An Investigation of the 'Anorexia of Ageing'

A Thesis Submitted By

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For The Degree Of

Doctor Of Philosophy

Department Of Medicine
University Of Adelaide

December, 2000

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SUMMARY

Ageing is associated with a progressive decline in appetite and energy intake. Because the latter is often greater than the decrease in energy expenditure that also occurs with normal ageing, involuntary weight loss frequently occurs. This 'physiological' reduction in appetite and energy intake, termed the "anorexia of ageing", may also predispose individuals to pathological weight loss and malnutrition, which represent major causes of morbidity and mortality in the elderly. The cause(s) of this physiological anorexia are poorly defined.

The studies presented in this thesis address some of the mechanisms which may potentially contribute to the physiological anorexia of ageing, as suggested by previous animal and human studies. The studies reported in Chapters 8-11 are a logical progression from a study conducted during the authors' Honours Degree. In that initial study, which is described in Appendix A, the effects of intraduodenal (ID) infusion of lipid and glucose on appetite, pyloric motility and subsequent energy intake were evaluated in young and older, healthy, men. In the young, ID lipid suppressed hunger to a greater extent than ID glucose; in contrast ID lipid did not appear to reduce hunger in the older subjects. The stimulation of phasic, but not tonic, pyloric pressure waves by intraduodenal lipid infusion was greater in older than young men. There was a greater suppression of energy intake by fat than glucose in the young men, but no difference in the effects of the two nutrients in older men. These observations suggested that effects of ID nutrients on appetite are impaired in the healthy elderly. The enhanced pyloric response to ID lipid may potentially lead to slower gastric emptying.

There is some evidence that ageing is associated with an enhanced endogenous release of gastrointestinal satiety hormones, specifically cholecystokinin (CCK). The effects of ageing on endogenous release of CCK, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) in response to intraduodenal lipid and glucose infusion were evaluated using blood samples collected during the initial study described above. Plasma CCK concentrations were higher in older than young subjects at both baseline, and in response to the ID lipid infusion. There was no difference in plasma GLP-1 or PYY concentrations between the age groups. The decrease in hunger during ID lipid was inversely related to the increase in CCK, GLP-1 and PYY in young, but not older, subjects. During ID lipid infusion the increase in isolated pyloric pressure waves (IPPW) frequency was positively related to plasma GLP-1 and PYY and the increase in

IPPW amplitude was positively related to CCK in the older, but not young, subjects while the increase in IPPW amplitude and pyloric tone was inversely related to GLP-1 and PYY in the young subjects. These observations indicate for the first time that human ageing is associated with an increase in circulating CCK concentrations which may potentially contribute to slowing of gastric emptying, increased pyloric motility and reduction in appetite

As stated previously, in the initial Honours study, there was a greater suppression of energy intake by ID fat than glucose in the young men, but no difference between the effects of the two nutrients in older men. Due to the absence of a control (saline) infusion in that study, however, it was not known whether this age-related difference was due to an increased satiating effect of ID glucose, or a reduction in the satiating effect of fat, in older compared to young men ie potential differences in the effects of ID glucose and lipid between the two groups could not be evaluated. A second study was therefore conducted, to investigate the effects of intraduodenal (ID) infusion of saline, glucose and lipid on appetite, blood glucose and gastrointestinal hormone release, gastric myoelectrical activity and energy intake in young and older healthy men. ID lipid suppressed energy intake in both the young and older men, whereas ID glucose suppressed energy intake only in the older men. The blood glucose and insulin responses to ID glucose were greater in older, than young men, with no difference in GLP-1 or GIP responses to any of the infusions. There was a greater increase in the EGG power ratio, both during and following, ID glucose in young than older subjects, and an attenuation of the EGG frequency by ID nutrients in older, but not young, men. These observations suggest that ageing is associated with nutrient-specific changes in appetite, hormonal and gastric myoelectrical responses to ID nutrients. An enhanced satiating effects of ID carbohydrate may contribute to the anorexia of ageing.

Studies in rodents have shown that the suppressive effects of exogenous CCK on appetite are enhanced in older compared to young animals. The effects of intravenous infusion of two doses of CCK-8 on appetite and energy intake were evaluated in healthy older and young humans. Older subjects ate less of a test meal during all treatment infusions than young subjects, and the suppression of energy intake by intravenous CCK-8 infusions was greater in older than young subjects. Plasma concentrations of endogenous CCK (greater than 12 amino acids) were suppressed by CCK-8 infusion in both the older and young subjects, indicative of an autocrine negative feedback mechanism that is involved in the regulation of endogenous CCK

secretion. Plasma CCK-8 concentrations rose more during the infusions in the older than young subjects, possibly explaining the greater suppression of energy intake by the CCK-8 infusions in older subjects. Nevertheless, when corrected for the higher CCK levels, there was no significant difference in the magnitude of the suppression of energy intake for a given change in plasma CCK-8 concentration from baseline immediately before the meal between the two age groups. These results indicate that the sensitivity to the suppressive effects of exogenous CCK-8 is retained in the healthy elderly. Given that healthy ageing is associated with increased fasting and lipid-induced plasma CCK concentrations, increased CCK activity may play a role in the "anorexia of ageing".

Impaired gastric relaxation and accomodation to a meal may be associated with gastrointestinal symptoms and early satiation in patients with functional dyspepsia and diabetes mellitus. The effect of ageing on proximal gastric sensory and motor function has not been evaluated previously. A study was conducted to investigate the effects of ageing on fasting gastric compliance, the perception of gastric distension, and gastric accommodation to a meal. During both isobaric and isovolumetric distensions the pressure-volume relationship did not differ significantly between older and young subjects. During gastric distensions perceptions of fullness, abdominal discomfort and bloating were less in older than young subjects, whereas the perception of hunger was less in the young compared to older subjects. While there was no effect of age on energy intake and the size of a non-standardised test meal on the "barostat day", the "tube-only" day and the "control day" (no nasogastric tube), young subjects ate less at the meal on the "barostat day" compared to the "tube-only day" and the "control day" and less on the "tube-only day" compared to the "control day". In contrast, there was no effect of the different study conditions on energy intake in the older subjects and they ate similar amounts on the "tube-only day" and the "control day". Following the meal on the "barostat day", the maximum intrabag volume occurred later in the older compared with the young subjects. These observations indicate that healthy ageing is associated with decreased perception of gastric distension without any change in fasting gastric compliance, suggest that gastric tone late in the postprandial period may be less when compared to the young. The observation that the presence of a nasogastric tube inhibited food intake in young, but not older subjects, suggests that the control of energy intake is less sensitive to external stimuli in older than young subjects.

Studies in animals indicate the stimulation of feeding by endogenous opioids may be attenuated by "ageing". The effects of intravenous infusion of two doses of the opioid antagonist, naloxone, on appetite and energy intake were evaluated in young and older healthy subjects. In both age groups naloxone had no significant effect on perceptions of hunger, fullness or nausea, but increased drowsiness compared to the control infusion. Naloxone infusion reduced energy intake at an ad libitum meal compared to control, with no difference between the doses in both young and older subjects. The magnitude of this suppression was slightly, but not significantly, greater in the young than older subjects, reflecting a trend to reduced suppression in older women. These observations suggest that healthy older adults retain their sensitivity to the suppressive effects of naloxone on energy intake, although possible gender differences in this sensitivity warrant further investigation. A decline in opioid activity is unlikely to contribute substantially to the physiological "anorexia of ageing".

Ageing is associated with slight, but significant slowing of gastric emptying (GE) and impaired postprandial glucose homeostasis. In young healthy subjects dietary glucose supplementation increases the rate of gastric emptying of a glucose meal and enhances the postprandial plasma insulin and GIP response. The effects of dietary glucose supplementation on gastric emptying, postprandial blood glucose homeostasis, and appetite after a glucose/oil "preload" were evaluated in healthy older volunteers. Glucose supplementation accelerated GE of glucose, but not oil; there was a trend for an increase in GIP, no change in GLP-1, an earlier insulin peak and a subsequent reduction in blood glucose. Glucose supplementation had no effect on energy intake so that energy intake was greater during the glucose supplemented diet. Appetite ratings and energy intake at the buffet meal were also not affected by glucose supplementation. This study indicates that in the healthy elderly, glucose supplementation accelerates GE of glucose, but not fat, modifies postprandial blood glucose homeostasis and increases total energy intake.

Postprandial hypotension represents a major cause of falls and increased morbidity in the elderly. A study was conducted to determine whether slowing of gastric emptying and glucose absorption with guar gum would reduce the fall in blood pressure after an oral glucose load in healthy older subjects. The magnitude of the falls in systolic, diastolic and mean arterial blood pressure were less, and gastric emptying slower after guar gum. Blood glucose, insulin and 3-O-methyl-D-glucose (3-OMG; a non-absorbable glucose analogue) concentrations were reduced by guar gum. 3-OMG

concentrations were inversely related to the intragastric retention of glucose and blood pressure was inversely related to 3-OMG after the drink without guar. The blood glucose concentration was related to 3-OMG. The results establish that guar gum reduces the magnitude of the fall in blood pressure after oral glucose in older subjects. Slowing of gastric emptying and glucose absorption may represent a novel approach to the treatment of postprandial hypotension in the elderly.

Malnutrition is a common clinical problem in the elderly, but it remains largely unrecognised in community-dwelling persons. The prevalence of malnutrition, and relationships between nutritional status [assessed using the Mini-Nutritional Assessment (MNA)] and scores on the SF-36 Health Survey® (SF-36), Standardised Mini-Mental State Examination, Geriatric Depression Scale were evaluated in 250 Domiciliary Care 'functionally dependent' recipients. Risk factors including living status (ie alone/spouse or other), the amount of domiciliary care/ formal care (hr/month) received, medical history, number of medications, and recent hospital admissions (number of days within the last 12 months) were also recorded. In total, 59.6% were well-nourished 37.2% were 'at risk' of malnutrition and 4.4% were malnourished according to the MNA. The independent predictors of poor nutritional status were (i) a history of respiratory disease; (ii) receipt of 'Meals on Wheels'; (iii) an increased number of days spent in hospital in the past 12 months; and according to the SF-36 (iv) role limitation due to emotional problems; (v) physical functioning; (vi) general health perception and (vii) mental health. The prevalence of being 'at risk' of malnutrition was high in this population, therefore the 'functionally dependent' community-dwelling elderly may represent a subset of the elderly population who may benefit from routine screening for prevention or treatment of malnutrition.

STATEMENT OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution (except the study described in Appendix A which formed the basis of my Honours degree) and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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

Signed:

Caroline Gabrielle MacIntosh
December 2000

DEDICATION

To Alistair,

My husband and best friend.

For your constant love, support, patience and belief in me through the roller-coaster of a life it has been the past four years.

I could not have done it without you.

Thank you

ACKNOWLEDGMENTS

To the following people, I am extremely grateful for your fellowship, guidance and support throughout my time in the Department of Medicine, Royal Adelaide Hospital.

To my supervisors, Dr. Ian Chapman and Prof. Michael Horowitz; thank you for your support throughout this period in my life. There have been times when I may have pushed the boundaries of patience for you both, but I will always be grateful for your guidance and willingness to pass on your knowledge. Not only did you teach me a great deal about conducting research studies, improving my oral presentations and refining my writing skills, you have also taught me a lot about myself and given me the motivation and confidence to exceed beyond my own belief in my abilities.

To Ian, I am especially grateful for your encouragement, guidance and support throughout the past four years and for those frequent and always amusing comments that made the whole process that little bit less serious.

To Michael, thank you for introducing me to clinical research during a vacation scholarship in early 1996, and for your continued support and motivation over the past 5 years; with your persistence and encouragement, no longer am I the shy and apprehensive (but determined) person when we first met.

To my parents, I am truly grateful for your love, support and reassurance not only during the past five years, but throughout my life. To Mum, thank you for being there to listen to my grievances and keeping me informed of the tranquillity of country life.

To the rest of my family; Matthew and Amanda Cook, James and Lynda Cook and Louise and Gerard Lynch, as well as my nephews (Dion, Liam, Cameron, Dylon, Callum and Tyson) and nieces (Emily, Alecia, Michaely, and Lauren) thank you for welcoming me 'home' to the country when I needed a break from city life for a couple of days. You all remind me of the simple joys in life.

To my mother-in-law, Rossie and sister-in-law, Anna, for your continuing friendship and reassurance over the years, I am very grateful.

To my good friends, Ms. Jesia Berry and Ms. Alissa Lemar, many thanks for those fortnightly lunches, weekend dinners, movies and nights out, forcing me to forget my thesis, at least for a little while. To my boarding school friends who I have managed to keep in contact with since leaving school; Megan, Leonie, Sarah, Emma, Christina and Natalie, thank you for your friendship, and especially for those great girls-only nights out.

To Dr. Karen Jones, Paul Jansen Senior Lecturer, former fellow Ph.D. student, thank you for initially giving me the encouragement and inspiration to take on a Ph.D. Many thanks for teaching me the fundamentals of gastric emptying measurement and analysis for two of the studies reported in this thesis, and especially for your friendship and patience; you always had a solution to all those little problems with statistics, graphing etc. that unexpectedly occurred. I am also very grateful for your advice not only in the writing of this thesis, but about life in general, ie interior decorating, wine collecting, pregnancy and motherhood (for future reference).

To Dr. Jane Andrews, Gastroenterologist and former fellow Ph.D. student, thank you for teaching me manometry when I first started in research, for your continued friendship and support throughout and for that odd baby-sitting request which gave me a break from study. I am also particularly grateful for your advice and great time-saving tips in the writing of this thesis.

To Prof. John Morley (St Louis, Missouri); although most communication was via fax and email, with the occasional visit to Adelaide during the cricket season, thank you for your enthusiastic remarks and continuing interest and guidance in the conduct of studies and reviewing of manuscripts for submission to journals. I am extremely grateful.

To Prof. Andre Smout and Dr. Mark Verhagen (Utrecht, The Netherlands); many thanks for teaching me the fundamentals of electrogastrographic measurement and analysis from which I have gained some understanding of the complexities and limitations of assessing the electrical activity of the stomach.

To Dr. Chris Rayner, Gastroenterologist in training, fellow Ph.D. student and barostat expert, for teaching me about the basics of gastric barostat techniques and for measurement and analysis of gastric compliance data for one of the studies reported in this thesis, I am extremely grateful.

To Prof Jan Jansen, (Nijmegen, The Netherlands); thank you for your assistance with the assays for measurement of cholecystokinin (CCK) for two of the studies reported in this thesis.

To Ms. Judith Wishart, Research Officer and gut peptide assay extraordinaire; many thanks for conducting the assays for measurement of gut peptides for four of the studies reported in this thesis. In particular, for completing the cholecystokinin assay within such a short time frame so that I could complete my thesis on time. Thank you also to Dr. Howard Morris for your contribution to the completion of these assays.

To Drs. Robert Penhall (Geriatrician) Mandy Callary (Geriatric registrar) and Anita Comacchio (Dietitian); many thanks for your assistance with the survey of Domiciliary Care recipients.

To past and present administrative and secretarial staff of the Department of Medicine; Ms. Briony Lane, Ms. Kate Reinhart and Ms. Sue Suter, and Miss Laureen Graham, I am very grateful for putting up with those frantic requests for more paper, the photocopy card, organising a courier etc. and for the chats about life outside the Department.

To Honours' student, Ms. Katherine Beckoff and 4th Year Medicine Students, Ms. Nusha Davani and Ms. Jessica Sheehan; many thanks for your assistance in conducting two of the studies reported in this thesis, and for your friendship and your eagerness to complete the tasks at hand.

To past and present fellow Ph.D. students, Ms. Perdita Hope and Ms. Rosalie Vozzo, Ms. Natalie Luscombe, Ms. Franca Scopascasa and Ms. Monica Kwiatek; thank you for your friendship and support during the time that we have been working in the Department, especially for coffee across the road and chats about life after the thesis.

To other past and present research staff of the Department of Medicine, Ms. Melanie Berry, Ms. Elizabeth Goble, Ms. Rochelle Botten, Ms. Antonietta Russo and Ms. Julie Stevens; as well as Scott Clark, Tim Crichton and Michael Edmonds, many thanks for your friendly conversations about things other than work and for your assistance throughout the time we were working together in the Department. Also to the other

senior staff, Dr. Rob Fraser, Gastroenterologist and Dr. Gary Wittert, Endocrinologist, your well wishes and words of wisdom have been much appreciated.
To the recent visiting research fellows, Drs. Christine Feinle (Germany), Deidre O'Donovan (Ireland) and Kerstin Sturm (Germany), although I haven't known you very long, many thanks for your friendliness and support in these past few months. To Kerstin, I am especially grateful for your extremely enthusiastic approach in taking on the completion of an ongoing study, that I had commenced earlier this year.

To the research staff of Department of Gastrointestinal Medicine in Q7, RAH; to Ms. Selena Doran and Mr. Marcus Tippett for your assistance with manometry studies during my Honours' year and including Mrs. Dora D' Amateo for your continuing friendship and support over these past few years.

Last, but certainly not least, I am indebted to all of the subjects, particularly the older volunteers, who participated in the nine studies reported in this thesis. Without their enthusiasm and patience and in some cases, willingness to endure periods of some discomfort during these studies, none of this work could have been possible.
Thank you.

PUBLICATIONS ARISING FROM THE THESIS

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MacIntosh CG, JE Morley, Wishart J, Morris H, Jansen JBMJ, Horowitz M, Chapman IM. Effect of exogenous CCK-8 on appetite, food intake, plasma leptin and endogenous CCK in healthy older and young subjects. J Clin Invest (submitted for publication).

