

**Appetite, food intake and ageing:
The role of the gut**

**A thesis submitted by
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**For the degree of Doctor of Philosophy
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Dedication

I dedicate this thesis to my darling husband and children, Lyndon, Lucy and Callum Parker. This would not have been possible without you.

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Thesis summary

This thesis is concerned with gastrointestinal mechanisms and the changes that occur with age that may affect food intake and appetite. Studies are presented that evaluate the contribution of these mechanisms to the control of appetite in older persons. In particular, the use and validation of visual analogue scales in these studies and gastrointestinal aspects of appetite control, the effects of intraduodenal and gastric infusions of fat versus carbohydrate, relationships between gastric antral area and appetite (using ultrasound measurements) and the effect of accelerating gastric emptying (with domperidone) on appetite and food intake are addressed.

Life expectancy is increasing both in Australia and overseas. Many health initiatives focus on decreasing food intake due to the increasing prevalence of obesity in our society, however reduced nutritional intake can also contribute to illness and death in older people. Ageing is associated with changes in gastrointestinal function affecting food intake. The spiralling costs of healthcare highlight the need for promotion and maintenance of healthy lifestyle choices, especially adequate nutritional intake, in older persons.

Healthy ageing is associated with decreased appetite and food intake, the so-called 'anorexia of ageing'. This anorexia of ageing is a part of the normal process of ageing in people who do not suffer physical, psychiatric or social disorders. From young to old adult (18-70 years) the average daily food intake falls by approximately 30%. This reduction is probably in response to a normal decline in activity, however, when our food intake decreases more than our exercise levels weight loss, usually muscle, occurs. Unlike fat, loss of muscle has adverse effects including decreases in strength and increases in falls and fractures, loss of independence and increased risk of protein energy malnutrition, which in turn increases acute and chronic illness, hospitalisation and death. There are many explanations for changes in appetite and food intake in older people including sensory (taste and smell changes, dental abnormalities) and social (poverty, loneliness, institutionalisation) factors.

Appetite regulation in humans is complex. Although many of the mechanisms are unclear, alterations in gastrointestinal responses to food ingestion are important in appetite (how hungry or full we feel) and food intake changes with age. Changes occur with increasing age in functions of the stomach and small intestine, including how quickly food and fluids leave the stomach and enter the small intestine to begin digestion, and changes in the way food is distributed and retained within the three sections of the stomach. These changes can influence appetite by increasing fullness and decreasing hunger. In addition, small intestinal hormones associated with appetite regulation increase with age and there is a greater sensitivity to their effects. The interaction of nutrients with gastrointestinal tract receptors stimulates the release of satiety hormones, including cholecystinin (CCK), glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP) and amylin, and inhibits the release of ghrelin, which stimulates feeding.

Visual analogue scales (VAS) are widely used in appetite research involving adults of all ages, yet the reproducibility and validity of these scales to evaluate appetite has not been assessed in older subjects. In two studies discussed in Chapter 4, retrospective (to determine the relation of appetite sensations to food intake) and prospective (to determine reproducibility), VAS were evaluated as a measurement of appetite in healthy older subjects. The retrospective study was a combined analysis of four single-blind, randomised, controlled appetite studies undertaken in young and older subjects and the prospective study was an assessment in healthy older men and women aged 65-85 years. Perceptions of appetite (i.e. hunger and fullness) were assessed by 100mm visual analogue scales administered at regular intervals. Food intake was quantified from food intake at a test meal.

In the retrospective study, food intake at the test meal was positively related to perceptions of hunger, drowsiness, and calmness and inversely related to ratings of fullness taken immediately before the meal in both older and young subjects. In the prospective study, VAS measures of appetite were found to have comparable reproducibility and validity in older subjects to reported values in young adults. These observations confirm that food intake is related to perceptions of hunger and fullness as assessed by VAS in healthy older and young subjects, and suggest that sensations, not obviously associated with appetite, including 'drowsiness' and 'calmness', are also associated with food intake.

Gastric distension reduces food intake, and antral rather than proximal, gastric distension may be the dominant mechanism in the initiation of appetite-related sensations. To evaluate the age-related changes in appetite, food intake, gastrointestinal hormone concentrations and antral area healthy young and older subjects were administered oral yoghurt preloads and water (control) 60 minutes prior to a buffet meal (Chapter 5). Antral area was greater after the nutrient preloads than after water ($P = 0.001$) and larger in the older than young subjects ($P = 0.005$). Hunger ($r = -0.59$, $P < 0.001$) and food intake ($r = -0.90$, $P < 0.001$) were inversely, and fullness directly ($r = 0.66$, $P < 0.001$), related to antral area in both age groups. In healthy older and young subjects the suppression of subsequent food intake was nutrient-dependent and both satiation (meal termination) and satiety (time to subsequent meal consumption) were related to antral area, and antral distension.

The effect of intragastric and intraduodenal administration of macronutrients, such as fat and carbohydrate, on appetite and food intake may be influenced by age. In Chapter 6, intragastric infusions of water (0 kcal), carbohydrate and fat (both 343 kcal), were delivered over 5 minutes to older and young men. Food intake was assessed at a buffet meal. Carbohydrate infusion suppressed food intake significantly more than the fat infusion (23 v 10%, $P = 0.005$), and this was so in both young (25 v 14%, $P = 0.03$) and older (21 v 7%, $P = 0.05$) men. These results were compared to equienergetic, equivolaemic fat and carbohydrate solutions delivered into the small intestine of older and young men. Fat inhibited food intake significantly more than carbohydrate in the young men (26 v 5%, $P < 0.001$) whilst the suppressive effects of fat and carbohydrate were similar in the older men (21 v 22%, $P = 0.05$). This suggests that with increasing age regional differences in the gastrointestinal tract play both distinct and interacting roles in appetite regulation.

Ghrelin is a recently identified peptide hormone secreted primarily from the gastric mucosa. It plays a role in energy balance by stimulating appetite, thereby increasing food intake and enhancing weight gain and fat mass deposition. Plasma ghrelin concentrations increase with fasting and are suppressed by nutrient intake. In Chapter 7, the contribution of both the stomach and small intestine in postprandial suppression of ghrelin was assessed. On three separate days, glucose (300 kcal) and water (C, 0 kcal) were infused slowly over 150 minutes into the stomach (IG), and an equienergetic infusion of glucose was infused into the small intestine (ID) of older men and women. Ghrelin was suppressed following both glucose infusions compared to control (ID 25% and IG 19% v C, $P < .00001$). There was no difference in the degree of suppression between the two glucose infusions ($P = 0.2$). These results suggest that although the primary source of ghrelin is the gastric mucosa, small intestinal exposure is largely responsible for ghrelin suppression in humans. The effect of age on the suppression of ghrelin in response to nutrient intake is unclear.

Domperidone, a prokinetic drug, is a predominantly peripheral acting dopamine₂-receptor antagonist known to accelerate gastric emptying. It has been reported to be effective in the treatment of gastrointestinal symptoms associated with non-ulcer dyspepsia, Parkinson's disease and diabetic gastroparesis. The acute effects of domperidone on perceptions of appetite and food intake in healthy older people may include a reduction in antral distension (as a result of more rapid gastric emptying and pharmacologically-induced gastric 'relaxation') and meal-related symptoms, and increases food intake at a subsequent meal.

In Chapter 8, 10 older men were treated with either domperidone or placebo and food intake, appetite and antral area assessed after a yoghurt preload. There were no differences in appetite scores of food intake between the study days. There was a decrease in antral area and an increase in blood glucose concentrations on the domperidone day, suggesting an increase in gastric emptying, although this difference was not significant. Further studies are required to evaluate the effects of this prokinetic agent on gastric emptying and food intake in older subjects.

As the causes of the anorexia of ageing are still largely unknown, the aim of this research was to examine the effects of ageing on appetite, food intake and gastrointestinal function. This research will provide further insight into the 'physiological' anorexia of ageing and management of the frail elderly, whether resident at home, in acute or long-term care.

Declaration of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference is made in the text.

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

Barbara Parker
December 2004

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Mrs Selena Doran (Department of Medicine, Royal Adelaide Hospital) provided instruction and assistance in the studies presented in Chapters 7 and 8.

Ms Judith Wishart (Department of Medicine, Royal Adelaide Hospital) conducted the plasma insulin, cholecystokinin, glucagon-like peptide-1 and ghrelin assays reported in Chapters 5, 6 and 7.

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Publications

The material in this thesis formed the basis for the publications listed below:

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Parker BA, Ludher AK, Khai Loon, Tan, Horowitz M, Chapman IM (2004). Reproducibility and validity of visual analogue scales in assessment of appetite sensations and food intake in healthy older subjects. *Appetite* 43,227-233.

Parker BA, Chapman, I (2004). Food intake and ageing- the role of the gut. Review. *Mechanisms of Development and Ageing* 125, 859-866.

Parker BA, Doran S, Wishart J, Horowitz M, Chapman I (2004). Effect of small intestinal glucose exposure on ghrelin suppression in healthy older men and women. *Clinical Endocrinology* In Press.

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Appetite, food intake and ageing: The role of the gut

Chapter 1

The 'anorexia of ageing' and protein energy malnutrition

1.1 Introduction

Life expectancy for both men and women in developed and developing countries is increasing. Many health initiatives focus on decreasing food intake as a response to the rising incidence of obesity in both young and older people, however reduced nutritional intake can also contribute to illness and death in older people. Ageing is associated with changes in gastrointestinal function affecting food intake. The spiralling costs of healthcare highlight the need for promotion and maintenance of healthy lifestyle choices, especially adequate nutritional intake, in older persons.

1.1.2 Epidemiology

Population ageing is defined as 'an increasing median age of a population or an alteration in the age structure of a population, so that elderly people are increasingly represented within a country's overall age structure' (Shrestha 2000). The elderly population in the United States is expected to reach 65 million people (25% of the population) by the year 2020 (Jackson 1999; MacIntosh, Morley et al. 2000). However, the greatest numbers of older persons are expected to live in developing countries such as China, India and Indonesia (Morley and Kraenzle 1994; MacIntosh, Morley et al. 2000), probably reflecting an increase in medical knowledge and health care programmes as well as declining fertility rates (Shrestha 2000).

Like many other countries Australia is facing fundamental changes in its population structure. Three reasons have emerged to explain this- 1) growing longevity, 2) declining fertility and 3) 'baby boomer' progression (Australian Bureau of Statistics (ABS) 2003). Between 1977 and 1997, life expectancy, in Australia, for men at age 65 increased from 13 to 16 years, and for women from 17 to 20 years (ABS 1992; Australian Institute of Health and Welfare (AIHW) 2000). The life expectancy at birth has increased from 66.1 years in

1947 to 76.2 years in 1999 for men and from 70.6 years to 81.8 years over the same period for women (ABS 2003). In 1976, the total fertility rate (TFR) fell below replacement level (2.1 births per woman) and a record low of 1.7 births per woman occurred in 1999. This trend is expected to continue and the TFR is predicted to fall further still.

The peak of the large 'baby boomer' generation (born between 1946 and 1966) will be entering the over 65-year age group between 2011 and 2031. These factors have contributed to an ageing population, which may be categorised in two ways. The term 'numerical ageing' refers to 'the absolute increase in the number of people aged over 65 years'. In Australia, the number of people over 65 years is expected to grow from 2.3 million in 1999 to between 6.2 million and 7.9 million by 2051 (ABS 2003). In addition, 'structural ageing' refers to 'the relative increase, or growing proportion, of older people within the total population'. This is a direct effect of falling fertility, as the proportion of people under 15 years falls, then the proportion of people aged over 65 years increases (ABS 2003).

The demographic trend is towards an older population. By 2051 the proportion of people in Australia aged over 65 years is predicted to be about a quarter of the population, double the proportion reported in 1999 (ABS 2003). Between June 2002 and 2003, the number of people aged over 65 years comprised 12.8% of the population. The greatest population increase in this time was for the over 85-year age group where the number increased from 276,900 to 289,500, a growth rate of 4.6%. This group has grown by 165% over the last twenty years compared to total population growth. The over 85 age group is expected to almost quadruple as a proportion of the population, from 1.3% today to around 5% by 2051.

At advanced ages women outnumber men; however, the gender differential in life expectancy appears to be decreasing. In 1999, the difference (in favour of women) was 5.6 years compared with 6.3 years in 1989. The narrowing of this gap reflects the faster downward trend of the male death rate compared with the female death rate (DeLooper 2001). The National Strategy for an Ageing Australia, undertaken as part of the

International Year of Older Persons in 1999, identified several key factors important for promoting healthy ageing (AIHW 2000). These included maintaining physical and mental health, engaging in physical activity, preventing falls and injury, and maintaining adequate nutrition.

1.1.3 Impact of an ageing population

Reduced nutritional status has been shown to be a major predictor of mortality in older men and women (Fried, Kronmal et al. 1998; Duerksen, Yeo et al. 2000; Newman, Yanez et al. 2001). Normal ageing is associated with a number of significant changes in gastrointestinal function, which may impact on daily energy intake. Promoting and maintaining adequate nutritional intake in older persons is essential, since reduced nutritional status contributes to some of the adverse outcomes associated with ageing (Morley and Silver 1988; Mowe, Bohmer et al. 1994).

The present level of demand for residential, respite and day-care services for the elderly will accelerate as our population continues to age. This already overburdened system has resulted in an increase in the number of older people relying on community services for nutritional and home help. Industrialised countries, including Australia, Britain and the United States, currently spend between one-third and one-half of total health care budgets on the elderly (Anderson and Hussey 2000). Nearly half of lifetime health care expenditure is incurred after 65 years of age (Alemayehu and Warner 2004). In many instances, however, health budgets are not keeping pace with an ageing population and contributions to this area have decreased (Seshamani and Gray 2002) resulting in inadequate management and treatment of age-related illness.

1.2 Energy intake in the elderly

Total energy intake has been shown to decline with age. National Health and Nutrition Examination Survey (NHANES III) data in America shows that throughout the lifespan energy intake decreases for both men and women from the onset of adulthood until 80 years (Briefel, McDowell et al. 1995). Information collected between 1988-1991 shows an average reduction in daily energy intake of 1321 calories in men and 629 calories in women (Briefel, McDowell et al. 1995). The absolute values of daily energy intake in each age group may have increased during the last ten years, however, recent data point to similar reductions in daily energy intake of 1200kcal in men and 800 kcal in women between these age groups (Wakimoto and Block 2001).

In addition, a longitudinal study conducted in New Mexico reviewed daily energy intake in 156 people aged 64 to 91 years, and reported reductions in daily energy intake of 25.1 kcal/day per year in men and in 19.3 kcal/day per year women (Koehler 1994). Ageing may also affect macronutrient choices in food consumption. Older people have been reported to eat a greater percentage of daily energy as carbohydrate and less as fat (De Castro 1993; Morley 1997) (Chapter 2). In addition, nutrient and vitamin intakes are often below recommended daily allowances (Foote, Giuliano et al. 2000).

1.3 Factors affecting adequate energy intake with age

1.3.1 Homeostatic regulation of ageing

The challenge of maintaining adequate energy intake and weight in old age is increased by impaired homeostatic regulation of a number of factors (MacIntosh, Morley et al. 2000). Healthy older people have been shown to be less able to regulate weight in the face of fluctuations in food intake (Roberts, Fuss et al. 1994). The ability to control energy intake in the face of stressors is essential for developing effective methods to prevent undesirable weight loss in older people (Roberts, Fuss et al. 1994).

Roberts et al, 1994 have shown that following a period of imposed reduced energy intake resulting in weight loss, older people are less able than young people to make compensatory increases in their energy intake and regain the lost weight (Roberts, Fuss et al. 1994). For example, a 21-day period of surplus energy intake, 1000kcal/day above weight maintenance resulted in weight gain in groups of healthy old and young men. When both groups were offered food *ad libitum* and assessed for a further 46 days, the old men continued to overeat and failed to return to their basal weight, whereas young men were able to regulate their food intake and returned to basal weight. Conversely, after a 21-day period of reduced energy intake resulting in weight loss, the old men continued to undereat and were unable to return to their basal weight during the subsequent 46 days of *ad libitum* diet, whereas young men overate in the *ad libitum* period and returned to their previous weight (Roberts, Fuss et al. 1994).

Further evidence for an age-related alteration in energy intake is provided by appetite studies that focus on differences in food intake and appetite sensations between older and young subjects. Rolls et al (1995) found that healthy older men ingest less energy during a single meal than young men. At baseline, when offered a buffet meal and invited to eat freely, the old men consumed significantly less energy than the young men. This finding was consistent with visual analogue scale results that showed the old men were less hungry and more full at the beginning of the meal (Rolls, Dimeo et al. 1995).

Accurate energy compensation of nutrient intake may also decline with nutritional status in older people. For example, food intake was suppressed by a nutrient preload in well-nourished older and young women but not in undernourished older women (Sturm, MacIntosh et al. 2003). When healthy young and older women were given an oral nutrient preload, food intake was less at a subsequent meal compared to when no preload (control) was given. In contrast, older malnourished women did not adjust their food intake, relative to control, to compensate for the additional energy consumed (Sturm, MacIntosh et al.

2003). This finding is supported by Beck et al (2002) who observed that an oral supplement given to older malnourished nursing home residents increases total daily energy intake ($P < 0.001$) without suppressing energy intake from normal food (Beck, Ovesen et al. 2002).

1.3.2 Sensory and social factors

Weight loss that often accompanies psychological or physical illnesses common in older people may predispose to the development of pathological anorexia and malnutrition. Alterations in sensory and social factors serve as additional stresses and result in inadequate dietary intake in older people. Some of these factors include age-related taste and smell deficits (Morley 1997; Mathey, Siebelink et al. 2001; Mulligan, Moreau et al. 2002), impairments in sensory specific satiety (awareness of change in palatability of food associated with consumption of a variety of foods) (Rolls, Kim et al. 1991), poor dentition, decreased manual dexterity, decreased cognitive ability, poverty, depression, chronic illness and social isolation (Morley 1997; Ritchie, Burgio et al. 1997; Sheiham and Steele 2001; Marshall, Warren et al. 2002; Marcenés, Steele et al. 2003).

1.3.3 Body mass distribution changes with age

Normal ageing is also associated with a decline in energy expenditure (Morley 1997). For many the decrease in energy intake with age is greater than the decrease in energy expenditure and weight loss occurs (Rolls 1992; Morley 1997). This weight loss may be desirable in the majority of adults who experience an increase in body mass, which is predominantly fat, in middle age (Steen 1988). In general however the decline in body weight after about 70 years is predominantly lean tissue (Steen 1988; Rolls 1992; Evans and Campbell 1993; Morley 1997; Nair 2000). This age-related loss of muscle mass, or sarcopenia, is associated with a decline in strength, the consequences of which may include changes in functional status leading to falls, fractures and loss of independence (Morley 1997; Tenover 1998; Roshan, Nader et al. 1999; AIHW 2001; Volpi, Sheffield-Moore et al. 2001) (see Chapter 2).

In Australia in 1998, 1,014 elderly people (65 years and older) died from accidental falls. Falls accounted for 54% of injury-related hospitalisations for this age group and 48% of accidental falls leading to hospitalisation occurred in the home (AIHW 2001). Age-related weight loss also predisposes this group to the development of protein-energy malnutrition (PEM), further exacerbating muscle loss and worsening existing medical conditions (Lovat 1996; Morley 1997; Omran and Morley 2000).

1.4 Prevalence of protein energy malnutrition

Protein energy malnutrition (PEM) results from insufficient protein and reduced energy intake (Mion, McDowell et al. 1994; Omran and Morley 2000) and is a common worldwide problem in the elderly. In the USA, up to 15% of community dwelling older persons, 5-12% of homebound patients and 20-65% of hospitalised patients are reported as suffering from PEM (Seiler 2001). In a recent Australian study the prevalence of malnutrition in inpatients at two Sydney teaching hospitals was found to be 36% and of these, only 36% had been previously identified as malnourished (Middleton, Nazarenko et al. 2001). The malnourished patients were predominantly over the age of 65 years and found to have a significantly longer time in hospital than the well-nourished patients. Mortality at 12-months was also significantly greater in malnourished than well-nourished patients (29.7% and 10.1 % respectively) (Middleton, Nazarenko et al. 2001).

The prevalence of malnutrition in 850 patients admitted to four English hospitals was evaluated using anthropometric measurements and patient-reported recent weight loss. The authors found 20% of patients admitted were malnourished and this finding was associated with significantly longer hospitalisations, more infections and greater disease severity (Edington, Boorman et al. 2000). Thomas et al 2002, determined using the Mini Nutritional Assessment (MNA), that of 837 patients admitted to a subacute centre in St Louis, only 8% were well-nourished, 29% were malnourished and 63% were at risk of malnutrition (Thomas, Zdrowski et al. 2002). In a study in a similar setting, of 65 patients older than 65

years, approximately 50% were malnourished (Visvanathan, Penhall et al. 2004), and in a collaborative study performed in patients admitted to 33 hospitals in and around the Chicago area, 50% of patients able to be assessed (60% unable to be assessed) were at risk of malnutrition (Kamath, Lawler et al. 1986).

A study conducted by this department used the MNA to determine the nutritional status of 250 elderly community dwelling men and women in Adelaide, and found that nearly half of those assessed were either malnourished (5%) or at risk of malnutrition (38%). During the next twelve months these combined two groups were significantly more likely to be admitted to hospital (45.1% vs 29.1%, $P < 0.05$), to be hospitalised for more than one month (16.1% vs 4.7%, $P < 0.005$), to suffer falls (41.9% vs 26%, $P < 0.05$) and to lose weight (44.1% vs 16.5%, $P < 0.001$) than the well nourished group (Visvanathan, Macintosh et al. 2003).

Saletti et al (1999) determined nutritional status in elderly (over the age of 70 years) community dwelling Swedish people. Two thirds (62%) were assessed as having suspected malnutrition despite the provision of home care services (Saletti, Johansson et al. 1999). In a similar study conducted in Denmark, 61 patients over the age of 65 years were recruited from general practices. At baseline 38% of patients were found to be 'at risk' of malnutrition. Thirty-four out of the 61 patients (56%) attended the scheduled 6-month follow up visit. The lower participation in this visit was predominantly due to non-attendance by previously identified 'at risk' patients (44% vs 18%, $0.05 < P < 0.01$). The authors found a non-significant higher prevalence of acute diseases, hospitalisations and start of home care in the malnourished than well-nourished group (Beck, Ovesen et al. 2001) (Table 1.1).

Table 1.1 Summary of studies in community and hospital facilities investigating the prevalence of protein-energy malnutrition in older people.

Study	Sample size	Percentage of PEM	Setting	Follow-up
Visvanathan et al (2004)	65	35-43%	Sub-acute care	LOS
Visvanathan at al (2003)	250	5*-38 [†] %	Community	12 months
Thomas et al (2002)	837	29*-63 [†] %	Sub-acute care	LOS
Middleton et al (2001)	819	36%	Acute care	12 months
Beck et al (2001)	61	38%	Community	6 months
Edington et al (2000)	850	20%	Acute care	LOS
Saletti et al (1999)	80	62%	Community	N/a
Mion et al (1994)		20-65%	Acute care	N/a
		15%	Community	N/a
		5-12%	Homebound	N/a

*, malnourished; [†] 'at risk' of malnutrition; LOS, length of stay; N/a, not applicable

1.4.1 Protein energy malnutrition and diagnosis

Protein energy malnutrition (PEM) is found in a large percentage of the elderly cared for in nursing homes (Potter, Langhorne et al. 1998), and nutritional surveys have shown an increased risk of deficiencies in both institutionalised and non-institutionalised elderly groups (Jensen, Friedmann et al. 2001). PEM is often diagnosed by low body weight or excessive involuntary weight loss occurring over a short period of time and abnormal biochemical indicators, including low albumin (Delmi, Rapin et al. 1990; Tkatch, Rapin et al. 1992; Murphy, Brooks et al. 2000; Lumbers, New et al. 2001).

The mini-nutritional assessment (MNA) questionnaire has been validated for use in the elderly and is widely used to determine PEM (Guigoz, Vellas et al. 1996; Cohendy, Gros et al. 1999; Guigoz and Vellas 1999; Vellas, Guigoz et al. 1999; Murphy, Brooks et al. 2000). This tool provides an index of nutritional status without undertaking biochemical or clinical assessments. The MNA test is composed of simple measurements and brief questions and can be completed in about 10 minutes. It comprises data from 1) anthropometric measurements (height, weight and weight loss); 2) global sources (six questions related to lifestyle, medication and mobility); 3) dietary information (eight questions related to

number of meals, food and fluid intake and feeding independence) and 4) subjective information (self-perception of health and nutrition) (Guigoz, Vellas et al. 1996). The score categorises elderly subjects as either well nourished, at risk of malnutrition or malnourished. It has been compared with an extensive nutritional assessment, undertaken to assess PEM, which includes complete anthropometric, clinical biochemistry and dietary parameters, and found to have a sensitivity of 96%, specificity of 98%, and predictive value of 97% in predicting PEM (Vellas, Guigoz et al. 1999).

Recently this method has been evaluated in an Australian sub-acute care setting. Sixty-five older patients admitted to medical, geriatric and orthopaedic rehabilitation units were assessed for nutritional status within 48 hours using the MNA, a 'rapid screen' revised MNA tool consisting of two factors (low BMI and weight loss as assessed by MNA criteria) and a Standardised Nutritional Assessment (SNA) based on the usual clinical practices of the facilities dietitians and including biochemical analysis (Visvanathan, Penhall et al. 2004). The study found a high percentage of patients were malnourished as assessed by all the tools. Both the MNA and 'rapid screen' tools were able to identify undernourished individuals when compared with the SNA. The authors conclude that these screening tools provide alternatives to suit the resources of institutions and as such can be integrated into admission assessments to facilitate greater identification of patients at risk of nutritional deficits (Visvanathan, Penhall et al. 2004).

1.4.2 Possible interventions in protein energy malnutrition

As indicated above, reduced nutritional status in the elderly is common and predisposes to the development of various diseases. It increases the severity and worsens the outcome of many diseases when they do develop (Lumbers, New et al. 2001; Morley 2001). Early recognition of malnutrition, and subsequent nutritional intervention, may therefore provide a means of preventing or minimising the adverse effects of some diseases affecting older persons (Mion, McDowell et al. 1994).

Sullivan et al (1991) evaluated 109 patients between admission to a Geriatric Rehabilitation Unit (GRU) and one-year post discharge, and found a strong correlation between the amount of involuntary weight loss and the risk of subsequent morbidity or mortality, providing evidence for the importance of nutritional status in predicting inpatient and post discharge mortality (Sullivan, Walls et al. 1991). These data are supported by the previous finding that the level of 'protein- calorie undernutrition' on admission to hospital correlates with the risk of developing an infectious complication or a major life-threatening complication or dying while in hospital (Sullivan, Patch et al. 1990).

1.4.3 Oral supplementation and outcomes in PEM

Daily oral nutritional (often high protein) supplementation has been shown to result in increased energy intake with associated increases in body weight, increases in functional status and decreases in the incidence of falls in malnourished subjects (Abbasi and Rudman 1994; Gray-Donald, Payette et al. 1994; de Jong, Chin et al. 1999; Lauque, Arnaud-Battandier et al. 2000). Potter et al (1998) reviewed 32 randomised controlled trials of oral or enteral protein supplementation in malnourished adults (Potter, Langhorne et al. 1998). In these studies, which included 1670 subjects, the main outcome measures were change in body weight, change in mid-arm circumference and mortality. The subjects receiving nutritional supplementation showed consistently improved changes in body weight and anthropometry compared with controls. There was a reduced mortality rate (when 83% of studies with similar methodology were analysed) in treatment compared to control groups, however, the inclusion of studies that did not meet methodological criteria reduced this result to a non-significant trend. This review did not address the effect of supplementation on functional status (Potter, Langhorne et al. 1998).

Evidence from studies in elderly patients who have sustained hip and neck of femur fractures indicates that malnutrition increases the likelihood of falls and fracture, and leads to more unfavourable outcomes, including infection, cardiac and respiratory complications

and death (Delmi, Rapin et al. 1990; Tkatch, Rapin et al. 1992; Schurch, Rizzoli et al. 1998; Lumbers, New et al. 2001). When daily oral supplements (250ml, 20g protein, 254kcal) were administered for approximately 32-38 days in two studies an improvement was found in the rate of complications and death (Delmi, Rapin et al. 1990; Tkatch, Rapin et al. 1992). Sixty-two patients admitted for fracture of the proximal femur received either 250 kcal/day of an oral supplement containing protein (n = 33) or 250 kcal/day of an oral supplement not containing protein (n = 29) for 38 days. Rate of complications and death, after 7 months, were significantly lower in the protein-supplemented group versus the control group (52 % v 80%, $P < 0.05$). Length of stay in a rehabilitation facility was also significantly decreased in the protein supplemented group compared to control (69 v 102 days, $P < 0.05$) (Tkatch, Rapin et al. 1992). In a similar study in 59 elderly patients with femoral neck fractures, rates of complications and death were decreased in patients given an oral supplement compared to patients given no supplement (44% v 87%). Six months treatment (250kcal, 20g protein/day) improved severe clinical outcomes in 82 elderly subjects, with recent osteoporotic hip fracture. Outcomes included reduced length of stay in a rehabilitation hospital ($P = 0.018$) and attenuation of proximal femur bone loss ($P = 0.029$), but no difference in muscle strength was observed at a six-month post-treatment follow-up (Schurch, Rizzoli et al. 1998).

The influence of 10 days of oral protein supplementation (398kcal, 30g protein) on body composition [assessed by dual energy x-ray absorptiometry (DEXA)] and whole-body protein kinetics was assessed in a group of 17 elderly malnourished hospital inpatients and 12 healthy young medical student subjects. A control group of 6 malnourished elderly who did not receive the supplement was used for comparison. Supplemented subjects had a significantly greater fat free mass gain than the control group (1.3 and 0.6 kg respectively, age effect, $P < 0.05$; diet effect, $P < 0.02$), and increased protein synthesis (0.6 and 0.2, age effect, $P < 0.05$), which the authors suggest may produce beneficial functional effects that were not assessed in this study (Bos, Benamouzig et al. 2000).

More recent studies confirm the findings that nutritional supplementation may decrease length of hospital stay and complications but found no effect on functional status (Espauella, Guyer et al. 2000; Neumann, Friedmann et al. 2004). Older patients admitted to an acute care hospital for hip fractures were given either an oral protein drink (n = 85) or placebo (n = 86) (derived mainly from carbohydrate) for 60 days and assessed for mortality, complications, mobility and independence of activities of daily living (Espauella, Guyer et al. 2000). No differences were observed at a 6 month post fracture follow-up in return to functional status or mortality, however, the protein supplemented group experienced fewer in hospital (P = 0.05) and total complications (P = 0.04) than the control group (Espauella, Guyer et al. 2000). In a similar study, 46 older patients undergoing surgical repair of a hip fracture were randomised to either a standard protein (17.8g/d) or a high protein (30g/d) supplement drink for 28 days. Outcome measures included length of stay, functional status (change in mobility) and biochemical analysis (serum albumin, prealbumin and C-reactive protein). There was an increase in protein intake (P < 0.048) and a greater improvement in serum albumin (P < 0.019), fibre, calcium, vitamin K and phosphorous in the high protein supplemented group. Length of stay was reduced in the group with the higher protein intake although this did not reach statistical significance. There were no differences in complications or mobility (assessed by questionnaire and exercise tolerance) between groups (Neumann, Friedmann et al. 2004).

Improvements in weight with oral protein supplementation has also been shown in community living older people without effect on functional status (Faxen-Irving, Andren-Olsson et al. 2002). Body mass composition and ADL status was assessed in 36 community-assisted living older residents. At baseline, 19% of the participants had a BMI \leq 20 kg/m² and 44% had a BMI \leq 23 kg/m² and cognitive function was low (assessed by minimal state examination). Oral protein supplements were given daily for 6 months to 22 residents and personnel in contact with this group were educated in nutrition. The remaining 14 residents served as controls. Body weight in the supplemented group increased by 3.4 ± 3 kg but there was no change in the controls (P = 0.001). There was no effect of treatment on cognitive function and ADL status declined in both groups (Faxen-Irving, Andren-Olsson et al. 2002). (Summarized in Table 1.2).

Table 1.2 Summary of nutritional intervention studies in older subjects.

Study	Subjects	Treatment	Energy (kcal)/ volume (ml)	Protein (g)/day	Duration	Results
Neumann et al (2004)	24 [†] (Ensure) 22 [†] (Boost)	Ensure Oral protein-rich drink (Boost)	250/200 240/200	17.8 30	28 days	↑LOS in Ensure group ↑Se albumin in Boost group No difference in functional status
Espauella et al (2000)	85 [†] (treatment) 86 [†] (control)	Oral High-Protein drink	149/200 155/200	20	60 days	↓complications in treatment group No difference in functional status
Bos et al (2000)	17* (treatment) 6* (control)	Oral High-Protein drink	398/400	30	10 days	↑FFM in treatment group (1.3 v 0.1 kg)
Lauque et al (2000)	19 [†] (control) 22# (no treatment) 19# (treatment) 28* (treatment)	Oral Soup Fruit Drink Dessert	120/200 200/200 200/200 150/150	10 7.5 15 12	30 days	↑BW in malnourished ↑MNA score
Schürch et al (1998)	41 (treatment) 41 (control)	Oral High-Protein drink	250	20	6 months	↓rehabilitation in treatment group ↓bone loss in treatment group
Gray-Donald et al (1995)	25# (treatment) 25# (control)	Ensure [‡] Ensure-Plus [‡] Ensure high fibre [‡] ([‡] chosen by subjects)	250/470 250/470 350/470	8.74 13 9.4	12 weeks	↑BW in treatment group No change in functional status
Tkatch et al (1992)	33# (treatment + protein) 29# (treatment – protein)	Oral vitamin and mineral supplement ±Protein	254/250	20.4	38 days	↓mortality ↓complications ↓LOS in protein group
Delmi et al (1990)	27# (treatment) 32# (control)	Oral High-Protein drink	254/250	20	32 days	↓mortality ↓complications in treatment group

* malnourished; # 'at risk' of malnutrition; [†] well-nourished; FFM, fat-free mass; BW, body weight; MNA, Mini Nutritional Assessment; LOS, length of stay

1.4.4 Functional benefits of oral supplementation

Although the greatest benefit, at least in the short term, from increasing daily energy intake with oral supplementation may be an increase in body weight, there is some evidence that this intervention improves functional status measures, such as muscle strength and rate of falls (de Jong, Chin et al. 1999). Daily oral protein supplementation ($\approx 400\text{kcal}$) given for 60 days has been shown to result in increased energy intake ($P < 0.001$), with associated increases in body weight and grip strength and improvements in MNA score in a supplemented group, in malnourished nursing home residents and those at risk of malnutrition when compared to non-supplemented groups (Lauque, Arnaud-Battandier et al. 2000).

Similarly, in two studies conducted in the frail homebound elderly where subjects were given daily oral supplements ($\approx 500\text{ kcal}$) for 12 weeks and weekly dietician reviews, weight gain was significantly greater in the supplemented group. Functional status was determined by handgrip strength alone. Supplementation resulted in a daily energy intake increase of 390 kcal and an average corresponding weight gain of 1.25kg which was associated with increased handgrip strength ($r = 0.75$, $P = 0.002$) (Gray-Donald, Payette et al. 1994). In a follow-up study with the same supplementation regimen, functional status was determined by both handgrip strength and the number of falls over a 12 week period. A significant weight gain in the supplemented group ($2.1 \text{ v } 0.6\text{ kg}$, $P < 0.01$) was not associated with an improvement in handgrip strength, but the number of falls was significantly less in the supplemented than unsupplemented group (Gray-Donald, Payette et al. 1995).

1.5 Sarcopenia and possible interventions

In older adults decreases in appetite and energy intake may lead to unintentional weight loss. As previously discussed, loss of 5% or more of usual body weight is associated with decreased functional ability and increased morbidity and mortality (Morley 1997; Wallace and Schwartz 2002). The weight loss that generally occurs after about 70 years of age is

predominantly lean mass (Steen 1988; Nair 2000). Sarcopenia, or diminished reserves of muscle, leads to reductions in strength, power and endurance (Lindle, Metter et al. 1997; Wallace and Schwartz 2002), is associated with functional impairment and may play a role in the pathogenesis of frailty (Morley, Baumgartner et al. 2001).

Circulating androgen concentrations decline in both men and women with increasing age, and there is increasing evidence that this may contribute to the development of sarcopenia (Roubenoff, Rall et al. 1998; Melton, Khosla et al. 2000; Morley 2000; Morley 2001; Volpi, Sheffield-Moore et al. 2001), decline in functional status (Zumoff, Strain et al. 1995; Davis and Burger 1998; Tenover 1998; Davis 1999; Tenover 1999; Burger, Dudley et al. 2000; Morley 2001) and PEM. The hormonal changes that occur with age in men occur over a long period of time and are more subtle than the profound changes in ovarian function that occur in women at menopause (Morley 2000). In men, testosterone levels decline due to a decrease in Leydig cell numbers and the activity of enzymes in the metabolic pathway governing testosterone production (Roubenoff, Rall et al. 1998; Morley 2000). Data from longitudinal studies suggests that between 39-70 years testosterone levels decrease 0.4% per year with associated decreases in bioavailable testosterone, free testosterone and increases in sex-hormone binding globulin of 1.0%, 1.2% and 1.2 % per year respectively (Feldman, Longcope et al. 2002). Androgen replacement therapy (ART) is advocated for men with symptoms of marked androgen deficiency (Davis, McCloud et al. 1995; Tenover 1999; Melton, Khosla et al. 2000) but there is considerable controversy about the indications for treatment in borderline deficiency, such as occurs with normal ageing.

1.5.1 Intervention

Strategies to address the decline in body weight often involve nutritional supplementation (discussed above) however increasing energy intake alone may be inadequate. Intervention with replacement therapies may arrest this documented decline and improve muscle mass and strength in this age group.

In one study, older hypogonadal men ($n = 8$) were administered intramuscular testosterone enanthate (200mg/ml) every 2 weeks for 3 months and were assessed for serum testosterone, body composition, hand strength and balance. Six older hypogonadal men served as a control group (no treatment). The men who received testosterone had a significant increase in serum testosterone and bioavailable testosterone concentration and hand strength compared to controls (Morley, Perry et al. 1993). Snyder et al (1999), administered testosterone or placebo patches to men aged 65 years and older ($n = 108$) to investigate the effect of testosterone on body composition and muscle strength. There was a significant decrease in fat mass ($-0.3 \pm 0.5\text{kg}$) ($P = 0.001$) and a significant increase in lean mass ($1.9 \pm 0.3 \text{ kg}$) ($P < 0.001$) in testosterone treated men compared to placebo. However, these changes in body composition did not effect muscle strength (Snyder, Peachey et al. 1999).

In a similar study, conducted by this department, examining the effects of administering oral testosterone undecanoate in 76 elderly, borderline androgen deficient men, a significant increase in fat free mass (FFM, difference at 12 months 3.1%) and a significant decrease in fat mass (FM, difference at 12 months 5.7%) was observed in the treated group compared to the control group. No effect on muscle strength was observed (Wittert, Chapman et al. 2003). Similar studies with various forms of androgen replacement have been done in healthy older men, but although benefits may be seen in muscle mass and strength there is, as yet, no consensus that this leads to improvements in functional status (Tenover 1998; Morley 2000) and clinical outcomes. This may be because such studies have been conducted in healthy older men. Targeting more frail older men (eg those who are malnourished) for testosterone therapy may be more likely to produce functional benefits.

In postmenopausal women testosterone, administered with oestrogen, increases bone mineral density (BMD) and improves energy levels, libido and general well-being (Roubenoff, Rall et al. 1998; Castelo-Branco, Vicente et al. 2000; Davis, Walker et al.

2000; Davis and Tran 2001). Administration of oestrogen alone reduces body fat and LDL cholesterol and, when administered with testosterone, increases FFM (Davis, Walker et al. 2000). In addition, androgen replacement alone effects body composition and weight in postmenopausal women by increasing FFM and reducing FM (Gruber, Sator et al. 1998). There is no consensus about the use of ART in elderly men and women. The effect of such treatment on adverse outcomes of sarcopenia and PEM in this age group have yet to be determined.

Oral nutritional supplementation may increase body weight in malnourished older people but there is no consensus that this is associated with increases in muscle strength or functional status. Intervention studies evaluating the effect of androgen and nutritional supplementation are required to determine the effect on body weight, muscle strength and subsequent functional status with the aim of improving health status and independence.

A recent study evaluated the effects of a high protein supplement (200ml/day, 20g protein/day) drink alone or in combination with the anabolic steroid nandrolone decanoate on body composition (weight and lean body mass (LBM) assessed by DEXA), activities of daily living (ADL) status and health-related quality of life (questionnaire) after a femoral neck fracture (Tidermark, Ponzer et al. 2004). Sixty older women identified as 'at risk' of malnutrition ($BMI = 20.4 \pm 2 \text{ kg/m}^2$) were randomised to receive one of the following treatments for 6 months: high protein drink alone ($n = 20$), high protein drink in combination with nandrolone decanoate ($n = 20$) or no treatment [$n = 20$ control]. At 6 months LBM had decreased in the control and supplement alone group but there was no change in the combined supplement and nandrolone group ($P < 0.05$, combined group v control and supplement group). ADL status declined significantly in the control group but there was no difference between the two treatment groups ($P < 0.005$ control v supplement and combination group). In addition, the decline in health-related quality of life was attenuated in the combined supplement and nandrolone group ($P < 0.05$, combined group v

control and supplement group). At 12 months follow-up there were no differences between the groups for any variable aside from a trend toward maintained ADL function in the combined supplement nandrolone group. There was no difference at 12 months in hospitalisation between the groups (Tidermark, Ponzer et al. 2004). This research suggests that there may be additional benefits, both functional and in body mass composition, of adding anabolic steroids to nutritional supplementation in older people.

1.6 Conclusion

Physiological changes in gastrointestinal function that occur with ageing may have a significant impact on appetite and food intake in those over the age of 65 years. When these factors are combined with adverse social and psychological aspects of ageing many of this group are at risk of developing protein-energy malnutrition and sarcopenia. These conditions have been found to increase the risk of acute and chronic illness, hospitalisation and loss of independence in this group leading to a greater dependence on community and health care facilities. Elderly patients living in nursing homes, in the community or on acute care admission to hospital are not regularly identified as malnourished or ‘at risk’ of malnutrition and this highlights the need for incorporating nutritional screening and perhaps intervention in these settings. In addition, further studies are required to determine the effectiveness of combined protein supplement and androgen hormone treatments on body composition, strength and functional status in older people.

Chapter 2

Gastrointestinal regulation of appetite and changes with ageing

2.1 Introduction

Under-nutrition is common in older people and has been implicated in the development and progression of chronic diseases commonly affecting the elderly, as well as increasing mortality (Fried, Kronmal et al. 1998; Duerksen, Yeo et al. 2000). The causes for this reduction in energy intake are multifactorial and, in addition to many social and sensory factors, include changes in both central and peripheral appetite mechanisms. Normal ageing is associated with a number of significant changes in gastrointestinal function, which may impact on daily energy intake. This chapter will focus on age-related changes in gastrointestinal function as they affect appetite and food intake. These changes include altered activity of and sensitivity to endocrine factors involved in the control of appetite and feeding, and alterations to gastrointestinal motor control of gastric emptying, the size of the gastric antrum and intragastric distribution of food. Several studies in this thesis examine the gastrointestinal control of feeding (Chapters 5, 6, 7 and 8).

2.2 Appetite regulation and changes with ageing

Regulation of energy intake is thought to occur through the action of short, medium and long term feedback mechanisms, linking energy intake, usage and storage to feedback inhibition of food intake (French and Yeomans 2001). According to Blundell & Halford, appetite represents three levels of events and processes. These levels they describe as psychological events (hunger perception, cravings, hedonic sensations) and behavioural operations (meals, snacks, energy and macronutrient intakes); peripheral physiology and metabolic events; and neurotransmitter and metabolic interactions in the brain (Blundell and Halford 1994).

Short term appetite is controlled by a central feeding drive and a peripheral satiety system, which includes inputs from mechano- and chemo-receptors that respond to the presence of food in the gastrointestinal tract (Havel 2001) (Figure 2.1). Gastrointestinal satiety signals originate from gastric distension, which reduces food intake, and the interaction of nutrients with gastrointestinal tract receptors that stimulate the release of satiety hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP) and amylin, and inhibit the release of ghrelin (Morley and Levine 1983; Morley 1990; Lavin, Wittert et al. 1998; Nakazato, Murakami et al. 2001), which stimulates feeding (Nakazato, Murakami et al. 2001; Shintani, Ogawa et al. 2001).

These hormones play a role in both satiation (meal termination) and satiety (time to subsequent meal consumption), and may have a different function than long-term regulators of energy balance such as insulin and leptin (Havel 2001) (Figure 2.2).

