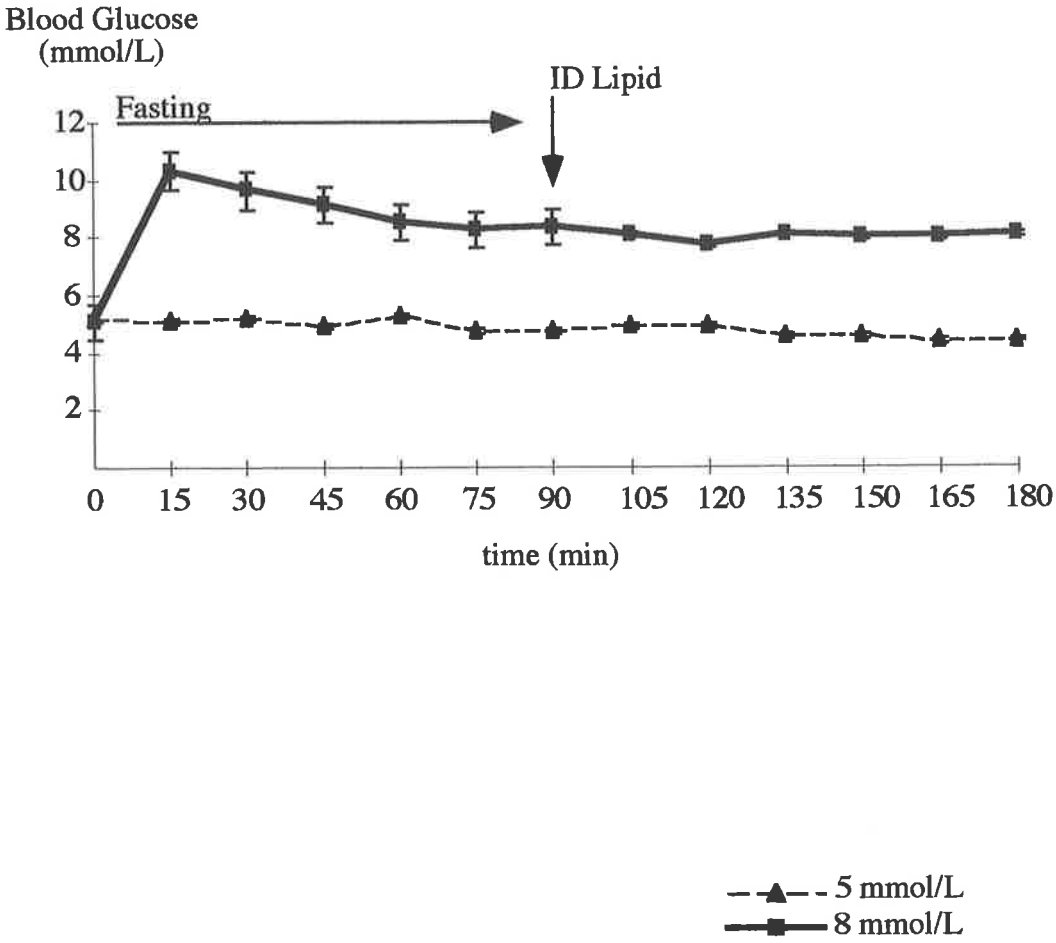


Figure 9A.2
Plasma Glucose concentrations on the two separate study days, during each of the 90 min periods (fasting and ID lipid). Data are mean +/- SE. (section 9A.3)

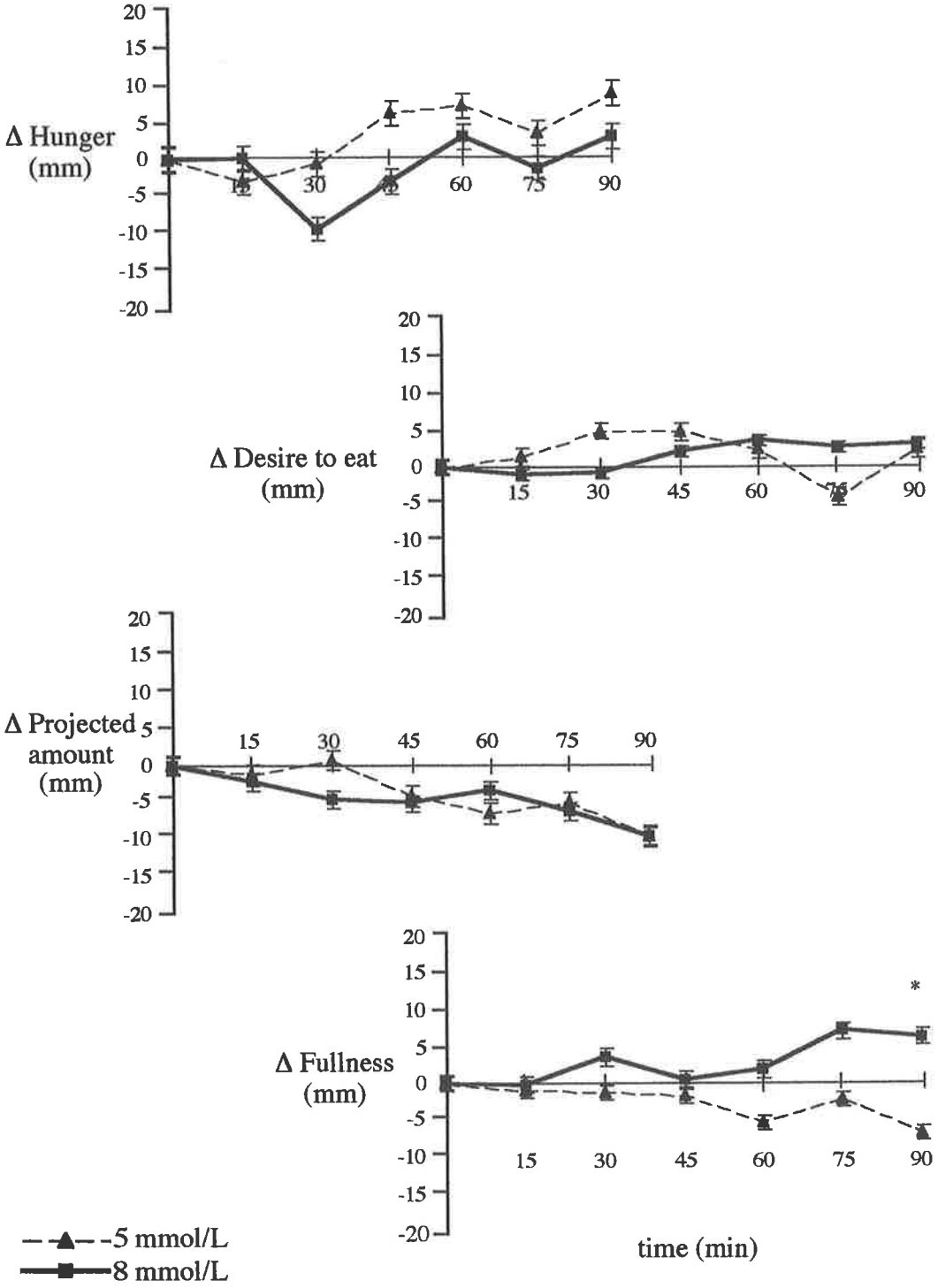


Figure 9A.3
Change in appetite ratings during fasting. No significant differences were observed for hunger, desire to eat or projected consumption; between blood glucose concentrations of 5 and 8 mmol/L, or with respect to baseline. Fullness, however, was greater at a blood glucose of 8 vs 5 mmol/L (*P = 0.01 by ANOVA for whole curves), although neither was significantly different from baseline. Data are mean +/- SE. (see 9A.3.1)

= 0.16), whilst it increased between fasting and ID lipid (P < 0.001) at a blood glucose of 5 mmol/L. In post-hoc analysis, the absolute score for fullness was not significantly higher in the last 45 mins of ID lipid at a blood glucose of 5 compared to 8 mmol/L (20.31 ± 6.2 vs 10.06 ± 3.8 mm; P = 0.12). Nausea ratings were greater at a blood glucose of 8 than 5 mmol/L during ID lipid (P = 0.002). This resulted from an increase in nausea between fasting and ID lipid at a blood glucose of 8 mmol/L (P = 0.003) as there was no change in nausea between fasting and ID lipid at a blood glucose of 5 mmol/L (P = 0.77).

9A.3.2 Antropyloric pressures

During the 90 min fasting period, there were fewer antral pressure waves at a blood glucose of 8 compared to 5 mmol/L (87.6 ± 14.3 vs 38.4 ± 12.8, P = 0.018). There was, however, no difference between a blood glucose of 5 and 8 mmol/L in the total number of IPPWs (20.38 ± 7.25 vs 24.8 ± 4.77, P = 0.41). During fasting pyloric tone did not change from baseline, nor did it vary between the two blood glucose levels.

During ID lipid, antral waves were suppressed compared to the fasting state at both blood glucose concentrations (5 mmol/L: 87.6 ± 14.3 vs 14.4 ± 8.7; P = 0.006; and 8 mmol/L: 38.4 ± 12.8 vs 3.1 ± 2.1; P = 0.03), with no difference in the number of antral waves noted between the two blood glucose levels during ID lipid (P = 0.26). Compared to the fasting state, ID lipid stimulated IPPWs at both blood glucose levels (5 mmol/L: 20.38 ± 7.25 vs 88.38 ± 15.55; P = 0.01, and 8 mmol/L: 24.8 ± 4.77 vs 65.38 ± 11.17; P = 0.019). The total number of IPPWs during ID lipid was not significantly different between the two blood glucose levels (5 mmol/L: 88.38 ± 15.55 vs 8 mmol/L: 65.38 ± 11.17; P = 0.09). However, the temporal patterning of IPPWs differed between blood glucose of 5 and 8 mmol/L. Initially the rate of IPPWs was higher at blood glucose of 8 mmol/L (P = 0.035). However, during the last 20 min of ID lipid, the frequency of IPPWs was greater at a blood glucose of 5 mmol/L than at 8 mmol/L (P < 0.04), (Figure 9A.5). The amplitude of IPPWs was greater at blood glucose of 5 mmol/L than 8 mmol/L (P < 0.001 for whole curve), (Figure 9A.5).

Pyloric tone rose in response to ID lipid at both blood glucose levels. However there was attenuation in this response after 55 min, giving an overall non-significant change from baseline by ANOVA (5 mmol/L vs baseline: P = 0.35, 8 mmol/L vs baseline:- P = 0.068 for whole curves). Although the initial rise in pyloric tone was greater at a blood glucose level of 8 than 5 mmol/L, this was not significant (Figure 9A.6).

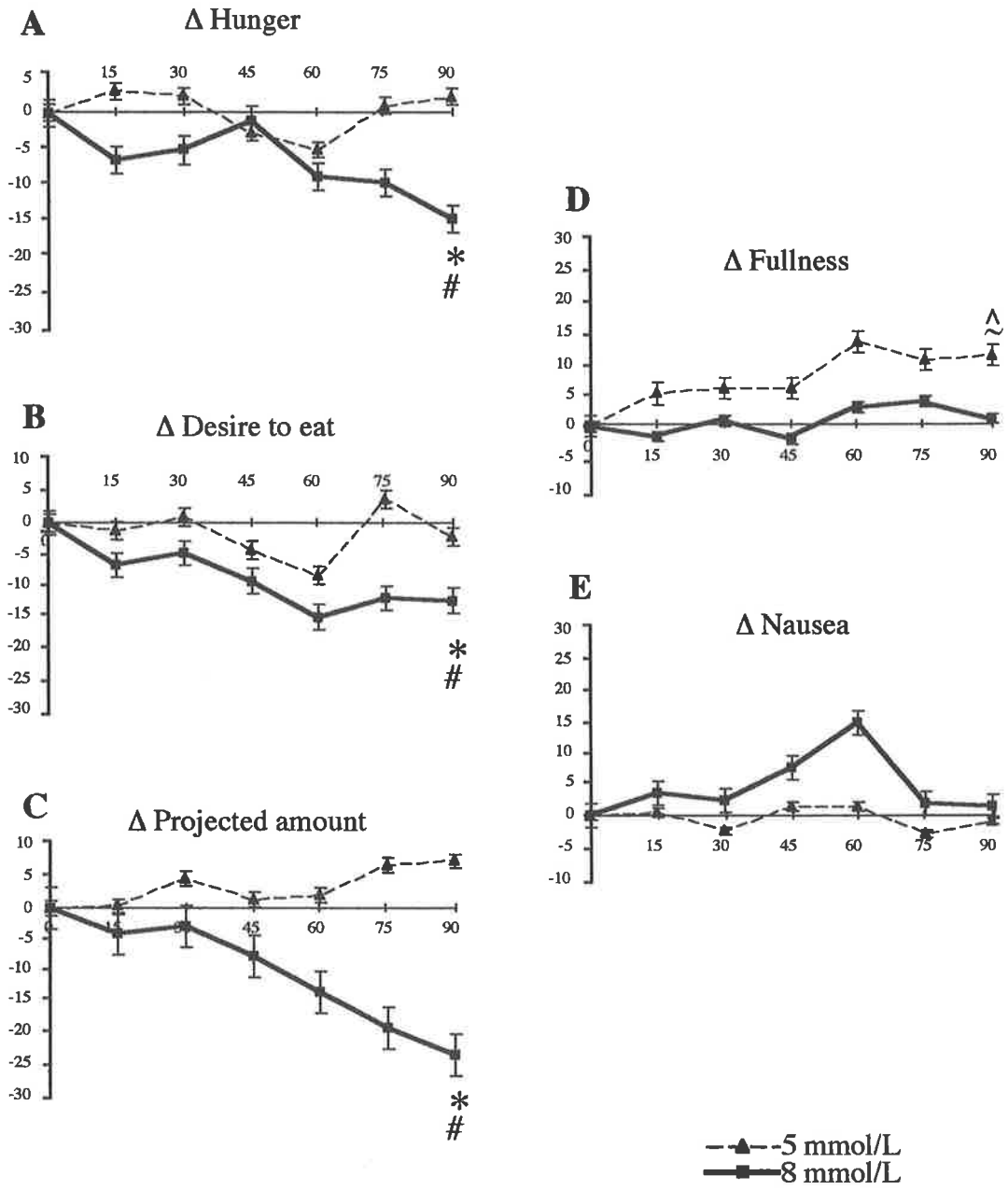


Figure 9A.4
Change in appetite during ID lipid. During ID lipid, perception of hunger (A), desire to eat (B), and projected consumption (C) all decreased at a blood glucose of 8 vs 5 mmol/L (*P = 0.02, 0.04 and < 0.001 for A,B and C respectively, for whole curves by ANOVA); they also decreased compared to baseline (#P = 0.034, 0.0001 and 0.0002, respectively). Surprisingly, fullness (D) was greater at blood glucose of 5 vs 8 mmol/L(^P < 0.001 by ANOVA for whole curves) and compared with baseline (~P = 0.0003). Nausea (E) was greater at a blood glucose of 8 vs 5 mmol/L (P = 0.002) and compared with baseline (P = 0.001). x axis represents time in min; and y axis represents change in ratings in mm. (section 9A.3.1).

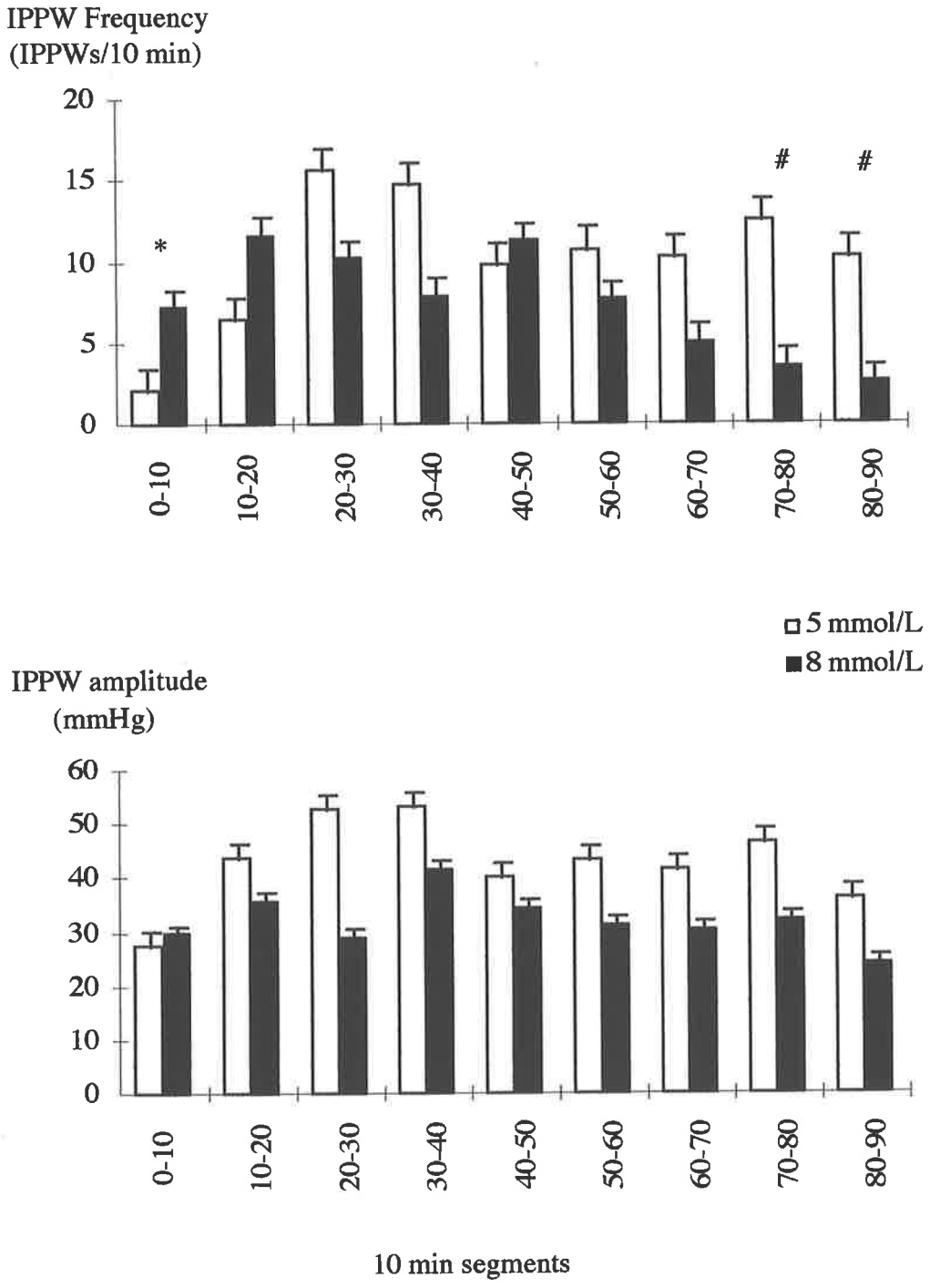


Figure 9A.5
Frequency and amplitude of IPPWs, during ID lipid are shown in 10 min segments. Initially the frequency is greater at a blood glucose of 8 vs 5 mmol/L (*P = 0.035); however, over time the response varies, so that toward the end of the infusion, the frequency is greater at 5 vs 8 mmol/L (#P < 0.04). The amplitude of IPPWs during ID lipid is greater at the lower blood glucose level (P < 0.001; 5 vs 8 mmol/L for whole curves by ANOVA). Data are mean +/- SE. (section 9A.3.2).

9A.4 DISCUSSION

It is well established that marked hyperglycaemia has major effects on gastrointestinal motor (MacGregor et al 1976; Fraser et al 1990 & 1991; De Boer et al 1992a, 1993a & b, 1994; Hasler et al 1995; Sims et al 1995; Hebbard et al 1996a & b, 1997; Russo et al 1996; Maleki et al 1997; Samsom et al 1997;) and sensory (Chey et al 1995a; Hebbard et al 1996a & b, 1997; Jones et al 1997a; Maleki et al 1997; Russo et al 1997) function. In contrast, there is only limited information about the potential impact of variation of the blood glucose concentration within the physiological range on gut function (Barnett & Owyang 1988; Hasler et al 1995; Boeckxstaens et al 1997; Schvarcz et al 1997). These results now show that both distal gastric motor function and perception of appetite and nausea are affected by modest changes in blood glucose within the physiological range. Moreover, these effects are modified by the presence of nutrient (lipid) within the small intestine, suggesting synergy between the blood glucose concentration and small intestinal nutrient receptor stimulation in the modulation of gastric motor and sensory function.

Specifically, it has been demonstrated for the first time, that at a blood glucose of 8 mmol/L when compared to 5 mmol/L; (i) *During fasting*, fullness is increased and, (ii) *During ID lipid*, appetite is reduced and nausea increased; and both the temporal patterning and amplitude of IPPWs are altered. The suppression of antral pressure waves by physiological hyperglycaemia has been previously reported (Hasler et al 1995).

9A.4.1 Appetite

Short-term regulation of appetite is multifactorial (Chapters 1 & 4), involving oral sensory stimulation (Rolls et al 1981), gastric distension (Bergmann et al 1992) and the interaction of nutrients with the small intestine (Chapters 8A, B & C; Lavin et al 1996). In the current study this has been simplified, delivering nutrients directly to the small intestine and bypassing the oral and gastric regions. Similar models have been previously used (Chapters 8A, B & C; Lavin et al 1996), with consistent observations. The rate of caloric delivery (1.5 Kcal/min) used in this study is slightly less than in these previous studies (2 - 2.9 Kcal/min), and was selected to reduce the incidence of nausea noted at higher rates (see below). It remains, however, within the normal caloric range for gastric emptying (Moore et al 1984) and is sufficient to convert motility from the fasting to the fed pattern, although its effect on appetite alone -

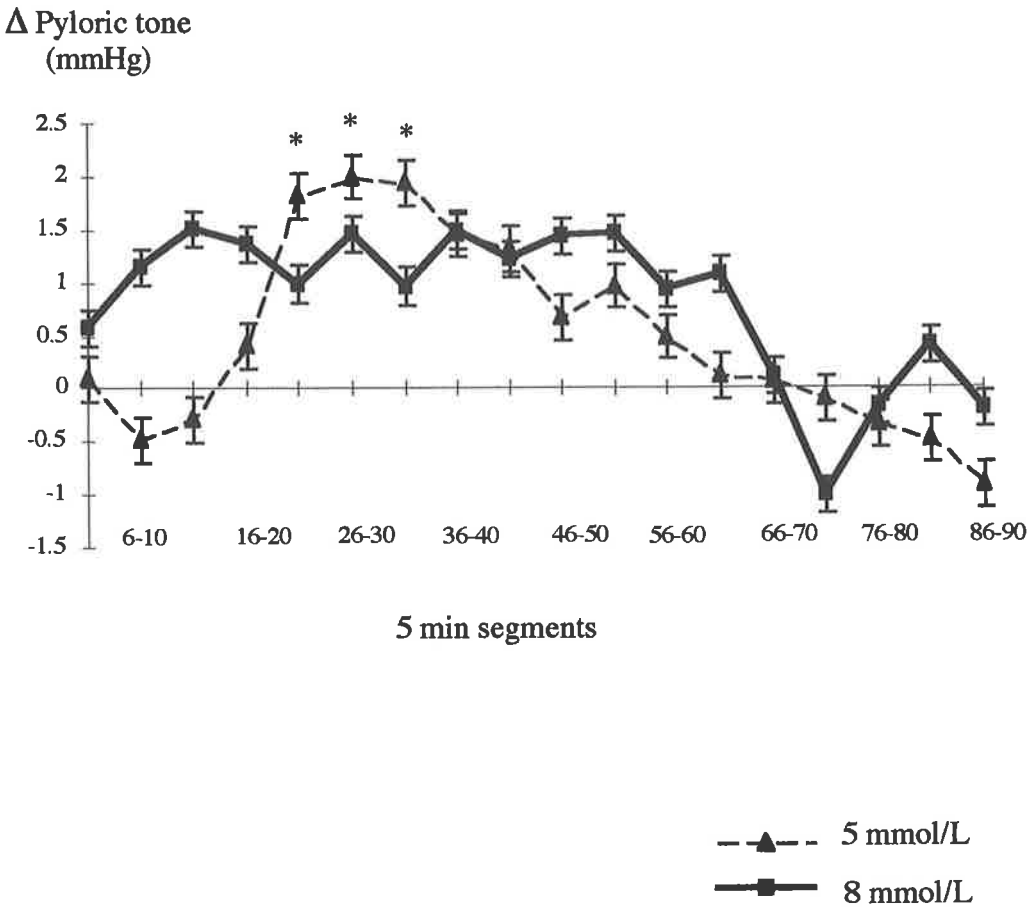


Figure 9A.6
Change from baseline in pyloric tone during ID lipid (mean +/- SE). Pyloric tone rose early during lipid infusion at both blood glucose levels, but then fell as the lipid infusion continued, giving a non-significant change from baseline over the 90 min period (5 mmol/L vs baseline, P = 0.35; 8mmol/L vs baseline, P = 0.068, by ANOVA for whole curves). At individual timepoints, however, tone did differ from basal (*P < 0.05). There was no significant difference between the response at a blood glucose of 5 vs 8 mmol/L, although the response at 8 mmol/L appeared to be greater. (section 9A.3.2).

without concurrent manipulation of blood glucose - has not been evaluated. This latter issue, however, was not the main focus of the study, but rather the perception of appetite (and antropyloric motility) during physiological hyperglycaemia and whether the presence of nutrients in the small intestine modified these responses. The order of the fasting period and ID lipid infusion was not randomised, for practical reasons, hence the possibility of an order effect arises. To minimise this, a new baseline was calculated immediately preceding the ID lipid infusion, so that any changes occurring during the lipid infusion would be clearly related to differences between blood glucose levels during lipid and not simply carry-over effects from the fasting period.

Little difference in appetite ratings was apparent between a blood glucose of 5 and 8 mmol/L in the fasting period. This is consistent with a previous study which suggested that modest elevations of blood glucose concentration alone do not effect appetite (Lavin et al 1996). During ID lipid infusion, all positive symptoms of appetite - hunger, desire to eat and projected consumption were less at a blood glucose of 8 compared to 5 mmol/L, indicative of a synergy between blood glucose concentration and stimulation of small intestinal nutrient receptors in modulating appetite. The finding of increased fullness, in the fasting state, at a blood glucose of 8 than 5 mmol/L, is consistent with previous fasting data in diabetic subjects (Jones et al 1997a). The fact that, during ID lipid, there was a greater change in fullness at blood glucose of 5 than 8 mmol/L suggests that both the glycaemic state and the presence of small intestinal nutrients are capable of signalling fullness.