CHAPTER 1

Anorexia in the Elderly

1.1 INTRODUCTION

In 1868, Henry Purdon wrote of the residents of an infirmary in Belfast:

> "Decay of Nature, or Senile Marasmus, has the greatest number of deaths attributed to it. Their ages vary from 69 to 92 years. The inmates affected with this gradual wasting of the body, which approaches very slowly, have usually their mental facilities clear and unclouded till the last, but complain of loss of appetite, bowels costive, pulse small, quick and weak, and sleepless nights, feel no pain, and look at death with seeming indifference and carelessness, in many cases as a happy release. With regards to treatment, medicines are of little use." (Purdon 1868).

This quotation illustrates the fact that severe anorexia leading to marked weight-loss and protein-energy malnutrition in older adults is not a recent phenomenon. In fact, despite the enormous medical advances in the twentieth century, particularly in the last three decades, anorexia and protein-energy malnutrition remains a major cause of morbidity and mortality in the elderly. The causes of anorexia in the elderly are varied and include psychological, physical and social factors. There is also evidence that ageing is associated with a 'physiological' anorexia, which may predispose to the development of severe anorexia.

The purpose of this chapter is to review the demographic trend of ageing in society (section 1.2), as well as the patterns of food intake (section 1.3), aetiology of pathological anorexia (section 1.6), and the prevalence, clinical significance and potential treatment of protein energy malnutrition (section 1.7) in the elderly. Particular emphasis is placed on the 'physiological' anorexia of ageing (section 1.4), which represents the primary focus of this thesis. Current knowledge about the central and peripheral mechanisms involved in the regulation of appetite are discussed in Chapters 2 and 3, respectively. The known age-related effects on these appetite regulatory mechanisms are discussed in Chapter 4 in relation to their potential role in the physiological anorexia of ageing.

1.2 AGEING IN SOCIETY

In the last 100 years there has been a dramatic, and continuing, increase in both the number and proportion of people living into old age, particularly in western countries. In the United States the elderly population is expected to increase by 8% over the next 20 years, so that by the year 2020, 65 million people (24.6% of the population) will be over 60 years of age (Jackson 1999). Similar predictions have been made for Australia; 24% of the population will be more than 65 years of age by the year 2051 (Australian Bureau of Statistics 1997) compared to 12% in 2000. The largest absolute growth in the numbers of older persons, however, will occur in the developing countries such as China, Indonesia, the Indian subcontinent and Mexico (Morley 1994c). Furthermore, there will be a marked increase in the number of persons living beyond the age of 85 years ie. the old-old (Morley 1994c). These dramatic demographic trends are inevitably associated with a substantial socioeconomic burden, not only on the individual, but also on governments, since the use of healthcare increases with age.

Poor nutritional status has been implicated in the development and progression of chronic medical disorders which commonly affect the elderly, including osteoporosis, cardiovascular disease, diabetes mellitus and cancer (Morley 1996a). An increased understanding of the factors which contribute to poor nutrition in the elderly should, accordingly, facilitate the development of appropriate preventive and treatment strategies and improve the health of older people.

1.3 EFFECT OF AGEING ON PATTERNS OF FOOD INTAKE IN THE ELDERLY

Although food intake varies widely between individuals of a given age, ageing is, on average, associated with a decline in energy intake. In the cross-sectional National Health and Nutrition Examination Survey (NHANES I), conducted in the USA in 1971, approximately 16% of the population over the age of 60 yr consumed less than 4182 kJ per day (Abraham 1977), an energy intake which is associated with radical weight loss diets often undertaken by young adults. A more recent NHANES (III) study, conducted in 1989, and based on single 24-hr diet recall, reported an average decline in energy intake between the ages of 20 and 80 yr of 5524 kJ/day in men and 2630 kJ/day in women (Figure 1.1)(Briefel et al 1995).

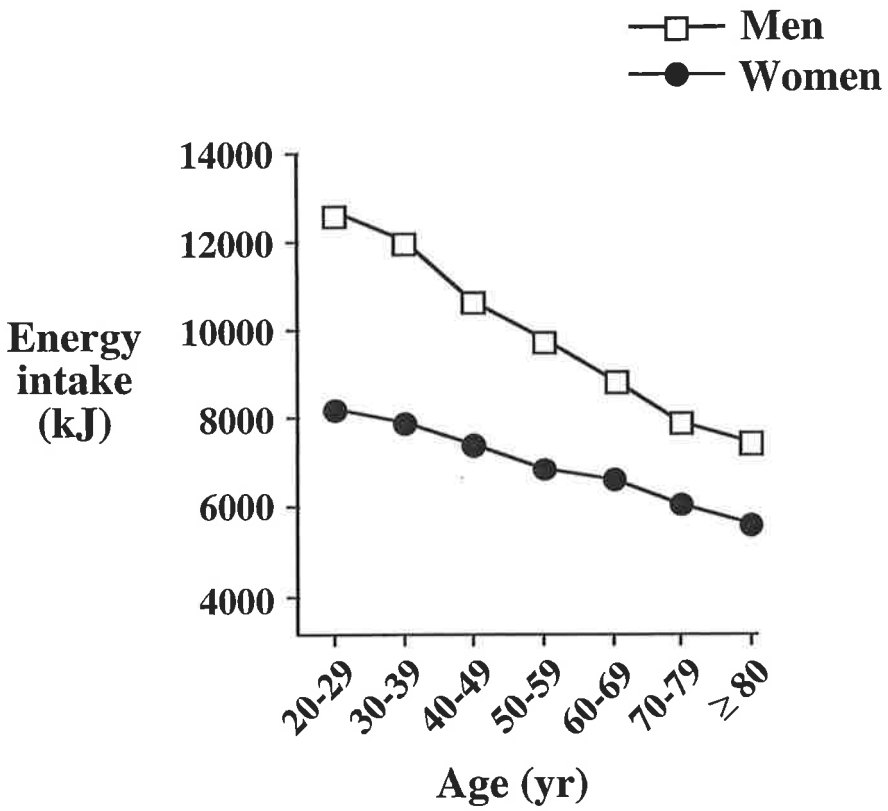

Figure 1.1: Daily energy intake (kJ) based on single 24-hr dietary recall for men and women (n= 14801) aged 20 years and older from the third National Health and Nutrition Survey, US, 1988-1991 (Briefel et al, 1995).

Other cross-sectional, as well as longitudinal studies have demonstrated this decline in energy intake with age (Hallfisch et al 1990, Sjogren et al 1994, Koehler et al 1994). For example, the Baltimore Longitudinal study, which followed-up free-living males aged 20 to 99 yr, reported that in men aged 46-56 yr (n= 53) and 57-67 yr (n= 8) energy intake per kilogram of body weight decreased by an average of 17.2 and 23.8 kJ per kg, respectively during the 30 years of the study (Hallfisch et al 1990). A Swedish study of 98 men and women found that there was an overall decrease in energy intake of 2551 kJ/day in men and 1840 kJ/day in women between the ages of 70 and 76 yr (Sjogren et al 1994). A longitudinal study conducted in New Mexico, USA, of 156 persons aged 64-91 yr, reported a decrease of 80.7 kJ/day/yr in women and 105.0 kJ/day/yr in men (Koehler et al 1994) during 7 years of follow-up.

These studies have consistently shown an age-related decline in energy intake at a population level, but most have been conducted in developed, western societies (Hallfisch et al 1990, Sjogren et al 1994, Koehler et al 1994) and not all have included both men and women (Hallfisch et al 1990). The decline in energy intake in less developed countries and in different subjects groups, therefore, remains to be defined.

There is evidence that the decline in energy intake in older persons is also associated with changes in the macronutrient composition of food eaten (Sjogren et al 1994, Hallfisch et al 1990, Koehler et al 1994). Sjogren et al (1994) reported a decrease in the percentage of energy from protein, whereas others have shown a decrease in the percentage of energy intake obtained from fat, in particular saturated fat (Koehler et al 1994) and an increase in percentage of energy from carbohydrate (Hallfisch et al 1990, Morley 1997, de Castro 1993) with age. This alteration in macronutrient composition of diet with ageing may largely be a reflection of changes in the food availability as well as public education relating to the potential health risks and benefits of diet. On the other hand, the decline in dietary fat intake observed in these studies (Koehler et al 1994, Hallfisch et al 1990, Morley 1997, de Castro 1993) may simply be a result of survival bias of those consuming a low-fat diet. According to Patterson et al (1996), who assessed food frequency questionnaires in over 7,000 women (aged 50-79 yr) enrolled in the Women's Health Initiative in the USA, low-fat dietary practices (eg trimming fat from meat and using low fat dairy products) are widespread in the older population. Although a reduction in dietary fat intake may be beneficial in older persons who are overweight or have cardiovascular disease, as discussed, many elderly consume too little food and therefore low-fat choices may make it even more difficult to

maintain a positive energy balance. Furthermore, alterations in the macronutrient content of the diet in the elderly may be due, in part, to changes in oro-sensory function (Chapter 3.2.1) or in sensitivity to the satiating effects of specific macronutrients (see Chapter 3.4 and Chapter 9).

1.4 THE 'PHYSIOLOGICAL' ANOREXIA OF AGEING

The population-based studies summarised previously, which have demonstrated an age-related reduction in energy intake, by definition include older people who are ill as a result of medical or psychiatric disorders, as well as residents in nursing homes or other institutions. It is therefore unclear whether this decline in food intake is physiological or pathological. There is, however, evidence that there is also an age-related decline in energy intake in *healthy, ambulant, non-institutionalised* older people, and this has been termed the "anorexia of ageing" (Morley 1997). It is intuitively likely that the combination of this "physiological" anorexia with the anorectic effects of social (1.6.1), psychological (1.6.2), physical (1.6.3) and medical (1.6.4) problems which become increasingly frequent with ageing, predisposes to pathological anorexia and malnutrition (Wurtman et al 1988, de Castro 1993, Clarkston et al 1997, Roberts et al 1994, Rolls et al 1995b). Wurtman et al (1988) showed that when 45 healthy elderly (65-94 yr) and 41 healthy young (21-35 yr) adults from Massachusetts USA were studied under identical conditions, the elderly ingested approximately 30% less energy than young adults, particularly of fat (Figure 1.2). In addition, the elderly consumed about 85% of their energy during meals compared with 72% in the young subjects, and the total number of snacks consumed per day in the young was about twice that of the elderly (Wurtman et al 1988). De Castro et al (1993), who assessed 7 day diet diaries in 307 healthy adults in Georgia USA, aged 20-80 yr, reported that healthy ageing was associated with a reduction in energy intake of approximately 12%, reduced meal size, slower rates of eating, fewer snacks between meals and less physical activity. Others have found that healthy older persons are less hungry (Figure 1.3) and more rapidly satiated after, eating a standard meal than younger persons (Clarkston et al 1997, Roberts et al 1994, Rolls 1995b).

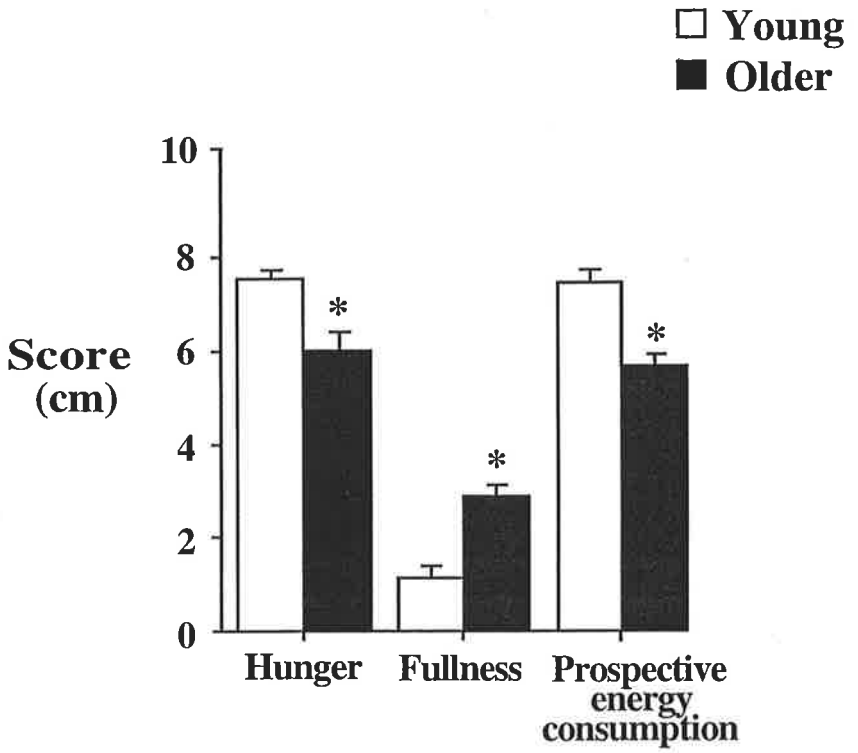

Figure 1.2: Mean appetite scores after an overnight fast in 16 older and 16 young healthy subjects, as assessed by 10 cm visual analogue scales (VAS) (see Chapter 6.3.1) (Rolls et al 1995). *P<0.05 vs young.

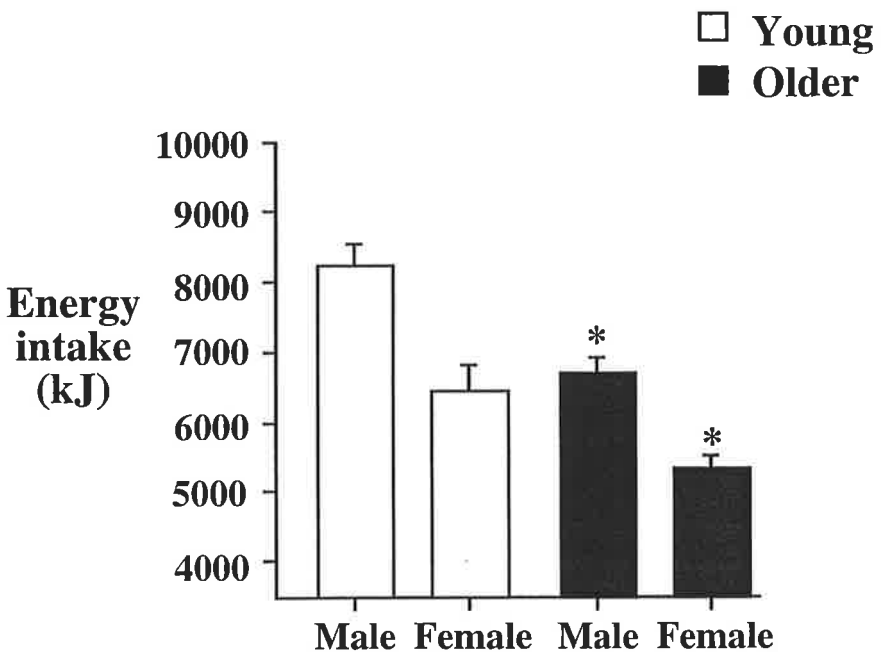

Figure 1.3: Average daily energy intake (kJ), over a four day period under identical conditions, in 45 young (21 male/24 female; mean age 27 yr) and 45 older (21 male/24 female; mean age 74 yr)(Wurtman et al, 1988). *P< 0.01 vs young of same gender.

Much of the observed decrease in energy intake is probably attributable to the decline in energy expenditure that also occurs during normal ageing (Morley 1997). For example, Tzankoff et al (1977), who studied a group of 959 men from Maryland USA enrolled in the Baltimore Longitudinal Study, reported that there is a reduction in the basal oxygen consumption of approximately 33% between the ages of 20 and 90 yr (Figure 1.4). This decline in metabolic rate is also reflected by the reported decline in the amount of physical activity and exercise with ageing. In many individuals, however, the decrease in energy intake is greater than the decrease in energy expenditure, so body weight is lost. Wallace et al (1995) reported, in 247 community-dwelling males aged over 65 yr living in Washington USA, that the annual incidence of involuntary weight loss of 4% or more of initial body weight was 13.1%, and of 5% or more was 7.8 %. This decline in body weight, particularly after the age of 70 yr, as measured by body mass index (BMI) (kg/m^2), has been well documented in population-based cross-sectional (Kuskowska-Wolk & Rossner 1990, Aloia et al 1996, Silver et al 1993, Baumgartner et al 1998) and longitudinal (Steen 1988, Chumlea et al 1988) studies. This age-related decline in body weight may be desirable in the majority of adults in whom body mass increases, as a result of increased body fat, during middle age, that is, 'the middle age spread' (Steen 1988); because of the high, and increasing, prevalence of obesity in western countries mean body weight of the elderly remains within the recommended range or decreases into it (Rumpel et al 1993, Steen 1988).

The relationship of BMI to in-hospital mortality is typically a U-shaped curve (Potter et al 1988) (Figure 1.5), with the highest mortality rates among those people with a BMI greater than 35 kg/m^2 or less than 19 kg/m^2. It has, however, been suggested that age may 'accentuate' the relationship between BMI and mortality rate at the lower extreme (less than 19 kg/m^2), since loss of lean body tissue (sarcopenia) accounts for a substantial amount of the decline in body weight after the age of 60 yr (Evans & Campbell 1993). It has been estimated that individuals lose up to 3 kg of lean body mass per decade after the age of 50 yr (Dwyer 1993). Sarcopenia is associated with adverse metabolic, physiologic and functional impairments and disability, including increased risk of falls, as well as protein energy malnutrition (Baumgartner et al 1998). Individuals who are already lean are clearly most at risk of sarcopenia (Rumpel et al 1993, Allison et al 1998). Moreover, the decline in lean mass with ageing appears to differ between genders; men have a greater residual muscle mass and seem to tolerate age-related sarcopenia better than women, who may reach the 'frailty threshold' more quickly (Walston & Fried 1999).