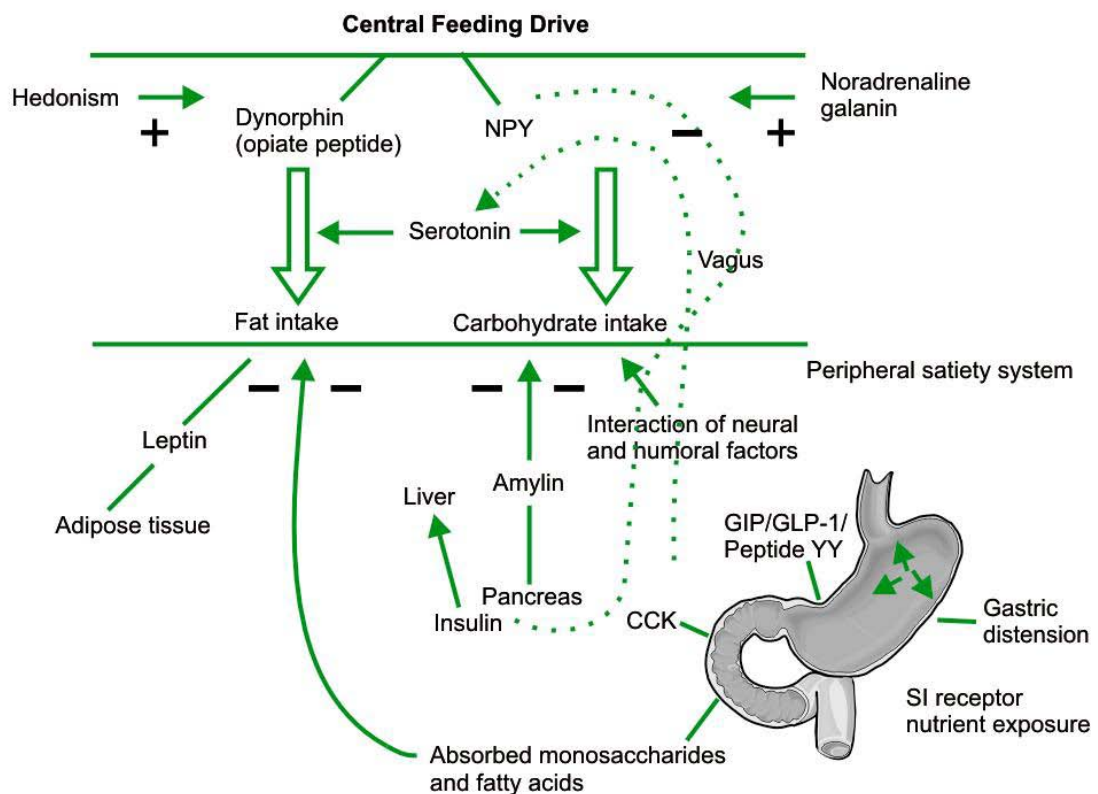


Figure 2.1 Overview of mechanisms involved in appetite regulation in humans (adapted from Morley 1990)

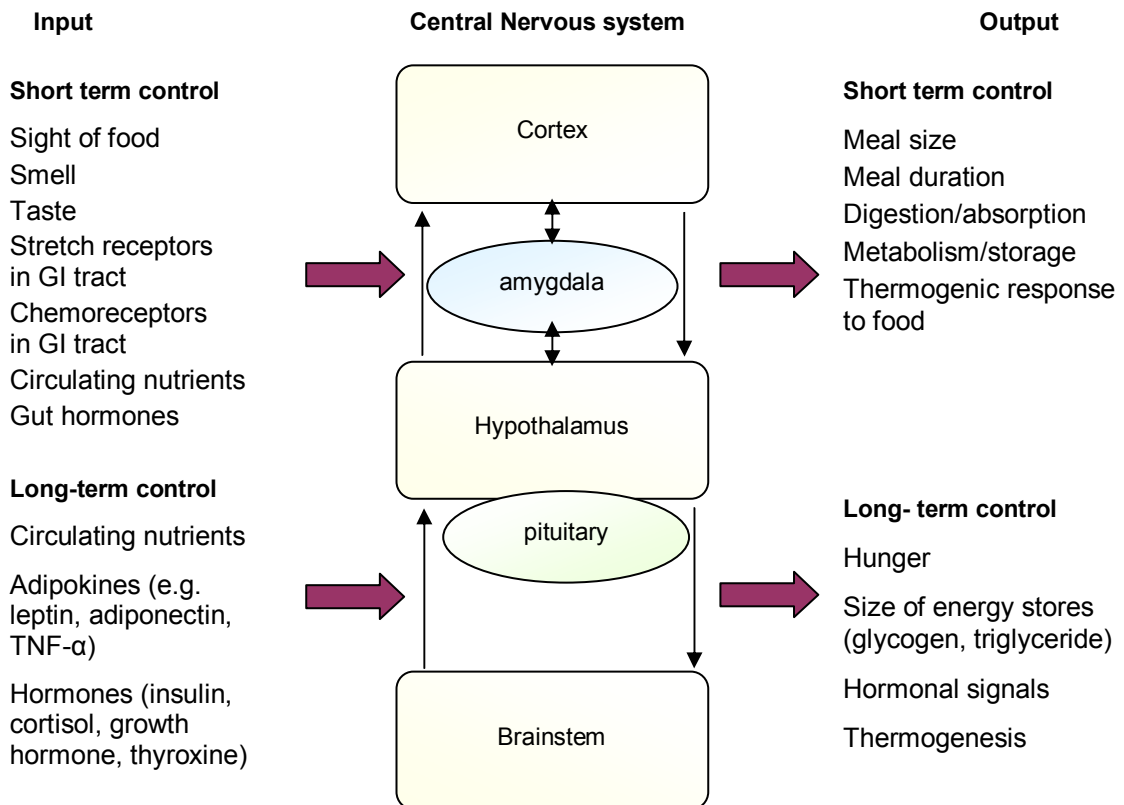


Figure 2.2 Short and long term factors influencing energy balance (Wilding 2002)

2.3 Changes in gastric factors in response to ageing

Appetite regulation is affected by ageing (Clarkston, Pantano et al. 1997; Morley 1997; Lavin, Wittert et al. 1998; MacIntosh, Morley et al. 2001; Chapman, MacIntosh et al. 2002; Donini, Savina et al. 2003; Sturm, MacIntosh et al. 2003). Healthy older subjects are less hungry (MacIntosh, Morley et al. 2001; Chapman, MacIntosh et al. 2002; Sturm, MacIntosh et al. 2003) and, probably experience greater postprandial fullness than healthy younger subjects (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997). Signals originating in the upper gastrointestinal tract (stomach and small intestine) play a major role in the perception of appetite sensations and the control of food intake in older people (Morley 1997). Interrelated factors include the rate of gastric emptying, intragastric meal distribution, fasting gastric compliance and gastric accommodation. The stomach is divided into three sections based on anatomical, histological and functional distinctions. The fundus is proximal, above and just below the oesophageal opening. The body encompasses the

middle, and the distal portion, known as the antrum, lies above the pyloric sphincter. Gastric contents are emptied from the antrum through the pylorus into the duodenum (Figure 2.3).

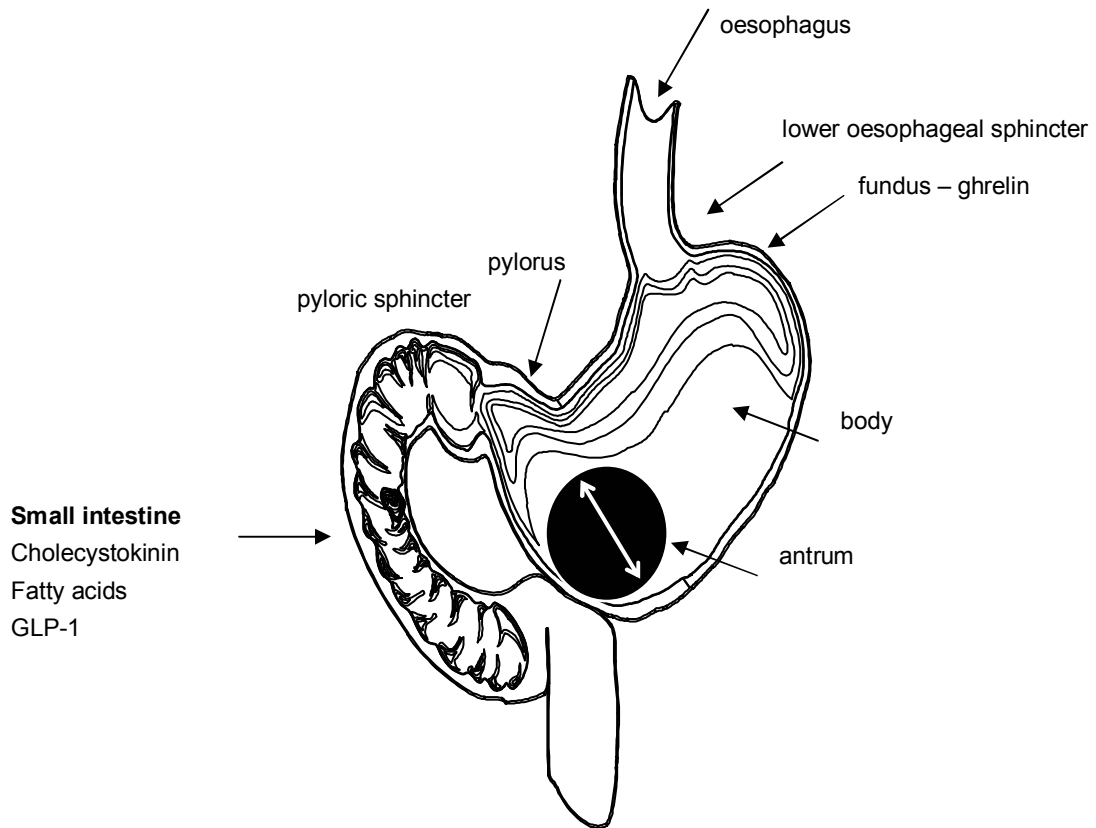


Figure 2.3 Structure of the stomach.

With ageing there is a decline in the compliance of the (proximal) fundus of the stomach (Sun, Doran et al. 1998) – the ability to distend in response to a given pressure. This results in more rapid filling of the distal gastric antrum than in young adults (Clarkston, Pantano et al. 1997), in whom the food is often held high in the gastric fundus until just before it is emptied into the small intestine. Antral distension seems to be particularly effective in signalling the central nervous system to terminate the meal (Jones, Doran et al. 1997); the degree of antral distension has been shown to be directly proportional to the development of satiation after a meal (Jones, Doran et al. 1997).

Motor function of the gastrointestinal tract changes with ageing (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997; Rayner, MacIntosh et al. 2000). One such change is a modest slowing of gastric emptying (Horowitz, Maddern et al. 1984; Kupfer, Heppell et al. 1985; Wegener, Borsch et al. 1988; Clarkston, Pantano et al. 1997). The effects of changes in the rate of gastric emptying of food on meal cessation and the initiation of subsequent meals are complex. While, on the one hand, delayed gastric emptying has the effect of prolonging retention of food in the stomach and thus inducing satiation by increasing and prolonging gastric distension (Rayner, MacIntosh et al. 2000), it also delays the onset of powerful satiety signals initiated by entry of nutrients into the small bowel. On balance, however, available evidence suggests that slowing of gastric emptying, as occurs with normal ageing, has the effect of reducing appetite and food intake (Clarkston, Pantano et al. 1997).

The use of gold-standard radioactive scintigraphic techniques to measure gastric emptying (Gilja, Hausken et al. 1999) is limited by their complexity and the level of radiation exposure. Ultrasound measures of gastric emptying have been developed recently and show considerable promise. Ultrasonography of the gastric antrum has been validated against scintigraphy as an alternate method for measuring gastric emptying (Bolondi, Bortolotti et al. 1985; Holt, Cervantes et al. 1986; Hveem, Jones et al. 1996; Darwiche, Almer et al. 1999) and the reproducibility of this method determined in young subjects (Ricci, Bontempo et al. 1993). There is also a close relationship between ultrasound measurements of antral area and the rate of gastric emptying of nutrient and non-nutrient liquids in older subjects (correlation coefficients greater than 0.9 (Sturm, Parker et al. 2004)) allowing the former to be used to measure the latter. The limitations of this procedure however, include an inability to determine transpyloric flow and the contribution of the proximal stomach, which may aid in further understanding of intragastric food distribution.

Gastric antral area after eating is also related to appetite sensations and probably subsequent voluntary food intake. Our group has shown a highly significant positive relationship, after an oral nutrient pre-load, between fullness ratings and antral area and inverse relationship between hunger and antral area in both young and older adults (Sturm, Parker et al. 2004). We have also shown that voluntary food intake 60 minutes after a pre-load is significantly related to antral area (as assessed by ultrasound) in healthy older and young subjects ($r = -0.9$, $P < 0.0001$), with older subjects having larger antral areas ($P = 0.002$) (Sturm, Parker et al. 2004). These findings highlight the role of food movement from the proximal to the distal stomach in producing feelings of fullness and suppressing hunger and food intake. It seems likely that age-related changes in gastric motility favour earlier distal distribution of food after a meal in older people than young adults, hence accentuating these effects. Further careful studies of the effects of ageing on intragastric food distribution, and its relationship to gastric emptying, appetite and voluntary food intake, are needed to clarify these effects.

2.4 Effect of ageing on gastrointestinal hormone activity

2.4.1 Cholecystokinin

Cholecystokinin (CCK) is found in both the brain and the gastrointestinal (GI) tract and acts both centrally and peripherally (Moran 2000). CCK is released from the endocrine cells of the duodenum and jejunum in response to the ingestion of fats and amino acids (Matzinger, Gutzwiller et al. 1999). Some of the secreted CCK enters the blood and stimulates the exocrine pancreas and liver/gallbladder to secrete appropriate enzymes into the duodenum to facilitate the digestive process (Woods 2004). Research into the actions of the duodenal peptide CCK has established it as a powerful satiety signal (Kissileff, Pi-Sunyer et al. 1981; Muurahainen, Kissileff et al. 1988; Moran and Schwartz 1994; Moran 2000; Woods 2004). CCK stimulates gallbladder contraction and pancreatic exocrine enzyme secretion, causes a delay in gastric emptying and inhibits food intake (Lieverse, Masclee et al. 1995;

Matzinger, Gutzwiller et al. 1999; Moran 2000). The latter effect is probably physiological, as administration of CCK-A receptor antagonists has been shown to increase food intake in both animal (Bado, Durieux et al. 1991; Bellissimo and Anderson 2003) and human (Beglinger, Degen et al. 2001) studies.

Circulating CCK concentrations increase with healthy ageing, both fasting and in response to nutrient stimuli, but are comparable in under-nourished and well-nourished older people (Sturm, MacIntosh et al. 2003). In obesity and anorexia nervosa, where abnormalities are found in the control of appetite and hormonal secretion, fasting CCK concentrations are decreased in young women with anorexia nervosa and increased in young obese women compared to young normal weight controls probably in response to rather than causative of these conditions (Baranowska, Radzikowska et al. 2000). There is evidence that older people may be more sensitive than young adults to the satiating effects of CCK (Berthelemy, Berthelemy et al. 1992; MacIntosh, Morley et al. 2001). Infusions of CCK have been shown to produce dose-dependent suppression of energy intake compared with control ($P < 0.001$). Low dose CCK-8 (1 ng/kg/hr) and high dose CCK-8 (3 ng/kg/hr) infusions to healthy older and young adults were associated with a mean 7.7% and 35.2% suppression respectively of energy intake, compared with the control infusion. The mean suppression in older subjects was significantly greater and double (32 vs 16%) that in young subjects due to a reduction in both the duration and rate of eating (MacIntosh, Morley et al. 2001). Higher circulating CCK levels and increased sensitivity to the satiating effects of CCK in older people together support increased CCK activity as a possible cause of the anorexia of ageing.

Endogenous circulating CCK concentrations increase in response to nutrient ingestion and interaction with receptors in the small intestine (Degen, Matzinger et al. 2001). It has been suggested that increased gastric distension induced by slowing of gastric emptying may be a mechanism by which CCK reduces food intake (Moran and McHugh 1982). Intraperitoneal

(IP) injection and intravenous (IV) infusion of CCK-8 slowed gastric emptying in rhesus monkeys comparable to that produced by intraduodenal nutrient. Four treatments were administered -intravenous (IV) CCK infusion (3 pmol/kg/min) with and without an intraduodenal infusion of 150 ml saline, intraduodenal infusion of 150 ml saline and IV infusion of saline. Intravenous CCK infusion with 150 ml intraduodenal saline produced a greater suppression of food intake than any of the other treatments. The dose of CCK shown to delay gastric emptying did not affect feeding unless gastric distension with saline occurred simultaneously, suggesting that the satiating effect of CCK depends on both of these factors (Moran and McHugh 1982). It was reported in healthy young human subjects that when CCK-8 was infused intravenously, intake of a pasta meal was significantly lower after a 500g soup but not after a 100g preload, again suggesting that gastric distension is important for a satiating effect of CCK (Muurahainen, Kissileff et al. 1991). However, Nolan et al 2003 argue that a nutrient preload, such as soup, cannot be considered solely as a gastric distending stimuli as ingestion of a nutrient preload, such as tomato soup, stimulates release of endogenous CCK thereby confounding the outcome (Nolan, Guss et al. 2003). In order to address this effect, Kissileff et al, 2003 infused intravenous CCK-8 in healthy human subjects with and without gastric distension by balloon and compared the CCK response to identical studies with saline infusions. The CCK-8 infusion with gastric distension produced a greater suppression of a liquid meal compared to both CCK-8 infusion without gastric distension and saline infusion with distension (Kissileff, Carretta et al. 2003).

Higher baseline concentrations and increased sensitivity to the effects of CCK in older people suggest there may be a role for the use of CCK blocking agents, in the form of CCK-A receptor antagonists, to increase appetite and food intake in this age group. Several studies, using Loxiglumide (Lox), a specific CCK-A receptor antagonist available for human use, have been conducted to investigate the actions of CCK on appetite and food intake. In healthy normal weight (n = 7) and obese (n = 7) young women, Lox has been

shown to have no significant effect on food intake (Lieverse, Masclee et al. 1995). In this study Lox (10 mg/kg/hr) or saline was administered intravenously for 165 minutes during which time volunteers were given a milkshake [(132 kcal/300 ml) 60 minutes following infusion commencement]) and a test meal [(banana slices) 90 minutes following infusion commencement]. Food intake and VAS ratings of appetite were measured. Food intake was higher following the Lox infusion than saline infusion in both groups (lean 359 ± 39 g vs 333 ± 31 g; and obese 313 ± 54 g vs 301 ± 45 g, respectively) but this difference was not significant. There was no effect of Lox on ratings of hunger or fullness for either lean or obese subjects or when the data for the two groups were analysed together (Lieverse, Masclee et al. 1995). The p values for this study were not reported, however there was a higher intake of food following the Lox infusion and the low subject numbers may account for the lack of significance.

The outcome of this study is in agreement with Drewe et al (1992) who found that Lox had no influence on hunger feelings or food intake in lean, healthy subjects (Drewe, Gadiant et al. 1992). Ten healthy young subjects administered intravenous Lox (10 mg/kg/hr) or saline with or without intrajejunal perfusions of fat (50% corn oil and 3% albumin) and saline (four-period latin square design) were assessed for food intake, plasma CCK concentrations and hunger and fullness. When assessed separately from the Lox data, saline infusions reduced the amount of food intake (361 ± 31 g vs. 454 ± 35 g; $P < 0.05$), when intrajejunal fat was perfused compared with intrajejunal saline. There was no difference in food intake or appetite scores during the lox infusions (\pm fat and saline) (Drewe, Gadiant et al. 1992).

This finding is supported by observations of a three-day study investigating the effects of oral loxiglumide on food intake (test meal day three of treatment) and appetite ratings during this meal. Oral Lox (tablet) or placebo was administered three times daily (for three days), 15 minutes prior to main meals, in healthy normal weight male and female subjects ($n = 11$). There was no effect of treatment on food intake or feelings of hunger and fullness

compared to placebo (French, Bergin et al. 1994). Again, subject numbers in these studies were small and it is possible that a greater sample or a higher dose of Lox may have produced a significant increase in food intake. However, taken together these studies suggest that other factors may be involved in CCK-induced satiety or may override the effects of a CCK antagonist.

Alternatively, there is evidence from other studies involving greater subject numbers that CCK-A receptor antagonists increase food intake and hunger (Gutzwiller, Drewe et al. 2000; Beglinger, Degen et al. 2001). In 10 healthy young normal weight men and women infusions of Lox [at the same rate as a previous study (Drewe, Gadiant et al. 1992)], or saline, was infused for 120 minutes. Subjects were offered an *ad libitum* cold meal (sandwiches, dessert) after 60 minutes and allowed to eat for one hour until the end of the infusion. Lox infusions resulted in a 10% increase in energy intake (kcal) ($P < 0.004$). Immediately prior to the test meal hunger ratings were higher during Lox infusion than during saline infusion ($P < 0.05$), and this effect was maintained for 45 minutes. Following the test meal a reduced feeling of fullness was observed with the Lox infusion ($P < 0.05$) (Beglinger, Degen et al. 2001). When CCK-8 is infused, in young healthy men ($n = 32$), in conjunction with Lox (10 mg/kg/hr), the expected satiating effects of CCK-8 are attenuated. In this four-treatment crossover design subjects, received either intravenous Lox or saline infusions for 120 minutes and either lox or saline bolus (0.75ug infused at a rate of 1ml/min over 10 min) following a milkshake preload. Subjects were allowed to eat [as for (Beglinger, Degen et al. 2001)] for the final 60 minutes of the infusions. CCK-8, when infused alone or with saline, significantly reduced food intake ($P < 0.001$) and hunger ($P < 0.05$) in healthy young men. When Lox was infused with CCK-8 or saline, food intake and hunger ratings were significantly increased (Gutzwiller, Drewe et al. 2000).

The conflicting results in these studies may reflect differences in methodology. All the studies were undertaken in young lean subjects (apart from a comparison with young obese subjects that yielded no differences (Lieverse, Masclee et al. 1995)), and used the same doses of Lox for infusion. However, when no effect of treatment was observed some differences in methodology was apparent. For example, a longer infusion time (Lieverse, Masclee et al. 1995), an oral intervention in the form of a tablet (French, Bergin et al. 1994), a concomitant intrajejunal fat perfusion (Drewe, Gadiant et al. 1992) and small subject numbers were employed in studies (Drewe, Gadiant et al. 1992; Lieverse, Masclee et al. 1995) which reported no effect on food intake. The contribution of any of these factors is unknown, however, not all studies of intravenous administration of CCK find consistent effects on appetite and food intake in humans (Schick, Schusdziarra et al. 1991; Lieverse, Jansen et al. 1993) and the same may apply to studies using CCK-A receptor antagonists. Nevertheless, although the effects of administering CCK-A receptor antagonists to older people have not been reported, some of these findings in young adults suggest that there may be a role for CCK-A receptor antagonists in increasing appetite and food intake in older under-nourished and at-risk people.

2.4.2 Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide produced in and secreted from the L-cells of the intestinal mucosa into the circulation after food intake (Naslund, Bogefors et al. 1999). Oral glucose is a potent stimulant of GLP-1 secretion (Kong, Chapman et al. 1999). GLP-1 has been shown to stimulate biosynthesis of insulin, suppress glucagon release, delay gastric emptying and enhance peripheral glucose disposal (Nauck, Niedereichholz et al. 1997; Edwards, Todd et al. 1999).

In humans, GLP-1 slows liquid and solid gastric emptying (Nauck, Niedereichholz et al. 1997; Long, Sutton et al. 1999; Naslund, Bogefors et al. 1999). When GLP-1 (7-36) amide in doses of 0.4, 0.8 or 1.2 pmol/kg/min, GLP-1 (7-37) 1.2 pmol/kg/min or placebo was

infused intravenously for 270 minutes, liquid gastric emptying was dose dependently slowed by GLP-1 (7-36) amide with the 1.2 pmol/kg/min dose producing the greatest effect ($P < 0.0001$). Following intravenous infusion of GLP-1 or saline in normal volunteers, solid gastric emptying is inhibited ($P < 0.01$). In normal men subjects both liquid (400 ml water) and solid (buffet meal) gastric emptying and food intake were measured following intravenous infusion of GLP-1 (1.2 pmol/kg/min) or saline. Gastric emptying was significantly delayed during the GLP-1 infusion and, although food intake was unaffected by GLP-1 infusion, subjects tended to be less hungry after the buffet meal ($P < 0.09$) (Long, Sutton et al. 1999).

In humans there is evidence that GLP-1 plays a role in controlling appetite and energy intake in both normal and obese subjects (Gutzwiller, Goke et al. 1999; Hellstrom and Naslund 2001). In normal weight young men, graded intravenous doses of GLP-1 (0.37, 0.75, 1.5 pmol/kg/min) dose dependently reduced the amount of food eaten at a test meal compared to control ($P < 0.01$). The maximal reduction in food consumption with the highest dose of GLP-1 (1.5 pmol/kg/min) amounted to a decrease in energy intake of 32% ($P < 0.001$) (Gutzwiller, Goke et al. 1999). Similar results have been found with infusion of GLP-1 in obese men, where physiological doses of GLP-1 suppressed ratings of hunger and prospective food consumption compared to a saline infusion ($P < 0.05$) and slowed gastric emptying ($P < 0.0001$) although food intake was unaffected (Flint, Raben et al. 2001). In overweight subjects with Type 2 diabetes mellitus, intravenous infusion of GLP-1 (1.5 pmol/kg/min) increased fullness ($P = 0.028$), reduced hunger ($P = 0.026$) and reduced food intake by 27% ($P = 0.034$) compared with a saline infusion (Gutzwiller, Drewe et al. 1999).

Little is known about the effects of GLP-1 on appetite sensations and food intake in older men and women. No difference in plasma GLP-1 concentrations between young and older subjects after either an overnight fast or intraduodenal infusions of lipid and glucose were identified in a study conducted by this department (MacIntosh, Andrews et al. 1999). More

research is required to investigate the effect of GLP-1 on regulating appetite and food intake in older subjects and to determine the impact of this peptide hormone on the anorexia of ageing.

2.4.3 Ghrelin

Ghrelin, a 28 amino-acid peptide hormone, is produced mainly by enteroendocrine cells within the mucosal epithelial layer of the stomach (Tschop, Smiley et al. 2000), as well as at other sites including the hypothalamus. It stimulates feeding and growth hormone release (Kojima, Hosoda et al. 1999; Hosoda, Kojima et al. 2000; Takaya, Ariyasu et al. 2000; Tschop, Smiley et al. 2000). Growth hormone is the most abundant hormone produced by the anterior pituitary and is secreted throughout life in healthy humans. It's primary action is to stimulate growth in childhood, however, GH also exerts metabolic effects, unrelated to growth, on the liver, adipose tissue, and muscle, such as conservation of carbohydrates and mobilization of fat stores (Camina, Carreira et al. 2003; Spiliotis 2003).

Ghrelin acts through the growth hormone secretagogue receptor (GHS-R), G-protein-coupled receptors, for which natural ligands until recently were unknown (Kojima, Hosoda et al. 1999; Date, Kojima et al. 2000). Kojima et al (1999) first isolated ghrelin from rat stomach and identified it as an endogenous ligand specific for GHS-R. This ligand was given its name from the Proto-Indo-European word of 'ghre', which means grow, and 'relin' as it had GH-releasing activities (Kojima, Hosoda et al. 1999). Although produced mainly in the stomach, ghrelin is expressed in many other organs such as bowel, kidney, heart, pancreas and testis. In addition to the involvement of ghrelin in the regulation of GH secretion, it has other effects such as gastric motility and acid secretion control, influence on endocrine pancreatic function, glucose metabolism, cardiovascular activity and antiproliferative effects in neoplastic thyroid, mammary and lung cell lines (Muccioli, Tschop et al. 2002; Volante, Allia et al. 2002; De Ambrogi, Volpe et al. 2003).

Ghrelin plays a role in energy balance by stimulating appetite, thereby increasing food intake and enhancing weight gain and fat mass deposition. There is evidence from animal studies that intracerebroventricular (ICV) and intraperitoneal (i.p) ghrelin administration dose-dependently stimulates food intake, which in the longer term is associated with weight gain (Wren, Small et al. 2000; Wren, Small et al. 2001). These results are supported by studies in both healthy people (Wren, Seal et al. 2001) and in cancer patients who report loss of appetite (Neary, Small et al. 2004). Infusions of ghrelin increased food intake, compared with a saline infusion in both groups of subjects. This finding was associated with higher levels of meal appreciation (Neary, Small et al. 2004) and increased hunger scores (Wren, Seal et al. 2001), assessed by visual analogue scales, suggesting ghrelin may be an effective treatment for loss of appetite. A possible mechanism for the increased food intake may be due to a more rapid emptying of gastric contents, however, evidence of accelerated gastric emptying following ghrelin infusion in mice (Dornonville de la Cour, Lindstrom et al. 2004) but not humans (Wren, Seal et al. 2001) requires further examination.

Circulating ghrelin concentrations increase with fasting and with diet-induced weight loss in obese subjects (Cummings, Weigle et al. 2002), and are elevated in underweight, undernourished young (anorexia nervosa (Cummings, Weigle et al. 2002; Rigamonti, Pincelli et al. 2002)) and older subjects (Sturm, MacIntosh et al. 2003). This is probably due to a combination of reduced body weight, as ghrelin is negatively correlated with body mass index (BMI) (Haqq, Farooqi et al. 2003; Purnell, Weigle et al. 2003; Tanaka, Naruo et al. 2003; Tritos, Kokkinos et al. 2003) and energy intake (Rigamonti, Pincelli et al. 2002; Tolle, Kadem et al. 2003). In contrast, circulating concentrations are suppressed following food intake, particularly fat and carbohydrate (Monteleone, Bencivenga et al. 2003), and in positive energy states such as obesity (Marzullo, Verti et al. 2004). The fall in ghrelin concentrations following food intake may be unaffected (Nakai, Hosoda et al. 2003) or impaired in undernourished (Nedvidkova, Krykorkova et al. 2003) and obese people

(Cummings, Weigle et al. 2002; English, Ghatei et al. 2002). These changes are consistent with compensatory responses to, rather than causes of, these altered nutritional states. However, there is some evidence of a ghrelin-gene mutation (affecting the mature ghrelin product) in obese persons affecting both fasting and postprandial ghrelin concentrations thereby playing a role in the aetiology of obesity (Ukkola, Ravussin et al. 2001). It remains unclear if reduced ghrelin activity contributes significantly to the anorexia and weight loss in markedly under-nourished older subjects. The effects of ageing and under-nutrition on sensitivity to ghrelin have not been reported, and ghrelin resistance may occur in these states.

In human studies, ghrelin concentrations have been shown to be dependent on nutrient selection (Erdmann, Lippel et al. 2003; Monteleone, Bencivenga et al. 2003). There is evidence that fat and carbohydrate ingestion may reduce ghrelin concentrations, while the role of protein has not been clarified. For example, circulating ghrelin concentrations were significantly reduced following consumption of carbohydrate compared with fat in young healthy women. There was a greater suppression of hunger by the carbohydrate meal, and plasma ghrelin changes were positively associated with hunger changes (Monteleone, Bencivenga et al. 2003). Supporting these findings, Greenman et al (2004), observed that following a 75g oral glucose challenge ghrelin concentrations were suppressed from baseline levels of 538 ± 107 pg/ml to 328 ± 38 pg/ml after 120 minutes ($P < 0.0001$). Obesity affected the ghrelin response to glucose over time such that levels started to return to baseline levels at 30 minutes in obese and 120 minutes in lean subjects ($P = 0.02$). In addition, plasma ghrelin concentrations were consistently higher in women than men ($P = 0.03$). Similarly, ghrelin concentrations declined following a 150g/400kcal lipid load from a baseline of 728 ± 205 pg/ml to 533 ± 114 pg/ml at 120 minutes ($P = 0.04$). Protein exerted no effect on ghrelin concentrations (Greenman, Golani et al. 2004).

There is also evidence that the time course of ghrelin suppression following food intake, i.e. how long it takes for the maximum suppression to occur, differs depending on the macronutrient. For example, ghrelin concentrations were measured in young healthy subjects fed, on separate days, an oral glucose drink, and each of a solid carbohydrate, high fat and high protein meal. Carbohydrate and fat decreased, and protein increased, plasma ghrelin concentrations. After the solid carbohydrate-rich test meal ghrelin concentrations fell from 559 ± 59.3 pg/ml to a nadir of 449 ± 47.4 pg/ml within 60 minutes ($p < 0.05$). Following an oral glucose load (75 g in 300 ml water), a similar decrease was observed ($p < 0.05$). A fat-rich meal also decreased plasma ghrelin concentrations ($p < 0.05$), but more slowly with a nadir towards the end of the study period at 180 minutes (Erdmann, Lippel et al. 2003).

Findings from a recent study, in which rats received intragastric infusions of 25% glucose (infused at 1 ml/min) or water (12 ml), with gastric emptying proceeding either normally or prevented by inflation of a pyloric cuff, suggest that the suppression of plasma ghrelin concentrations after nutrient ingestion is dependent on the exposure of the nutrient with the small intestine and not the stomach (Williams, Cummings et al. 2003). When normal gastric emptying was permitted, glucose infusions, compared with water, reduced ghrelin concentrations by approximately 50% ($P < 0.05$) at 32 minutes post infusion. An equivalent nutrient infusion increased ghrelin concentrations, compared with baseline, when the pylorus was closed but this effect was not significant, indicating that gastric chemosensation is not a sufficient trigger for the ghrelin response (Williams, Cummings et al. 2003). In support, an earlier study in rats, in which ghrelin concentrations were measured following intravenous glucose infusion, concluded that neither a rise in blood glucose nor the presence of nutrients in the stomach contribute to meal related ghrelin inhibition (McCowen, Maykel et al. 2002). The effect of small intestinal and gastric exposure to glucose on ghrelin suppression in human subjects is discussed in Chapter 7.

The effect of healthy ageing on circulating ghrelin concentrations has not yet been clarified, despite some evidence of an age-related decline in plasma ghrelin concentrations (Rigamonti, Pincelli et al. 2002; Sturm, MacIntosh et al. 2003). Two small studies have reported circulating ghrelin concentrations 20% (Sturm, MacIntosh et al. 2003) and 35% (Rigamonti, Pincelli et al. 2002) lower in healthy older than young adults, the latter reduction statistically significant. However, increasing body fat is associated with decreasing ghrelin concentrations, and the older subjects had higher body mass indices (BMIs) than the young subjects in both studies. Neither study included detailed body composition analysis, so the higher ghrelin levels in older subjects may have been due to differences in body composition rather than increasing age itself.

Evidence is conflicting regarding ghrelin and body mass index. Purnell et al, (2003) found no relationship of ghrelin to components of body mass i.e. percentage body fat, total fat mass, abdominal fat mass or lean mass and concluded that ghrelin concentrations best reflect body weight. In addition, they found a positive correlation with ghrelin and age but no differences between men and women (Purnell, Weigle et al. 2003). Greenman et al (2004), found inverse relationships between ghrelin concentrations and body mass index ($r = -0.047$, $P = 0.02$), waist circumference ($r = -0.58$, $P = 0.003$) and waist/hip ratio ($r = -0.56$, $P = 0.046$), but no relationship between age and ghrelin concentrations in 24 subjects (age range 26-74 years) following an oral glucose (75g), lipid (150g cream/ 400 kcal) or protein load (200g low fat chicken breast). Ghrelin concentrations were suppressed by both the glucose and lipid loads and this response was modulated by gender in that females exhibited higher concentrations following the glucose ($P < 0.0001$) and lipid ($P < 0.03$) loads (Greenman, Golani et al. 2004). Further studies involving body composition analysis are needed to assess the effects of ageing on ghrelin.

2.4.4 Amylin

Islet amyloid polypeptide (IAPP, amylin), a neuroendocrine hormone produced by pancreatic beta cells, is co-secreted with insulin in response to food or glucose intake (Reidelberger, Arnelo et al. 2001). In animal studies, amylin has been shown to inhibit postprandial glucagon secretion, slow gastric emptying and reduce food intake (Arnelo, Blevins et al. 1996; Bhavsar, Watkins et al. 1998). Chronic administration in rodents has been found to result in reductions in food intake and weight loss (Arnelo, Blevins et al. 1996). When amylin and CCK-8 were co-injected into mice after an overnight fast, food intake in the subsequent 30 minutes was reduced by 91%, an amount greater than the suppression produced by either injection alone (CCK $71 \pm 7\%$, amylin $57 \pm 6\%$). Analysis revealed a marked synergy between amylin and CCK in reducing food intake, such that statistically ineffective doses of amylin and CCK, when combined, evoked near-maximal inhibition of food intake. Since amylin and CCK are both secreted in response to nutrient intake, the synergy between them could indicate a shared role in appetite control (Bhavsar, Watkins et al. 1998).

Pramlintide, a stable, soluble analogue of human amylin is currently under clinical investigation as possible treatment of Type 1 and Type 2 diabetes mellitus. No studies have examined the effect of amylin on feeding behaviour in humans although indirect evidence from studies in Type 1 and 2 diabetes mellitus suggests that it may have an effect on appetite. Pramlintide, when administered in Type 2 diabetes mellitus produces improvements in glycaemic control that are accompanied by a significant reduction in weight (Ratner, Want et al. 2002). This finding, and the delayed gastric emptying compared with placebo seen in Type 1 diabetes (Kong, Stubbs et al. 1998), suggests that Pramlintide may decrease appetite but currently there is no evidence that amylin suppresses food intake in humans. There is limited evidence that amylin secretion or effect is altered by ageing in either animal (Morley, Morley et al. 1993) or human studies (Mitsukawa, Takemura et al.

1992; Edwards, Perry et al. 1996). Intraperitoneal administration of amylin has been shown to be slightly more potent in suppressing food intake in young than older rats (Morley, Morley et al. 1993) but this effect was not significant. In humans, basal plasma amylin concentrations, and those recorded following a 75g oral glucose load, were not different between older and young adults (Mitsukawa, Takemura et al. 1992; Edwards, Perry et al. 1996).

2.4.5 Other pancreatic and gastrointestinal satiety hormones

In addition to CCK there are a number of other peptides that act to decrease food intake (Table 2.1). Gastrin releasing peptide (GRP) is a peptide released from the antrum of the stomach that has been shown to decrease food intake in animal and human studies (Stein and Woods 1982; Gutzwiller, Drewe et al. 1994; Lieverse, Jansen et al. 1995). In young men, intravenous infusions of GRP at concentrations that produce physiological effects (such as stimulation of acid or gallbladder contraction) have been shown to decrease food intake, compared to saline infusions (Gutzwiller, Drewe et al. 1994). Peptide YY is a 36 amino acid peptide synthesized and secreted from the L cells of the gastrointestinal tract and released in response to food intake (Batterham and Bloom 2003; McGowan and Bloom 2004). Postprandial concentrations are affected by macronutrient composition such that higher concentrations are seen following fat ingestion than CHO or protein (Adrian, Ferri et al. 1985; Lin and Chey 2003). PYY infusion reduces food intake in animal studies (Batterham, Cowley et al. 2002; Challis, Pinnock et al. 2003) and in normal weight human subjects (Batterham, Cowley et al. 2002). This effect is not altered by obesity even though endogenous concentrations are reduced compared with lean subjects (Batterham, Cohen et al. 2003). Other peptides that play a role in inhibition of food intake include apolipoprotein A-IV, a glycoprotein secreted by the small intestine (Tso, Liu et al. 2001) and somatostatin, a peptide closely related to GRP (Lotter, Krinsky et al. 1981). There are no studies evaluating the effects of these satiety hormones on appetite and food intake in older people.

Table 2.1 Gastrointestinal peptides that may influence food intake (Taken from Woods 2004)

Reduce food intake	Increase food intake
CCK	Ghrelin
Bombesin family-bombesin, GRP and neuromedin B	
Glucagon	
Glucagon-like peptide-1	
Glucagon-like peptide-2	
Apolipoprotein A-IV	
Amylin	
Somatostatin	
Enterostatin	
Peptide YY (3-36)	

The release of CCK and other gastrointestinal hormones such as GLP-1 and amylin and the suppression of ghrelin in response to small intestinal nutrients may contribute to the reduction in appetite and food intake observed in older people. However, the effect these hormones and the contribution, if any, of antagonists to these hormones on satiety and food intake in older subjects has not been established. Similarly, the contribution of these mechanisms to the ‘physiological’ anorexia associated with ageing is unclear.

2.5 Differential responses to macronutrients

2.5.1 Effect of oral preloads on appetite and food intake

This thesis will consider the differential effect of fat and carbohydrate on appetite and food intake in both young and older people. Together these two macronutrients comprise more than 70% of the energy consumed by most people. Whilst the evidence for a greater satiating effect of protein compared to fat and carbohydrate (CHO) is well documented (Poppitt, McCormack et al. 1998; Baba, Sawaya et al. 1999; Latner and Schwartz 1999; Skov, Toubro et al. 1999) there appears to be little consensus regarding the differential effects of fat and carbohydrate on appetite and food intake.

In studies conducted in young normal weight or obese volunteers, CHO has been shown to be as satiating if not more so than fat. For example, evidence of a similar effect of CHO and fat on food intake is provided by the following study in which appetite and food intake were measured 5 hours after different macronutrient breakfasts in young normal weight men ($n = 10$) and women ($n = 10$). Breakfast meals were of similar energy density and fibre content but rich in either protein (32% of energy), fat (65% of energy), CHO (65% of energy) or alcohol (23% of energy). Men ate significantly more than women at each meal ($P < 0.0001$) but there were no significant differences in between macronutrient meals in food intake or ratings of hunger in either sex (Raben, Agerholm-Larsen et al. 2003). In another study, high fat (58% E, 234 kcal) high CHO (84% E, 240 kcal) or high protein (77% E, 237 kcal) snacks were offered to young normal weight men 240 minutes after a standard lunch. An *ad libitum* buffet dinner meal was provided on request. The high CHO snack was significantly more palatable ($P < 0.03$) compared to fat and protein however, no difference was found for food intake between treatments. In addition, there was no effect of macronutrient type on ratings of hunger (Marmonier, Chapelot et al. 2000).

Similar findings were reported in a study in which healthy young men were fed 714 kcal yoghurts high in either fat (HF), CHO (HCHO) or protein (HP) or no preload and then allowed to eat *ad libitum* (from foods provided in the clinic setting) for the remainder of the day (7 hours). Food intake was suppressed by the HP ($29 \pm 10\%$), HF ($20 \pm 9\%$) and HCHO ($17 \pm 6\%$) compared with no preload ($P = 0.01$), without a difference between CHO and fat, and hunger decreased irrespective of the macronutrient composition (effect of time, $P < 0.01$) (Vozzo, Wittert et al. 2003). Rolls et al, 1991 gave equivoalaemic yoghurts high in CHO (81% CHO) and fat (65% fat), and containing similar levels of protein, to normal weight young men ($n = 14$) and women ($n = 14$). A self-selection meal was served at three different times (30 min, 90 min and 180 min) following the preloads. There was no significant difference between the preloads for food intake regardless of the time the meal was served in either men or women (Rolls, Kim et al. 1991).

There is some evidence for a greater satiating effect of CHO compared to fat in young people. Blundell et al (1993) studied sixteen lean, healthy young men who consumed equienergetic (803 kcal) breakfasts supplemented with fat or CHO or a breakfast without supplementation (control, 440 kcal). Subjects were blind to the additional supplementation. Food intake was quantified at an *ad libitum* meal (sandwiches) presented 90 minutes later and appetite ratings assessed. The CHO-supplemented breakfast suppressed ratings of hunger and increased ratings of fullness compared with the fat-supplemented breakfast ($P = 0.024$). Food intake after the CHO-supplemented breakfast was 31% (235 kcal) lower than the fat-supplemented breakfast ($P = 0.02$) (Blundell, Burley et al. 1993).

This finding is supported by a study in which young obese women ($n = 13$) were offered either a high fat or high carbohydrate lunch and high fat or high carbohydrate snack (whole foods) on four separate days. The participants were not informed to the exact nature of the dietary manipulation. When offered the high CHO foods subjects ate less (628 ± 167 sd vs 1051 ± 298 sd, $P < 0.001$) but there was no effect on VAS ratings of hunger (Green, Wales et al. 2000). In another study, 14 young normal weight subjects were fed one of four equienergetic breakfasts (high fat \pm fibre, high CHO \pm fibre; 485 kcal). Food intake and appetite ratings were assessed for the remainder of the day. The high CHO plus fibre breakfast was less palatable and reduced food intake significantly at lunch ($P < 0.05$). Both fat meals were more palatable but less satiating than CHO meals and resulted in increased food intake. The average total daily intake was greater after the fat meal than after the high fibre CHO meal ($P < 0.05$) (Holt, Delargy et al. 1999).

2.5.2 Ageing and macronutrient choice

It is unclear from cross-sectional and longitudinal studies if macronutrient consumption changes with age, despite many studies reporting alterations in vitamin and mineral intake in older people (Posner, Smigelski et al. 1987; Ryan, Craig et al. 1992; Amorim Cruz, Moreiras et al. 1996; Nydhal, Andersson et al. 2003). Older people have been reported to eat a greater percentage of daily energy as carbohydrate and less as fat (Elahi, Elahi et al. 1983; Hallfrisch, Muller et al. 1990; De Castro 1993; Morley 1997), whilst some studies

report little or no change in the percentage of daily energy from carbohydrate, but report decreases in fat intake and increases in protein intake (Garry, Hunt et al. 1992; Fernyhough, Horwath et al. 1999) or reductions in all nutrients (Sjogren, Osterberg et al. 1994).

The relative satiating effects of different macronutrients may change with age in oral preload studies. For example, in one study (Wilson, Purushothaman et al. 2002) food intake and appetite ratings of 15 older (78 ± 2 yrs) and 15 young (29 ± 1.5 yrs) volunteers were measured after four oral liquid preloads consisting of either a high fat, high carbohydrate or high protein blend. The preloads were administered within either 5 or ≥ 60 minutes (on request from the volunteer) of a test meal. Mean energy consumption of the test meals was significantly lower in the older than in the younger subjects ($P = 0.001$), as was mean absolute macronutrient consumption (kJ) of fat ($P = 0.002$) and CHO ($P = 0.001$). There was no difference in ratings of hunger or fullness but the interval between the preload and the request for the test meal was longer in the older than young subjects after the high protein and high fat preloads than high CHO preloads ($P = 0.001$) (Wilson, Purushothaman et al. 2002).

In another study (Ryan, Salle et al. 2004) undernourished ($BMI = 20.3 \pm 3 \text{ kg/m}^2$) hospitalised older people (77 ± 8 yrs) were offered isoenergetic (250 kcal) high fat or high CHO supplements or no supplement (control) with breakfast for three consecutive days. Mean food intake (including supplement energy) significantly increased following nutrient supplementation compared to control but there was no difference in food intake following the different nutrient supplements (high fat 1660 kcal/d, high CHO 1644/d vs control 1447 kcal/d, $P = 0.004$). Compared to controls, supplemented subjects experienced reduced hunger ($P = 0.07$) but there was no difference between appetite ratings in the fat and CHO groups (Ryan, Salle et al. 2004). Rolls et al (1995) compared the effects of yoghurt preloads differing in macronutrient content (high fat, high energy, 500 kcal; high CHO, high energy, 510 kcal or no preload) on food intake in 16 older (68.9 ± 1.6 years) and 16 young ($24.3 \pm$

1.2 years) men. Compensation for energy consumed in the preloads was less precise in the older men but there were no differences in food intake between the age groups for high fat and high CHO yoghurts at a buffet meal given 30 minutes later (Rolls, Dimeo et al. 1995).

Overall these results suggest that older people eat less and are less likely to be able to compensate at a subsequent meal for energy consumed in a preload than young people. However, the same inconsistencies in results from studies evaluating the effects of macronutrients i.e. fat and carbohydrate on food intake that occurs in young subjects are also present in older subjects suggesting that age is not a factor.

2.5.3 Effect of intragastric and intraduodenal macronutrient infusion

Studies in which young adult subjects have been given gastric or intestinal fat and CHO infusion have also failed to yield consistent results about the relative satiating effects of these macronutrients. For example, Cecil et al, (1998) infused 500ml/1000kcal (2 kcal/ml) intragastric preloads of either fat (Intralipid) or CHO (53.4% glucose) to healthy young men over 15 minutes. At a test meal provided 90 minutes following the completion of the infusions, there was no difference in food intake between treatments (fat, 755 kcal vs CHO, 737 kcal, $P = 0.93$). In addition there were no differences in ratings of hunger and fullness between treatments ($P = 0.07$) (Cecil, Castiglione et al. 1998).