Nausea was profoundly affected by the combination of elevated blood glucose and the presence of lipid in the small intestine. In previous studies we have noted a high incidence of nausea when nutrients are delivered to the small intestine at caloric rates greater than 2 Kcal/min -, and also when the blood glucose concentration is elevated to pathological levels -~12-15 mmol/L - (unpublished observations). This study confirms that blood glucose and small intestinal nutrients have a synergistic effect in this regard, leading to nausea at a physiologically normal blood glucose level and at a lower rate of caloric delivery than we have previously noted. Although this has not been previously reported, other examples of synergy between the presence of small intestinal nutrients and other stimuli include distension (Feinle et al 1996; Hebbard et al 1996b) and vection (Feinle et al 1995a). Abnormalities of gastric electrical control activity are known to be increased by both marked hyperglycaemia (~14 mmol/L) and ID lipid (Hebbard et al 1997) and may be important in the aetiology of nausea.

In light of these apparent interactions between glycaemic state and the presence of small intestinal nutrients in healthy volunteers it would be of interest to evaluate patients with functional gastrointestinal symptoms in whom symptoms are commonly augmented by food. These patients are known to have heightened sensitivity to other gut stimuli such as distension and electrical stimulation (Accarino et al 1995; Troncon et al 1995).

9A.4.2 Antropyloric Pressures

Both marked and physiological hyperglycaemia suppress antral pressure waves (Barnett & Owyang 1988; Hasler et al 1995; Samsom et al 1997) and slow gastric emptying (Fraser et al 1990; Schvarcz et al 1997); pathological hyperglycaemia stimulates phasic and tonic pyloric pressures (Fraser et al 1991). These motor mechanisms are associated with slowing of gastric emptying (4.2.1.2). The findings of fewer antral waves in the fasting state at the higher blood glucose are consistent with previous observations (Barnett & Owyang 1988; Hasler et al 1995). This study did not, however, show more fasting IPPWs at the higher blood glucose as previously noted (Fraser et al 1991), although in this earlier study blood glucose was greater than 12 mmol/L.

It is well documented that small intestinal nutrient infusion stimulates IPPWs, elevates pyloric tone and suppresses antral waves (Fraser et al 1990 & 1991; Heddle et al 1988a & b, 1989). Although not statistically significant, the fact that fewer IPPWs were stimulated by ID lipid at the higher blood glucose was somewhat unexpected. It should be recognised however, that the effect of hyperglycaemia on gut function is not always predictable with regional variations at the same blood glucose level and variation within regions depending on the degree of hyperglycaemia induced both being reported. At a blood glucose of 8 mmol/L there is slowing of gastric emptying (Schvarcz et al 1997), but no effect on anorectal motility (Russo et al 1997), and oesophageal peristaltic velocity is both accelerated (Boeckxstaens et al 1997) and slowed (De Boer et al 1992a) in response to varying degrees of hyperglycaemia (8 and 15 mmol/L respectively). On closer inspection of the data from the current study, it is apparent that the stimulation of IPPWs is a time dependent phenomenon, with more IPPWs early at a blood glucose of 8 mmol/L and more IPPWs later with a blood glucose of 5 mmol/L. Variability in the temporal patterning of IPPWs has been previously reported (Fraser et al 1992a). Pyloric tone was no different between a blood glucose of 5 and 8 mmol/L during fasting or ID lipid. However, it did appear to rise from baseline during the lipid infusion, as previously reported (Fraser et al 1991;

Chapter 8B & C). The fact that the elevation of tone in response to lipid was not more clear-cut in this study is likely to represent a type 2 error.

The rate of gastric emptying is dependent on integrated motor activity in the proximal stomach, antrum, pylorus, and proximal small intestine (Chapter 6; Horowitz et al 1994). Although this study did not evaluate gastric emptying, it has been demonstrated to be faster at a blood glucose of 4 than 8 mmol/L (Schvarcz et al 1997). Because the IPPWs and pyloric tone were similar at both blood glucose levels, it is difficult to attribute the slowing of gastric emptying to changes in pyloric motility, despite the number of IPPWs (presumably as a measure of pyloric resistance) being negatively correlated with the rate of gastric emptying (Sun et al 1997a). It is possible that the demonstrated antral hypomotility at a blood glucose of 8 mmol/L is primarily responsible for retarding gastric emptying, although a more compliant proximal stomach may also contribute to delayed gastric emptying by retaining gastric contents in this region (Jones et al 1995). Proximal gastric motor and sensory function during physiological hyperglycaemia is thus the subject of the next study (Chapter 9B).

The mechanisms mediating the synergy between blood glucose concentrations and the presence of nutrients in the small intestine in modulating gastric motor and sensory functions were not addressed in the current study. Hyperglycaemia (~15 mmol/L) is known to reduce vagal efferent tone (Lam et al 1993 & 1997) and to lead to suppression of the release of some gastrointestinal hormones including gastrin, and pancreatic polypeptide (DeBoer et al 1993a; Lam et al 1993). The role of increased insulin levels during hyperglycaemia is controversial but insulin's role is probably minor given that many of the changes in gut function observed during hyperglycaemia also occur in patients with type 1 diabetes, who are insulin-deficient (Fraser et al 1990; Samsom et al 1997). During euglycaemia, cholinergic transmission is known to be necessary for the production of IPPWs (Fraser et al 1992a) and, during hypoglycaemia, atropine prevents the acceleration of gastric emptying (Schvarcz et al 1995b). This emphasises the importance of cholinergic transmission in acutely regulating gastric motor function, regardless of the blood glucose level. Less is known, however, about how signals from nutrient receptors in the small intestine may respond to variations in the blood glucose level, and whether they alter cholinergic outflow to the antropyloric region. It is, however, known that the secretion of CCK in response to ID lipid is not affected hyperglycaemia (De Boer et al 1993b). It is possible that blood glucose levels sensed centrally may modulate perception of appetite, but from these results (and Lavin et al 1996), this only seems to occur if an additional stimulus, such as small intestinal nutrient, is present.

CHAPTER 9B

Physiological Hyperglycaemia does not Alter Proximal Gastric Compliance or the Perception of Gastric Distension in Healthy Volunteers

9B.1 INTRODUCTION

As discussed earlier (1.3.2.2, 4.2.2,4.3.2, 9.1, 9A.1, 9A.4), marked hyperglycaemia (>12 mmol/l) is known to affect gastric motor and sensory function. As demonstrated in 9A (and discussed in 4.2.2), physiological hyperglycaemia also has significant influence on perception of appetite and antropyloric motor function. This study was performed to determine whether physiological hyperglycaemia affects proximal gastric compliance and/or the perception of gastric distension.

9B.2 MATERIALS AND METHODS

9B.2.1 Subjects

10 healthy volunteers were studied (9 male; median age 27 yr, range 19 - 39; body mass index 23 kg/m^2, range 21 - 27). None of the subjects had a history of systemic or gastrointestinal disease, nor was taking medication. Smoking was prohibited on the study day.

9B.2.2 Experimental design

In each subject, fasting gastric compliance and sensations induced by distension of the proximal stomach were measured when the blood glucose concentration was maintained at both 4 mmol/L and 9 mmol/L. The two studies were performed on one day, using a single blind, randomised, crossover design.

After an overnight fast, at around 10 AM a catheter incorporating a distal polyethylene bag was inserted through an anaesthetised nostril and positioned in the proximal stomach. 500 ml of air was introduced to unfold the bag in a controlled manner using an electronic barostat. The bag was then completely deflated to a pressure of 0 mmHg with respect to atmospheric. Subjects were seated in a semi-kneeling position, in a

specially designed chair, with their torso upright (Hebbard et al 1996b). Their arms were allowed to rest on a table that was adjusted to elbow height. Intravenous cannulae were inserted into an antecubital vein in each arm, one for intravenous infusion of dextrose or saline, and the other for obtaining blood samples. Before each series of distensions, the pressure required to overcome intra-abdominal pressure (the minimal distension pressure {MDP}) was determined, by increasing the intragastric bag pressure by steps of 1 mmHg and identifying the lowest pressure at which continuous respiratory fluctuations of intrabag volume were detected (Hebbard et al 1996b).

The blood glucose concentration was stabilised at either 4 or 9 mmol/L as described in 9A.2.2. As perception of gastric distension is related to both pressure and volume (Novitol et al 1995; Hebbard et al 1996b), both pressure- and volume-determined distensions were performed. After the blood glucose had been maintained at the desired concentration for 30 min, a set of distensions at steps of fixed volume (isovolumetric) and another set of distensions at steps of fixed pressure (isobaric) were performed, in random order. The duration of each volume or pressure step was 3 min. Isovolumetric distensions were performed using steps of 100 ml, and isobaric distensions using steps of 1 mmHg, starting at MDP - 1 mmHg. The measured intrabag volumes were corrected on-line for air compression, by applying an experimentally determined correction factor (1.848 ml/mmHg)(Samsom et al 1995; Whitehead & Delvaux 1997). Distensions were stopped at an intragastric bag pressure of 20 mmHg, an intragastric bag volume of 800 ml, or if the subject reported marked discomfort.

Between isovolumetric and isobaric distensions, the intragastric bag was deflated completely, and there was a rest period of at least 10 min. When both sets of distensions had been performed, the blood glucose concentration was stabilised to the alternative level for 30 min, and the both sets of distensions were repeated; again with a 10 min rest between each set.

9B.2.3 Performance of gastric distension

The barostat used and the methods for positioning the intragastric bag, and recording of pressures were as described in Chapter 7 (7.2.4). For comparison of isobaric distensions, differences in MDP were corrected for by subtraction of the MDP from the pressure at each step (Samsom et al 1995). For the comparison of isovolumetric distensions, differences in MDP were corrected for by subtraction of the pressure in the bag at a volume of 100 ml for each distension.

9B.2.4 Evaluation of sensation and appetite

Perceptions of fullness, abdominal discomfort, nausea, bloating, hunger and desire to eat were scored by each subject twice in the 5 min before each series of distensions, and in the last min of each distension step, using VAS (7.2.7). (Hebbard et al 1996b).

9B.2.5 Statistical analysis

Data relating to pressure, volume and sensation were evaluated, using analysis of variance with repeated measures (SPSS for Windows 7.0), after logarithmic transformation of the data, without correcting for missing data. Both order of distensions (isobaric or isovolumetric first) and blood glucose concentration (4 or 9 mmol/L first) were included in the analysis as between-subject factors. The Wilcoxon signed rank test was used for individual comparisons. A P value < 0.05 was regarded as significant, and data are presented as median values with interquartile ranges; except for sensation scores, which are mean values ± SE, as they were normally distributed.

9B.3 RESULTS

All 10 subjects completed the study. The blood glucose concentration was maintained in the desired range; the median blood glucose (measured every 10 min during distensions) was 4.4 mmol/L (range 3.8-4.9) during euglycaemia, and 9.3 mmol/L (8.7-9.8) during hyperglycaemia. The mean rest period between isobaric and isovolumetric distensions was 17.4 min (range 10-22) during euglycaemia and 19.4 min (10-30) during hyperglycaemia; and the mean period of stabilisation when changing to the alternative blood glucose level was 39 min (range 17-60).

During isovolumetric distensions, the maximal volume of 800 ml was reached in 8 subjects during euglycaemia and in 9 subjects during hyperglycaemia. In 2 subjects the isovolumetric distension was stopped at 700 ml, due to the occurrence of marked discomfort. Isobaric distensions were not completed in 4 subjects during euglycaemia and in 3 subjects during hyperglycaemia because discomfort was reported. In the other isobaric distensions the maximum bag volume (800 ml) was reached. Nine subjects reached a pressure of 7 mmHg above MDP. No effects were found for either the order of distensions or for the sequence of hyper- and euglycaemia in the analysis of pressure-volume relationships and sensations.

9B.3.1 Pressure-volume relationships

The pressure-volume relationships during the isovolumetric and isobaric distensions are shown in Figure 9B.1. Intrabag pressure rose as volume was increased ($P < 0.001$); similarly an increase in volume was observed when intrabag pressure was increased ($P < 0.001$). During isovolumetric distensions, there was no difference in intragastric pressure after inflation of 100 ml of air between hyperglycaemia (6.5[range 5.2-7.1] mmHg) and euglycaemia (6.3[5.0-6.9] mmHg). There was no difference in the pressure/volume relationship during either set of distensions between blood glucose concentrations.

9B.3.2 Perception of gastric distension and appetite

Sensations of fullness, nausea, abdominal discomfort, and bloating increased with increasing volume ($P \leq 0.002$) and pressure ($P \leq 0.006$) during both euglycaemia and hyperglycaemia (Figure 9B.2). In contrast, there was no effect of either volume or pressure on sensations of hunger or desire to eat (Figure 9B.3). There were no differences in any sensation scores between euglycaemia and hyperglycaemia (Figures 9B.2 & 3).

9B.4 DISCUSSION

This study shows that in the fasted state (i) physiological hyperglycaemia does not affect either proximal gastric compliance or sensory response to proximal gastric distension, and (ii) proximal gastric distension does not affect sensations of hunger or desire to eat. Previous studies have demonstrated in normal subjects that marked hyperglycaemia (~15 mmol/L) increases the compliance of the proximal stomach during gastric distension both in the fasted state (Hebbard et al 1996a) and during intraduodenal (ID) lipid infusion (Hebbard et al 1996b). Using the same technique, the current study indicates that physiological hyperglycaemia does not alter proximal gastric compliance in the fasted state. These findings might be explained by the gastric fundus having a higher threshold for hyperglycaemia before motor patterns are altered. A blood glucose level of 9 mmol/L might be below the threshold required, which appears similar to the influence of hyperglycaemia on anorectal function (Russo et al 1997), but contrasts with the effects on the distal stomach (Chapter 9A) and gallbladder (De Boer et al 1993a).

These findings do not rule out an effect of physiological hyperglycaemia on the response to gastric distension under postprandial conditions, as intestino-gastric feed-

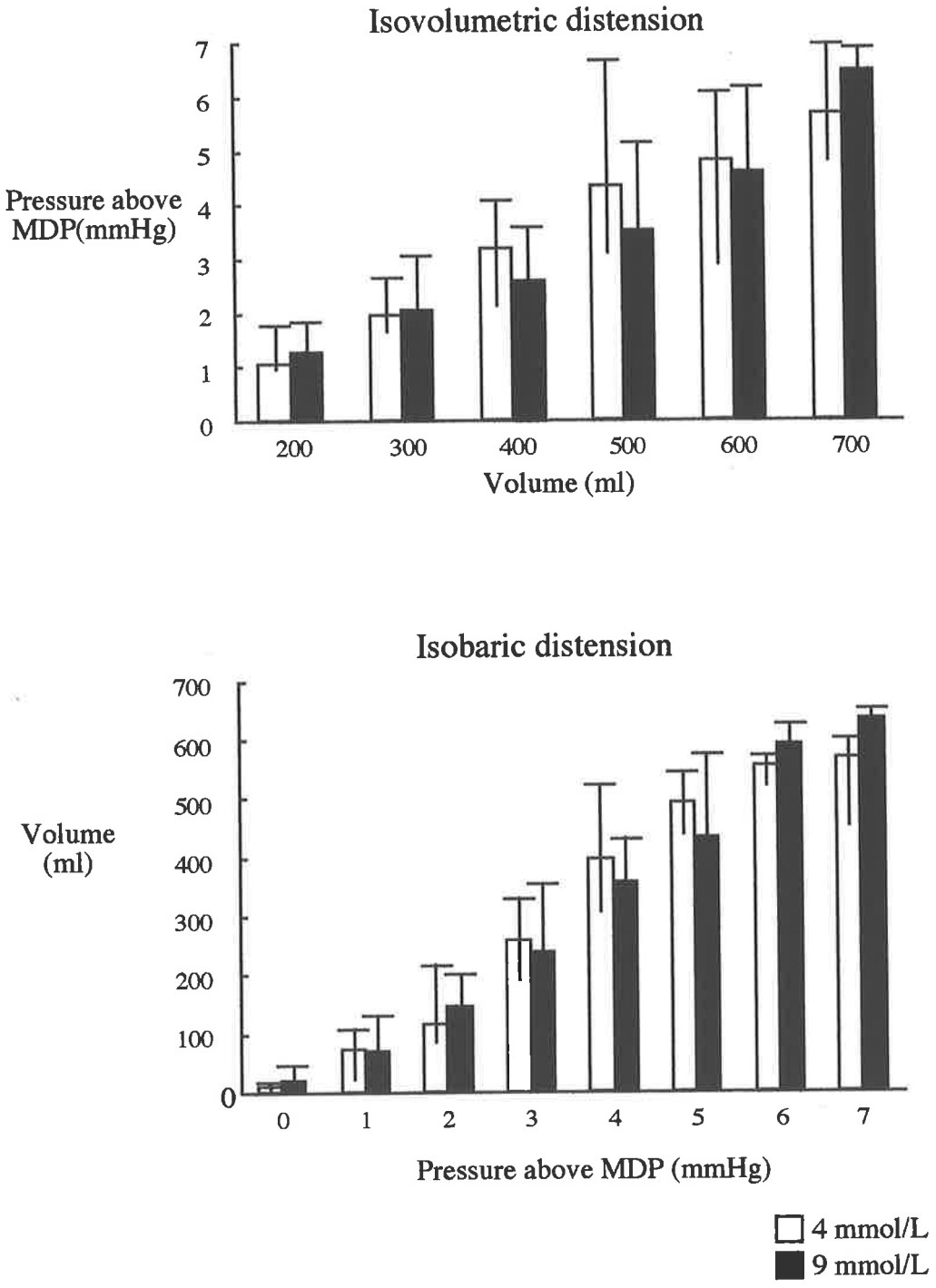


Figure 9B.1
Pressure-volume relationships during euglycaemia and hyperglycaemia. During isovolumetric distention (above), intrabag pressure increased during both euglycaemia and hyperglyceamia ($P < 0.001$), and there was no difference in the responses. Pressures are corrected for the pressure at 100 ml of air insufflation. During isobaric distention (below), intrabag volume increased during both euglycaemia and hyperglycaemia ($P < 0.001$), and there was no difference between responses. Pressures are corrected for the minimal distending pressure. Data are median values and interquartile ranges. (section 9B.3.1).

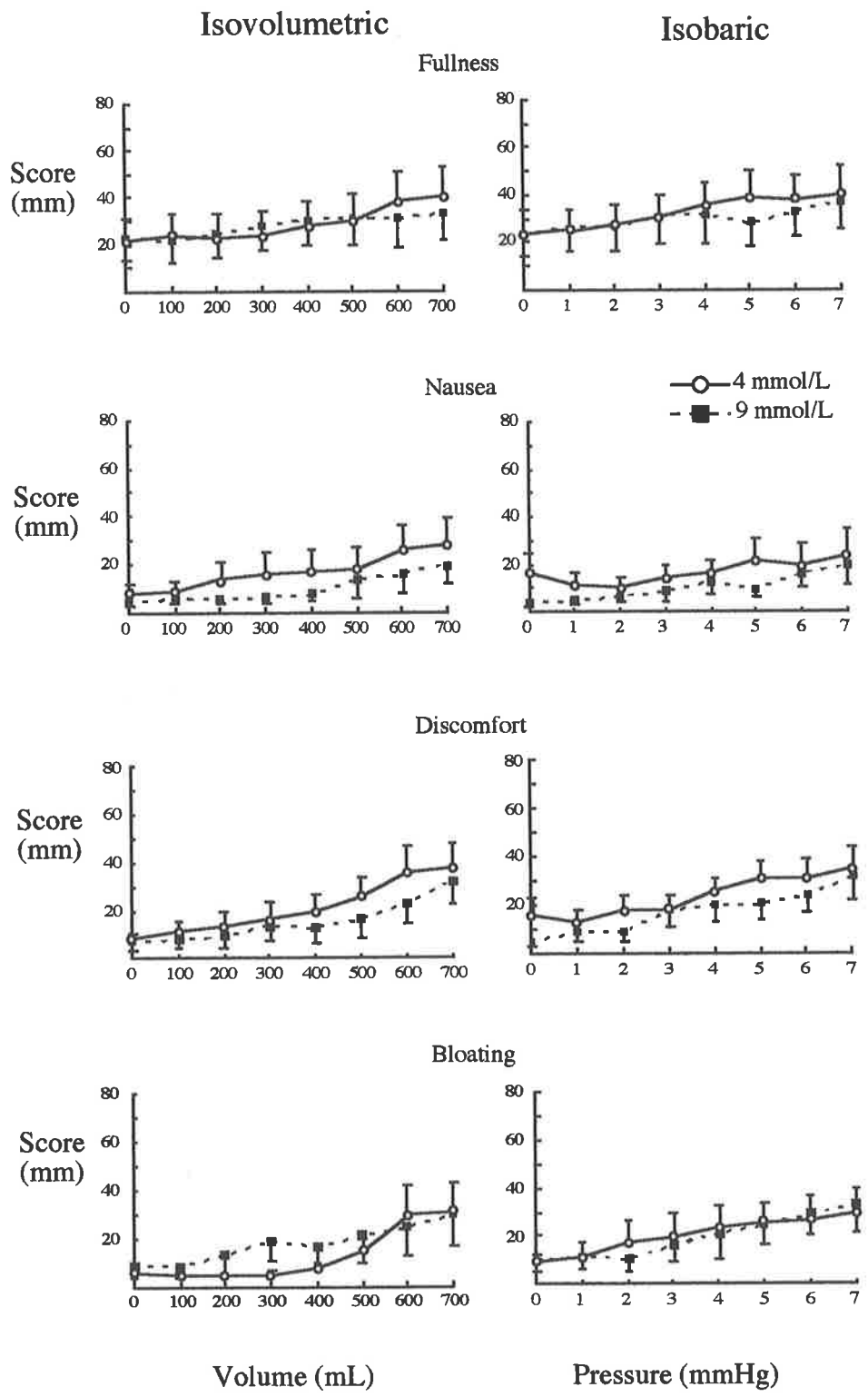


Figure 9B.2
Sensations recorded during isovolumetric (left) and isobaric (right) distentions at blood glucoe concentrations of 4 and 9 mmol/L. The sensations of fullness, nausea, abdominal discomfort and bloating increased with increasing intrabag volume ($P < 0.002$ for all sensations), and with increasing pressure ($P < 0.006$). There were no differences between euglycaemia and hyperglycaemia. (section 9B.3.2).

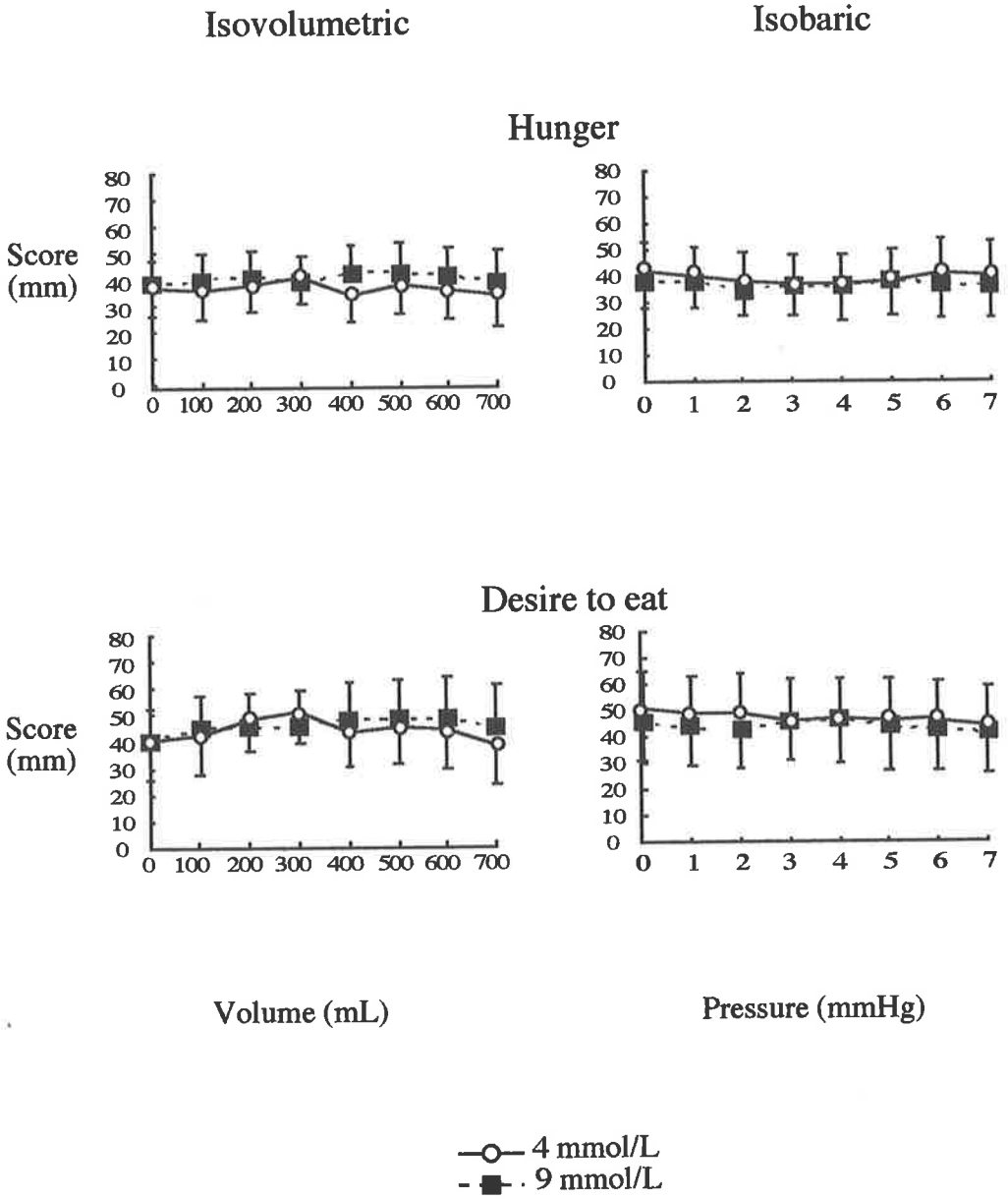


Figure 9B.3
Sensations recorded during isovolumetric (left) and isobaric (right) distentions at blood glucose concentrations of 4 and 9 mmol/L. The sensations of hunger and desire to eat were not affected by increasing pressure or volume, nor were they influenced by blood glucose concentration. 9B.3.2

back mechanisms are involved in the regulation of postprandial fundic tone (Azpiroz et al 1985a & b, 1986 & 1990; Hebbard et al 1996b). Moreover, in the preceding study (9A), a synergy between nutrient-mediated signals and glycaemic state has been demonstrated. It is possible that proximal gastric compliance is increased at a blood glucose of ~9 mmol/L postprandially, compared to 4 mmol/L and this would be consistent with the slowing of gastric emptying documented in these conditions (Schvarcz et al 1997). Previous studies have demonstrated a degree of synergy between gastric distension and the presence of nutrients in the small intestine, at least as far as the perception of gastric distension is concerned (Barbera et al 1995b; Feinle et al 1996). In the preceding study (Chapter 9A), it has been shown that during ID lipid, physiological hyperglycaemia increases nausea and decreases hunger, when compared to euglycaemia. Postprandially several hormones are released from the duodenum, such as CCK and serotonin, and these may play a role in the synergistic effect. Administration of CCK-antagonist loxiglumide (Feinle et al 1996) and serotonin antagonist ondansetron (Feinle & Read 1996) reduce the perception of gastric distension. With these additional, nutrient-induced, mechanisms at work, differences in the response to distension at a blood glucose of 9 vs 4 mmol/L may be observed. Although this would be a technically more demanding study, it would be of interest.