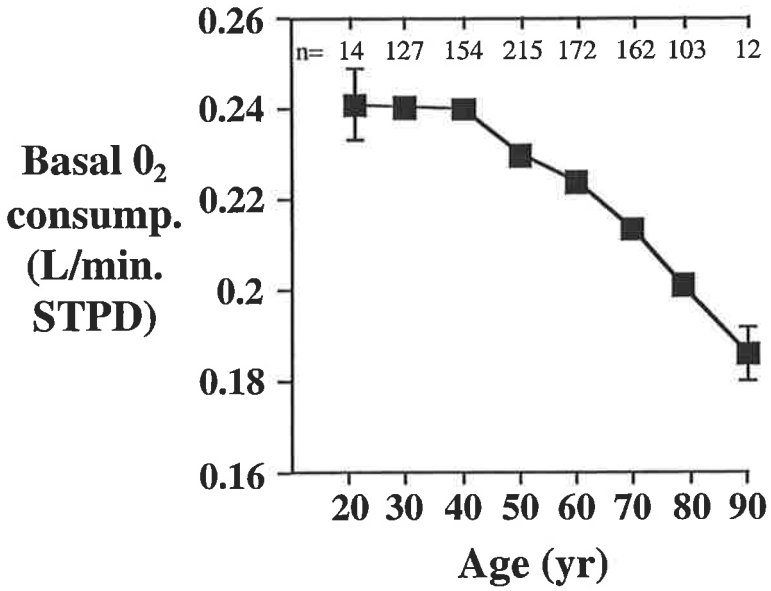

Figure 1.4: Basal oxygen consumption (mean ± SEM), a marker of energy expenditure, for 959 men participating in the Baltimore Longitudinal Study grouped by age (Tzankoff et al 1977).

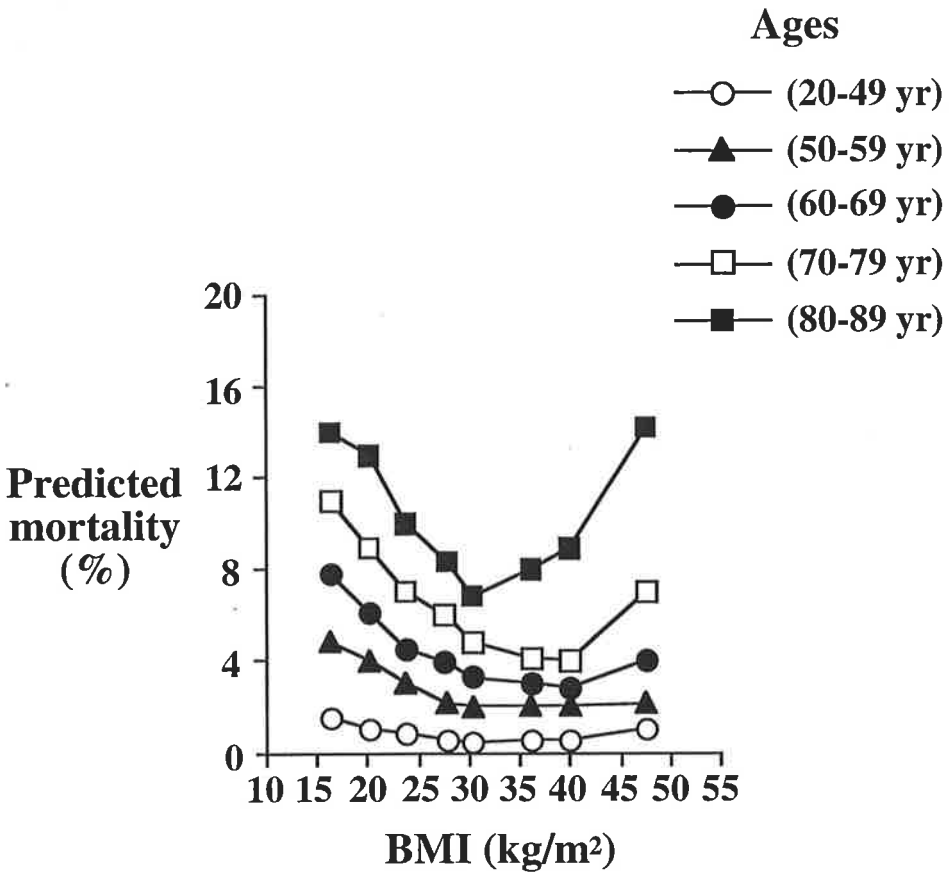

Figure 1.5: Predicted probability of death (mortality) as a function of body mass index (BMI) for 8428 patients (aged 20-99 yrs) admitted to the Nebraska Medical Center hospital, Nebraska, between 1979-1983. Predicted probability was calculated from the logistic model for each five age groups, Plotted points are mean values (Redrawn with permission from the Journal of Gerontology; Potter et al, 1988).

1.5 AGE-RELATED IMPAIRMENT OF HOMEOSTATIC REGULATION OF ENERGY INTAKE AND BODY WEIGHT

One of the characteristics of ageing is a decreased ability to maintain physiological homeostasis in the face of changes in either the internal or external environment (Schlenker 1993). The general decline in physical and psychological systems with ageing may possibly reflect this change in responsiveness. For example, by 80 years of age, there is a significant decline in the physiological functions of the heart and nervous system compared to young adulthood, ie. a 30% reduction in cardiac output, and a 15% decrease in neuronal conduction (Schlenker 1993). Recent studies, in both animals and humans, have demonstrated an age-related impairment in the homeostatic regulation of energy intake and body weight, which has the potential to augment the adverse effects of the anorexia of ageing. Felgines et al (1999) recently reported that during a 6 week period of dietary restriction (50% of average intake during baseline diet) in adult (3-mo old) rats, there was a rapid decrease in body weight during the first 2 weeks which then stabilised, whereas in aged (22-mo old) rats weight loss continued throughout the 6 weeks and was significantly greater than in the younger animals. Furthermore, compared to the 3-mo old rats, aged rats showed more marked visceral protein depletion and deterioration of nitrogen balance during dietary restriction (Felgines et al 1999).

Healthy older people also have a reduced ability to compensate for modifications to their diet compared to young adults (Roberts et al 1994, Rolls 1995b). For example, Roberts et al (1994) imposed a 21 day period of overfeeding on healthy young (mean age 24 yr) and older (mean age 70 yr) men (Figure 1.6); during this time both groups gained weight. Following this, both group were allowed to eat ad libitum. During the ad libitum period the young men ate less than their baseline (pre-overfeeding) intake and their weight quickly returned to normal (Figure 1.6A). In contrast, the older men continued to overeat and did not lose the weight they had gained. Perhaps more importantly, following a 21-day period of underfeeding, older men continued to eat less and did not regain the lost weight after ad libitum intake resumed, whereas the young men overate and quickly regained the lost weight (Roberts et al 1996) (Figure 1.6B). Furthermore, Rolls et al (1995b) showed that healthy elderly (aged 60-84 yr) men have a reduced capacity to compensate at subsequent meals for variations in the macronutrient and energy content of food eaten. In a study where they administered blinded yoghurt preloads of varying energy and fat content and assessed ad libitum food intake at a self-selected lunch, the elderly men consistently overate by between 10

and 30% after the low fat/low energy, high fat/high energy and high carbohydrate/high energy preloads compared to baseline (no preload), whereas young (aged 18-35 yr) men adjusted their intake more accurately to within 10% of their baseline intake (Rolls 1995b). These findings are consistent with those of Phillips et al (1984) who reported that older men are less thirsty and drink less, after 24 hr fluid deprivation, than young men, suggesting that the elderly have a reduced ability to detect and respond to dehydration (Phillips et al 1984).

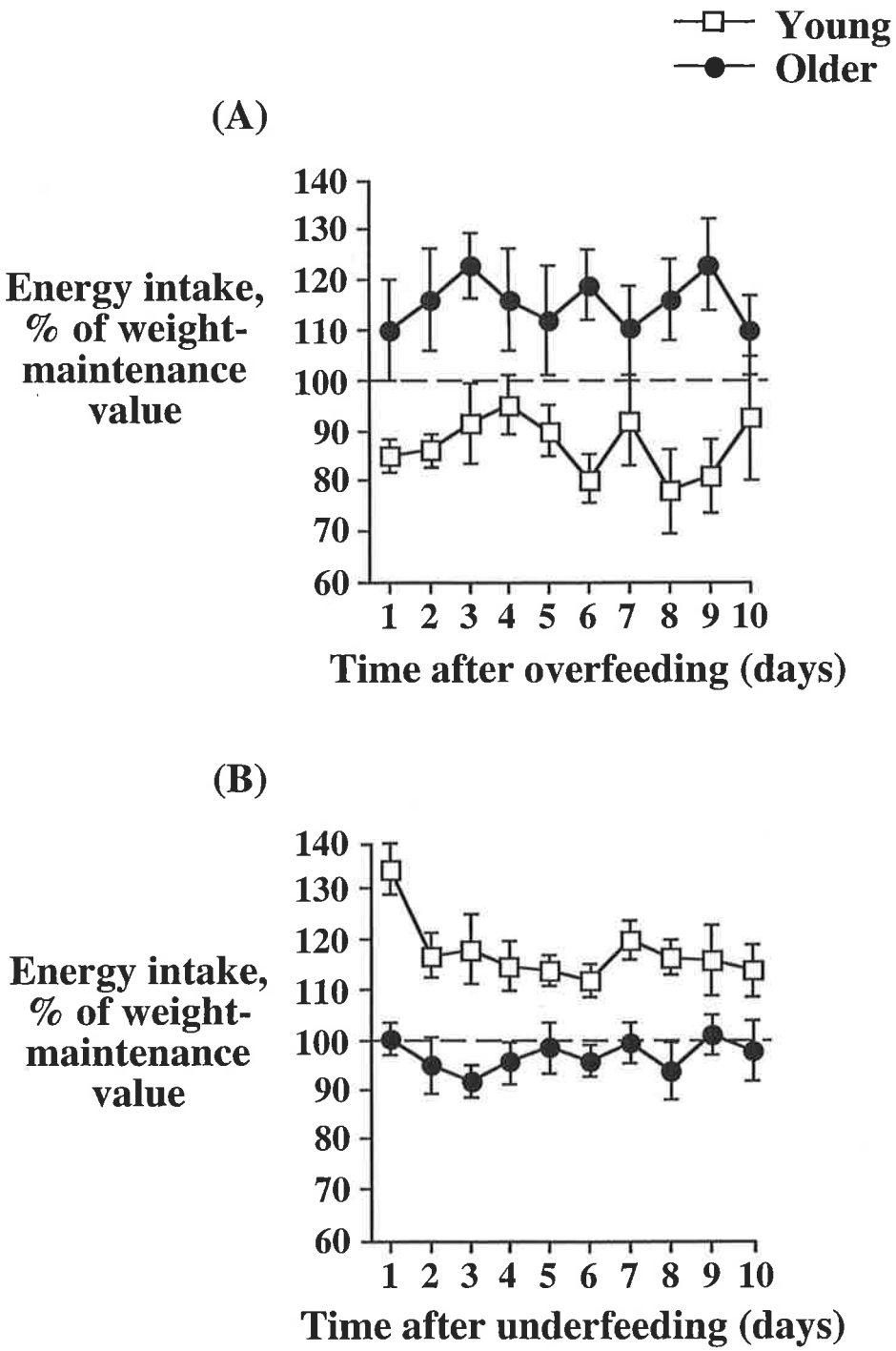

Figure 1.6: **(A)** Voluntary energy intake during a 10-day period following overfeeding in younger (n=7) and older (n= 9) men and **(B)** voluntary energy intake during a 10-day period following underfeeding in younger (n=10) and older (n= 9) men. Values are mean (± SEM) expressed as a percentage of the initial (phase 1) weight maintenance energy requirements (indicated by the grey horizontal line). The change in energy intake relative to initial weight maintenance requirements were significantly different between the age groups, with young subjects losing more weight after overfeeding and gaining more weight after underfeeding than older subjects.

1.6 AETIOLOGY OF PATHOLOGICAL ANOREXIA

The physiological anorexia of ageing may augment the adverse effects of a number of factors which contribute to weight loss in the elderly; these factors include social, psychological, physical, and medical factors, the majority of which are responsive to treatment (summarised in Table 1.1).

1.6.1 *Social factors*

An important social factor that contributes to decreased food intake in the elderly is poverty. Some older individuals, even in the wealthiest countries, have limited financial means, making it difficult to afford food of good nutritional quality. Many also live alone; social isolation may lead to a decreased appetite and energy intake in the elderly. Social isolation and loneliness were both associated with dietary inadequacies in a group of 61 independently living individuals, aged 60-94 yr, in Tennessee, USA (Walker & Beachene 1991). Moreover, elderly individuals tend to consume more food (up to 50% more) when eating in the company of friends than when eating alone (de Castro 1993).

1.6.2 *Psychological factors*

Depression, often associated with bereavement and the deterioration of social networks, is a common problem in older people and a significant cause of loss of appetite in some (Blaum et al 1995, Katz et al 1993, Wade 1994). Depression is often associated with feelings of hopelessness, fatigue and lack of interest in life and living, the so-called 'failure to thrive" (Newburn & Krowchuk 1994). Brodaty et al (1997) reported that, among 285 patients diagnosed with major depression in a psychiatric ward in Australia, the older patients (≥60 yr) experienced more severe loss of appetite and body weight as a result of their illness than younger (<60 yr) patients. In extreme cases individuals may even give up eating as a passive means of suicide (Newburn & Krowchuk 1994) Alcoholism, which is associated with depression and loneliness, may also influence appetite and energy intake in the elderly. Dementia may contribute to reduced food intake in the elderly, because individuals simply forget to eat. Sandman et al (1987) reported that as many as 50% of the institutionalised patients they had studied in Umea, Sweden with dementia had protein-energy malnutrition.

1.6.3 *Physical factors*

Physical factors, such as poor dentition and ill-fitting dentures, may limit the type and quantity of food eaten by older persons (Wilson et al 1998). Sahyoun et al (1988)

found that half of 260 nursing home patients, aged 60-101 yr, studied in Boston USA, complained of problems with chewing, biting, and swallowing, and that the requirement for dentures was associated with poor protein intake in both men and women. Mojon et al (1999), who studied a group of 324 institutionalised frail older adults in Geneva Switzerland, reported that both body mass index (BMI) and serum albumin were lower in the elderly with compromised dentition (ie full or partial dentures, five or fewer natural teeth or the presence of mobile teeth) compared to those who had adequate oral functional status. A dry mouth and reduced saliva are also common complaints in the elderly (Dormenval et al 1999) and may potentially reduce taste and appetite. Dormenval et al (1999) reported that among 99 elderly non-psychiatric patients (mean age 83 yr) in a geriatric hospital in Geneva, Switzerland, lack of appetite was associated with a stimulated salivary flow less than or equal to 0.5 ml/min compared to >1.0 ml/min in young healthy adults. Reduced saliva production is associated with a number of diseases (eg: Parkinson's disease) as well as some medications (eg: diuretics) frequently prescribed to older persons. Atrophic glossitis (absence of papillae in more than 50% of the tongue) also occurs frequently in the elderly (Bohmer a& Mowe 2000) and has been associated with reductions in BMI, triceps skinfold thickness, mid-arm circumference, muscle strength, as well as serum concentrations of cholesterol, ascorbic acid, cholecalcidiol and vitamin B12. Immobility, impaired balance, tremor, and visual impairment may also affect the capacity of an older person to shop for, prepare and consume, food.

Table 1.1 Nonphysiologic causes of anorexia in older persons

Social factors
Poverty
Inability to shop
Inability to prepare and cook meals
Inability to feed one-self
Living alone/ social isolation / lack of social support network
Failure to cater to ethnic food preferences in institutionalised individuals
Psychological factors
Alcoholism

Bereavement
Depression
Dementia/ Alzheimer's disease
Cholesterol phobia

Physical factors

Reduced mobility
Decreased saliva
Poor dentition, ill-fitting dentures
Atrophic glossitis
Tremor
Impaired vision
Impaired balance

Medical factors

Cancer
Alcoholism
Cardiac failure
Chronic obstructive pulmonary disease
Infection
Dysphagia
Rheumatoid arthritis
Parkinson's disease
Hypermetabolism (eg. hyperthyroidism)
Malabsorption syndromes
Dyspepsia
Vomiting/ diarrhoea/constipation

Medications

- anti-infectives
- antineoplastics
- antirheumatics
- cardiovascular agents
- CNS agents
- gastrointestinal agents

- diuretic agents
- pulmonary agents
- antihypertensive agents
- nutritional supplements

1.6.4 *Medical factors*

Medical conditions that are common in the elderly, including gastrointestinal disease (Katelaris et al 1993), malabsorption syndromes, acute and chronic infection, cardiac failure and hypermetabolism (ie hyperthyroidism) (Morley 1997) often result in anorexia, micronutrient deficiencies (Russell 1986) and increased energy requirements (summarised in Table 1.2).

Malignancy (Wallace et al 1995), infection and rheumatoid arthritis (Roubenoff et al 1994) probably produce their anorexic effects by the release of cytokines [eg. interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-a)]. Cytokines have been shown to decrease food intake and reduce body weight via a number of central and peripheral pathways (Yeh &Schuster 1999).

Ageing may itself be a form of stress. It is associated with "stress-like" changes in circulating hormones; increased cortisol and catecholamines and decreased sex hormones and growth hormone (Morley 1997). Increased secretion of cortisol and catecholamines may in turn stimulate the release of IL-6 and TNF-a (Yeh &Schuster 1999), whereas sex hormones inhibit IL-6. Serum IL-1 and IL-6 levels are elevated in older people with cachexia (Anker et al 1997, Roubenoff et al 1994, Liao et al 1993, Cederholm et al 1997, Roubenoff et al 1998), while plasma IL-6 concentrations apparently increase as a function of normal ageing (Ershler et al 1993) and correlate inversely with levels of functional ability in elderly people (Cohen et al 1997). Increased cytokine levels, due to the "stress" of ageing per se, or the amplified stressful effects of other superimposed pathologies, may thus provide an explanation for some of the decline in appetite and body weight which occurs in many older people.