Alternatively, there is evidence from both intragastric and intraduodenal infusion studies that fat and CHO may exert differing effects on food intake and appetite in older and young subjects. In a single-blind study, 6 young men received 250ml/500kcal intragastric infusions of fat (20% Intralipid), CHO (50% dextrose) or saline administered over either 15 minutes (rapid) or 210 minutes (slow) (Shide, Caballero et al. 1995). Following the rapid infusion food intake was non-significantly less after the CHO than fat (964 ± 166 vs 1142 ± 185 , $P > 0.05$). However, following the slow infusion food intake was less after the fat than CHO infusion (1245 ± 82 vs 1409 ± 150 , $P < 0.05$). There was no difference between the

two infusions on ratings of hunger or fullness (Shide, Caballero et al. 1995). These results (Shide, Caballero et al. 1995) suggest that duodenal exposure to nutrients together with the distension provided by the rapid infusion may affect food intake from CHO, whilst the interaction of fat with duodenal receptors in the absence of gastric distension (as in the slow infusions), may be more important in suppressing food intake due to fat.

The result from the slow infusion is consistent with those reported in a previous intraduodenal study (MacIntosh, Horowitz et al. 2001) in which intraduodenal infusion of 343kcal of fat, in the form of a triglyceride emulsion (Intralipid), suppressed the perception of hunger and food intake at a subsequent test meal more than an isoenergetic infusion of carbohydrate (glucose) (Cook, Andrews et al. 1997; Chapman, Goble et al. 1999; MacIntosh, Horowitz et al. 2001).

In that study, 13 young (23.7 ± 1.4 years) and 13 older men (72.1 ± 1.6 years) received equi energetic (343 kcal), intraduodenal (ID) infusions of fat (10% Intralipid) and glucose (25% glucose) or saline (control) over 120 minutes (MacIntosh, Horowitz et al. 2001). A buffet meal was offered at the end of the infusion and subjects were invited to eat for 30 minutes. Food intake and VAS scores of appetite were measured. Fat inhibited food intake non-significantly more than the carbohydrate infusion (24 vs 13% $P = 0.3$), and significantly more in the young men (26 vs 5% $P < 0.001$). In contrast, the suppressive effects of fat and glucose were similar in older men (21 vs 22% $P = 0.5$), suggesting an increased satiating effect of ID carbohydrates in the elderly (MacIntosh, Horowitz et al. 2001). This latter observation suggests that an enhanced satiating effect of carbohydrate may contribute to the anorexia of ageing.

Orosensory factors, such as palatability, have been shown to be important in determining the appetite and food intake response to different macronutrients (Geliebter 1979; Green, Wales et al. 2000). The discrepancies observed between intraduodenal, intragastric and oral administration of nutrients may reflect the fact that direct infusion of nutrients into the

duodenum bypasses these prepyloric mechanisms that influence the satiating effects of intraduodenal carbohydrate or lipid (Chapman, Goble et al. 1999). It remains unclear whether the gastrointestinal satiety response to different macronutrients does change with age. Further investigation is required and the differential effect of intragastric infusion of CHO and fat is discussed in Chapter 6.

2.5.4 Fatty acids

Fat is a major constituent of the Western diet, comprising 20-50% of energy intake for most people. Its primary function is to supply energy (Robinson 1972). Most dietary fats are triglycerides. Their digestive products, free fatty acids (FFA), appear to be the critical stimulus for many physiologic responses to fat ingestion that arise from the small intestine, including the suppression of hunger and food intake and the stimulation of fullness and CCK release (Masclee, Jansen et al. 1989; Feinle, O'Donovan et al. 2003). Most fatty acids in foods and in the body have straight, even-numbered carbon chains. Short-chain fatty acids contain 4 to 6 carbon atoms, medium-chain fatty acids contain 8 to 12 carbon atoms, and long-chain fatty acids contain more than 12 carbon atoms (C12) (Robinson 1972).

There is now persuasive evidence that the gastrointestinal response to ingested fatty acids varies with the length of the carbon chain. In an early study, Hunt and Knox (1968) demonstrated a relationship between gastric emptying rate and fatty acid chain length in young adults. Gastric emptying was determined by aspiration of stomach contents 20-30 minutes following intragastric administration of an 1 litre aqueous solution containing either short (< 12 carbon atoms) or long chain fatty (> 12 carbon atoms) acids. The solution containing fatty acids > 12 carbon atoms was approximately 4 times more effective at delaying gastric emptying than solutions with < 10 carbon atoms (Hunt and Knox 1968). In addition, Meyer et al, (1998) have demonstrated that intraduodenal infusion of C12 and C18 (long chain), but not C8 or C10 (short chain), fatty acids suppressed sham feeding in rats (Meyer, Hlinka et al. 1998).

In humans also, there is evidence that the satiating effect of fatty acids increases at a chain length of C12 or greater. In young adults, duodenal infusions of long chain (C18) but not short chain, fatty acids reduce subsequent food intake (Matzinger, Degen et al. 2000) whilst an intraduodenal C12 emulsion with gastric distension (using a barostat device) reduced hunger more and increased fullness more on hunger is decreased and on fullness than an equal energy and volume emulsion consisting of fatty acids with chain length of <12 C (Feinle, Rades et al. 2001). It has been recently demonstrated that intraduodenal infusion of C12 in young men suppresses food intake and hunger more than equienergetic C10 (Feltrin, Little et al. 2004). In addition, although both C10 and C12 stimulated release of CCK – the latter finding consistent with previous animal and human data (McLaughlin, Lomax et al. 1998; Meyer, Hlinka et al. 1998; French, Conlon et al. 2000; Lawton, Delargy et al. 2000) - the response was greater during the C12 infusion (Feltrin, Little et al. 2004). This suggests a relationship between fatty acid-induced CCK release and food suppression.

Available evidence suggests an increase in circulating fatty acid concentrations with normal ageing in humans (Bonadonna, Groop et al. 1994; Kuriki, Nagaya et al. 2002), particularly in association with increased fat stores and inactivity. This increase may therefore contribute to the increase in circulating CCK concentrations and decreased appetite that accompany normal ageing. To our knowledge, no studies have compared the effects of oral administration of fatty acids on appetite and food intake in older or young subjects.

2.6 Conclusion

Physiological changes in gastro-intestinal function that occur with ageing may have a significant impact on appetite and food intake in older people. When these factors are combined with adverse social and psychological aspects of ageing many of this group are at risk of developing sarcopenia and protein-energy malnutrition. Further understanding of the mechanisms responsible for a decrease in appetite with age is warranted.

Chapter 3

Common methodologies

3.1 Introduction

This chapter will discuss the methods that are common to the studies presented in this thesis. These procedures have been used in previous studies both in our department and by other groups, and are accepted methods of assessing appetite and eating behaviour. In addition, screening instruments, biochemical analyses, assessment of gastrointestinal hormone concentrations and statistical analyses will be examined. Where a new technique has been established (i.e. analysis of plasma ghrelin concentrations, Chapter 7), this will be reported in detail in the relevant chapter.

3.2 Subjects

Healthy young (age range 18-35 years) men (Chapters 5 and 6) and older (age range 65-85 years) men and women (Chapters 4–8) were recruited by advertisements placed in the hospital and university environs and, where necessary in the local newspapers. Comparisons were made between older and young subjects in some studies (Chapters 4, 5 and 6) in order to determine age-related changes in outcome variables. Older men and women were used (Chapters 4 and 7) in order to determine differences in gender in outcome variables in this age group. Prior to participation each subject was assessed for 'dietary restraint' using Factor 1 of the Three-Factor Eating Questionnaire (Stunkard and Messick 1985). Subjects who recorded a score of >11 , indicating they were restrained eaters (unlikely to eat freely), were excluded. Older subjects were assessed for cognitive ability and depression using the Standardised Mini-Mental State Examination (score <25 excluded) (Folstein, Folstein et al. 1975; Molloy, Alemayehu et al. 1991) and Geriatric Depression Scale (score >13 excluded) (Yesavage 1988), respectively. Young subjects were assessed for depression based on a self-reported history only. Subjects were excluded if (i)

they had significant gastrointestinal symptoms or known history of upper gastrointestinal disease, recurrent peptic ulcer, oesophageal or gastric surgery, (ii) were currently taking drugs which alter gastrointestinal motor function, body weight or appetite (e.g. prokinetic agents, gastric antisecretory, anti-cholinergic, anti-histamine or antidepressant medications), (iii) have a history of disease known to affect gastric motility, e.g. diabetes mellitus, connective tissue disease, neuroendocrine tumours, (iv) the investigator was unable to locate gastric antrum on ultrasound at screening visit (Chapter 5, 6 and 8), or (v) a smoker (> 10 cigarettes/day) and alcohol consumption of > 20g/day.

Height and weight was measured for each subject and all subjects were within the range 20-28 kg/m² for body mass index (BMI) and were not more than 5% below their ideal body weight for height (MacIntosh, Horowitz et al. 2001). When medication was administered as part of the treatment (Chapter 8), or as a safety measure to ensure the health of the older subjects (Chapters 5 and 6), blood samples were undertaken to test for abnormalities in thyroid stimulating hormone (TSH) (range < 0.35mU/L or > 3mU/L), fasting plasma glucose (>7 mmol/L), serum creatinine (> 0.12 mmol/L) and serum ALT or AST (greater than 3 times upper limit of normal). Abnormal results excluded the subjects from participation in the studies and a follow-up medical examination was arranged with the subject's own medical practitioner. A dietary history was obtained and subjects completed a food diary prior to participation to determine their usual intake. Subjects were asked to refrain from alcohol for 24 hours prior to each study day and to maintain their usual physical activity during the study. Written, informed consent was obtained from every subject prior to participation in studies. All subjects were informed of their right to withdraw from studies at any time. Subjects were offered an honorarium for their participation.

3.2.1 Geriatric Depression Scale (Appendix I)

In each study, older subjects were assessed at screening for depression using the Geriatric Depression Scale (GDS) (Yesavage 1988). The GDS consists of 30 questions designed to assess the characteristics of depression in elderly people living independently. It was originally developed and validated using the criteria of researchers and clinicians involved in geriatric psychiatry and gerontology (Yesavage, Brink et al. 1982). The sensitivity and specificity of the GDS was evaluated in a study with two samples of elderly people; 60 subjects complaining of depression, and 40 reportedly not affected by depression (Yesavage, Brink et al. 1982). A score of 11 (out of 30) correctly classified 84% of depressed elderly people (sensitivity) and 95% of those not affected by depression (specificity). At a cut-off score of 14, the sensitivity was 80%, and specificity was 100%. Accordingly, a score of 0-10 is considered normal and 11 or more as a possible indicator of depression (Montorio and Izal 1996). The GDS has subsequently been validated against the Zung Self Rating Scale for Depression (Zung 1965) and the Hamilton Depression Rating Scale (Hamilton 1967) and significant correlations found between the classification criteria for depression ('no', 'mild' and 'severe') and each of the three scales (Montorio and Izal 1996).

3.2.2 Standardised Mini-Mental State Examination (Appendix II)

The Mini-Mental State Examination was developed to assess cognitive function in the clinical setting and is widely used (Tombaugh and McIntyre 1992). The tasks focus on broad cognitive aspects of mental functioning such as, orientation, registration, calculation, attention and memory. A number of factors can influence the score on the MMSE including education, cultural environment and sensory impairments (Folstein, Folstein et al. 1975), however high validity and reliability values were found when compared to standard psychological scales (Burns, Bergmann et al. 1998). The MMSE has been modified by either extending or reducing the number of questions. A standardised version imposed strict

guidelines for administration and scoring to improve the reliability of this tool (Molloy, Alemayehu et al. 1991). This test, used in the screening process in this thesis, can take as little as 5 minutes to perform with a cognitively intact subject. A score of 24-30 indicates no cognitive impairment; 20-23 mild cognitive impairment or dementia; 10-19 moderate dementia; and 0-9 severe dementia (Molloy, Alemayehu et al. 1991).

3.3 Ethics approval

All protocols were approved by the Royal Adelaide Hospital Research Ethics Committee (Chapters 4-8) and the Royal Adelaide Hospital Investigational Drug Sub-committee (Chapter 8), prior to recruitment of subjects.

3.4 Study environment

All studies presented in this thesis were conducted in clinical research study rooms in the University of Adelaide, Department of Medicine at the Royal Adelaide Hospital. These rooms were designed to enable each aspect of the study to be conducted within the room and subject's only left the room to use the toilet. Therefore, subjects were isolated from stimulation from the external environment. During studies, subjects were allowed to read (but not about food or related topics), listen to the radio or pre-recorded music, study or do crafts. Where applicable, subjects were allowed to move about the room freely (Chapter 4). To allow for correct placement of the catheter in intraduodenal intubation studies (Chapter 7) subjects were kept in the supine position, or the head of the bed was elevated to 30° for comfort. For intragastric intubation studies (Chapters 6 and 7) subjects were seated at $\approx 90^\circ$ during intubation and for the duration of the infusions. To facilitate accurate ultrasound images (Chapters 5, 6 and 8) subjects were seated at $\approx 90^\circ$ at all times. During timed preloads and buffet meals subjects were not allowed to read, converse with investigators or listen to the radio. To reproduce normal eating arrangements subjects were seated at a table for meals.

3.5 Preload preparation and administration

3.5.1 Yoghurt preloads

Liquid and solid meals empty at different rates from the stomach (Collins, Houghton et al. 1991). Preloads derived from pure nutrients, such as glucose and some forms of fat, are not typical of normal meals and usually empty more rapidly from the stomach (de Graaf, Hulshof et al. 1992; Chapman, Goble et al. 1999). For this purpose the yoghurt preloads used in this thesis (Chapters 5 and 8) were administered as a liquid (resembling a milkshake or ‘smoothie’), in order to facilitate emptying from the stomach during the time between the preload and subsequent meal. In addition, liquids allow for clearer ultrasound images of the gastric antrum.

In order that subjects would be unable to distinguish between the energy content of the yoghurt preloads (Chapter 5) they were designed so that all sensory characteristics, that is sight, smell, taste and texture, would be similar. The low-dose (250 kcal) and high-dose (750 kcal) preload compositions are detailed in Table 5.1. The preload in Chapter 8 (500 kcal) was made using double the low-dose preload ingredients. The composition of this preload is detailed in Table 8.1.

The preloads were prepared in the research kitchen using commercially available ingredients. Each yoghurt contained plain low (Traditional Natural Yoghurt, Australia Co-operative foods Ltd, Lidcombe NSW, Australia) and high (Yoplait Greek Style, Australia Co-operative foods Ltd, Lidcombe NSW, Australia) fat yoghurts as the base ingredient. Cornflour (White Wings Foods, Macquarie Park, NSW, Australia) and glucose powder (Glucodin energy powder, Boots healthcare, North Ryde, NSW, Australia) were used as the carbohydrate source. Sunflower oil (Meadow Lea Foods td, Mascot, NSW, Australia) and cream were used as the fat source. Pro-mod supplement (Ross Products Division, Abbott Laboratories, Ohio, USA) was used to supply protein and gelatine (Davis Gelatine Australia Pty Ltd, Qld, Australia) to ensure a similar consistency between the two preloads. All

yoghurts were made fresh on the morning of the study and served within 5 minutes of removal from storage at 4°C.

3.5.2 Triglyceride and glucose solutions

Commercially available Intralipid® 10% (Kabi Pharmacia Ltd., Milton Keynes, UK) containing 10% triglycerides as fractionated soybean oil (50g/500ml), 1.2g fractionated egg phospholipids and 2.25g glyceryl anhydrous, was used as the fat infusate in the study described in Chapter 6. In addition, 25% glucose (Baxter Healthcare, Qld, Australia) was used as the carbohydrate infusate in the studies described in Chapters 6 and 7.

3.6 Techniques

3.6.1 Assessment of food intake and appetite

3.6.1.1 Three factor eating restraint questionnaire (Appendix III)

Studies presented in this thesis evaluated appetite sensations, such as hunger, fullness and desire to eat, and food intake in response to a specific intervention. It was important that the intervention provided the only stimulus affecting the amount of food eaten at a subsequent test meal. All subjects were therefore assessed for ‘normal’ eating behaviour. Subjects were assessed for dietary restraint, and required to be an ‘unrestrained eater’ (i.e. when presented with food, if hungry, this person is likely to eat freely). In all studies subjects were assessed for ‘dietary restraint’ using the Three-Factor Eating Questionnaire (Stunkard and Messick 1985). This questionnaire is still commonly used to measure ‘abnormal’ eating behaviour.

The Three-Factor Eating Questionnaire (TFEQ) was based on the 10-item Restraint Scale (Herman and Mack 1975) and the Latent Obesity Questionnaire (Pudel, Metzдорff et al. 1975). It consists of 51 questions relating to eating and dietary behaviour and was devised to measure three factors related to eating habits; Factor 1 ‘cognitive restraint of eating’, Factor 2 ‘disinhibition’ and Factor 3 ‘hunger’. The reproducibility of this scale was assessed

in ‘unrestrained eaters’ (n = 45) and ‘restrained eaters (dieters)’ (n = 53). The reported test-retest reliability (a measure of the capacity to provide the same measurement on separate occasions) was high (0.8 was considered acceptable) for each of the three factors; restraint (0.93), disinhibition (0.91) and hunger (0.85). The reliability of restraint measures was slightly different between the ‘dieters’ (0.79) and ‘unrestrained eaters’ (0.92) and there was an association between weight and restraint scores, such that heavier people recorded higher scores ($r = 0.2$, $P < 0.01$).

The factor measured in each study in this thesis was Factor 1 ‘cognitive restraint of eating’. This factor is defined as ‘the tendency of individuals to restrict their food intake in order to control their body weight (Herman and Mack 1975). In the original Stunkard and Messick (1985) study, mean \pm SEM Factor 1 ‘cognitive restraint of eating’ scores for ‘restrained’ and ‘unrestrained’ eaters were 14.3 ± 3.6 v 6.0 ± 5.5 ; respectively. Therefore, in all studies in this thesis subjects who recorded a score of >11 , (within the range of TFEQ ‘restrained eaters’) probably indicating they were unlikely to eat freely, were excluded. This score was also based on the cut-off scores used in previous appetite studies in healthy older and young subjects (Rolls, Kim-Harris et al. 1994; Rolls, Dimeo et al. 1995; MacIntosh, Horowitz et al. 2001).

3.6.1.2 Three-day food diary (Appendix IV)

Measurement of dietary intake is problematic and different assessment methods may lead to different results in individual subjects (Mertz, Tsui et al. 1991; Asbeck, Mast et al. 2002). Underreporting is an acknowledged source of measurement error in studies assessing food intake (Livingstone 1995; Lissner, Heitmann et al. 1998). A variety of methods that evaluate food intake have been previously validated; 24 hour recalls (Beaton, Milner et al. 1979), 1 day records (Sempos, Johnson et al. 1985) and 2 day records (Hartman, Brown et al. 1990). Both 7-day and 3-day diet records are a rigorous and widely accepted method of assessing short-term food intake (Bingham 1994; Bathalon, Tucker et al. 2000), however,

food intake recording becomes less accurate toward the end of a 7-day food diary. A 3-day record can provide a comparable estimate of dietary intake, particularly if at least one weekend day is included, and lessens the chance of inaccuracy due to declining subject compliance (Gersovitz, Madden et al. 1978). Accordingly, this method was considered the most appropriate means of determining baseline dietary information and, prior to participation in a study all subjects completed a 3-day diet diary.

Subjects were asked to complete the provided 3-day food diary over three consecutive days (including one weekend day). Detailed instructions and examples were provided so that subjects understood the need for accurate weighing and recording of all food and drink consumed over this period. Where possible, subjects were asked to provide recipes and methods of cooking. The average daily energy (kcal) and macronutrient intake was determined using Foodworks Nutritional software (Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia) and this information enabled comparison of dietary intake between older and young subjects.

3.6.1.3 Visual analogue scale

Appetite is subjective and as such is difficult to quantify. Sensations of appetite such as hunger, fullness and desire to eat are influenced by many factors including individual physiology and psychology (de Castro and Plunkett 2002), and external cues such as prior food intake, sensory stimulation from food (Rolls, Rowe et al. 1981) and social interaction during eating (de Castro 1991). The most common form of assessment in appetite studies is the visual analogue scale (VAS). Clinical research studies using VAS to assess appetite have shown highly significant correlations between these ratings and food intake in young subjects (Pi-Sunyer, Kissileff et al. 1982; Rolls, Hetherington et al. 1988; Mattes 1990). The reproducibility of VAS ratings of appetite and their ability to predict subsequent food intake has also been demonstrated in young adult subjects in previous studies [e.g. (Mattes 1990; Flint, Raben et al. 2000; Stubbs, Hughes et al. 2000)], and visual analogue scales are useful

in assessing subjective appetite sensations, such as hunger, fullness and nausea in this age group (Sepple and Read 1989).

Despite the wide-ranging use of VAS in appetite research involving adults of all ages, the reproducibility and validity of these scales to evaluate appetite had not been assessed in older subjects. The results of two studies, a retrospective combined analysis of four single-blind, randomised, controlled appetite studies with older and young subjects evaluating relationships between food intake and VAS scores of appetite and ‘non-appetite’ sensations, and a prospective study, based on and compared to a previous study in young subjects, evaluating the reproducibility and validity of VAS as a measurement of appetite in healthy older subjects are reported in Chapter 4.

In this thesis, all studies have employed VAS to assess appetite and ‘non-appetite’ sensations. In general, eight variables were evaluated: hunger, fullness, nausea, drowsiness, anxiety, satiety, desire to eat and prospective consumption. Other variables were inserted in VAS (Chapter 5 and 6) in order that subjects would be blind to the real purpose of the exercise (Appendix V). The validated VAS (Chapter 4) was used in Chapters 7 and 8 (Appendix VI). Subjects were familiarized with these scales prior to the commencement of the study. On each 100mm line an appetite (hunger, fullness, nausea) or mood (anxiety, drowsiness) sensation was paired with the opposing sensation or mood, (for example, ‘hungry’ and ‘not hungry’ or ‘calm’ and ‘anxious’). Subjects were requested to make a vertical mark on each line that best matched how they were feeling at the time. Each score was determined by measuring the distance from the left side of the line to the mark. To determine ‘prospective consumption’ subjects were asked - “How much food do you think you could eat?” with the answer in the range from ‘None’ to ‘A large amount’. Similarly, to determine ‘desire to eat’ subjects were asked - “How strong is your desire to eat?” with the answer in the range from ‘Weak’ to ‘Strong’.

3.6.1.4 Food intake

Each study in this thesis employed an intervention (oral, intragastric or intraduodenal preload) designed to have an effect on subsequent food intake. Prior to participation subjects were canvassed regarding their food preferences and allergies. If subjects were unable to eat the provided foods they were excluded from the study. On each occasion subjects were presented with a buffet meal in excess of what they would be expected to eat. Therefore, the amount of food eaten would be determined by the subject and not by the quantity of food available. Subjects were invited to eat until they were comfortably full, up to a limit of 30 minutes in each study.

In Chapters 5, 6, 7 and 8 subjects were offered a cold buffet meal. The buffet meal included various food items: bread, cheese, chicken, ham, yoghurt, custard, fruits, juices and flavoured milk, of predetermined caloric content. The composition and energy content of supplied foods is in Table 3.1. In Chapter 4 (prospective study) subjects were offered a hot pasta meal (Table 3.2) and the meals supplied in the retrospective study are given in Table 4.1.

The total amount of food eaten at the meal was measured by weighing food items separately to the nearest 0.1 gram before and after eating (Cook, Andrews et al. 1997). Energy content (kcal) and macronutrient composition of the meal was determined using Foodworks Nutritional software (Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia) (Cook, Andrews et al. 1997; Parker, Sturm et al. 2004).

3.6.2 Assessment of gastrointestinal hormone concentrations

Blood was collected in ice-chilled ethylenediamine tetra-acetic acid (EDTA) tubes containing 400-kIU aprotinin (Trasylol[®], Bayer Australia) per milliliter blood for measurement of plasma cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), insulin and ghrelin concentrations. Plasma was separated by centrifugation (3200 rpm for 15 min at 4°C) within 10 min of collection and stored at -70°C until assayed.

3.6.2.1 Blood glucose concentrations

Blood glucose was measured at the bedside using a portable blood glucose meter (MediSense Precision Q·I·D[®] System, Abbott Laboratories, MediSense Products Inc., Bedford, MA, USA). The accuracy of this method has been confirmed using the hexokinase technique (Horowitz, Maddox et al. 1991).

3.6.2.2 Plasma cholecystokinin (CCK) concentrations

Plasma CCK concentrations (pmol/l) were measured after ethanol extraction using a radioimmunoassay, as described previously (Santangelo, Peracchi et al. 1998; MacIntosh, Morley et al. 2001). A commercially available antibody (C258, Lot 105H4852; Sigma Chemical, St Louis, MO, USA) raised in rabbits against synthetic sulphated CCK-8 was employed. This antibody binds to all CCK peptides containing the sulphated tyrosine residue in position 7, shows a 26% cross-reactivity with unsulphated CCK-8 and less than 2 % cross-reactivity with human gastrin, and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation (CV) was 9% and the interassay was 27% with a sensitivity of 2.5 pmol/l. The sensitivity of the assay relates to its reliable detection limit.

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

Plasma GLP-1 concentrations (pmol/l) were measured by radioimmunoassay (Wishart, Horowitz et al. 1998) using a previously described method (Orskov, Jeppesen et al. 1991). Ethanol extraction of plasma samples was undertaken using an antibody, supplied by Professor S.R Bloom (Hammersmith Hospital, London, UK) that does not cross-react with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides and has been demonstrated by chromatography to measure intact GLP-1₍₇₋₃₆₎ amide. It is likely that this antibody also reacts with the degraded form of GLP-1₍₉₋₃₆₎ amide. Intra-assay CV was 17% and interassay CV was 18%, with a sensitivity of 1.5 pmol/l.

3.6.2.4 Plasma insulin concentrations

Plasma insulin (mU/ml) was measured using the Abbott Imx Microparticle Enzyme Immunoassay (Abbott Laboratories, Diagnostic Division, Dainabot, Tokyo, Japan) (Chapman, Goble et al. 1998). The detection limit of the assay was 1.0 mU/l; the interassay coefficients of variation were 4.5 % at 8.3 mU/l and 3.4 % at 40.4 mU/l.

3.6.2.5 Plasma ghrelin concentrations

Measurement of plasma ghrelin concentrations is described in detail in Chapter 7.3.4.

3.6.3 Measurement of antral area

Ultrasound has been shown to be useful in both clinical gastroenterology and clinical studies to study gastric accommodation (Berstad, Hausken et al. 1996). In addition, two-dimensional ultrasonography is used to assess gastric emptying in both healthy subjects (Bolondi, Bortolotti et al. 1985; Holt, Cervantes et al. 1986; Marzio, Giacobbe et al. 1989; Duan, Zheng et al. 1993; Darwiche, Almer et al. 1999), and in subjects with altered gastric emptying patterns (Darwiche, Almer et al.; Darwiche, Bjorgell et al. 2003). Scintigraphy is considered the gold standard tool for assessing gastric emptying. Ultrasound measurements of the gastric antrum, used to determine gastric emptying, have been shown to correlate highly with scintigraphic measurements (Holt, Cervantes et al. 1986). Ultrasound is non-invasive and does not require exposure to radioactive material and thus may be considered a reliable alternative to scintigraphy.

Measurements of antral area (Chapters 5, 6 and 8) were performed with an Aloka SSD-650 CL Ultrasound Machine (ALOKA Co., LTD. Tokyo) using either a 3.5 or 5 MHz sector transducer. Antral area (cm²) was measured with a calliper and calculation program built into the ultrasound machine. To optimise precision, the transducer was positioned vertically to obtain a parasagittal image of the antrum with the superior mesenteric vein and the abdominal aorta in a longitudinal section and measurements performed at the end of

inspiration, as described previously (Hveem, Jones et al. 1996; Jones, Doran et al. 1997). In Chapter 5, the time at which antral area had decreased to 75% of maximum (T75%) was used as an index of gastric emptying (Hveem, Jones et al. 1996).

3.6.4 Intraduodenal and intragastric intubation

3.6.4.1 Intraduodenal intubation

For the intraduodenal infusions in Chapter 7 a 17-channel manometric catheter (Dentsleeve, Adelaide, Australia) was introduced into the duodenum, via an anaesthetised nostril. This catheter consisted of 16 side holes spaced at 1.5 cm intervals (Figure 3.1). Six side holes (channels 1-6) were positioned in the gastric antrum, a 4.5 cm sleeve sensor (channel 7), with two channels on the back of the sleeve (channels 8 and 9), were positioned across the pylorus and seven channels were positioned in the duodenum (channels 10-16). A further channel used for intraduodenal infusions was positioned 11.75 cm distal to the end of the sleeve sensor. Following intubation the sleeve assembly was allowed to pass through the pylorus into the duodenum by peristalsis. The catheter was considered to be in the correct position when the sleeve sensor straddled the pylorus. The time for this to occur differed between subjects but if the catheter was not in position 90 minutes after intubation the study was abandoned and the subject asked to return on another day. Correct positioning of the catheter was monitored continuously throughout the infusion by measuring the transmucosal potential difference (TMPD) across the stomach (- 40 mV) and duodenum (\approx 0 mV), using a reference electrode (20 G intravenous cannula filled with sterile saline) placed subcutaneously in the forearm (Collins, Houghton et al. 1991; Edelbroek, Horowitz et al. 1992).

3.6.4.2 Intragastric intubation

On the intragastric study days (Chapters 6 and 7) subjects were seated upright for insertion of an intragastric catheter (10Fr, 91cm, Viasys Medsystems, Wheeling, Illinois, USA) via an anaesthetised nostril into the stomach, assisted by swallowing a glass (\approx 150 ml) of

water. Once the tube was in position it was held in place by taping it firmly to the subject's nose. For each subject, insertion length (to the infusion exit port) was determined as the distance from the tip of the nose to the earlobe and then to the base of the sternum. Correct placement was determined by auscultation after air injection. Subjects rested for 30 minutes to allow time for the water to pass out of the stomach and for the subject to be comfortable with the tube insitu.

3.6.5 Statistical analyses

For all studies results are shown as means \pm SEM. In each case, a P value < 0.05 was considered statistically significant. The statistical analyses for each study are given in detail in the relevant chapter. In each study, data following the buffet meal were not analysed, because the amount of food eaten was variable.

3.7 Conclusions

Assessing appetite and food intake in human subjects is complex. This requires that clinical research studies are conducted according to careful guidelines to ensure accurate data collection. The methods presented in this chapter are all previously validated and accepted forms of assessment of dietary intake, appetite and eating habits. In each study, a careful screening process was undertaken to ensure that each subject group (older and young) was representative of these age groups and, as such, the results could be extrapolated to a wider population.

Table 3.1 Composition of the cold buffet meal

Food items	Amount served (g)	Energy content (kcal)	Fat (g)	CHO (g)	Protein (g)
Wholemeal bread, 4 slices ^a	125	310	3.6	50	12.6
White bread, 4 slices ^a	125	308	2.9	56.4	11.8
Ham, sliced ^b	100	108	3.6	0	18.8
Chicken, sliced ^c	100	161	7.0	0	24.6
Cheese, sliced ^d	85	342	28.3	0.9	21.9
Tomato, sliced	100	13	0.1	1.9	1.0
Lettuce	100	6	0	0.4	0.9
Cucumber, sliced	100	10	0.1	1.9	0.5
Strawberry yoghurt ^e	200	230	6.2	33.8	9.4
Fruit salad ^f	140	82	0.1	19.3	0.6
Chocolate custard ^g	150	158	5.3	22.7	4.8
Apple	170	85	0.2	21.3	0.5
Banana	190	162	0.2	37.8	3.2
Orange juice ^h	500	190	5.0	42.5	5.0
Iced coffee ⁱ	300	426	10.2	61.8	21.0
Margarine ^j	20	145	16.4	0.1	0.1
Mayonnaise ^k	20	74	6.5	4.0	0.2
Total	2525	2812	95.7	354.6	136.9

^aSunblest, Tiptop, Australia; ^bDeli leg ham, Woolworths, Australia; ^cVirginian chicken, Woolworths, Australia; ^dCoon Tasty Cheese Slices, Australia Cooperative Foods Ltd., Australia; ^eYoplait, National Foods Ltd., Australia; ^fGoulburn Valley, SPC, Ardmona Operations Ltd., Australia; ^gYogo, National Foods Ltd., Australia; ^hDaily Juice Company, Australia; ⁱFarmers Union. Balemar Pty Ltd., Australia; ^jFlora, Unilever Australasia, Australia; ^kKraft, Kraft Foods Ltd., Australia; CHO, carbohydrate; g, grams

Table 3.2 Composition of the hot buffet meal

Food items	Amount served (g)	Energy content (kcal)	Fat (g)	CHO (g)	Protein (g)
Lasagne, meat, commercial ^a	340	310	3.6	50	12.6
Bread Roll, white ^b	50	143	19.4	42.8	25.5
Fruit salad ^c	140	82	0.1	19.3	0.6
Chocolate custard ^d	150	158	5.3	22.7	4.8
Apple	170	85	0.2	21.3	0.5
Banana	190	162	0.2	37.8	3.2
Orange juice ^e	500	190	5.0	42.5	5.0
Iced coffee ^f	300	426	10.2	61.8	21.0
Margarine ^g	20	145	16.4	0.1	0.1
Total	1860	1701	60.4	298.3	73.3

^aDolmio Master Foods of Australia, Wyong, NSW, Australia; ^bBread roll crusty 6 pack, Woolworths, Australia; ^cGoulburn Valley, SPC, Ardmona Operations Ltd., Australia; ^dYogo, National Foods Ltd., Australia; ^eDaily Juice Company, Australia; ^fFarmers Union. Balemar Pty Ltd., Australia; ^gFlora, Unilever Australasia, Australia; CHO, carbohydrate; g, grams

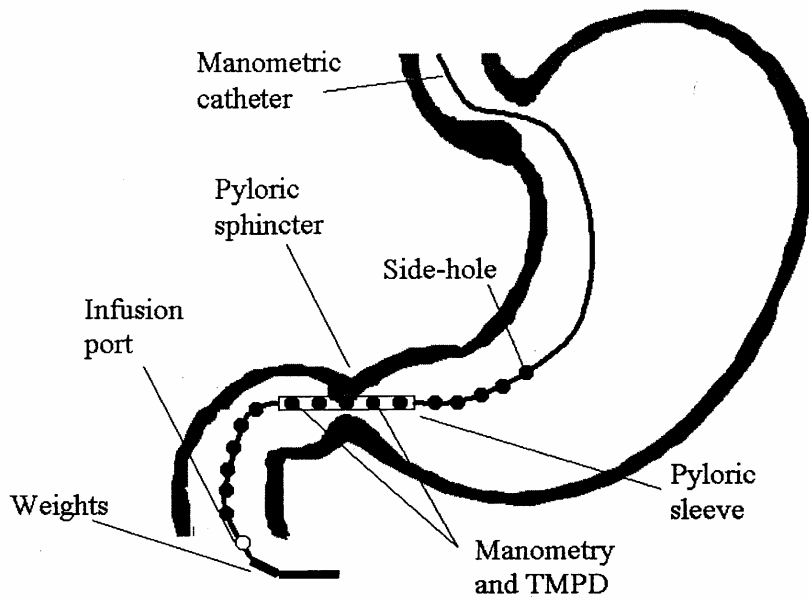


Figure 3.1 Diagram of multi-lumen catheter used in the intraduodenal nutrient infusion study (Chapter 7). Side holes are 1.5 cm apart. An infusion port was located 14 cm distal to the middle of the pyloric sleeve. TMPD, transmucosal potential difference.

Chapter 4

Reproducibility and validity of visual analogue scales in assessment of appetite and food intake in healthy older and young subjects

4.1 Summary

Visual analogue scales (VAS) are widely used in appetite research involving adults of all ages, yet the reproducibility and validity of these scales to evaluate appetite has not been assessed in older subjects. This chapter will report the results of two studies, (i) a retrospective combined analysis of four single-blind, randomised, controlled appetite studies with older and young subjects evaluating relationships between food intake and VAS scores of appetite and 'non-appetite' sensations (Study 1), and (ii) a prospective study, based on and compared to a previous study in young subjects, evaluating the reproducibility and validity of VAS as a measurement of appetite in healthy older subjects (Study 2).

Study 1: 45 healthy young men ($n = 24$) and women ($n = 21$) aged 18-35 years and 45 healthy older men ($n = 24$) and women ($n = 21$) aged 65-85 years received oral, intraduodenal or intravenous administration of treatments, which suppressed food intake. Up to 90 minutes after treatment a test meal was offered and subjects ate freely for between 30-60 minutes. Relationships between food intake at the test meal and ratings of appetite and 'non-appetite' sensations were determined using Pearson product moment correlation coefficients. Study 2: 32 healthy older men ($n = 16$) and women ($n = 16$) aged 65-85 years received on two identical study days a standardised breakfast and 4 hours later were offered lunch from which they could eat *ad libitum* for up to 30 minutes. Validity was determined by Pearson product moment correlation coefficients between VAS scores of appetite and food intake. Reproducibility was determined for VAS ratings of appetite between the two visits of the study and reported as the coefficient of repeatability. In each study perceptions of appetite were assessed by 100 mm visual analogue scales administered at regular intervals and food intake (kcal) at the subsequent test meal calculated.

In the retrospective study, food intake at the test meal was positively related to perceptions of hunger, drowsiness, and calmness at both baseline and premeal ($r > 0.16$, $P < 0.05$), and inversely related to premeal ratings of fullness ($r > 0.2$, $P < 0.05$) in both older and young subjects. Food intake was related to VAS ratings at least as strongly, if not more so, in older as in young subjects.

In the prospective study the optimal time point for assessment of both reproducibility and validity was just before the meal (4-hr mean values). In assessing reproducibility, 4-hr mean coefficients of repeatability (21-38mm) and correlation coefficients were similar to those reported previously in young adults. In assessing validity, significant relationships of similar strength to those reported in young subjects were observed between food intake and 4-hr mean VAS ratings of appetite (hunger [$r = 0.67$, $P < 0.001$], desire to eat [$r = 0.68$, $P < 0.001$], prospective consumption [$r = 0.71$, $P < 0.001$]).

Taken together, these results suggest that visual analogue scale measures of appetite have comparable reproducibility and validity in older subjects to reported values in young adults. These observations confirm that food intake is related to perceptions of hunger and fullness as assessed by VAS in healthy older and young subjects.

4.2 Introduction

Visual analogue scales (VAS) have been widely used in clinical research to assess appetite (Cecil, Castiglione et al. 1998; Wilson, Purushothaman et al. 2002). Several studies have assessed the relationship between appetite ratings and food intake at a subsequent meal in young subjects i.e. validity (Pi-Sunyer, Kissileff et al. 1982; Rolls, Hetherington et al. 1988; Mattes 1990) and shown highly significant correlations. The reproducibility of VAS ratings of appetite and their ability to predict subsequent food intake has also been demonstrated in young adult subjects in previous studies [e.g. (Mattes 1990; Flint, Raben et al. 2000; Stubbs, Hughes et al. 2000)], and visual analogue scales are useful in assessing subjective appetite sensations, such as hunger, fullness and nausea in this age group (Sepple and Read 1989).

There is increasing interest in the effect of ageing on appetite and feeding behaviour, particularly the decline in food intake that accompanies normal ageing [‘the anorexia of ageing’ (Morley 1997)] and its possible adverse affects (Morley and Silver 1988; MacIntosh, Morley et al. 2000). Although visual analogue scales are often used to evaluate appetite in older people they have not been formally evaluated for reliability or related to eating behaviour in this age group. The relationship between appetite ratings and food intake may differ between older and young adults. For example, age-related changes in cognitive, sensory and gastrointestinal function may affect both perceptions of appetite and desire to eat as well as food intake in older people (Wurtman, Lieberman et al. 1988; Rolls, Dimeo et al. 1995; Clarkston, Pantano et al. 1997; Donini, Savina et al. 2003). Alterations in appetite that accompany normal ageing include a diminished perception of hunger both at fasting and after meals exhibited by lower scores on ratings anchored at ‘extremely hungry’, and increased fullness ratings with an associated reduction in food intake (Rolls 1992; Briefel, McDowell et al. 1995; Rolls, Dimeo et al. 1995; Morley 1997).

Despite increasing interest in appetite regulation in the elderly and frequent use of VAS in feeding studies in this age group, the relationship between VAS ratings of appetite and food intake has not been assessed in older people. In addition, mood and sensations that are not obviously linked to food intake, such as anxiety, depression and drowsiness, may be affected by food intake (De Castro 1993; Wells, Read et al. 1995; Wells, Read et al. 1997). Conversely, mood states may potentially influence and be related to food intake. There is little information on the latter relationships in young subjects (Christensen 1993; Patel and Schlundt 2001), and none that we know of in older people.

This chapter will report the results of two studies; (i) a retrospective combined analysis of four single-blind, randomised, controlled appetite studies with older and young subjects, and (ii) a prospective study, undertaken to determine the value of VAS as a measurement of appetite in healthy older subjects. The two hypotheses explored were that the relationships

between VAS ratings of appetite and food intake in older people are similar to those reported previously for young people (Flint, Raben et al. 2000), and the same relationships between VAS ratings of appetite and ‘non-appetite’ or ‘mood’ sensations and food intake apply for both young and old people, making it appropriate to use VAS in appetite studies in both age groups.

4.3 Research design and methods

4.3.1 Common methodologies

Identical inclusion and exclusion criteria, (previously described in Chapter 3.2), applied for each study and each subject gave informed, written consent. In both studies, appetite and other sensations were assessed using 100mm visual analogue scales (previously described in Chapter 3.6.1). Subjects were familiarized with these scales prior to the commencement of the study. In each test meal the total amount of food eaten at the test meal was measured to the nearest 0.1 gram, by weighing food items separately before and after eating (Cook, Andrews et al. 1997). Energy content (kcal) and macronutrient composition of the meal was determined using dietary calculation programs (Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia (Cook, Andrews et al. 1997; Andrews, Doran et al. 1998).

4.3.2 Experimental design (Study 1)

4.3.2.1 Protocol (Table 4.1)

The results of four appetite studies conducted in the University of Adelaide Department of Medicine were combined for analysis (MacIntosh, Horowitz et al. 2001)^a (MacIntosh, Sheehan et al. 2001)^b (MacIntosh, Morley et al. 2001)^c (Sturm 2002)^d. These studies were chosen as they were all studies we have performed in the last 4 years that compared food intake and appetite sensations (i.e. hunger, fullness) in healthy older and young subjects in response to an intervention that significantly affected food intake. The within-subject changes in appetite and food intake permitted analysis of correlations between these parameters in older and young subjects. In total, 45 healthy young men (n = 24) and women

(n = 21) aged 18-35 years and 45 healthy older men (n = 24) and women (n = 21) aged 65-85 years were studied. All studies compared responses in older subjects to an equal number of gender- matched young subjects.

Each study was designed to evaluate the effect of an intervention on appetite and food intake in young and older men and women. Treatments (oral pre-load, intravenous or intraduodenal infusion), and in some cases test meals differed between the four studies. In each study during, or up to 90 minutes after, the intervention subjects were offered a test meal (cold buffet in Study 1, 2, & 4 or hot meal in Study 3), in excess of what they would be expected to consume, and invited to eat freely (Lavin, Wittert et al. 1996).

4.3.2.2 Visual analogue scales

Appetite and other sensations were assessed using 100 mm visual analogue scales (Sepple and Read 1989). Seven variables, hunger, fullness, nausea, drowsiness, calmness, desire to eat and prospective consumption, were common to the VAS in all four studies. These scales were administered at intervals of 10-30 minutes during studies. Baseline appetite values were calculated from the mean of the two time-points immediately prior to treatment and premeal data as the mean of two time-points immediately prior to consumption of the test meal. Change from baseline was calculated by subtracting baseline data from premeal data.

4.3.2.3 Food intake

Foods consumed at the test meals in Study 1, 2 and 4 included wholemeal and white bread, ham, processed chicken and cheddar cheese, tomato, cucumber and lettuce, fresh fruit, chocolate custard and low-fat strawberry yoghurt, flavoured milk and fruit juice. Subjects in Study 3 were offered a tomato based sauce and pasta dish. Energy content (kcal) and macronutrient composition of the meal was determined using Diet-1 Nutrient Calculation Software (Study 3), Diet-4 Nutrient Calculation Software (Study 1 & 2) or Foodworks 2.10 (Study 4), Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia (Cook, Andrews et al. 1997; Andrews, Doran et al. 1998).

4.3.2.4 Statistical analyses

Data from the four studies were combined for analysis and presented as means \pm SEM. Food intake and macronutrient composition between older and young subjects in individual studies were compared by unpaired-t tests. VAS ratings in old and young subjects were compared by unpaired-t tests as the data were normally distributed. Pearson product moment correlations were calculated between energy intake at the buffet meal and mean VAS ratings of hunger, fullness, nausea, calmness, drowsiness, desire to eat and prospective consumption at baseline, premeal and change-from-baseline using SPSS for Windows 10.0 statistical software (Chicago, USA). VAS ratings found to be significantly related to energy intake using the Pearson correlation were then included in a multiple regression analysis (Forwards entry method) to establish independent determinants of food intake. Significance was accepted as $P < 0.05$.

4.3.2.5 Results-Visual analogue scale ratings (Table 4.2)

Baseline, change-from-baseline and premeal VAS ratings for each age group are shown in Table 4.2. At baseline older subjects rated themselves as less hungry, nauseous and drowsy, with less desire to eat and lower prospective consumption scores than young subjects. Premeal, older subjects also rated themselves as less hungry and drowsy, with less desire to eat and lower prospective consumption scores than young subjects. Hunger and prospective consumption ratings increased between baseline and premeal (change-from-baseline) more in older than young subjects. There were no significant differences between groups for change-from-baseline ratings for hunger, fullness, nausea, calmness and drowsiness.

4.3.2.6 Results-Relations between VAS ratings and food intake at the study meal (Table 4.3)

In older people the amount of food eaten was positively related to baseline fullness and baseline and premeal hunger, drowsiness, calmness, desire to eat and prospective consumption, and inversely related to premeal fullness. There was a positive relationship between food eaten and change-from-baseline desire to eat and prospective consumption,

and an inverse relationship with change-from-baseline fullness. In young subjects the amount of food eaten was positively related to baseline drowsiness and calmness and premeal calmness and prospective consumption, while it was inversely related to premeal and change-from-baseline fullness. There were no significant relationships between energy intake at the study meal and baseline, change-from-baseline or premeal VAS ratings for nausea.