The current findings highlight, once again, the complex relationship between fullness and hunger, showing that one is not simply the reciprocal of the other. Whilst gastric distension increased perception of fullness, hunger and desire to eat did not diminish.. These findings are consistent with previous observations that proximal gastric distension requires concurrent small intestinal nutrient stimulation to induce feelings of satiety and/or satiation (Barbera et al 1995b; Feinle et al 1996; Feinle & Read 1996). Jones et al (1997b) showed that postprandial fullness is closely related to antral distension but hunger is not, also suggesting that fullness and hunger are mediated by different mechanisms.

9.2 CONCLUSION

Gastric motor and sensory function is known to be influenced by a number of common stimuli, including small intestinal nutrients (Chapters 8B & C; Feinle et al 1996; Fraser et al 1992a; Hebbard et al 1996b, 1997; Heddle et al 1988a & c, 1989; Lavin et al 1996; Russo et al 1996), cold stress (Fone et al 1990), pathological hyperglycaemia (Fraser et al 1990, 1991; Hasler et al 1995; Hebbard et al 1996a& b, 1997), and motion (Feinle et al 1995a). These results (9A) now demonstrate, for the first time, a synergy between physiological variation of blood glucose and the presence of small intestinal nutrients. The importance of this observation lies in the fact that these two stimuli not only occur commonly, but also commonly occur simultaneously. This therefore represents a novel insight into the physiological mechanisms involved in gastric motor and sensory function. The second study (9B) has shown that physiological hyperglycaemia does not, however, change the pressure-volume relationship or gastric perception in the fasted state, and confirms that gastric distension in the fasted state does not result in typical meal-like fullness (Feinle et al 1995b & 1996). The hierarchy, relative magnitude and specificity of the interaction between glycaemic state and intestinal nutrients should be addressed in future studies and the effects of physiological hyperglycaemia on the proximal stomach should be re-evaluated during intestinal nutrient infusion.

CHAPTER 10

Effect of a Lacto-ovo Vegetarian Diet on Fasting Small Intestinal Motility

10.1 INTRODUCTION

It is well documented that dietary changes modify gastric emptying and small intestinal (SI) transit (Chapters 1, 4 & 8B). The alteration of gastric emptying and small intestinal transit has been documented in response to subsequent nutrient intake, particularly intake of the specific nutrient whose dietary content was manipulated (1.3.1 & 4.2.4). The rate of gastric emptying is also altered by changes in total caloric intake, with a reduction in caloric intake slowing and, an increase, accelerating the rate of emptying (4.2.4). Altering the physical state or temperature of food also modulates the rate of gastric emptying (4.2.4). These changes are presumably mediated by variations in the magnitude of the inhibitory feedback from the small intestine, in response to nutrients. "Fasting" small intestinal motility re-emerges when 80-90 % of

gastric contents have emptied (Sarna 1985; Sepple & Read 1989; Husebye 1999), when nutrient is still present in the small intestine. However, the potential effect of dietary change on fasting motility has not been studied.

High fibre diets are generally thought to lead to more rapid whole gut transit time (Gogler 1976; Spiller 1994; Probert et al 1995), and vegetarian diets are generally higher in dietary fibre than the typical Western diet (Gogler 1976; Alexander et al 1994; Wolever & Jenkins 1997). In the motility laboratory at the Royal Adelaide Hospital, vegetarian subjects have not been included in recent studies conducted in healthy volunteers, due to occasional observations of unusual fasting small intestinal motility recorded (extremely prolonged duration of the interdigestive motor cycle [IDMC] and atypically long periods of both phase I and phase II) in co-incidentally vegetarian subjects recruited as "healthy normals" (unpublished observations). There are no studies which have specifically examined small intestinal motility in vegetarian, as compared to omnivorous, subjects.

This study was therefore performed to determine whether (i) there are substantial differences in fasting small intestinal motility in vegetarian subjects compared to omnivores and, (ii) whether acutely switching to a vegetarian diet affects fasting small intestinal motility.

10.2 MATERIALS AND METHODS

10.2.1 Subjects

Nine healthy lacto-ovo vegetarians (7F & 2M) and 9 omnivores (controls) (7F, 2M) were recruited by advertisement. The mean age and mean body mass index (BMI) of the vegetarian and control subjects were similar (AGE: vegetarians 22 [range 18-43] years vs controls 23 [19-30] years) (BMI: vegetarians 22.5 [range 18-24] kg/m^2 vs controls 24.1 [21.5-28.5] kg/m^2). No subject reported any symptoms of gastrointestinal disease nor had undergone gastrointestinal surgery. All female subjects in both groups were taking oral contraceptives, and continued them during the study. Two control volunteers were smokers and refrained from smoking for 24 hr preceding each study. Subjects were initially contacted by telephone to determine suitability; and then attended the motility laboratory where the experimental protocol and diet diary were further explained.

10.2.2 Experimental design

Both control and vegetarian subjects completed a diet diary prior to undergoing a single day manometric study (Day 1 for controls = C1). Control subjects then commenced a lacto-ovo vegetarian diet for 14 days, completed a second diet diary, and underwent a second identical manometric study (C2). (Figure 10.1).

On each manometric study day, subjects arrived at the hospital at 8.30 AM after an overnight fast, and were intubated as described in 7.2.1. The manometric assembly used (*Dentsleeve*, Wayville, SA) had an external diameter of 4.4 mm, and comprised ten 0.75 mm lumina, 9 of which were used for pressure measurement, and one to inflate a distal balloon to aid positioning of the assembly. (Figure 10.2). The 2 most proximal sideholes were 5 cm apart and positioned in the antrum. The third sidehole (10 cm from the second antral sidehole) was positioned in the duodenum. Its position was verified by observing the gradient of transmucosal potential difference (TMPD) across the gastroduodenal junction (7.2.3). Seven sideholes at 10 cm intervals recorded pressures along the small intestine (Figure 10.2). Once the assembly was correctly positioned, fasting pressures were recorded from the onset of the first phase III in any of the 9 pressure recording channels and recorded for 3 complete cycles of the IDMC or for a maximum of 5 hr (Figure 10.1). Each subject had a least one full IDMC recorded during each study .

10.2.3 Dietary assessment

A lacto-ovo vegetarian was classified as a person who had refrained from eating either red or white meat or fish for a period of a least 6 months preceding the study; eggs and dairy products were permitted. Both vegetarian and control subjects were instructed to maintain their usual eating habits and to keep a 5-day diet diary (3 weekdays, Saturday and Sunday) documenting the weight, time of eating, method of cooking and type of food they consumed as well as the volume and type of fluid, prior to undergoing the initial manometric study. Control subjects kept a second 5 day diet diary from days 9 to 14 of the period of vegetarian diet prior to undergoing the second manometric study. Control subjects were provided with information on a lacto-ovo vegetarian diet prior to the 14 days of dietary intervention (sourced from *The Vegetarian Society of South Australia*, Adelaide, SA). Diet diary records were analysed for total kilojoule intake and quantities of protein, fat, carbohydrates, fibre, calcium, iron and alcohol (*Diet 4*, Xyris software, Australia).

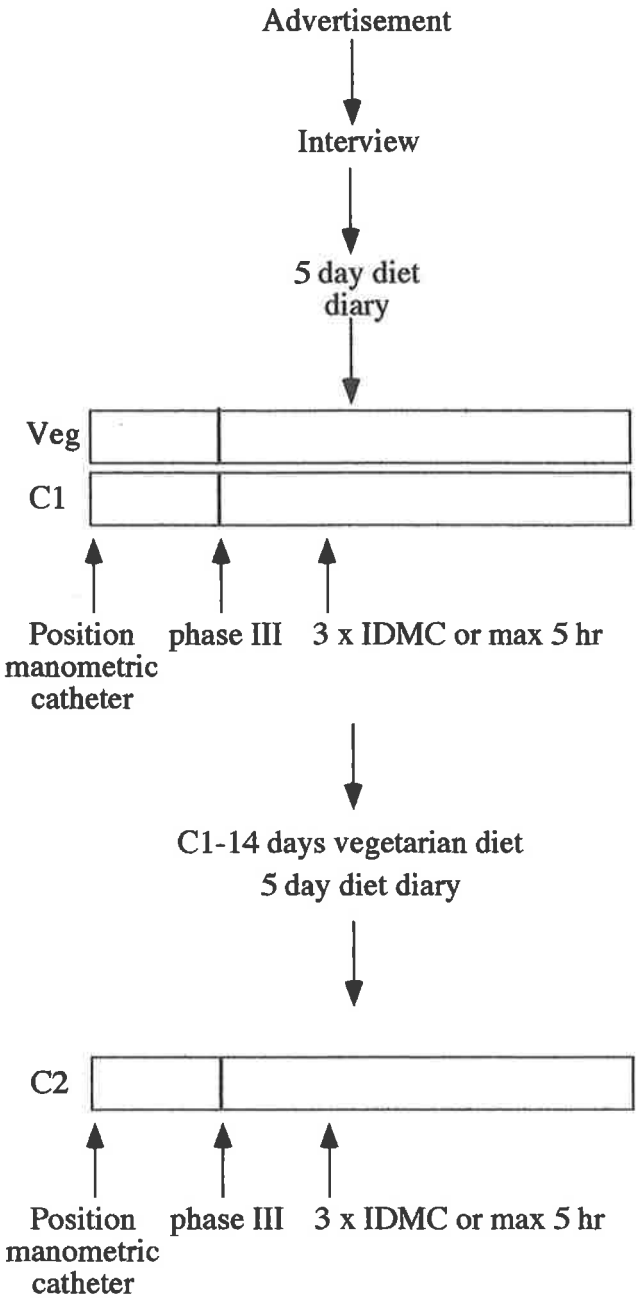


Figure 10.1
Schema of Experimental design (10.3.2).

10.2.4 Manometric data

Intraluminal pressures were recorded and stored at 10 Hz using customised software (*HAD*, Dr GS Hebbard, Adelaide, Australia), written in Labview (*National Instruments*, Houston, Texas, USA) on a Power Macintosh 7100/80 (*Apple Corp*, Cupertino, USA). Recordings were converted from *HAD* to AcqKnowledge vers. 3.2.7 (*Biopac*, California, USA) for display and analysis.

Pressure waves were manually identified and the number of pressure waves counted for each channel. Pressure waves were only scored when the assembly was correctly positioned, according to standard TMPD criteria (Heddle et al 1988b). The patterning and organisation of pressure waves were defined consistent with existing literature (Sarna 1985; Russo et al 1996 & 1999a; Husebye 1999):

- Phase I - motor quiescence (< 3 pressure waves/10 min of > 5 mm/Hg in amplitude)
- Phase III - pressure waves > 5 mm/Hg in amplitude, at a frequency ≥ 10 waves/min for ≥ 2 mins, extending over ≥ 4 sideholes (40 cm)
- Phase III-like "burst activity" - activity similar to phase III but in < 4 sideholes
- Phase II - periods when phase I, phase III and phase III like "burst activity" were absent.
- IDMC length - time from the onset of one phase III to the onset of the next phase III

As a substantial number of IDMCs do not exhibit an antral phase III, and commence in the jejunum rather than the proximal duodenum (Kellow et al 1986), the periodicity of the IDMC was calculated from the time between phase III activities in channel 5 which was positioned in the proximal jejunum (Figure 10.2).

The cycle length; duration of phases I, II and III; the number of phase III activities; and the amount of phase III-like "burst activity" were compared between the chronic vegetarians and omnivores and in the omnivores consuming their usual diet and after 14 days of a vegetarian diet.

10.2.5 Statistical analyses

Dietary and manometry data were analysed using *Statview* 4.1 (*Abacus Concepts*, Berkeley, California, USA). To correct for the variable number of IDMCs between subjects, a single mean value was determined for manometric data in each subject. Unpaired data between vegetarians and controls (C1 & C2) was compared using Mann-Whitney U test. Paired data (between C1 and C2) was compared using

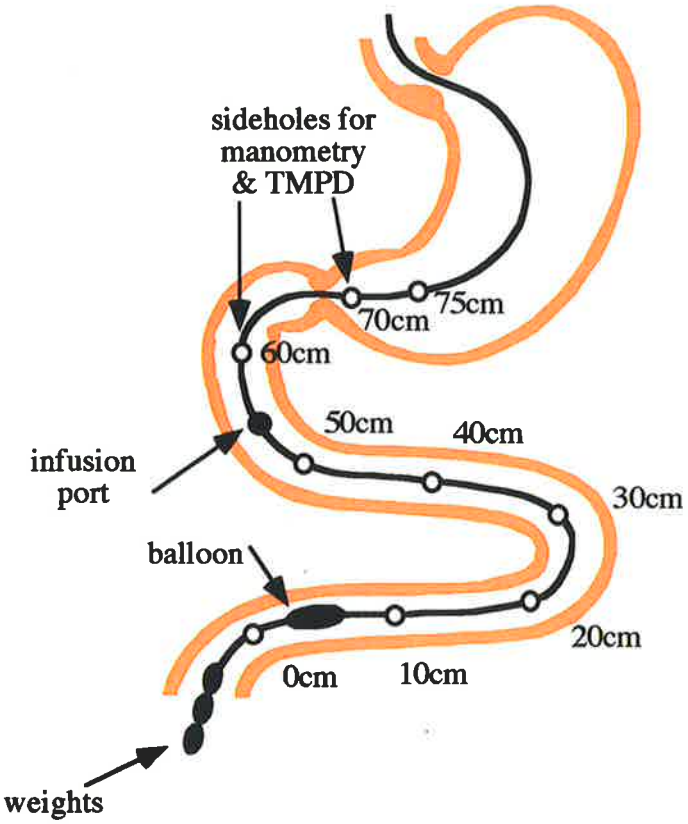


Figure 10.2
Manometric assembly used to study small intestinal pressures. For detail see 10.3.2.

Wilcoxon signed rank test. Correlations between cycle length and fibre intake were made using regression analysis. A P value of < 0.05 was considered as significant. Data are presented as means ± SE unless otherwise stated.

10.3 RESULTS

All subjects completed the protocol and the manometric study was well tolerated. The mean duration between the 2 manometric studies in control subjects was 17.7 (range 14-42 days). Compliance with the vegetarian diet in control subjects was excellent. One subject inadvertently consumed meat during the 14 day intervention period. Her second manometric study was repeated after a further 14 day period of vegetarian diet (following an intervening 14 day period of her usual diet).

10.3.1 Dietary data

10.3.1.1 Chronic vegetarians vs chronic omnivores

There were no significant differences in energy, protein, fat, carbohydrate, calcium or iron intake between chronic omnivores (C1) and vegetarian subjects. There was a trend for the fibre intake to be greater in the vegetarians, although this did not reach statistical significance ($P = 0.058$). There was no significant difference in alcohol consumption between omnivores and vegetarians (Table 10.1).

10.3.1.2 Chronic omnivores vs omnivores consuming a vegetarian diet

There were no significant differences in energy, protein, fat, carbohydrate, calcium or iron intake between omnivores on their usual diet and omnivores eating a vegetarian diet. There was a trend for alcohol consumption to be less in omnivores eating a vegetarian diet, but this did not reach statistical significance ($P = 0.068$). Despite acute adoption of a vegetarian diet, fibre intake was not significantly different between omnivores on their usual diet and omnivores eating a vegetarian diet (Table 10.1).

10.3.1.3 Chronic vegetarians vs omnivores consuming a vegetarian diet

There were no significant differences in energy, protein, fat, carbohydrate, calcium or iron intake between vegetarians and omnivores eating a vegetarian diet. There was no difference in alcohol consumption in vegetarians and omnivores eating a vegetarian diet

(P = 0.20). There was no significant difference in fibre intake in omnivores eating a vegetarian diet compared to vegetarian subjects (P = 0.10) (Table 10.1).

Table 10.1. Intake Assessed by 5-Day Diet Diaries in the Three Subject Groups

	Veg	C1	C2
Energy (Kcal/day)	2003 ± 168	2152 ± 209	1999 ± 233
Fibre (g/day)	29 ± 3 *	20 ± 3	21 ± 3
Alcohol (mg/day)	18.6 ± 7.6	12.6 ± 6.2 **	6.5 ± 4.2
Protein (g/day)	68 ± 5	76 ± 6	62 ± 9
Fat (g/day)	64 ± 4	77 ± 9	72 ± 11
Carbohyd. (g/day)	261 ± 32	271 ± 31	266 ± 33
Calcium (mg/day)	840 ± 98	865 ± 111	872 ± 86
Iron (mg/day)	13 ± 1	12 ± 2	11 ± 2

* P = 0.058 vs C1; ** P = 0.068 vs C2.

10.3.2 Fasting small intestinal motility

A total of 79 complete IDMCs were recorded; 25 (range 1-5) in the vegetarian subjects and 54 in the controls (C1: 25 [range 1-5], C2: 29 [range 2-5]). The number of phase III activities with an antral component was no different between groups (vegetarians: 14/25, C1: 10/25 and C2: 12/29). There was no correlation between IDMC length and fibre intake in either vegetarians (P = 0.4), or omnivores during either C1 (P = 0.8), or C2 (P = 0.14) studies when analysed separately, or when analysed as a single group (P = 0.28).

10.3.2.1 Chronic vegetarians vs chronic omnivores

There were no major differences in the cycle length, or duration of individual components of the IDMC between long standing vegetarians and omnivores (C1) (Table 10.2).

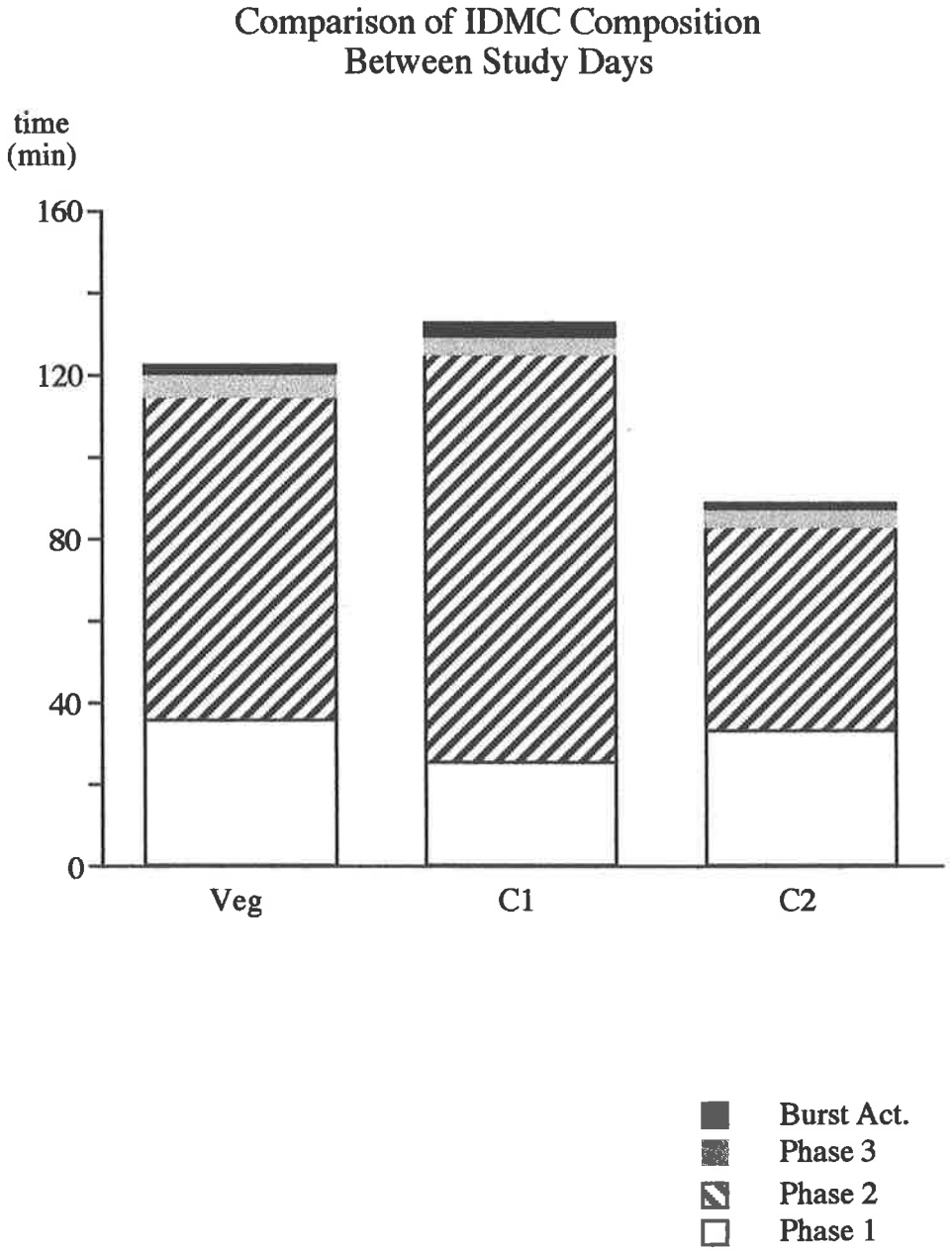


Figure 10.3
Graphical representation of composition of the IDMC in each of the three subjects groups. Cycle length is reduced in Controls eating a vegetarian diet compared to Controls eating their usual diet (P = 0.021) and chronic vegetarians (P = 0.068). For detail see Table 10.2, and section 10.3.2

10.3.2.2 Chronic omnivores vs omnivores consuming a vegetarian diet

Following acute adoption of a vegetarian diet, the IDMC length in controls was reduced compared to that on their usual omnivorous diet (C1: 128 ± 19 vs C2: 86 ± 12 min, P = 0.021), this was predominantly the result of a non-significant reduction in Phase II duration (C1: 99 ± 20 vs C2: 50 ± 8 min, P = 0.066) (Table 10.2).

10.3.2.3 Chronic vegetarians vs omnivores consuming a vegetarian diet

Following acute adoption of a vegetarian diet, IDMC cycle length in controls was less than in the chronic vegetarian subjects (C2: 85.9 vs Veg: 120.7 min), although this difference did not reach statistical significance (P = 0.068). This appeared to be related to a non-significant reduction in Phase II duration in C2 (C2: 49.7 vs Veg: 78.9 min; P = 0.12) (Table 10.2).

Table 10.2. Composition of IDMC

	Veg	C1	C2
Cycle length (min)	120.7 ± 11.8	127.9 ± 19.1	85.9 ± 11.7#
Phase I (min)	35.6 ± 8.4	25.3 ± 3.8	32.9 ± 6.3
Phase II (min)	78.9 ± 12.2	99.1 ± 20.4	49.7 ± 8.4*
Phase III (min)	5.2 ± 0.8	4.8 ± 0.3	4.3 ± 0.4
No of Phase III	2.8 ± 0.4	2.7 ± 0.2	3.0 ± 0.2
Burst activity (min)	2.1 ± 0.0	3.1 ± 0.9	1.8 ± 0.6

P = 0.021 vs C1 & 0.068 vs Veg; * P = 0.066 vs C1 & 0.12 vs Veg.

10.4 DISCUSSION

This study has demonstrated that there are no major differences in fasting motor activity between longstanding lacto-ovo vegetarians and omnivores. Acute adoption of a vegetarian diet, however, appeared to cause a reduction in IDMC length by ~1/3, attributable in part to a shorter duration of Phase II.

Although these were not the anticipated findings, the fact that there was no difference in motor patterns between the chronic omnivores and vegetarian subjects suggests that, despite wide inter- and intra- subject variability, large differences in IDMC duration and organisation do not occur with a chronic lacto-ovo vegetarian diet. Importantly, if one is only concerned with assessing basic IDMC patterning, this data now enables one to confidently apply standard criteria to the fasting motility of lacto-ovo vegetarians. These findings do not, however, exclude the possibility of more subtle differences in motility and it may be necessary to study a substantially larger number of subjects to determine this. It is also possible that differences in patterns of small intestinal motility between chronic vegetarians and omnivores would be seen in response to nutrients, rather than in the fasting state, as in other studies differences between groups have been accentuated by the presence of small intestinal nutrients (Chapter 9A, Hebbard et al 1996b &1997).

Although unexpected, the observed change in fasting motility with acute dietary change is consistent with other studies (Chapter 8B; Cunningham et al 1991a & b; Brown et al 1994; Horowitz et al 1996; Shi et al 1997) which have demonstrated alterations in gastric and small intestinal motor function in response to dietary manipulations. In these previous studies, motor function in response to a subsequent load of the manipulated nutrient was altered. The novel observation in the current study is that acute dietary manipulation also appears to alter fasting motility in humans. Disruption of the patterning of the IDMC due to dietary alteration has been previously reported in dogs by Georgieva et al (1985). In their study, however, there were large macronutrient differences between dietary regimes. Somewhat surprisingly, in the current study, there were no significant differences detected in macronutrient composition of the diet between omnivores and longstanding lacto-ovo vegetarians.

In the current study it is difficult to identify a mechanism by which the apparent adaptation in cycle length occurred, as the macronutrient composition of the diet did not vary with the acute adoption of a vegetarian diet. Notwithstanding this, both mechanical and chemical mechanisms need to be considered. Other authors have found fibre intake to be higher, and fat and protein intake to be less (Alexander et al 1994; Johansson et al 1998), and an increased rate of zinc, iron, copper and selenium deficiency in vegetarians (Donovan & Gibson 1995; Kadrabova et al 1995). This discrepancy may reflect a tendency for vegetarians in our society to consume a diet which is equivalently refined to that consumed by omnivores, and for omnivores to consume significant amounts of non-meat foods. In particular, in the current study, both vegetarians and chronic omnivores had a lower intake of protein (14.6 & 15.4 %

of energy) and fat (30.9 & 32.1 %) compared to published population norms of ~21 % for protein and 34 % for fat (see Chapter 8B). These intakes of protein and fat were also lower than those found in a group of all male subjects in Chapter 8B. It is possible that the technique of dietary quantification which was used here lacked the precision to detect important differences in intake between subject groups. For instance, it was not possible to determine the specific types of dietary fibre consumed from the data collection and analysis in the present study. This is potentially important, as specific fibre types may have different effects on gastrointestinal transit (Spiller 1994). The lack of major dietary differences between these chronic vegetarians and omnivores suggests that caution is required in interpreting the effects of dietary constituents from published studies and, that greater dietary differences may exist within defined dietary groups than between them.

Overall, in the current study, there was no correlation between fibre intake and IDMC cycle length. This appears to be in conflict with the generally accepted concept that fibre reduces whole gut transit time (Gogler 1976; Davies et al 1986; Spiller 1994). However, the major proportion of whole gut transit time relates to colonic transit which is unaffected by the small intestinal IDMC. Moreover, fasting motility is responsible for transport of non-digestible intestinal contents, with postprandial motility having a substantial influence on transit of chyme. Additionally, fibre added to nutrients has been reported to slow small intestinal transit, by increasing the length of intestine exposed to nutrients and presumably intensifying the inhibitory motor feedback from the distal gut (Lin et al 1997).