The prevalence of atrophic gastritis and *Helicobacter pylori* infection increases with age (Saltzman & Russell 1998, Katelaris et al 1993). The decrease in gastric acid secretion often associated with atrophic gastritis and *Helicobacter pylori* infection (Katelaris et al

1993) and a decrease in active intestinal transport (Schumann 1999) may lead to malabsorption of vitamin B12, vitamin K, folate, calcium, iron and zinc (Saltzman & Russell 1998, Schumann 1999). Deficiencies of vitamin B12, folate and zinc have been associated with loss of appetite (Russell 1986). Constipation is also a common complaint in the elderly and may cause gastrointestinal symptoms, including decreased appetite, and lead to abuse of laxatives which may compromise nutrient absorption (Schlenker 1993).

Postprandial hypotension or the sudden fall in blood pressure following food ingestion, particularly after eating foods that are high in carbohydrate, occurs frequently in the elderly. In most elderly persons, meal-associated declines in blood pressure are modest and asymptomatic, however, in elderly patients with hypertension, Parkinson's disease or autonomic dysfunction and particularly those in nursing homes, the postprandial decline in blood pressure may be of sufficient magnitude to cause dizziness or syncope and subsequent falls (Jansen et al 1995a) (see Chapter 5). Postprandial hypotension may indirectly lead to malnutrition, by causing electrolyte imbalance due to insulin release, or aspiration pneumonia in individuals with cognitive problems or dysphagia (Morley & Silver 1995). The regulation of postprandial blood pressure may be related to the rate of gastric emptying which is altered in the elderly (see Chapter 4.3 and 14).

1.6.4.1 Medications

The elderly are major users of prescription medications, a number of which can affect taste or cause malabsorption of nutrients, gastrointestinal symptoms and loss of appetite. Furthermore, the elderly frequently take multiple medications, which increases the risk of drug interactions that may cause anorexia. For example, digoxin and some forms of cancer chemotherapy can cause nausea, vomiting and loss of appetite (Roe 1988). Other medications can deplete mineral stores; penicillamine induces zinc depletion which can lead to loss of taste acuity and decreased food intake (Roe DA, 1988, Schumann 1999), while high doses of aluminium or magnesium hydroxide antacids deplete phosphate and potassium stores, potentially leading to muscle weakness and anorexia (Roe 1988). The use of diuretics, antihypertensives and CNS or sedative medications, may also reduce energy intake indirectly by causing electrolyte abnormalities, hypotension or confusion (Morley & Silver 1995).

1.7 PROTEIN ENERGY MALNUTRITION IN THE ELDERLY

Protein energy malnutrition occurs frequently in the elderly, but is often unrecognised, particularly in those living in institutions. The prevalence varies depending on the type of institution ie nursing home vs hospital, of the study population. The methods used to define this condition are discussed in more detail in Chapter 6.5.

1.7.1 *Prevalence*

Studies in both the US and Australia have reported that up to 15% of community-dwelling and home-bound elderly (Mion et al 1994, Thorslund et al 1990), between 23 and 62%, of hospitalised patients (Wilson et al 1998, Zador & Truswell 1987, Marshman et al 1980, Wood et al 1985, Bacon 1995, Low & Cameron 1997) and up to 85% (Pinchofsky-Devin & Kaminski 1986, Shaver et al 1980) of nursing homes residents (Mion et al 1994), have protein energy malnutrition.

Many of the surveys conducted both overseas and in Australia have focussed on the institutionalised elderly, even though a large majority of the elderly population are not dependent on institutional care. The Australian Longitudinal Study of Ageing (ALSA), conducted in a randomly selected sample of both community-dwelling and institutionalised people over 70 yr living in Adelaide, has assessed nutrient intakes and factors influencing choices using self-completed questionnaires including the ANSI (12 question) checklist and a semi quantified food frequency questionnaire (Cobiac & Syrette 1995). According to the results of the ANSI checklist, in a sample of 940 non-institutionalised subjects from this study, 30% of the population were classified at high risk, 20.6% at moderate and 49.5% at low risk of developing nutritional problems. There is some suggestion from previous studies that the 'functionally dependent' elderly, ie those who receive in-home Domiciliary care and/or "Meals on Wheels" have a lower dietary intake (Lipschitz et al 1985, Payette et al 1995) and below-normal indices of nutritional status (Morgan et al 1986), compared to free living elderly and therefore could be considered as a high risk group for malnutrition (Payette et al 1995). If this were so, they may represent a subset of the elderly population who could be targeted for prevention and treatment of malnutrition. The prevalence of malnutrition in a sample of elderly individuals (n= 300) who receive domiciliary care, in South Australia, using the Geriatric Mini Nutritional Assessment (see Chapter 6.5.3.3) (Guigoz et al 1994, Guigoz et al 1996) is evaluated in Chapter 15.

1.7.2 *Clinical significance of PEM in the elderly*

Protein energy malnutrition is associated with impaired muscle function, decreased bone mass, immune dysfunction, anaemia, reduced cognitive function, poor wound healing, delayed recovery from surgery and, ultimately, increased morbidity and mortality (detailed in Table 1.2). Epidemiological studies have demonstrated that protein energy malnutrition is a strong, independent, predictor of mortality in elderly people regardless of whether they live in the community (Campbell et al 1990, Mattila et al 1986) or in a nursing home (Morley & Silver 1995), are patients in a hospital (Cederholm et al 1995; Marton et al 1981, Rabinovitz et al 1986), or have been discharged from hospital in the last 1-2 years (Sullivan et al 1991, Wallace et al 1995).

Table 1.2 Effects of weight loss and protein energy malnutrition on function in the elderly - [adapted from Morley et al (1996a)].

↓ muscle function
↓ muscle relaxation
↓ muscle mass
↓ muscle strength
↑ risk of fracture
↓ bone mass
↑ incidence of falls
↓ functional status
↓ immune function
↑ increased risk of infection
↓ delayed skin hypersensitivity
↑ T cell lymphocytopenia
↓ synthesis of interleukin-2
↓ cytolytic cell activity
↓ response to influenza vaccination
Anaemia
Poor wound healing
Fatigue
Pneumonia
Delayed recovery from surgery
↓ cognitive function
↓ cardiac output

↓ intravascular fluid (dehydration)
↑ incidence of pressure sores
↓ maximal breathing capacity
↑ hospital admission and length of stay
↑ mortality

The increased mortality rate in elderly people with protein energy malnutrition per se is further increased when other medical diseases are present. For example, in a Swedish study of 205 patients >70 yr without cancer, the 9 month mortality rate after admission to a medical ward was 44% in 41 malnourished patients without cardiac failure, 18% in 164 well-nourished patients without cardiac failure, but 80% in 10 malnourished patients with congestive heart failure (Cederholm et al 1995). Fiaccadori et al (1999) reported that among 309 patients with acute renal failure admitted to a renal ward in the USA, the presence of severe malnutrition was associated with a 62% in-hospital mortality rate compared to 18% mortality rate in well-nourished patients. Davalos et al (1996) in a study of 104 patients who had suffered acute stroke (cerebral accident), found that those patients who were malnourished had an increased risk of death, or disability at follow-up, compared to those who were well nourished at the time of their stroke.

1.7.3 *Treatment*

There are a number of potential treatments available for protein energy malnutrition in the elderly; the success of these treatments is dependent on the severity of malnutrition and presence of related co-morbidities.

1.7.3.1 Nutrient/vitamin supplementation, and exercise

The simplest way of treating malnutrition in undernourished older people is to encourage and educate those who are willing and able, to eat more. There is evidence that nutritional supplementation is effective. For example, dietary protein supplementation, given for a 60 day period, increased daily protein and energy intake, body weight and nutritional status in a group of nursing home patients in Toulouse, France who were malnourished, or at risk of malnutrition, when compared to the non-supplemented group (Lauque et al 2000). Tkatch et al (1992) assessed the mortality rate following administration of an oral nutritional supplement, containing protein (20.4g), mineral salts (calcium, magnesium and phosphorus) and vitamins A & D3, daily for a

mean of 38 days, in 33 elderly patients (mean age 82 yr) with hip fracture admitted to a hospital in Geneva. They reported that the mortality rate over a 7 month period was 28% lower than in a group of patients (n= 29) who received the same nutritional supplement without protein over the same period of time (Tkatch et al 1992). Furthermore, in a study of 60 elderly long-term-care residents in Ohio, USA, residents who were given a 1255 kJ nutritional supplement (which contained 15g protein, 40g carbohydrate, 9g fat and 25% of the recommended daily intake (RDI) for all vitamins/minerals) 3 times daily over a 6 week period, increased their total energy and macro- and micro nutrient intake, without displacing energy or nutrients from their normal diet compared to residents who received no supplement (Turic et al 1998).

The efficacy of oral nutritional supplementation in the elderly, is probably attributable, at least in part, to the age-related impairment of homeostatic regulation of food intake (Section 1.5), such that older people reduce their energy intake from non-supplement sources less in compensation for the supplement than do young adults. The potential mechanisms influenced by dietary supplementation are examined in more detail in Chapter 13, where the effect of dietary glucose supplementation for 10 days on food intake, gastric emptying, gastrointestinal hormone release and appetite in response to a glucose/oil preload in 8 healthy older subjects is investigated.

Not all studies have reported positive effects of oral nutritional supplementation in the elderly. Gray-Donald et al (1995) reported that frail elderly people, living at home, in Québec, Canada, gained weight during 12 weeks of oral protein supplementation (2.1 vs 0.6 kg), but this was not associated with changes in handgrip strength, general well-being, or perception of health. Hogarth et al (1996) reported that mental status, Barthel (activities of daily living) score or length of hospital stay significantly improved in a group of elderly medical patients in London, England, following dietary glucose supplementation with and without the addition of vitamins compared to a placebo supplemented group.

Other studies suggest that vitamin and trace element supplementation may be beneficial in both institutionalised and healthy older people. For example, prophylactic use of calcium and vitamin D3 (cholecalciferol) has been shown to reduce the rate of hip fracture by 43% in institutionalised older persons in Lyon, France (Chapuy et al 1992). Vitamin and trace element supplementation also reduces the incidence of minor infections in healthy elderly subjects (Chandra 1992).

Increasing the physical activity and strength of older persons through aerobic exercise and resistance training has the potential to increase metabolic rate, and therefore appetite, as well as slow the decline in muscle mass normally associated with ageing. Horber et al (1996) reported that in trained (defined as regular physical activity for more than 10 years with a jogging distance of at least 30 km per week) older men (aged 67 ± 1 yr) lean mass was similar to untrained young men (aged 31 ± 2 yr) (56.0 ± 1.0 kg vs 56.4 ±1.0 kg) and a mean of 3.5 kg greater than in untrained older men (aged 69 ± 1 yr). In the older trained men, carbohydrate oxidation was higher and fat oxidation less than in the other two groups (Horber et al 1996). A recent study by Morio et al (Morio et al 1998), in 13 elderly sedentary subjects (aged 63 ± 2 yr), however, reported only a transient increase in sleeping metabolic rate, basal metabolic rate and diet-induced thermogenesis after 7 weeks of progressive endurance training, and this was not maintained over the 14 weeks of training. A combination of resistance training and protein supplementation may be more beneficial than either alone, as indicated by the results of a study performed by Fiatarone et al (1994), in frail elderly men (n= 37) and women (n= 63) aged 72-98 yr from Boston, USA. A nutritional supplement (240 ml, 1506 kJ [60% carbohydrate, 23% fat, 17% soy-based protein]), combined with lower extremity resistance training (3 x 45 min sessions per/week) over 10 weeks, increased bilateral hip and knee extensor muscle strength by ~138 ± 8% and type II (fast-twitch) thigh muscle area by ~3 ± 3% compared to that of the placebo group. Total dietary energy intake was also approximately 1550 kJ greater than the control group in subjects who received both the supplement and exercise training and this lead to a 1.8 % increase in body weight (Fiatarone et al 1994).

Improving the pleasurable qualities of food ie taste and smell (Chapter 3.2), may potentially stimulate an increase in appetite and food intake in the elderly, but there is little information about this. Schiffman et al (1993) assessed the effectiveness of flavour-enhanced foods, given over a 3 week period, and had limited success in improving appetite and body weight in elderly nursing home patients. Patients consumed more of the flavour-enhanced foods compared to the control period, but the overall macronutrient content and energy intake of their diet and individuals' body weight did not increase during flavour-enhanced diet (Schiffman et al 1993). Exposure to a flavour-enhanced diet may, therefore, be of limited success in increasing overall intake or may require more than 3 weeks to be effective.

Enteral feeding via a nasogastric tube may be used when swallowing difficulties or dysphagia occur in malnourished patients. There is an improvement in clinical outcome in patients recovering from hip fracture when enteral feeding is given in addition to oral supplementation (Delmi et al 1990). Enteral feeding also assists weight gain, improves biochemical nutritional markers and reduces mortality in patients with alcoholic liver disease (Kearns et al 1992). Total parenteral nutrition (TPN) is administered only when the gut is no longer functional, and the use of TPN is limited since it has a higher complication rate and is more expensive than enteral feeding (Morley 1997). There may, however, be a place for short-term parenteral nutrition in malnourished older persons who are hospitalised or in a nursing home who have an insufficient oral intake and impaired gastrointestinal motility (Kamel et al 1998).

1.7.3.2 Medications

None of the currently available medications has a major role in safely stimulating appetite in the elderly. Corticosteroids have been used as appetite stimulants in palliative care patients, and cause some body weight gain, but this is primarily through increases in fat mass and fluid retention, and these drugs have many side effects (Morley 1997). Jackobs et al (1999) reported recently that 20 out of 27 underweight nursing home residents in Ohio USA, aged 75-100 yr, gained between 2.0-3.8% of their body weight following administration of the hormonal antineoplastic agent, megestrol acetate (40 mg), for one month, however, its longer-term effects on body weight are not known. Megestrol acetate is associated with some adverse effects including thromboembolic disorders (thrombophlebitis and pulmonary embolism), hypertension, heart failure, nausea, vomiting and fluid retention. Anti-depressant medications increase appetite and promote weight gain in older persons suffering depression (Fitten et al 1989), but have not been shown to be beneficial in those who are not. Most other appetite-stimulating drugs that have been used to treat anorexia in the elderly have been of limited success and are associated with major side-effects, such as sedation, dizziness, nausea and delirium (Morley 1997). They include duranabinol (tetrahydrocannabinol), metaclobemide and cyproheptadine (Morley 1997). There may be a role for CCK antagonists, such as loxiglumide, in increasing food intake in the elderly (see Chapter 10), but this has not yet been evaluated.

There is some evidence that the relative growth hormone deficiency which affects up to half of elderly persons may be a cause of the reduction in lean body mass observed with ageing (Borst et al 1994). Growth hormone supplementation causes weight gain in

malnourished elderly patients (Borst et al 1994). There is, however, no evidence for improvements in morbidity or mortality with growth hormone supplementation (Morley 1997); moreover a recent large study in catabolic intensive care subjects reported an increased mortality in patients receiving high doses of growth hormone, mainly due to complications such as multiple organ failure, sepsis or uncontrolled infection (Takala et al 1999).

Anabolic steroids have been shown to increase muscle mass (Sheffield-Moore et al 1999) and strength (Bhasin et al 1996) in young adults, particularly in individuals who are hypogonadal (Strawford et al 1999, Tenover 1998). Testosterone has been used in older people, with some success. For example, Snyder et al (1999) recently reported, in men aged over 65 yr, that testosterone treatment for 36 months increases bone mineral density in those subjects with low pretreatment serum testosterone concentrations. In a cross-sectional study of 121 healthy men aged 65-97 yr, enrolled in the New Mexico Aging Process study, serum testosterone was a major predictor of muscle mass and strength (Baumgartner et al 1999b) and has also been found to predict functional status (Morley 1999b). Furthermore, with the exception of an increased hematocrit leading to polycythemia, there have been few side effects associated with testosterone therapy in older persons (Morley 1997). Anabolic steroids have also been successfully used to increase body weight in patients with AIDS and cancer cachexia (Strawford et al 1999, Dobs et al 1999) and increase tissue healing after severe burns (Demling 1999).

While specific medications may, therefore, be beneficial in stimulating appetite in elderly persons with severe anorexia the extent of their benefit particularly in patients with existing co-morbidities remains to be determined.

1.8 CONCLUSIONS

Increasing the quality of life and reducing morbidity in the older adult is becoming increasingly important in our rapidly ageing society. Anorexia and the associated weight loss and malnutrition are major causes of morbidity and mortality in the elderly. The reduced function of the homeostatic mechanisms controlling energy intake and body weight with ageing and the physiological anorexia of ageing, particularly when combined with pathological anorexia can result in loss of weight and malnutrition in older people. The causes of the physiological anorexia of ageing are, however, poorly

understood. Investigation of some of the mechanisms that may potentially contribute to the physiological anorexia of ageing are detailed in Chapters 8-14 of this thesis.

CHAPTER 2

Role of Central Mechanisms in Appetite Regulation

2.1 INTRODUCTION

The regulation of appetite is extremely complex, and poorly understood, involving the interaction of the components of the central feeding system (the cortex, limbic system and midbrain), as well as inputs from peripheral organs that convey neural and hormonal signals relating to taste and smell, gut sensation, adipose tissue mass, plasma nutrient levels and endocrine function (Morley 1987) (Figure 2.1). It is the interplay between these signals which monitors not only energy intake, but also the quality of various foods, so that adequate quantities of macro- and micro-nutrients are ingested. The purpose of this chapter is to review the central factors involved in the regulation of appetite, with particular focus on what is known about the role of endogenous opioids, as the latter issue was investigated in the study described in Chapter 10. The peripheral mechanisms involved in the regulation of appetite are discussed in the following chapter (Chapter 3).