4.3.2.7 Results-Determinants of food intake

Young subjects Multiple regression analysis of the determinants of food intake revealed a significant relationship ($R^2 = 0.11$, $P < 0.0001$) with pre-meal ratings of calmness (positive) and fullness (inverse). The contribution of premeal ratings of calmness was greater ($P = 0.002$) than premeal ratings of fullness ($P = 0.03$).

Old subjects Multiple regression analysis of the determinants of food intake revealed a significant relationship ($R^2 = 0.25$, $P < 0.0001$) with premeal prospective consumption and hunger ratings (positive). The contribution of premeal prospective consumption ratings was greater ($P < 0.0001$) than premeal ratings of hunger ($P = 0.02$).

4.4 Experimental design (Study 2)

4.3.3.1 Protocol Study 2 (Table 4.4)

The protocol is based on, and almost identical to that conducted by Flint et al, 2000 (Flint, Raben et al. 2000) in young adult subjects. Thirty-two healthy older men ($n = 16$) and women ($n = 16$), aged 65-85 years were studied. Subjects attended the department on two days (Visit 1 and Visit 2) separated by one week, at 0900 after an overnight fast (except for water) of at least 10 hours. The protocol was identical for each study visit. Following a 30 minute baseline period subjects were served a standardised breakfast ($t = 0$ min) consisting of 3 slices of white toast, 2 x 14g pots of strawberry jam, 1 x 10g canola margarine and 1 x 300ml unsweetened orange juice (total energy intake 485 kcal, 2.03 MJ) and asked to finish all food and drink in 15 minutes.

4.3.3.2 Visual analogue scales

Visual analogue scales were completed at baseline ($t = -30, -15, 0$ min), immediately following breakfast ($t = 15$ min) and then at 30-minute intervals ($t = 45, 75, 105, 135, 165, 195, 225$ and 255 min) until lunch ($t = 255$ min) and when subjects finished eating lunch ($t = 315$ min). Eight variables were evaluated: hunger, fullness, nausea, drowsiness, anxiety, satiety, desire to eat and prospective consumption. Baseline appetite values were calculated from the mean of the three time-points immediately prior to breakfast ($t = -30, -15, 0$ min) and 4-hour mean scores from the mean of nine measurements between the end of breakfast and the start of lunch. The pre-lunch measure was the VAS completed immediately prior to lunch and the pre-post difference values refer to the difference between scores immediately prior to and following lunch.

4.3.3.3 Food intake

On each study day subjects were offered the same food items for lunch; beef lasagne, mixed vegetables, bread roll, margarine, fresh fruit, flavoured milk and fruit juice, in excess of what they would be expected to consume, and invited to eat freely for up to 60 minutes (Lavin, Wittert et al. 1996). During each visit subjects were allowed to read, do craft and writing activities and move freely around the clinic room as desired. Energy content (kcal) and macronutrient composition of the lunch was determined using Foodworks 2.10, Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia (Cook, Andrews et al. 1997; Parker, Sturm et al. 2004).

4.3.3.4 Statistical analyses

Subject characteristics are presented as means \pm SEM. Age, weight, height, Three Factor Eating Questionnaire (TFEQ) and Body mass index (BMI) were compared in men and women by one-way analysis of variance (ANOVA). Appetite sensations at all time points and food intake at lunch on the two study days were compared by paired-t tests. The Bonferroni test was used to correct for multiple comparisons and the P-values adjusted

accordingly. Validity was determined by Pearson product moment correlations between energy intake at the buffet meal and mean VAS ratings at 'baseline', '4-hr mean', 'pre-lunch' and 'pre-post difference' for Visit 1 and 2 and for the mean of both days. Reproducibility was assessed by (i) The Coefficient of Repeatability (CR), as described by Bland and Altman (Bland and Altman 1995) and used by Flint et al, 2000 (Flint, Raben et al. 2000), that is, 2 x the standard deviation of the mean of the differences between Visit 1 and Visit 2 was calculated for 'baseline' values, '4-hr means' and peaks/nadirs of the appetite ratings, (ii) the Coefficient of Variation (CV) of these differences ($[\text{SD}/\text{mean} \times 100]\%$) and (iii) correlation analysis by linear regression. All calculations were performed using SPSS for Windows 10.0 statistical software (Chicago, USA). Significance was accepted as $P < 0.05$.

4.3.3.5 Results-Reproducibility of appetite scores (Figure 4.1 & Table 4.5)

Absolute ratings of hunger, fullness, nausea, satiety, drowsiness, anxiety, desire to eat and prospective consumption are presented. As expected, hunger, desire to eat and prospective consumption decreased and fullness and satiety increased following both meals. No differences between visits at baseline, 4-hr mean, pre-lunch or pre-post difference were found for hunger, nausea, drowsiness, anxiety, desire to eat or prospective consumption. Subjects reported feeling more full at baseline on Visit 1 than Visit 2 ($P < 0.0001$), and greater satiety scores were observed on Visit 1 than Visit 2 for pre-lunch ($P = 0.03$) and pre-post difference ($P = 0.04$) values. No differences between visits were found for satiety ratings at baseline or 4-hr mean. Reproducibility results for baseline, 4-hr mean and peak/nadir values are presented in Table 2. The coefficient of repeatability (CR) values are reported together with previously reported values for young adults where available (Flint, Raben et al. 2000).

4.3.3.6 Results-Validity (Table 4.4 and Table 4.6)

Food intake data are presented in Table 4.4. Mean food intake at Visit 1 was 1214 ± 64 kcal and at Visit 2 1217 ± 68 kcal ($P = 0.97$). Men ate more on both study days than women ($P < 0.0001$) and at Visit 1 men had a higher percentage carbohydrate intake and lower percentage protein intake than the women.

Relationships between food intake and appetite scores at Visit 1 and Visit 2 are presented in Table 4.6, together with reported values for young adults where available. Strong positive relationships between food intake and baseline, 4-hr mean, pre-lunch and pre-post difference scores were observed for hunger, desire to eat and prospective consumption at Visit 1, Visit 2 and the mean of the two visit days. The strongest relationship with food intake for all four variables was with the 4-hr mean ratings, with highly significant relationships between food intake and hunger, fullness, desire to eat and prospective consumption, all with P values < 0.005 , and r values > 0.5 , and greater than those reported in young adults. An inverse relationship was found between food intake and scores for fullness at baseline and 4-hr mean. The strongest inverse relationship for fullness was observed at the 4-hr mean time point. There was an inverse relationship between food intake and pre-post difference scores for satiety at Visit 1 and for the mean of both days. No relationship between food intake and scores for nausea, drowsy, anxiety or satiety were found for baseline, 4-hr mean or pre-lunch values.

4.5 Discussion

The main aim of these two studies was to determine the relationships between food intake and visual analogue scale (VAS) ratings of appetite sensations, such as hunger and fullness, in healthy older subjects. We were interested in whether the same relationships existed between food intake and ratings of appetite in older subjects as previously reported in young subjects. We were also interested in the relationship between “non-appetite” VAS ratings of moods and sensations and food intake both in older and young subjects.

4.5.1 Validity

In Study 1, we found that hunger ratings were a poor predictor of test meal food intake in young subjects. This contrasts with several (Flint, Raben et al. 2000) but not all (Raben, Tagliabue et al. 1995) previous studies, in which significant positive relationships between hunger and food intake were found. Perceptions of fullness were a more useful predictor of food intake than hunger in young subjects in our study, with significant inverse relationships, as in the previous validation study (Flint, Raben et al. 2000). Nevertheless, although statistically significant, these associations were relatively weak.

In this study, VAS ratings were more closely associated with subsequent test meal food intake in older than young adult subjects. Fifteen VAS rating parameters, including baseline and pre-meal hunger, fullness, drowsiness, calmness, desire to eat and prospective consumption, were significantly related to food intake in older subjects, compared to only six in the younger subjects (Table 4.3). No VAS parameter was significantly related to food intake in young but not older subjects, whereas the converse was true for four parameters. Of the fifteen relationships that were significant in at least one age group, the r-values for the correlations were higher in the older than young subjects in all but one, in which they were very similar (pre meal fullness; -0.21 young vs -0.195 older). Therefore, although we made no direct statistical comparison of the strength of the correlations between the age groups, it seems that VAS ratings relate as closely (and possibly more so) to food intake in old as young subjects, and it is at least as appropriate to use these scales in studies involving older people.

In Study 2, we found a significant positive relationship between food intake at the test meal and perceptions of hunger, desire to eat and prospective consumption at all time-points, with the strongest relationships for the 4-hr mean ratings, all with r values ≥ 0.67 . There were also significant inverse relationships between fullness and food intake for all time-points for all these measures; the strongest relationship (highest r value) was again for the 4-

hr mean ratings. These relationships are stronger than those reported in young men by Flint et al, (2000) (Flint, Raben et al. 2000), and those found in the retrospective study in both older and young subjects. There was a trend for an inverse relationship between food intake and ratings of satiety for 4-hr mean ($P = 0.08$) and pre-lunch ratings of fullness ($P = 0.1$) for the older subjects in comparison to significant inverse relationships for the young subjects possibly due to the power of the sample size to detect this association.

No time-point clearly emerges from Study 1 as the best in terms of VAS ratings and their relation to food intake at the test meal. It may even be that different time points are better for different sensations. In this study, by univariate analysis, subsequent food intake was most closely related to drowsiness ratings assessed at baseline, fullness ratings assessed as change-from-baseline, and calmness, hunger, desire to eat and prospective consumption ratings assessed premeal, although the latter three may be measuring the same sensation. Nevertheless, premeal ratings were most often those with the highest r-values in the univariate analyses, and multivariate analyses indicated that pre-meal VAS ratings were those significantly related to food intake in both young and older subjects. It therefore seems, perhaps not surprisingly, that the VAS ratings measured immediately before the test meal are most closely associated with the amount of food eaten at that meal.

An interesting finding of this study was the significant positive associations between ratings of drowsiness and calmness and subsequent *ad libitum* food intake. These associations were strongest for drowsiness ratings at baseline and for calmness ratings pre-meal. Indeed, as indicated by the results of the multivariate analyses and the r-values of the univariate analyses, pre-meal calmness was the VAS rating most closely related to food intake in young subjects in this study, more so than both hunger and fullness. Even though hunger and prospective consumption were the sensations most closely associated with food intake in older subjects, there were still significant positive correlations between drowsiness and calmness ratings and subsequent food intake. We are unaware of previous reports of such

associations and can only speculate as to the cause. Although it seems to make sense that a feeling of calmness and relaxation just prior to a meal might translate to increased food intake at that meal, it is perhaps surprising that this is a stronger predictor than either fullness or hunger ratings in young subjects. This finding, including the differential effects of age, warrants closer examination.

In the prospective study (Study 2), although the reproducibility of drowsiness and anxiety measures was similar to other measures (eg fullness), there was no relationship between the scores at any time-point and subsequent food intake. The different methodologies employed in these two studies may account for this disparity. In the retrospective analysis we combined data from four studies that compared food intake and appetite in response to an intervention. Treatments (oral preload, intravenous or intraduodenal infusion), and, in some cases, test meals, differed between the four studies. In contrast the prospective study was designed specifically to investigate food intake and appetite under standard conditions. Subjects did not undergo any invasive treatments and were fed familiar foods in a setting more conducive to eating. There is, therefore, a greater likelihood that the findings of the prospective study reflect the relationship between food intake and these sensations. However, the relationship between ‘non-appetite’ sensations and food intake may warrant further research.

Our finding that food intake in older subjects is related at least as closely to hunger, fullness and other ‘non-appetite’ ratings in older as in young adult subjects, suggests that increases or decreases in these sensations rather than the ability to respond to them, is mainly responsible for ageing-related reductions in food intake (Rolls 1992; Morley 1997). Indeed, healthy older people in this and other studies consistently rate themselves as less hungry than do healthy young subjects (Wurtman, Lieberman et al. 1988; Rolls, Dimeo et al. 1995; Clarkston, Pantano et al. 1997). In the combined analysis (Study 1) and the individual studies from which the data are taken, we have not found that old people feel more full than young adults, although others have reported this (Wurtman, Lieberman et al. 1988; Rolls,

Dimeo et al. 1995; Clarkston, Pantano et al. 1997). Although ratings of desire to eat and prospective consumption increase more from baseline to pre-meal (change-from-baseline) in older than young subjects, this may reflect their significantly lower baseline values, rather than the effects of the intervention, as their pre-meal ratings remain significantly below that of the young subjects.

4.5.2 Reproducibility

We have replicated, in older subjects, the study by Flint et al, 2000 (Flint, Raben et al. 2000), of visual analogue scale (VAS) measures of appetite and have found similar values to young subjects for measures of reproducibility and validity. Measures of reproducibility were similar to each other at baseline, 4-hr mean and peak time points, with no clear superior time point. We found coefficient of repeatability (CR) values within the range of 22-31mm for fasting ratings of hunger, fullness and prospective consumption. Although it is not possible to directly compare results, due to differences in subject numbers and minor differences in study design, they compare favourably to those obtained by Flint et al, (2000) (Flint, Raben et al. 2000) and Raben et al (1995) (Raben, Tagliabue et al. 1995), who reported fasting ranges of 24-28mm and 29-37mm respectively, for the same variables in young subjects. Fasting satiety scores were also similar for all studies, with CR values of 36mm (current study), 30mm (Flint, Raben et al. 2000), and 38mm (Raben, Tagliabue et al. 1995).

Mean appetite ratings (obtained by averaging scores between breakfast and lunch) produced CR values of 22-28mm for hunger, fullness and prospective consumption compared with ranges of 17-24mm (Flint, Raben et al. 2000) and 14-38mm (Raben, Tagliabue et al. 1995). There was, however, a marked difference between studies for satiety scores at this time-point. Scores were comparable between the Flint and Raben studies, with CR values of 20mm and 21mm respectively, however, we found a large CR of 38mm. This large CR value was much greater than those of the other appetite ratings at this time-point and may be explained by the difficulty the older subjects reported in assessing this aspect of their

appetite. In many cases the older subjects were not familiar with the term satiety and, to avoid prompting their responses, subjects were given the explanation for this term only once at the beginning of each study day. Moreover, there was a significant difference for the results of this variable between Visit 1 and Visit 2 and this wide range of ratings may account for the poor reproducibility.

4.6 Conclusion

These studies were conducted to test the reproducibility and validity of visual analogue scale measurements of appetite and 'non-appetite' sensations in an older population. Although our CR values are relatively large for some variables they are comparable to those reported in young adults and measures of validity were at least as good, if not better (Flint, Raben et al. 2000). VAS appear at least as effective in assessing appetite sensations and predicting subsequent food intake in healthy older as in young adults. The reproducibility and validity of VAS in other settings has yet to be determined, however, we conclude that they are a valid tool for use in clinical laboratory appetite studies.

Table 4.1 (studies 1-4) Subject characteristics of young (18-35 years) and older (65-85 years) subjects

Study 1	MacIntosh et al, 2001a	
	Old	Young
<i>Age group</i>	n=13	n=13
<i>Age (years)</i>	72.0 ± 5.8	23.7 ± 5.1
<i>Body Mass Index (BMI)(kg/m²)</i>	25.6 ± 2.3	23.7 ± 3.4
<i>Method</i>	Intraduodenal 25% glucose (0.95kcal/ml), 10% Intralipid (1.1kcal/ml) or saline @ 2.86 kcal/min for 2 hrs on separate days	
<i>Test meal</i>	Cold buffet meal offered on completion. Allowed to eat freely for 30 mins	
<i>Results</i>	Lipid suppressed food intake more than glucose in young, but not older subjects	
<i>Energy intake (kJ)</i>	3344 ± 224	3825 ± 236
<i>Macronutrient composition (% energy)</i>	%protein 15.4 ± 0.5 % fat 42.1 ± 1.0 % CHO 42.6 ± 0.8	%protein 13.8 ± 0.4 % fat 38.2 ± 1.0 % CHO 48.1 ± 1.0
Study 2	MacIntosh et al, 2001b	
	Old	Young
<i>Age group</i>	n=12	n=12
<i>Age (years)</i>	72.4 ± 4.8	22.8 ± 2.4
<i>Body Mass Index (BMI)(kg/m²)</i>	25.1 ± 1.6	24.6 ± 2.4
<i>Method</i>	Intravenous naloxone *LD- bolus 27µg/kg/ continuous 50 µg/kg/hr or **HD- bolus 54.5µg/kg/ continuous 100 µg/kg/hr for 150 mins on separate days	
<i>Test meal</i>	Cold buffet meal offered 90 mins after start of infusion. Allowed to eat freely for 60 mins	
<i>Results</i>	Naloxone suppressed food intake, with no difference between older and young subjects	
<i>Energy intake (kJ)</i>	¹ 3609 ± 176	¹ 5501 ± 357
<i>Macronutrient composition (% energy)</i>	^a %protein 15.4 ± 0.5 ^b % fat 42.1 ± 1.0 ^c % CHO 42.6 ± 0.8	^a %protein 13.8 ± 0.4 ^b % fat 38.2 ± 1.0 ^c % CHO 48.1 ± 1.0

Study 3		MacIntosh et al, 2001c	
	Old	Young	
<i>Age group</i>	n=12	n=12	
<i>Age (years)</i>	71.2 ± 4.5	22.6 ± 4.1	
<i>Body Mass Index (BMI)(kg/m²)</i>	23.9 ± 2.5	23.3 ± 2.7	
<i>Method</i>	Intravenous cholecystokinin (CCK-8)* LD-1 ng/kg/min, **HD- 3 ng/kg/min or saline for 25 mins on separate days		
<i>Test meal</i>	Hot pasta meal offered 10 mins after start of infusion. Allowed to eat freely for 30 mins		
<i>Results</i>	CCK-8 suppressed food intake more in older than young subjects		
<i>Energy intake (kJ)</i>	² 2141 ± 211	² 3603 ± 261	
<i>Macronutrient composition (% energy)</i>	%protein 19.1 ± 1.4 ^d % fat 20.3 ± 2.2 ^e % CHO 60.8 ± 3.0		
		%protein 17.4 ± 0.5 ^d % fat 13.8 ± 0.8 ^e % CHO 68.8 ± 0.9	
Study 4		Sturm et al, 2002	
	Old	Young	
<i>Age group</i>	n=8	n=8	
<i>Age (years)</i>	77.0 ± 2.6	22.0 ± 3.5	
<i>Body Mass Index (BMI)(kg/m²)</i>	23.7 ± 2.7	20.5 ± 1.0	
<i>Method</i>	Oral icecream-1176kJ, 40% CHO, 50% fat, 10% protein; eaten over 5 mins or no preload		
<i>Test meal</i>	Cold buffet meal offered 90 mins after completion of preload. Allowed to eat freely for 30 mins		
<i>Results</i>	The preload suppressed food intake more in young than older subjects		
<i>Energy intake (kJ)</i>	³ 2099 ± 151	³ 3166 ± 243	
<i>Macronutrient composition (% energy)</i>	%protein 19.0 ± 0.6 % fat 33.1 ± 2.1 % CHO 45.0 ± 1.9		
		%protein 19.0 ± 0.8 % fat 32.7 ± 1.2 % CHO 45.6 ± 1.3	

Data presented as mean ± SD for age and BMI; mean ± SEM for energy intake and macronutrient composition

* low-dose, ** high-dose, CHO- carbohydrate; older vs young: ¹ P < 0.0001; ² P < 0.0001;

³ P = 0.0008; ^a P = 0.02; ^b P = 0.005; ^c P < 0.0001; ^d P = 0.008; ^e P = 0.01

Table 4.2 Visual analogue ratings (baseline, premeal and change-from-baseline) in old (n=45) and young (n=45) subjects. Comparison between age groups by unpaired (2-tailed) t-test

Parameter	Age	Mean \pm SEM	Significance (p value)
Hunger baseline	Old	49.3 \pm 2.5	0.01
	Young	57.5 \pm 2.1	
Hunger pre-meal	Old	49.1 \pm 2.5	0.02
	Young	57.0 \pm 2.3	
Change-from-baseline hunger	Old	-0.24 \pm 2.4	0.92
	Young	-0.56 \pm 1.7	
Full baseline	Old	25.6 \pm 1.7	0.92
	Young	23.0 \pm 1.6	
Full pre-meal	Old	33.0 \pm 2.0	0.09
	Young	28.6 \pm 1.7	
Change-from-baseline full	Old	7.4 \pm 2.1	0.52
	Young	5.6 \pm 1.7	
Nausea baseline	Old	7.3 \pm 0.95	0.005
	Young	12.2 \pm 1.4	
Nausea pre-meal	Old	10.1 \pm 1.2	0.18
	Young	12.7 \pm 1.5	
Change-from-baseline nausea	Old	2.8 \pm 1.2	0.17
	Young	0.49 \pm 1.2	
Calm baseline	Old	63.4 \pm 3.5	0.84
	Young	62.4 \pm 3.4	
Calm pre-meal	Old	62.7 \pm 3.4	0.86
	Young	61.8 \pm 3.5	
Change-from-baseline calm	Old	-0.8 \pm 1.0	0.92
	Young	-0.7 \pm 0.8	
Drowsy baseline	Old	39.9 \pm 3.1	0.002
	Young	52.1 \pm 2.3	
Drowsy pre-meal	Old	38.0 \pm 2.7	0.005
	Young	48.6 \pm 2.5	
Change-from-baseline drowsy	Old	-1.9 \pm 2.5	0.61
	Young	-3.6 \pm 2.2	
Desire to eat baseline	Old	38.7 \pm 2.1	0.0001
	Young	60.0 \pm 1.8	
Desire to eat pre-meal	Old	45.6 \pm 2.2	0.0001
	Young	61.0 \pm 1.9	
Change-from-baseline desire to eat	Old	6.9 \pm 1.98	0.02
	Young	1.0 \pm 1.6	
Prospective consumption baseline	Old	38.7 \pm 1.8	0.0001
	Young	61.5 \pm 1.5	
Prospective consumption pre-meal	Old	44.9 \pm 1.96	0.0001
	Young	61.7 \pm 1.8	
Change-from-baseline prospective consumption	Old	6.2 \pm 1.6	0.005
	Young	0.17 \pm 1.4	

Table 4.3 Correlation between VAS sensations and food intake in all subjects

Parameter		Baseline	Premeal	Change from baseline
Hunger	Old	r=0.20, P=0.02	r=0.33, P<0.0001	r=0.13, P=0.16
	Young	r=0.09, P=0.34	r=0.12, P=0.19	r=0.06, P=0.54
Fullness	Old	r=0.21, P=0.02	r=-0.195, P=0.03	r=-0.4, P<0.0001
	Young	r=0.07, P=0.43	r=-0.21, P=0.02	r=-0.27, P=0.002
Nausea	Old	r=0.02, P=0.82	r=0.002, P=0.98	r=-0.01, P=0.87
	Young	r=-0.12, P=0.18	r=-0.15, P=0.09	r=-0.05, P=0.59
Drowsy	Old	r=0.27, P=0.002	r=0.18, P=0.041	r=-0.14, P=0.12
	Young	r=0.21, P=0.02	r=0.17, P=0.06	r=-0.19, P=0.83
Calm	Old	r=0.34, P<0.0001	r=0.36, P<0.0001	r=0.03, P=0.73
	Young	r=0.25, P=0.004	r=0.28, P=0.001	r=0.16, P=0.06
Desire to eat	Old	r=0.22, P=0.01	r=0.38, P<0.0001	r=0.197, P=0.03
	Young	r=0.04, P=0.68	r=0.06, P=0.49	r=0.03, P=0.72
Prospective consumption	Old	r=0.34, P<0.0001	r=0.47, P<0.0001	r=0.22, P=0.01
	Young	r=0.095, P=0.30	r=0.19, P=0.034	r=0.14, P=0.11

Table 4.4 Subject characteristics and food intake at study lunch (Mean \pm SEM)

	Visit	Men	Women	Total
Age (yrs)		72.3 \pm 0.9	75.1 \pm 1.1	73.7 \pm 0.8
Height (m)		1.73 \pm 0.02*	1.58 \pm 0.02*	1.68 \pm 0.02
Weight (kg)		75.0 \pm 2.4*	62.6 \pm 2.2*	68.8 \pm 1.9
BMI (kg/m ²)		25.0 \pm 0.5	24.8 \pm 0.7	24.9 \pm 0.4
TFEQ		6.9 \pm 1.0	6.0 \pm 1.0	6.5 \pm 0.7
Food intake (kcal)	1	1423 \pm 70.6*	1004 \pm 76.7*	1214 \pm 63.6
	2	1451 \pm 83.1*	983 \pm 71.5*	1217 \pm 68.4
Food intake (MJ)	1	5.95 \pm 0.3*	4.2 \pm 0.3*	5.1 \pm 0.3
	2	6.1 \pm 0.4*	4.1 \pm 0.3*	5.1 \pm 0.3
Protein (g)	1	59.1 \pm 2.9* [#]	45.2 \pm 3.2*	52.1 \pm 2.5
	2	59.4 \pm 3.6* [#]	42.7 \pm 3.1*	51.0 \pm 2.8
CHO (g)	1	202.2 \pm 10.9*	138.1 \pm 12.2*	170.1 \pm 9.9
	2	208.2 \pm 12.6*	137.7 \pm 11.1*	172.9 \pm 10.4
Fat (g)	1	43.5 \pm 2.1*	31.2 \pm 2.0*	37.4 \pm 1.8
	2	43.8 \pm 2.4*	30.2 \pm 2.0*	37.0 \pm 2.0
Protein (%)	1	16.9 \pm 0.4* [#]	18.4 \pm 0.5*	17.7 \pm 0.3 [#]
	2	16.6 \pm 0.3 [#]	17.7 \pm 0.5	17.2 \pm 0.3 [#]
CHO (%)	1	54.2 \pm 0.6*	52.0 \pm 0.7*	53.1 \pm 0.5
	2	54.7 \pm 0.5	53.1 \pm 0.9	53.9 \pm 0.5
Fat (%)	1	27.1 \pm 0.4	27.9 \pm 0.6	27.5 \pm 0.4
	2	26.9 \pm 0.6	27.5 \pm 0.9	27.2 \pm 0.5

*P < 0.05 men vs women; [#]P < 0.05 Visit 1 vs Visit 2; CHO, carbohydrate; g, grams; BMI, body mass index; TFEQ, Three Factor Eating Questionnaire

Table 4.5 Reproducibility of appetite scores between the two study visits (n = 32)

	Mean difference (mm)	CV%	CR (mm)	r
Baseline				
Hunger	5	16	30.9 (28)	0.81** (0.26)
Fullness	-7.8	47	21.5 (27)	0.82** (0.16)
Nausea	2	60	23.7	0.48*
Drowsy	-2	53	24.3	0.66**
Satiety	-5	44	35.5 (30)	0.69** (0.17)
Anxiety	0.4	41	21.9	0.67**
Desire to eat	5	16	33.7	0.76**
Prospective consumption	2	13	28.6 (24)	0.75** (0.47)*
4hr mean				
Hunger	0.2	17	26.1 (24)	0.76** (0.61)**
Fullness	1.5	24	27.5 (18)	0.58** (0.81)**
Nausea	0.1	63	18.5	0.64**
Drowsy	1	54	28.7	0.62**
Satiety	-7	32	38 (20)	0.68** (0.73)**
Anxiety	8.2	42	16.9	0.69**
Desire to eat	-2	16	25.7	0.80**
Prospective consumption	-2	13	21.1 (17)	0.85** (0.87)**
Peak				
Hunger	-2.1	10	24.7	0.85**
Fullness	3.0	7	36.6 (41)	0.51* (0.43)*
Nausea	0.5	69	25.6	0.67**
Drowsy	5.5	96	44.9	0.26*
Satiety	-0.1	6	17.7 (33)	0.87** (0.61)**
Anxiety	9.4	61	56.0	0.08
Desire to eat	-1.3	12	29.1	0.80**
Prospective consumption	0.8	11	29.7	0.74**
Nadir				
Hunger	-0.5	66	5.1 (41)	0.71** (0.54)**
Fullness	0.9	53	35.5	0.47*
Nausea	0.3	51	21.9	0.43*
Drowsy	0.3	54	19.5	0.63**
Satiety	14.3	62	53.6	0.48*
Anxiety	3.0	45	24.5	0.45*
Desire to eat	0.2	74	6.8	0.56**
Prospective consumption	-0.2	53	8.4 (26)	0.72** (0.81)**

Mean difference: mean of (Visit 1- Visit 2). * P < 0.05, ** P < 0.01,

CR: coefficient of repeatability (2sd); r: Pearson product-moment correlation coefficient; CV: coefficient of variation (%)

Data in young adults from Flint, Raben, Blundell & Astrup (2000) (n=55)- presented in italics

Table 4.6 Validity: correlation coefficients of food intake at lunch (kcal) and VAS scores at (1) baseline, (2) 4hr mean, (3) pre-lunch and (4) pre-post difference.

				r ^a
	Visit 1	Visit 2	Mean of Visit 1 & 2	Reported in young adults
Baseline				
Hunger	r = 0.44, P = 0.01*	r = 0.53, P = 0.002**	r = 0.53, P = 0.002**	
Fullness	r = -0.40, P = 0.02*	r = -0.397, P = 0.03*	r = -0.41, P = 0.02*	
Nausea	r = 0.01, P = 0.98	r = 0.06, P = 0.75	r = 0.03, P = 0.89	
Drowsy	r = 0.05, P = 0.78	r = -0.17, P = 0.36	r = -0.05, P = 0.77	
Anxiety	r = -0.07, P = 0.70	r = -0.09, P = 0.61	r = -0.10, P = 0.59	
Satiety	r = -0.11, P = 0.54	r = -0.11, P = 0.55	r = -0.12, P = 0.52	
Desire to eat	r = 0.55, P = 0.001**	r = 0.57, P = 0.001**	r = 0.62, P < 0.0001**	
Prospective consumption	r = 0.58, P < 0.0001**	r = 0.68, P < 0.0001**	r = 0.69, P < 0.0001**	
4hr mean				
Hunger	r = 0.59, P < 0.0001**	r = 0.63, P < 0.0001**	r = 0.67, P < 0.0001**	0.50**
Fullness	r = -0.49, P = 0.004**	r = -0.49, P = 0.005**	r = -0.53, P = 0.002**	-0.52**
Nausea	r = -0.05, P = 0.80	r = 0.05, P = 0.79	r = -0.01, P = 0.95	
Drowsy	r = 0.02, P = 0.93	r = -0.24, P = 0.18	r = -0.12, P = 0.50	
Anxiety	r = 0.02, P = 0.91	r = -0.28, P = 0.13	r = -0.12, P = 0.51	
Satiety	r = -0.27, P = 0.14	r = -0.28, P = 0.12	r = -0.32, P = 0.08	-0.52**
Desire to eat	r = 0.61, P < 0.0001**	r = 0.68, P < 0.0001**	r = 0.68, P < 0.0001**	
Prospective consumption	r = 0.64, P < 0.0001**	r = 0.71, P < 0.0001**	r = 0.71, P < 0.0001**	0.53**
Prelunch				
Hunger	r = 0.43, P = 0.01*	r = 0.46, P = 0.009**	r = 0.46, P = 0.008**	0.32*
Fullness	r = -0.11, P = 0.54	r = -0.42, P = 0.02*	r = -0.297, P = 0.1	-0.43**
Nausea	r = -0.07, P = 0.69	r = 0.13, P = 0.48	r = 0.03, P = 0.86	
Drowsy	r = 0.08, P = 0.67	r = -0.22, P = 0.22	r = -0.14, P = 0.45	
Anxiety	r = 0.25, P = 0.16	r = -0.06, P = 0.76	r = 0.19, P = 0.296	
Satiety	r = -0.07, P = 0.71	r = -0.35, P = 0.05	r = -0.19, P = 0.297	-0.42**
Desire to eat	r = 0.47, P = 0.007**	r = 0.53, P = 0.002**	r = 0.54, P = 0.002**	
Prospective consumption	r = 0.51, P = 0.003**	r = 0.62, P < 0.0001**	r = 0.60, P < 0.0001**	0.39**

continued

				<i>r</i> ^a
	Visit 1	Visit 2	Mean of Visit 1 & 2	Reported in young adults
Pre-post difference				
Hunger	<i>r</i> = 0.44, <i>P</i> = 0.01*	<i>r</i> = 0.46, <i>P</i> = 0.008**	<i>r</i> = 0.46, <i>P</i> = 0.008**	<i>0.33*</i>
Fullness	<i>r</i> = -0.29, <i>P</i> = 0.10	<i>r</i> = -0.41, <i>P</i> = 0.02*	<i>r</i> = -0.42, <i>P</i> = 0.02*	<i>0.40**</i>
Nausea	<i>r</i> = -0.12, <i>P</i> = 0.53	<i>r</i> = 0.29, <i>P</i> = 0.10	<i>r</i> = 0.14, <i>P</i> = 0.46	
Drowsy	<i>r</i> = 0.15, <i>P</i> = 0.41	<i>r</i> = -0.08, <i>P</i> = 0.65	<i>r</i> = 0.09, <i>P</i> = 0.63	
Anxiety	<i>r</i> = 0.23, <i>P</i> = 0.21	<i>r</i> = 0.08, <i>P</i> = 0.68	<i>r</i> = 0.25, <i>P</i> = 0.16	
Satiety	<i>r</i> = -0.23, <i>P</i> = 0.20	<i>r</i> = -0.50, <i>P</i> = 0.003**	<i>r</i> = -0.38, <i>P</i> = 0.03*	<i>0.39**</i>
Desire to eat	<i>r</i> = 0.49, <i>P</i> = 0.005**	<i>r</i> = 0.53, <i>P</i> = 0.002**	<i>r</i> = 0.54, <i>P</i> = 0.001**	
Prospective consumption	<i>r</i> = 0.53, <i>P</i> = 0.002**	<i>r</i> = 0.62, <i>P</i> < 0.0001**	<i>r</i> = 0.62, <i>P</i> < 0.0001**	<i>0.35**</i>

**P* < 0.05, ** *P* < 0.01. Baseline: mean of three ratings taken immediately before breakfast; 4hr mean: mean of ratings between breakfast and lunch (4 hours); pre-lunch: the rating immediately before lunch; pre-post difference: the difference between ratings before and after lunch; *r*: Pearson product-moment correlation coefficient; ^a *Data in young adults from Flint, Raben, Blundell & Astrup (2000) (n=55)- presented in italics*

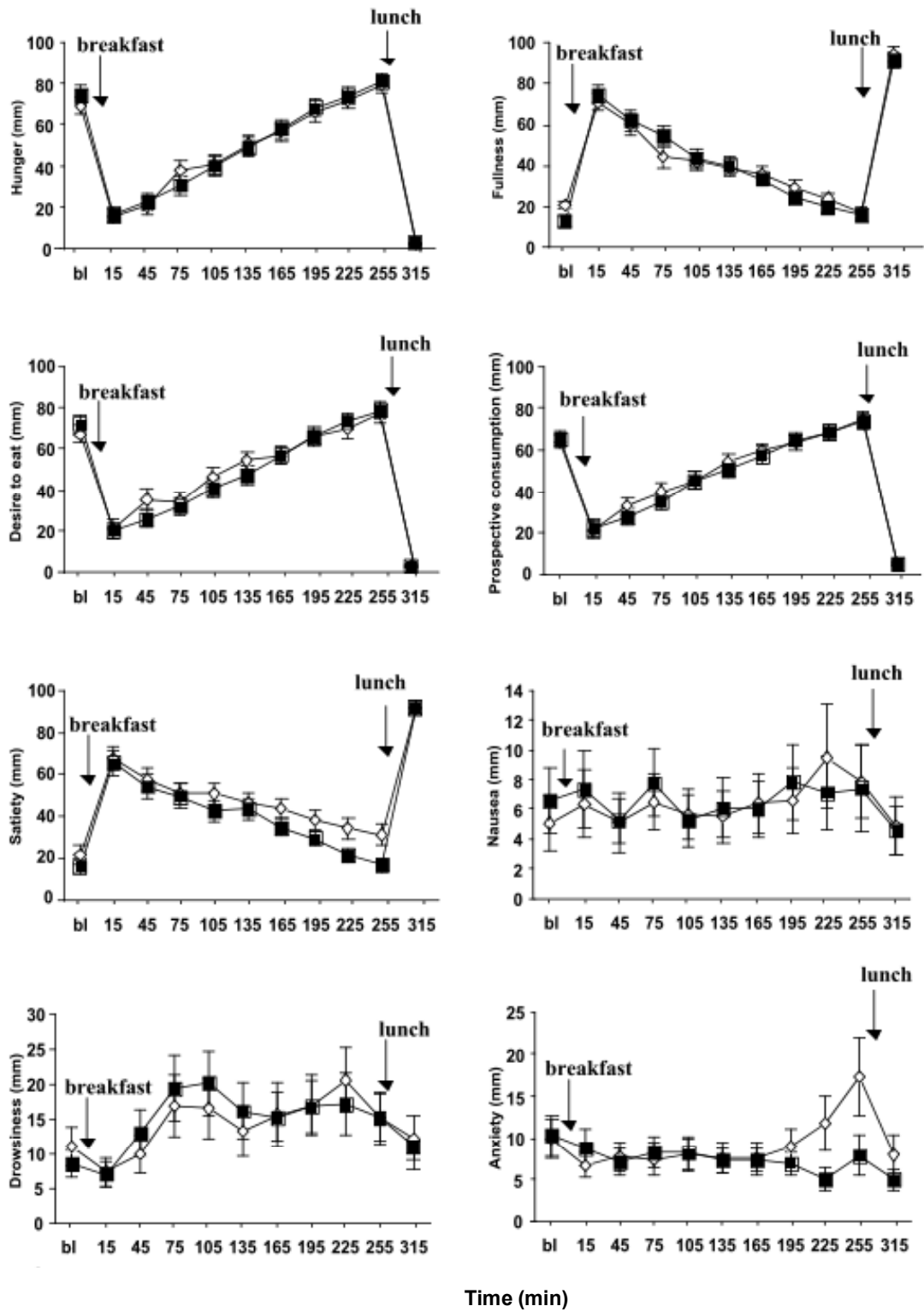


Figure 4.1 Ratings of appetite and ‘non-appetite’ sensations after two identical breakfast test meals in healthy older men and women (n=32). Data presented as means ± SEM. bl = baseline (the mean of three timepoints prior to breakfast). The arrow indicates the initiation of both test meals [breakfast (t = 0 - 15 min) and lunch (t = 255 – 315 min)]. Visit 1 = ■, Visit 2 = (◇).

Chapter 5

Food intake and appetite are related to antral area in healthy young and older subjects

5.1 Summary

The gastrointestinal factors that modulate food intake remain poorly defined. Gastric distension reduces food intake and there is evidence that antral, as opposed to proximal, gastric distension may be the dominant mechanism in the induction of appetite-related sensations. Healthy ageing is associated with reduced appetite. The aim of this study was to examine the effects of different energy preloads on appetite, plasma cholecystokinin, antral area (ultrasound) and subsequent food intake in healthy older and young subjects.

On three separate days, 12 young (23.9 ± 1.4 yr) and 12 older (74.4 ± 1.2 yr) subjects consumed a 400 ml drink containing either 0 kcal (water), 250 kcal or 750 kcal, 70 min before a buffet-style meal. Hunger was less in the older than the young subjects ($P < 0.001$). Both nutrient pre-loads reduced hunger and increased fullness when compared with water ($P < 0.02$), and older subjects were more full than the young ($P < 0.05$). Antral area was greater after the nutrient pre-loads than water ($P = 0.001$), and greater in the older than the young ($P = 0.005$). In both groups, food intake was suppressed in an energy-dependent manner ($P = 0.008$). Plasma cholecystokinin was greater in the older subjects ($P = 0.003$). Immediately before the meal, hunger ($r = -0.59$, $P < 0.001$) and food intake ($r = -0.90$, $P < 0.001$) were inversely, and fullness ($r = 0.66$, $P < 0.001$) directly, related to antral area. Antral area, but not plasma cholecystokinin, was a predictor of subsequent food intake.

In healthy young and older subjects: (i) the suppression of subsequent energy intake by a liquid pre-load is nutrient-dependent and comparable, and (ii) both satiation and satiety are related to antral area, and (presumably) antral distension.

5.2 Introduction

Gastrointestinal factors that may decrease food intake include gastric distension, exposure of small intestine receptors to nutrient, and hormones, such as cholecystokinin (CCK) (Kissileff, Pi-Sunyer et al. 1981; Read, French et al. 1994; MacIntosh, Horowitz et al. 2001). Gastric distension reduces food intake in young and obese subjects (Geliebter 1988; Geliebter, Westreich et al. 1988) and may mediate the effects of gut hormones on food intake (Kissileff, Carretta et al. 2003). Mechanical properties and neural innervation vary in different regions of the stomach (Horowitz and Dent 1991) and it is uncertain whether the site of gastric distension is important in mediating appetite-related sensations. In some studies using gastric balloon distension, the site of distension was not precisely defined (Geliebter 1988; Geliebter, Westreich et al. 1988). In young subjects, distension of the proximal stomach, using a barostat device, increases the perception of fullness (Hebbard, Samsom et al. 1996; Feinle, Grundy et al. 1997), but effects on food intake have not been evaluated. There is also evidence, albeit inconsistent, in both healthy subjects and patients with functional dyspepsia, that energy intake is influenced by proximal gastric accommodation (Tack, Piessevaux et al. 1998; Boeckxstaens, Hirsch et al. 2001). In contrast, observations in young subjects established that the perception of postprandial fullness is closely related to antral content or area, and not to the content of the proximal stomach (Hveem, Jones et al. 1996; Jones, Doran et al. 1997; Santangelo, Peracchi et al. 1998). Hence antral, rather than proximal gastric, distension may be the dominant intragastric mechanism in the induction of appetite-related sensations. The relationship between energy intake and antral area has not been evaluated.

When potential gastrointestinal factors that may contribute to the ‘anorexia of ageing’ are considered, ageing may be associated with an increased sensitivity to the satiating effects of cholecystokinin (CCK) (Masclee, Geusken et al. 1988; MacIntosh, Morley et al. 2001). Moreover, plasma CCK concentrations are increased in both healthy (Masclee, Geusken et

al. 1988; MacIntosh, Andrews et al. 1999) and malnourished (Berthelemy, Bouisson et al. 1992) older subjects. Other gastrointestinal peptides, perhaps particularly peptide YY (Batterham, Cohen et al. 2003), may suppress food intake; however, there does not appear to be any effect of ageing on glucagon-like-peptide-1, gastric inhibitory polypeptide or peptide YY (Masclee, Geusken et al. 1988; MacIntosh, Morley et al. 2001). Fasting plasma ghrelin concentrations are higher in malnourished older subjects than in healthy older and healthy young subjects, but there is no difference between the two healthy groups (Sturm, MacIntosh et al. 2003). Whereas ghrelin may stimulate food intake (Tschop, Smiley et al. 2000), these observations suggest that a reduction in ghrelin activity is not a cause of the anorexia of ageing. The sensitivity of the small intestine to nutrients does not appear to be affected by healthy ageing (Cook, Andrews et al. 1997; MacIntosh, Horowitz et al. 2001). The perception of proximal gastric distension is reduced, rather than increased, in healthy, older subjects (Rayner, MacIntosh et al. 2000). It is unclear whether the effects of oral nutrients on perceptions of appetite or energy intake are modified by ageing (Roberts, Fuss et al. 1994; Rolls, Dimeo et al. 1995; Beckoff, MacIntosh et al. 2001; Chapman, MacIntosh et al. 2002), nor is it known whether these responses can be accounted for by antral area, the overall rate of gastric emptying, plasma CCK, or all of these factors.

The aims of this study were to evaluate in healthy young and older subjects the effects of different energy preloads on appetite, gastric antral area, gastric emptying, plasma CCK concentrations, and the relationships between these parameters with subsequent food intake. The primary hypothesis was that food intake is related to antral area.

5.3 Research design and methods

5.3.1 Subjects

Subjects were recruited according to criteria in Chapter 3.2. The cohort comprised 24 healthy volunteers divided into two groups of 12 each (6M, 6F). The older subjects had a mean (\pm SD) age 74.4 ± 1.2 yr (range: 67-83 yrs) and a body mass index (BMI; kg/m^2) of 24.1 ± 0.5 kg/m^2 (range: 21.1-27.1). The young subjects had a mean age 23.9 ± 1.4 yr

(range: 18-33 yrs) and a BMI of $23.2 \pm 0.6 \text{ kg/m}^2$ (range: 21.0-27.0). No subject was $> 5 \%$ below, or above, his or her ideal body weight (MacIntosh, Horowitz et al. 2001). Each older subject was selected so their BMI was matched to within 1 kg/m^2 of that of a young subject – accordingly, the BMI of the two groups did not differ significantly. The study protocol was approved by the Ethics Committee of the Royal Adelaide Hospital, and each subject gave written, informed consent.

5.3.2 Experimental design

Subjects underwent three studies in randomised order; and each study day was separated by at least a week. Subjects arrived at 0900 after a 12-hour overnight fast (from food and fluid, except water). Subjects were allowed one standard glass of water (200 ml) on the morning of the test, but not within 2 hours of arrival at the laboratory. Ultrasound measurements were used to confirm that the stomach was empty at baseline. On arrival subjects were seated comfortably on a bed (upright, in a relaxed position, so that the angle between the upper and the lower part of the body was $\sim 90^\circ$; with a pillow placed under their knees). An intravenous cannula was placed in an antecubital vein for blood sampling. Subjects then rested for 20 min. At $t = 0$ min each subject drank a 400 ml preload (composition given below) within 10 minutes. The subjects were blinded to the composition of the energy preloads, blinding to the water was not possible. At $t = 70$ min (i.e. 60 min after completion of ingestion of the preload) each subject was offered a standard, buffet-style meal, containing food in excess of what they would normally eat, and invited to eat as much as they wished over 30 minutes until they felt comfortably full ($t = 70$ -100 min). An interval of 60 minutes between the preload and subsequent meal was chosen on the basis of previous studies (Rolls, Kim et al. 1991). Visual analogue scales (VAS) were administered, and venous blood samples were taken, at $t = -10$ min, immediately before and after ingestion of the preload and then every 10 minutes from $t = 10$ min to $t = 160$ min, excluding the period of the buffet meal ($t = 70$ -100 min). Measurements of antral area were made using ultrasound at $t = -10$ min, immediately before and after ingestion of the preload and then

every 5 minutes from $t = 10$ min to $t = 160$ min, excluding the period of the buffet meal ($t = 70$ -100 min). Subjects were monitored over the period $t = 110$ -160 min but the data was not included in the statistical analysis.

5.3.3 Preloads (Table 5.1)

On the control day the preload was 400 ml spring water (0 kcal, room temperature). On the other two days the preloads differed in energy content (250 kcal on the low-dose day and 750 kcal on the high-dose day), but had the same macronutrient distribution (13 % protein, 33 % fat and 54 % carbohydrate). Both nutrient preloads were yoghurt-type drinks of similar odour, taste, palatability, consistency and sweetness. A detailed description of preloads can be found in Chapter 3.5.