Total caloric intake affects some aspects of gastrointestinal motility (1.3.1 & 4.2.4). However, energy intake in the current study was no different between any subject group, providing evidence that the vegetarian subjects studied were not using their chosen diet to mask restrained eating and dieting behaviour, as has been previously reported by Martins et al (1999). Subtle changes in nutrient intake could potentially affect neurotransmission within the small intestine. Nitric oxide (NO) is known to be important in modulating many aspects of gastrointestinal motility (Luiking et al 1998), and may trigger Phase III activity (Russo et al 1999a). L-arginine, an NO precursor, is an amino acid present in the diet. It is possible that in adopting a vegetarian diet, omnivores consumed a larger quantity of L-arginine, however, the dietary data from the current study, is not sufficiently detailed to enable this to be ascertained. Although there was a non-significant trend for alcohol to be consumed in lesser amounts in C2 as compared to both C1 and vegetarian subjects, this is unlikely to account for the shortening of the IDMC, as alcohol hastens gastrointestinal transit (Probert et al 1995).

In the current study, the order of the 2 manometric studies C1 and C2 was not randomised as the experiment was originally designed to evaluate potential differences between vegetarians and controls. The second control study (on vegetarian diet) was intended to control for subject specific (rather than diet specific) factors between omnivores and vegetarians. It is possible the reduction in cycle length is due to an order effect, although this appears unlikely. In numerous studies of small intestinal motility reported in humans involving serial manometric recordings (Sarna 1985; Husebye 1999) a reduction in cycle length with prolonged (Kumar et al 1989) or repeated (Russo et al 1996; von Schonfeld et al 1998; Gielkens et al 1999) recordings has not been documented. The substantial variations in cycle length within and between individuals (Dooley et al 1992) make it unlikely that this difference has arisen by chance. Moreover, Bjornsson & Abrahamsson (1995) found no difference in motility indices for phases II and III or for duration or migration of phase III between tube-tolerant and tube-naive subjects. However, this issue has not been specifically addressed, thus to clarify this issue further, data from a previously published study from our motility laboratory, in which each of 8 subjects had 2 manometric studies, (Russo et al 1996) was reanalysed with no order effect in IDMC length found ($P = 0.21$, by paired t-test).

It is unlikely that the menstrual cycle influenced these results, even though female subjects did not have their manometric studies scheduled to all occur in the same phase of the menstrual cycle. The effect of the menstrual cycle on small intestinal motility is controversial, and it is unclear whether important differences in gastrointestinal motility exist between phases of the menstrual cycle (Jackson et al 1994; Wedmann et al 1995; Degen & Phillips 1996; Mohiuddin et al 1999). Bowel motions are consistently reported as being looser and more frequent during menstruation (Heitkemper et al 1988), but no subject was studied whilst menstruating.

The finding of a shorter IDMC with an acute vegetarian diet, but no difference with its chronic consumption compared to an omnivorous diet suggests that the IDMC may have a set point, to which it returns with time. Further studies are required to examine this possibility. The introduction of a nutrient infusion arm in manometric studies on both control and vegetarian subjects and randomisation of the order of both the dietary periods and manometric studies in the control subjects would be desirable. Restudying the omnivores eating a vegetarian diet after a longer time period of continued consumption of the vegetarian diet would also be of substantial interest.

10.5 CONCLUSION

There is no major difference in fasting motor activity between these longstanding vegetarians and omnivores. Acute adoption of vegetarian diet is associated with a reduction in IDMC length by approximately 30 %, suggesting that acute dietary modification may alter fasting gastrointestinal motility. The lack of difference in IDMC length between the chronic vegetarians and omnivores suggests that this effect may be short lived, with the IDMC length returning to a "set point" with continued exposure to a vegetarian diet.

CHAPTER 11

High Resolution Manometry of the Human Duodenum During Fasting and Lipid Infusion

11.1 INTRODUCTION

The human duodenum is a short but highly specialised region of the gastrointestinal tract. Its motor activity plays a role in retarding the rate of gastric emptying (Rao et al 1996b) and regulating the orderly delivery of chyme to the remainder of the small intestine (Malbert & Ruckebusch 1989). Rather than being merely a passive conduit, these mechanical functions appear to be highly modulated by luminal feedback control mechanisms (Meeroff et al 1975; Shirazi et al 1988; Rao et al 1996b). The duodenum also subserves an important sensory function (Meeroff et al 1975; Parr et al 1987; Lin et al 1994a), containing both chemo and mechanoreceptors (Cervero 1994) which, when stimulated, modulate the rate of gastric emptying directly by feedback onto gastric motor functions (De Ponti et al 1987; Heddle et al 1988a; Allescher et al 1989) and, indirectly, by varying duodenal resistance to gastric emptying (Shirazi et al 1988). These effects are mediated by neural (Allescher et al 1989) and humoral (Fraser et al 1993; Shi et al 1997) means. Absence or "malfunction" of the duodenum is associated

with disordered gastric emptying and dyspeptic symptoms (Lin 1994), due to the resulting mismatch between gastric emptying and subsequent digestion/absorption, attesting to the vital regulatory role of the duodenum.

Despite the recognised importance of the duodenum in humans, there is little detailed knowledge of its motor function in health. In part, this is a result of technical limitations. Most information on normal human upper gastrointestinal motility concentrates on the oesophagus, stomach and small intestine. The relatively few manometric studies which have focussed on duodenal motility in humans are limited in their temporospatial resolution due to the relatively restricted number of recording sites placed in the duodenum (Borgstrom & Arborelius 1975a & 1978; Thompson et al 1982; Thompson & Wingate 1988; Rao et al 1996b; Castedal et al 1998). Scintigraphic studies are limited by the low spatiotemporal resolution of this technique (Quon et al 1989), and fluoroscopic studies by the length of radiation exposure permissible in volunteers (Rao et al 1996b). In other regions of the gut, pressure patterns are known to vary over short distances (Sun et al 1997b); moreover, closely spaced pressure recordings have been found invaluable in defining some of the pressure/flow relationships in the oesophagus (Brasseur 1993) . We have therefore sought to perform duodenal manometry with high spatio-temporal resolution.

Luminal manometry is the most direct method of assessing the forces applied to luminal contents by motor events and closely spaced (1.5 - 2 cm) manometry gives better spatial resolution of these forces. High spatial resolution manometry has been facilitated by the recent development of fine bore silicone rubber assemblies capable of recording intraluminal pressures concurrently from up to 21 channels. In this study, one such assembly, with an array of 18 sideholes at 1.5 cm intervals, was used to record duodenal pressures in healthy subjects along the whole length of the duodenum. High temporal resolution was obtained by employing a computer based system recording data at 10 Hz.

The aims of this study were (i) to describe normal motor patterns in the human duodenum of healthy subjects and (ii) to examine the relationship between motor patterns and nutrient delivery.

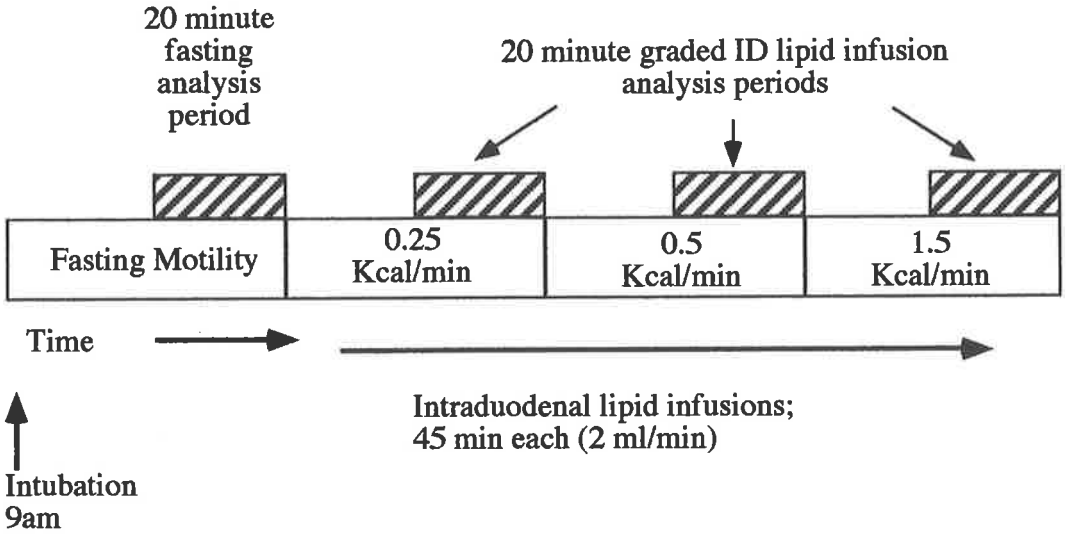


Figure 11.1
Protocol for high resolution duodenal manometry study (section 11.2.2).

11.2 METHODS

11.2.1 Subjects

Nine volunteers (6 male, 3 female), aged 21 - 39 (mean 30) years, were recruited by advertisement. No volunteer had any history of upper gastrointestinal disease or surgery, nor was taking any medication. Their mean body mass index was 24.2 Kg.m-2 (range 21.1 - 27.8), and they were all nonsmokers.

11.2.2 Protocol

The protocol is illustrated in figure 11.1. After an overnight fast each subject attended the motility laboratory between 8:30 and 9 am. The manometric assembly (as illustrated in figure 10.2) was introduced via an anaesthetised nostril into the stomach. The position of the assembly was continuously monitored taking advantage of the known transmucosal potential difference (TMPD) across the gastroduodenal junction (7.2.3). When the two most distal sideholes were located in the duodenum, a small (10 ml) balloon was inflated to speed the passage of the remainder of the assembly into the duodenum. Once the manometric assembly was correctly positioned, fasting motility was recorded until the occurrence of a duodenal phase III migrating motor complex (MMC) of the interdigestive motor cycle (IDMC) (Sarna 1985). Within five minutes of duodenal phase III cessation, a graded infusion of a triglyceride (lipid) emulsion (Intralipid 10%, Kabi Pharmacia), into the proximal duodenum was commenced. It was diluted with normal saline to deliver 0.25, 0.5 and 1.5 Kcal/min at 2 ml/min, for 45 min at each caloric rate. These caloric rates of intraduodenal (ID) lipid were selected as lipid at rates 1 Kcal/min and greater has been shown to stimulate pyloric motility and slow gastric emptying (Heddle et al 1988a).

Subjects were recumbent, and in order to minimise the occurrence of pressures arising external to the gut, they were requested to refrain from any avoidable movement during each of the four recording periods.

11.2.3 Positioning of the Manometric Assembly

TMPD was monitored as previously described (7.2.3). Initially the two most distal sideholes of the assembly were perfused with saline and used to record TMPD. When these were both noted to be in the duodenum according to established TMPD criteria

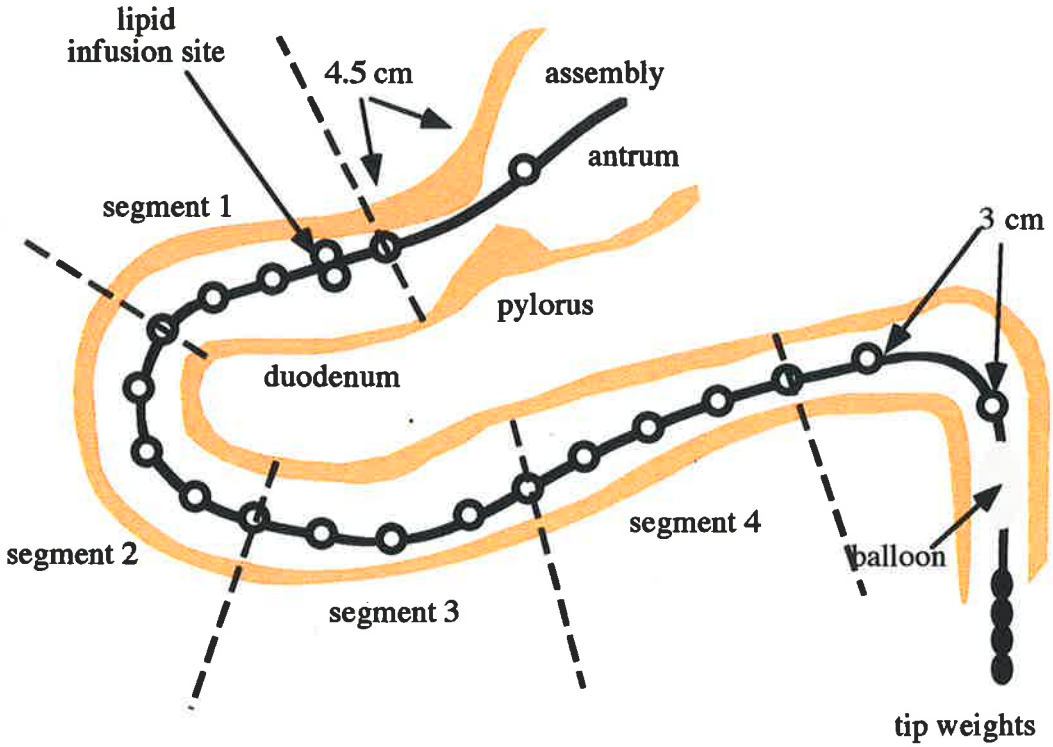


Figure 11.2
Schematic showing the manometric assembly used, with the lipid infusion site and sideholes for pressure recording indicated. All intervals between sideholes are 1.5 cm unless otherwise marked. The arbitrary division of the duodenum into four equal, 6 cm segments, is indicated. (sections 11.2.2, 11.2.3 & 11.2.4)

(Heddle et al 1988a), the next two, more proximal, sideholes were used to record TMPD in order to monitor their passage across the gastroduodenal junction. Dual point TMPD recording was made progressively from each more proximal pair of sideholes in this fashion, until only one sidehole remained in the antrum, with 19 in the duodenum. The TMPD gradient between the two most proximal sideholes was then monitored and maintained throughout the remainder of the study by adjustments in the assembly's position as necessary.

11.2.4 Manometric Recordings

The manometric assembly used (Figure 11.2) had an external diameter of 4 mm. It contained 21 lumina of 0.5 mm internal diameter, one being used for infusion of lipid into the proximal duodenum and 20 for manometry. An additional 0.9 mm lumen was used for inflation of the balloon at the tip of the assembly. The 4.5 cm interval between the first two manometric sideholes straddled the pylorus, the interval between the two most distal sideholes was 3 cm, and the remaining 17 intervals were 1.5 cm each. Thus, the sidehole chain spanned the duodenum. The manometric channels were perfused at 0.15 ml/min with distilled degassed water, or saline (whilst used for TMPD recording), which gave pressure rise rates of at least 150 mmHg/s. Data were recorded on-line at 10 Hz with a Power Macintosh (7100/80, Apple Corp., Cupertino, USA) using software developed in-house (MAD - Prof CH Malbert), written in LabView (National Instruments, Tx, USA), and logged direct to disk for later analysis. In each subject, 4 separate data files were generated, one for the fasting period, and one for each rate of ID lipid infusion.

11.2.5 Data Analysis

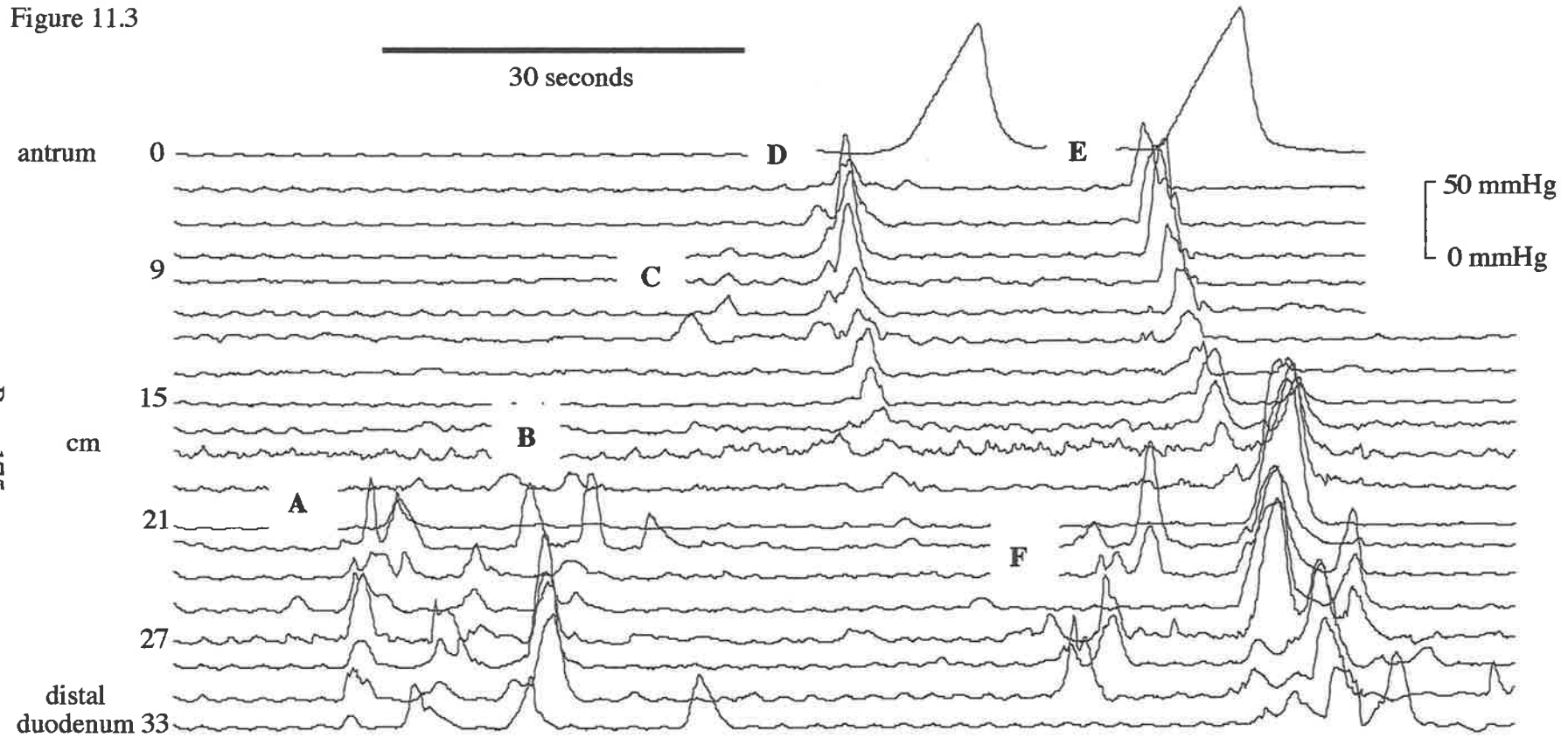
The analysis focused on the timing and patterning of pressure wave (PW) sequences and as we were primarily interested in studying intraluminal pressures likely to be important in determining intraluminal flows, we elected not to take account of stationary PWs. Pressures were only included in the analysis when the assembly was correctly positioned according to established TMPD criteria (Heddle et al 1988a). Pressures with a pattern characteristic of those arising external to the gut, due to straining or movement, were excluded. In order to make standardised comparisons between the 4 analysis periods (fasting and the 3 different lipid infusion rates), 20 minutes of each condition was selected. The last 20 minutes of phase II was selected as the fasting data, as it

superficially resembles postprandial motility (irregular frequent contractions) and can be reproducibly identified (retrospectively) in each subject. In order to allow each given rate of ID lipid some time to exert its effect, data analysis at each rate is restricted to the last 20 min of each lipid infusion (min 25-45) (see Figure 11.1).

The analysis was performed in a semi-automated fashion. Previously validated (Mathis & Malbert 1995 & 1998), custom-written software (MAD - Prof CH Malbert) detected pressure waves, and PW sequences in Labview 3.0.1 (National Instruments, Tx, USA). A PW was first defined by its peak, as a rise in pressure from baseline of > 6 mmHg lasting between 0.8 and 7 seconds. The onset of the PW was then defined as the first point at which the PW was greater than baseline by more than 1%. On the basis of the slow wave frequency in the human duodenum, a PW sequence was defined as the occurrence of pressure waves in two or more adjacent channels, which had onsets within +/- 3 seconds of each other. PW sequences could therefore involve between two and 20 recording points (a span varying from 1.5 to 33 cm). Although this time window appears long, it will encompass sequences within the reported range for small intestinal propagation velocities (0.39 - 4.51 cm/s) (Ehrlein & Schemann 1992). Moreover, it is known that contractions cause shortening of ~1-2 cm along the intestine (Sarna 1993) and, whilst the intestine shortens, the interval between sideholes does not. In effect this window thus is likely to include waves with a lower limit of propagation velocity around ~1 cm/s; which is also within the range quoted (Husebye 1999). PW sequences were then sorted manually by length and predominant direction of travel. The direction of travel of very short PW sequences involving only two sideholes (1.5 cm) was not considered. PW sequences were defined as travelling in either a purely or predominantly antegrade or retrograde direction by examining the time interval between each pair of sideholes which a PW sequence traversed. If a majority of these intervals was positive, it was scored as antegrade, if negative, as retrograde. PW sequences which converged on or spread from a site were not analysed separately and were likewise allocated a direction based on their predominant component. In this analysis, no account is taken of either "synchronous" PW sequences or those with bidirectional components of equal length.

This automated identification of PW sequences, and assignation of direction was validated by comparison with manual analysis of the first 2 minutes of the analysis period in 8 (of 36) randomly selected 20 minute recording periods from 6 subjects (4 during fasting and 4 during ID lipid).

Figure 11.3



Although this 2 minute segment is atypical because of the high number of longer PW sequences seen, it economically illustrates a number of PW patterns. (A) a 9 cm long predominantly synchronous onset PW sequence; (B) a couple of isolated PW's occur prior to a longer PW sequence with retrograde and synchronous components;(C) a predominantly retrograde PW sequence over 3 channels; (D) a long antegrade PW sequence; (E) a rare very long predominantly antegrade PW sequence which sweeps down the entire duodenum, and (F) several short (1.5 - 4.5 cm) predominantly antegrade PW sequences. (section 11.3)

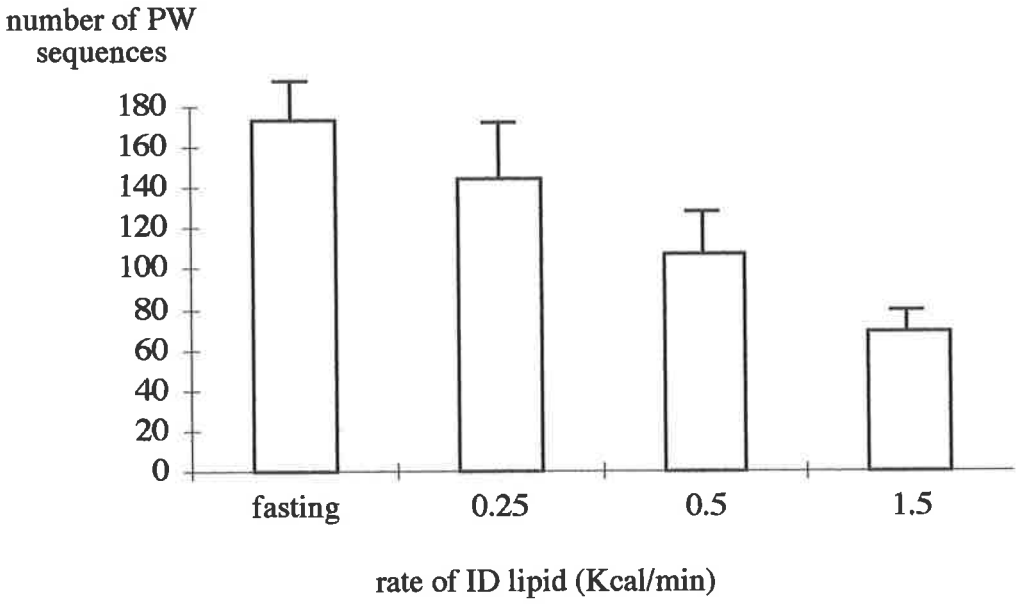
For analysis of the regional occurrence and site of origin of PW sequences, the duodenum was divided into four equal 6 cm segments, comprised of 4 continuous 1.5 cm intervals, as illustrated in figure 11.2. Data from each interval were combined within segments, and averaged. In order to maintain equal segment lengths, data from the last (seventeenth) 1.5 cm interval was excluded from this analysis. The total number of PW sequences, those traversing each segment, their start site, length, and predominant direction of travel during each of these 20 min periods were assessed and compared between fasting (late phase II of the IDMC) and the 3 rates of ID lipid.

Statistical analysis was performed with repeated measures analysis of variance (ANOVA) in SuperAnova (ABACUS Concepts, Calif, USA). All observations were matched between subjects. A P value of less than 0.05 was regarded as significant. Data are presented as mean ± SEM, unless otherwise stated.

11.3 RESULTS

The study was well tolerated by all volunteers, with no adverse effects noted. Recordings were satisfactorily completed in 9 subjects for all 4 analysis periods (fasting, 0.25, 0.5 and 1.5 Kcal/min). In one volunteer, a duodenal phase III episode occurred towards the end of the 0.5 Kcal/min lipid infusion. In this case, the 10 minutes preceding phase III were used for analysis, and the values doubled to simulate a 20 minute period. In the remaining 8 volunteers, complete data sets were obtained, yielding matched data from 9 subjects for all 4 analysis periods. Some typical motor patterns recorded are presented in figure 11.3.