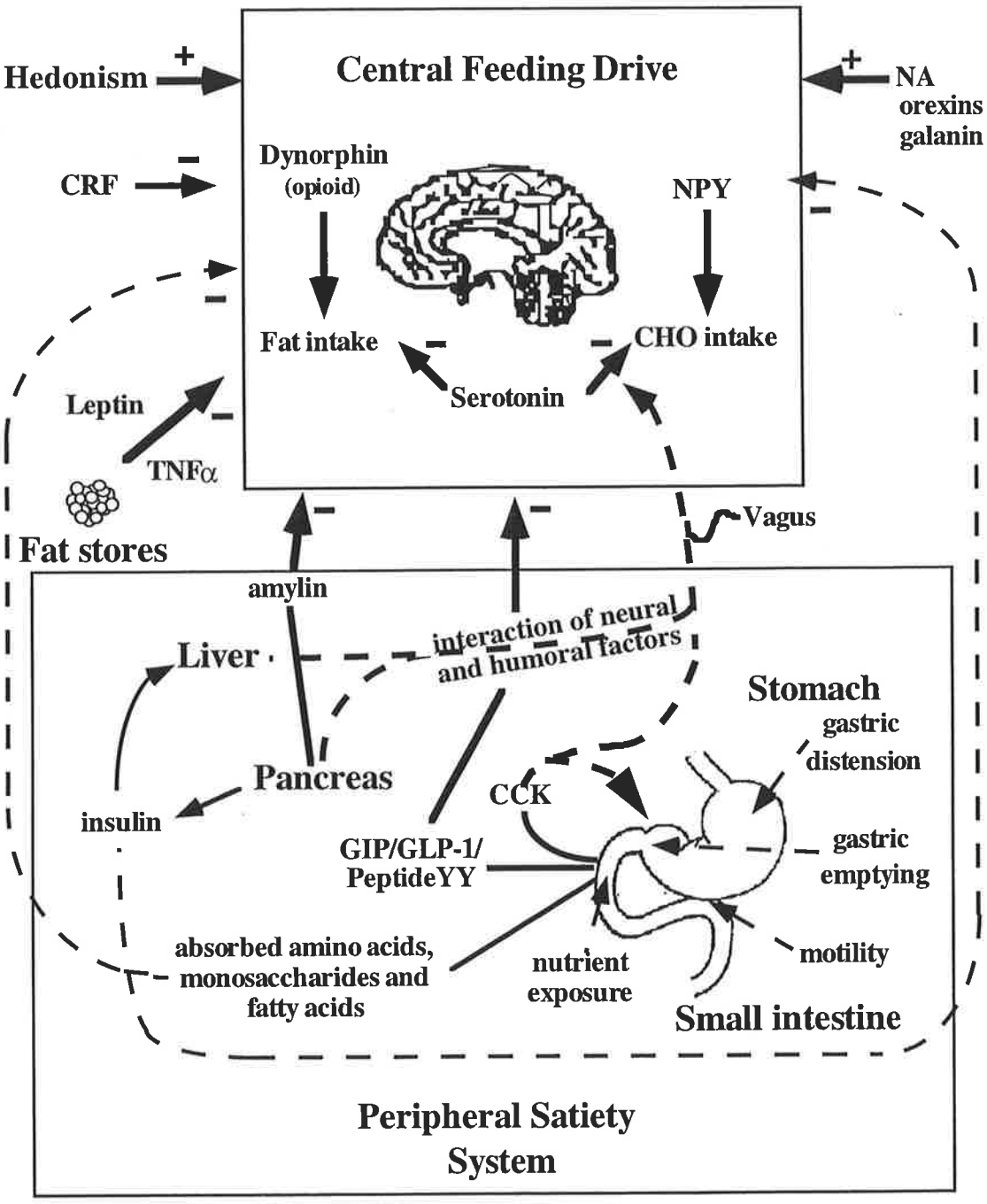

Figure 2.1: Model of appetite regulation

2.2 THE CENTRAL CONTROL OF APPETITE

Central mechanisms play a major role in the short-term regulation of food intake [reviewed in (Morley 1987, Kalra et al 1999)]. Much of the evidence relating to the mechanisms involved in the central regulation of feeding comes from animal studies, given the invasive nature of experiments, for example, intracerebroventricular injection of neuropeptides to examine the neurotransmitter pathways involved in the stimulation and inhibition of appetite. Such experimental manipulation of particular neural pathways, even in animals, also may not yield the expected results, as there are numerous redundancies in the complex pathways controlling feeding; ie certain neural pathways or transmitters may act to compensate for a pathway or neuropeptide that has been chemically or physically blocked. Furthermore, information about the physiological role of most of the neuropeptides is limited due to the absence of specific antagonists for them. Except where due reference is made the majority of evidence discussed is extrapolated from animals experiments.

2.2.1 *Anatomical regions*

The hypothalamus is the primary area of the brain involved in the stimulation of feeding, and acts as a transducer of multiple sensory inputs from other areas of the brain, as well as the periphery (Morley 1987, Kalra et al 1999). Traditionally, it was thought that separate centres within the brain controlled appetite and satiation; a feeding centre in the ventrolateral hypothalamus was responsible for the initiation of eating and a satiety centre in the ventromedial hypothalamus responsible for producing satiation (Ballinger 1994). Evidence for this concept was derived from early studies in rats in which lesions of the lateral hypothalamus caused aphagia, whereas electrical stimulation of this area induced feeding and weight gain (Hetherington & Ranson 1942). Subsequent studies demonstrated that electrical stimulation of the ventrolateral hypothalamus not only increases feeding, but enhances various anabolic functions, including insulin release, hepatic glucagon synthesis and gastric acid secretion (Powley & Laughton 1981). In rats, lesions within the ventromedial hypothalamus induce hyperphagia, and obesity (Hetherington & Ranson 1942), while electrical stimulation of this region is associated with increases in various catabolic functions, including glycogenolysis, and the release of glucagon (Powley & Laughton 1981).

This traditional concept of the central regulation of feeding is, however, now recognised to represent an over-simplification. In particular, more recent studies have demonstrated that other sites in the central nervous system can modulate appetite and

feeding behaviour. For example, the importance of the paraventricular nucleus (PVN) of the hypothalamus was established by the demonstration that the feeding response to intraventricular injection of norepinephrine is attenuated after bilateral damage to the PVN (Leibowitz et al 1983). Furthermore, the ventromedial hypothalamus is associated with projections from the pontine-midbrain area (serotonergic raphe nuclei tract) and the ventral noradrenergic bundle (Ungerstedt et al 1971), and the lateral hypothalamus is associated with the dopaminergic nigrostriatal tract (Ungerstedt et al 1971, Fibiger et al 1973). The serotonergic raphe nuclei tract and dopaminergic nigrostriatal tract are thought to be responsible for the integration of reward or pleasure stimuli associated with food in the brain (Morley 1987). The role of the nucleus of the solitary tract in the regulation of feeding was established by Hyde & Miselis (1983), who demonstrated that destruction of the area postrema/caudal medial nucleus of the solitary tract caused hypophagia and chronic weight loss in rats. The nucleus of the solitary tract may be particularly important in the integration of vagally-mediated inputs from the tongue, liver and gastrointestinal tract (Morley 1987).

A recent study in humans using the technique of functional magnetic resonance imaging (fMRI) has provided further insights into the neural processes within particular regions of the brain during food ingestion *in vivo* (Liu et al 2000). This study demonstrated that there is a peak of neural activity within the supplementary motor area, somatosensory cortex, cerebellum, anterior cingulate and orbitofrontal cortex within 2 minutes of ingesting a glucose drink, suggesting that these regions may be involved in the integration of sensory and visceral signals, as well as signals associated with appetite, taste and olfaction (Liu et al 2000). A subsequent peak in neural activity occurs ~10 minutes after glucose ingestion and within the medial hypothalamus. The amplitude of this peak is inversely related to the plasma insulin concentration, suggesting that there is a dynamic interaction between the hypothalamic neural response and the peripherally mediated biochemical signal ie. insulin, in response to glucose ingestion.

2.3 NEUROTRANSMITTERS

A large number of neurotransmitters within the brain and periphery mediate signals involved in the regulation of food intake. They include the endogenous opioids (section 2.3.1), noradrenaline neuropeptide Y (NPY)(section 2.3.2) dopamine, melanin-concentrating hormone, nitric oxide (NO)(section 2.3.2), the orexins, galanin and, possibly, ghrelin (3.6.7) and the anorexigenic or inhibitory effects of corticotropin-

releasing factor, serotonin, cholecystokinin (CCK)(Chapter 3.6.1) and, possibly, insulin (Chapter 3.6.2) (Morley et al 1983, McHugh & Moran 1986, Morley 1987, Heinrichs et al 1993, Schick et al 1993, Dube et al 1994). Table 2.1 summarises the excitatory and inhibitory effects on food intake of many of the known centrally located neurotransmitters. A detailed discussion of the role of each of these neurotransmitters is beyond the scope of this thesis [for review see (Morley 1987, Kalra et al 1999)]. Many of these neurotransmitters have both central and peripheral actions. For example, the gut peptides CCK, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotrophic polypeptide (GIP), peptide YY (PYY), amylin and the recently identified peptide ghrelin, which are secreted within the gastrointestinal tract and influence gastrointestinal motor and sensory function, also affect appetite when administered centrally. The role of these gut peptides in the regulation of food intake is discussed in more detail in Chapter 3.6. The following discussion is limited to the endogenous opioid feeding system, and a brief discussion of some of the other important neurotransmitters, including, neuropeptide Y, nitric oxide and leptin.

Table 2.1 Neurotransmitters implicated in the central regulation of food intake.

↑ Food Intake	↓ Food Intake
Endogenous opioids*	CRF/urocortin*/sauvagine
Neuropeptide Y	GI peptides-CCK*/GLP-1*
Melanin-concentrating hormone	Insulin*/IGF-1
Galanin	Glucagon
GHRH	Neurotensin
Orexins	Isatin
Oxytocin	Dopamine
Nitric oxide*	Oestrogen
Peptide YY (PYY)*	α-melanocortin stimulating-hormone
Norepinephrine	Neuromedin

Table 2.1 cont.

↑ Food Intake	↓ Food Intake
Dopamine	Oxytocin (short term)
Motilin	Leptin*
Histamine	Serotonin*
Thyroid hormone	Isatin
Testosterone	Exendin*
cytokines	Bombesin*/GRP
ghrelin*	Amylin/CGRP

* indicates both central and peripheral effects

2.3.1 *Endogenous opioids*

Endogenous opioids play a role in mediating the short-term sensory reward response to food (Gosnell et al 1983, Morley 1987). Opioids are believed to act directly on the hypothalamus, amygdala and nucleus accumbens to enhance appetite, food intake and fluid intake (Morley 1987, Levine et al 1985). Elevated levels of the endogenous opioid, β-endorphin, have been found in the pituitary of genetically obese rats and mice and in the plasma of genetically obese rats (Margules et al 1978). Other neurochemicals such as neuropeptide-Y (section 2.3.3) (Rudski et al 1996, Morley et al 1983) and dopamine (Morley et al 1983) are thought to interact with endogenous opioids to modulate feeding behaviour. Endogenous opioids are also involved in the control of other physiological processes, such as pain perception, reproductive function, thermoregulation, thirst and immune function (Morley 1987).

The major endogenous opioid involved in stimulating feeding is believed to be the kappa (κ) opioid receptor ligand, dynorphin (Cooper et al 1985a, Levine et al 1985, Papadouka & Carr 1994). There is evidence, however, that the opioid receptor subtypes: delta (δ) (Gosnell et al 1986) and mu (μ) (Levine et al 1987) and the ORL1 (Opioid Receptor like 1) receptor, with its recently identified endogenous ligand,

nociceptin or orphanin FQ (structurally-related to the endogenous opioid dynorphin A) (Meunier 1997, Stratford et al 1997), are also involved in the control of food intake. Microinjections of nociceptin into either the ventromedial hypothalamus or nucleus accumbens increases food intake in rats (Stratford et al 1997). Other studies have demonstrated effects of opioid agonists on food intake in both animals (Tepperman & Hirst 1983, Morley et al 1983, Giraudo et al 1993, Baile et al 1984). For example, Gosnell et al (1986) showed that intracerebroventricular injections of dynorphin 1-17, [D-ala2MePhe4,-Gly-ol5], enkephalin (DAMGO) and [Dser2, leu5] enkephalin-thr6 (DSLET), which are selective agonists at κ, μ and δ opioid receptors, respectively, at doses in the 3-10 nmol range stimulate food intake in non-deprived rats by up to 38%.

Studies in humans have reported that plasma and cerebrospinal fluid levels of β-endorphin are increased in obese individuals (Atkinson 1987) and there are also data indicating that plasma levels of endogenous opiate alkaloids may be elevated in patients with anorexia and bulimia nervosa (Marrazzi et al 1997). In addition, Morley et al (1985) found that intravenous infusion of butorphonal tartrate (predominantly a κ receptor agonist) increased food intake by 46%, in young, normal-weight, adult humans.

Possibly, the most convincing evidence for the role of endogenous opioids in stimulating food intake is derived from studies that have used opioid antagonists. These antagonists are thought to act centrally by blocking specific opiate receptors, influencing other neurotransmitter systems and modifying neuroendocrine activity (de Zwaan & Mitchell 1992). Peripheral administration of the opioid antagonist naloxone, which predominantly acts on μ and δ receptors and weakly at the κ opioid receptor, reduces food intake in a variety of animals including tigers, wolves, slugs and rodents, following food-deprivation [for review see (Morley et al 1983)]. Furthermore, a number of studies have shown that opioid antagonists suppress feeding induced by administration of opioid agonists [for review see (Morley et al 1983)]. For example, Giraudo et al (1998) found in the rat that feeding induced by the opioid agonist DAMGO was blocked by administration of naltrexone (multiple receptor antagonist) into the central nucleus of the amygdala and paraventricular nucleus.

A number of studies have also demonstrated the suppressive effects of various opioid antagonists on food intake in humans (Drewnowski et al 1995)[for review see (de Zwaan & Mitchell 1992)]. For example, Trenchard et al (1983) reported a dose-

dependent suppression of cumulative food intake following an intravenous (iv) bolus injection of naloxone compared with a placebo injection in 12 young normal weight subjects (6 men and 6 women); naloxone at a dose of 0.8 mg failed to reduce food intake whereas a dose of 1.6 mg reduced food intake from an automated dispenser by approximately 25%. Cohen et al (1985) found that an iv bolus injection of naloxone in a dose of 2 mg/kg reduced food intake from meals given 2.75 and 7.75 hours after drug administration in 7 (5 men and 2 women) normal subjects by approximately 28% (Figure 2.2). In both of these studies, however, there was no significant effect of the antagonist on subjective feelings of hunger or fullness (Cohen et al 1985; Trenchard et al 1983). Other antagonists such as naltrexone (2.5 mg) (Bertino et al 1991) and nalmefene (50 mg)(predominantly δ and κ receptor antagonist) (Yeomans et al 1990), given as a single oral dose, also reduce food intake by 20-30% (Bertino et al 1991, Yeomans et al 1990).

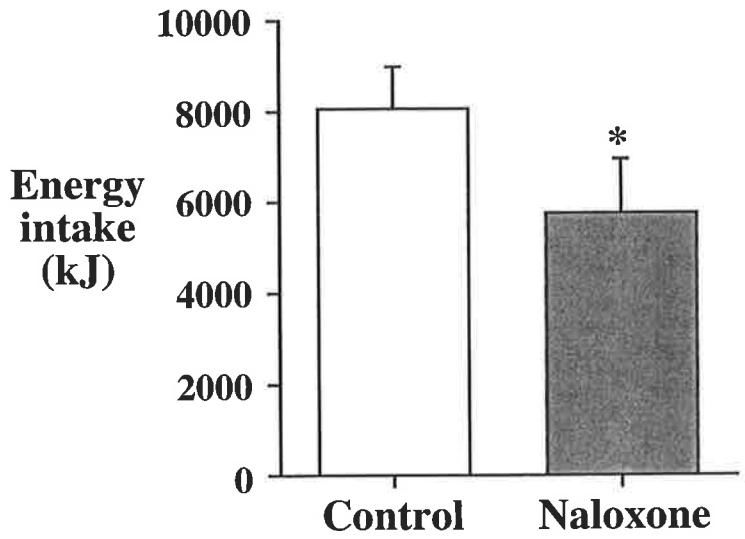

Figure 2.2: Mean energy intake (kJ) during lunch and dinner (combined) 2.75 hr and 7.75 hr,respectively, after iv saline or naloxone (2mg/kg) bolus infusion in fasted healthy young (19-51 yr) subjects (Cohen et al 1985). *P <0.02 vs control.