5.3.4 Antral area and gastric emptying

Measurements of antral area were performed with an ultrasound machine (Aloka SSD-650 CL, ALOKA Co., LTD. Tokyo) as described in Chapter 3.6.3. Antral area at baseline and at $t = 70$ min (immediately before the start of the buffet meal) were used to evaluate relationships with baseline ratings of appetite and energy intake at the buffet meal, respectively. The time at which antral area had decreased to 75% of maximum (T75%) was used as an index of gastric emptying (Hveem, Jones et al. 1996).

5.3.5 Appetite and food intake

Sensations of hunger and fullness were rated by each subject using 100mm visual analogue scales (VAS), as described previously (Sepple and Read 1989). For further description and validation of VAS see Chapter 3.6.1 and Chapter 4, respectively. Food intake, as assessed by the three-day food diary maintained before entry into the study, and the amount of food consumed at the buffet meal (including its macronutrient distribution), was quantified using Foodworks 2.10, Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia (MacIntosh, Horowitz et al. 2001). For further description of foods and quantification of food intake see Chapter 3.6.1.

5.3.6 Blood glucose, plasma insulin and plasma CCK concentrations

Blood glucose, plasma insulin and plasma cholecystokinin concentrations were measured as previously described in Chapter 3.

5.3.7 Statistical analyses

For visual analogue scores, blood glucose and gastrointestinal hormone concentrations and antral area, baseline was calculated as the mean of $t = -10$ and 0 min values. Comparisons between single characteristics of the two age groups (eg. BMI, baseline symptom scores, and fasting blood hormone concentrations) were performed using one-way analysis of variance (SuperANOVA Version 1.11 (Abacus Concepts Inc., California, USA). Repeated measures analysis of covariance (ANCOVA, SAS Version 8.2 (SAS Institute Inc., SAS, North Carolina, USA), with time ($t = 10-70$ min) and treatment (preloads) as within-subject factors, and age group (older vs young) as a between-subject factor, and baseline as a covariate, was performed for ratings of appetite, blood glucose, plasma insulin and gastrointestinal hormone concentrations and antral area.

Differences in mean food intake and macronutrient content of the buffet meal were analysed by repeated-measures two-way ANOVA, with treatment as the within-subject factor and age group as the between-subject factor. When significant effects were observed, pairwise comparisons of adjusted means were performed using Student's t-test and adjusted for multiple comparisons using the Bonferroni (Holms adjustment, ANCOVA) and stepdown Bonferroni (ANOVA) adjustments. Data following the buffet meal ($t = 110-160$ min) were not analysed, because the amount of food eaten was variable.

Within subject correlations accounting for repeated measures across treatments were calculated for each combination of food intake (kcal) and antral area, CCK, gastric emptying (T75%) and sensations of hunger and fullness using methodology specified by Bland and Altman (1995) (Bland and Altman 1995). When relationships were found

between the above variables, multiple regression analysis was performed to establish determinants of hunger, fullness and energy intake by taking into account effects of other possible determinants (SAS Version 8.2, SAS Institute Inc., North Carolina, USA). Where no significant relationship was observed, results were omitted. Results are shown as means \pm SEM. A P value < 0.05 was considered statistically significant.

5.4 Results

All of the studies were well tolerated without untoward events. Measurements of antral area were technically adequate in all cases. Food intake immediately before the study (3-day food diary) was 23 % lower in the older than in the young subjects (1860 ± 123 kcal/day vs 2430 ± 204 kcal/day, respectively, $P = 0.007$). There were no differences between the two age groups in the proportions of carbohydrate, fat or protein ingested. There was no significant effect of gender on the results (data not shown).

5.4.1 Hunger ratings (Figure 5.1)

5.4.1.1 Effect of preload.

In both age groups there was a significant reduction in hunger ($t =$ baseline to 10 min, treatment effect: $P < 0.001$) after both the low-dose (compared with control, $P < 0.001$) and high-dose (compared with control, $P = 0.019$) preloads without a significant difference between the two nutrient preloads in the magnitude of this reduction ($P = 0.10$). Scores for hunger were significantly lower ($t = 10-70$ min) on both the low-dose ($P = 0.003$) and high-dose ($P < 0.001$) days than on the control day (treatment effect: $P < 0.001$) in both age groups, without any difference between the two nutrient preloads.

5.4.1.2 Effect of buffet meal.

Ingestion of the buffet meal was associated with a reduction in hunger under all three conditions in both groups ($P < 0.001$). The magnitude of the decrease ($t = 100-70$ min) was greater on the control day than on the low-dose ($P < 0.001$) and high-dose ($P < 0.001$) days, without a difference between the two nutrient preloads.

5.4.1.3 Effect of age group.

Hunger ratings were significantly ($P < 0.001$) lower in the older than in the young subjects both at baseline (47.3 ± 4.8 mm vs 68.6 ± 3.8 mm, respectively) and after ingestion of the preloads. The magnitude of the decrease in hunger after the low-dose and high-dose preloads did not differ significantly between the two groups.

5.4.2 Fullness ratings (Figure 5.1)

5.4.2.1 Effect of preload.

Fullness increased significantly in both age groups after ingestion of the preload ($t = \text{baseline-10 min}$, treatment effect: $P < 0.001$); the magnitude of this increase was significantly ($P < 0.001$) greater with the low-dose and high-dose preloads than with the control preload and significantly ($P = 0.03$) greater with the high-dose preload than with the low-dose preload. Scores for fullness were significantly greater on both the low-dose ($P = 0.003$) and high-dose ($P < 0.001$) days than on the control day (treatment effect: $P < 0.001$), and there was a non-significant trend ($P = 0.08$) for this increase to be greater with the high-dose preload than with the low-dose preload.

5.4.2.2 Effect of buffet meal.

Meal ingestion was associated with an increase in fullness in both groups for all three treatments ($P < 0.001$); the magnitude of this increase ($t = 100-70$ min) was greater on the control than on the low-dose ($P = 0.005$) and high-dose ($P < 0.001$) days, without any difference between the two nutrient preloads.

5.4.2.3 Effect of age group.

There was no significant difference in fullness between the two age groups at baseline (older: 16.5 ± 2.6 mm vs young: 15.1 ± 1.8 mm), or after preload ingestion. The magnitude of the increase in fullness differed significantly between the age groups ($P = 0.049$); the older subjects were more full after the preloads. There was no treatment x age group interaction.

5.4.3 Food intake (Figure 5.2)

There was an energy-dependent suppression of food intake by the preloads (treatment effect: $P = 0.008$) in both age groups. The magnitude of this suppression did not differ significantly between the age groups, irrespective of whether food intake was expressed in absolute terms or as a percentage of that on the control day: in the young subjects, the values were $80.4 \pm 3.1\%$ on the low-dose day and $61.1 \pm 4.8\%$ on the high-dose day; in the older subjects, the values were $84.8 \pm 3.2\%$ on the low-dose day and $66.8 \pm 3.7\%$ on the high-dose day. On the control day, food intake was less, albeit not significantly so, in the older than in the young subjects (1077 ± 121 kcal vs 1274 ± 102 kcal, respectively). The macronutrient distribution did not differ significantly between the two groups, nor was there a difference between the three preloads (data not shown).

5.4.4 Antral area and gastric emptying (Figures 5.3 and 5.4)

5.4.4.1 Effect of preload.

There was a significant increase in both age groups in antral area after the preloads ($t = \text{baseline-10 min}$, treatment effect: $P = 0.04$). The magnitude of this increase was significantly greater on the low-dose ($P = 0.001$) and high-dose ($P = 0.002$) days than on the control day, without any difference between the two nutrient preloads. Antral area was greater after ingestion of both the low-dose and high-dose ($P < 0.001$ for both age groups) preloads than of the control, and it tended to be greater after ingestion of the high-dose preload than after the low-dose preload ($P = 0.095$).

5.4.4.2 Effect of age group

There was no difference between the older and young subjects in antral area at baseline (3.8 ± 0.1 cm² vs 3.6 ± 0.1 cm²; respectively). After the preload, antral area was significantly greater in the older than in the young subjects ($P = 0.002$; age group x time interaction: $P = 0.006$), irrespective of the preload. Antral area at $t = 70$ min did not differ between the two

age groups after the control (older: $3.8 \pm 0.3 \text{ cm}^2$ vs young: $3.2 \pm 0.2 \text{ cm}^2$), or after the high-dose pre-load (older: $7.8 \pm 0.5 \text{ cm}^2$ vs young: $7.2 \pm 0.5 \text{ cm}^2$). However, it was significantly ($P = 0.03$) greater in the older subjects than in the young subjects after the low-dose preload ($6.14 \pm 0.26 \text{ cm}^2$ vs young: $5.1 \pm 0.3 \text{ cm}^2$, respectively; treatment effect: $P < 0.001$; effect of age group: $P = 0.005$), even though there was no age group x treatment interaction.

There was an energy-dependent slowing of gastric emptying (T75%) in both groups (treatment effect: $P < 0.001$) but there was no significant difference between the two age groups.

5.4.5 Blood glucose and plasma insulin concentrations (Figure 5.5)

5.4.5.1 Blood glucose concentrations

There was no significant difference between older and young subjects in baseline blood glucose concentrations ($6.0 \pm 0.1 \text{ mmol/l}$ vs $5.9 \pm 0.1 \text{ mmol/l}$; respectively). In both age groups blood glucose concentrations increased significantly ($P < 0.001$) after ingestion of the two nutrient preloads as compared with the control; there was no significant difference between the two nutrient preloads. Whereas the magnitude of the increase in blood glucose concentrations after ingestion of the nutrient preloads did not differ significantly between the two age groups, this increase was more sustained in the older subjects than in the young subjects (low-dose and high-dose compared with control, $P < 0.001$; age group x time x treatment interaction: $P = 0.03$).

5.4.5.2 Plasma insulin concentrations

Plasma insulin concentrations increased significantly ($P < 0.001$) in both age groups after ingestion of the two nutrient preloads as compared with the control, and there was no significant difference in the magnitude of this increase with the two preloads. There was also no significant difference in the magnitude of this increase between the two age groups.

5.4.6 Plasma CCK concentrations (Figure 5.6)

5.4.6.1 Effect of preload.

There was a significant ($P < 0.001$) increase in both age groups in plasma CCK after ingestion of the nutrient preloads as compared with the control. There was no significant difference in the magnitude of this increase between the two nutrient preloads ($P = 0.09$).

5.4.6.2 Effect of age group

Plasma CCK concentrations were significantly higher in the older than in the young subjects at baseline (4.7 ± 0.7 pmol/l vs 2.2 ± 0.3 pmol/l; respectively: $P = 0.003$) and after preload ingestion ($P = 0.001$). The magnitude of the increase in plasma CCK immediately after ingestion of the nutrient pre-loads did not differ significantly between the two age groups.

5.4.7 Relations between appetite, food intake, plasma CCK and antral area

5.4.7.1 Hunger and fullness vs antral area (Figure 5.7, Table 5.2)

There was no relationship between baseline scores of hunger or fullness with baseline antral area (data not shown). There were significant inverse relationships between the score for hunger at $t = 70$ min and the mean score for hunger (mean $t = 10-70$ min) with both antral area at $t = 70$ min (i.e., immediately before the buffet meal) and the mean antral area (mean $t = 10-70$ min). There were significant positive relationships between the score for fullness at $t = 70$ min and the mean score for fullness (mean $t = 10-70$ min) with both antral area at $t = 70$ min and mean antral area (mean $t = 10-70$ min).

5.4.7.2 Food intake vs antral area (Figure 5.8, Table 5.2)

There were significant inverse relationships between food intake at the buffet meal and antral area at $t = 70$ min and between food intake and antral area at $t = 100$ min (i.e. at the end of the buffet meal). Similarly, there were significant relationships between food intake

at the buffet meal and the magnitude of increase ($t = 100-70$ min) in antral area (data not shown). There was also a significant inverse relationship between food intake at the buffet meal and gastric emptying (T75%).

5.4.7.3 Hunger and fullness vs CCK (Table 5.2)

After ingestion of the preloads, there was an inverse relationship between plasma CCK concentrations and scores for hunger, and there was a positive relationship between plasma CCK concentrations and scores for fullness. There was also an inverse relationship between food intake at the buffet meal and plasma CCK at $t = 70$ min.

5.4.7.4 Predictors of hunger, fullness and food intake (Table 5.3)

Multiple regression analysis of the combined data revealed that both antral area and plasma CCK concentrations at $t = 70$ min were predictors of hunger. In addition, it showed that antral area at $t = 70$ min was the only predictor of fullness, and that both antral area at $t = 70$ min and mean antral area (between $t = 10-70$ min) were predictors of food intake.

5.5 Discussion

This study evaluated the effects of liquid preloads of varying energy content on perceptions of hunger and fullness (satiation), antral area, plasma cholecystokinin and subsequent food intake (satiety), in healthy young, and older, adults. It has confirmed that: (i) in young adults antral area, gastric emptying, and food intake are dependent on the nutrient content of a preload and that satiety is inversely related to antral area (Hunt, Smith et al. 1985; Jones, Doran et al. 1997; Santangelo, Peracchi et al. 1998) and (ii) ageing is associated with a reduction in preprandial hunger (Wurtman, Lieberman et al. 1988; Clarkston, Pantano et al. 1997; MacIntosh, Horowitz et al. 2001), and an increase in plasma cholecystokinin (Maslee, Geusken et al. 1988; MacIntosh, Andrews et al. 1999; MacIntosh, Morley et al. 2001). Novel observations are that in older subjects after ingestion of a nutrient-containing liquid: (i) antral area is greater than that in young subjects; (ii) hunger is inversely, and fullness directly, related to antral area; and (iii) the suppression of hunger and food intake is

comparable to that observed in the young. However, arguably the most important observation is that in both age groups satiety was related to antral area (and presumably, antral distension), so that preprandial antral area accounted for ~34 % of the variance in subsequent food intake.

In considering the gastrointestinal mechanisms involved in the regulation of appetite, it is important to make a distinction between satiation and satiety. Satiation refers to the process that controls the size of a meal by terminating the period of eating, whereas satiety can be described as the state following a meal during which hunger is dampened and the urge to consume food is inhibited (Blundell 1991). The effects of oral nutrients on both postprandial fullness (Bergmann, Chassany et al. 1992; Horowitz, Jones et al. 1993; Benini, Brighenti et al. 1994; Jones, Doran et al. 1997) and subsequent food intake (Beckoff, MacIntosh et al. 2001) correlate relatively poorly with the overall rate of gastric emptying (which of necessity is associated with distension of both the proximal and distal stomach), supporting the concept that the site of gastric distension may be important in triggering both satiation and satiety. Gastric distension with a balloon suppresses food intake, but in these studies the positioning of the balloon was not precise (Geliebter 1988; Geliebter, Westreich et al. 1988).

More recent studies of the relationship between upper gastrointestinal sensations (including those of appetite) and gastric distension have focussed on proximal gastric motor and sensory function, assessed with a barostat technique, with a particular emphasis on the pathophysiology of functional dyspepsia (Hebbard, Samsom et al. 1996; Feinle, Grundy et al. 1997; Tack, Piessevaux et al. 1998; Boeckxstaens, Hirsch et al. 2001). A limitation of these studies is the recognition that the barostat balloon, when positioned in the proximal stomach, may affect distal stomach motility (Mundt, Hausken et al. 2002; Mundt, Samsom et al. 2002). An alternative possibility, that the distal stomach has an important role in the aetiology of appetite related sensations, dyspeptic symptoms and food intake, has hitherto received little attention.

Our group initially reported in young, healthy subjects that after ingestion of a glucose drink the perception of fullness is closely related to antral area measured ultrasonographically, as well as the content of the distal stomach measured by scintigraphy, but not to the content of the proximal stomach (Jones, Doran et al. 1997); similar observations were made subsequently by others using solid meals (Santangelo, Peracchi et al. 1998). Moreover, in a recent study, reported in abstract form, in which gastric volume was quantified with three-dimensional ultrasound in healthy young subjects, the perception of fullness after a drink was closely related to antral size relative to total gastric volume (Mundt, Samsom et al. 2002). It is of interest that functional dyspepsia is associated with a wide antrum and increased antral filling (Hausken and Berstad 1992; Scott, Kellow et al. 1993; Troncon, Bennett et al. 1994). Accordingly, the current study confirms that satiation is related to antral distension in young subjects and establishes that this is also the case in healthy older subjects. The relationship between subsequent food intake and antral area or content has not been evaluated previously.

Our group (Hveem, Jones et al. 1996) and others (Holt, Cervantes et al. 1986; Marzio, Giacobbe et al. 1989) have established that there is a close concordance between scintigraphic and ultrasound measurements of liquid gastric emptying, although the latter is derived from antral area. In normal subjects after ingestion of solid (Collins, Houghton et al. 1991) and nutrient liquid (Hveem, Jones et al. 1996) meals, antral content is relatively stable for 30-60 min, while there is a progressive reduction in the content of the proximal stomach (Malbert, Mathis et al. 1997). Although the demonstrated correlations do not necessarily imply causality, our observations suggest that antral distension, rather than the overall rate of gastric emptying or the content of the proximal stomach, is a major determinant of satiety, as well as satiation. The mechanisms mediating the effects of antral distension on satiation and satiety also remain to be defined. The perception of 'fullness' is likely to, at least in part, reflect the activation of gastric "stretch" receptors by gastric

distension (Blackshaw and Grundy 1993); there are known to be substantial variations in patterns of mechanoreceptor activity within the stomach (Andrews, Grundy et al. 1980). A number of hormones, which may affect energy intake, are released from the antrum.

In healthy, young subjects oral nutrients suppress subsequent food intake (Read, French et al. 1994; Rolls, Dimeo et al. 1995) and the rate of gastric emptying is dependent on the energy content of a meal (Hunt, Smith et al. 1985; Horowitz and Dent 1991). Healthy ageing is associated with abnormalities in a number of gastrointestinal mechanisms that are potentially relevant to appetite regulation, including diminished perception of proximal gastric distension (Rayner, MacIntosh et al. 2000), delayed gastric accommodation (Rayner, MacIntosh et al. 2000), an increased satiating effect of small intestinal glucose when compared to lipid (MacIntosh, Horowitz et al. 2001) as well as increased stimulation of phasic pyloric pressure waves by intraduodenal lipid (Cook, Andrews et al. 1997). Studies by ourselves and others (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997) suggest that ageing may also be associated with slowing of gastric emptying, but the magnitude of any difference is small, and observations have been inconsistent (Tougas, Eaker et al. 2000). It is, therefore, not surprising that no difference in gastric emptying between the two age groups was evident in this study. We have, however, demonstrated that after a nutrient preload antral area is greater in older subjects and this may potentially account for the increase in postprandial fullness. The mechanism(s) underlying the increase in antral area is unknown.

The effect of oral nutrients on food intake has the potential to offer insights into the pathophysiology of the anorexia of ageing, as well as potential approaches to its treatment. As in previous studies (MacIntosh, Morley et al. 2001), hunger was less in the older than subjects; the absence of a significant difference in food intake is likely to represent a type 2 error – immediately before the study food intake was some 23 % lower in older subjects. We have demonstrated that there is a strong correlation between hunger, as assessed by

visual analogue questionnaires, and subsequent food intake in healthy older subjects (Parker, Sturm et al. 2004). Rolls et al (Rolls, Dimeo et al. 1995) reported that energy compensation in response to different yoghurt pre-loads is less precise in older than young men, so that the suppression of food intake is relatively greater in the young.

Other studies also suggest that the precision of energy intake regulation declines with age (Roberts, Fuss et al. 1994). In contrast, Zandstra et al (Zandstra, Mathey et al. 2000), reported that the capacity to regulate food intake after a nutrient pre-load does not differ between children, young adults and older subjects, consistent with our observations. These discrepant results may be attributable to differences in study design, particularly the volume and composition of the preload(s) and the time interval between the preload and the meal. For example, in the study by Rolls et al (Rolls, Dimeo et al. 1995) the time period between the preload and the test meal was 30 minutes compared to 90 minutes in the study by Zandstra et al (Zandstra, Mathey et al. 2000) and 60 minutes in our study.

Cholecystokinin, which is released from the small intestine after ingestion of nutrients appears to stimulate both satiation and satiety (Kissileff, Pi-Sunyer et al. 1981; Lieverse, Jansen et al. 1994; MacIntosh, Morley et al. 2001; Chapman, MacIntosh et al. 2002; Kissileff, Carretta et al. 2003). For example, in young adults intravenous infusion of CCK in 'physiological' concentrations results in a dose-dependent suppression of energy intake (MacIntosh, Morley et al. 2001), which may be mediated by stimulation of vagal afferent activity, slowing of gastric emptying and/or an increase in gastric 'sensitivity' (Kissileff, Pi-Sunyer et al. 1981; MacIntosh, Morley et al. 2001; Kissileff, Carretta et al. 2003). In young subjects the suppression of food intake by exogenous CCK is enhanced by gastric distension (Kissileff, Carretta et al. 2003).

We have confirmed that both fasting and postprandial CCK is increased in healthy, older subjects (Khalil, Walker et al. 1985; Masclee, Geusken et al. 1988; MacIntosh, Andrews et al. 1999; MacIntosh, Morley et al. 2001). As it is clear that the effect of exogenous CCK on

appetite is maintained (and may be increased) in older subjects (MacIntosh, Morley et al. 2001), this is consistent with a role for CCK in the anorexia of ageing. The observed increase in blood glucose and plasma insulin concentrations after oral nutrients in older when compared to young subjects is indicative of the impaired glucose tolerance and insulin resistance associated with ageing. While a role for either glucose or insulin in the anorexia of ageing cannot be discounted, this appears unlikely (Chapman, Goble et al. 1998; Russell, Horowitz et al. 2001).

5.6 Conclusion

This study has confirmed previous findings in young people that antral area and food intake are dependent on the nutrient content of a preload, and that ageing is associated with a reduction in fasting hunger and an increase in plasma CCK concentrations. In addition, despite a difference in antral area between older and young subjects following nutrient preloads, satiety is related to antral area in both age groups. Further studies are required to confirm the contribution of gastrointestinal mechanisms in age-related changes in appetite and food intake.

Table 5.1 Composition of low (250 kcal) and high (750 kcal) energy preloads

Foods	Low dose Energy (kcal)	High dose Energy (kcal)	% Protein	% CHO	% Fat	% Others
Total	263	745	13	54	33	
Yoghurt Greek Style (high fat) ^a	86	282	18	28	50	4
Yoghurt (reduced fat, plain) ^b	13	41	29	41	21	9
Cornflour ^c	19	62	0	93	1	6
Cream (Fat > 35%) ^d	10	20	2	3	95	0
Golden syrup ^e	44	139	1	99	0	0
Sunflower oil ^f	27	80	0	0	100	0
Promod® (protein powder) ^g	N/A	23	0	0	0	0
Nonfat skim milk ^h	29	N/A	42	51	2	5
Glucose powder	27	98	0	97	0	3
Water	0	N/A	0	0	0	0
Gelatine ⁱ	6	N/A	100	0	0	0
Aspartame 951 (artificial sweetener) ^k	2	N/A	3	91	0	6

Calculated using Foodworks Version 2.10, Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia; Others, percentage of energy due to organic acids, fibre, alcohol etc; CHO, carbohydrate; ^a Australia Co-operative Foods Ltd, Dairy Farmers, Birnie Ave, Lidcombe NSW 2141; ^b Traditional Natural yoghurt 99.8% Fat Free, Australia Co-operative Foods Ltd, Dairy Farmers, Birnie Ave, Lidcombe NSW 2141; ^c White Wings Foods, 75 Talavera Rd, Macquarie Park, NSW 2133, Australia; ^d Pura Cream, National Foods Ltd, 5 Queens Rd, Melbourne, Victoria 3004, Australia; ^e Smiths, 399 Archerfield Rd, Richlands, Brisbane 4077, Australia; ^f Meadow Lea Foods Ltd, 514 Gardeners Rd, Mascot NSW 2020, Australia; ^g Ross Products Division, Abbott Laboratories, Columbus, Ohio USA; ^h Pura Skimmer, Farmers Union, National Foods Ltd, 5 Queens Rd, Melbourne, Victoria 3004, Australia; ⁱ Davis Gelatine Australia Pty Ltd, Sunny Hills, Flood Road, Josephville via Beaudesert QLD 4285; ^k Merisant Australia Pty Ltd, 20 Clarke St, Crows Nest, NSW 2065 Australia.

Table 5.2 Relationships between appetite scores, antral area (AA), gastric emptying and plasma cholecystokinin (CCK) in older (n = 12) and young (n = 12) subjects

	r	P
Relationship between hunger and AA, gastric emptying and CCK		
Hunger (at 70 min) vs AA (at 70 min)	-0.59	<0.0001
Hunger (at 70 min) vs AA (10–70 min)	-0.59	<0.0001
Hunger (10–70 min) vs AA (at 70 min)	-0.63	<0.0001
Hunger (10–70 min) vs AA (10–70 min)	-0.68	<0.0001
Hunger (at 70 min) vs T75%	-0.39	0.006
Hunger (10–70 min) vs T75%	-0.33	0.02
Hunger (at 70 min) vs CCK (at 70 min)	-0.54	<0.0001
Hunger (at 70 min) vs CCK (10–70 min)	-0.59	<0.0001
Hunger (10–70 min) vs CCK (at 70 min)	-0.63	<0.0001
Hunger (10–70 min) vs CCK (10–70 min)	-0.67	<0.0001
Relationship between fullness and AA, gastric emptying and CCK		
Fullness (at 70 min) vs AA (at 70 min)	0.66	<0.0001
Fullness (at 70 min) vs AA (10–70 min)	0.61	<0.0001
Fullness (10–70 min) vs AA (at 70 min)	0.73	<0.0001
Fullness (10–70 min) vs AA (10–70 min)	0.69	<0.0001
Fullness (at 70 min) vs T75%	0.31	0.03
Fullness (10–70 min) vs T75%	0.39	0.005
Fullness (at 70 min) vs CCK (at 70 min)	0.54	<0.0001
Fullness (at 70 min) vs CCK (10–70 min)	0.58	<0.0001
Fullness (10–70 min) vs CCK (at 70 min)	0.67	<0.0001
Fullness (10–70 min) vs CCK (10–70 min)	0.71	<0.0001
Relationship between food intake and AA, gastric emptying, CCK and hunger and fullness		
Food intake vs AA (at 70 min)	-0.90	<0.0001
Food intake vs AA (10–70 min)	-0.83	<0.0001
Food intake vs T75%	-0.51	0.0002
Food intake vs CCK (at 70 min)	-0.66	<0.0001
Food intake vs CCK (10–70 min)	-0.66	<0.0001
Food intake vs hunger (at 70 min)	0.60	<0.0001
Food intake vs hunger (10–70 min)	0.62	<0.0001
Food intake vs fullness (at 70 min)	-0.66	<0.0001
Food intake vs fullness (10–70 min)	-0.75	<0.0001

T75%, time at which antral area had decreased to 75% of maximum, a measure of gastric emptying; food intake, food intake (kcal) at test meal; (10-70 min), mean of 7 time points immediately following preload up to and including immediately before test meal; (at 70 min), value at 70 minutes immediately prior to test meal; r, within subjects correlations accounting for repeated measures across treatments (SAS Institute Inc., North Carolina, USA). Significance accepted at P<0.05.

Table 5.3 Predictors of food intake (kcal), hunger and fullness derived from multiple regression analysis in older (n = 12) and young (n = 12) subjects.

Variable	Predictor	Combined	
		β	P
Hunger	AA (at 70 min)	-0.26	0.03
	CCK (at 70 min)	-0.37	0.002
Fullness	AA (at 70 min)	0.64	0.0001
Food intake	AA (at 70 min)	-1.1	0.0001
	AA (10-70 min)	0.54	0.005

AA, antral area; CCK, cholecystokinin; Food intake, food intake (kcal) at test meal; (10-70 min), mean of 7 time points immediately following preload up to and including immediately before test meal; (at 70 min), value at 70 minutes immediately prior to test meal; Multiple linear regression analysis to derive Pearson correlation estimates using Statview Version 5.0 (SAS Institute Inc., North Carolina, USA). Significance accepted at $P < 0.05$.

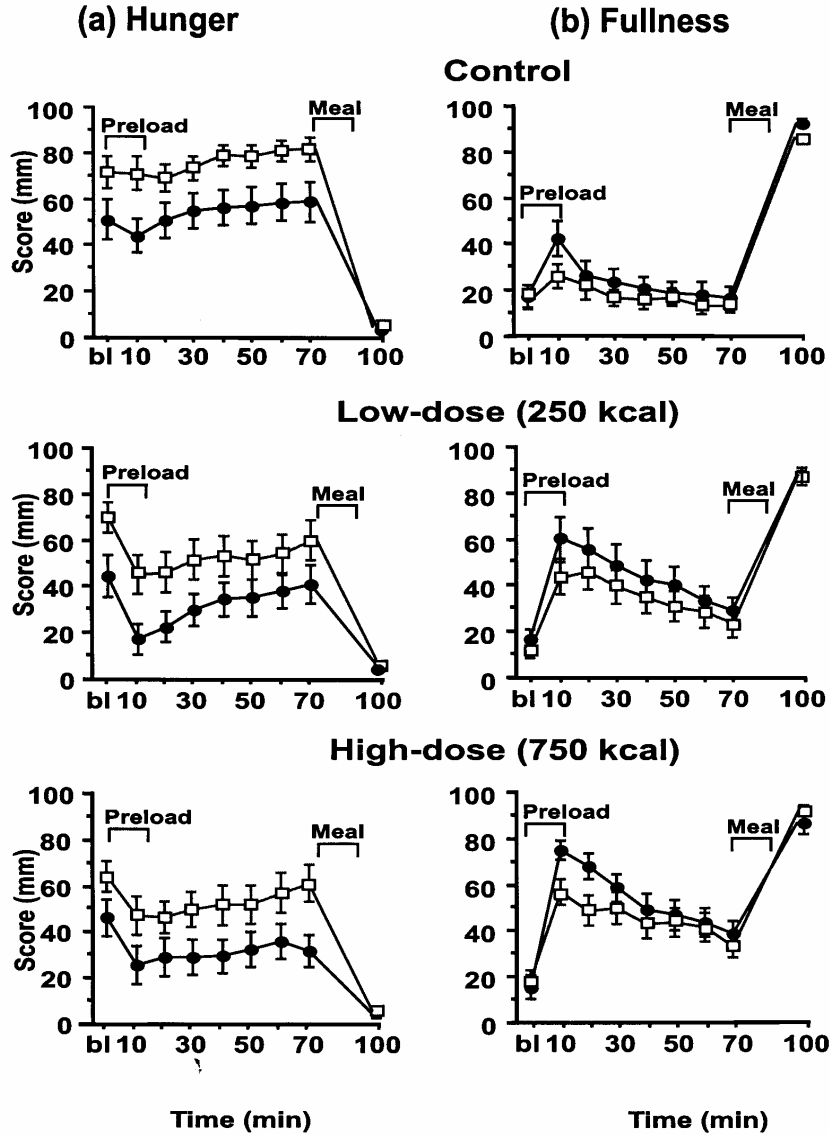


Figure 5.1 Mean \pm SEM absolute ratings of (a) hunger and (b) fullness, in young ($n = 12$, \square) and older ($n = 12$, \bullet) subjects who received preloads of either water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Analysed by repeated-measures analysis of covariance with baseline (bl) as a covariate. (a) At baseline, hunger ratings were significantly ($P < 0.001$) lower in the older than in the young subjects. In both age groups, there was a significant reduction in hunger after the nutrient preloads compared to control ($P < 0.001$) but no significant difference between the two nutrient preloads. (b) There was no significant difference in fullness ratings between the two age groups at baseline. There was a significant difference in fullness ratings between the age groups after the preloads ($P = 0.049$); the older subjects had greater fullness ratings. In both age groups, ratings for fullness were significantly greater after the nutrient preloads than after the control (treatment effect: $P < 0.001$; low-dose compared with control, $P = 0.003$; high-dose compared with control, $P < 0.001$), and there was a trend for the magnitude of this increase to be greater with the high-dose preload than with the low-dose preload ($P = 0.08$).

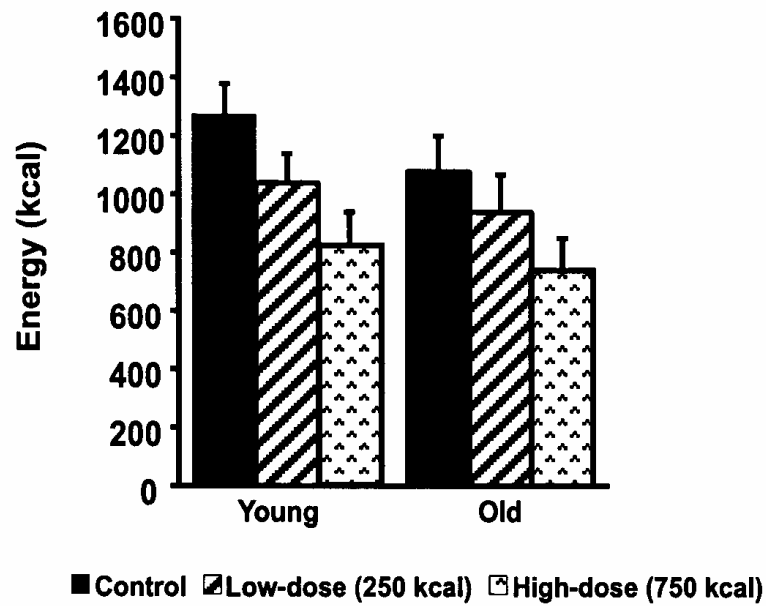


Figure 5.2 Mean \pm SEM differences in food intake (kcal) at the buffet meal between young ($n = 12$) and older ($n = 12$) subjects who received preloads of water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Analysed by repeated-measures two-way ANOVA with stepdown Bonferroni correction to adjust for multiple comparisons. There was no age \times treatment effect. In both age groups there was an energy-dependent suppression of food intake by both nutrient preloads in comparison with the control (treatment effect: $P = 0.008$). The magnitude of this suppression did not differ between the age groups.

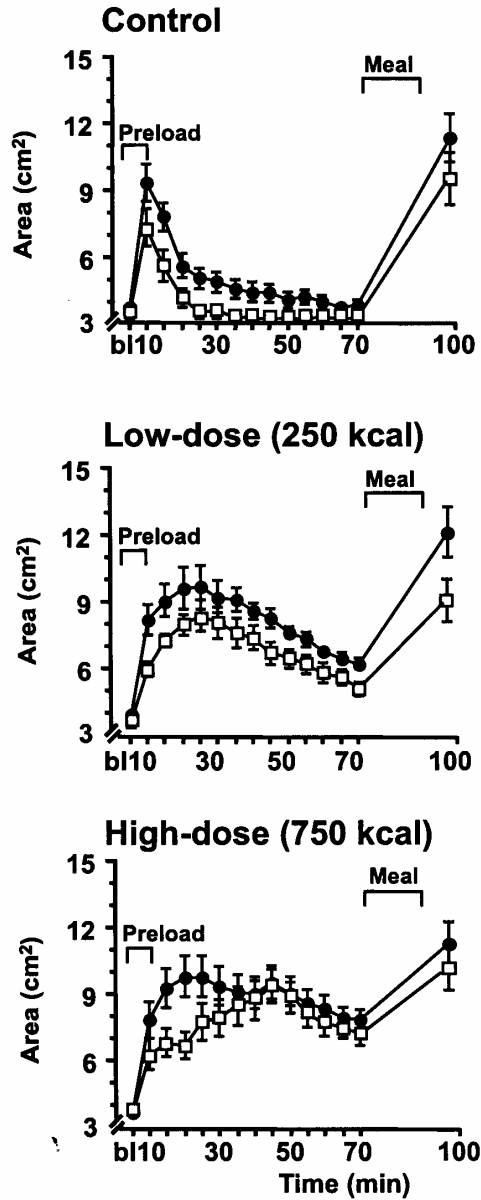


Figure 5.3 Mean \pm SEM antral area, in young ($n = 12$, \square) and older ($n = 12$, \bullet) subjects who received preloads of water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Analysed by repeated-measures analysis of covariance with baseline (bl) as a covariate. There was no significant difference in antral area at baseline between the older and young subjects. After nutrient preload ingestion, antral area was significantly greater in both age groups ($P < 0.001$) than after ingestion of the control. Antral area was significantly greater in the older than the young subjects after preload ingestion (age group \times time interaction: $P = 0.0006$) irrespective of the preload.

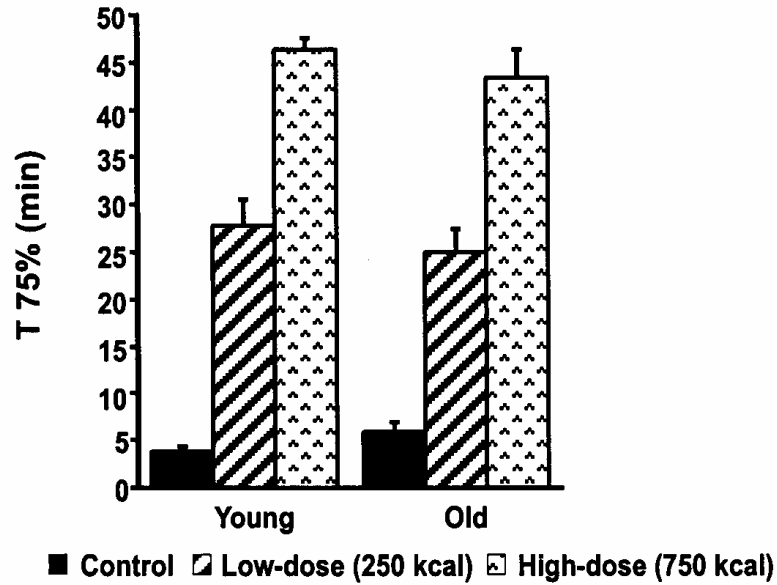


Figure 5.4 Mean \pm SEM gastric emptying (T75%: time for antral area to decrease to 75 % of maximum) between young ($n = 12$) and older ($n = 12$) subjects who received preloads of water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Analysed by repeated-measures two-way ANOVA with stepdown Bonferroni correction to adjust for multiple comparisons. There was no age \times treatment effect. There was an energy- dependent slowing of gastric emptying (T75%) in both groups after the low- and high- dose preloads as compared with T75% after the control (treatment effect: $P < 0.001$); there was no significant difference between the age groups.

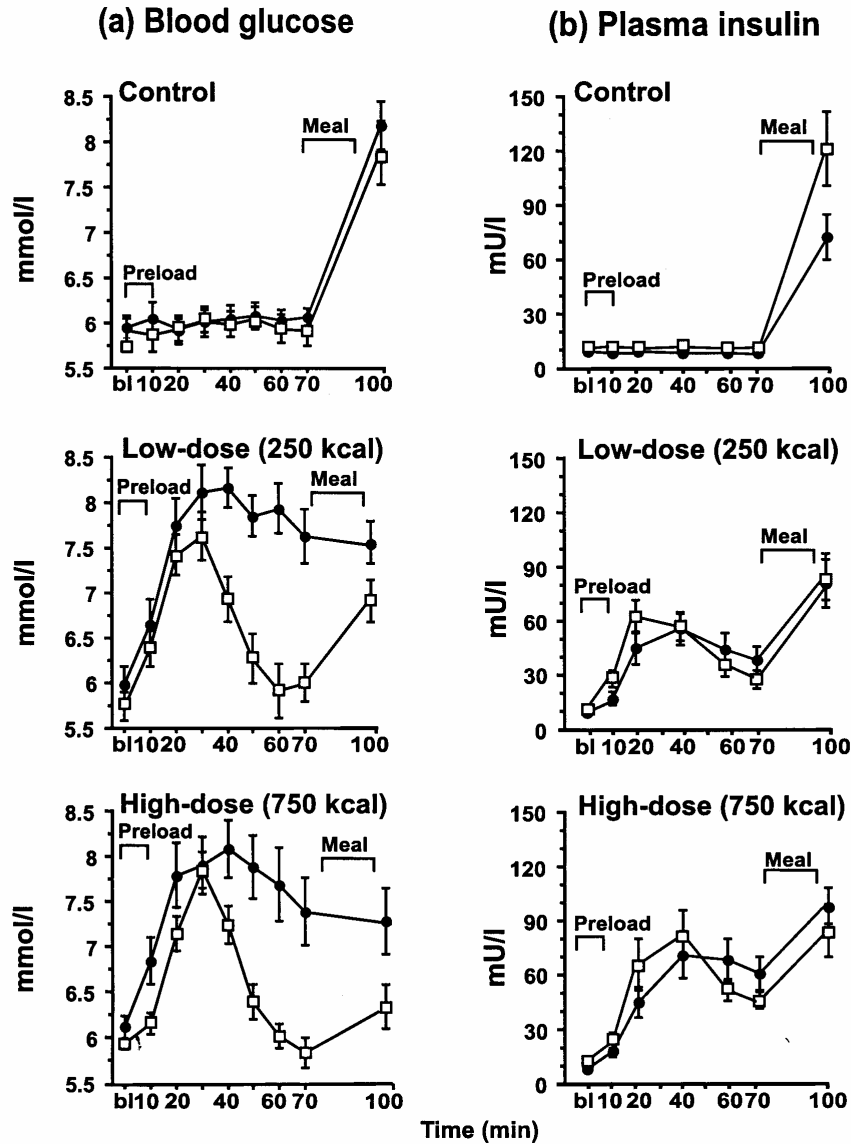


Figure 5.5 Mean \pm SEM (a) blood glucose concentrations (mmol/l) and (b) plasma insulin concentrations (mU/l), in young ($n = 12$, \square) and older ($n = 12$, \bullet) subjects who received preloads of either water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Analysed by repeated-measures analysis of covariance with baseline (bl) as a covariate. (a) There was no significant difference in baseline blood glucose concentrations between older and young subjects. In both age groups, blood glucose concentrations increased after ingestion of the two nutrient preloads. The magnitude of this increase did not differ significantly between the two age groups, but this increase was more sustained in the older than in the young subjects (treatment effect: $P < 0.001$; low-dose and high-dose compared with control, $P < 0.001$; age group \times time interaction \times treatment interaction: $P = 0.03$). (b) Plasma insulin concentrations increased in both age groups after ingestion of the two nutrient preloads as compared with control ($P < 0.001$), and there was no significant difference between the two age groups in the magnitude of this increase.

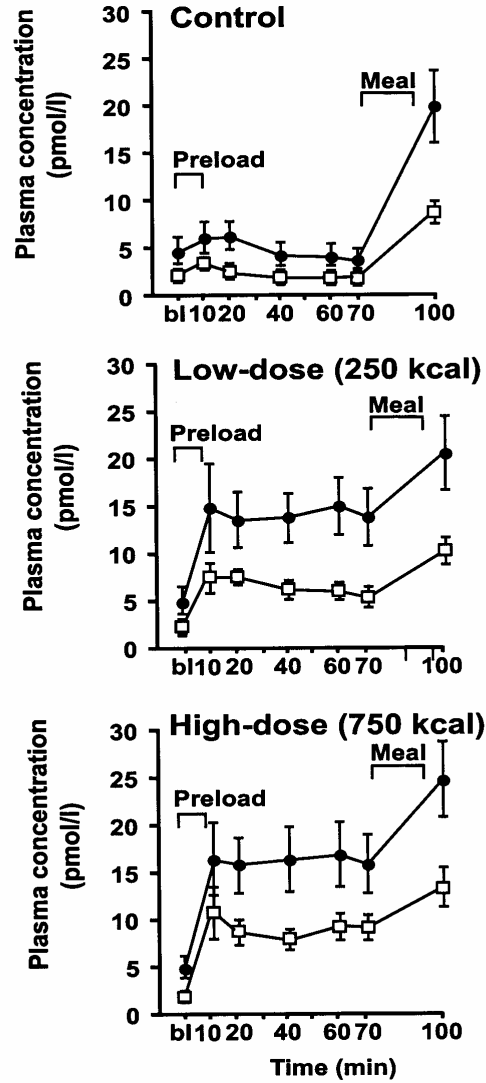


Figure 5.6 Mean \pm SEM plasma CCK concentrations (pmol/l) in young ($n = 12$, \square) and older ($n = 12$, \bullet) subjects who received preloads of water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Analysed by repeated-measures analysis of covariance with baseline (bl) as a covariate. Baseline CCK concentrations were significantly higher in the older than in the young subjects ($P = 0.003$). After preload ingestion CCK concentrations were significantly higher in the older than in the young subjects for all three treatments ($P = 0.001$). In both age groups there was a significant ($P < 0.001$) increase in plasma CCK concentrations after the nutrient preloads as compared with the control, there was no significant difference in the magnitude of this increase between the two nutrient preloads ($P = 0.09$).

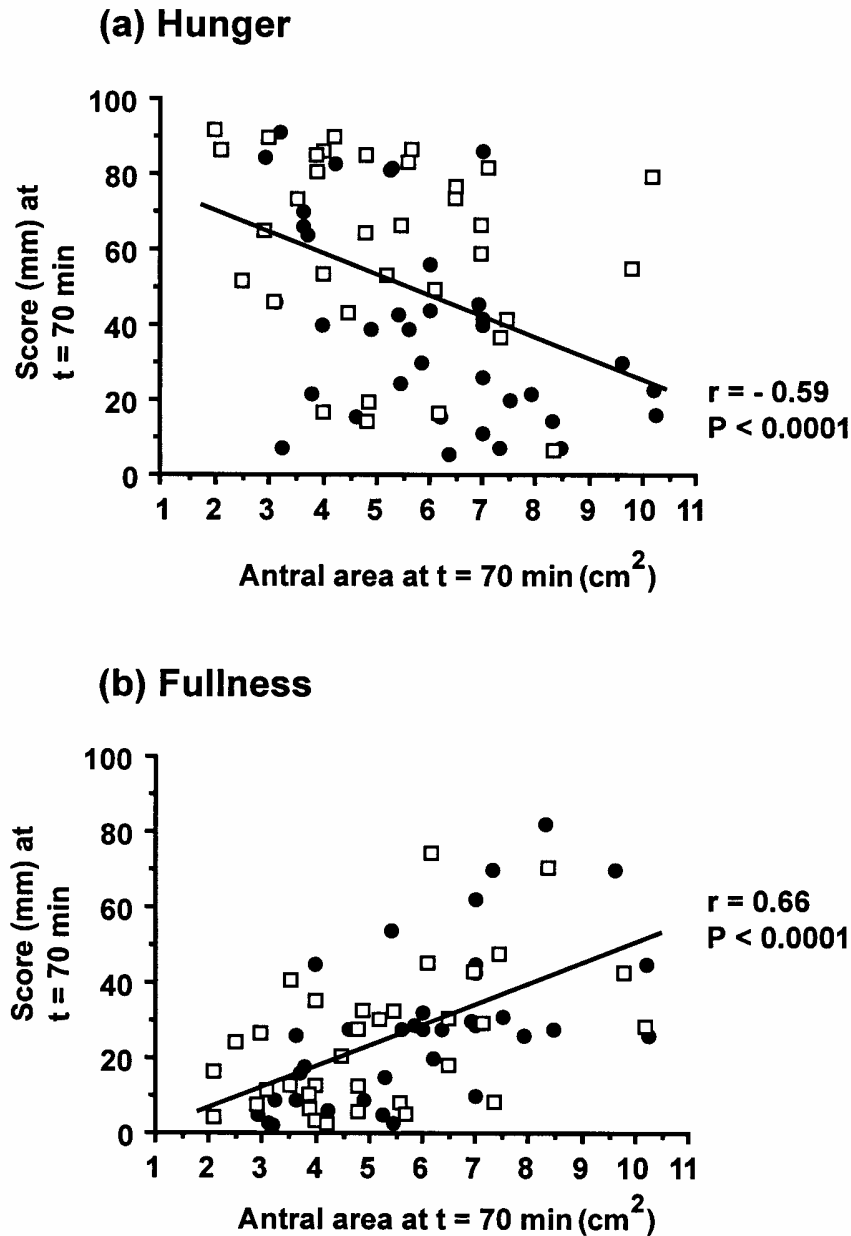


Figure 5.7 Relationship between (a) hunger and (b) fullness at t = 70 min (immediately before the buffet meal) with antral area (cm²) at t = 70 min in young (n = 12, □) and older (n = 12, ●) subjects who received preloads of water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Within-subject correlations accounting for repeated measures across treatments were calculated for each combination of antral area and sensations of hunger and fullness by using the method of Bland & Altman (1995) (Bland and Altman 1995). (a) There were significant inverse relationships between hunger ratings and antral area ($r = -0.59$, $P < 0.0001$). (b) There were significant relationships between ratings of fullness and antral area ($r = 0.66$, $P < 0.0001$).