Two minute segments of data from 8 of the 36 analysis periods were used to validate the computerised detection of PW sequences and assignation of their direction of travel by comparison with the manual analysis. The manual analysis detected 64 PW sequences, and the computerised analysis 103. There was concordance between the two analysis methods for directional information in 93% of instances. The discrepancy in the number of PW sequences was nearly all accounted for by errors in the manual analysis. Two categories of variation between the automated and manual analyses accounted for most (37/39) of these discrepancies; (i) eight longer PW sequences scored manually were broken up (correctly) by the automated analysis into twenty-three shorter PW sequences, where a wave within the PW sequence failed to meet the strict analysis criteria, and (ii) twenty-two short (21 of 1.5 cm, and 1 of 3 cm) PW sequences



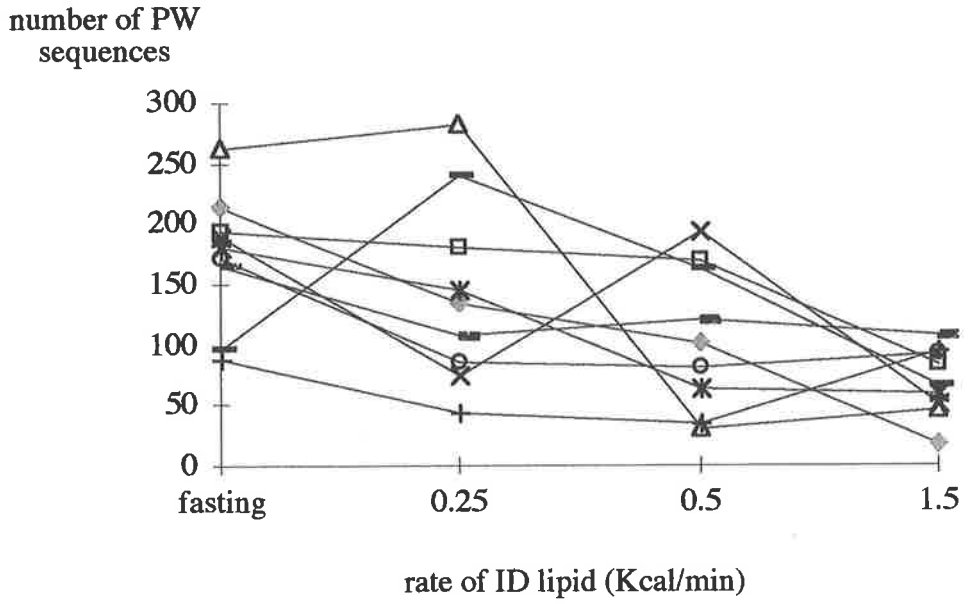


Figure 11.4
Group data (above) (mean +/- SEM), and individual data (below) for the overall number of PW sequences during each of the four 20 min analysis periods (P for linear effect < 0.001). (section 11.3.1)

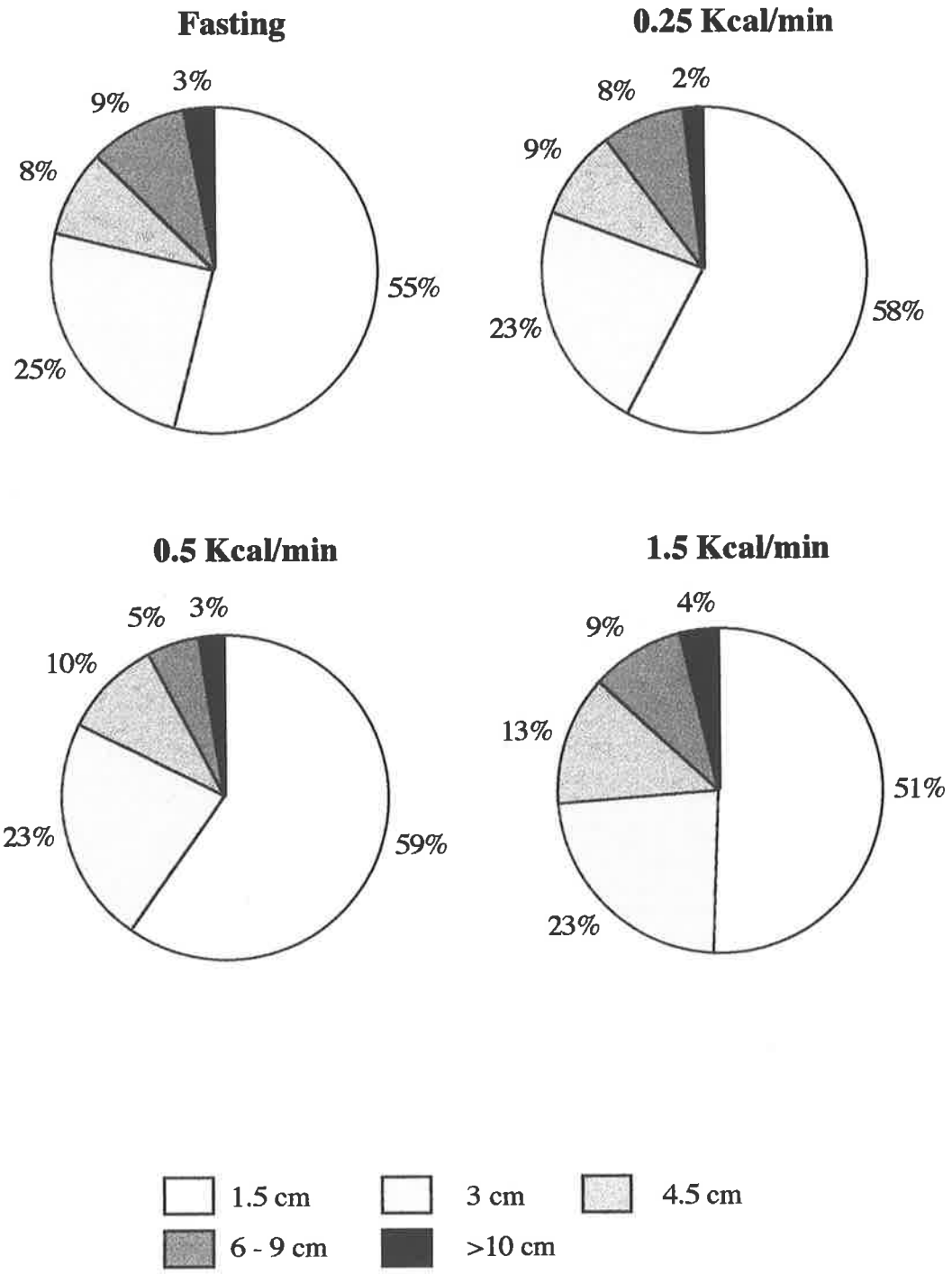


Figure 11.5
The frequency distribution (%) of length of PW sequences is shown for late phase II and for the three rates of ID lipid infusion. There are no differences between fasting and any of the infusion rates. (section 11.3.2)

ignored in the manual analysis (because of low, but above threshold PWs) were correctly identified by the automated method. In two cases, the automated analysis appeared to have incorrectly scored a 1.5 cm PW sequence not identified manually. On the basis of this comparison, and review of discrepant findings, the automated analysis had an accuracy of > 98% in identification of PW sequences.

11.3.1 Number of PW sequences

The greatest number of PW sequences occurred during fasting. There was a dose-dependent suppression of PW sequences as the rate of delivery of ID lipid increased (P for linear contrast effect < 0.001), so that at the highest rate of ID lipid (1.5 Kcal/min) the number of PW sequences was reduced by 60% compared to fasting (see Figure 11.4).

11.3.2 Frequency of PW sequence lengths

Despite the large differences in the total number of PW sequences among the twenty minute analysis periods (see above and Figure 11.4), PW sequence length was not affected by ID lipid (P = 0.44), (see Figure 11.5). The vast majority (87 - 90%) of PW sequences traversed 1.5-4.5 cm. PW sequences which traversed 6-9 cm accounted for 5 to 10%, and those of 10.5 cm and longer made up only 2 to 4% of all sequences. Although long PW sequences comprised only a small proportion of the total, their occurrence was such that during the 20 minute analysis periods most volunteers had at least one PW sequence which spanned a substantial portion of the duodenum (as shown in Figure 11.3E).

11.3.3 Site of PW sequences

The numbers of PW sequences that traversed each of the 4 duodenal segments are shown in figure 11.6. These varied significantly both by duodenal segment, and by rate of ID lipid infusion (by ANOVA, effect by segment P = 0.0016, effect by rate P = 0.0086, and interaction between segment & rate P = 0.0009). These differences were largely brought about by regional variation in the occurrence of PW sequences along the duodenum during all 3 rates of lipid infusion. During ID lipid, fewer PW sequences occurred proximally, compared to distally, and this effect was greatest as the

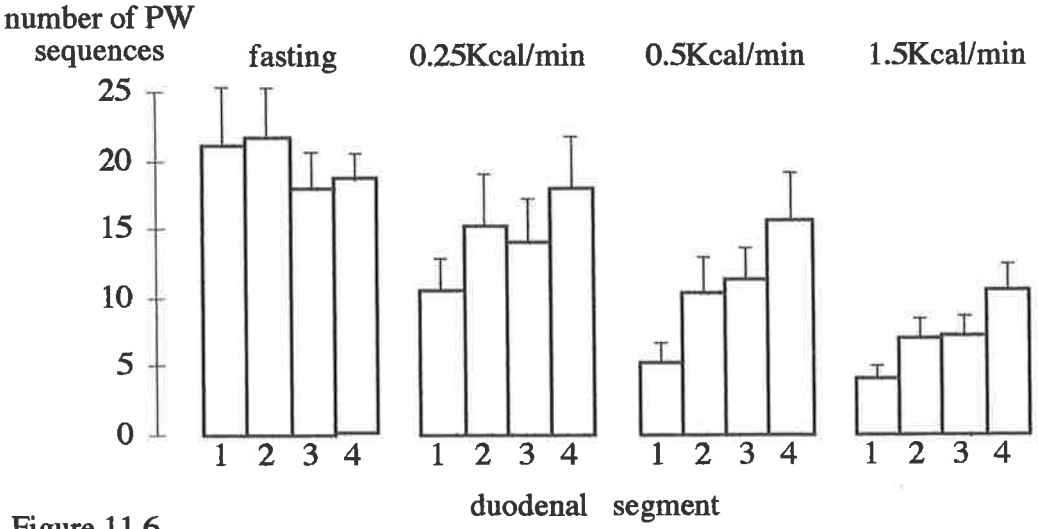


Figure 11.6
Numbers (mean +/- SEM) of PW sequences traversing each segment are shown, by rate of ID lipid infusion. By ANOVA, there is an effect by segment, P = 0.0016, an effect by rate of ID lipid, P = 0.0086, and an interaction between these, P = 0.0009. When compared to fasting, ID lipid caused a dose-related reduction of PW sequences overall, and a differentially greater reduction of proximal PW sequences. During fasting, the rate of occurrence of PW sequences was similar along the length of the duodenum. (section 11.3.3)

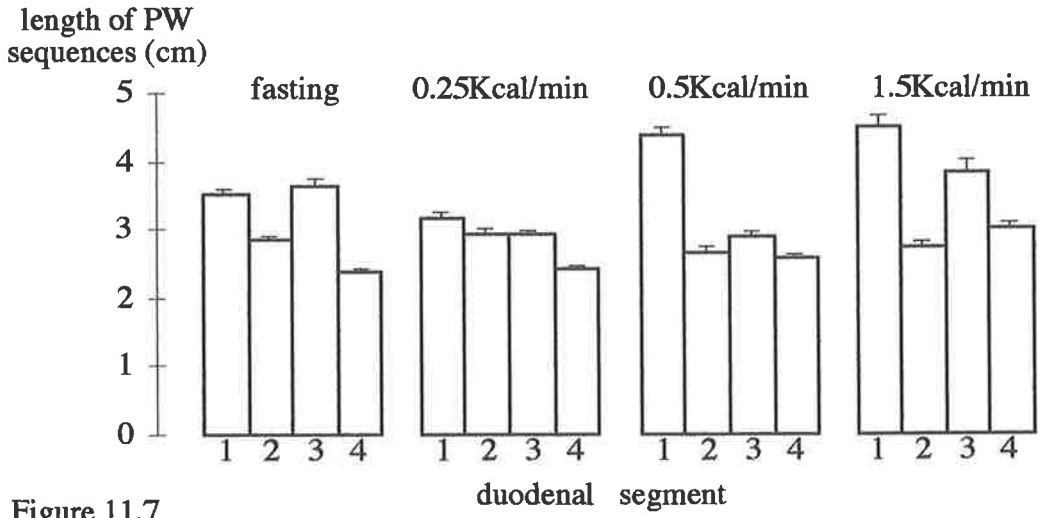


Figure 11.7
Length (mean +/-SEM) of PW sequences is shown. ID lipid had no effect on the mean length of PW sequences (P = NS, by ANOVA). However, there was an effect on PW sequence length by segment (P = 0.0001, by ANOVA), mainly because PW sequences in segment 1 were slightly longer than those starting in other segments. (section 11.3.3)

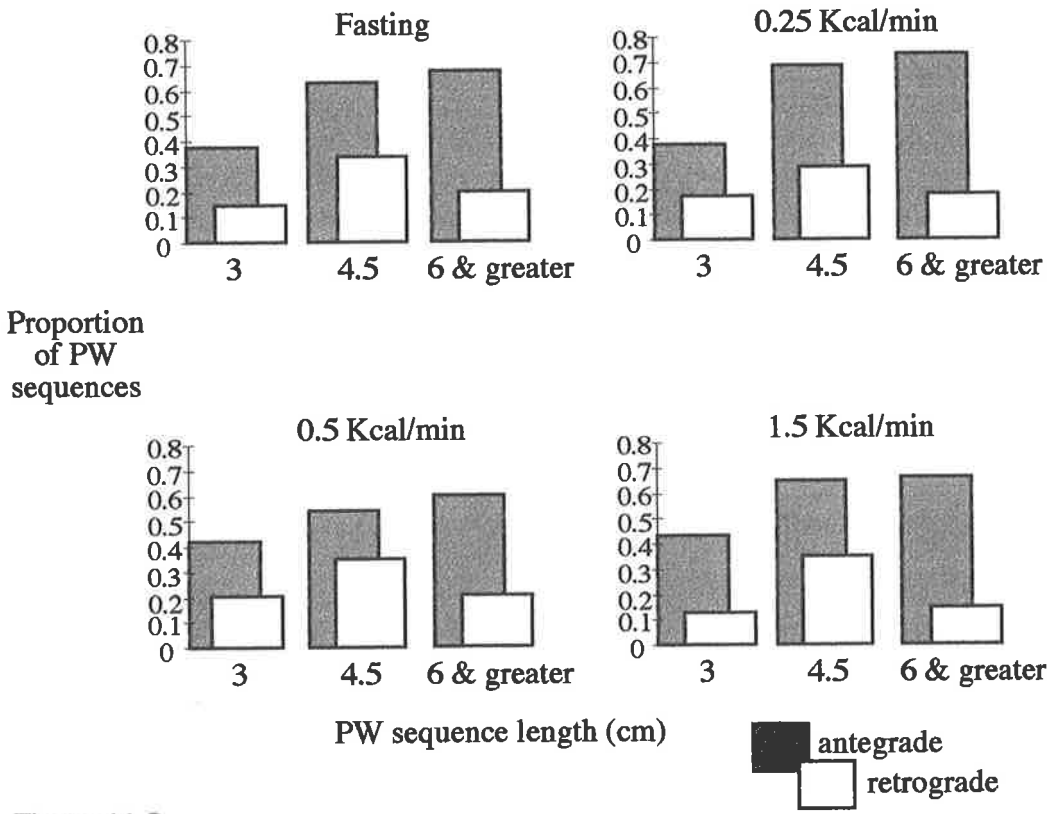


Figure 11.8
The predominant direction of travel of PW sequences for the 4 conditions tested is shown (mean proportions for each length examined). Proportions are used because of the large differences in the number of PW sequences (figure 11.4). At all distances evaluated, and at all rates of ID lipid, the proportion of antegrade PW sequences was higher than retrograde sequences. (section 11.3.4)

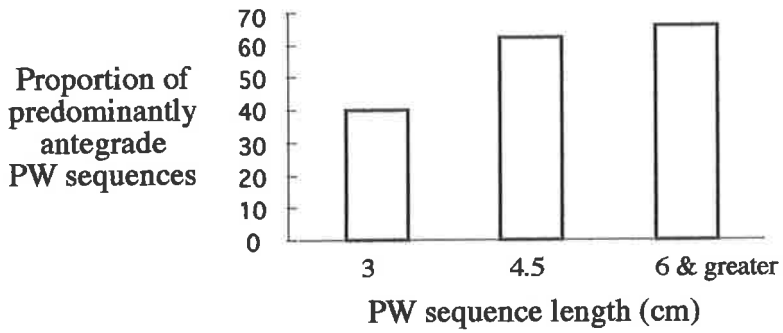


Figure 11.9
As the length of PW sequences increased, the proportion of purely or predominantly antegrade sequences also increased (P for linear effect = 0.0001) (section 11.3.4)

rate of ID lipid increased. By contrast, during fasting the rate of occurrence of PW sequences was similar across the 4 duodenal segments.

The regional variation (by duodenal segment) in the occurrence of PW sequences was largely accounted for by variation in the site of origin of PW sequences. Site of origin varied by both duodenal segment, and rate of ID lipid (by ANOVA, P for segment < 0.01, P for rate < 0.01, but P for interaction = 0.056). Again the same trends (as above) were noted, with fewer PW sequences starting proximally, particularly at the higher rates of ID lipid. In contrast, as shown in figure 11.7, the distance travelled by PW sequences was not different between fasting and ID lipid at any rate of ID lipid infusion. PW sequences which started proximally tended to be slightly longer than those starting more distally.

11.3.4 Direction of PW sequences

The predominant direction of travel of PW sequences is shown in figure 11.8. Direction varied by the length of PW sequences, but not by rate of ID lipid infusion. At all lengths, and at all rates of ID lipid, antegrade PW sequences were more frequent than retrograde sequences. As the length of PW sequences increased, a greater proportion of sequences were antegrade for all conditions tested (Proportion antegrade: 3 cm 39.9 ± 3%; 4.5 cm 62.0 ± 4%; ≥ 6 cm 66.0 ± 4%; P for linear effect = 0.0001) (Figure 11.9).

11.4 DISCUSSION

This is the first study to present high temporospatial resolution pressure recordings from the full length of the human duodenum, and gives an important base on which a better understanding of duodenal mechanics can be built. Potentially important differences between the fasting and fed states have been demonstrated. The major findings are; (i) a greater number of PW sequences occurs during phase II of the IDMC, compared to during ID lipid infusion; (ii) the suppression of PW sequences associated with ID lipid is dose-dependent; (iii) the frequency of duodenal PW sequences show a regional variation during ID lipid infusion which is not seen during fasting, with fewer PW sequences proximally, than distally; (iv) regardless of their site of origin, or whether nutrient was being delivered, most duodenal PW sequences were relatively short, traversing only a mean distance of ~3 cm; and (v) the majority of PW

sequences of all lengths was purely or predominantly antegrade under all conditions tested, with a linear trend for a greater proportion of PW sequences to travel in an antegrade direction as PW sequence length increased. Thus, rather than being a passive conduit, there is substantial variation and complexity in the patterning of duodenal pressures

The order of lipid infusions was not randomised as this would have meant either a four day study (introducing other potential problems such as standardising diet over this period), or a protocol so long that few subjects would have completed. Without long delays (~2 hr post each dose) waiting for the re-emergence of phase III as a marker of fasting motility, it would not be possible to give lower caloric rates after high caloric rates on the same day without the risk of carry-over effects. The changes in motility can be safely said not to represent simply a time effect as, over the study period (3-5 hr), one would have expected to see cyclical occurrence of phase III if lipid were not present. Prolonged infusion of saline would not be expected to interrupt the IDMC (Husebye 1999). Although all subjects began receiving lipid in phase I, suppression of duodenal pressure waves by ID lipid (at 1.1 Kcal/min) has been previously reported in humans in differing phases of the IDMC by Heddle et al (1988a), making it unlikely that the phase of the IDMC during which lipid was commenced affected our results. After a variable period of lipid infusion (~20-30 Kcal), phases of the IDMC are no longer relevant, as one is examining postprandial motility (see below).

Previous studies of human duodenal motor function have had to choose between closely spaced pressure recordings of part of the duodenum, or broader sidehole spacing to cover the whole organ largely because of technical limitations on the number of recording points. Without the 1.5 cm spacing and total organ coverage used in the current study, a substantial proportion of the PW sequences would not have been categorised correctly, as 74 - 82% of PW sequences were only 1.5 - 3 cm in length. With broader sidehole spacing, some spatially unrelated short PW sequences which occurred close together in time would be misclassified as a single longer sequence; and the infrequent, longer PW sequences could not have been reliably identified. With our approach, it is possible to resolve the PW onset patterns accurately in both time and space, which is likely to have important consequences for propulsion.

The interval between the uppermost recording points on our manometric assembly was 4.5 cm. The pylorus was positioned between these points, so it is possible that, for some of the recording time, pressures from up to the first 3 cm of the duodenum were

not registered, due to the maximal tolerated distal displacement of the assembly, on the basis of the TMPD criteria used for documentation of the assembly position. Even if we failed to record continuously from the first 3 cm of the duodenum, this does not alter our findings substantially. Moreover, we used an unusually rigorous process to continuously document assembly position, by monitoring dual point TMPD. This ensures complete confidence that the highest recording point in the duodenum, was, in fact, in the duodenum without relying on pressure wave criteria. Pressure wave criteria for confirmation of assembly position has frequently been used in other studies. It is a somewhat circular method of wave-form definition and suffers from problems of what is "truly" antral or duodenal. In addition, it leads to uncertainties about assembly position when pressure waves are not occurring frequently.

The automated analysis used in the present study enabled the processing of large amounts of data, from multiple channels with high temporal resolution - an approach not practical with manual analysis. Given the importance of high spatio-temporal resolution of pressures to gaining an understanding of duodenal mechanics, some form of automated analysis is vital to progress in this field. The automated analysis used here performed well, both in its ability to identify manually scored PW sequences and to correctly assign direction. The discrepancies between the manual and automated analyses were virtually all due to inaccuracies in the manual interpretation. The automated method is consistent and reproducible, allowing accurate comparisons to be made between recordings. Although manual analysis has been previously held up as the "gold standard", with large amounts of data, human error due to misjudgment and fatigue are increasingly likely and, whilst discretion in analysis based on experience holds some appeal in theory, it leads to inconsistency in data handling, rendering data sets non-comparable.

High resolution manometric recordings in the oesophagus, combined with fluoroscopy have led to a clear understanding of the mechanics of flows and the significance of intraluminal pressures in creating these flows (Brasseur 1993) and, given the tubular nature of both the oesophagus and duodenum, it is reasonable to propose that the relationships between flow and spatiotemporal pressure patterns will be similar in these two organs. We thus believe that the duodenal pressure patterns observed in the present study are likely to be the major determinants of duodenal intraluminal flow. As duodenal motor characteristics differ somewhat from the oesophagus, the exact pressure/flow relationships still need to be studied directly in the duodenum. However, it is likely that the short PW sequences we recorded are analogous to "mixing"

contractions described previously (Borgstrom & Arborelius 1975a & 1978), and that longer PW sequences are responsible for movement of intraluminal contents in both directions over greater distances as observed by Borgstrom and Arborelius (1975a & 1978). In the dog, pulsatile flow at the pyloric level is transformed within ~5 cm to smooth, non-pulsatile flow in the duodenum (Malbert & Ruckebusch 1989). This is due to the resistive characteristics of the duodenum, and it is likely that retrograde PW sequences noted in the current study, are one of the mechanisms which contribute to this resistive function.

Castedal et al (1998) have also recorded pressures at 1.5 cm intervals, but only from the first 6 cm or so of the duodenum. They found that a significant number of PW sequences had bidirectional components, rather than being simply unidirectional. Our recordings in the current study have confirmed this, and have defined the spatial relationships of these pressure patterns to the rest of the duodenum. However, unlike Castedal et al (1998), we did not find that predominantly retrograde PW sequences exceeded the proportion of predominantly antegrade sequences in any comparison. This may be due to differences in the analysis technique used, as they examined direction of travel by start site, whereas we examined direction of travel, by length and by rate of ID lipid infusion. Other differences in methodology between their study (Castedal et al 1998) and ours include their restriction in analysis to sequences of ≥ 6 cm, and their use of peaks rather than pressure wave onset times to establish propagation patterns. Another recent high resolution manometric study in the duodenum, has confirmed our finding of regional variation in duodenal motor patterning, and also found the motor response to vary by stimulus (Schwartz et al 1999). Borgstrom et al (1978) recorded pressures from a greater proportion of the duodenum, but with much lower spatial resolution. During fasting, we found a higher proportion of predominantly antegrade (current study 51% vs Borgstrom et al 40%), and a lower proportion of "static" (synchronous or equal directional components) (current study 19% vs Borgstrom et al 31%) PW sequences than they and, during ID lipid infusion, we observed a far higher proportion of predominantly antegrade (current study 61% vs Borgstrom et al 19%) and a much lesser proportion of predominantly static PW sequences (current study 15.5% vs Borgstrom et al 51%) with the proportion of retrograde PW sequences similar between studies at 23.5% and 29% respectively. These differences are likely to arise primarily from the different spatial resolution in manometric recordings between these 2 studies as discussed earlier.

Some investigators have asserted that duodenal flows are intermittent and bidirectional (Schulze-Delrieu 1992; Rao et al 1996b). This is consistent both with the pattern of occurrence of intraluminal pressures we recorded, and with our hypothesis that intraluminal pressures determine flows. This hypothesis would ideally be addressed by concurrent high resolution recordings of both intraluminal pressures and flows. At present this is not possible in human volunteers, although a number of groups are working on potential methodology to enable this (Hausken et al 1992; Silny et al 1993; Boulby et al 1997; Chapter 12). Given the intermittent nature of duodenal flows, if one seeks to understand the relationship between pressures and flows, it is important to use techniques which enable sufficient temporospatial resolution of the data.

Some limited data exist in which duodenal flows and pressures, or movement of contents, have been found to interrelate. A combination of concurrent high resolution manometry with scintigraphy lead Samsom et al (1999) to conclude that the transit of chyme through the proximal small intestine in humans is related to the number of propagated PW sequences. However, because of the limitations in temporal resolution of the scintigraphic technique, one cannot relate discrete episodes of flow to individual PW sequences. In dogs, Furukawa and Hatano (1998) have shown a clear relationship between retrograde small intestinal contractions and intraluminal flow, contractions travelling in an orad direction preceded emesis, and transported intestinal contents in a retrograde direction. In humans, simultaneous manometric and videofluoroscopic studies, referred to above (Borgstrom & Arborelius 1975a & 1978), have categorised manometric PW sequences as "stationary" (mixing), "antegrade" or "retrograde". However, in these studies, only 4 manometric channels at 3 cm intervals in the proximal half of the duodenum were used.

Duodenal motor activity may influence gastric emptying in a number of ways (Camilleri 1997). Rapid clearance is thought to facilitate emptying, delayed clearance to impede emptying (Rao et al 1996b; Nguyen et al 1997) and duodenogastric reflux returns content to the stomach, also effectively slowing emptying (Castedal et al 1998). Although net duodenal flow is antegrade, in humans, retrograde flow is also known to occur, particularly in the proximal duodenum, both during the fed (Hausken et al 1992) and the fasted state (Castedal et al 1997a & b). Our data, with a predominance of antegrade, but a significant proportion of retrograde sequences observed, would support this contention. Although with only an eighth of the sequences over a twenty minute period observed in our study being predominantly (or purely) retrograde, it does not appear likely that postprandial duodenogastric reflux is a major determinant of gastric

emptying rate (Castedal et al 1998) under these conditions. Other duodenal motor patterns associated with slowing of gastric emptying in humans include dilatation and reduction of propagating pressure waves, leading to delayed duodenal clearance, and prolonged tonic occlusion giving high resistance to further gastric outflow (Rao et al 1996b). As our study examined pressures, but not diameter, we cannot entirely clarify this matter, although we recorded a dose-dependent reduction in PW sequences during ID lipid infusion, which would tend to support Rao et al's (1996b) findings. When considering the influence of the duodenum on gastric emptying, it must be remembered that the pyloric sphincter lies at the junction of these two organs. Thus, the timing of pyloric opening relative to the sequencing of duodenal motor events is likely to be pivotal in determining whether or not transpyloric flow occurs.