A few studies have investigated the effects of opioid antagonists on food intake in subjects other than young healthy adults. The responses of obese subjects to intravenous naloxone infusions appears to be comparable to adults of normal weight, although the data are somewhat inconsistent [for review see (de Zwaan & Mitchell 1992)]. For example, Atkinson et al (1982) evaluated the effects of naloxone (2 mg and 15 mg iv) on food intake in 14 obese and 5 lean young subjects. They were able to demonstrate a reduction in food intake of 29% during the higher dose naloxone compared to lower dose naloxone and control infusion in the obese subjects, but failed to show any reduction in food intake with naloxone in the lean subjects (Atkinson et al 1982). In contrast, Malcolm et al (1985) and Mitchell et al (1987) reported in young obese subjects that the amount of weight lost during oral administration of naltrexone, at doses of 200 mg/day and 300 mg/day for 8 weeks, respectively, was similar to that observed after placebo. Atkinson et al (1985), however, reported that weight loss was greater during oral administration of naltrexone in a dose of 50 mg/kg for 8 weeks compared to the placebo in female, but not male obese subjects. Food intake was not assessed during any of these studies. The suppressive effects of opioid antagonists on food intake have also been shown in patients with Prader-Willi Syndrome (Kyriakides et al 1980), as well as patients with bulimia nervosa (Mitchell et al 1986) [for review see (de Zwaan & Mitchell 1992)]. Most studies have evaluated either all men or all women, and with the exception of the study by Atkinson et al (1985) when both male and female subjects were included, the potential effect of gender on the impact of opioid antagonism on food intake was no evaluated. The effect of gender, if any, on the response to opioid antagonism is, therefore, uncertain. This issue is investigated in Chapter 10.

As well as stimulating food intake, opioid peptides have also been implicated in the modulation of food choice and the hedonic responses to food (Giraudo et al 1993, Cooper et al 1985b, Drewnowski et al 1995, Drewnowski et al 1992). It has been proposed that they increase the intake of high fat, high sugar foods preferentially (Gosnell et al 1983, Morley 1987). The suppression of food intake by naloxone appears to be enhanced when animals are offered a more palatable (Rudski et al 1997, Shabir et al 1999) or preferred (Glass et al 1996, Hope et al 1997) diet. For example, in the marsupial *Sminthopsis crassicaudata,* suppression of food intake induced by intraperitoneal injection of naloxone predominantly resulted from suppression of the intake of the animal's preferred diet of mealworms, rather than the less preferred, laboratory diet (Hope et al 1997). Furthermore, in non-deprived rats consumption of

highly palatable foods results in a substantial fall in the amount of β-endorphin and an increase in opioid binding within the hypothalamus (Dum et al 1983).

Previous studies of the effects of opioid antagonists on food choice in humans have, however, yielded inconsistent results (Drewnowski et al 1992, Yeomans et al 1997, Arbisi et al 1999, Cohen et al 1985). Yeomans et al (1997) found that oral naltrexone (50 mg) acutely decreased the amount of energy consumed of a meal of pasta with either a high-fat cheese sauce or low-fat tomato-based sauce by approximately 14 and 23 %, respectively, in healthy young men. The reduction in energy intake was associated with greater reductions in the rated pleasantness of both foods and an overall reduction in eating rate. Drewnowski et al (1992) reported that preferences for high fat/high carbohydrate foods were decreased during naloxone infusion in both binge-eating (n= 20) and non binge-eating (n= 21) women, but that the carbohydrate, protein and fat content of food eaten at a test meal was reduced only in the binge-eaters. In contrast, Cohen et al (1985) studied 7 (5 male and 2 female) young healthy adults and reported that naloxone reduced the intake of foods high in fat and protein by approximately 34% compared to the saline infusion, but had no effect on intake of foods high in carbohydrate (Figure 2.3). Arbisi et al (1999) reported that oral naltrexone (50 mg) decreased the attractiveness of sweet, but not salty, bitter or sour tastes, in 18 healthy women. The effect of opioid antagonism on the palatability of foods is evaluated in Chapter 10.

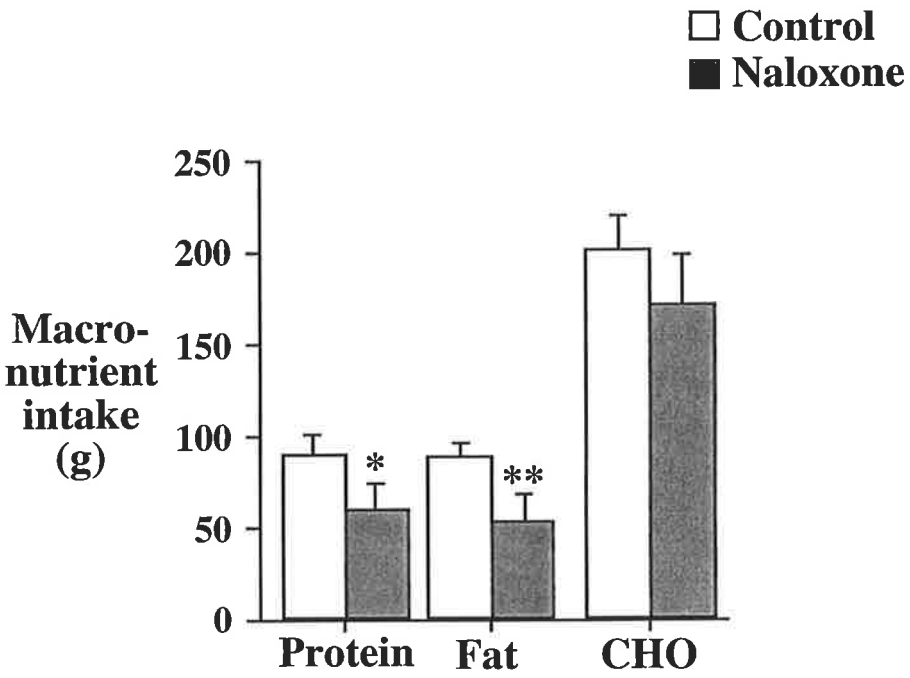

Figure 2.3: Macronutrient content (g) of food consumed at lunch and dinner (combined) 2.75 hr and 7.75 hr, respectively, after iv saline or naloxone (2mg/kg) bolus infusion in fasted 7 normal subjects (Cohen et al 1985) *P<0.02 vs control; **P <0.05 vs control

There is evidence that opioid activity is altered with ageing and that this may play a role in disordered appetite regulation. This issue is addressed in more detail in Chapter 4.2.1 and further investigated in Chapter 10.

2.3.2 *Nitric oxide (NO)*

Nitric oxide is a short-lived gas produced endogenously by the action of endothelial, neuronal and inducible nitric oxide synthase (NOS) on the amino acid L-arginine. The production of NO is widespread and its numerous, often powerful, physiological effects have been the subject of considerable recent research. Data in animals strongly support a role for nitric oxide as a modulator of the effects of a number of neurotransmitters including leptin (section 2.3.4) (Morley et al 1999a, Calapai et al 1998) and neuropeptide Y (section 2.3.3)(NPY) (Morley et al 1999a) involved in the control of feeding. Various L-arginine analogues act as competitive inhibitors of nitric oxide synthase (NOS) and have been used to study the effects of endogenous NO; their administration is associated with reductions in short-term food intake in rats (Squadrito et al 1993), chickens (Choi et al 1994) and the marsupial, *Sminthopsis crassicaudata* (Vozzo et al 1999b) and weight loss in mice (Morley & Flood 1992). Lean, but not genetically obese, rodents develop tolerance to the anorectic effects of NOS inhibitors (Squadrito et al 1993, Morley & Flood 1994a) and in the obese ob/ob mouse NOS and NOS mRNA levels are elevated in the hypothalamus. Consequently, it has been hypothesised that NO stimulates feeding and that obesity may be associated with increased NO tone. NO is also found widely in the gut where it appears to have an important role in the regulation of fundal relaxation after a meal (see Chapter 3.3.1) as well as gastric motility (see Chapter 3.5.2), which may in turn influence food intake. The impact of age on NO activity is discussed in Chapter 4.2.2.

2.3.3 *Neuropeptide Y*

Neuropeptide Y (NPY) is a 36 amino acid peptide hormone which is a potent stimulus of food intake. NPY is synthesised in the peripheral nervous system and widely in the brain, particularly in the neurons of the arcuate nucleus. Injection of NPY into the PVN increases food intake in a number of animals (Morley et al 1987). In the rat, fasting increases production of NPY in the arcuate nucleus, and release in the PVN, but not in other hypothalamic areas. These changes are reversed by refeeding and may represent an important mechanism in the hyperphagia that initially accompanies refeeding after fasting. The effects of NPY and also leptin (section 2.3.4) may be secondary to increased NO (section 2.3.2) turnover (Morley et al 1999a, Calapai et al 1999).

Despite apparently compelling evidence that NPY stimulates feeding, somewhat surprisingly, NPY knockout mice are relatively normal (Hollopeter et al 1998). These knockout mice eat normally, are not underweight and have normal thyrotropic, gonadotropic and corticotropic axes (Erickson et al 1997). They are, however, twice as sensitive as wild type mice to the satiating effects of leptin (Hollopeter et al 1998), and reduced NPY activity partially ameliorates the obesity and endocrine abnormalities of leptin deficiency in ob/ob mice (Palmiter et al 1998). Genetic leptin deficiency also increases NPY gene transcription in the arcuate nucleus. These observations indicate that the actions of NPY and leptin are closely related, so that each inhibits the action of the other. There is also evidence that the interaction of NPY with insulin (Chapter 3.6.2) (Schwartz et al 1992) and the melanocortin system (Kask et al 1998) may be important in the central control of feeding. The effects of ageing on NPY activity are discussed in Chapter 4.2.2, in relation to its potential role in the physiological anorexia of ageing.

2.3.4 *Leptin*

Leptin is a recently discovered peptide hormone which is produced predominantly in adipose tissue so that its blood concentrations are directly related to the size of fat stores [for review see (Kalra et al 1999)]. Leptin acts mainly in the hypothalamus to inhibit feeding. Numerous studies have shown that both central and peripheral administration of leptin decreases food intake in rodents [for review see (Kalra et al 1999)]. Congenitally leptin deficient, ob/ob, mice are obese due to a combination of increased food intake and reduced energy expenditure (Halaas et al 1995, Pelleymounter et al 1995). Administration of leptin, reverses these effects, as well as the infertility and other hormonal defects associated with this condition (Mounzih et al 1997).

Studies in humans have demonstrated that fasting (Maffei et al 1995) decreases leptin secretion, whereas overfeeding (Kolaczynski et al 1996) enhances its secretion. Congenital leptin deficiency has been identified as a very rare cause of morbid obesity in humans, associated with hyperphagia and infertility, but not, apparently, reduced energy expenditure (Montague et al 1997). Leptin treatment produces substantial weight loss in these people (Farooqi et al 1999). Most obese people, however, have elevated circulating leptin concentrations, consistent with their increased fat mass. Indeed, "leptin resistance" is probably a feature of most human obesity and the administration of leptin to obese people appears to result in only minor weight loss (Heymsfield et al

1999). Leptin treatment may, however, be effective after weight loss in helping prevent fat re-accumulation.

Leptin interacts with a number of other neurotransmitters including NPY (section 2.3.2) and GLP-1 (Chapter 3.6.3). There is evidence from a study in rats that leptin is also present in the stomach, and that feeding and exogenous administration of CCK-8 both decrease leptin cell immunoreactivity and leptin content of the fundic epithelium, with a concomitant increase in plasma leptin concentrations (Bado et al 1998). These observations indicate that gastric leptin may be involved in mediating the effects of CCK on satiety (Bado et al 1998). Furthermore, Matson et al (1997) have shown that intraperitoneal administration of both leptin and CCK reduces total daily energy intake significantly more than either given independently, suggesting that leptin and CCK may synergistically interact to control food intake at least in the long-term. The effect of exogenous CCK on plasma levels of leptin in humans is not known. This issue is investigated in Chapter 10.

2.4 CONCLUSIONS

The central modulation of feeding involves an intricate network of neural pathways which act to stimulate feeding. Important components of this are endogenous opioids and the neuropeptide Y feeding system. Alterations in the activity (ie. concentrations, receptor binding affinity or sensitivity) of particular central neurotransmitters may potentially contribute to the age-related reduction in appetite and food intake. Current knowledge, much of which is derived from animal studies, about some of the possible central factors which may contribute to the physiological anorexia of ageing, are discussed in Chapter 4.2. A study designed to determine whether ageing is associated with a reduced opioid feeding drive is detailed in Chapter 10.

CHAPTER 3

Role of Peripheral Mechanisms in the Regulation of Appetite

3.1 INTRODUCTION

The peripheral satiety system is driven largely by signals from the gastrointestinal tract. These signals are thought to be mediated by both neural and humoral mechanisms. The purpose of this chapter is to review information relating to the gastrointestinal mechanisms involved in the short-term control of feeding, including the role of oropharyngeal and intragastric sensation, gastric emptying, small intestinal nutrient mediated feedback and gut peptides evidence derived from studies in both animals and in healthy young adult humans. The role of absorbed nutrients (amino acids, monosaccharides and fatty acids) in the short-term control of appetite is also discussed. The effects of ageing on the peripheral mechanisms involved in the control of appetite are discussed in Chapter 4.3 in relation to their potential role in the physiological anorexia of ageing.

There are a number of gastrointestinal mechanisms which optimise the relationship between food ingestion with the delivery, digestion and absorption of nutrients. Nutrients interact with the gastrointestinal tract along its length and it is the summation of these interactions which contributes to the termination of a meal. In considering the relative contribution of the different regions of the proximal gut (mouth ie. oropharyngeal sensory function, stomach ie. gastric motility and gastric emptying and small intestine) in the regulation of satiation each these of regions will be discussed separately.

3.2 OROPHARYNGEAL SENSORY FUNCTION

The presence of food in the mouth influences both the amount of food consumed and food selection (Rolls et al 1982). Studies in animals suggest that oro-sensory mechanisms do not per se induce satiety or contribute to the termination of a meal (Koopmans et al 1981). For example, dogs equipped with oesophageal fistulae, through which ingested food drains without entering the stomach ("sham feeding"), eat continuously until exhausted (Janowitz & Grossman 1949). However, when "sham

feeding" is combined with the direct infusion of nutrients into either the stomach (Berkun et al 1952, Janowitz & Grossman 1949) or small intestine (Antin et al 1977), food intake is suppressed. These findings suggest that orosensory stimulation magnifies the satiating effects of intragastric and intestinal nutrients (see sections 3.4 and 3.5). Consistent with this, a recent study in young healthy humans showed that oral ingestion of 425 ml tomato soup (1673 kJ) suppressed hunger and increased fullness to a greater extent than intragastric or intraduodenal administration (Cecil et al 1998). Furthermore, in that study gastric emptying of soup was slower after oral ingestion when compared to intragastric (either covert or overt) infusions (Cecil et al 1998), indicating that the presence of food in the mouth and/ or swallowing may enhance gastrointestinal satiety mechanisms by slowing gastric emptying. This concept is supported by animal studies. In rats gastric emptying of fat is faster following intragastric infusion when compared to oral ingestion (Kaplan et al 1997, Ramirez 1985). In sham fed rats oropharyngeal stimulation enhances the effect of exogenous administration of cholecystokinin (CCK) on satiety (Forsyth et al 1985). There is also evidence, in both animals (Ramirez 1985) and humans (Mattes 1996), that oral stimulation may influence the metabolism of ingested nutrients, which in turn may influence satiety. For example, Mattes (1996) demonstrated that the postprandial metabolism of fat (50 g safflower oil) is accelerated by concurrent oronasal exposure to high-fat food when compared to a low-fat food (which had been masticated and then expectorated without being swallowed).

The pleasurable qualities of food are also important in the orosensory response. In general those foods that are the most pleasant tasting are consumed preferentially (Warwick et al 1993). For example, rats over-consume foods that are highly palatable and avoid quinine-adulterated foods (Morley 1987). In contrast, there is evidence that more palatable 'tasty' versions of high-fat and high-carbohydrate meals may be more satiating than nutritionally identical 'bland' meals (Warwick et al 1993). There is also evidence that specific macronutrient oro-sensory abilities exist in humans. Both animals (Rolls et al 1999) and humans (Mattes 1996) also appear to have specific abilities to 'sense' fat, through its texture and odour (Rolls et al 1999), as well as different intensities of sweetness (de Graaf et al 1993). Myers & Epstein (1997) demonstrated a significantly faster rate of 'salivary habituation' when subjects consumed a high-fat preload compared to a low fat preload, whereas the carbohydrate content of the preload did not influence the rate of habituation.

Rolls et al (Rolls et al 1981, Rolls et al 1982) have proposed the concept of "sensory-specific satiety", and suggested that this has an important influence on the amount and type of food ingested. Sensory-specific satiety is defined as the decline in pleasantness of the taste of a particular food after it has been consumed, leading to a decrease in its subsequent consumption, with a tendency to shift consumption to other food choices during a meal (Rolls et al 1991). This promotes the intake of a more varied, nutritionally balanced diet (Rolls et al 1991, Rolls et al 1981, Rolls et al 1982). Sensory-specific satiety primarily influences food selection and plays only a minor role in the regulation of satiation.

Oral exposure to food alone, therefore, does not appear to play a direct role in terminating food intake; rather it influences food choice, nutrient metabolism and augments the generation of satiety signals from the stomach and small intestine. Alterations in orosensory function may potentially contribute to changes in food intake or food choice. This is issue discussed further in relation to the age-related changes in taste and smell in Chapter 4.3.

3.3 STOMACH

Functionally, the stomach can be divided into three regions, each with specific mechanical characteristics; ie the proximal stomach (fundus and body), the distal stomach (antrum) and the pylorus (Figure 3.1). The coordinated motor activity of these regions facilitates gastric emptying (section 3.5.2).