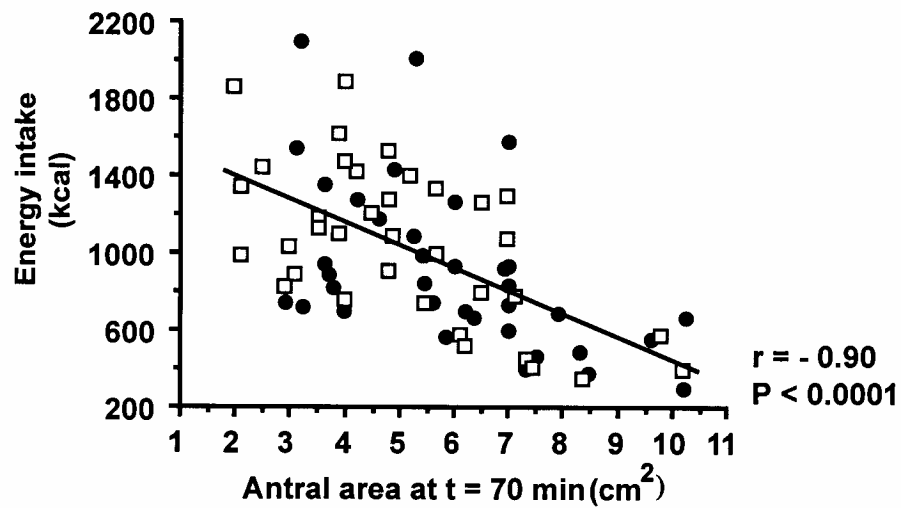


Figure 5.8 Relationship between food intake at a buffet meal (kcal) and antral area (cm²) at $t = 70$ min (immediately before the buffet meal) in young ($n = 12$, \square) and older ($n = 12$, \bullet) subjects who received preloads of water (0 kcal, control), 250 kcal (low-dose) or 750 kcal (high-dose). Within-subject correlations accounting for repeated measures across treatments were calculated for each combination of food intake (kcal) and antral area by using the method of Bland and Altman (1995) (Bland and Altman 1995). There were significant inverse relationships between food intake at the buffet meal and the premeal antral area ($r = -0.90$, $P < 0.0001$).

Chapter 6

Effects of intra-gastric carbohydrate and fat on food intake and appetite in healthy older and young men.

6.1 Summary

Healthy ageing is associated with decreased appetite and reduced energy intake, predisposing to the development of under-nutrition in the elderly. The mechanisms responsible for these effects of ageing are poorly understood, but may include alterations in gastrointestinal responses to food ingestion.

Twelve older (72.8 ± 1.1 yrs) and 12 young (22.3 ± 0.9 yrs) healthy, normal weight men received, on separate days in single-blind, randomized fashion, one of three equivo-laemic (360 ml) intra-gastric infusions of water (control), glucose (carbohydrate) and fat (both 343 kcal), delivered over 5 minutes. Food intake was assessed at a buffet meal served 45 minutes later. Subjects were invited to eat freely for up to 30 minutes until they were comfortably full. These results were compared to those from a previous study in which identical equi-energetic, equivo-laemic fat and carbohydrate solutions and saline (control) were infused over 120 minutes into the small intestine of older and young men.

In the intra-duodenal study ratings of appetite were evaluated in response to the three infusions, and food intake determined from a buffet meal served at the conclusion of the infusions. Fat inhibited food intake significantly more than carbohydrate in the young men (26% vs 5%, $P < 0.001$) whilst the suppressive effects of fat and carbohydrate were similar in the older men (21% vs 22%, $P = 0.05$). Hunger ratings were lower both at baseline and throughout the three infusions in older than young men, but there was no effect of the infusions on fullness ratings in either age group.

In the present study, intra-gastric carbohydrate infusion suppressed food intake compared to control significantly more than fat infusion (23 vs 10%, $P = 0.005$), and this was so in both young (25 vs 14% $P = 0.03$) and older (21 vs 7% $P = 0.05$) men. Hunger ratings were unaffected by nutrient infusions in either age group. Older men were more full following the carbohydrate than fat infusion ($P = 0.04$), whereas there was a trend for young men to be more full after the fat than carbohydrate infusion (age \times treatment effect, $P = 0.054$).

Infusion of carbohydrate into the stomach is significantly more suppressive of food intake than fat in both older and young men, whereas when delivered into the duodenum fat is more suppressive than carbohydrate in young subjects and equally suppressive in older subjects. Ageing appears to affect the response to carbohydrate but not fat, with an enhancement of the satiating effects of intraduodenal carbohydrate in older men. The causes and implications of these differences require further study.

6.2 Introduction

Appetite regulation is affected by ageing (Clarkston, Pantano et al. 1997; Morley 1997; Donini, Savina et al. 2003) and healthy ageing is associated with a reduced perception of gastric distension (Rayner, MacIntosh et al. 2000) and slight slowing of gastric emptying (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997). Healthy older people tend to be less hungry (Wurtman, Lieberman et al. 1988; MacIntosh, Horowitz et al. 2001; Chapman, MacIntosh et al. 2002), and probably experience greater postprandial fullness than young subjects (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997).

In young men, postprandial fullness, but not hunger, has been shown to be closely related to gastric antral area (Hveem, Jones et al. 1996; Jones, Doran et al. 1997). It has been recently shown that antral area, as determined by ultrasound, following an oral

liquid preload, is greater in older than young subjects, and that hunger is inversely, and fullness directly, related to antral area (Sturm, Parker et al. 2004) These observations suggest that antral distension may be a major determinant of satiety (Sturm, Parker et al. 2004).

The satiating effects of different macronutrients may change with age. In young healthy men, intraduodenal infusion of 343kcal of fat, in the form of a triglyceride emulsion (Intralipid), suppresses the perception of hunger and food intake at a subsequent test meal more than an equienergetic infusion of carbohydrate [(CHO) glucose] (Cook, Andrews et al. 1997; Chapman, Goble et al. 1999; MacIntosh, Horowitz et al. 2001). In addition, there is no difference between the satiating effects of these macronutrients when the same infusions are given to older men, due to an age-related enhanced satiating effect of glucose rather than a diminished satiating effect of lipid (MacIntosh, Horowitz et al. 2001).

The responses to intraduodenal nutrient infusions appear to differ from those to oral or intra-gastric administration. In young adults oral and intra-gastric administration of carbohydrate has been shown consistently to be at least as satiating as fat (Rolls, Kim-Harris et al. 1994; Cecil, Castiglione et al. 1998) if not more so (Blundell, Burley et al. 1993). However differences in the way these macronutrients were administered may account for the discrepancies in results between these studies. For example, Blundell et al (1993), administered breakfasts consisting of whole foods supplemented with either fat or carbohydrate in comparison with Rolls et al (1994) who fed their subjects yoghurt preloads (Rolls, Kim-Harris et al. 1994) and Cecil et al (1998), who infused intra-gastric solutions of fat (Intralipid) and carbohydrate (glucose) (Cecil, Castiglione et al. 1998). Whole foods and the contribution or absence of orosensory inputs may influence the satiating effects of carbohydrate or lipid (Chapman, Goble et al. 1999) and exert

different effects than pure nutrients administered as an oral or intra-gastric preload. Alternatively it is unclear if the contribution of gastric distension as occurs in an intra-gastric bolus alters the satiating effect of carbohydrate and fat.

The differential effect of fat and carbohydrate and the contribution of any of these factors to appetite and food intake in older subjects are uncertain. The present study was designed to examine the effect of equienergetic, equivolaemic intra-gastric preloads on appetite, food intake, antral area and the release of gastrointestinal hormones and in healthy older and young individuals. To eliminate differences in infusates and also to explore effects of age identified in the intraduodenal study (MacIntosh, Horowitz et al. 2001) on responses to different macronutrients, the same nutrients were given. In addition, the contribution of gastric distension to the proposed differential satiating effects of fat and carbohydrate was evaluated by a rapid bolus intra-gastric load. This was compared with a slow infusion administered as in the previous intraduodenal study. The primary hypothesis to be addressed was that intra-gastric carbohydrate is more satiating than fat in older than young adults.

6.3 Research design and methods

6.3.1 Subjects

Subjects were recruited according to Chapter 3.2. Twelve healthy older (age range 65-85 years) and twelve healthy young (age range 18-35 years) men were studied (Table 6.1). Each older subject was selected so that their BMI was matched to within 1 kg/m² of that of a young subject- consequently the BMI of the two groups was not different (P = 0.08). The Research Ethics Committee of the Royal Adelaide Hospital approved all of the studies and each subject gave written, informed consent.

6.3.2 Experimental design

Subjects attended the clinic for three study days in randomised order. Each study day was separated by between 3 and 7 days. Subjects were instructed not to undertake any vigorous exercise or drink alcohol in the 24 hours before each study and to fast overnight (from food and fluid except water) for at least 10 hours. They were allowed one standard glass (200ml) of water on the morning of the study, but not within two hours of arrival at the clinic. Ultrasound measurements were used to confirm that the stomach was empty at baseline.

The protocol was identical for each study visit. On arrival subjects were seated upright on a bed so that the angle between the upper and lower part of the body was $\sim 90^\circ$ (Sturm, Parker et al. 2004). A nasogastric catheter (10Fr, 91cm, Viasys Medsystems, Wheeling, Illinois, USA) was introduced into the stomach via an anaesthetised nostril, aided by swallowing a glass of water, and under local anaesthetic, a cannula inserted in a forearm vein for blood sampling. Following a rest period of 60 min, to allow the water to pass out of the stomach and for the subject to be comfortable with the tube, baseline blood samples were taken (to calculate mean basal levels) at $t = -15$ min and $t = 0$ min and subjects were asked to complete visual analogue scales. From $t = 0$ to $t = 5$ min subjects received, in a single-blind fashion and random order, one of three equivaemic (360ml) intra-gastric infusions at 72ml/min of: 1) fat: 10% Intralipid 300mOsmol/kg (1.1 kcal/ml), 312ml Intralipid + 48ml H₂O [343 kcal]; 2) carbohydrate: 25% glucose 1390mOsmol/kg (0.95 kcal/ml) [343kcal]; and 3) control: water. Immediately following the infusion at $t = 5$ min subjects were extubated. At $t = 50$ min subjects were offered a cold buffet meal, containing food in excess of what they would normally be expected to eat, and allowed to eat as much as they wished for up to 30 minutes until they felt comfortably full (Lavin, Wittert et al. 1996; Cook, Andrews et al. 1997; Lavin, Wittert et al. 1998).

Blood samples were taken immediately before and after the infusion ($t = -15, 0, 5, 10$ min), every ten minutes from $t = 10$ min to the buffet meal ($t = 50$ min), and then every 15 minutes until the conclusion of the study ($t = 140$ min). Ultrasound measurements of antral area were made (described below) immediately before and after the infusion, at 5-minute intervals ($t = -15, 0, 5$ min) until $t = 20$ min, every ten minutes from $t = 20$ min to the buffet meal ($t = 50$ min), and then every 15 minutes until the conclusion of the study ($t = 140$ min). At the conclusion of the study the cannula was removed and the subject discharged.

6.3.3 Assessment of appetite and energy intake

Appetite and other sensations were assessed using 100mm visual analogue scales (Sepple and Read 1989). Visual analogue scales (VAS) were completed at baseline ($t = -15, t = 0$ min), immediately following the infusion (at $t = 5$ and $t = 10$ min), at 10-minute intervals ($t = 20, 30, 40, 50$ min) until the buffet meal ($t = 50$ min), and then every 15 minutes until the conclusion of the study ($t = 140$ min). For further description and validation of VAS see Chapter 3.6.1 and Chapter 4, respectively.

The total amount of food eaten at lunch (Table 3.1) was calculated. For further description of food intake methods see Chapter 3.6.1.

6.3.4 Measurement of antral area

Measurements of antral area were performed with an Aloka SSD-650 CL Ultrasound Machine (ALOKA Co., LTD. Tokyo), according to methods described in Chapter 3.6.3.

6.3.5 Blood glucose and gastrointestinal hormone concentrations

Blood glucose concentrations and plasma cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) concentrations were measured using methods previously described in Chapter 3.6.2.

6.3.6 Statistical analyses

For visual analogue scores, blood glucose, plasma cholecystokinin and antral area, 'baseline' was calculated as the mean of $t = -15$ and 0 min values. Comparisons between single characteristics of the two age groups (eg. BMI, baseline symptom scores, and fasting blood hormone concentrations) were performed using one-way analysis of variance (ANOVA, SuperANOVA Version 1.11 (Abacus Concepts Inc., California, USA). Repeated measures analysis of covariance (ANCOVA, SAS Version 8.2 (SAS Institute Inc., North Carolina, USA), with time ($t = 5 - 50$ min) and treatment (infusions) as within-subject factors, and age group (older vs young) as a between-subject factor, and baseline as a covariate, was performed for ratings of appetite, blood glucose, plasma CCK and antral area.

Differences in mean energy intake and macronutrient content of the buffet meal were analysed by repeated-measures two-way ANOVA, with treatment as the within-subject factor and age group as the between-subject factor. When significant effects were observed, pairwise comparisons of adjusted means were performed using Student's t -test and adjusted for multiple comparisons using the Bonferroni (Holms adjustment) (ANCOVA) and stepdown Bonferroni (ANOVA) adjustments. Data following the buffet meal ($t = 80 - 140$ min) were not analysed, because the amount of food eaten was variable.

Data was analysed according to methods described in Chapter 3.6.5. Within subject correlations accounting for repeated measures across treatments were calculated for each combination of energy intake (kcal) and antral area, CCK and sensations of hunger and fullness at both $t = 50$ min ('premeal' - immediately before the buffet meal) and for mean values, using methodology specified by Bland and Altman (1995) (Bland and Altman 1995). Mean values of CCK, and sensations of hunger and fullness were determined by the average of 6 time points (5, 10, 20, 30, 40, 50 min) between the preload and the test meal. Mean values of antral area were determined by the average of 7 time points (5, 10, 15, 20, 30, 40, 50 min) between the preload and the test meal. Results are shown as means \pm SEM. A P value < 0.05 was considered statistically significant.

6.4 Results

6.4.1 Food intake (Figure 6.1)

Food intake before the study, assessed by three-day food diaries, was 5% lower in the older than in the young subjects (2036 ± 157 kcal/d and 2146 ± 305 kcal/d, respectively; $P = 0.7$). There was no difference between older and young subjects in the percentage of daily protein, fat or carbohydrate consumed.

The younger subjects ate slightly more than the older subjects on all three study days, but this difference was not significant ($P = 0.38$). There was a significant effect of food intake at the buffet meal ($P = 0.0002$), with intake after the carbohydrate significantly less than after both fat ($P = 0.005$) and control ($P < 0.001$), but intake after fat not significantly different to that on the control day ($P = 0.14$). Compared to control day, the suppression after carbohydrate was 23 % and after fat 10%. The suppressive effect on food intake of the two nutrients was not different in the two age groups (age \times treatment effect, $P = 0.5$), with 25% and 21% suppression after carbohydrate, and 14%

and 7% suppression after fat, in the younger and older subjects respectively to control. Food intake was lower after carbohydrate than fat in both young ($P = 0.03$) and older men ($P = 0.05$).

6.4.2 Appetite

6.4.2.1 Hunger (Figure 6.2)

There were no differences at baseline in hunger ratings between the age groups ($P = 0.6$) or different infusion days ($P = 0.6$). Hunger decreased immediately after the infusions and then tended to increase until the start of the buffet meal, in both age groups ($P = 0.059$). Hunger ratings did not differ between age groups ($P = 0.6$) or infusions ($P = 0.7$) (age x treatment x time interaction, $P = 0.9$).

6.4.2.2 Fullness (Figure 6.2)

There were no differences at baseline in fullness ratings between the age groups ($P = 0.5$) or infusion days ($P = 0.9$). Young subjects were more full, immediately after and up to 10 min following infusions, than the older subjects (age x time, $P = 0.02$). Following the fat infusion there was a trend for the younger subjects to be more full than following the carbohydrate infusion (age x treatment effect, $P = 0.054$). Older subjects were more full following the carbohydrate infusion than the fat infusion ($P = 0.04$).

6.4.2.3 Prospective consumption and desire to eat

There were no differences at baseline for ratings of prospective consumption between the age groups ($P = 0.6$) or infusion days ($P = 0.7$). Ratings of prospective consumption increased over time following the infusions with no difference between older and young subjects (effect of time, $P = 0.002$). Baseline ratings of desire to eat (older 67 ± 4.6 mm vs young 79 ± 2.0 mm, $P = 0.02$) differed between older and young subjects. No

differences in baseline data between infusions ($P = 0.9$) were found for ratings of desire to eat. There was a trend toward a difference between older and young subjects for ratings of desire to eat following the nutrient infusions (age x treatment interaction, $P = 0.052$) (data not shown).

6.4.2.4 Nausea and drowsiness

Baseline ratings of nausea were higher in young than older subjects (older 10 ± 2.7 mm vs young 17 ± 2.4 mm, $P = 0.03$). No differences in baseline data between infusions ($P = 0.4$) were found for ratings of nausea. There was a difference between older and young subjects for ratings of nausea following the infusions, such that older subjects were less nauseous than young subjects (older 11.1 ± 1.7 mm vs young 21.7 ± 1.5 mm, effect of age, $P = 0.02$) with no difference between infusions (treatment effect, $P = 0.42$) (data not shown). Baseline ratings of drowsiness were higher in young than older subjects (older 21 ± 4.7 mm vs, young 34 ± 3.7 mm, $P = 0.04$), but not between infusions ($P = 0.7$). There was a difference between older and young subjects for drowsy ratings following the infusions, such that older subjects were less drowsy than young subjects (older 14.9 ± 0.9 mm vs young 32.7 ± 1.1 mm, effect of age, $P = 0.03$) with no difference between infusions (treatment effect, $P = 0.39$) (data not shown).

6.4.3 Gastric Antral Area (Figure 6.3)

There were no differences at baseline in antral area between age groups (older 3.2 ± 0.17 cm² vs young 3.6 ± 0.17 cm², $P = 0.07$) or infusion days ($P = 0.4$). There was an increase in antral area size following both nutrient infusions compared to control, with no difference between older and young subjects (treatment x time effect, $P = 0.005$; treatment x age, $P = 0.2$; treatment x time x age, $P = 0.4$). Antral area decreased between the end of the infusion and buffet meal in both age groups following the control infusion but not following either nutrient infusion (control v carbohydrate, $P <$

0.001; control v fat, $P < 0.001$; carbohydrate v fat, NS). There was a trend toward a larger antral area size immediately following the infusions in young compared to older subjects (older $7.5 \pm 1.7 \text{ cm}^2$ vs young $10.1 \pm 2.0 \text{ cm}^2$, age x time, $P = 0.054$).

6.4.4 Blood glucose and gastrointestinal hormone concentrations

6.4.4.1 Plasma CCK concentrations (Figure 6.4)

Baseline plasma CCK concentrations in older subjects were approximately double those in young subjects (older $9.9 \pm 1.8 \text{ pmol/l}$ vs young $4.7 \pm 0.38 \text{ pmol/l}$, $P = 0.005$). There was no difference in baseline data between infusion days ($P = 0.98$). There was a greater increase in plasma CCK concentrations following both nutrient infusions than following control (treatment x time effect, $P = 0.003$), with no difference between the age groups (effect of age, $P = 0.16$; age x treatment, $P = 0.19$). Mean plasma CCK concentrations were 49% higher following carbohydrate ($P = 0.02$) and 92% higher following fat infusions ($P < 0.0001$) than following control. Mean plasma CCK concentrations were 20% greater following the fat than carbohydrate infusions ($P = 0.1$).

6.4.4.2 Plasma GLP-1 concentrations (Figure 6.5)

Baseline plasma GLP-1 concentrations were higher in young than older subjects (older $8.9 \pm 0.5 \text{ pmol/l}$ vs young $12.7 \pm 1.2 \text{ pmol/l}$, $P = 0.006$). There was no difference in baseline data between infusion days ($P = 0.7$). GLP-1 concentrations increased following both carbohydrate and fat infusions, but not following control (treatment x time effect, $P = 0.003$). There was a difference in the response to the infusions between older and young subjects (age x treatment effect, $P = 0.03$), with concentrations higher following the carbohydrate than fat infusion in the older ($P = 0.046$) but not younger subjects ($P = 0.8$).

6.4.4.2 Blood glucose concentrations (Figure 6.5)

There was no difference in blood glucose concentrations at baseline between older and young subjects ($P = 0.07$) or infusion days ($P = 0.8$). Blood glucose concentrations were higher than control following the carbohydrate infusion but not the fat infusion (treatment effect, $P < 0.001$). Blood glucose concentrations were higher in older than young subjects following the carbohydrate infusion but there was no difference in the response between older and young subjects following the fat and control infusion (age x treatment effect, $P < 0.001$).

6.4.5 Relationships between appetite ratings, food intake, antral area and cholecystokinin

There was a weak inverse relationship between food intake and mean CCK value [(mean of $t = 5-50$ min between the infusion and buffet meal) ($r = - 0.35$, $P = 0.02$)]. There were no relationships between food intake at the buffet meal and premeal (timepoint immediately before the buffet meal, $t = 50$ min) or mean (mean $t = 5- 50$ min) ratings of hunger or fullness, measurements of antral area or plasma cholecystokinin concentrations. There were no relationships between premeal and mean ratings of hunger and fullness and premeal and mean measurements of antral area or plasma cholecystokinin concentrations (data not shown).

6.5 Discussion

The design of this intra gastric infusion study was based on that of a previous study (MacIntosh, Horowitz et al. 2001), in which identical fat and carbohydrate solutions to those in this study were infused into the duodenum (ID) rather than the stomach. One aim was to examine the results of both studies, to determine if the relative effects of fat and carbohydrate on appetite and food intake were the same after rapid IG administration as after ID administration. In the previous ID study, thirteen young (23.7

± 1.4 years) and 13 older (72.1 ± 1.6 years) men received equi-energetic (343 kcal), equivolaemic (360ml) ID infusions of fat (10% Intralipid) and carbohydrate (25% glucose) or saline (control) over 120 minutes. A buffet meal was offered at the end of the infusion and subjects were invited to eat for 30 minutes until they were comfortably full. Food intake after ID fat infusions was significantly less than that after control infusions ($P < 0.01$), whereas intake after carbohydrate infusion was not, with the fat infusions inhibiting food intake non-significantly more than carbohydrate infusions (24 vs 13% $P = 0.3$). In young men ID fat infusion suppressed food intake significantly more than did carbohydrate infusion (26 vs 5% $P < 0.001$), whereas the suppressive effects of fat and carbohydrate were similar in older men (21 vs 22% $P = 0.5$). These responses to ID nutrients suggested that the satiating effects of carbohydrates might increase with age, prompting the present study of the effects of ageing on responses to fat and carbohydrate administered higher in the gastrointestinal tract.

The major finding of the present study was that intra-gastric administration of carbohydrate (CHO) suppressed food intake more than an equienergetic, equivolaemic fat administration in both older and young men, with the responses very similar in the two age groups. The finding that the satiating effect of intra-gastric carbohydrate was not greater in older than young men, as hypothesized, does not support an age-related increase in the satiating effects of carbohydrate as a cause of the anorexia of ageing.

Although it is not strictly valid to compare the relative satiating effects of ID and IG nutrients between the IG and ID studies, this is an interesting exercise. Intra-gastric carbohydrate was more satiating than ID carbohydrate (23 vs 13%), whereas ID fat was more satiating than IG fat (24 vs 10%). The reason(s) for the different relative satiating effects of intra-gastric and intraduodenal nutrients is not clear, with a number of possible explanations. Firstly, the intra-gastric infusions were given over five minutes whereas

the ID infusions were given over 120 min. The resulting gastric distension from the relatively rapid gastric administration is likely to have increased the satiating effect of intra-gastric nutrients. Non-nutrient gastric distension, such as induced with a barostat or intra-gastric balloon, has been shown to reduce food intake per se (Geliebter, Westreich et al. 1988; Rayner, MacIntosh et al. 2000) and enhance the satiating effect of simultaneously administered gastric nutrients (Castiglione, Read et al. 1998). It would be interesting to compare the effect of the same nutrient solutions used in this study given as a slow intra-gastric versus rapid intra-gastric infusions. The effect of gastric distension may explain why gastric carbohydrate was more satiating than ID carbohydrate, but not, however, why intra-gastric fat appears to be less satiating than ID fat.

The lower satiating effect of intra-gastric fat in this study compared to ID fat in the previous study, may be because gastric distension enhances the satiating effect of carbohydrate, more than that of fat, or that there was little emptying of fat from the stomach into the duodenum (where nutrients are thought to exert most of their GI satiating effects) in the 50 minutes between the start of the infusion and the test meal. The satiating effects of the gastric distension may therefore have not been enough to counteract the reduced duodenal fat exposure in the IG versus ID study. This would suggest that less of the fat solution emptied from the stomach in this study than did the carbohydrate solution. This may have been the case. Although fat and CHO have previously been shown to empty from the stomach at the same rate when delivered with the same energy content and volume (Hunt and Stubbs 1975; McHugh and Moran 1979), and therefore deliver nutrients at the same rate to the duodenum, the emptying of fat solutions from the stomach is dependent on body position (Horowitz, Jones et al. 1993).

Fat solutions have been shown to disperse in the stomach into two layers, a top layer of oil and a bottom aqueous layer (Jian, Vigneron et al. 1982; Meyer, Mayer et al. 1986). When subjects are sitting upright, as in the current study, the aqueous phase, which contains very little energy, empties first producing little small intestinal nutrient stimulation. The high-energy oil layer is retained and only empties when the gastric volume is quite low. As the maximal mean rate of gastric emptying during the first hour after a meal is about 3 kcal/min (i.e. at most 180 of the 343 kcal in the infusion solutions and less if the aqueous later emptied first), this may have been the situation in the present study, with a substantial portion of the fat solution not entering the duodenum before the test meal was eaten.

Antral area has been validated as a measure of gastric emptying rate and was not significantly different after the two nutrient infusions in this study. The ultrasound technique used to measure antral area is not able to determine the nature of the fluid remaining in the stomach (and distending the antrum), however, and therefore could not determine whether the gastric emptying after the fat infusion was preferentially of an aqueous layer. Furthermore, there was a trend, albeit not significant, for antral area to be greater after fat than carbohydrate solution infusion, particularly in the young subjects, suggesting that gastric emptying of the fat solution may have been somewhat slower.

There is little consensus regarding the relative satiating effects of dietary fat and carbohydrate. The results of a number of studies comparing the effects of oral, intra-gastric or intraduodenal carbohydrate and fat on food intake are summarised in Table 6.2. There is limited evidence that intraduodenal fat infusion suppresses food intake more than carbohydrate, however, oral and intra-gastric studies alternately report similar suppressive effects of fat and carbohydrate, or a greater suppression of food intake by carbohydrate.

In this study we report a greater decrease in food intake following carbohydrate infusion. This finding differs from the results of two previous IG studies in young men (Shide, Caballero et al. 1995; Cecil, Castiglione et al. 1998). The discrepancies in results may reflect the differing methodologies between these studies. For example, in the study by Shide et al (1995), a ‘slow’ infusion administered over 210 minutes was associated with a subsequent suppressive effect of fat on food intake, in comparison with the ‘rapid’ infusion, over 15 minutes, which was associated with a greater, albeit non-significant, suppression of CHO (Shide, Caballero et al. 1995). This latter result supports the findings of the current study. The contribution of gastric distension from the volume delivered in a shorter time (5-15 min v 210 min) is unknown, however in the study detailed in Chapter 7, in which older men were given similar carbohydrate infusions (315ml 300kcal 25% glucose) slowly over 150 minutes, there was a smaller suppression of food intake with infusions into both the stomach (8.3%) and duodenum (4.5%) than in this study, indicating that the contribution of gastric distension may be important in the reduction of food intake.

The slow infusion (Shide, Caballero et al. 1995) compares with the results of the previous ID study (MacIntosh, Horowitz et al. 2001) where fat suppressed food intake more than carbohydrate. The detected difference between CHO and fat in the present study may reflect sample size. A larger number of subjects in the previous rapid IG study (n = 6) (Shide, Caballero et al. 1995) may afford adequate power to detect a difference between these two macronutrient infusions. Alternately, variations in infusion composition (energy, volume), the timing of the meal with respect to the preload (30, 45, and 90 min) and the length of infusion (5, 15 and 210 min) may explain the disparity in results between these studies.

We have confirmed results from previous studies, showing that CCK concentrations are higher at baseline and following nutrient intake in older than younger subjects (MacIntosh, Morley et al. 2001; Sturm, Parker et al. 2004). We have previously reported that antral area size is larger in older than young subjects and greater after oral nutrient preloads than water (Sturm, Parker et al. 2004), whereas in this study we did not find larger antral areas in older than young subjects. We also observed no relation between antral area and energy intake or appetite scores as previously seen (Sturm, Parker et al. 2004). The different results in the two studies may reflect different measurement methods (the different relative baseline values in the two studies suggest this is at least partly the case), different nutrient preparations and possibly also the result of giving the nutrient preloads orally in the previous study and intra-gastrically via a tube in the present study. It may be that putting food in the mouth and then swallowing it, as opposed to having it infused down a tube into the stomach, has an effect on antral area. Further studies would be required to clarify this.

6.6 Conclusion

Whilst the regulation of appetite in humans is complex it is clear that signals from the gastrointestinal system are important (Havel 2001). Our novel finding that intra-gastric CHO preloads suppress food intake to a greater extent than equivo-laemic, equi-energetic fat preloads with no effect on ratings of hunger or fullness requires further investigation. Infusing identical nutrient solutions, we have demonstrated gastrointestinal tract regional and age-specific effects of carbohydrates and fat on appetite and food intake. When infused into the stomach, carbohydrate is significantly more suppressive of food intake than fat, whereas when delivered into the duodenum fat is more suppressive than carbohydrate in young subjects and equally suppressive in older subjects. Ageing may affect the response to carbohydrates but not fat, with an enhancement of the satiating effects of intra-duodenal carbohydrate in older men. The causes and implications of these differences require further study.

Table 6.1 Baseline characteristics and inclusion criteria for older (n = 12) and young (n = 12) men

Parameter	Older	Young
Age (yrs)	72.8 ± 1.1	22.3 ± 0.9
Height (m)	1.7 ± 0.02	1.8 ± 0.02
Weight (kg)	71.8 ± 1.9	75.4 ± 3.7
BMI (kg/m ²)	24.7 ± 0.5	23.1 ± 0.8
TFEQ	8 ± 0.9	4 ± 0.6*
Food (kcal/d)	2036 ± 157	2146 ± 305*
Protein (%) [†]	16.6 ± 0.7	17.3 ± 1.2
CHO (%) [†]	46.8 ± 1.9	47.6 ± 2.5
Fat (%) [†]	31.8 ± 1.8	30.1 ± 2.0

TFEQ, Factor 1 'cognitive restraint score' of the Three Factor Eating Questionnaire; Food, daily intake quantified from three-day food diary; CHO, carbohydrate; [†] daily percentage protein, carbohydrate and fat intake; BMI, body mass index Means ± SEM, * P < 0.05 older v young

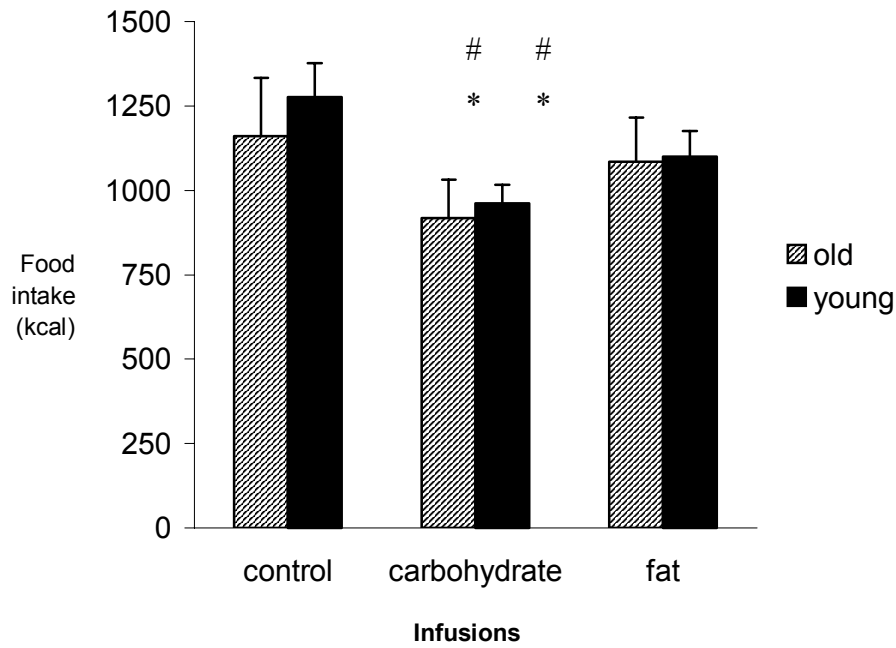


Figure 6.1 Mean \pm SEM of food intake at the buffet meal in older ($n = 12$) and young ($n = 12$) men who received 5 minute intra-gastric infusions of water (control, 0 kcal, 360 ml), carbohydrate (25% glucose, 343 kcal, 360ml) or fat (10% Intralipid, 343 kcal, 360ml) 45 minutes before a buffet meal ($t = 50-80$ min). The young subjects ate slightly more than the older subjects on all three study days, but this difference was not significant ($P = 0.38$). There was a difference between the infusions but no difference between the older and young subjects (age \times treatment effect, $P = 0.5$; treatment effect, $P = 0.0002$). The carbohydrate infusion suppressed food intake significantly more than the fat infusion (23% vs 10%, $P = 0.005$), and this was so in both young (25% vs 14% $P=0.03$) and older (21 vs 7% $P=0.05$) men. There was no difference between the amount eaten at the buffet meal following the control and fat infusions ($P = 0.14$). * $P < 0.0001$ v control; # $P = 0.005$ v fat

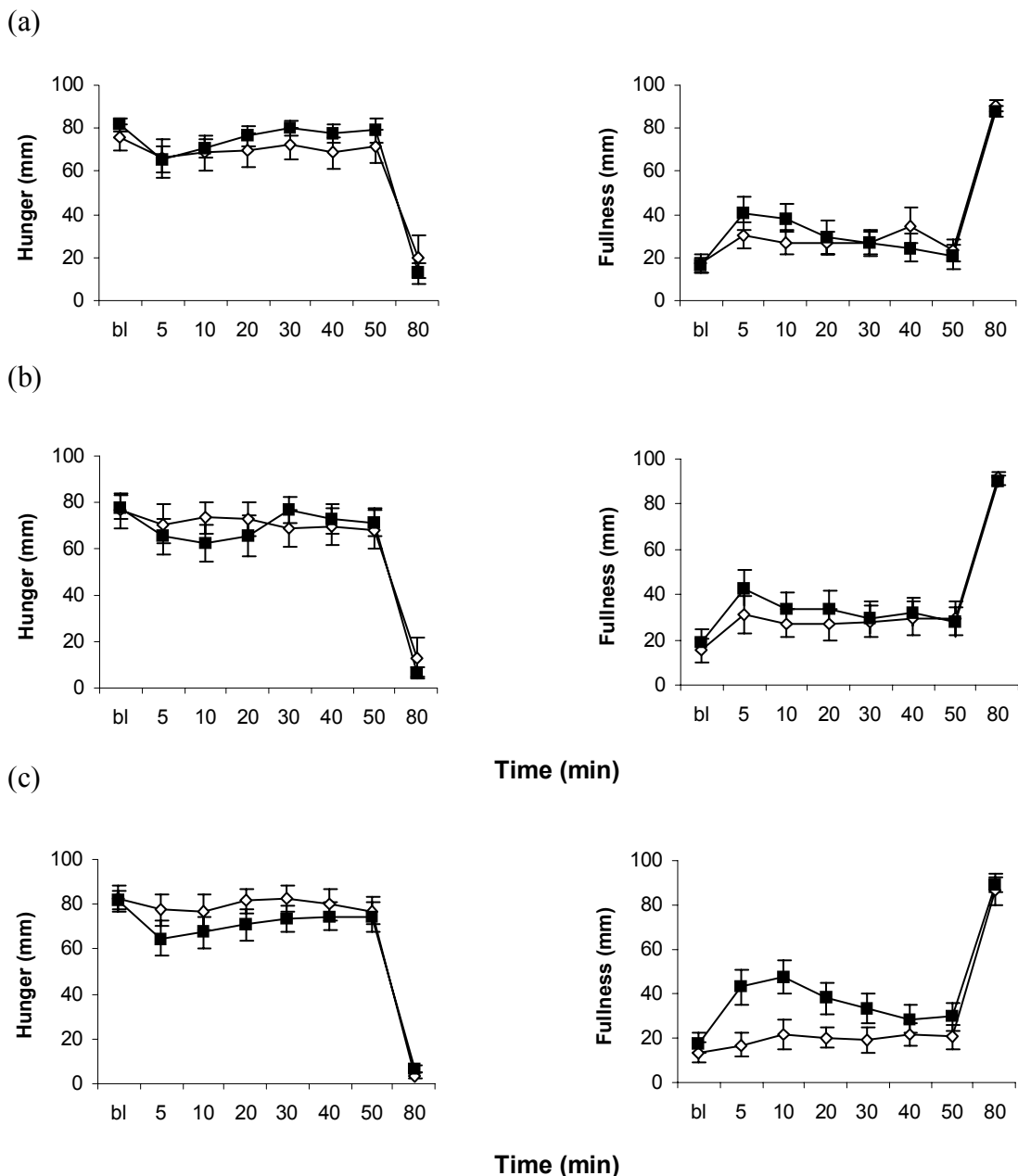


Figure 6.2 Mean \pm SEM VAS ratings of (1) hunger and (2) fullness in older ($n = 12$, Δ) and young ($n = 12$, \blacksquare) men who received 5 minute ($t = 0-5$ min) intragastric infusions of (a) water (control, 0 kcal, 360 ml), (b) carbohydrate (25% glucose, 343 kcal, 360ml) or (c) fat (10% Intralipid, 343 kcal, 360ml) 45 minutes before a buffet meal ($t = 50-80$ min). (1) Hunger ratings decreased immediately after infusions and then tended to increase until the start of the buffet meal, in both age groups ($P = 0.059$). (2) Young subjects were more full, immediately after and up to 10 min following infusions, than older subjects (*age x time, $P = 0.02$). There was a trend for the younger subjects to be more full after fat than carbohydrate infusions (age x treatment effect, $P = 0.054$). Older subjects were more full following the carbohydrate infusion than the fat infusion (# $P = 0.04$).

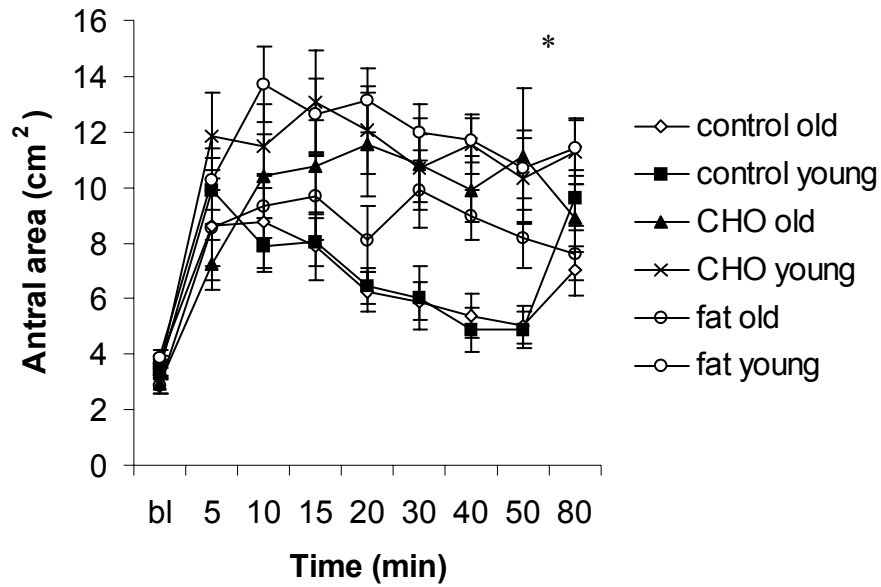
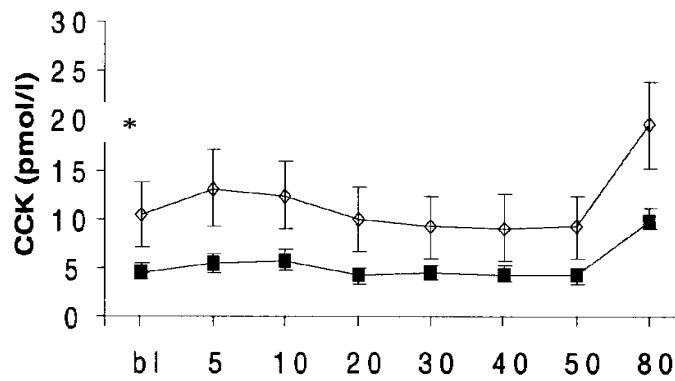
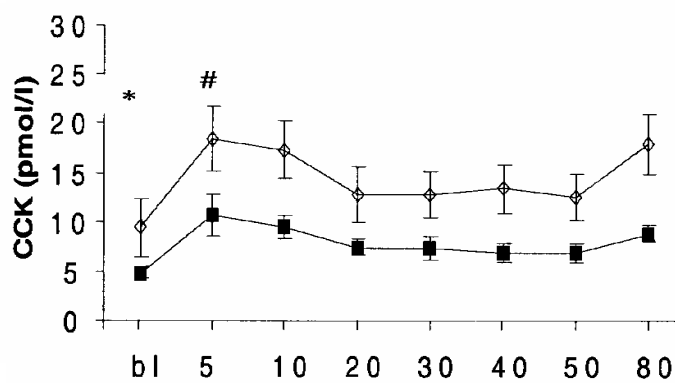


Figure 6.3 Mean \pm SEM of antral area in older ($n = 12$) and young ($n = 12$) men who received 5 minute ($t = 0-5\text{min}$) intra-gastric infusions of water (control, 0 kcal, 360 ml), carbohydrate (25% glucose, 343 kcal, 360ml) or fat (10% Intralipid, 343 kcal, 360ml) 45 minutes before a buffet meal ($t = 50-80$ min). Antral area decreased between the end of the infusion and buffet meal in both age groups following the control infusion but not following either nutrient infusion (*treatment x time effect, $P = 0.005$; treatment x age $P = 0.2$; treatment x time x age, $P = 0.4$). There was a trend toward a larger antral area size immediately following the infusions in young compared to older subjects (age x time, $P = 0.054$).

(a)



(b)



(c)

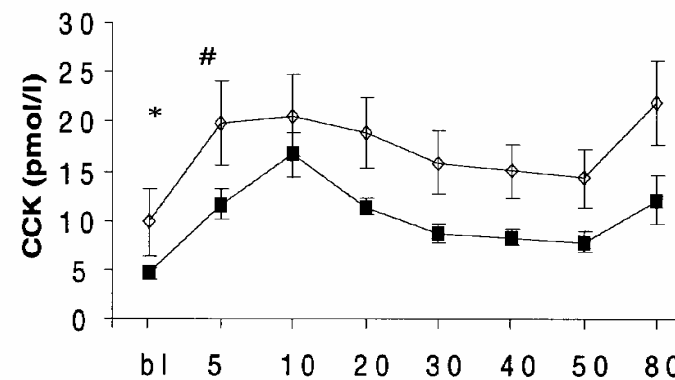


Figure 6.4 Mean \pm SEM plasma CCK concentrations in older ($n = 12$, \diamond) and young ($n = 12$, \blacksquare) men who received 5 minute ($t = \text{bl} - 5\text{min}$) intragastric infusions of (a) water (control, 0 kcal, 360 ml), (b) carbohydrate (25% glucose, 343 kcal, 360ml) and (c) fat (10% Intralipid, 343 kcal, 360ml) 45 minutes before a buffet meal ($t = 50\text{-}80$ min). Baseline plasma CCK concentrations were 2x higher in older than young subjects, $*P = 0.005$. Plasma CCK concentrations increased following both nutrient infusions ($\#$ treatment \times time effect, $P = 0.003$) with no difference between the age groups ($P = 0.16$). Mean plasma CCK concentrations were 49% higher following carbohydrate ($P = 0.02$) and 92% higher following fat infusions ($P < 0.0001$) than control. Mean plasma CCK concentrations were non-significantly greater (20%) following the fat than carbohydrate infusions ($P = 0.1$).