The occurrence of phase III of the IDMC during the 0.5 Kcal/min lipid infusion in one subject raises the likelihood that, in this subject, at that time, a postprandial motility pattern had not yet been induced. It is however, known that ingestion of a meal takes ~10-20 min to interrupt the small intestinal IDMC in progress at the time of eating (Sarna 1985). At the time of this phase III activity, the subject would have received ~25 Kcal lipid, which in caloric terms corresponds to a period of ~10 -15 min post ingestion of a meal if one accepts a rate of gastric emptying ~1.5-2.5 Kcal/min (Hunt et al 1985). This would then place this event within the previously described phenomenon. Presumably a time lag exists between the first presence of small intestinal nutrients and IDMC interruption whilst sufficient mucosal receptors, and quanta of small intestinal hormones are recruited to induce a "fed" motor pattern.

The fact that the duration of IDMC interruption is proportional to the caloric load of a meal (reviewed in Sarna 1985) also adds biological plausibility to the dose-related suppression of duodenal PW sequences. Moreover, the extent of small intestinal nutrient exposure determines the magnitude of small intestinal nutrient-mediated feedback (Lin et al 1990b) and, as the dose of nutrient given increases, so will the area of intestine exposed increase, because of saturation of absorption capacity (Lin 1994). Hence providing an interrelationship between dose given and extent of small intestine exposed, which may well account for the increasing suppression of PW sequences seen in the current study as dose of lipid increased. Huge et al (1995), reported a similar dose effect in the jejunum of minipigs, with motility index and propagation distance decreasing with increasing rates of nutrient delivery. They concurrently measured flow, and were able to document that these changes in motility were associated with a decreased flow rate and increased transit time.

CHAPTER 12

Validation of a Novel Luminal Flow Velocimeter With Video Fluoroscopy and Manometry in the Human Oesophagus.

12.1 INTRODUCTION

The overall aboral direction of flow within the gut is the net result of individual episodes of flow which occur in both directions (Cannon 1902). Relatively little is known about these patterns of flow, and the patterns of luminal pressures which cause them. It is now possible to monitor intraluminal pressures with a spatial resolution as close as 1 mm between each of up to 21 recording sideholes. Exact temporal resolution of these pressures is also possible, due to the capacity of computer-based recording systems for high frequency data acquisition. Concurrent recording of intraluminal flow with a similar temporal resolution is essential in determining how these individual pressure events relate to luminal flow (6.2.1 & 6.2.1). Previously it has not been possible to monitor pressures and intraluminal flows simultaneously in humans with a temporal resolution of less than one second, although suitable methods exist for use in animals (Malbert et al 1987). The methods currently available for evaluation of luminal flows in humans are discussed in Chapter 2 (2.7.2 & 2.7.4). They include radiographic contrast or marker studies, Doppler-ultrasonography, scintigraphy, MRI and impedance plethysmography. Each of these techniques has substantial limitations with respect to either the length of observation permissible, reliability of the measurement and/or the spatial/temporal resolution which can currently be achieved.

In this chapter the development of a laser-Doppler velocimeter, suitable for use in the gut lumen, which measures the polarity and velocity of liquid movement, with a temporal resolution between 4-7 Hz is described. It has been embedded in a multi-lumen silicone rubber manometric assembly (Dentsleeve, Wayville, SA, Australia) to allow concurrent measurement of intraluminal pressures. The potential advantages of this device include complete electrical insulation, high frequency detection of flows and the absence of radiation exposure.

The aims of these studies were to;

i) perform safety and bench testing of the instrument,
ii) validate the laser-Doppler signal *in vivo* as an indicator of flow by comparing the velocimeter signal with fluoroscopically observed flow in the human oesophagus
iii) define more precisely the patterns of oesophageal luminal flows during swallowing, and
iv) if possible, determine the relationships between intraluminal flows and pressures.

The initial human validation study was performed in the oesophagus because of the ease of triggering episodes of bolus flow predictably and the ability to observe them fluoroscopically. The analysis of oesophageal luminal pressures and flows which occurred during 50 barium swallows, recorded with concurrent video barium fluoroscopy, laser-Doppler velocimetry, and water-perfused multi-channel manometry, are reported here.

12.2 MATERIALS AND METHODS

12.2.1 Design of the Velocimeter System

12.2.1.1 Design and system overview

A schematic of the equipment is illustrated in figure 12.1. Light from a helium-neon laser (633 nm) is split into two beams, with half the beam transmitted down an optical fibre which has its distal end embedded in a manometric assembly. The optical fibre has an external diameter of 125 μm; however, the transmitted light is unimodal as it is restricted to a 4 μm core within the centre of the fibre. The transmitted light enters the gut lumen and is reflected, and Doppler-shifted, by the passing particles it encounters. Some of this reflected light is recaptured by a second optical fibre within the assembly which transmits it back to a photodiode in which the frequency of the reflected light is compared with that of the original beam.

Direction of flow is determined by beating the reference beam with a pulse of known frequency (+80 MHz) from an acousto-optic modulator, so that the direction of frequency shifts of the reflected light is evident. This frequency shift is processed, initially in a spectrum analyser (a specially modified and screened UHF/VHF spectrum analyser Cat # K7620, Dick Smith, Australia), and then on-line by a computer (Apple Macintosh IIci, Apple Corp., Cupertino, USA), using Labview 3.1.1 based (National Instruments, Austin, Texas, USA) computer analysis software custom-written in-house (by Prof CH Malbert). The signal processing software converts the Doppler-shift observed into both a velocity and direction of flow.

The system was developed to detect velocities in the range of 30-120 mm/s. The characteristics of the components limited the velocity sampling frequency to 4-7 Hz. The velocity signal was logged concurrently with the manometric data on a second computer (Power Macintosh 7100/80, Apple Corp., Cupertino, USA). The signal

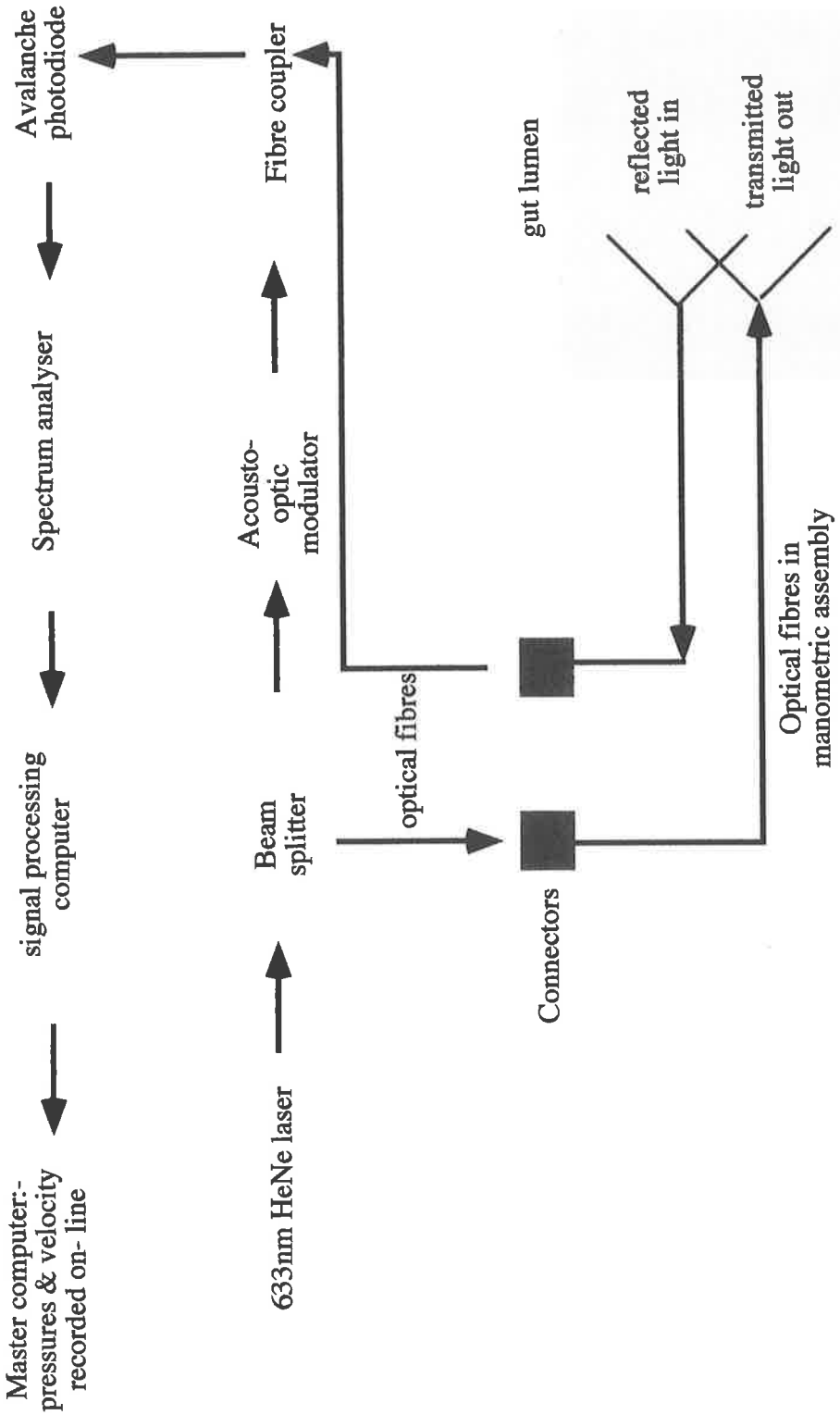


Figure 12.1
Schematic of the laser-Doppler velocimeter. For detailed description, see section 12.2.1.1

processing gave a ~0.4 s internal delay from actual velocity measurement to logging which was corrected prior to analysis.

12.2.1.2 Laser sensor tip

Three separate sensor tips were used (i) a simple design was used for the bench calibration consisting merely of the two optical fibres held at a fixed angle (~3^0) to each other with adhesive, and (ii) for the concurrent manometry and velocimetry, the optical fibres were incorporated into a multi-channel manometric assembly (such as in Figure 12.2). The fibres pass down the shaft of the extrusion within individual channels until they reach the last few millimetres of their course. At that point they enter a common space formed by drilling a hole perpendicular to the side of the extrusion. An acrylic plug was moulded around the two optical fibres which were held in correct position by a jig. The plug with the embedded optical fibres was then glued into the trephined hole in the manometric assembly. The optical fibres curved towards the side of the extrusion at an angle of 30^0, and were angled relative to each other (~3^0) so that their light sampling/projecting areas overlapped 1-2 mm from the side of the assembly as illustrated (Figure 12.2). This area of overlap determined the zone of luminal contents within which velocity measurements were made. During the moulding process, the luminal ends of the fibres were made to protrude ~1 mm from the side wall of the assembly. The tips of the fibres were encased in a mound of epoxy glue, and this mound was ground down with the ends of the optical fibres to optimise the focus and overlap of the fibres' cones of projection. This process was assessed visually by evaluating the fibres' cones of projection, and directly by verifying the sensor's ability to detect flow during the course of its manufacture. For use in pig gut (12.2.2.2), an assembly able to record at 6 sideholes was used. In the human oesophagus (12.2.4) pressures were recorded at up to 20 sideholes.

12.2.1.3 Software

Information from the spectrum analyser was processed on-line. The software's major functions were to; (i) remove the residual spectral component of the incident laser light, (ii) detect the Doppler-shift, and (iii) calculate the mean velocity.

Briefly, the software acquired in burst the full spectrum supplied by the analog spectrum analyser, processed all the information comprised in each data set and, finally, sent the computed velocity to a digital to analog converter. Each sweep of the spectrum analyser, which took 5 ms, was digitised at 5 KHz with a spectral

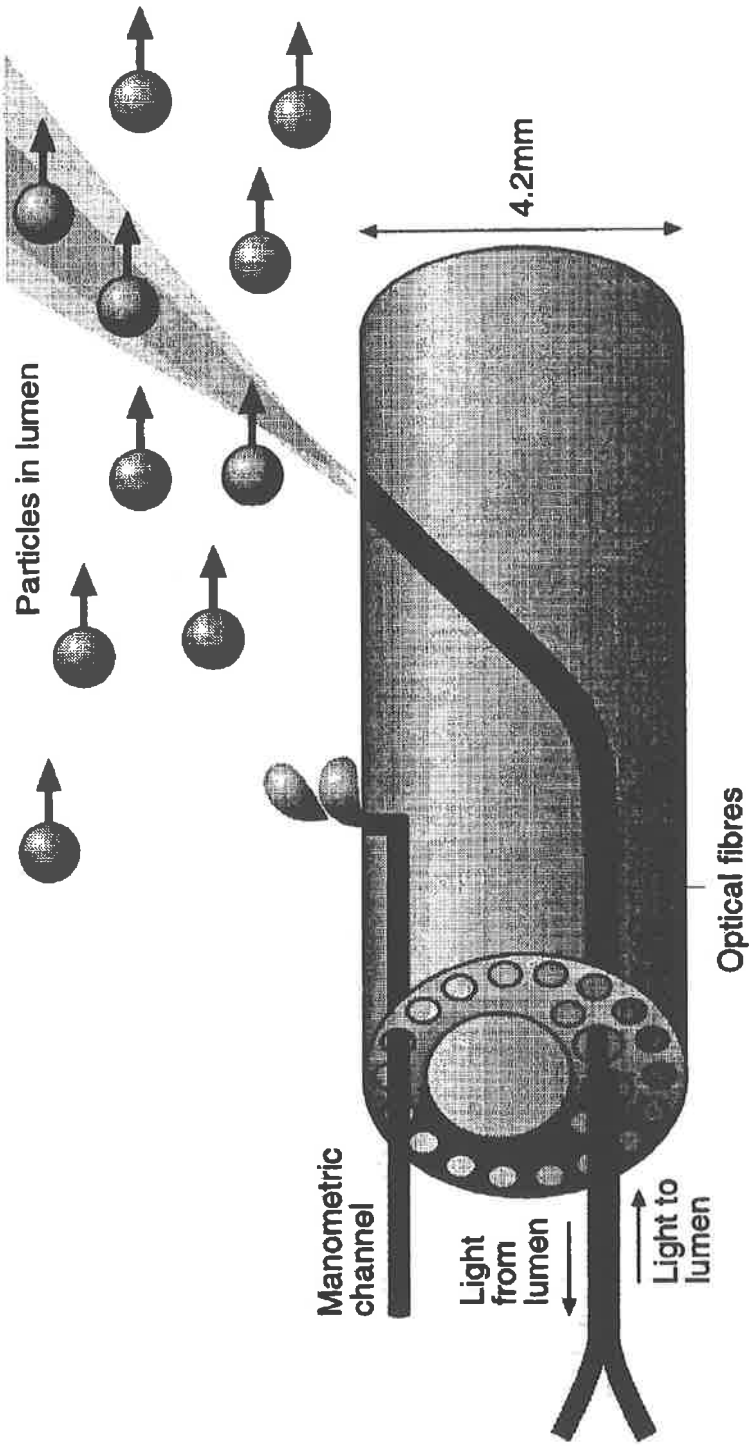


Figure 12.2
Schematic of the manometric-velocimetric assembly, illustrating the arrangement of the optical fibres within the assembly and the area within which velocity measurements are made. See section 12.2.1.2 for further detail.

resolution of 10 Hz. This set of data was then subtracted, point by point, from a similar set acquired with a zero velocity (null template). Since, in the absence of fluid motion, the only spectral component is the residual spectral component of the incident laser light, the digital subtraction resulted in the removal of this residual spectral component present in the original signal. Any shift in the centre frequency of the residual spectral component was corrected for by recalculation of the null template every 120 seconds. This repeated subtraction procedure is important because of the relatively infrequent occurrence of intraluminal flow. At the pyloric level, for example, flow episodes last for only a brief period of each contractile cycle (Malbert & Ruckebusch 1991) and without this continual correction of the null signal, it would not be possible to distinguish flow episodes from shift in the centre frequency over time.

When the signal had been processed to the point where the only frequency component present was the Doppler-shifted frequency, the software identified the position of the maximal and minimal values of the signal. A linear regression was constructed using all the data points present between these two extremes The mid-value of this regression line represented the actual mean velocity. Once converted from Hz to $m.s^{-1}$, this value was presented to the digital to analog converter and a new acquisition from the spectrum analyser commenced.

12.2.2 Bench testing

12.2.2.1 Turntable validation

A circular disk with three circumferential grooves cut into it at varying distances from the centre was mounted on a turntable and rotated at set speeds, generating movement of known velocities in liquid (dilute milk or lipid emulsion) within the grooves. The number of revolutions of the disk was verified by marking a point on its perimeter and counting revolutions within a fixed time interval. The velocity of the movement of the liquid in each groove was thus calculated. The laser sensor tip was held static in a jig and submerged in the centre of the liquid, whilst the disk was rotated, and the laser-Doppler velocimeter signal recorded. This recorded velocity was then compared to the calculated velocity of the liquid. Measurements were made after the fluid had been rotated for 1-2 minutes so that the velocity of the liquid within the groove was equal to that of the groove itself.

12.2.2.2 Concurrent velocimetry-manometry in pig gut

To verify that incorporation of the laser-Doppler sensor and fibres into a multi-channel manometric assembly was possible, and would yield concurrent pressure and fluid particle velocity recordings, the fibres were incorporated into a 6 channel assembly, and a sensor tip fashioned (12.2.1.2). As illustrated in 12.3, this combined assembly was then inserted into a length of excised pig gut. One end of the gut was tied closed around the assembly. A small cannula was inserted through the tie to allow liquid to be instilled. The other end of the gut was left open. A moveable constricting band, fashioned from the finger of a latex glove, was used to simulate a phasic contraction moving along the section of gut, causing constriction of the lumen and movement of contents. Pressures and particle velocity were then recorded.

12.2.3 Safety Assessments

12.2.3.1 Calculations

The energy output of the laser is 15 mW (in free space). However, due to the beam splitter and coupling losses, the maximum power which can be emitted at the fibre tip is 1.5 mW (given the beam diameter of 4 μm). With regard to eye safety, at a distance of 100 mm from the eye (the beam cannot be focused any closer), the beam diameter spreads out to 15 mm, and the intensity of energy falls well within the limit of 25 Wm^{-2} for a Class 3A laser product (Standards Australia 1991). With regard to the likelihood of tissue burn, the maximal permitted exposure, under this Standard, for a duration of 10 seconds or more, is 2 Kwm^{-2}. If direct (continuous) tissue contact with the sensor tip is assumed, the velocimeter delivers energy with a calculated intensity of 120 KWm^{-2}. However, at a distance of only 3.25 mm in air, or 6 mm in water, from the sensor tip, the intensity falls under the 2 Kwm^{-2} limit. Importantly, it should be emphasised that two other factors mitigate against the likelihood of a tissue burn with prolonged use of the velocimeter: i) the known ability of tissues, and local blood flow, to act as a heat sink and ii) the narrowness of the 4 μm beam which makes it highly unlikely that it will remain in direct contact with the exact same patch of tissue, in a living organism, for any substantial period of time given the mobility of tissue.

12.2.3.2 Direct Tissue Assessment

Because of the theoretical possibility of tissue burns resulting from use of the velocimeter, it was evaluated in vivo, in anaesthetised rats. The sensor tip (as used in

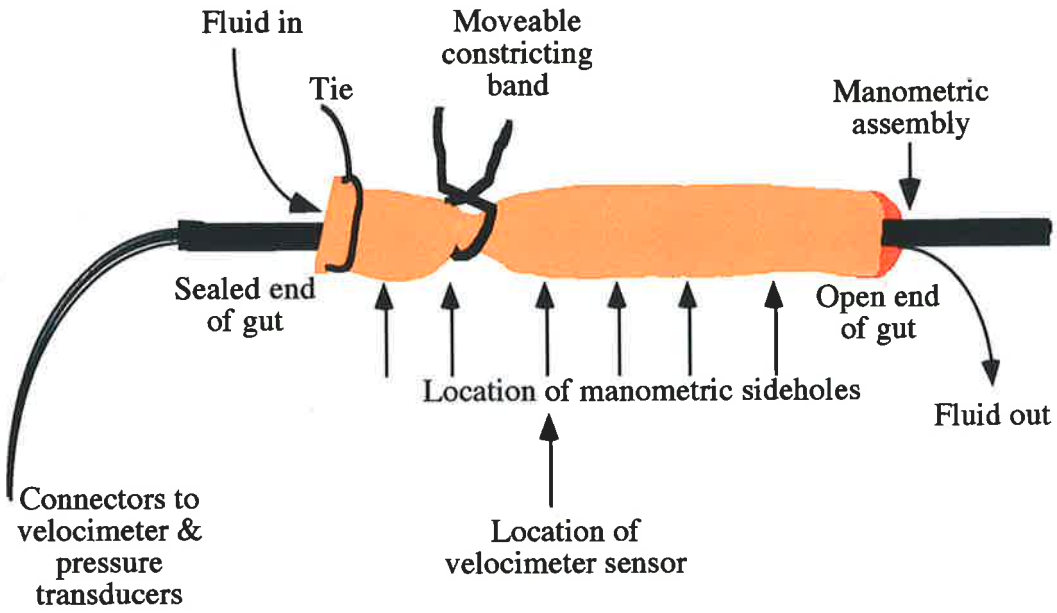


Figure 12.3
Schematic showing experimental set-up for performing concurrent manometry and velocimetry in a length of excised pig gut. The locations of the manometric sideholes and the velocimeter sensor are indicated by the vertical arrows. One end of the pig gut was tied to prevent liquid efflux, and fluid was instilled into this end by a small plastic cannula connected to a 5 ml syringe. Fluid flow could then be created within the length of gut by moving the constricting band along it.

12.2.2.1) was inserted via a gastrotomy and placed in contact with the proximal intestinal mucosa for varying periods (30, 60 & 120 min). Between one and three observations were made per animal for four animals. The sensor was held stationary by use of a jig and its position could be clearly recognised by the light visible through the intestinal wall. The position of the light was marked by a suture on the serosal surface. Three animals were sacrificed immediately following the experiment, yielding two observations for each time period. A further animal was allowed to recover for 3 days and then sacrificed. Tissue was examined macroscopically and three circumferential sections at 2-5 mm intervals were taken at each level marked by the sutures. Sections were fixed and stained with haematoxylin and eosin prior to light microscopy and the histological examination was performed by a qualified pathologist.

12.2.4 Human Study

12.2.4.1 Subjects

Eight healthy subjects (6M, 2F) aged from 19 to 39 years were recruited by advertisement. None had any upper gastrointestinal symptoms, nor were they taking any regular medication.

12.2.4.2 Protocol

The subjects attended the Radiology Department after a 6 hour fast. The recording assembly was introduced transnasally after local anaesthetic spray had been applied. The volunteers then stood upright in front of the fluoroscopy tube and the velocimeter sensor was positioned according to the pressure patterns recorded by the array of sideholes which straddled it. Initially the sensor was positioned ~ 5 cm above the lower oesophageal sphincter, and later withdrawn to lie ~ 5 cm below the upper oesophageal sphincter. With the assembly in each of these 2 positions, the volunteers swallowed 15 mL of half strength liquid barium (Polibar, E-Z-EM Inc, New York, USA) on command, up to a maximum of 5 swallows per site. During each swallow, concurrent recordings of video fluoroscopy, velocimetry and manometry were made. Radiation exposure was strictly monitored and limited to a total of 70 s. Between barium swallows, volunteers drank water to clear the sensor and oesophagus of residual contrast.

12.2.4.3 Manometry

The recording assembly was a 23 lumen silicone rubber extrusion, with an external diameter of 4.2 mm (Dentsleeve, Wayville, SA, Australia). Two lumina were used to carry the transmitting and receiving optical fibres which led back up to the light source and fibre-coupler respectively (Figure 12.1). A chain of 20 sideholes at 1.5 cm intervals was used to record pressures via 0.4 mm diameter lumina. The laser-Doppler sensor tip was installed 7.5 cm proximal to the most distal manometric sidehole. Manometric channels were each perfused at 0.15 mL/min, giving pressure rise rates of at least 160 mmHg.s^{-1}. Data were acquired at 50 Hz in a custom written program (HAD, GS Hebbard, Dept GI Med, RAH) in Labview 3.1.1 (National Instruments, Austin, Texas, USA), averaged to 10 Hz, digitised, and logged on the master computer (Power Macintosh, 7100/80, Apple Corp., Cupertino, USA).

12.2.4.4 Velocimetry

Velocimetry measurements were made at 4-7 Hz and logged concurrently with the manometric recording on the master computer.

12.2.4.5 Radiology

All views were appropriately coned and shielded. The level of the laser sensor tip was rendered radiologically opaque by tantalum wires inserted within channels not being used for manometry at that level. A lateral oblique projection was used for swallows with the probe in the lower oesophagus, aiming to include the lower oesophageal sphincter and diaphragmatic hiatus. When the sensor was in the upper oesophagus, a slightly oblique PA projection was used, with the neck and thoracic inlet in view. The swallows were recorded direct to video tape at 30 frames per second. No still frames were used.

12.2.4.6 Synchronisation

A timing device (TD-100-S, Provideo Systems, Adelaide, Australia) generated and simultaneously sent a number, at 10 Hz, to both the video tape (fluoroscopy) and to the master computer (which logged both manometry and velocimetry data). This numerical code was then visible on review of both the video and combined manometric and velocity data, enabling corresponding events to be correlated to within 0.1 s.

12.2.4.7 Data Definitions and Analysis

The recordings of pressures and velocity were translated into AcqKnowledge (Biopac, Goleta, California, USA) for display and analysis. Video fluoroscopic images of the barium flow patterns were analysed separately from the manometric and velocity data. Two observers each independently recorded the timing of 12 items for each swallow; 4 from fluoroscopy, 5 from velocimetry and 3 from manometry .

The fluoroscopic items scored were: (i) initial upward movement of the velocimeter sensor on swallowing (taken as the swallow reference time), (ii) first appearance of contrast at the level of the velocimeter sensor, (iii) departure of the trailing edge of the column of barium from the velocimeter sensor and (iv) lumen occlusion at the level of the velocimeter sensor.

The items scored from the velocimetry were: the onsets of (i) the initial and (ii) the major velocimeter signal associated with each swallow, (iii) the duration of the major signal, (iv) the peak velocity of the major signal and (v) the number of signals associated with each swallow. By virtue of the signal processing on-line, the velocimeter signal in the absence of flow was steady at zero $m.s^{-1}$. Deflections were thus easily recognised and were defined as a clear departure of the velocimeter signal from the baseline by 10 $m.s^{-1}$ or more for 0.5 s or longer. The major signal was defined as the deflection during which the greatest velocity was measured by the velocimeter and, if the same peak velocity was recorded in more than one deflection for a given swallow, the deflection with the longest duration was judged to be the major signal.

The manometric items scored were: (i) the onset of the swallow-induced pharyngeal pressure wave, (ii) the onset of the oesophageal body common cavity pressure rise which occurs with entry of liquid boluses into the oesophagus prior to the onset of the peristaltic pressure wave and (iii) the onset of the major upstroke of the oesophageal body peristaltic pressure wave at the level of the velocimeter sensor.