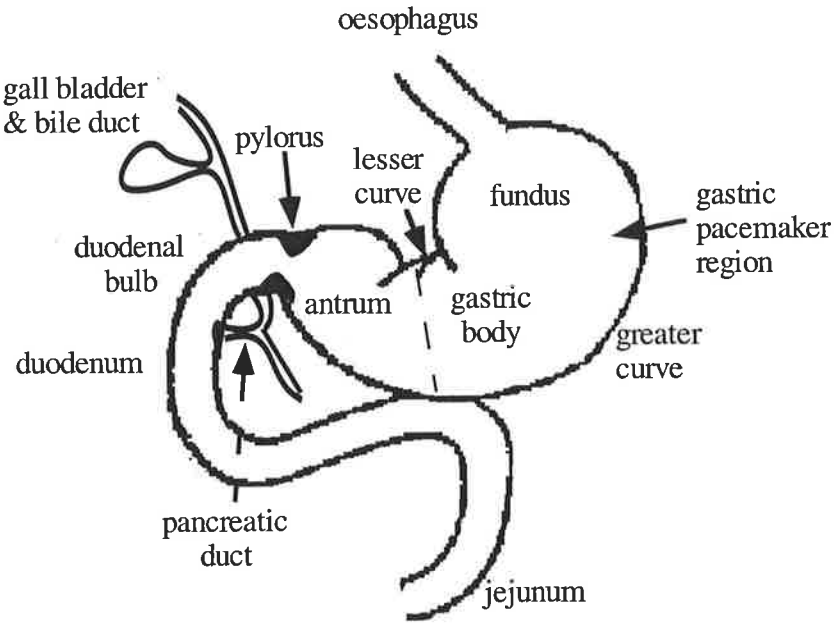

Figure 3.1: The basic anatomy of the stomach and duodenal region. See text for details (section 3.3).

3.3.1 *Proximal stomach*

The smooth muscle cells of the proximal stomach have a low resting membrane potential and are continuously in a state of partial tonic contraction that is well suited to its primary functions; a temporary reservoir for food, to control and maintain intragastric pressure (ie fundic tone) and to propel food into the distal stomach (Hasler 1999). The two main reflex processes which mediate these functions of the proximal stomach are receptive relaxation and gastric accomodation. Receptive relaxation is the reduction in proximal gastric tone, triggered by mechanical stimulation of the throat and oesophagus, which occurs during swallowing (Hasler 1999). Gastric accomodation refers to the increase in proximal gastric volume in response to gastric distension or after ingestion of food and, unlike receptive relaxation, this response requires no pharyngeal or oesophageal stimulation. Gastric accomodation allows the stomach to accomodate relatively large volumes (up to 2 L) with relatively little increase in intra-gastric pressure (Weisbrodt 1984). The smooth muscle of the body of the stomach has a lower resistance to stretch than the distal stomach (antrum) and therefore facilitates this gastric accomodation response to a meal. The vagus nerves are the major efferent pathways involved in receptive relaxation (Jansson 1969). Both receptive relaxation and gastric accomodation responses are mediated via noncholinergic and nonadrenergic pathways (Jansson 1969). The neurotransmitters that have a dominant role in these responses are vasoactive inhibitory peptide (VIP) (Hasler 1999) and nitric oxide (NO) (Arakawa et al 1997). For example, Coulie et al (1999) showed that intravenous bolus administration of the NO synthase inhibitor, *N* $^{\omega}$-nitro-L-arginine methyl ester (L-NAME) in fasted cats causes a significant reduction in fundic volume, which is restored completely following administration of the NOS substrate L-arginine. The use of the barostat technique, which involves monitoring the pressure and volume changes within a distensible air-filled bag placed in the proximal stomach, allows assessment of the receptive relaxation and accomodation responses of the stomach. This technique is described in Chapter 7.4.2.

Proximal gastric tone is also influenced by stimulation of other regions of the gastrointestinal tract. It is reduced by duodenal (Rouillon et al 1991) and colonic mechanical distension (Sims et al 1995), as well as by the infusion of nutrients and acid into the small intestine (Azpiroz & Malagelada 1985) (see section 3.6.2).

Impaired adaptive relaxation during and after meal ingestion may increase the activation of gastric mechanoreceptors located in the stomach wall and the perception of gastric

distension thus leading to early satiation. An increased perception of gastric distension in evident in some patients with functional dyspepsia, who also exhibit an impaired gastric accomodation to a meal (Tack et al 1998). There is some evidence that proximal gastric motor function may be affected by ageing. This issue is discussed in Chapter 4.2 and investigated in the study described in Chapter 11.

3.3.2 *Distal stomach*

The antrum plays a major role in the grinding of solid food into small particles and the delivery of "liquefied" gastric contents into the small intestine. Unlike the tonic contraction of the proximal stomach, the distal stomach exhibits phasic contractile activity. This contractile activity is controlled by the rhythmic electrical depolarisation, ie. pacesetter potential or gastric slow wave, generated in the greater curvature of the stomach (Hasler 1999) which is modulated by feedback signals from the small intestine (see section 3.6.2). The interstitial cells of Cajal, which exhibit rhythmic depolarisation's in the small intestine and colon, may play a role in generating gastric slow waves (Hasler 1999). The pacemaker usually discharges at a rate of approximately 3 per minute, and determines the maximum frequency of phasic contractions. However, not every pacemaker discharge results in a phasic contraction. The activity of the pacemaker can be measured by surface electrodes, so-called electrogastrography (EGG); this technique is described in Chapter 7.2. Disturbances of the gastric electrical rhythm, particularly an increased prevalence of abnormally fast rhythms (tachygastria) have been documented in conditions, such as nausea during pregnancy, motion sickness, anorexia nervosa and gastroparesis (Hasler 1999). Contractions usually begin in the middle of the body (corpus) of the stomach and propagate towards the pylorus with an increase in both velocity and amplitude as they travel distally, (Tougas et al 1992). The velocity of propagation of the gastric electrical slow wave is approximately 0.5 cm/sec in the corpus and increases to 4 cm/sec in the antrum (Hasler 1999). The patterns of postprandial antral contractions/pressure waves are complex. They serve to either empty gastric contents if the pylorus is open, or grind and mix food when the pylorus is closed. The frequency, amplitude and duration postprandial antral contractions ('fed pattern') are dependent on both the physical and chemical properties of a meal. For example, the amplitude of antral contractions is higher after ingestion of a solid, when compared to a homogenised meal (Rees et al 1979).

In the fasted state the contractile activity of the antrum is markedly different from that of the fed state. The pattern of activity during the fasting state is known as the migrating motor complex (MMC). It consists of three phases; phase I is a period of motor quiescence which lasts for 40-60 min; phase II is characterised by a period of irregular contractions and usually lasts between 20 and 40 min; phase III is characterised by motor activity, usually at the maximum rate of ~3/min, that lasts for 5-10 min and is largely responsible for 'sweeping' undigested food matter and dead epithelial cells from the stomach into the small intestine (Hasler 1999).

Studies in young adult human subjects have indicated that the contractile activity of the antrum, like the proximal stomach is affected by stimulation of other regions of the gut. For example, distension of the fundus increases the frequency of antral contractions (Andrews et al 1980) whereas distension of the duodenum (Edelbroek et al 1994) or infusion of nutrients or acid into the small intestine (Heddle et al 1989, Heddle et al 1988a) (see section 3.6.2) suppress phasic antral pressure waves.

3.3.3 *Pylorus*

The pylorus consists of thick smooth muscle layers (longitudinal and circular) and dense connective tissue underlying a redundant, highly folded mucosa, which narrows the diameter of the gastric lumen (Hasler 1999). The function of the pylorus is to act as a mechanical stricture inhibiting the passage of large particles even in the absence of contractions (Hasler 1999). The timing of pyloric closure plays an important role in determining the rate of gastric emptying and is modulated by small intestinal feedback (see section 3.6.2). The neural and humoral mechanisms responsible for the stimulation of pyloric motility are discussed in more detail in section 3.6.2.

The motility patterns of the pylorus are characterised by changes in the basal pyloric pressure (or pyloric tone) and the frequency of phasic pyloric pressure waves, also known as isolated pyloric pressure waves (IPPW's), which varies between the fasting and fed state. For example, during phase III of the MMC the pylorus is open to allow residual chyme to be passed into the duodenum [for review see (Hasler 1999)]. After meal ingestion, the motor patterns of the pylorus are complex, ie involving prolonged periods of closure, to allow sufficient grinding of food by the antrum, interrupted by brief intervals, approximately twice a minute, during which the contents of the antrum are passed into the intestine at a rate optimal for absorption (Hasler 1999).

Both neural and humoral factors are thought to be responsible for the stimulation of the pylorus after meal ingestion (Fraser et al 1992, Fraser et al 1993). Nutrients (fat, glucose and amino acids) and hydrochloric acid infused into the small intestine increase basal pyloric tone and the frequency of phasic pyloric pressure waves (Heddle et al 1988a, Heddle et al 1988b). This response is mediated, at least in part, via muscarinic mechanisms; since intravenous administration of atropine (15 μg/kg) inhibits the increase in pyloric contractions induced by both intraduodenal lipid (Fraser et al 1992) and glucose (Fone et al 1989). Intravenous administration of cholecystokinin (CCK) (Fraser et al 1993) and GLP-1 (Schirra et al 2000) stimulates phasic and tonic pyloric motility and inhibits antroduodenal motility in healthy young subjects. The increase in pyloric motility induced by intraduodenal lipid infusion is attenuated following administration of the nitric oxide (NO) donor glycerol trinitrate, indicating a role for NO in the regulation of pyloric motility (Sun et al 1996). Several other agents such as VIP, galanin, serotonin, prostaglandin E_1 and peptide histidine-isoleucine are also known to relax the pylorus (Hasler 1999).

3.3.4 *Neural innervation of the stomach*

The stomach is richly innervated by extrinsic nerve fibers - which relay information to and from the extragastrointestinal ganglia, the spinal cord, and the central nervous system (CNS) - and by intrinsic nerves within the gastric wall [for review see (Hasler 1999)]. The vagus and splanchnic nerves, which contain efferent fibers that modulate motility, and afferent fibers that relay sensory information via the vagus nerves, and the enteric and autonomic nervous systems from the gut to the CNS, are responsible for the extrinsic innervation of the stomach. The stimulation of gastric smooth muscle motility and gastric secretion is primarily mediated via the activation of nicotinic receptors on efferent vagal cholinergic neurons, whereas inhibition of these functions is mediated via vasoactive intestinal peptide (VIP) and nitric oxide (NO) (Hasler 1999). Afferent sensory fibers respond to mechanical (stroking) as well as chemical (eg; hydrochloric acid) stimulation (Hasler 1999). Gastric distension, active gastric contractions and exposure to high and low pH or temperature all activate mechanoreceptors in the gastric smooth muscle wall (Hasler 1999). There is evidence that those afferent fibers which signal gastric distension, mediate sensations of fullness after a meal via CCK (Feinle et al 1998, Feinle et al 1997, McLaughlin et al 1999).

The myenteric plexus provides the major intrinsic innervation, to the gastric smooth muscle, regulating physiologic motor patterns in the absence of extrinsic input (Hasler

1999). Excitatory myenteric neurons, which contain acetylcholine or tachykinins (Substance P and neurokinin A) and possibly serotonin, project directly into the circular smooth muscle layer of the stomach wall. Inhibitory myenteric neurons, which contain VIP and NO, project in an aboral direction are thought to be involved in the relaxation phase of gastrointestinal motility. Inhibitory roles have also been proposed for endogenous opioids and ATP (Hasler 1999).

3.4 ROLE OF GASTRIC DISTENSION IN SATIATION

Sensory signals induced by gastric distension as a result of the presence of food in the stomach contribute, in part, to the initial sensations of fullness during a meal, and are mediated by mechanoreceptors situated within the stomach wall via vagal afferent fibers. These distension signals are the result of a temporary reduction in gastric wall "tension" ie. gastric accomodation (see section 3.3.1 and 3.3.4), rather than an "elongation" of the stomach (like that of a balloon as it stretches) caused by the arrival of food into the proximal stomach (Azpiroz & Malagelada 1985, Lepionka et al 1997). Distension of the stomach using a balloon inhibits feeding in sham fed dogs with oesophageal fistulae (Janowitz & Grossman 1949). In young obese and nonobese humans, non-nutrient gastric distension, induced by a latex balloon, acutely reduces food intake by up to 30%. Barostat techniques were not, however, used in those studies and so gastric pressure could not be controlled and therefore increased during these distensions, cancelling out the consequences of gastric accomodation (Geliebter 1988). Chronic gastric distension leads to short-term weight loss in non-dieting rodents (Northway et al 1992) and humans (Geliebter et al 1990). The site of gastric distension may be relevant (Hveem et al 1996, Jones et al 1997a). A recent study by Jones et al (Jones et al 1997a), suggests that the extent of filling and distension of the distal stomach (antrum) may be more closely related to fullness and satiation than distension of the proximal stomach. After ingestion of a 350 ml 20% glucose drink, post-prandial sensations of fullness were related to both antral area as measured by ultrasound, and the content of the distal stomach measured scintigraphically, but not related to the content of the proximal or total stomach (Jones et al 1997). This relationship between postprandial fullness and antral area has been replicated in a more recent study in young healthy men (Santangelo et al 1998).

Recent evidence suggests that the effects of gastric distension per se on food intake may be only important in inhibiting food intake when relatively large volumes of food are

present in the stomach. Studies by Geliebter (1988) suggest that a volume of 400 ml or more is required to produce a significant suppression of food intake in both lean and obese subjects. Furthermore, Houpt et al (1994) showed in pigs that during spontaneous feeding and drinking, intragastric pressure is maintained at a relatively constant level and only increases above this level when the animals eat large volumes of food after 16-18 hr of food deprivation. Recent studies have suggested that the effects of gastric distension on satiation are modulated by chemical (and possibly mechanical) stimulation of the small intestine. This is illustrated by Feinle et al (1996), who reported that a pleasant sensation of postprandial fullness is induced when a lipid emulsion is infused into the small intestine at the same time as the proximal stomach is distended by a balloon, whereas discomfort results from the same gastric distension in the fasting state. The effects of small intestinal nutrients on the perception of gastric distension are thought to be mediated, at least in part, by the release of gut peptides including CCK (see section 3.6.1). For example, Melton et al (1992) showed that intravenous infusion of CCK enhanced the satiating effects of gastric balloon distension in young, healthy women. Stimulation of small intestinal receptors by nutrients appears to be the major inhibitory feedback signals which induces satiation at the smaller volumes typically consumed in an individual meal, while distension comes into play at larger volumes, mainly as a safety brake to prevent harmful over-distension (Phillips & Powley 1996).

There is evidence that sensitivity to proximal gastric distension is enhanced in a number of conditions, including type 1 diabetes (Samsom et al 1995, Rayner et al 2000) and functional dyspepsia (Mearin et al 1999). Isobaric balloon distension induces more gastrointestinal sensations in these patients than in healthy young subjects (Samsom et al 1995, Mearin et al 1999). There is some evidence that ageing may be associated with an alteration in gastric sensory function. This is discussed in Chapter 4.3.1 and is investigated in the study described in Chapter 11.

The rate of gastric emptying plays an important role in the regulation of appetite since emptying rate is potentially a major factor in determining the extent of gastric distension, the duration of small intestinal nutrient exposure and the extent to which the small intestine is exposed to nutrients. As small intestinal feedback plays a primary role in the regulation of gastric motor function and, therefore, the rate of gastric emptying this is discussed in more detail in section 3.5.2.

3.5 SMALL INTESTINE

The presence of nutrients in the small intestine has both direct and indirect effects on appetite, although this distinction is somewhat artificial since the small intestinal mechanisms which trigger satiation and other sensations and also slow gastric emptying are comparable.

3.5.1 *Direct effects of nutrients on satiation*

The presence of nutrients in the small intestine suppresses food intake in both animals (Meyer et al 1998a-c) and humans (Castiglione et al 1998, Chapman et al 1999, Feinle et al 1998, Lavin et al 1997). In humans, direct infusion of nutrients into the small intestine also decreases sensations of hunger and increases sensations of fullness in young lean (Castiglione et al 1998, Chapman et al 1999, Lavin et al 1997) and obese (Chapman et al 1999) adults. As discussed, the perception of gastric distension is enhanced during small intestinal nutrient infusion (Feinle et al 1996). It has been postulated that the return of hunger after a meal is associated directly with a decrease in small intestinal exposure to nutrients (Sepple & Read 1989). The pivotal role of small intestinal chemoreceptor mechanisms in short-term appetite regulation was demonstrated by Welch et al (1985), who reported in normal subjects that the infusion of fat (50% corn oil and 3% albumin) at a rate of 1.2 ml/min for a total of 75 min (1547 kJ) into the ileum reduced hunger, increased fullness and reduced subsequent food intake by ~30% at an ad libitum meal. In contrast, no effects on food intake were observed when the same lipid emulsion was infused intravenously (Welch et al 1985) (Figure 3.2). These observations were reinforced by Lavin et al (1997), who demonstrated in healthy volunteers that intraduodenal glucose infusion suppressed hunger, and increased both fullness and satiety, whereas intravenous glucose infusion (leading to identical blood glucose concentrations) had no effect on appetite.