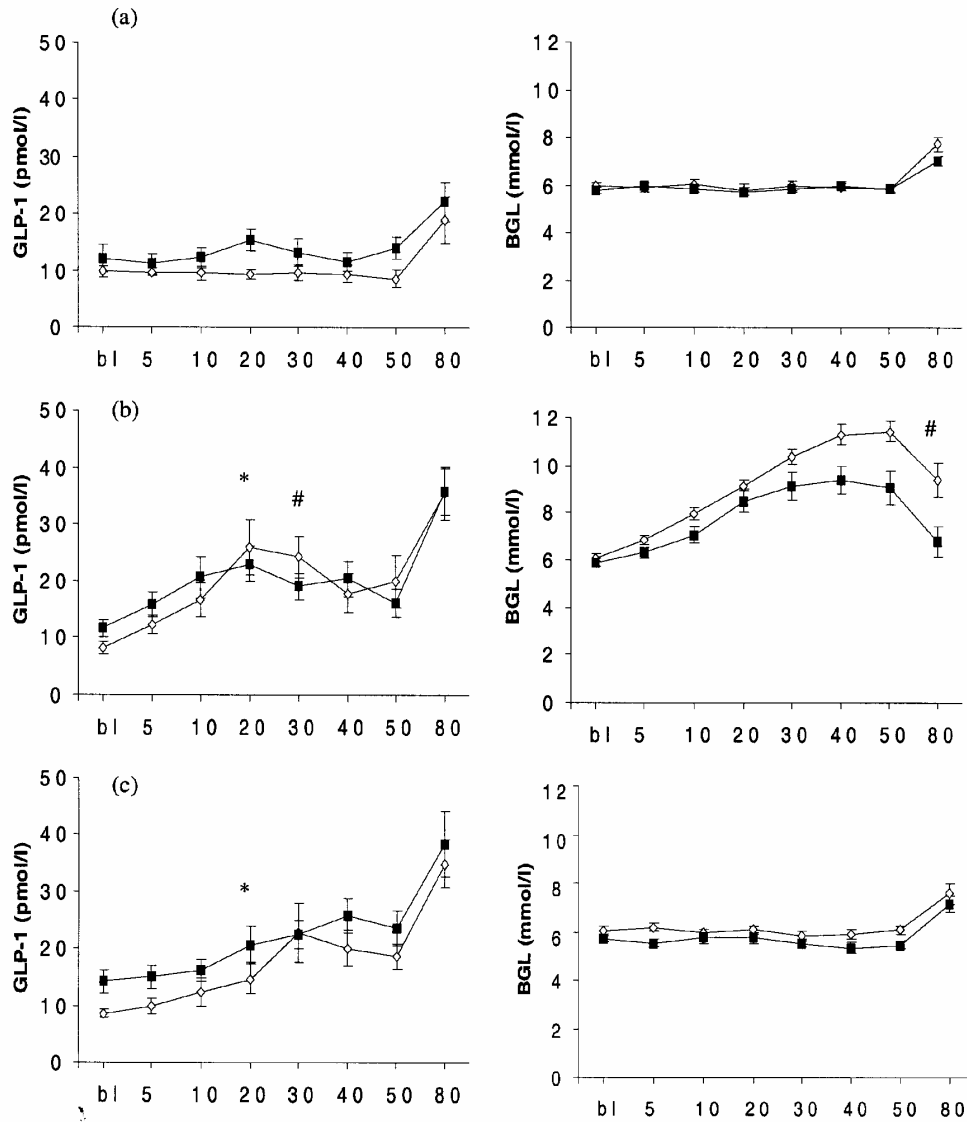


Figure 6.5 Mean \pm SEM plasma concentrations of (1) GLP-1 and (2) blood glucose in older ($n = 12$, \diamond) and young ($n = 12$, \blacksquare) men who received 5 minute ($t = \text{bl}-5\text{min}$) intragastric infusions of (a) water (control, 0 kcal, 360 ml), (b) carbohydrate (25% glucose, 343 kcal, 360ml) or (c) fat (10% Intralipid, 343 kcal, 360ml) 45 minutes before a buffet meal ($t = 50-80$ min). (1) Plasma GLP-1 concentrations increased after carbohydrate and fat infusions but not control (*treatment x time effect, $P = 0.003$). Plasma GLP-1 concentrations were higher after carbohydrate than fat infusion in the older (# $P = 0.046$) but not younger subjects ($P = 0.8$) (age x treatment effect, $P = 0.03$). (2) Blood glucose concentrations were higher than control following the carbohydrate but not fat infusion (*treatment effect, $P < 0.001$). Blood glucose concentrations were higher in older than young subjects following the carbohydrate infusion but there was no difference in the response between older and young subjects following the fat and control infusion (#age x treatment effect, $P < 0.001$).

Table 6.2 Summary of research studies with equivoalaemic, equienergetic fat and carbohydrate preloads and the effect on food intake

Study	Subjects	Treatment	Energy of treatment (kcal)	Meal	Ingestion/infusion time (minutes)	Time interval between treatment & meal (minutes)	Result
Rolls et al, 1991*	14 young women 14 young men	High fat yoghurt (500g men, 350g women) High CHO (500g men, 350g women) No yoghurt	357 357	<i>ad libitum</i> cold buffet meal	15	30, 90 or 180	Equal suppression of food intake by high fat and high CHO yoghurts compared with control
Blundell et al, 1993*	16 young men	High fat breakfast High CHO breakfast Standard breakfast (control)	803 803 440				Equal food intake at high fat and high CHO breakfasts compared with control
Blundell et al, 1993*	12 young men	High fat breakfast High CHO breakfast Standard breakfast (control)	803 803 440	mixed sandwiches	15	90	31% suppression by CHO vs fat, P = 0.024
Rolls et al, 1995*	16 older men 16 young men	High fat yoghurt (500g) High CHO yoghurt (500g) Low fat/low CHO yoghurt (500g) No yoghurt	500 500 250	<i>ad libitum</i> cold buffet meal	15	30	Equal suppression of food intake by high fat and high CHO yoghurts compared with control
Raben et al, 2003*	10 young women 10 young men	High protein breakfast High fat breakfast High CHO breakfast High alcohol breakfast	595 (women), 714 (men)	<i>ad libitum</i> hot pasta meal	15	300	Equal suppression of food intake by high fat and high CHO yoghurts compared with control
Vozzo et al, 2003*	16 young men	High fat yoghurt (499g) High CHO yoghurt (511g) High protein yoghurt (514g) No yoghurt	714	<i>ad libitum</i> food intake remainder of the day			Equal suppression of food intake by high fat and high CHO yoghurts compared with control
Ryan et al, 2004*	10 mainourished men, 6 mainourished women	High fat supplement (250ml) High CHO supplement (250ml) No supplement	250 250	<i>ad libitum</i> lunch		270	No difference between supplements for food intake at lunch
Shide et al, 1995*	6 young men	High Fat yoghurt 500g High CHO yoghurt 500g No yoghurt	500 500	<i>ad libitum</i> cold buffet meal		30	Food intake non-significantly less after CHO yoghurt than fat yoghurt (690 ± 116 v 870 ± 212, P = NS)
Shide et al, 1995	6 young men	IV fat (20% Intralipid) 500ml IV CHO (20% dextrose) 500ml IV saline 500ml	500 500	<i>ad libitum</i> cold buffet meal	210	30	Equal suppression of food intake by fat and CHO infusions compared with control
Shide et al, 1995#	Slow infusion	IG fat (20% Intralipid) 250ml	500	<i>ad libitum</i> cold buffet	210	30	Fat infusion more suppressive of food

Study	Subjects	Treatment	Energy of treatment (kcal)	Meal	Ingestion/infusion time (minutes)	Time interval between treatment & meal (minutes)	Result
	6 young men	IG CHO (50% dextrose) 250ml IG saline 250ml	500	meal			intake than CHO infusion (1245 ± 82 v 1409 ± 150, P < 0.05)
Shide et al, 1995 [#]	Rapid infusion 6 young men	IG fat (20% Intralipid) 250ml IG CHO (50% dextrose) 250ml IG saline 250ml	500 500	ad libitum cold buffet meal	15	30	Food intake non-significantly less after CHO infusion than fat infusion (964 ± 166 v 1142 ± 185, P = NS)
Cecil et al, 1998 [#]	10 young men	IG 20 % Intralipid (500ml) IG 53.4% glucose (500ml)	1000 1000	ad libitum buffet meal	15	90	Equal suppression of food intake by fat and CHO infusions
MacIntosh et al, 2001 [†]	12 older men 12 young men	ID 10% Intralipid (360ml) ID 25% glucose (360ml) ID saline (360ml)	343 343	ad libitum buffet meal	120	immediately following infusion	Fat 30% more suppressive than CHO

*oral preload studies; # intragastric infusion studies; † intraduodenal infusion studies; CHO, carbohydrate

Chapter 7

Effect of small intestinal glucose exposure on suppression of ghrelin in healthy older men and women.

7.1 Summary

Ghrelin is a peptide hormone secreted primarily from the gastric mucosa. It plays a role in energy balance by stimulating appetite, thereby increasing food intake and enhancing weight gain and fat mass deposition. Plasma ghrelin concentrations increase with fasting and are suppressed by nutrient intake. To determine the contribution of the small intestine and stomach in postprandial suppression of ghrelin, equivolaemic (300kcal) intragastric (IG) and intraduodenal (ID) carbohydrate infusions were infused at 2kcal/min for a total volume of 315ml and compared to an intragastric control infusion of water infused at the same rate.

Twelve healthy older (age range 65-85 years) men (n = 7) and women (n = 5) were studied. Food intake was quantified at a buffet meal offered immediately following each 150-minute infusion and ratings of appetite assessed using visual analogue scales. Blood samples were taken to analyse plasma ghrelin and cholecystokinin concentrations.

Plasma ghrelin concentrations were different between the infusions (effect of treatment, $P < 0.0001$). There was a 25% suppression of mean plasma ghrelin concentrations following ID (2016 v 2686pg/ml, $P < 0.0001$) and a 19% suppression of mean plasma ghrelin concentrations following IG (2181 v 2686pg/ml, $P < 0.0001$) infusions compared to control. Mean plasma ghrelin concentrations were non-significantly (7.6%) lower following the ID than IG infusions (2016 v 2181pg/ml, $P = 0.2$). There was no difference between the treatments for the amount of food consumed at the buffet meal ($P = 0.88$). Although the primary source of ghrelin is the gastric mucosa, small intestinal exposure is necessary for ghrelin suppression in humans.

7.2 Introduction

On average, appetite and food intake decrease with ageing. This physiological ‘anorexia of ageing’ predisposes to the development of protein-energy malnutrition, which is associated with poor health outcomes (Morley 1997). Short term appetite is controlled by a central feeding drive and a peripheral satiety system, which includes inputs from mechano- and chemo-receptors that respond to the presence of food in the gastrointestinal tract (Morley 1997; Havel 2001). Signals originating in the upper gastrointestinal tract (stomach and small intestine) play a major role in the perception of appetite sensations and the control of food intake in older people (Morley 1997).

Gastrointestinal satiety signals originate from gastric distension, which reduces food intake, and the interaction of nutrients with gastrointestinal tract receptors that stimulate the release of satiety hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP) and amylin (Morley and Levine 1983; Morley 1990; Lavin, Wittert et al. 1998; Nakazato, Murakami et al. 2001), or inhibit the release of ghrelin which stimulates feeding (Nakazato, Murakami et al. 2001; Shintani, Ogawa et al. 2001). These hormones play a role in both satiation (meal termination) and satiety (time to subsequent meal consumption) (Havel 2001).

Ghrelin, a recently identified peptide hormone, is produced mainly by enteroendocrine cells within the mucosal epithelial layer of the stomach (Tschop, Smiley et al. 2000), as well as at other sites including the hypothalamus. It acts to stimulate feeding and growth hormone release (Kojima, Hosoda et al. 1999; Hosoda, Kojima et al. 2000; Takaya, Ariyasu et al. 2000; Tschop, Smiley et al. 2000). Circulating ghrelin concentrations increase with fasting (Cummings, Weigle et al. 2002) and with weight loss in obese subjects, and are elevated in undernourished young (Cummings, Weigle et al. 2002; Rigamonti, Pincelli et al. 2002) and older subjects (Sturm, MacIntosh et al. 2003). In contrast, plasma ghrelin concentrations decrease after ingestion of food particularly fat and carbohydrate (Monteleone, Bencivenga et al. 2003), while the role of protein has not been clarified (Erdmann, Lippl et al. 2003; Monteleone, Bencivenga et al. 2003).

The effect of healthy ageing on circulating ghrelin concentrations has not yet been clarified, despite evidence suggesting an age-related decline in plasma ghrelin concentrations (Rigamonti, Pincelli et al. 2002; Sturm, MacIntosh et al. 2003). Two small studies have reported circulating ghrelin concentrations 20% (Sturm, MacIntosh et al. 2003) and 35% (Rigamonti, Pincelli et al. 2002) lower in healthy older than young adults, the latter reduction statistically significant. However, increasing body fat, as indicated by BMI, is associated with decreasing ghrelin concentrations (Rigamonti, Pincelli et al. 2002; Greenman, Golani et al. 2004), and the older subjects had higher body mass indices (BMI) than the young subjects in both studies. Neither study included detailed body composition analysis, so the higher ghrelin levels in older subjects may have been due to differences in body composition. Nevertheless, these findings suggest that ghrelin activity may decrease with normal ageing and contribute to the physiological anorexia of ageing.

Findings from a recent study in rats suggest that ghrelin suppression is due to nutrient contact with the small intestine and not stomach (Williams, Cummings et al. 2003). The suppression of plasma ghrelin concentrations by IG glucose infusion was abolished when gastric emptying, and thus nutrient emptying into the small intestine, was prevented (Williams, Cummings et al. 2003). This suggests that, even though the majority of circulating ghrelin appears to originate from the stomach, nutrient exposure with the stomach has little or no effect on ghrelin secretion, which is modulated by feedback systems from the duodenum. As the gastrointestinal factors controlling ghrelin release have not been similarly investigated in humans, this study was designed to determine the origin of signals that lead to suppression of plasma ghrelin levels after nutrient ingestion and in particular the relative contribution of the stomach and small intestine. The present study evaluated the effect of equienergetic, equivolaemic carbohydrate (glucose) intragastric and intraduodenal infusions on plasma ghrelin concentrations, to test the hypothesis that food-induced suppression of ghrelin is mediated by small intestinal and not gastric nutrient exposure. The study was performed in healthy older subjects as part of the ongoing investigations of the factors controlling appetite and food intake in older people.

7.3 Research design and methods

7.3.1 Subjects

Subjects were recruited according to criteria described in Chapter 3.2. Twelve healthy older (age range 65-85 years) men (n = 7) and women (n = 5) were studied (Table 7.1). The Research Ethics Committee of the Royal Adelaide Hospital approved all of the studies and each subject gave written, informed consent.

7.3.2 Experimental design

Subjects attended the clinic for three study days in randomised order. Study days were separated by 3 to 7 days. Subjects were instructed not to undertake any vigorous exercise or drink alcohol in the 24 hours before each study, and to fast overnight (from food and fluid except water) for at least 10 hours. They were allowed one standard glass (200ml) of water on the morning of the study, but not within two hours of arrival at the clinic.

The protocol was identical for each study visit. For the two intragastric (IG) study days subjects were seated upright for insertion of an intragastric catheter (10Fr, 91cm, Viasys Medsystems, Wheeling, Illinois, USA) via an anaesthetised nostril into the stomach, assisted by swallowing water. On both occasions the tube was held in place by taping it to the subject's nose. For each subject, insertion length (to the infusion exit port) was determined as the distance from the tip of the nose to the earlobe and then to the base of the sternum. Correct placement was determined by auscultation after air injection. Subjects then rested for 30 minutes to allow time for the water (approximately 150ml used to assist intubation) to pass out of the stomach and for the subject to be comfortable with the tube insitu. On the intraduodenal (ID) study day a 17-channel manometric catheter (Dentsleeve, Adelaide, Australia) was introduced into the duodenum, via an anaesthetised nostril. The tube was held in place by taping it to the subject's nose. The sleeve assembly was allowed to pass through the pylorus into the duodenum by peristalsis and its passage monitored by

measuring the transmucosal potential difference across the stomach and duodenum, using a reference electrode (20 G intravenous cannula filled with sterile saline) placed subcutaneously in the forearm (Edelbroek, Horowitz et al. 1992). (A detailed description of these methods is included in Chapter 3.6.4).

On each study day an intravenous cannula was inserted into an antecubital vein for regular blood sampling. A local anaesthetic injection under the skin minimised discomfort from both of these procedures. A baseline period of 30 minutes was undertaken during which time two blood samples were taken and VAS (see below) administered ($t = -15, 0$ min). Each subject received all three infusions (described below) and the administration of the treatments was single-blind. Blood samples and VAS were collected throughout the infusion (see below). At $t = 150$ min subjects were extubated and offered a cold buffet meal, and allowed to eat freely for up to 30 minutes until they were comfortably full (Lavin, Wittert et al. 1996; Cook, Andrews et al. 1997). After the meal ($t = 180$ min) one blood sample and VAS was collected and subjects were free to leave. Subjects were asked to keep a detailed diet diary for the remainder of the study day and to return for subsequent studies at least 3 days (and not longer than 10 days) later.

7.3.3 Infusions

Each study solution: 1) intraduodenal glucose infusion; 2) intragastric glucose infusion; and 3) intragastric water was infused over 150 minutes. The equivolaemic, equienergetic (315 ml, 300 kcal) carbohydrate infusions (IG and ID) were 25% glucose (1390mOsmol/kg) infused at 2 kcal/min, and the control infusion (IG) was 315ml of water infused at the same rate. Infusions were prepared in the departmental research kitchen.

7.3.4 Measurement of plasma ghrelin, cholecystokinin and blood glucose concentrations

7.3.4.1 Measurement of plasma ghrelin concentrations

Plasma ghrelin concentrations were measured at baseline ($t = 0$ min) and at $t = 30, 60, 90, 120$ minutes, at the completion of the infusion ($t = 150$ min) and meal ($t = 180$ min). Total ghrelin was measured by radioimmunoassay using an adaptation of a method developed by Prosearch International with a commercial antisera (RAST-4745, Bachem, Ca), which does not crossreact with secretin, orexin, motilin, galanin or VIP.

Human ghrelin was iodinated with an equimolar quantity of 125 iodine by the CT oxidisation method. Iodo-histidyl-ghrelin was separated from free 125 iodine and unlabelled ghrelin by reverse phase HPLC on a Phenomenex Jupiter C4 300A 5u column cat no. 00B-4167-EO 250 x 4.6 mm. The column was eluted isocratically with 27% acetonitrile in triethylamine phosphoric acid buffer pH 3.0 (Prosearch International, Victoria). Standards were serially diluted from ghrelin peptide (Phoenix Pharmaceuticals, Ca) in a range from 4 to 256 [pg/tube in buffer (50 mM phosphate pH 7.4 containing 10 mM EDTA and 2 g/L gelatin). Incubation was for 20-24 hours at 4°C and second antibody precipitation was used to separate the antibody bound peptide from free peptide. The second antibody was added at the time of the assay setup (100 μ L of sheep antirabbit antibody, Prosearch International, Victoria) and 100 μ L of 2% normal rabbit serum and 1m; 8% PEG 6,000 was added immediately prior to centrifugation. After centrifugation for 25 minutes at 4°C, tubes were decanted and counted on Crystal LKB gamma counter.

The minimum detectable level was 40 pg/ml, interassay CV was 23% and intraassay CV was 17%. The performance of this assay incorporating Bachem antisera was assessed by measuring ghrelin levels in 20 samples and comparing results to those obtained using the commercial ghrelin kit (Sturm, MacIntosh et al. 2003) (Phoenix Pharmaceuticals, Ca). Results are shown in Figure 7.1, ($r = 0.93, P < 0.0001$).

7.3.4.2 CCK and blood glucose concentrations

Venous blood samples (~ 10 ml) were drawn for the determination of blood glucose, plasma cholecystokinin concentrations and plasma ghrelin concentrations (MacIntosh, Andrews et al. 1999) A detailed description of these assays is included in Chapter 3.6.1. Plasma CCK was measured at baseline ($t = 0$ min) and at $t = 10, 20, 30, 45, 60, 90$ minutes, and at the completion of the infusion ($t = 150$ min). Blood glucose was measured at baseline ($t = -15$ min, $t = 0$ min), at $t = 10, 20, 30, 45, 60, 75, 90, 105, 120$ minutes, at the completion of the preload ($t = 150$ min) and at the completion of the test meal ($t = 180$ min). The total amount of blood taken each study visit was approximately 130ml.

7.3.5 Visual analogue scales

Ratings of hunger, fullness, nausea, satiety, desire to eat and prospective consumption, as well as sensations of drowsiness and anxiety were assessed using a VAS at the same time points as blood was collected. The total amount of food eaten at the buffet meal was calculated. A detailed description of these methods is included in Chapter 3.6.1. Validation of VAS is discussed in Chapter 4.

7.3.6 Statistical analyses

For visual analogue scores and blood glucose concentrations baseline⁷ was calculated as the mean of $t = -15$ and 0 min values. For plasma cholecystokinin and ghrelin concentrations, one value was measured for 'baseline' ($t = 0$ min). Comparisons between single characteristics of the two age groups (eg. BMI, baseline symptom scores, and fasting blood hormone concentrations) were performed using one-way analysis of variance (ANOVA, SuperANOVA Version 1.11 (Abacus Concepts Inc., California, USA).

Repeated measures analysis of covariance (ANCOVA, SAS Version 9 (SAS Institute Inc., North Carolina, USA), with time ($t = 5 - 150$ min) and treatment (infusions) as within-subject factors, and baseline as a covariate, was performed for ratings of appetite and blood

glucose, plasma CCK and plasma ghrelin concentrations. When significant effects were observed, pairwise comparisons of adjusted means were performed using Student's t-test and adjusted for multiple comparisons using the Bonferroni (Holms adjustment) (ANCOVA) and stepdown Bonferroni (ANOVA) adjustments. Mean values of ghrelin were determined by the average of 5 time points ($t = 30, 60, 90, 120$ and 150 min) between the start of the infusion and the buffet meal. Mean values of CCK were determined by the average of 7 time points ($t = 10, 20, 30, 45, 60, 90,$ and 150 min) between the start of the infusion and the buffet meal.

Data was analysed according to methods described in Chapter 3.6.5. Differences in mean energy intake was analysed by one-way ANOVA, with treatment as the within-subject factor using SPSS for Windows 11.0 statistical software (Chicago, USA). Data following the buffet meal ($t = 150 - 180$ min) were not analysed, because the amount of food eaten was variable. Pearson product-moment correlations were calculated between an average baseline plasma ghrelin concentration (to obtain one ghrelin value for each subject the mean of the three baseline ghrelin values (one per treatment) was calculated) and BMI using SPSS for Windows 11.0 statistical software (Chicago, USA). Results are shown as means \pm SEM. A P value < 0.05 was considered statistically significant.

7.4 Results

7.4.1 Food intake (Figure 7.2)

There was no difference between the treatments for the amount of food consumed at the buffet meal ($P = 0.88$). The IG glucose infusion suppressed food intake 8.3% more than the control infusion ($P = 0.86$), and 4% more than the ID glucose infusion ($P = 0.97$) and the ID glucose infusion suppressed food intake 4.5% more than the control infusion ($P = 0.96$).

7.4.2 Appetite

7.4.2.1 Hunger (Figure 7.3)

There were no differences in baseline ratings of hunger between study days or between men and women. Following commencement of infusions, ratings of hunger increased until the start of the buffet meal (effect of time, $P < 0.0001$). There was a difference between treatments for ratings of hunger (effect of treatment, $P = 0.001$; control v IG, $P = 0.02$; control v ID, $P = 0.0003$; IG v ID, $P = 0.3$) with the highest ratings during the control infusion (47.6 ± 3.7 mm, $P < 0.0001$), then the IG glucose infusion (40.3 ± 3.7 mm, $P < 0.0001$) and the lowest ratings during the ID glucose infusion (37.6 ± 3.7 mm, $P < 0.0001$).

7.4.2.2 Fullness (Figure 7.3)

There were no differences in baseline ratings of fullness between study days or between men and women. There was a difference between treatments for fullness ratings (effect of treatment, $P = 0.003$; control v IG, $P = 0.3$; control v ID, $P = 0.04$; IG v ID, $P = 0.001$) with the highest ratings during the IG glucose infusion (27.4 ± 3.8 mm), then the control infusion (23.9 ± 3.8 mm) and the lowest ratings during the ID glucose infusion (17.5 ± 3.8 mm).

7.4.2.3 Prospective consumption

There was no difference in baseline ratings of prospective consumption between study days. Baseline ratings of prospective consumption were significantly higher in men than women (47 ± 4.3 mm v 28 ± 4.8 mm, $P = 0.005$; respectively). Ratings of prospective consumption increased over time following the infusions (effect of time, $P = 0.0001$).

7.4.2.4 Desire to eat

Ratings of desire to eat increased over time between the start of the infusions until the start of the buffet meal (effect of time, $P < 0.0001$). There were no differences in baseline ratings of desire to eat between study days or between men and women. There was a difference

between treatments for ratings of desire to eat (effect of treatment, $P = 0.02$; control v IG, $P = 0.41$; control v ID, $P = 0.006$; IG v ID, $P = 0.06$) with the highest ratings during the control infusion (45.0 ± 4.1 mm), then the IG infusion (42.6 ± 4.1 mm) and the lowest ratings during the ID glucose infusion (37.6 ± 4.1 mm).

7.4.2.6 Nausea, anxiety and drowsiness

There were no differences in baseline ratings of nausea, drowsiness and anxiety between study days or between men and women. There was no difference between treatments or over time for ratings of nausea, drowsiness or anxiety (data not shown).

7.4.3 Assessment of blood glucose and plasma ghrelin and cholecystokinin concentrations

7.4.3.1 Plasma ghrelin concentrations (Figure 7.4)

There was no difference in baseline plasma ghrelin concentrations between study days (control, 2741 ± 495 pg/ml; IG glucose, 2506 ± 458 pg/ml; ID glucose, 2515 ± 517 pg/ml, $P = 0.93$). Baseline plasma ghrelin concentrations were significantly lower in men than women (1970 ± 288 pg/ml v 3453 ± 446 pg/ml, $P = 0.006$; respectively). There was a difference between treatments for plasma ghrelin concentrations (effect of treatment, $P < 0.0001$). Mean plasma ghrelin concentrations were 25% lower during ID glucose than during control infusions (2016 v 2686 pg/ml, $P < 0.0001$) and 19% lower during IG glucose than control (2181 v 2686 pg/ml, $P < 0.0001$). While the suppression of mean plasma ghrelin concentrations during the ID glucose infusion was slightly greater than during the IG glucose infusion this difference was not significant (25 v 19%, respectively; $P = 0.2$).

7.4.3.2 Plasma cholecystokinin concentrations (Figure 7.4)

There were no differences in baseline plasma CCK concentrations between study days or between men and women. During both nutrient infusions plasma CCK concentrations were higher than control (treatment x time, $P < 0.047$). There was an increase in plasma CCK

concentrations during both IG and ID infusions compared to control from 30 minutes to the start of the buffet meal ($P < 0.0001$). Mean plasma CCK concentrations were 50% higher following IG ($P < 0.0001$) and 63% higher following ID ($P < 0.0001$) infusions than control with no difference between the effects of the two glucose infusions.

7.4.3.3 Blood glucose concentrations

There were no differences in baseline blood glucose concentrations between study days or between men and women. Following both nutrient infusions blood glucose concentrations were higher than control (treatment x time, $P < 0.0001$). There was no difference in blood glucose concentrations between IG and ID infusions (data not shown).

7.4.4 Relationships between ghrelin concentrations and food intake, appetite, body mass distribution, gender and blood glucose

There was no relationship between plasma ghrelin concentrations and food intake at the buffet meal ($r = -0.04$, $P = 0.83$), or between plasma ghrelin concentrations and hunger ($r = -0.11$, $P = 0.53$) or fullness ($r = 0.17$, $P = 0.33$). There was an inverse relationship between mean plasma ghrelin concentrations and blood glucose ($r = -0.53$, $P < 0.0001$) and this was so for both men ($r = -0.46$, $P < 0.0001$) and women ($r = -0.65$, $P < 0.0001$). There was an inverse relationship between baseline plasma ghrelin concentrations and BMI in women ($r = -0.92$, $P = 0.03$) but not men ($r = 0.28$, $P = 0.7$).

7.5 Discussion

The main findings of this study are (i) both intragastric and intraduodenal infusion of glucose suppressed plasma ghrelin concentrations more than an equivolaemic intragastric infusion of water, (ii) there was no significant difference between the extent of suppression of ghrelin produced by intraduodenal and intragastric glucose, although there was a trend for ID glucose to be more suppressive, (iii) glucose infusions were associated with similar reductions in hunger compared with water infusions irrespective of whether administered

into the stomach or duodenum and (iv) intragastric infusions were associated with similar increases in fullness compared to intraduodenal infusion irrespective of whether water or glucose was infused into the stomach.

Further findings are that baseline plasma ghrelin concentrations were 43 % higher in women than men and negatively associated with body mass index in women and blood glucose concentrations in both men and women. These results confirm previous findings that fasting plasma ghrelin concentrations are negatively associated with body mass index (Greenman, Golani et al. 2004; Salbe, Tschop et al. 2004), however this is not reported in all studies (Broglio, Benso et al. 2003), and also confirms a gender differential in that women have higher basal concentrations than men (Greenman, Golani et al. 2004; Salbe, Tschop et al. 2004).

The results of this study indicate that small intestinal nutrient (glucose) exposure is required to produce a suppression of postprandial plasma ghrelin concentrations, despite ghrelin being primarily produced and secreted in the stomach (Tschop, Smiley et al. 2000). There was no difference in the suppression of plasma ghrelin concentrations when glucose was infused into the small intestine and stomach, indicating that glucose contact with the stomach lining added nothing to the decrease in ghrelin concentration produced by duodenal glucose exposure. This finding is consistent with the results of a study in rats (Williams, Cummings et al. 2003) that received intragastric infusions of 25 % glucose (infused at 1ml/min) or water, with gastric emptying proceeding either normally or prevented by inflation of a pyloric cuff. When gastric emptying was permitted there was a 50% suppression of plasma ghrelin concentrations by intragastric glucose compared to water. This suppression did not occur when gastric emptying was prevented. A recent study, also in rats, suggests that the inhibitory signals mediating postprandial ghrelin suppression are derived from the jejunum, rather than the duodenum or stomach (Overduin, Frayo et al. 2004); infusions of nutrients into all three regions in rats produced equivalent ghrelin suppression.

In the present study glucose was infused into the stomach at a rate of 2 kcal/min, commensurate with the reported average gastric emptying rate of glucose (Horowitz and Dent 1991). This method of delivery is not physiological, not just because of the direct infusion via a nasogastric tube, but also because dietary carbohydrate is usually taken more as a bolus, mixed with other macronutrients as part of food. It is possible that intragastric glucose would have suppressed ghrelin more if given as a bolus, with consequent gastric distension and greater initial contact of the glucose solution with the gastric mucosa. There is evidence, for example, that the suppression of plasma ghrelin concentrations occurs earlier following gastric than intravenous glucose administration (Nakagawa, Nagaya et al. 2002; Shiiya, Nakazato et al. 2002), suggesting that gastric distension may make some, as yet unknown, contribution to ghrelin suppression. If so, such a role is likely to be minor, as non-nutrient (water) gastric distension has been shown not to suppress ghrelin secretion in humans (Shiiya, Nakazato et al. 2002) and there was also no suppression of plasma ghrelin in the previous rat study when the gastric emptying of this glucose infusion was prevented by a pyloric balloon, an intervention which resulted in gastric distension in the presence of IG glucose.

There is, however, some evidence that gastric distension may contribute to the suppression of plasma ghrelin observed after intragastric nutrient loads (Erdmann, Lippl et al. 2003; Greenman, Golani et al. 2004), and it would be interesting to compare the effects on ghrelin of an intragastric glucose bolus to those of a slow glucose infusion (Williams, Cummings et al. 2003). Nevertheless the available evidence indicates that duodenal contact is a requirement for ghrelin suppression and gastric factors contribute little, if anything, to this process. Further studies evaluating the effect of glucose and gastric distension on ghrelin secretion are required.

The suppression of ghrelin following the glucose infusions was not associated with a reduction in food intake. Findings from recent studies indicate that ghrelin may not be an

essential regulator of food intake. For example, ghrelin-deficient mice have equivalent food intake and body weight to wild-type mice (Wortley, Anderson et al. 2004). Rather ghrelin may function as an initiator of feeding (Cummings, Purnell et al. 2001; Wortley, Anderson et al. 2004), in determining the type of fuel (i.e. fat or carbohydrate) used for maintenance of energy balance (Wortley, Anderson et al. 2004), and as a regulator of long-term, rather than acute food intake (Salbe, Tschop et al. 2004).

This study was performed in healthy older subjects. It would be interesting to also evaluate the effect in younger people. There is currently no conclusive evidence that circulating ghrelin concentrations change with age, or that the factors affecting them do. We have reported previously no difference in plasma ghrelin concentrations between healthy older and young women, either fasting or in response to a nutrient load (Sturm, MacIntosh et al. 2003). It seems likely, therefore, that the results of this study, which are consistent with those of previous studies in rodents, will apply across the full human age range.

7.6 Conclusion

This study has shown that suppression of plasma ghrelin concentrations is equivalent following intragastric and intraduodenal glucose infusions. Although the stomach is the main source of ghrelin, this finding suggests that exposure of the small intestine to nutrients is sufficient for food-induced suppression of plasma ghrelin concentrations in humans.

Table 7.1 Baseline characteristics and inclusion criteria for women (n = 5) and men (n = 7) and total (n = 12) subjects

	Women (n = 5)	Men (n = 7)	Total (n = 12)
Age (yrs)	72 ± 2.3	74 ± 1.3	73.2 ± 1.2
Height (m)	1.62 ± 0.03	1.72 ± 0.02	1.68 ± 0.02
Weight (kg)	62.4 ± 2.8	71.0 ± 2.5	67.4 ± 2.2
BMI (kg/m ²)	23.7 ± 0.9	23.9 ± 0.5	23.8 ± 0.4
TFEQ	6.4 ± 1.0	7.7 ± 1.4	7.2 ± 0.9
Food (kcal/d)	1799 ± 267	2068 ± 84	1956 ± 121
Protein (%) [†]	19.4 ± 0.5	16.1 ± 0.9	17.5 ± 0.7
CHO (%) [†]	37.2 ± 0.9	47.2 ± 2.2	43.0 ± 1.9
Fat (%) [†]	37.7 ± 2.5	32.5 ± 2.2	34.7 ± 1.7

TFEQ, Factor 1 'cognitive restraint score' of the Three factor eating questionnaire;

Food, daily intake quantified from three-day food diary; CHO, carbohydrate, [†] daily protein, carbohydrate and fat intake; BMI, body mass index

Means ± SEM, * P < 0.05 men v women

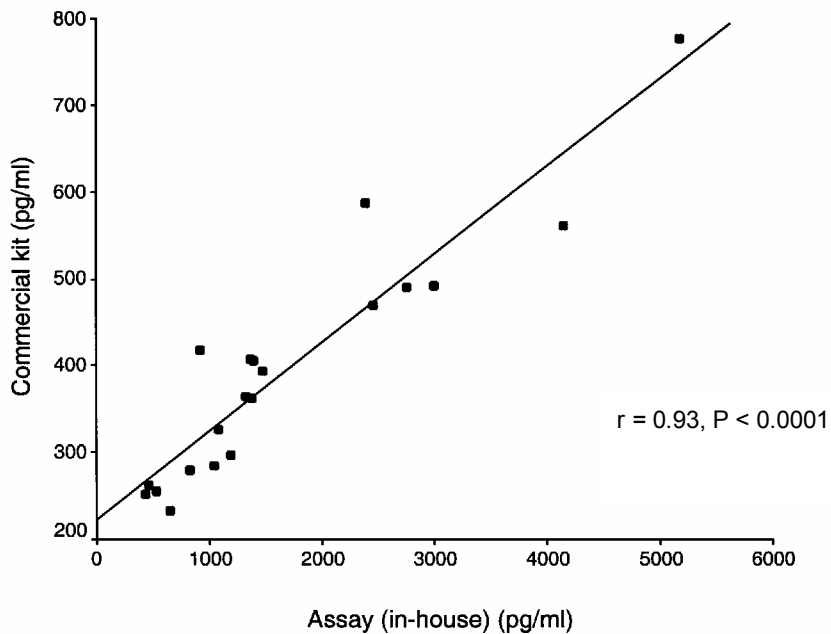


Figure 7.1 Correlation between plasma ghrelin concentrations (pg/ml) in 20 samples analysed with an assay incorporating Bachem antisera and comparing results to those obtained using the commercial ghrelin kit (Phoenix Pharmaceuticals, Ca). ($r = 0.93$, $P < 0.0001$). Phoenix, samples analysed using the commercial kit; standard, samples analysed using an in-house assay.

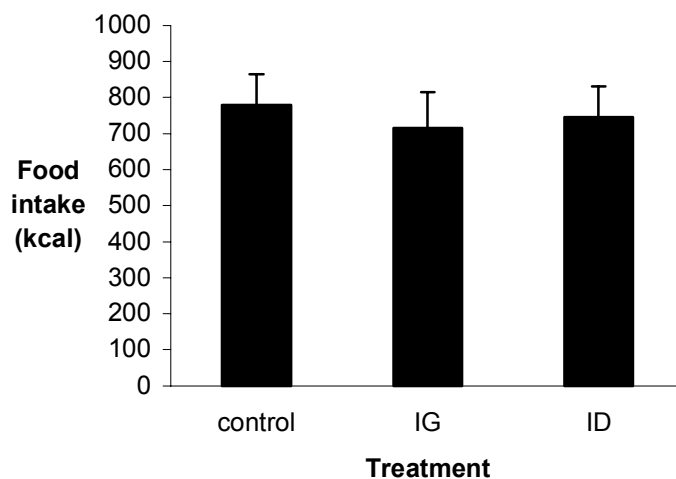


Figure 7.2 Mean \pm SEM differences in food intake at the buffet meal ($t = 150 - 180$ min) in 12 older subjects following 150 minutes of infusions of water (control, 0 kcal, 315ml of water at 2.1ml/min), IG (intra gastric, 25% glucose infusion 315ml at 2 kcal/min) or ID (intra duodenal, 25% glucose infusion 315ml at 2kcal/min). No differences were observed between treatments for food intake analysed by one-way ANOVA. Significance accepted as $P < 0.05$.

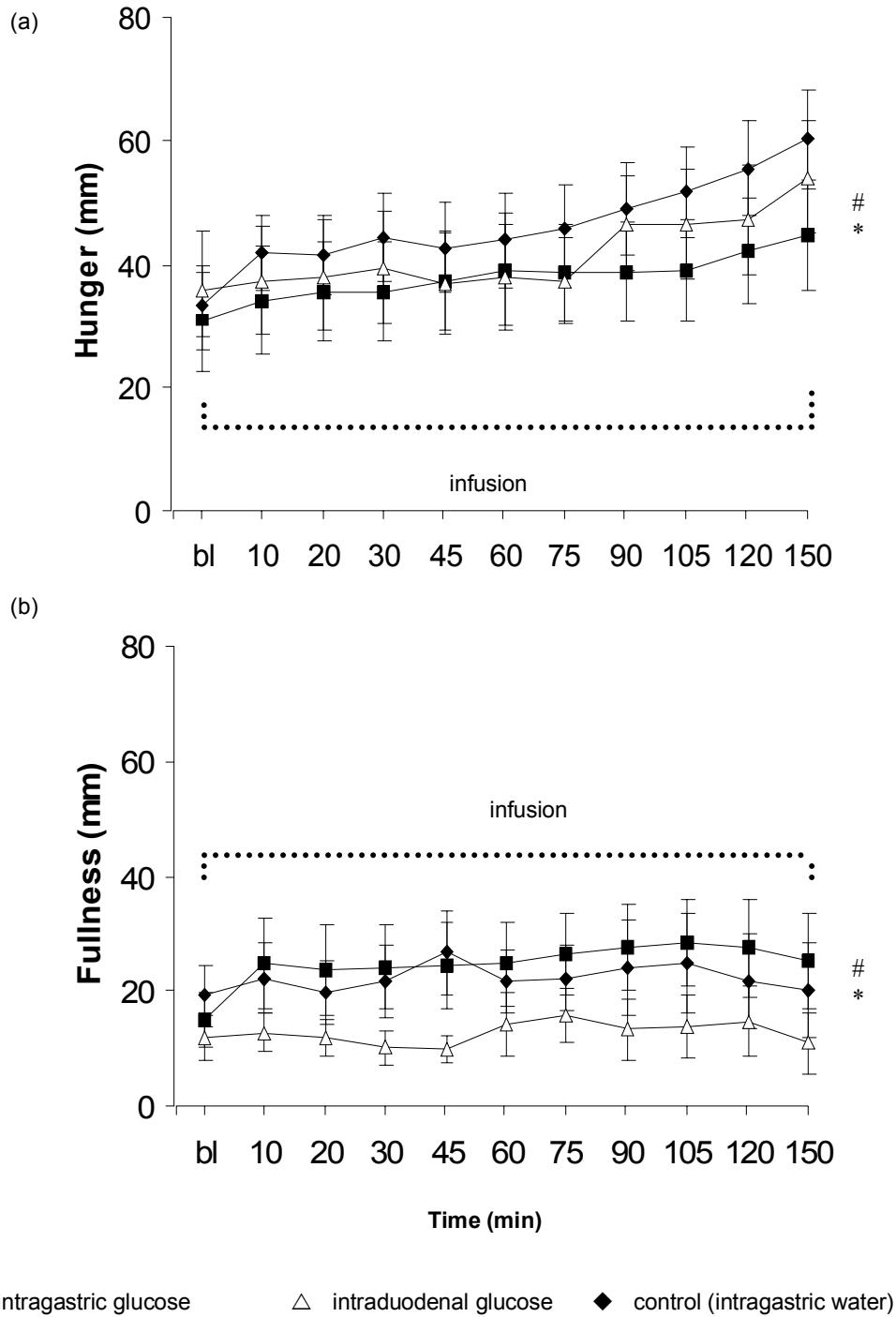


Figure 7.3 Mean ± SEM absolute ratings of (a) hunger and (b) fullness in 12 older subjects who received 150 minutes ($t = 0-150\text{min}$) of infusions of water (control - 0 kcal, 315ml of water at 2.1ml/min), IG (intra-gastric 25% glucose infusion 315ml at 2 kcal/min) or ID (intra-duodenal 25% glucose infusion 315ml at 2kcal/min) (a) effect of treatment, $P = 0.001$; *C v IG, $P = 0.02$; #C v ID, $P = 0.0003$; IG v ID, $P = 0.3$; and (b) effect of treatment, $P = 0.003$; C v IG, $P = 0.3$, *C v ID, $P = 0.04$; #IG v ID, $P = 0.001$; analysed by mixed model ANCOVA. Significance accepted as $P < 0.05$.

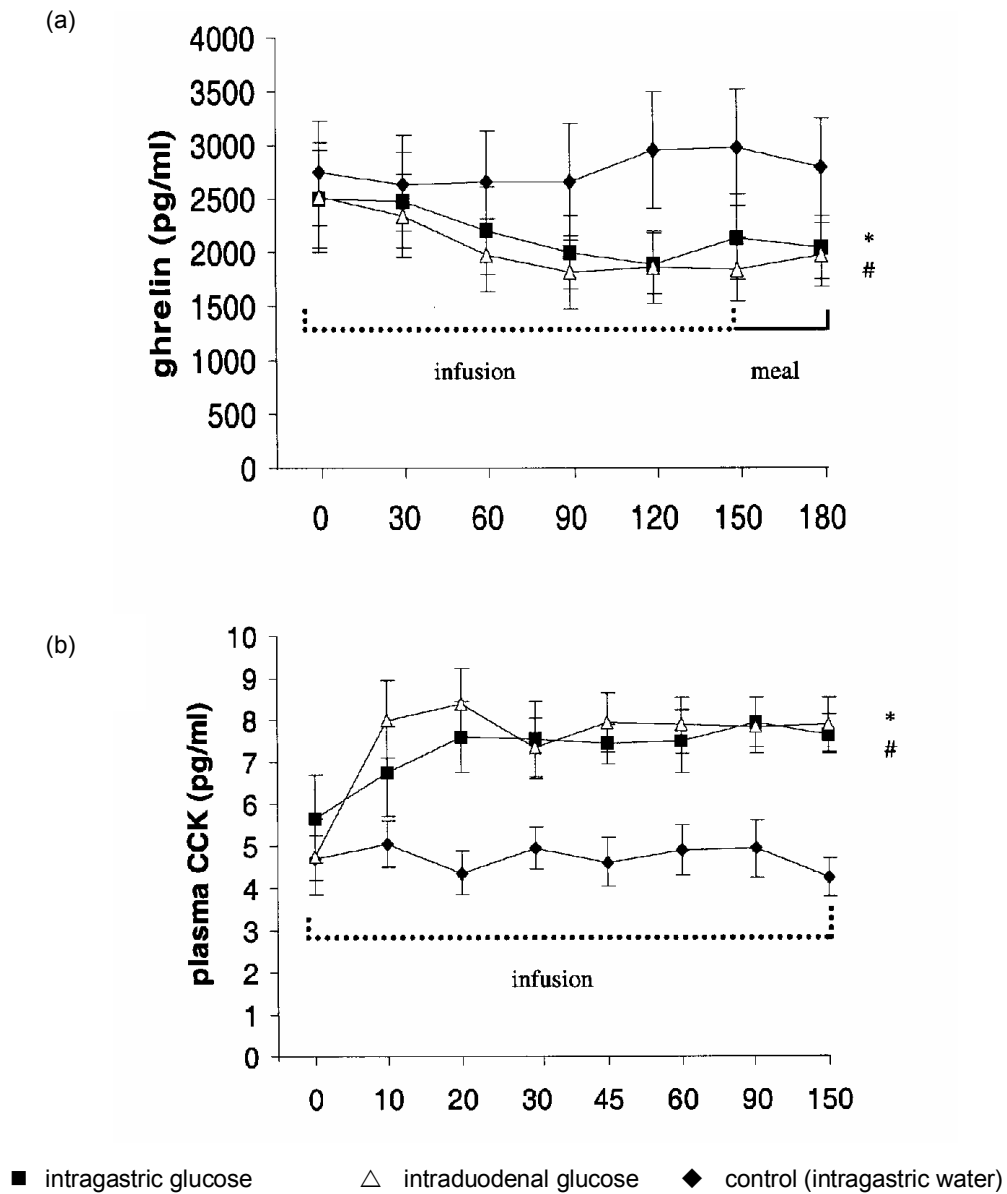


Figure 7.4 Mean \pm SEM plasma concentrations of (a) ghrelin and (b) cholecystokinin in 12 older subjects who received 150 (t = 0-150min) minutes of infusions of water (control - 0 kcal, 315ml of water at 2.1ml/min), IG (intragastric 25% glucose infusion 315ml at 2 kcal/min) or ID (intraduodenal 25% glucose infusion 315ml at 2kcal/min) (a) effect of treatment, $P < 0.0001$; *C v IG, $P < 0.0001$, #C v ID, $P < 0.0001$; IG v ID, $P = 0.2$) and (b) treatment x time effect, $P = 0.047$; *C v IG, $P < 0.0001$, #C v ID, $P < 0.0001$; analysed by mixed model ANCOVA. Significance accepted as $P < 0.05$.