12.2.4.8 Statistical analysis

Mean velocities and number of deflections were compared using an unpaired Z-test for comparisons of means. Differences in proportions were assessed with a 2x2

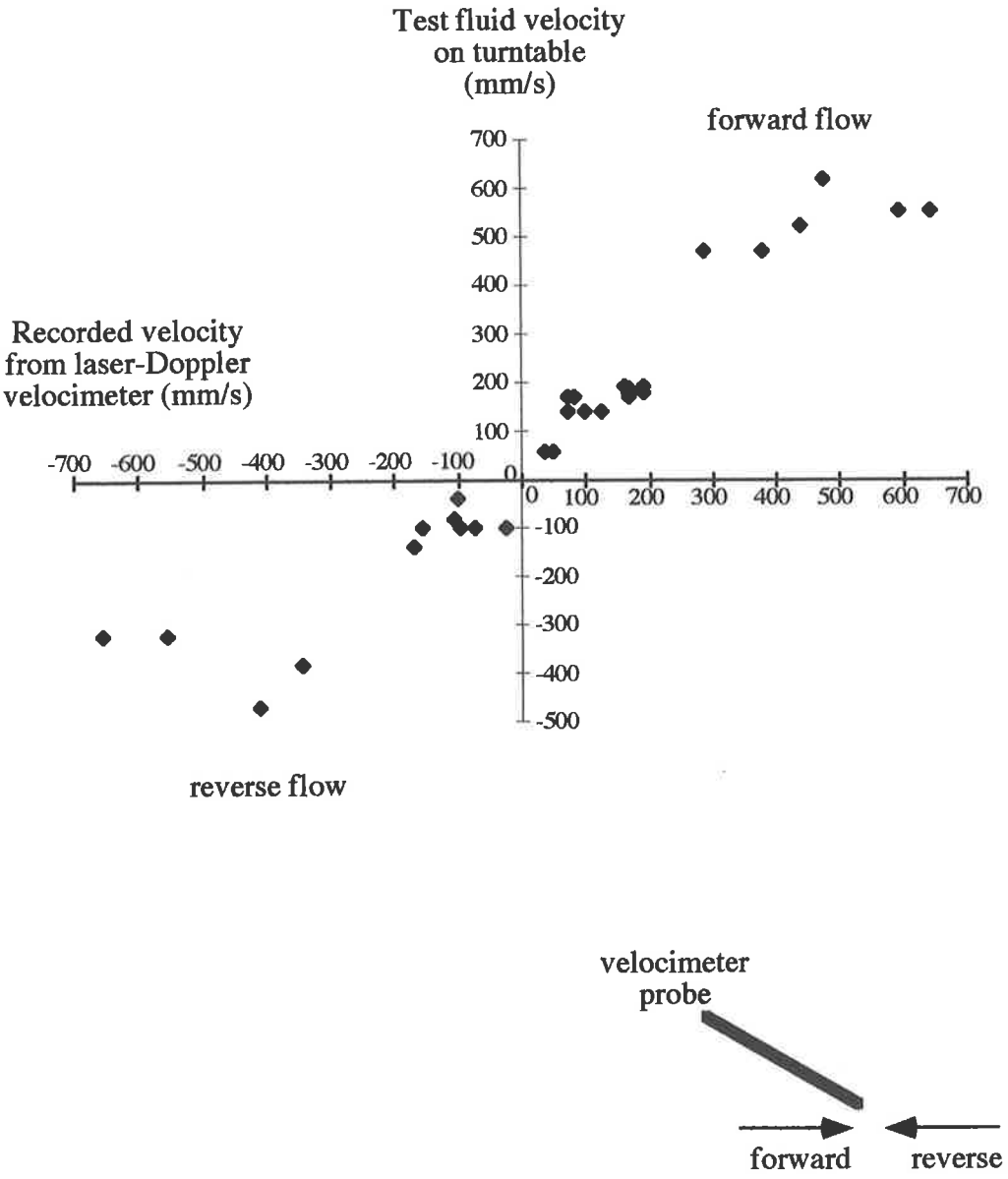


Figure 12.4
Data from calibration of velocimeter against the grooved disk: velocimeter reading (x-axis), is plotted against actual velocity of fluid within the grooves (y-axis), which is being rotated at a controlled rate by the turntable. See section 12.3.1.1.

contingency table and calculation of chi squared P-value. The level of statistical significance was taken to be at $P < 0.05$.

12.3 RESULTS

12.3.1 Bench testing

12.3.1.1 Turntable validation

A linear relationship was found between the known velocity of liquid on the turntable and the measured velocity with the laser-Doppler velocimeter for bidirectional fluid movements (Figure 12.4).

12.3.1.2 Pig gut velocimetry-manometry

With movement of the constricting band along the section of pig gut, pressures and velocity were repeatedly measured. Both direction and polarity of velocity were appropriately registered (Figure 12.5). The timing of the maximal velocity signal appeared to coincide with the peak pressure at the level of the sensor.

12.3.1.2 Tissue safety assessment

No evidence of perforation nor tissue damage could be identified macroscopically or on serial histological sections.

12.3.2 Human study

The protocol was well tolerated by all subjects and no adverse effects were detected. Fifty swallows were recorded with concurrent velocimetry, fluoroscopy and manometry in the 8 subjects, 24 swallows with the sensor situated in the proximal oesophagus, and 26 with it in the distal oesophagus. Each subject contributed between 5 and 10 swallows; from 2 to 5 with the sensor in the proximal, and 3 to 5 with it in the distal oesophagus. There was excellent interobserver agreement in the assignment of times to the fluoroscopic, velocimetric and manometric variables, with 89% concurrence to within ± 0.2 s, and 96% concurrence to within ± 0.4 s.

Figure 12.6 shows the typical combined manometric and velocimeter recordings from swallows with the sensor in the 2 sites. The velocimeter gave a signal between the time of initiation of a swallow and the time of lumen occlusion at the level of the

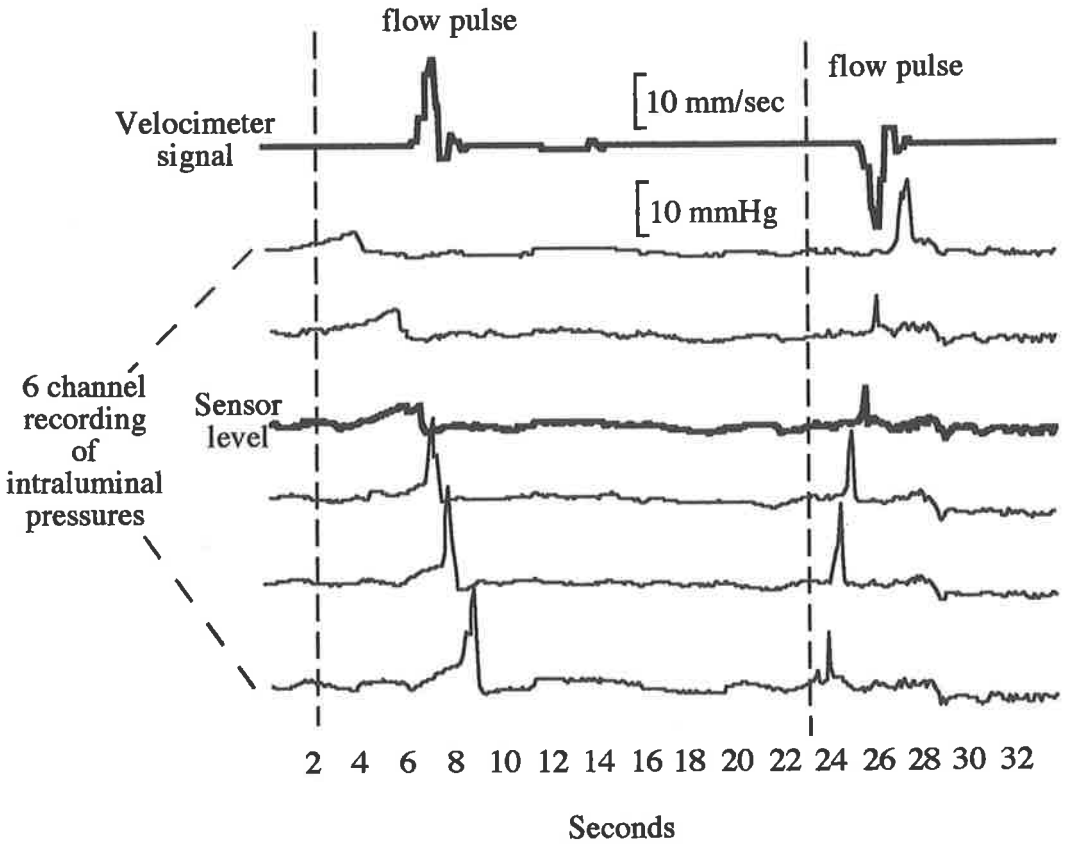


Figure 12.5
Representative section of data from the concurrent recording of pressures and velocimetry in an excised section of pig gut. Both antegrade and retrograde flow velocities are recorded in response to movements of the constricting band along the gut (section 12.3.2.2).

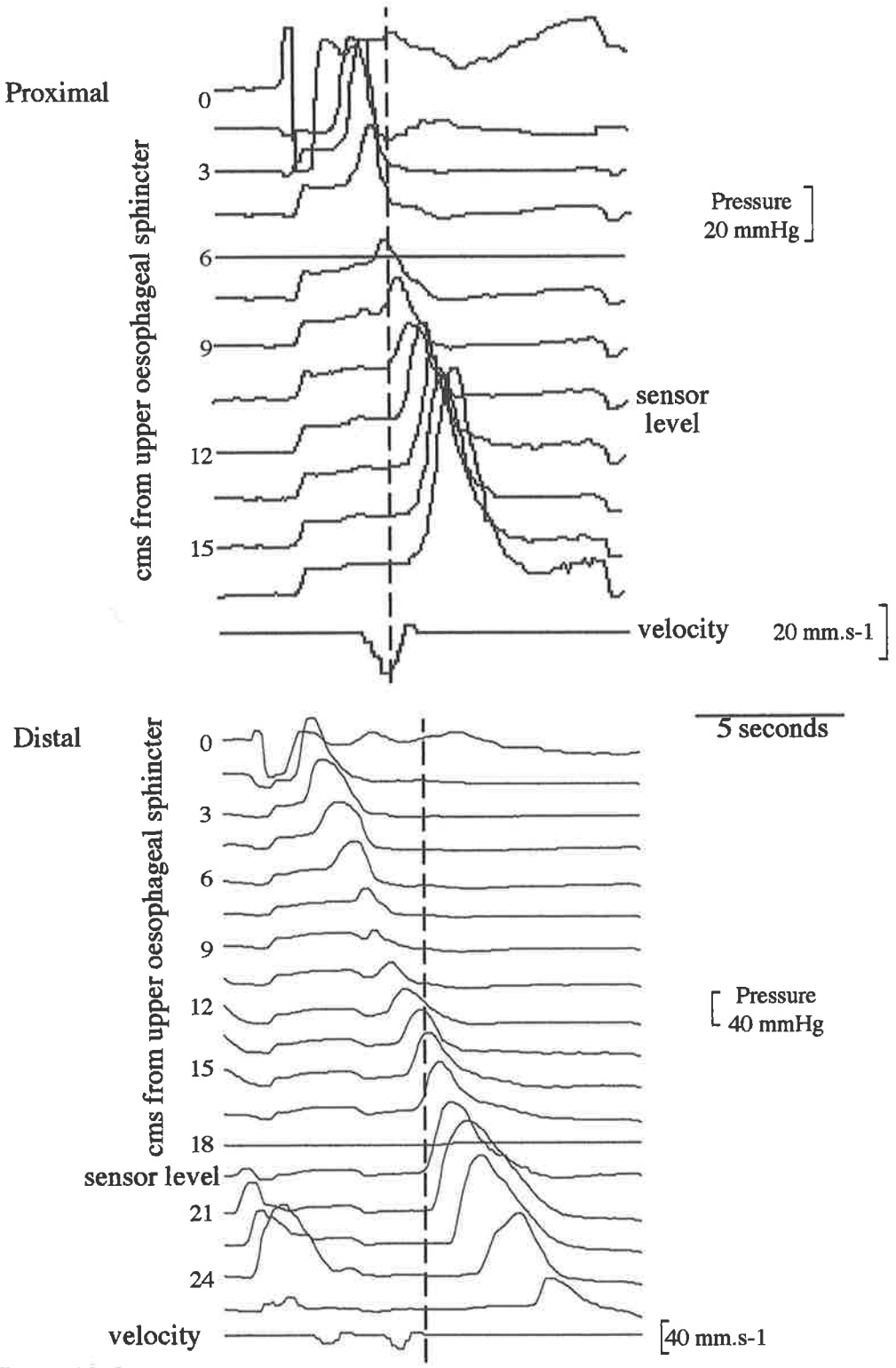


Figure 12.6
Concurrent pressures and velocimeter recordings during barium swallows, with the velocimeter sensor located in the proximal (above) and distal (below) oesophagus. Highest recording point shown is in the region of the upper oesophageal sphincter. The manometric channel at the level of the velocimeter sensor is indicated in each recording. The dashed vertical lines indicate the onset of the major upstroke of the peristaltic wave at the level of the sensor. (section 12.3.2).

sensor in 46 of the 50 swallows (92% for both proximal and distal sensor sites). In the remaining 4 swallows (in 3 subjects) there was no velocimeter signal (2 each with the sensor in the proximal and distal oesophagus). In 2 of these subjects the velocimeter subsequently registered a signal after the subjects drank 50-200 mL of water. The third subject was extubated directly following the swallow which did not register a velocimeter signal, as his X-ray exposure time did not allow further evaluation and the sensor was found to be heavily coated with barium. The velocimeter signal had between 1 and 13 deflections during each swallow, there being a greater number of deflections when the sensor was located in the distal as compared to the proximal oesophagus (4.3 vs 2.4, P = 0.001). No velocimeter flow signal has its onset after either fluoroscopic lumen occlusion or the onset of the major upstroke of the peristaltic pressure wave at the level of the sensor. Maximal flow rates recorded with the velocimeter did not differ between the proximal and distal oesophagus (76.7 vs 73.8 $mm.s^{-1}$, P = 0.4).

12.3.2.1 Comparisons between fluoroscopy and velocimetry

In half the swallows available for examination (22/44), the initial velocimeter signal commenced within ± 0.2 s of the upward movement of the assembly, before barium arrival at the sensor level (12 proximal, 10 distal). In the other half, the initial velocimeter signal occurred when contrast was at the level of the sensor (8 proximal, 14 distal). The relationship of the initial velocimeter signal to barium arrival at the sensor level could not be determined in 2 swallows, as the fluoroscopic recording commenced after barium reached the level of the sensor. Figure 12.7 gives a more detailed depiction of the temporal relationship between barium arrival at the level of the sensor and the initial velocimeter signal. The relationship between initial upward movement of the sensor and the initial velocimeter signal is shown in figure 12.8. In the proximal oesophagus, a greater proportion of swallows in which the timing of the initial velocimeter signal and barium arrival at the level of the sensor were closely temporally associated (± 0.5 s) occurred compared to the distal oesophagus, although this did not quite reach statistical significance (81% vs 54%, P = 0.057).

In 21 of 46 swallows during which the velocimeter recorded a signal, the initial and the major velocimeter signal were identical. The major velocimeter signal commenced prior to barium reaching the sensor in 13 swallows (10 proximal, 3 distal), whilst the sensor was within the column of barium in 28 swallows (9 proximal, 19 distal), or after the column had left the sensor (but traces remained on the mucosa) in 4 swallows (2 each proximal and distal). Figure 12.9 shows the

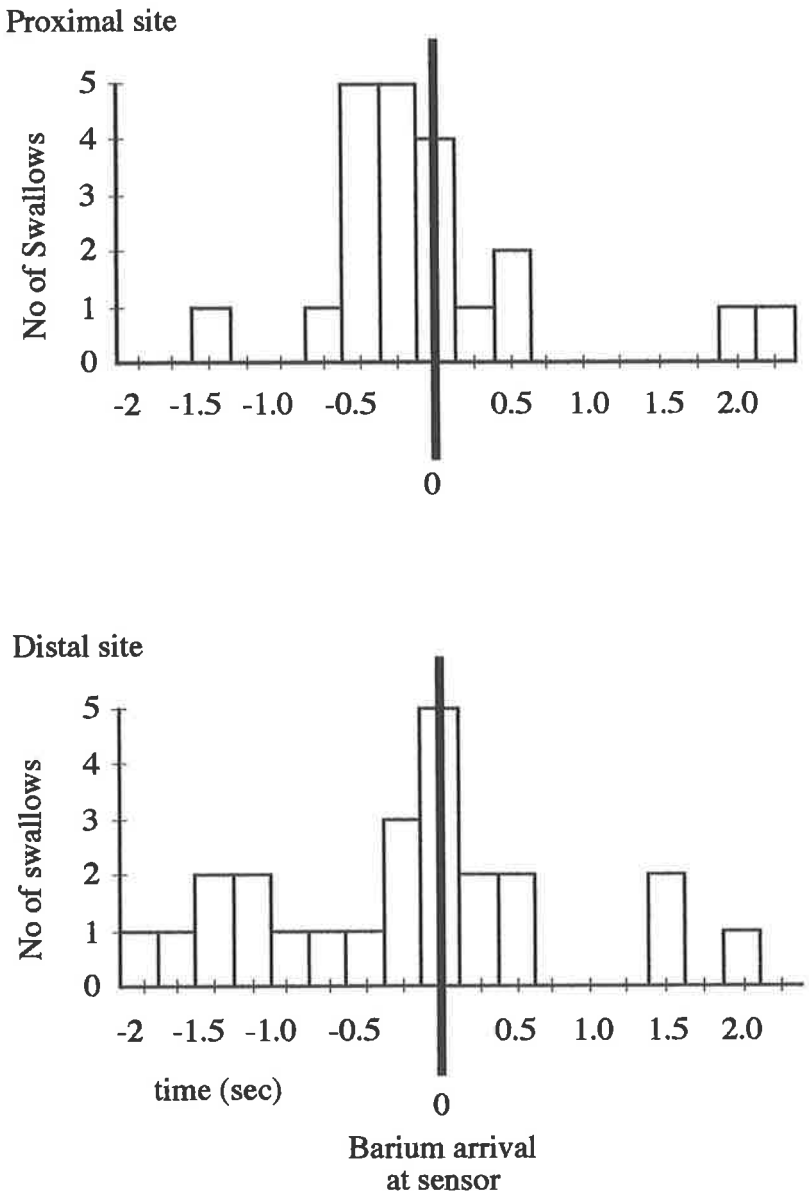


Figure 12.7
Relationship between onset of the initial velocimeter signal and visible arrival of barium at the level of the sensor is shown separately for the swallows with the sensor in the proximal (above) and distal (below) oesophagus. Bold vertical lines indicate the time of barium arrival. In the proximal oesophagus, there is close temporal clustering of velocimeter signal onsets around the time of visible barium (17/21 swallows have the onset of the initial velocimeter signal within +/- 0.5 s of the visible arrival of barium at the level of the sensor). This clustering is also seen with the sensor in the distal oesophagus, but is less impressive (13/24 swallows with initial signal and barium arrival within +/- 0.5 s of each other). Eight swallows with the sensor in the distal oesophagus had an initial velocimeter signal onset > 0.5 s before barium arrival. On fluoroscopic review of these swallows, the velocimeter signal is closely temporally related to upward catheter movement (see Figure 12.8). In the 5 swallows (2 proximal, 3 distal) in which the initial velocimeter signal onset was > 0.5 s after barium arrival at the sensor, fluoroscopic review showed that visible barium was still present and lumen occlusion had not yet occurred. (see 12.3.2.1).

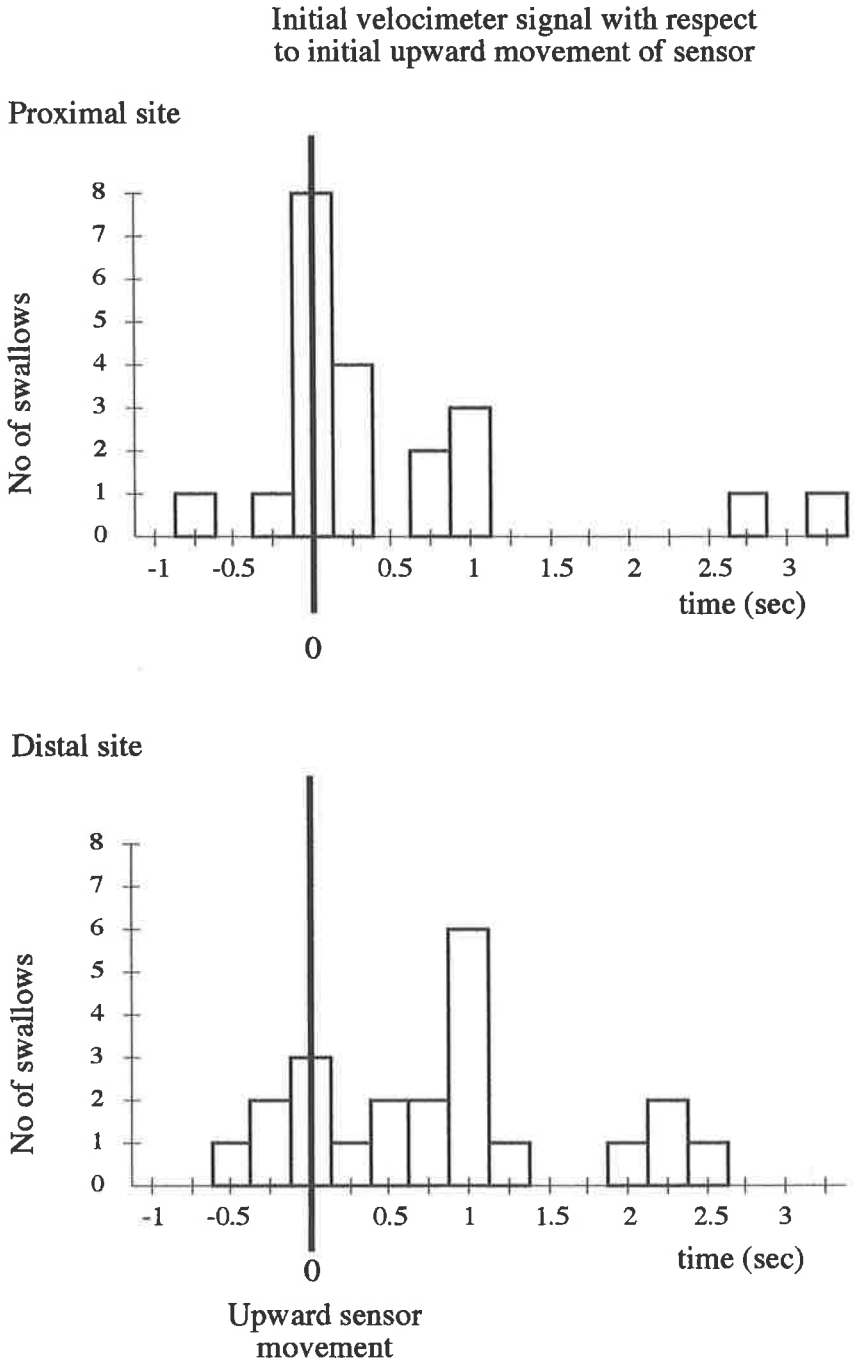


Figure 12.8
Relationship between onset of the initial velocimeter signal and initial upward movement of the sensor is shown separately for the swallows with the sensor in the proximal (above), and distal (below) oesophagus. Bold vertical lines indicate the timing of the initial upward movement of the sensor. In the proximal oesophagus, there is close temporal clustering of velocimeter signal onsets around the time of upward sensor movement (13/21 swallows within +/- 0.5 s). This relationship does not appear to hold true for the swallows with the sensor in the distal oesophagus. (section 12.3.2.1).

temporal relationship between the major velocimeter signal and barium arrival at the level of the sensor. Figure 12.10 shows the relationship between the major velocimeter signal and the initial upward movement of the sensor on swallowing. The major velocimeter signal and barium arrival at the level of the sensor were more closely temporally associated (± 0.5 s) for the proximal oesophageal recordings, compared to the distal oesophagus (71% vs 29%, $P < 0.005$). Swallows with the sensor situated in the distal oesophagus were more likely to have the major velocimeter signal commencing more than 0.5 s after barium arrival at the level of the sensor, compared to the proximal oesophagus (71% vs 24%, $P<0.002$).

In the proximal oesophagus, both the initial and the major velocimeter signal were closely temporally associated with the initial upward movement of the sensor and the arrival of barium at the level of the sensor (initial signal: 81% within ± 0.5 s of barium arrival; 62% within ± 0.5 s of upward movement, and major signal: 71% within ± 0.5 s of barium arrival; 67% within ± 0.5 s of upward movement). Whereas, in the distal oesophagus, neither initial nor major velocimeter signals appeared to be closely related to upward movement of the sensor (41% of initial signals and only 8% major signals within ± 0.5 s of upward movement). There was a shorter time interval between the initial upward movement of the sensor and the arrival of barium at the level of the sensor when the sensor was in the proximal, as compared to the distal, oesophagus (proximal mean 0.5 s {range 0.2 - 0.9 s} vs distal mean 1.0 s {range 0.4 - 2.5 s}, ($P < 0.001$).

12.3.2.2 Comparisons between manometry and velocimetry

The initial velocimeter signal had an onset within ± 0.5 s of the onset of the oesophageal common cavity pressure rise at the level of the velocimeter sensor in 28/46 swallows (16 proximal, 12 distal). The onset of the major velocimeter signal occurred during, or within ± 0.5 s of the onset of the oesophageal body common cavity pressure rise at the level of the velocimeter sensor in 36/46 swallows (16 proximal, 20 distal). No velocimeter signals had their onset after the onset of the major upstroke of the oesophageal peristaltic pressure wave at the level of the sensor.

When the manometric recordings of oesophageal body peristalsis were classified as normal (n=27) or abnormal (n=19) by established criteria (Kahrilas et al 1988), there was no difference in the number of velocimeter deflections between the normal (3.22 deflections) and the abnormal (3.58 deflections) swallows. Even when divided by sensor site, no difference in number of deflections was shown between the normal

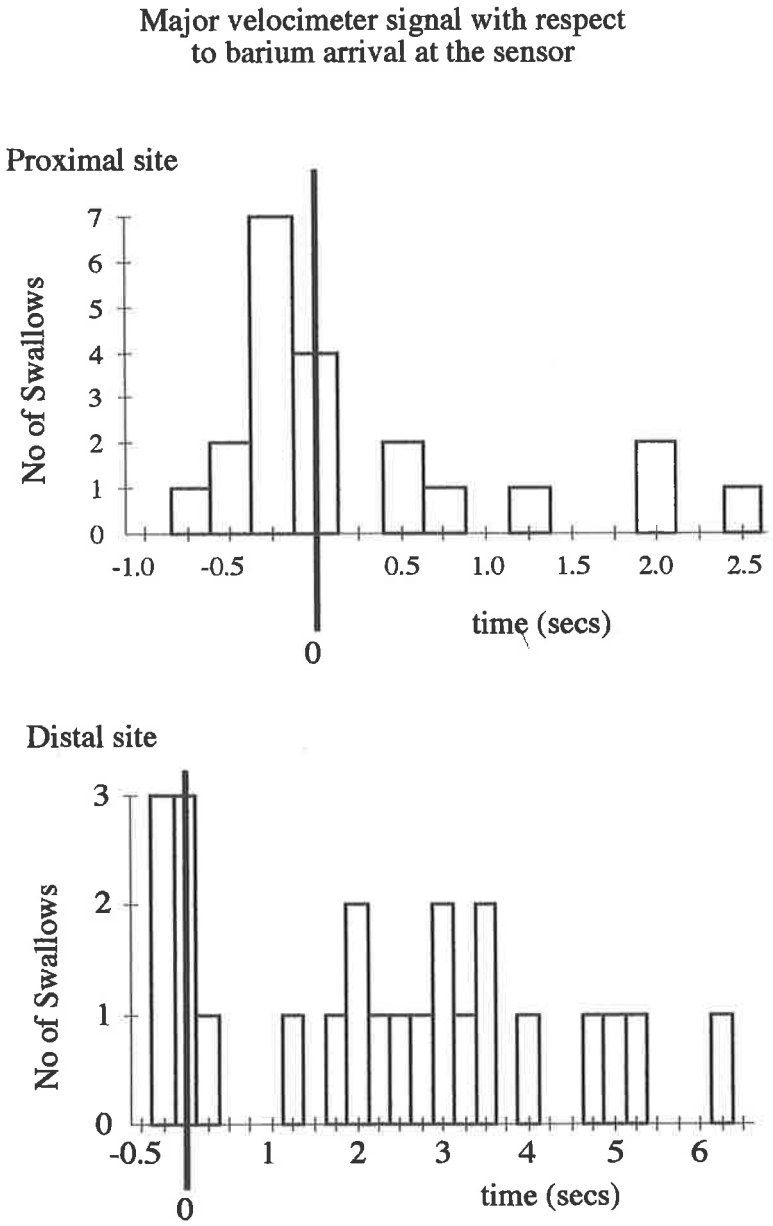


Figure 12.9
Relationship between the onset of the major velocimeter signal and visible arrival of barium at the level of the sensor is shown separately for swallows with the sensor in the proximal (above) and distal (below) oesophagus. Bold vertical lines indicate the arrival time of barium. In the proximal oesophagus, there is close temporal clustering of major velocimeter signalonsets around the time of barium arrival at the sensor (15/21 swallows have these 2 observations within +/- 0.5 s). In the distal oesophagus, this is seen to a far lesser extent (7/24 swallows within +/- 0.5 s), with the majority of the swallows (17/24), having the major velocimeter signal onset later. On review of the fluoroscopy in these swallows, maximal velocity was more frequently related to the emptying phase (as the barium column left the sensor) than the filling phase (as barium arrived at the sensor) of the bolus passage. In all instances, barium was still present and lumen occlusion had not yet occurred. (section 12.3.2.1).

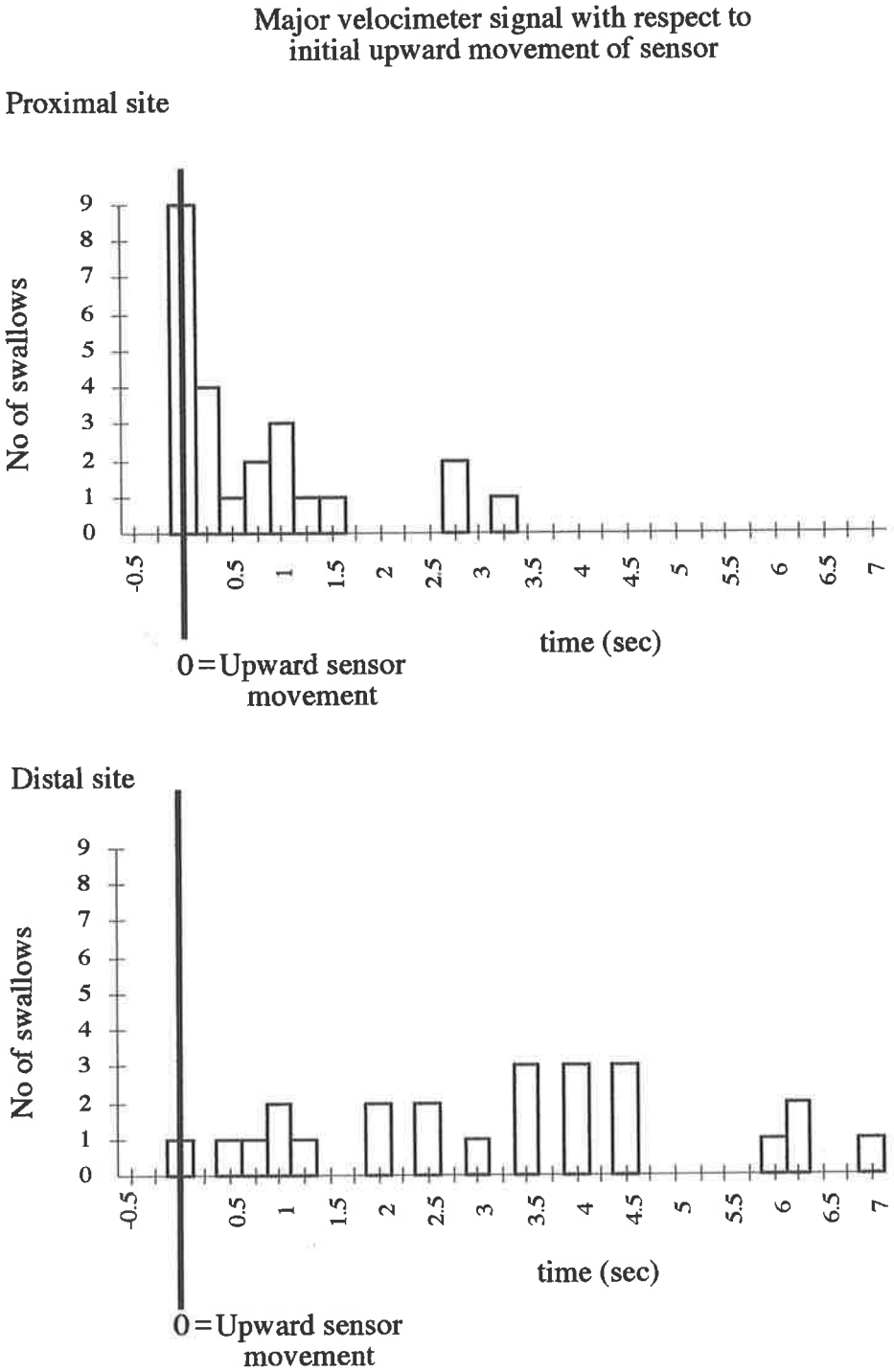


Figure 12.10
Relationship between onset of the major velocimeter signal and inital upward movement of the sensor is shown separately for swallows with the sensor in the proximal (above) and distal (below) oesophagus. Bold vertical lines show the timing of the initial upward movement of the sensor. In the proximal oesophagus the majority of the major velocimeter signals have their onsets within the 1.5 s following upward catheter movement. In the distal oesophagus, upward catheter movement appears to have no effect on the timing of the major velocimeter signal. (section 12.3.2.1).

(proximal 2.62; distal 3.79 deflections) and abnormal (prox 2.11; distal 4.9 deflections) swallows, (P for all > 0.05). There was also no difference in the maximal velocity recorded during manometrically normal (prox 69 $mm.s^{-1}$; distal 55.9 $mm.s^{-1}$) and abnormal (prox 80.1 $mm.s^{-1}$; distal 63.4 $mm.s^{-1}$) swallows (P both > 0.05).

12.4 DISCUSSION

The main aim in developing the instrument described here was to recognise individual episodes of luminal flow in the human gut, a capability which is not adequately provided by any established method. The development of a more complete understanding of the relationships between flow pulses and spatio-temporal patterns of intraluminal pressures depends on being able to recognise and monitor luminal flow episodes with high time resolution (6.2). In the present study, this laser-Doppler device has been shown to be safe, well tolerated and to accurately detect flow with a temporal resolution of less than a second. The in-vivo study provides strong direct evidence of the ability of the device to recognise intraluminal flow episodes under expected conditions of use, with a high detection rate (92%) for barium flow in the oesophagus as compared to fluoroscopy. Moreover, differences in flow patterns between the proximal and distal oesophagus, consistent with those seen on fluoroscopy, were detected.

For the initial testing in humans of the laser-Doppler velocimeter, the oesophagus was chosen, as movements of luminal contents could reliably be triggered and imaged fluoroscopically within acceptable radiation limits. Along with its ability to detect flow, the velocimeter also revealed apparent differences in flow patterns between the proximal and distal oesophagus. In particular, the flow pattern signalled by the velocimeter is more simple in the proximal oesophagus, with fewer flow pulses (deflections) per swallow. Fluoroscopic observation of the barium movement showed good agreement with the velocimeter recording, with straight-forward, unidirectional flow in the proximal oesophagus occurring soon after swallowing. The observation of barium flow in the distal oesophagus is consistent with those of Biancani and Behar (1995) who describe liquid flow occurring in two distinct phases; (i) during filling against a closed lower oesophageal sphincter and (ii) during emptying of the oesophagus after the opening of the sphincter. Moreover, there is a variable time interval, from swallow to swallow (and between subjects), between the filling and emptying flow phases in the distal oesophagus, during which to and fro movement of the column of barium can sometimes be seen. These fluoroscopically observed flow patterns in the distal oesophagus are likely to account for the greater

number of velocimeter signal deflections, which occurred over a longer time interval in the distal oesophagus.

In a number of swallows (22/44), the onset of the initial velocimeter signal appeared to precede fluoroscopic flow, particularly when the sensor was in the proximal oesophagus. On fluoroscopy, these "premature" flow signals were closely related to the rapid upward movement of the recording assembly on swallowing, although timing uncertainty (see below), precludes a definite conclusion. Initial, rapid upward movement (shortening) of the pharynx and oesophagus occurs on swallowing (Kahrilas et al 1988b) and, based on the current observations, rapid upward movement of the recording assembly also occurs. These two upward movements (of the probe and the oesophagus) are not necessarily synchronous, nor equal, as the assembly is not fixed to the oesophagus. As the velocimeter simply records movement of particles relative to the sensor, movement of either the recording assembly, or the mucosa, or an unequal movement between them, will generate a signal. The velocity of the upward movement of the sensor seen fluoroscopically is certainly sufficient to fall within the resolution of measurement of the velocimeter (>30 $mm.s^{-1}$). Formal evaluation under standardised conditions is warranted to clarify the issue of signals arising from relative movement between sensor and mucosa.

The differences between the two methods used to sense flow in this study are important, as they have influenced the data obtained. Fluoroscopy gives an indication of volumes flowing, and the movement of the leading and trailing edges of a bolus, whereas the velocimeter gives information on timing and velocity of flow in the immediate region of the sensor but does not signal volume. Mucosal coating from barium swallows also allows lumen occlusion to be fluoroscopically visualised when sufficient air contrast is present. Fluoroscopy is limited by the need for visual interpretation and the density of barium used. In this study, the barium used was half strength (to reduce sensor clogging), increasing the likelihood that the temporal resolution of the fluoroscopy was decreased. In contrast, interpretation of the laser-Doppler velocimeter's recording is less subjective. Taking these differences into account, the substantial concurrence of flow detected by the two methods in this study strongly supports the velocimeter's performance. The analysis of the *in vivo* data required somewhat subjective definitions of events. To counter this, the process was highly structured, with two observers independently evaluating the data sets. The high concordance between the analyses of the 2 observers strongly supports the validity of this approach.

We found no overall difference between manometrically "normal" and "abnormal" swallows in maximum velocity, or number of flow episodes recorded by the velocimeter, although there was a wide range of velocities and flow patterns recorded, attesting to the fact that flow patterns vary from one swallow to the next, and between individuals. It was somewhat surprising that maximal flow velocities, as measured by the velocimeter, were no different between proximal and distal sites as, on viewing the passage of contrast radiologically, the rate of advance of the bolus front appeared to be faster in the proximal oesophagus. It is likely that the shorter time from swallow to contrast appearance at the sensor level (0.2 - 0.9 s proximally vs 0.4 - 2.5 s distally) influenced our visual judgement of this. The lack of difference in velocities between the two sites may also partially relate to the differences between fluoroscopic and velocimetric measurement of flow (see above).

From the data, by both velocimetric and fluoroscopic assessment, oesophageal luminal flow was largely completed prior to the arrival of the peristaltic pressure wave at any given point. This is consistent with previous observations of liquid flow in the upright position (Biancani & Behar 1995), as gravity accounts for a large component of the flow. The spatio-temporal patterning of pressures in the oesophagus is, however, important in transport of solids regardless of posture. The causal relationship between pressures and flows is likely to be better understood when regions in which gravity plays a lesser role are evaluated, such as the pylorus and proximal small intestine. At the time of lumen occlusion judged fluoroscopically, some fluid movement was still being signalled by the velocimeter, although no velocimeter signals commenced after this time. This is likely to be due to flow in the thin layer of liquid left coating the mucosa as it is squeezed during lumen occlusion. If so, this is real flow, although of a small volume, again emphasising the different nature of flow monitoring between the two techniques as discussed earlier.

The characteristics of the instrumentation used unfortunately resulted in some timing uncertainty. The manometric data were recorded to disk at 10 Hz, whilst the velocimeter gave measurements at 4 -7 Hz due to limitations of componentry processing the signal. Consequently there was an unavoidable mismatch of the 2 signals which reduced the temporal resolution. In addition, the internal processing of the velocimeter signal, from measurement of the Doppler shift, to logging with the manometry, created a time lag between the velocimeter signal and the manometric data. Compensation for this time delay was complicated by the fact that it was not constant, but varied slightly from one swallow to the next (between ~0.3 - 0.6 s). As

it was not possible to retrospectively define this delay precisely for each swallow, an average delay of 0.4 sec was taken from 10 standardised (video recorded) movements performed in vitro, prior to analysis of the data. This figure obtained was then used as a best estimate to correct for these uncertainties in temporal resolution between the velocimetric and manometric recordings. Taking the above into consideration, events within ± 0.2 sec of each other may, in fact, be simultaneous. This uncertainty in timing can only be resolved by modifying the system so that the velocimeter measures at the same frequency as the manometry (10 Hz), and eliminating or standardising the internal delay in signal processing. These technical aspects are currently being addressed in further development of the instrument.

If the box containing the spectrum analyser was bumped or exposed to radiofrequency emitting devices the velocimeter system used in this study was noted to give signals when no flow was occurring at the sensor. These artefactual signals demanded that special care was taken during use of the device. This limitation is to be addressed, in future, by measures which will increase stability and shielding from external electromagnetic signals. The clogging of the sensor with barium is more of a nuisance than a serious technical problem and may be avoided in future by the use of other contrast strategies during instrument development. When the velocimeter is sufficiently validated to be used alone, a number of other solutions which are less likely to coat the sensor, such as dilute milk and lipid emulsions, can be used as the only absolute requirement is the presence of particles in the liquid to reflect the laser beam.

The novel instrument described here is capable of making a significant contribution to an improved understanding of human gastrointestinal mechanics. It is safe, well tolerated, and performs intraluminal flow measurements with a temporal resolution of better than one second. The initial experience described here indicates the need for enhancement of some aspects of its function. One anticipates that, once these enhancements have been achieved, the velocimeter will enable us to monitor, and so evaluate, pressure-flow relationships in the human upper gut.

CONCLUSION

This thesis provides novel insights into the interaction between nutrients and the motor and sensory function of the stomach and proximal small intestine. In addition, novel methodologies such as high resolution manometry and a laser-Doppler velocimeter have been developed and evaluated to further understanding of the interaction between motor events and intraluminal flows. Here, the major findings of the studies are reviewed, particularly with respect to their limitations and the priorities for future work.

From the literature review, it became apparent that, although there was an extensive body of knowledge about short-term regulation of appetite, little of the work had been performed in humans. Moreover, despite the recognition that signals from the gut play a major role in the regulation of appetite, there was a paucity of human studies which had concurrently evaluated both appetite and motor function in any detail. These issues formed a focus for several of the earlier studies (8B, 8C, 9A & 9B). The studies reported in Chapters 10 and 11, addressed novel questions relating to the gut motor response to nutrients. Chapter 10 includes the first study comparing fasting small intestinal motility between omnivores and vegetarians directly, whilst Chapter 11 provides the most comprehensive information to date on the pressure patterns along the length of the human duodenum during fasting and a progressively increasing small intestinal lipid infusion. The literature review reinforced the view that, apart from the oesophagus, very little is known about the moment-to-moment relationship of luminal flows to individual motor events in humans. Thus, Chapter 12 describes the development and testing of a novel laser-Doppler velocimeter which has the capability of relating flow the pressure events with high temporal resolution.

The study reported in Chapter 8A was designed to determine the means by which the infusion of glucose into the small intestine suppresses hunger and food intake. The novel observations were that, hyperinsulinaemia alone did not affect appetite, and the suppression of appetite by intraduodenal (ID) glucose was abolished by concurrent administration of the somatostatin analogue, octreotide. Thus, it now seems likely that gut hormones other than insulin mediate the satiety and satiation induced by ID glucose. Additionally, there was circumstantial evidence to implicate GLP-1 as an important satiety factor in this model. Unfortunately, because of the inability to specifically prevent further release of insulin in response to ID glucose, it has not been

entirely possible to exclude a contributory role for insulin. Although, the performance of a similar studies in subjects with type-1 insulin dependent diabetes would be a way of excluding insulin as a factor. The use of a specific GLP-1 antagonist would also be of interest to further test the role of GLP-1 in glucose mediated satiety and satiation. This study protocol could also be usefully applied to evaluate the effects on appetite of proteins and fats.

The relative potencies of fats vs carbohydrates in suppressing appetite and in stimulating aspects of antropyloric motility associated with the slowing of gastric emptying is controversial. Chapter 8B reports the first direct comparison of isocaloric ID infusions of glucose and lipid in terms of their motor and "sensory" effects. In healthy young males, lipid was found to exert a greater suppressant effect on appetite, and a more potent stimulatory effect on phasic pyloric motility than glucose. In this study, both nutrients were delivered directly to the small intestine, bypassing the pyloric motility. This may perhaps account for lipid's greater effect on appetite, as its greater stimulatory effect on the pylorus at this rate of small intestinal delivery, would usually then slow its rate of gastric emptying, to limit the length of small intestine exposed. This is important as absorption of an equivalent amount of fat requires a greater length of small intestine than glucose (Lin 1994), and length of gut exposed certainly determines the degree of delay in gastric emptying, and may also be related to the magnitude of appetite suppression. The time course over which appetite is assessed also probably contributes to the discrepancy in the literature regarding the relative magnitude of appetite suppression by carbohydrates vs fats. This could be addressed by extending the observation period following such a study, offering a buffet lunch at its completion and recording intake for the following 24 hours. Other factors involved in appetite regulation, made it possible that this result was not generalisable to women or to subjects with a BMI outside the normal range. Separate studies in these groups were then planned by others in this research group, with obese subjects also demonstrating a greater suppression of appetite by ID lipid (Chapman et al 1999).

Dietary change has been shown to modify some aspects of gastroduodenal motor function, these alterations seemed to largely occur as a specific response to the nutrient whose intake was altered. Unfortunately, in these previous studies, appetite was not evaluated, although as discussed in Chapter 4, changes in motility may well lead to changes in perception of appetite. Therefore in 8B, the effect of dietary supplementation with glucose on gastric motor and sensory function, in response to both ID glucose and ID lipid was evaluated. Adaptation of both motor function -

decreased pyloric tone in response to ID glucose - and the sensory response - attenuation of lipid's appetite-suppressant effect - was found. The motor adaptation was specific for the macronutrient given, whereas the sensory adaptation occurred across macronutrient class. This suggests that mechanisms mediating the motor adaptation to glucose are likely to involve relatively macronutrient-specific signals, such as small intestinal glucoreceptors. The fact that the appetite adaptation occurred across macronutrient classes implies a non-nutrient specific mechanism perhaps related to total caloric intake. Further studies addressing the issues of macronutrient specificity and adaptation to dietary change should include measurement of a large panel of candidate gastrointestinal hormones to clarify the means by which the changes documented are mediated.

Ageing is associated with impaired homeostasis, and there is strong evidence that dysregulation of appetite occurs even with healthy ageing. The gastrointestinal correlates of this are largely undocumented. In 8C it was hypothesised that the elderly may show an increased sensitivity to the presence of small intestinal nutrients, and that this may be mediated by increased sensitivity to the effects of gastrointestinal hormones. Healthy older males were documented to have decreased perception of hunger after an overnight fast, a lesser reduction in appetite ratings during ID infusion of lipid, and yet consumed a similar amount when presented with a meal when compared to younger subjects. Thus they exhibited a lesser "sensory" sensitivity to small intestinal nutrients than the young males. However, they showed an increased phasic pyloric response to ID lipid, which perhaps accounts for the slight slowing of gastric emptying seen with ageing. In contrast to the situation in rodents, healthy, older males appeared to have resistance to the satiating effect of CCK - with an elevated basal and stimulated level, but a lack of any correlation between CCK and either fullness or hunger. In view of these results, others in our group have gone on to evaluate the effects of dietary change (supplementation) in the elderly. This has the potential to provide a means of leading to increased intake in the elderly, perhaps protecting against further loss of appetite and weight, which are associated with increased morbidity and mortality.

After eating, blood glucose levels usually rise, and even small elevations in blood glucose concentrations are now known to effect the rate of gastric emptying. In Chapter 9, the relationship between such "physiological" levels of hyperglycaemia, such as occur postprandially, and gastric motor and sensory function were addressed. As it is technically difficult to examine proximal and distal gastric motility

concurrently, this was divided into two closely related protocols. In 9A, a synergy (particularly in terms of appetite suppression) was found to exist between blood glucose level and the presence of small intestinal nutrients. Compared to euglycaemia, physiological hyperglycaemia led to increased feelings of fullness during fasting, and decreased ratings of hunger during a low dose ID lipid infusion compared to euglycaemia. Pyloric tone was no different between eu- and hyperglycaemia, although the patterning of IPPWs varied. In 9B, gastric compliance was not affected by physiological hyperglycaemia, nor was the perception of gastric distention. Although subjects felt increasing balloon volumes as increasing levels of "bloating", "discomfort" and "fullness", this did not vary between eu- and hyperglycaemia, and moreover, increased perception of these sensations did not lead to decreased ratings of hunger or desire to eat. These observations suggest that important interactions exist between the motor and sensory signals generated by both postabsorptive (such as hyperglycaemia) and mucosal (nutrient "receptor") means. Moreover, they emphasise that gastric distension in the absence of small intestinal nutrients does not influence perception of appetite and, hunger and fullness are not simple opposites of one-another. In future studies, subjects should be asked to distinguish between fullness experienced as epigastric discomfort, and meal-like fullness, which are likely to be different sensations. In view of the synergy described here, future studies examining gastric motor and sensory function should report the blood glucose concentrations of subjects whenever small intestinal nutrients are present. Future studies with a specific CCK antagonist may help define the signals responsible for the synergy seen between glycaemic state and ID lipid, and additional separate protocols using both proteins and carbohydrates as the ID infusion during physiological hyperglycaemia are necessary to define the nutrient-specificity of the synergy seen.

Despite the potential for acute dietary change to alter postprandial motility, there have been no studies examining the potential differences in small intestinal motility between chronic vegetarians and omnivores. In Chapter 10, fasting small intestinal motility was found to be no different between longstanding lacto-ovo vegetarians and omnivores. This now allows one to confidently include vegetarian subjects in future fasting small intestinal studies, and to evaluate their fasting clinical manometry studies by standard criteria. The duration of the interdigestive motor cycle (IDMC) appeared to be shortened by acute adoption of a vegetarian diet. Why this should be so is uncertain. This finding requires confirmation. Future studies should examine the dietary components in greater detail, with particular respect to L-arginine content, and the specific types of dietary fibre consumed. In future studies a larger sample size, to

account for the variability within and between subjects in IDMC duration would be desirable. Examination of postprandial motor and sensory responses would also be of interest, given the evidence from Chapters 8 and 9 that small intestinal nutrients act in concert with other stimuli to modulate motor and sensory responses.

The absence of suitable methodology for measurement of intraluminal flows in humans limits current understanding of the second-by-second relationship between pressures and flows. Based on hydraulic principles, detailed assessment of pressures should assist in developing hypotheses regarding intraluminal flows. High resolution manometry of the human duodenum, in Chapter 11, revealed that the vast majority of pressure wave (PW) sequences are very short, travelling only 1.5-4.5 cm. These would be missed with standard measurement techniques. ID lipid infusion led to a dose-dependent suppression in the number of PW sequences seen, which then resulted in regional variation along the duodenum in the frequency of sequences. This contrasted to the situation during fasting when the frequency of PW sequences was similar along the duodenum. These differences, which can only be detected with high spatial resolution manometry along the length of the duodenum, are likely to have significant impact on intraluminal flow characteristics, and emphasise the need for recording of pressures at ~ 1.5 cm intervals in order to obtain a clearer understanding of small intestinal mechanics. Flow hypotheses, generated from data such as these, can then be examined when suitable methods for flow measurement become available (vide infra).

Chapter 12 reports on the development and initial human study of a novel laser-Doppler velocimeter, to measure intraluminal flows instantaneously. The analysis of 50 swallows concurrently recorded by velocimetry, fluoroscopy and manometry, performed in the human oesophagus was sufficiently reliable to enable recognition of differences in flow patterns between the proximal and distal oesophagus. The instrument was found to indicate the occurrence of flow reliably, although several practical limitations of the existing equipment, requiring future work, became evident. In particular, the temporal precision and resolution, of the instrument demand further enhancements, as does the exclusion of signals not attributable to flow, by more effective isolation of the individual components from each other and the environment. Upon addressing these design features, this instrument shows great promise, enabling for the first time, the prospect of measuring intraluminal flow in real-time in humans, with a temporal resolution suited to defining the precise motor correlates of individual flow episodes. The initial studies using the refined instrument are planned to evaluate

transpyloric flow in humans with high resolution manometry across the gastroduodenal junction.

Several new insights into the relationships between gut motility, nutrients, and how they are perceived have been presented. Much work still needs to be done, and several promising lines of investigation have been identified. From the work described here, the importance of concurrently assessing appetite perception and other sensations when evaluating motility is now recognised, as is the wisdom of employing the best spatial and temporal resolution available when examining pressure along a segment of gut. Further use of these approaches and techniques will substantially enhance our understanding of normal gastrointestinal physiology, which is likely to then enable more precise examination of common gastrointestinal pathology such as functional dyspepsia and irritable bowel syndrome.

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