Chapter 8

Effects of acute administration of domperidone on appetite and energy intake in healthy older men- a preliminary study

8.1 Summary

The study reported in Chapter 5 established, in healthy young and older subjects, that after a nutrient preload the perception of fullness is directly, and subsequent food intake inversely, related to antral area. The current, preliminary, study examined the hypothesis that the prokinetic drug, domperidone, would reduce antral area and thereby decrease fullness and increase food intake in healthy older subjects.

On two separate days, 10 healthy older men (age 73.7 ± 2.0 yr) were given either domperidone 40 mg dissolved in 50 ml water, or water alone, 60 minutes before a 400ml/500kcal yoghurt preload. After a further 60 minutes subjects were offered a buffet meal and invited to eat freely for up to 30 minutes. Antral area (measured by ultrasound), perceptions of appetite (visual analogues scales), and blood glucose concentrations were measured at 15-minute intervals. Food intake at the buffet meal was quantified.

After the preload there were increases in fullness ($P < 0.0001$), antral area ($P < 0.0001$) and blood glucose ($P < 0.0001$). At the time of ingestion of the buffet meal antral area had not decreased significantly from the maximum value. Domperidone had no effect on perceptions of appetite, antral area, blood glucose or food intake.

These preliminary observations suggest that domperidone when given acutely in a dose of 40mg, does not affect either antral area or appetite after a nutrient preload, in healthy older men.

8.2 Introduction

Gastric distension is known to inhibit food intake (Geliebter 1988; Sepple and Read 1989) and may be important in mediating the effects of gut hormones in appetite (Kissileff, Carretta et al. 2003). Recent studies indicate that the site of gastric distension may be important (Hveem, Jones et al. 1996; Jones, Doran et al. 1997). In Chapter 5 we reported (Sturm, Parker et al. 2004) that both the perception of fullness after a nutrient preload and subsequent food intake are related to antral area and, presumably, antral distension in healthy young and older subjects. There is evidence that prokinetic drugs, which increase the rate of gastric emptying, such as cisapride, and drugs which 'relax' the proximal stomach, such as sumatriptan, diminish postprandial fullness and reduce antral area/volume (Hausken and Berstad 1992; Eberl, Barnert et al. 1998). The effects of such agents on appetite in older subjects have hitherto not been evaluated.

Domperidone is a predominantly peripheral acting dopamine 2-receptor antagonist known to accelerate gastric emptying, particularly when the latter is delayed (Brogden, Carmine et al. 1982; Barone 1999). Gastric emptying of both solids and liquids has been reported to be accelerated by acute administration of domperidone in both healthy subjects (De Schepper, Wollaert et al. 1978) and in patients with gastroparesis (Corinaldesi, Stanghellini et al. 1983; Horowitz, Harding et al. 1985). In those studies involving healthy young subjects acceleration of gastric emptying of liquids has been observed after administration of oral doses of domperidone ranging from 10-20mg (Bateman, Gooptu et al. 1982; Valenzuela and Liu 1982; Tatsuta, Iishi et al. 1989) and the test meals included water, barium and cordial. The motor mechanisms underlying the acceleration of gastric emptying induced by domperidone remain poorly defined, but changes in proximal relaxation (Stanghellini, De Giorgio et al. 2004) and antroduodenal contractile activity (Baeyens, van de Velde et al. 1979; Weihrauch and Ehl 1981) are likely to be important. In healthy subjects and patients with disordered gastric emptying there is a gradual reduction in antral area/content following a meal (Sheiner, Quinlan et al. 1980; Collins, Horowitz et al. 1988; Hveem, Jones

et al. 1996; Sturm, Parker et al. 2004). Hence, acceleration of gastric emptying may be expected to be associated with an overall reduction in postprandial antral area. A number of prokinetic drugs, such as cisapride (which is now not available for clinical use in most countries) have been shown to also decrease fasting antral area (Hausken and Berstad 1992).

Domperidone has been shown to be effective in the treatment of gastrointestinal symptoms associated with functional dyspepsia, Parkinson's disease and diabetic gastroparesis (Horowitz, Harding et al. 1985; Duan, Zheng et al. 1993; Jost 1997; Soykan, Sarosiek et al. 1997; Patterson, Abell et al. 1999). It is generally well tolerated and unlike metoclopramide, adverse central nervous system (CNS) effects only occur rarely (Barone 1999; Patterson, Abell et al. 1999). The beneficial effects of domperidone on symptoms may also relate in part to central anti-emetic properties (Brogden, Carmine et al. 1982).

The aims of this preliminary study were to evaluate the acute effects of oral domperidone on perceptions of appetite and food intake in healthy older people. The broad hypothesis was that domperidone would reduce antral distension, induced by a nutrient preload, at least in part by accelerating gastric emptying and, thereby, reduce the perception of fullness and increase food intake at a subsequent meal.

8.3 Research design and methods

8.3.1 Subjects

Ten healthy older men (age range 65-85 years) were studied (Table 8.1). All subjects complied with the inclusion and exclusion criteria described in detail in Chapter 3.2. Subjects were informed that the purpose of the study was to assess the effects of domperidone on the distension of the stomach and the release of gastrointestinal hormones, and were, accordingly, unaware of the relevance of the assessment of appetite and food intake. The Research Ethics Committee of the Royal Adelaide Hospital approved the study and each subject gave written, informed consent.

8.3.2 Experimental design

Subjects attended the department at 0900h after a 12-hour overnight fast from food and fluids, except water, on two study days. Each study day was separated by between 3 and 7 days. Subjects were allowed one standard glass (200ml) of water before 0700h on the morning of the study, if they wished. Ultrasound was used to confirm that this had emptied from stomach.

Subjects were seated comfortably in an upright position that simulated normal eating conditions, and an intravenous cannula was inserted in an antecubital vein. After a 20-minute rest, each subject ingested either domperidone 40 mg (Jansen-Cilag Pty Ltd, North Ryde, NWS, Australia) dissolved in 50 ml of water or (on the control day) the same amount of water. On both days lemon juice (4 drops) was added to the water to disguise the taste of domperidone. Sixty minutes later, at $t = 0$ min, subjects consumed a 400ml (500 kcal) preload (detailed below) within 5 minutes. At $t = 60$ min subjects were offered a standard, buffet style meal containing food in excess of what they would normally eat, and invited to eat as much as desired over the next 30 minutes ($t = 60$ min to 90 min) until they were comfortably full. After the meal, subjects remained in the laboratory for a further 60 minutes (i.e. $t = 90$ to 150 min).

The energy intake of the buffet meal was quantified using Foodworks 2.10 software (Xyris Software Pty Ltd, Highgate Hill, Queensland, Australia). The interval of 60 minutes between the preload and the subsequent meal was selected on the basis of previous studies (Rolls, Kim et al. 1991; Sturm, Parker et al. 2004). Visual analogue scale (VAS) ratings of appetite and venous blood glucose concentrations were measured at 'baseline' i.e. immediately before ingestion of the preload ($t = -15$ and 0 min), and every 15 minutes from $t = 15$ min until $t = 150$ min, with the exception of $t = 75$ min when the subject was eating. Ultrasonographic measurements of antral area (using an Aloka SSD-650 CL Ultrasound Machine, ALOKA Co., LTD, Japan, see Chapter 3.6.3 for a detailed description) were performed before ingestion of the 'preload' ($t = -15$ and 0 min), and every 15 minutes from $t = 15$ min until $t = 150$ min, excluding $t = 75$ min.

8.3.3 Preload

The nutrient preload comprised a mixture of yoghurt, cream, milk, cornflour, golden syrup, sunflower oil and protein supplement with a macronutrient distribution of 15% protein, 30% fat and 55% carbohydrate (Table 8.2) (Sturm, Parker et al. 2004).

8.3.4 Visual analogue scales

Ratings of hunger, fullness, nausea, satiety, desire to eat and prospective consumption, as well as sensations of drowsiness and anxiety, were assessed using a VAS (Parker, Ludher et al. 2004). The amount of food eaten at the buffet meal and its macronutrient distribution were calculated and the energy content determined. Detailed descriptions of both the VAS and the quantification of food intake are provided in Chapter 3.2.

8.3.5 Statistical analyses

For visual analogue scores and blood glucose concentrations, 'baseline' was calculated as the mean of values at $t = -15$ and 0 min. Comparisons between single characteristics of the subjects (eg. BMI, baseline symptom scores, and fasting blood glucose concentrations) were performed using one-way analysis of variance (ANOVA, SuperANOVA Version 1.11 (Abacus Concepts Inc., California, USA). Repeated measures analysis of covariance (ANCOVA, SAS Version 9 (SAS Institute Inc., North Carolina, USA), with time ($t = 15 - 60$ min) and treatment (domperidone or control) as within-subject factors, and baseline as a covariate, was performed for ratings of appetite and blood glucose. When significant effects were observed, pairwise comparisons of adjusted means were performed using Student's t -test and adjusted for multiple comparisons using the Bonferroni (Holms adjustment) (ANCOVA) and stepdown Bonferroni (ANOVA) adjustments.

Differences in mean energy intake were analysed by one-way ANOVA, with treatment as the within-subject factor using SPSS for Windows 11.0 statistical software (Chicago, USA). Data following the buffet meal ($t = 90 - 150$ min) were not analysed, because the amount of food eaten was variable. Results are shown as means \pm SEM. A P value < 0.05 was considered statistically significant.

8.4 Results

All of the studies were well tolerated without untoward events. Measurements of antral area were technically adequate in all cases. Subjects were apparently unable to discriminate between domperidone and control.

8.4.1 Food intake (Figure 8.1)

There was no difference between study days in energy intake at the buffet meal (control 909 ± 83 kcal vs domperidone 908 ± 85 kcal, $P = 0.99$), or the macronutrient distribution of the food eaten (data not shown).

8.4.2 Visual analogue scales

8.4.2.1 Hunger (Figure 8.2)

There was no difference between the two days in baseline ratings of hunger ($P = 0.64$). There was a reduction in hunger ($t = \text{baseline to 15 min}$) after both control and domperidone ($P < 0.0001$, for both). Hunger increased between the preload and buffet meal ($t = 15 - 60$ min) with no difference between treatment and control (effect of time, $P < 0.0001$; treatment x time effect, $P = 0.96$).

8.4.2.2 Fullness (Figure 8.2)

There was no difference between the two days in baseline ratings of fullness. ($P = 0.78$). There was an increase in fullness ($t = \text{baseline to 15 min}$) after both control and domperidone ($P < 0.0001$, for both). Fullness decreased between the preload and buffet meal ($t = 15 - 60$ min) with no difference between treatment and control (effect of time, $P < 0.0001$; treatment x time effect, $P = 0.99$).

8.4.2.3 Desire to eat

There was no difference between the two days in baseline ratings of desire to eat ($P = 0.74$). Desire to eat increased between the preload and buffet meal ($t = 15 - 60$ min) with no difference between treatment and control (effect of time, $P < 0.0001$; treatment x time effect, $P = 0.98$) (data not shown).

8.4.2.4 Prospective consumption

There was no difference between the two days in baseline ratings of prospective consumption ($P = 0.78$). Prospective consumption increased between the preload and buffet meal ($t = 15 - 60$ min) with no difference in scores between treatment and control (effect of time, $P < 0.0001$; treatment x time effect, $P = 0.99$) (data not shown).

8.4.2.5 Nausea and drowsiness

Baseline ratings of nausea were not different between the two days ($P = 0.84$). There was no change in nausea over time or between treatments (treatment x time effect, $P = 0.7$). At baseline drowsiness was not different between study days ($P = 0.76$). There was no difference in scores for drowsiness over time or between treatments (treatment x time effect, $P = 0.9$) (data not shown).

8.4.3 Antral area (Figure 8.3)

There was no difference between the two days in baseline measurements of antral area ($P = 0.73$). There was an increase in antral area ($t =$ baseline to 15 min) after control and domperidone ($P < 0.0001$, for both). Following the preload ($t = 15 - 60$ min) mean antral area was less at 45 and 60 min after domperidone than after control, but this difference was not significant (treatment x time effect, $P = 0.93$). At $t = 60$ min antral area had not decreased significantly from its maximum (mean reduction from maximum: control $12.3 \pm 4.3\%$ vs domperidone $16.5 \pm 6.3\%$, $P = 0.57$).

8.4.4 Blood glucose (Figure 8.4)

There was no difference between the two days in baseline blood glucose ($P = 0.65$). On both days blood glucose increased between the preload and buffet meal ($t = 15 - 60$ min) with no difference in the effect of domperidone and control (effect of time, $P < 0.0001$; treatment x time effect, $P = 0.86$). Although mean blood glucose at 60 min was higher after domperidone this difference was not significant (domperidone 7.9 ± 0.7 vs control 7.1 ± 0.4 mmol/l, $P = 0.1$).

8.5 Discussion

In this preliminary study, acute administration of domperidone in a dose of 40mg had no effect on either perceptions of appetite, food intake, antral area or the glycaemic response to a yoghurt preload in healthy older subjects. Hence, the underlying hypothesis- that a pharmacological reduction in antral area would be associated with a reduction in fullness and an increase in subsequent food intake, was not addressed adequately. There are a number of factors which may account for this negative outcome including (i) the dose of domperidone used, (ii) the time interval between administration of domperidone and the buffet meal, (iii) the composition of the preload and (iv) the size of the cohort studied.

While previous studies have shown that acute oral and intravenous administration of domperidone increases the rate of gastric emptying of water, low nutrient liquids, and liquid barium in normal subjects (Baeyens, van de Velde et al. 1979; Broekaert 1979; Bateman, Gooptu et al. 1982), there is no information about the effect, if any, of domperidone on gastric emptying of high nutrient liquids. It is also well recognized that, in general, the magnitude of the acceleration of gastric emptying induced by prokinetic drugs is greater when gastric emptying is delayed (Harasawa and Miwa 1981; Valenzuela and Liu 1982; Horowitz and Dent 1991). Hence, it is difficult to overcome the regulatory mechanisms which control gastric emptying of nutrients in normal subjects - probably the most important of these is feedback inhibition generated by the interaction of nutrients with receptors in the small intestine (Lin, Doty et al. 1989; Lin, Moller et al. 1992).

In our study there was no evidence that domperidone accelerated gastric emptying of the preload, i.e. there were no differences in antral area, which is known to be a precise measure of gastric emptying of liquids (Bolondi, Bortolotti et al. 1985; Jones, Edelbroek et al. 1995). As mean antral area was slightly (albeit non-significantly) less at 30 and 60 minutes a type 2 error cannot be excluded, nevertheless if there was any effect on gastric emptying it would have been very small. The postprandial glycaemic response is known to be dependent on the rate of gastric emptying of carbohydrate (Rayner, Samsom et al. 2001) – even minor differences in the rate at which carbohydrate enters the small intestine may have a major effect on the rise in blood glucose (O'Donovan, Doran et al. 2004). Again, while blood glucose increased after the preload on both days, demonstrating that gastric emptying did occur, this was not affected by domperidone.

The fact that relatively little of the preload had emptied from the stomach at 60 minutes is, in retrospect, not surprising. In healthy older subjects gastric emptying may be slightly slower than in the young (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997) but usually still approximates an overall rate of 1-3 kcal/min (Brener, Hendrix et al. 1983; Horowitz, Edelbroek et al. 1993). If a rate of ~2 kcal/min is assumed in the current study at 60 minutes some 76% percent of the preload would be expected to still be in the stomach at that time, comparable to the non-significant mean decrease of 16.5% that was observed. Hence, the timing of the buffet meal (which was selected on the basis of our previous study in Chapter 5) was probably premature - a longer time period (perhaps 100 minutes) would have allowed for any effect of domperidone on gastric emptying to be evident and a significant reduction in antral area on both days. An alternative, and perhaps more likely explanation is that domperidone, in the dose used, does not accelerate gastric emptying of a high nutrient yoghurt preload in this group, as a result of small intestinal feedback. If this is the case effects are likely to be evident using a preload of lower nutrient content. These issues can only be resolved by further studies. It should also be recognized that the number

of subjects studied was relatively small, albeit comparable to those in other reports relating to the effects of domperidone on gastric emptying in healthy subjects (Broekaert 1979; Bateman, Goptu et al. 1982).

Based on the current observations and the considerations discussed above a further study is planned which will compare the effects of the same dose of domperidone on antral area, food intake and appetite in both healthy older and young subjects. In this way, age-related changes in gastric emptying/antral area and in the effect of domperidone will be able to be assessed. It is proposed that the nutrient yoghurt preload be of the same volume and composition used in this study, but of a lower energy content (250 kcal), and identical to the 'low-dose' preload in Chapter 5. This will enable direct comparisons to be made between the changes in antral area after this preload and those demonstrated previously in Chapter 5. In addition, the buffet meal will be offered later (at 100 minutes) to allow time for substantial emptying of the preload from the stomach so that the effects of domperidone on gastric emptying can be determined.

8.6 Conclusion

In healthy older men domperidone 40 mg had no effect on perceptions of appetite, food intake, antral area or blood glucose concentrations after a high nutrient preload. This result was perhaps unexpected given the documented effects of domperidone on gastric emptying in healthy young subjects and patients with gastroparesis. Effects related to age, sample size, the time of meal consumption and the energy content of the preload represent potential confounding variables. Further studies are planned to address these issues.

Table 8.1 Characteristics of subjects (n = 10)

Parameter	Total (n = 10)
Age (yrs)	73.7 ± 2.0
Height (m)	1.73 ± 0.02
Weight (kg)	74.7 ± 2.95
BMI (kg/m ²)	25.0 ± 0.6
TFEQ	6.5 ± 1.1
GDS	2.5 ± 1.0
MMSE	29.5 ± 0.3

TFEQ, Factor 1 'cognitive restraint score' of the Three factor eating questionnaire;

GDS, Geriatric Depression Scale; SMMSE, Mini-Mental State Examination; BMI, body mass index. Means ± SEM

Table 8.2 Composition of 400ml (500kcal) preload

Foods	Energy (kcal)	% Protein	% CHO	% Fat	% Others
Total	510	13	54	33	
Yoghurt Greek Style (high fat) ^a	172	18	28	50	4
Yoghurt (reduced fat, plain) ^b	26	29	41	21	9
Cornflour ^c	38	0	93	1	6
Cream (Fat > 35%) ^d	20	2	3	95	0
Golden syrup ^e	88	1	99	0	0
Sunflower oil ^f	54	0	0	100	0
Nonfat skim milk ^h	58	42	51	2	5
Glucose powder	54	0	97	0	3

Calculated using Foodworks Version 2.10, Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia; Others, percentage of energy due to organic acids, fibre, alcohol etc; CHO, carbohydrate; ^a Australia Co-operative Foods Ltd, Dairy Farmers, Birnie Ave, Lidcombe NSW 2141; ^b Traditional Natural yoghurt 99.8% Fat Free, Australia Co-operative Foods Ltd, Dairy Farmers, Birnie Ave, Lidcombe NSW 2141; ^c White Wings Foods, 75 Talavera Rd, Macquarie Park, NSW 2133, Australia; ^d Pura Cream, National Foods Ltd, 5 Queens Rd, Melbourne, Victoria 3004, Australia; ^e Smiths, 399 Archerfield Rd, Richlands, Brisbane 4077, Australia; ^f Meadow Lea Foods Ltd, 514 Gardeners Rd, Mascot NSW 2020, Australia; ^g Ross Products Division, Abbott Laboratories, Columbus, Ohio USA; ^h Pura Skimmer, Farmers Union, National Foods Ltd, 5 Queens Rd, Melbourne, Victoria 3004, Australia; ⁱ Davis Gelatine Australia Pty Ltd, Sunny Hills, Flood Road, Josephville via Beaudesert QLD 4285; ^k Merisant Australia Pty Ltd, 20 Clarke St, Crows Nest, NSW 2065 Australia.

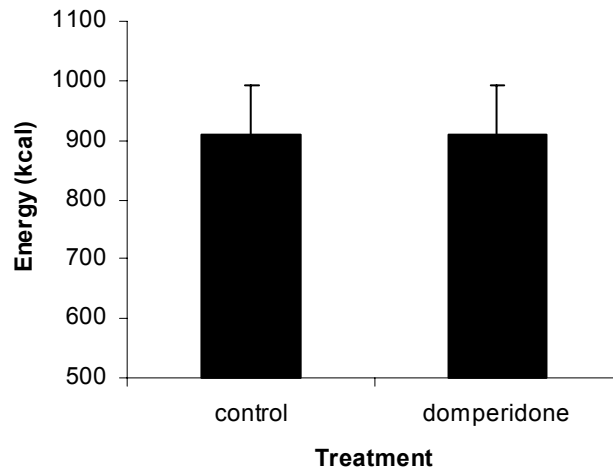


Figure 8.1 Mean \pm SEM ratings of food intake (kcal) at the buffet meal in 10 older (73.7 ± 2 yr) men who received a 400 ml (500 kcal) yoghurt preload 60 minutes after 40 mg oral domperidone or placebo. There is no difference in food intake between the two days.

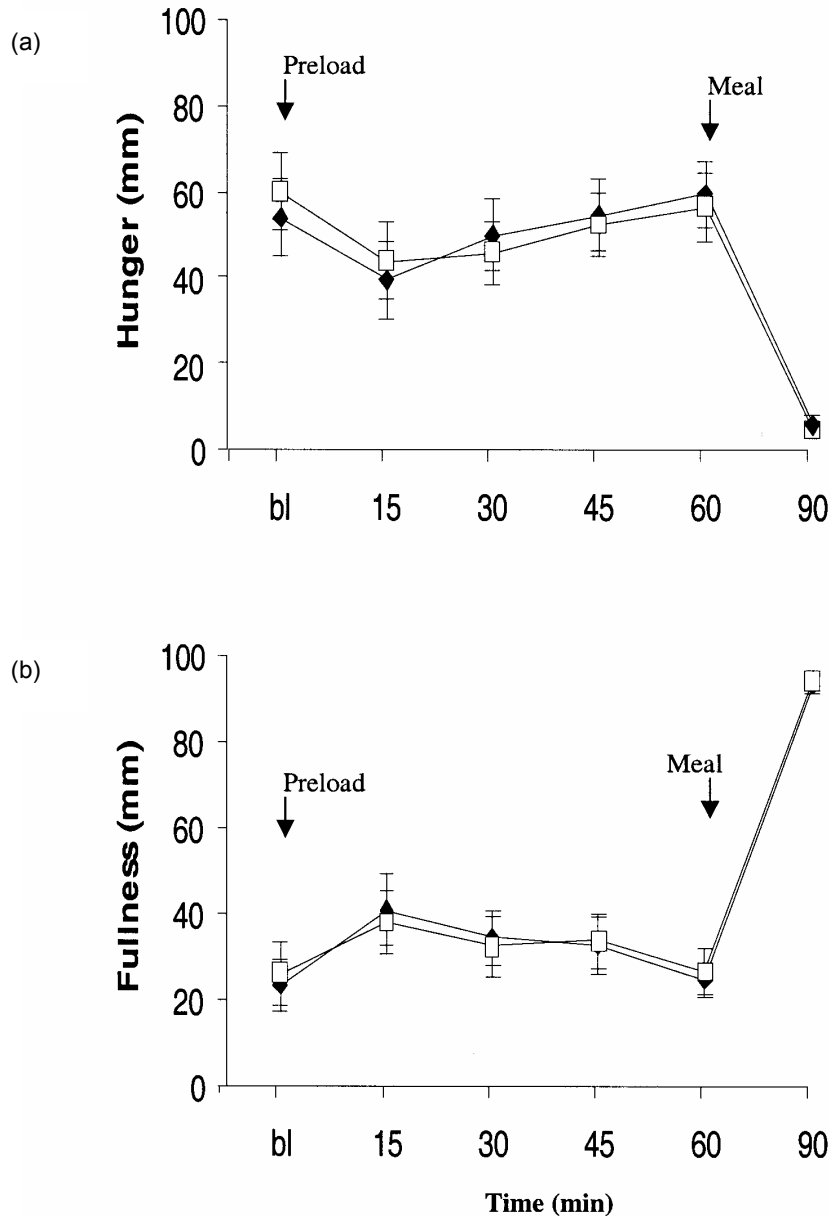


Figure 8.2 Ratings of (a) hunger and (b) fullness in 10 older men (73.7 ± 2 yr) who received a 400 ml (500 kcal) yoghurt preload 60 minutes after 40 mg oral domperidone (□) or placebo (♦). (a) There was no difference between the two days for baseline ratings of hunger ($P = 0.64$). There was a reduction in hunger scores ($t = \text{baseline to 15 min}$) after both control and treatment with domperidone ($P < 0.0001$). Ratings of hunger increased between the preload and buffet meal ($t = 15 - 60$ min) with no difference in scores between treatment and control (effect of time, $P < 0.0001$; treatment \times time effect, $P = 0.96$). (b) There was no difference between the two days for baseline ratings of fullness ($P = 0.78$). There was an increase in fullness scores ($t = \text{baseline to 15 min}$) after both control and treatment with domperidone ($P < 0.0001$). Ratings of fullness decreased between the preload and buffet meal ($t = 5 - 60$ min) with no difference in scores between treatment and control (effect of time, $P < 0.0001$; treatment \times time effect, $P = 0.99$). Data are mean \pm SEM.

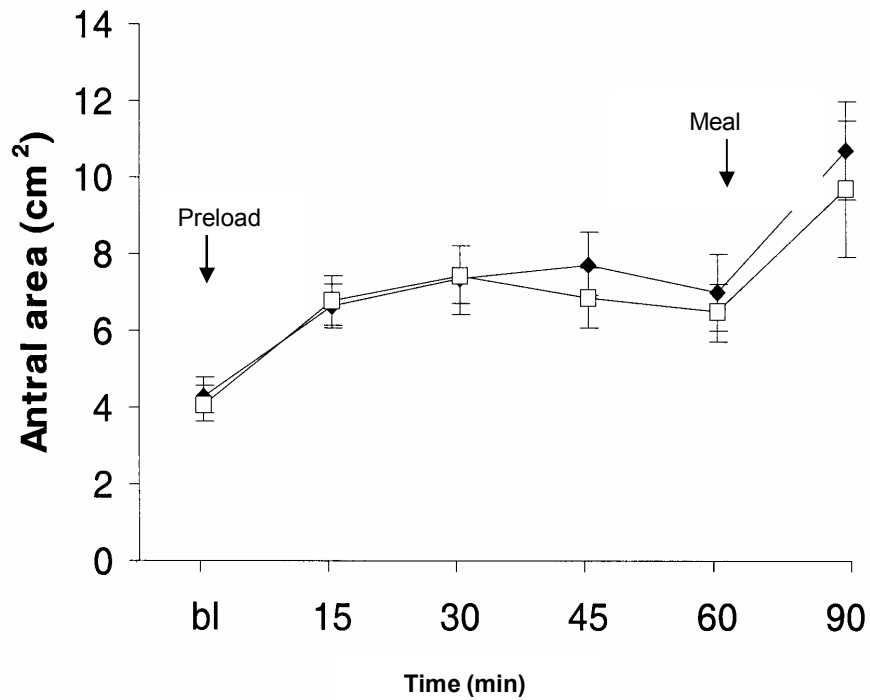


Figure 8.3 Antral area in 10 older men (73.7 ± 2 yr) who received a 400ml (500kcal) yoghurt preload 60 minutes after 40mg domperidone (\square) or placebo (\blacklozenge). There was no difference in baseline measurements of antral area ($P = 0.73$). There was an increase in antral area ($t = \text{baseline to 15 min}$) after control and treatment with domperidone ($P < 0.0001$). Following the preload ($t = 15 - 60$ min) antral area was less on the domperidone day but this difference was not significant (treatment \times time effect, $P = 0.93$). Analysed by mixed model ANCOVA. Data are mean \pm SEM.

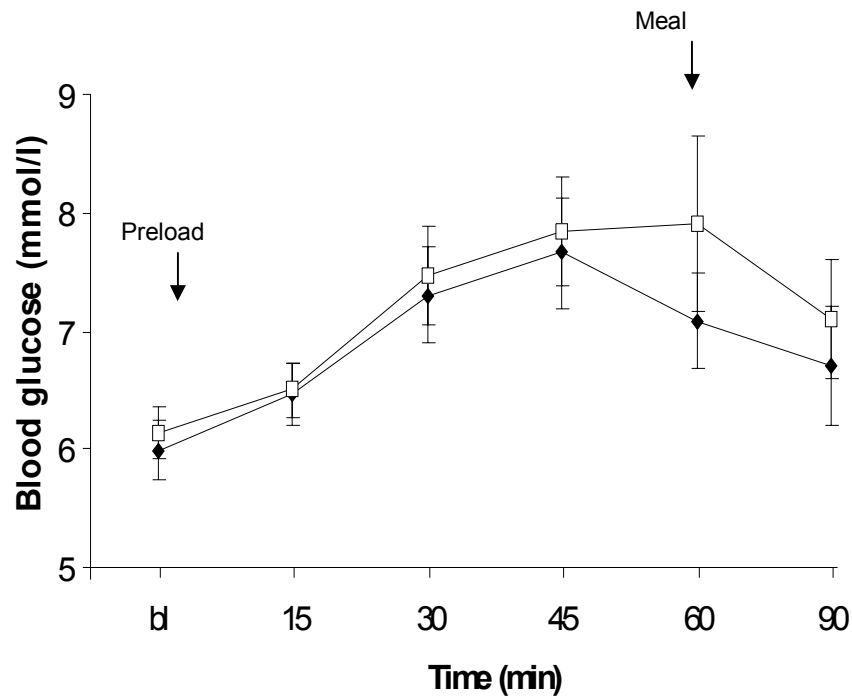


Figure 8.4 Blood glucose in 10 older men (73.7 ± 2 yr) who received a 400ml (500kcal) yoghurt preload 60 minutes after 40mg domperidone (\square) or placebo (\blacklozenge). There was no difference between the two days in baseline blood glucose ($P = 0.65$). There was an increase in blood glucose ($t =$ baseline to 15 min) after treatment with domperidone ($P = 0.04$ but not significantly after control ($P = 0.08$). Blood glucose increased between the preload and buffet meal ($t = 15 - 60$ min) with no difference between domperidone and control (*effect of time, $P < 0.0001$ compared to baseline; treatment x time effect, $P = 0.86$). Analysed by mixed model ANCOVA. Data are mean \pm SEM.

Chapter 9

Conclusion

Healthy ageing is associated with a reduction in appetite and food intake, termed the ‘anorexia of ageing’. In the face of competing stressors this physiological anorexia probably predisposes older people to malnutrition, weight loss, illness and death. Appetite regulation in humans is complex but it is clear that signals from the gastrointestinal system are important. A number of changes have been reported in both the stomach and small intestine with increasing age. These changes include an alteration in the distribution of food within the three compartments of the stomach, a slowing of contents from the distal stomach to the small intestine and the up or down regulation of gastrointestinal satiety hormones. The effect of these changes on appetite sensations, such as hunger and fullness and food intake in older people remains unclear. The aims of this thesis were to investigate the role of the stomach, in particular the distal stomach or gastric antrum and the effect of different macronutrients, such as fat and carbohydrate, on appetite, food intake and the release of gastrointestinal hormones in older subjects. Where possible these results were compared to those obtained in young subjects.

The most common form of assessment of appetite in clinical research studies is the visual analogue scale (VAS). This tool had previously been validated for use in appetite studies in a young but not older population. VAS were used in all studies in this thesis to assess appetite and ‘non-appetite’ sensations. Since appetite was a primary endpoint it was vital that this tool be validated for use in this age group. In two studies presented in Chapter 4, VAS were determined to be at least as effective in assessing subjective appetite and predicting subsequent food intake in healthy older as in previously reported studies in young adults. Thus, this validated scale was used to assess appetite sensations, such as hunger, fullness, desire to eat and prospective consumption, as well as ‘non-appetite’ sensations of anxiety and drowsiness, in Chapters 7 and 8 in older men and women. The

results obtained for the reproducibility of ‘satiety’ in older adults were much larger than that previously obtained in young adults. This was presumably due to the difficulty these subjects had in deciphering and applying the term, suggesting caution in the use of this term to describe appetite in this age group.

An ‘*a priori*’ hypothesis of this thesis was that an alteration in the distribution of food within the stomach with increasing age would influence appetite and food intake. Further, it was suggested that due to a decrease in gastric accommodation in the proximal stomach, food would be moved more rapidly to the distal stomach in older adults. It was predicted that food would remain longer in the gastric antrum and empty more slowly to the small intestine. In Chapter 5, information was provided that antral area was larger in older than young men after nutrient preloads, and more importantly that food intake immediately before a meal was related to antral area in older subjects. Although it had been previously reported that satiation was related to antral area in young men, this relationship in older men had not been previously established.

An acute oral dose (40mg) of domperidone, given 60 minutes before a nutrient preload, did not increase gastric emptying rate, as assessed by ultrasound measurements of antral area, or reduce food intake compared to placebo in healthy older men. Possible reasons for the lack of positive results in this study include a small sample, the use of ultrasound to assess gastric emptying and the time difference between the intervention and meal. These factors should be considered in any future studies investigating the use of domperidone on gastric emptying and food intake in both older and young subjects.

It should be recognized that although 2-dimensional ultrasonography of the gastric antrum has been validated as a proxy of gastric emptying a limitation of these studies is that we were not able to validate the measurements of antral area with other methods. For example, 3-dimensional ultrasonography allows for transpyloric flow assessment and scintigraphy for the simultaneous measurement of the proximal stomach, both of which would aid in

determining the concurrent contributions of the stomach and small intestine. In addition, a scintigraphic study would give further information about the distribution and emptying rates of different macronutrient liquids, such as fat and carbohydrate in Chapter 6, within the antrum.

This thesis has also provided new information regarding the effect of fat and carbohydrate on appetite and food intake in young and older subjects. There currently appears to be little consensus about the relative satiating effects of fat and carbohydrate when administered either as pure nutrients or whole foods, or delivered as an oral, intragastric or intraduodenal solution. A previous study by our group showed that fat was more satiating than carbohydrate in young men, and equally as satiating in older men, when infused to the small intestine. A novel finding is that carbohydrate is more satiating than fat in both older and young men when delivered as a rapid intragastric bolus. Taken together these results suggest that ageing may affect the response to carbohydrate but not fat. These studies used pure nutrients for the infusions and further studies using different compositions of carbohydrate and fat are required. In addition, administration of slow infusions to both the stomach and small intestine, and rapid infusions to the stomach will provide further information about the contribution of gastric distension and the relative satiating effects of these macronutrients in both older and young subjects.

In both studies (Chapters 5 and 6) evaluating the effects of a preload on appetite and food intake in healthy young and older men, previous findings that the small intestinal satiety hormone CCK is increased in older compared to young subjects both fasting and postprandial were confirmed. There were inverse relationships between plasma CCK concentrations and food intake in both older and young subjects providing further evidence of CCK as a satiety hormone. In Chapter 7, we have confirmed previous studies in rats that exposure of the small intestine is necessary for suppression of ghrelin concentrations. This finding provides confirmation that this effect is not species-specific. Although we also

found evidence of a relationship between ghrelin and gender, weight and blood glucose concentrations, these relationships are not unanimously found in studies by other groups and require further investigation.

This thesis has assessed the contribution of the gastrointestinal system, in particular the distal stomach, to the physiological 'anorexia of ageing'. The effect of changes in intragastric food distribution and the relative satiating effects of different macronutrients, such as fat and carbohydrate, to the reduction in food intake and appetite with increasing age remain uncertain. Further careful studies investigating the effect of these factors on appetite and food intake in older men and women are required.

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Appendices

APPENDIX I

Geriatric Depression Scale

Circle the answer on the same line for how you felt in this past week	Yes	No
1. Are you basically satisfied with your life?	0	1
2. Have you dropped many of your activities and interests?	1	0
3. Do you feel that your life is empty?	1	0
4. Do you often get bored?	1	0
5. Are you hopeful about the future?	0	1
6. Are you bothered by thoughts you cannot get out of your head?	1	0
7. Are you in good spirits most of the time?	0	1
8. Are you afraid that something bad will happen to you?	1	0
9. Are you happy most of the time?	0	1
10. Do you often feel helpless?	1	0
11. Do you often get restless and fidgety?	1	0
12. Do you prefer to stay at home, rather than going out and doing new things?	1	0
13. Do you frequently worry about the future?.....	1	0
14. Do you feel you have more problems with memory than most?.....	1	0
15. Do you think it's wonderful to be alive now?.....	0	1
16. Do you often feel down-hearted and blue?.....	1	0
17. Do you feel pretty worthless the way you are now?.....	1	0
18. Do you worry a lot about the past?.....	1	0
19. Do you find life very exciting?.....	0	1
20. Is it hard for you to get started on things?.....	1	0
21. Do you feel full of energy?.....	0	1
22. Do you feel that your situation is hopeless?.....	1	0

Appendix I

23. Do you think that most people are better off than you are?.....	1	0
24. Do you frequently get upset over things?.....	1	0
25. Do you frequently feel like crying?.....	1	0
26. Do you have trouble concentrating?.....	1	0
27. Do you enjoy getting up in the morning?.....	0	1
28. Do you prefer to avoid social gatherings?.....	1	0
29. Is it easy for you to make decisions?.....	0	1
30. Is your mind as clear as it used to be?.....	0	1

(Yesavage et al 1988)

APPENDIX II

Standardised Mini-Mental State Examination

	Maximum score	Score
ORIENTATION		
What is the: <year> <season> <date> <day> <month>?	5	()
Where are we: <state> <country> <town> <hospital> <floor>?	5	()
REGISTRATION		
Name 3 objects. Allow 1 second to say each. Then ask the patient all 3 after you have said them. Give 1 point for each correct answer. Then repeat until the patient learns all 3. Count the number of trials and record. Number of trials:.....	3	()
ATTENTION AND CALCULATION		
Serial 7's. Count forwards by seven. Stop after 5 answers 1 point for each correct. Alternatively spell 'world' backwards.	5	()
RECALL		
Repeat the 3 objects named above. Give 1 point for each correct answer.	5	()
LANGUAGE		
Name a pencil and watch	2	()
Repeat the following: 'No ifs, and or buts'	1	()
Follow a 3 stage command: 'take a piece of paper in your right hand, fold it in half, and put it on the floor.'	1	()
Read and obey the following: CLOSE YOUR EYES	1	()
Write a sentence	1	()
Copy a design	1	()
Assess level of consciousness along a continuum:		
<hr style="width: 100%;"/> Alert Drowsy Stupor Coma		
		<hr style="width: 100%;"/> Total 30 ()

(Molloy et al, 1991; Folstein et al, 1975)

APPENDIX III

Three-Factor Eating Questionnaire

One point is given for each item in Part I and for each item (numbered question) in Part II. The correct answer for the true/false items is underlined and beside it is a number of the factor that it measures. The direction of the question in Part II is determined by splitting the responses at the middle. If the item is labelled '+', those responses above the middle are given zero. Vice versa for those with a '-'. For example, anyone scoring 3 or 4 on the first item in Part II (item No. 37) would receive one point. Anyone scoring 1 or 2 would receive zero.

Part I

		Factor number
1.	When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	T F 2
2.	I usually eat too much at social occasions, like parties and picnics.	T F 2
3.	I am usually so hungry that I eat more than three times a day.	T F 3
4.	When I have had my quota of calories, I am usually good about not eating any more	T F 1
5.	Dieting is so hard for me because I just get too hungry.	T F 3
6.	I deliberately take small helpings as a means of controlling my weight.	T F 1
7.	Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	T F 2
8.	Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	T F 3
9.	When I feel anxious, I find myself eating.	T F 2
10.	Life is too short to worry about dieting.	T F 1
11.	Since my weight goes up and down, I have gone on reducing diets more than once.	T F 2
12.	I often feel so hungry that I just have to eat something.	T F 3
13.	When I am with someone who is overeating, I usually overeat too.	T F 2

- | | | | |
|-----|--|-----|---|
| 14. | I have a pretty good idea of the number of calories in common food. | T F | 1 |
| 15. | Sometimes when I start eating, I just can't seem to stop. | T F | 2 |
| 16. | It is not difficult for me to leave something on my plate. | T F | 2 |
| 17. | At certain times of the day, I get hungry because I have gotten used to eating then. | T F | 3 |
| 18. | While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. | T F | 1 |
| 19. | Being with someone who is eating often makes me hungry enough to eat also. | T F | 3 |
| 20. | When I feel blue, I often overeat. | T F | 2 |
| 21. | I enjoy eating too much to spoil it by counting calories or watching my weight. | T F | 1 |
| 22. | When I see a real delicacy, I often get so hungry that I have to eat right away. | T F | 3 |
| 23. | I often stop eating when I am not really full as a conscious means of limiting the amount that I eat. | T F | 1 |
| 24. | I get so hungry that my stomach often seems like a bottomless pit. | T F | 3 |
| 25. | My weight has hardly changed at all in the last ten years. | T F | 2 |
| 26. | I am always hungry so it is hard for me to stop eating before I finish the food on my plate. | T F | 3 |
| 27. | When I feel lonely, I console myself by eating. | T F | 2 |
| 28. | I consciously hold back at meals in order not to gain weight. | T F | 1 |
| 29. | I sometimes get very hungry late in the evening or at night. | T F | 3 |
| 30. | I eat anything I want anytime I want. | T F | 1 |
| 31. | Without even thinking about it, I take a long time to eat. | T F | 2 |
| 32. | I count calories as a conscious means of controlling my weight. | T F | 1 |
| 33. | I do not eat some foods because they make me fat. | T F | 1 |
| 34. | I am always hungry enough to eat at anytime. | T F | 3 |
| 35. | I pay a great deal of attention to changes in my figure. | T F | 1 |

36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods. T F 2

Part II

Directions: Please answer the following questions by circling the number above the response that is appropriate to you.

37. How often are you dieting in a conscious effort to control your weight?
 1 2 3 4
 rarely sometimes usually always +1
38. Would a weight fluctuation of 5 lbs affect the way you live your life?
 1 2 3 4
 not at all slightly moderately very much +1
39. How often do you feel hungry?
 1 2 3 4
 only at meal times sometimes between meals often between meals almost always -3
40. Do your feelings of guilt about overeating help you to control your food intake?
 1 2 3 4
 never rarely often always +1
41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours
 1 2 3 4
 easy slightly difficult moderately difficult very difficult +3
42. How conscious are you of what you are eating?
 1 2 3 4
 not at all slightly moderately very much +1
43. How frequently do you avoid 'stocking up' on tempting foods?
 1 2 3 4
 almost never seldom usually almost always +1
44. How likely are you to shop for low calorie foods?
 1 2 3 4
 unlikely slightly likely moderately likely very likely +1
45. Do you eat sensibly in front of others and splurge alone?
 1 2 3 4
 never rarely often always +2
46. How likely are you to consciously eat slowly in order to cut down on how much you eat?
 1 2 3 4
 unlikely slightly likely moderately likely very likely +1

47. How frequently do you skip dessert because you are no longer hungry?
 1 almost never 2 seldom 3 at least once a week 4 almost every day -3
48. How likely are you to consciously eat less than you want?
 1 unlikely 2 slightly likely 3 moderately likely 4 very likely +1
49. Do you go on eating binges though you are not hungry?
 1 never 2 rarely 3 sometimes 4 at least once a week +2
50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never giving in'), what number would you give yourself?
- 0
eat whatever you want, whenever you want it. +1
- 1
usually eat whatever you want, whenever you want it.
- 2
often eat whatever you want, whenever you want it.
- 3
often limit food intake, but often 'give in'.
- 4
usually limit food intake, rarely 'give in'.
- 5
constantly limiting food intake, never 'giving in'.
51. To what extent does this statement describe your eating behaviour? 'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'
- 1 not like me 2 a little like me 3 pretty good description of me 4 describes me perfectly. +2

(Stunkard and Messick, 1985)

APPENDIX IV

Three Day Food Diary

Name: _____

Please return to
Barbara Parker
Department of medicine, RAH
Tel: 8222 5039

Guidelines

1. This is a diary for you to record **everything** you eat and drink for **3** days.
2. To record in the food diary we would like you to **weigh** as many foods as practical.
Alternatively, use cup or spoon measures (metric) or common serves eg, **slice** of bread. **Do not** guess weights unless you are eating out and there is no alternative.
3. Record **everything** that you eat and drink from the time you get up in the morning until you go to bed at night. **Use a separate page for each day.**
4. Fill in the diary **immediately** after eating. Try to make your eating pattern as **typical** as possible.
5. Don't forget to record all **drinks and snacks** such as tea/coffee (with or without milk and sugar) or alcoholic and soft drinks.
6. Be as **specific** as possible. Specify the type of bread (white/wholemeal), the degree of fat trimming meat, type of margarine or oil and the type of milk (whole or skim).
7. If you follow a **recipe**, please record it in the food diary (see **example**).
8. Indicate the **method** of cooking eg; boiling or frying. Also indicate the type of oil used in frying (eg olive or canola etc).
9. List **separate foods on a different line** so that a ham sandwich should be recorded as; bread (type), margarine (type) and ham (type) - all on separate lines.
10. Please **take the diary with you** if you eat or drink anything outside of your home.
11. Use the **example** and **flow charts** given as a guide to record your foods and drinks.

Date: 20.5.00

Day of week: Saturday

Time	Description of food and drink consumed	Quantity	Measure
	EXAMPLE		
0700	Weetbix	3	Biscuit
	Milk (full cream)	1/2	cup
	Bread (white, toasted)	1	slice
	Margarine (low-salt, polyunsaturated)	2	tsp
	Orange juice (unsweetened)	1	glass
1000	Coffee (instant)	1	cup
	Sugar (white)	2	tsp
	Biscuits (milk arrowroot)	2	biscuit
1230	Bread (white)	2	slices
	Margarine (low-salt, polyunsaturated)	2	tsp
	Ham (shaved, shoulder)	1	slice
	Cheese (low-fat cheddar)	1	slice
1730	Steak (beef, grilled)	200	g
	Potato (with skin, baked)	200	g
	Beans (French, boiled)	60	g
	Bread (white)	1	roll
2200	Milk (full cream)	250	ml
	'milo'	2	tsp
	Biscuits (milk coffee)	2	biscuits
	EXAMPLE RECIPE		
Recipe	Tuna mornay	2	erves
	Tuna (in brine)	250	g
	Flour (plain, white)	1	tbsp
	Cheese (matured)	20	g
	Breadcrumbs (white)	1	tbsp
	Margarine (low-salt, polyunsaturated)	3	tsp
	Milk(skim)	1/2	cup