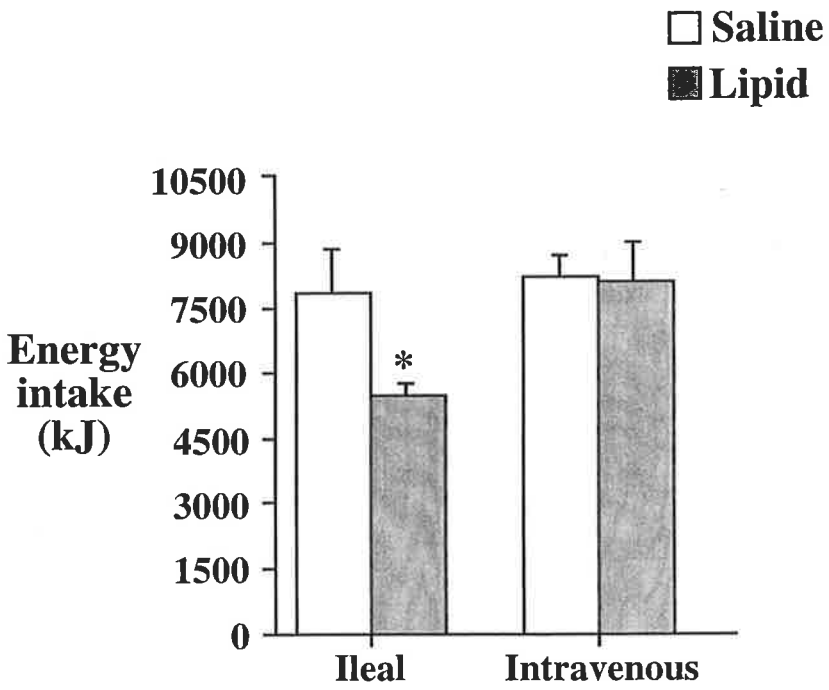

Figure 3.2: Effect of ileal (1.2 ml/min for 75 min) or intravenous (1.7 ml/min for 105 min) infusion of lipid (~1547 kJ) on total energy intake at a standard meal in 17 young healthy subjects; *P<0.05, (Welch et al 1985)

The suppressive effects of small intestinal nutrients are thought to reflect, in part, to the direct interaction of nutrients with specific chemoreceptors located on the intestinal lumen, and are mediated by both humoral (see section 3.6 for more detailed discussion of individual gut peptides) and neural mechanisms. The importance of humoral mediation of intestinal nutrient satiety was demonstrated in a study by Lavin et al (1998), who established that the suppressive effect of intraduodenal glucose infusion at a rate of 13.4 kJ/min was abolished when gastrointestinal hormone release was inhibited by simultaneous intravenous infusion of the somatostatin analogue, octreotide. Furthermore, Feinle et al (1996) showed that the "meal-like" sensations perceived during simultaneous gastric distension and small intestinal lipid infusion were reduced during intravenous infusion of the CCK-A receptor antagonist, loxiglumide. Small intestinal nutrients are also rapidly detected and signalled to the brain via visceral afferents. In support of this neural mediation, studies in animals have shown that the suppressive effects of intestinal carbohydrate and fat are abolished following peripheral administration of capsaicin, a neurotoxin that produces partial visceral deafferentation (Lucas & Sclafani 1996).

3.5.1.1 Region of intestine

The effects of small intestinal nutrients on appetite are dependent on the region and length of the small intestine exposed to nutrients as well as the duration of small intestinal nutrient exposure, and these factors may vary between animals and humans. In considering regional effects, Meyer et al (1998a) showed in the rat that infusions of nutrients into the duodenum, midgut and also the colon had similar satiating properties. Studies in humans, however, have shown that while overall food intake was not significantly different between infusions, infusion of fat into the jejunum results in a greater decrease in sensations of hunger and rate of food ingestion than infusion of an identical amount of fat into the terminal ileum (Welch et al 1988). Other studies have shown that infusion of nutrients into the duodenum also decreases sensations of hunger and increases sensations of fullness and satiety (Andrews et al 1998a, Lavin et al 1997, Chapman et al 1999). These findings suggest that hunger and satiety sensations induced by nutrient stimulation of the proximal gut may be mediated by different mechanisms to those induced by stimulation of the distal gut (Sepple & Read 1989).

3.5.1.2 Length of intestine

The length of intestine exposed to nutrients is also important in the small intestinal inhibitory feedback effects on appetite. For example, Meyer et al (1998c) reported that

in the rat the suppression of food intake by infusion of dodecanoate into the proximal gut was 3 fold higher when maltose was simultaneously infused into the distal small bowel than without maltose infusion. In rats, both the magnitude and duration of satiation of oral corn-oil preloads are correlated with the length and intensity of intestinal contact with lipolytic products (Meyer et al 1998b). Furthermore, when intestinal contact of oleate was limited to 35 cm of jejunum in a 'Thiry Vella Loop', its satiating potency was nearly abolished (Meyer et al 1998a). In humans, Castiglione et al (Castiglione et al 1998) demonstrated that intraduodenal infusions of lipid (20% Intralipid) for 15 min, 45 min and 90 min dose-dependently decreased energy intake by 126 kJ, 376 kJ and 753 kJ, respectively, compared to the control (2179 ± 335 kJ) in young healthy men (Figure 3.3). It was postulated that these observations reflected greater recruitment of small intestinal receptors (Castiglione et al 1998). The observation that the 15 min lipid infusion did not significantly reduce energy intake suggests that the area of intestine, the number of receptors recruited, the amount of energy present and/ or the duration that the intestine is exposed is critical for small-intestinal nutrient mediated inhibition of feeding. These factors are influenced by the rate of gastric emptying (see section 3.5.2). In considering the effects of duration of exposure of the small intestine to nutrients on satiation there appears to a threshold energy load above which small intestinal nutrients suppress food intake. In dogs, small intestinal infusions of oleate (fat) and dextrose polymer (carbohydrate) significantly reduced sham feeding at a energy concentration of 1.05 kJ/min for two hours, whereas peptone (protein solution) infusion did not significantly reduce sham feeding until a solution with 2.09 kJ/min was administered (Geoghegan et al 1997).

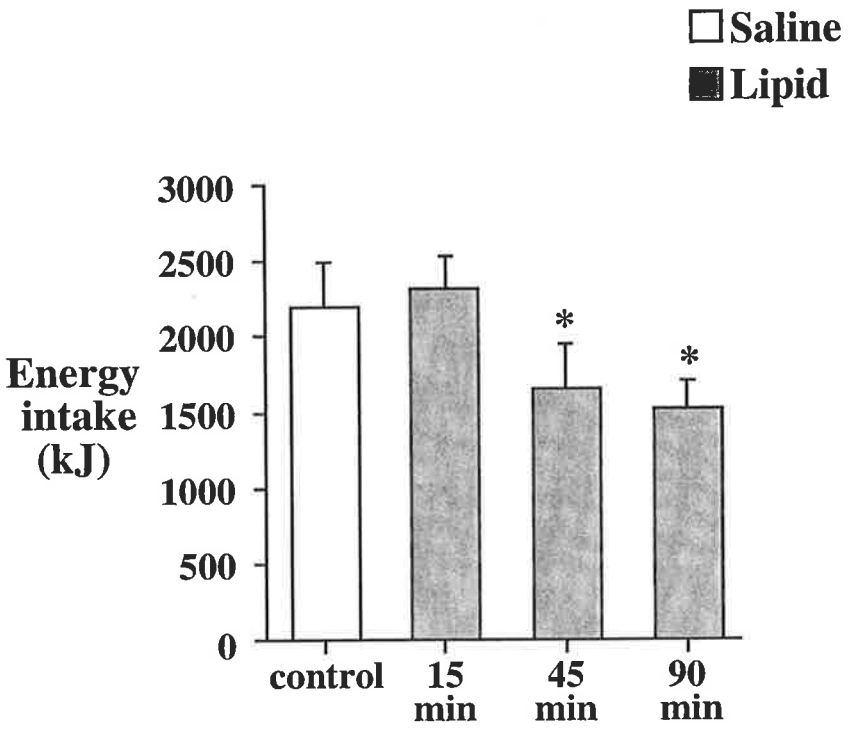

Figure 3.3: Effect of intraduodenal saline (control) or lipid infusions (20% Intralipid at 1.1 ml/min) for 15 min (126 kJ), 45 min (376 kJ) and 90 min (753 kJ) infusions on total energy intake at a standard meal in 8 young healthy men, (Castiglione et al 1998); *P<0.05 vs control.

3.5.1.4 Effects of different macronutrients

A number of studies have shown that different macronutrients have varying satiating potencies when administered into the small intestine. Intraduodenal infusion of fat is a more potent suppressor of both appetite (Andrews et al 1998a) and food intake (Chapman et al 1999) than isoenergetic infusions of glucose in young lean (Andrews et al 1998a, Chapman et al 1999) and obese (Chapman et al 1999) subjects. This contrasts with several studies comparing the satiating effects between macronutrients that have been administered either orally or intragastrically. Some studies have demonstrated that oral carbohydrate is more satiating than oral fat ingestion (Blundell et al 1995, Shah & Garg 1996, Holt et al 1999)[for review see (Rolls 1995a)]. For example, Blundell et al (Blundell et al 1995) showed that a high carbohydrate breakfast (1.52 MJ) suppressed hunger and energy intake 90 min later but had no effect on intake at a meal at 270 min, whereas an equi-energetic high fat breakfast had no effect on appetite or energy intake at either time-point. Other studies, however, have found no difference in the satiating effects of oral carbohydrate and fat on appetite and energy intake in healthy young subjects (Poppitt et al 1998, Romon et al 1999). Poppitt et al (1998) also reported that energy intake at a meal 90 min after a high-protein oral preload was significantly less than after isoenergetic high-fat and high-carbohydrate preloads in 12 lean women. This was consistent with the findings by Cecil et al (1998), who demonstrated that the amount of food consumed at a meal 90 min after isoenergetic intragastric infusions of fat and glucose was similar in young healthy subjects. In contrast, Shide et al (1995a) reported that rapid (15 min) intragastric infusions of fat and carbohydrate and slow (3.5 hr) infusions of fat suppressed energy intake at a meal 30 min after the end of the infusions. The discrepancies between the results of these studies may relate to differences in the mode of delivery of the nutrients, ie. oral vs intragastric vs intraduodenal, differences in the taste or preference of orally administered nutrients, or variations in the rate of gastric emptying of oral and intragastric macronutrients. It may also be that any differences between the effects of fat and carbohydrate on appetite are time dependent, so that the time interval between intake and evaluation of appetite, which has varied somewhat between 15 min (Shide et al 1995a) and 3.5 hr (Shide et al 1995a) in previous studies, is critical. Moreover, differences in the physical nature of the preloads administered (eg. whether incorporated in a meal (Poppitt et al 1998) or delivered as a liquid (Shide et al 1995) may have been responsible for these inconsistent results. The relative suppressive effects of fat and glucose when delivered intraduodenally, thus removing the effects of oral nutrients exposure and variations in the rate of gastric emptying are investigated further in Chapter 9.

There is evidence that subtle modifications in the chemical structure of carbohydrates, fats and proteins may profoundly modify their effects on satiation (Meyer et al 1998a), but this will not be considered in this thesis as only one form of fat (intralipid) and one form of carbohydrate (glucose) were administered in these studies.

3.5.2 Indirect effects of nutrients on satiation

Small intestinal nutrient feedback plays a major role in regulating the rate of gastric emptying. Infusions of various nutrients, including fat (Heddle et al 1988a), carbohydrate (Heddle et al 1988b, Brener et al 1983), protein (Shi et al 1997) and mixed nutrient meals (Hunt 1980) into the small intestine all retard gastric emptying. For example, in young adults intraduodenal triglyceride infusion when given at a rate of about 16.7 kJ/min inhibits gastric emptying which resumes when the lipid infusion is stopped (Heddle et al 1988a). Slowing gastric emptying may influence appetite by both prolonging gastric distension and increasing the duration of contact of nutrients with small intestinal receptors. (French & Read 1994). Consistent with this is the observation that sensations of fullness persist for longer after ingestion of fatty soup which contains non-nutrient guar gum, an agent which slows gastric emptying, than when the soup not containing guar is ingested (French & Read 1994).

The motor mechanisms by which nutrients in the small intestine or mechanical stimulation by balloon distension slow gastric emptying are multiple. They include an increase in both basal pyloric tone and the frequency of phasic pyloric pressures (IPPW's) (Heddle et al 1988a), suppression of antral pressure waves (Heddle et al 1989), a decrease in fundic tone (ie. gastric accomodation) (Heddle et al 1989), increased duodenal motility, ie random isolated or clusters (2-4 cycle/min) of non-propagating pressure waves (Rao et al 1996), and the generation of pressure waves of high amplitude that are retroperistaltic in nature (Castedal et al 1998). The stimulation of basal pyloric tone and frequency of IPPW's by intraduodenal nutrients (Heddle et al 1989) has been shown to prevent transpyloric flow (Tougas et al 1992), and is likely to be the most important of the mechanisms referred to above. Gastric emptying of nutrients is slower on average in healthy elderly than healthy young adults (see Chapter 4.3.2), and this may, potentially result from increased small intestinal nutrient-mediated feedback in the elderly (Clarkston et al 1997). The effects of ageing on the antropyloroduodenal motor response to intraduodenal lipid infusion are described in Appendix A.

Modifications in the gastric pacemaker rhythm may be responsible for the alteration in gastric motor function and slowing of gastric emptying that occur during intraduodenal nutrient stimulation. For example, intraduodenal lipid infusion reduces the frequency of the gastric pacemaker in rats (Melone & Mei 1991). In young, healthy humans intraduodenal infusion of glucose is associated with increase in power or amplitude of the gastric myoelectrical signal, as measured using electrogastrography (EGG) (see Chapter 7.2). This may reflect the stimulatory effect of intraduodenal nutrients on the intensity of pyloric contractions (Verhagen et al 1998). It is not known whether the effects of intraduodenal nutrients on gastric myoelectrical activity vary between nutrient classes. Little is known about the effects of ageing on gastric myoelectrical activity (see Chapter 4.4.2). The effect of intraduodenal lipid and glucose on gastric electrical activity measured by EGG in young and older subjects is evaluated in Chapter 9.

The alterations in gastropyloroduodenal motor activity which retard gastric emptying are mediated by both neural (ie vagal afferents) and hormonal mechanisms (McLaughlin et al 1999, Raybould et al 1994, Meyer et al 1998a, Meyer et al 1998b). Blockade of vagal and spinal sensory neurons by systemic capsaicin administration attenuates the slowing of gastric emptying produced by small intestinal nutrient infusion in animals (Raybould 1991, Holzer et al 1994). The slowing of gastric emptying induced by intestinal protein and lipid is attenuated by administration of the CCK antagonists L364,718 (Raybould 1991) and devazepide (Holzer et al 1994), respectively. Administration of the GLP-1 antagonist, exendin, enhances gastric emptying of glucose in rats (Imeryuz et al 1997). Other hormones thought to slow gastric emptying include secretin (Raybould et al 1994, Naslund et al 1999b) and peptide YY (PYY)(see section 3.6.4) (Savage et al 1987).

Consistent with the effects on satiation, the extent of slowing of gastric emptying by small intestinal nutrient exposure is dependent on both the region and length of small intestine which is exposed to nutrient (Lin et al 1995a, Lin et al 1995b). For example, Cooke (1977) reported that that glucose, highly osmotic substances (eg: NaCl) and fat all delayed gastric emptying in dogs when perfused into the proximal jejunum, but had no effect when confined to the proximal duodenum. Lin et al (Lin et al 1995a), showed that when the exposure of the canine intestine to a 1.0 M glucose solution was confined to the proximal 65 cm, gastric emptying was inhibited by only 50-60% of the maximal inhibition observed when the more distal 85 cm was also exposed. In a similar study the same group demonstrated that sodium oleate was more potent at inhibiting gastric

emptying when infused into the proximal than in the distal small bowel (Lin et al 1995b).

There is also evidence that different macronutrients have distinct effects on gastropyloroduodenal motor function. For example, Kumar et al (1993) reported that among isoenergetic, iso-osmolar infusions of oleic acid (fat), maltose (carbohydrate) and casein hydrolysate (protein), oleic acid was the most potent in suppressing antral motor function and slowing gastric emptying in dogs. Moreover, Azpiroz & Malagelada (1985) showed that intestinal lipid caused greater gastric relaxation than an equi-energetic load of carbohydrate in dogs. Similarly, in humans, fat appears to be more potent than both carbohydrate and protein in suppressing antral pressure waves, inducing fundic relaxation, increasing basal pyloric tone and frequency of IPPW's, and slowing gastric emptying (Heddle et al 1988a, Heddle et al 1988b, Feinle et al 1997). A recent study by Andrews et al (1998a) confirmed that intraduodenal infusion of lipid increased the frequency of isolated pyloric pressure waves to a greater extent than an isoenergetic intraduodenal glucose infusion. The stimulation of basal pyloric tone, however, was not significantly different during the lipid and glucose infusions, indicating that phasic and tonic pyloric motility is regulated by different pathways (Andrews et al 1998a). The effects of different macronutrients on gastric electrical activity are not known and this issue is investigated in Chapter 9. The more potent effects of intraduodenal fat on pyloric motility are concordant with its powerful suppressive effects on appetite (Andrews et al 1998a). Within a macronutrient class, the chemical structure of specific macronutrients may also affect the extent to which they slow gastric emptying (Meyer 1998a, Carney et al 1994). Relationships between the change in pyloric motility and subjective appetite ratings in response to intraduodenal glucose and lipid infusion in young and older subjects are addressed in Chapter

8.3.5.2.1 Effects of previous patterns of nutrient intake on gastric emptying and appetite

Changes in diet can modify the rate of gastric emptying (Corvilain et al 1995, Cunningham et al 1991b, Horowitz et al 1996, Shi et al 1997). Gastric emptying appears to be related to the amount of energy consumed. Reduction in energy intake is associated with slowing of the rate of gastric emptying in animals (Robinson et al 1988), normal weight and obese young subjects (Corvilain et al 1995) and patients with anorexia nervosa or bulimia nervosa (Stacher et al 1986, Rigaud et al 1988, Robinson et al 1988a). There is evidence that once normal dietary intake is restored in patients

with anorexia (Rigaud et al 1988, Robinson et al 1988a) as well as in underweight patients with bulimia nervosa (Robinson et al 1988a), gastric emptying, improves. Although appetite was not assessed in these studies, there is anecdotal evidence that symptoms of early satiation and nausea in patients with anorexia nervosa are diminished once adequate intake is maintained. Gastric emptying is more rapid than normal in obese subjects on a high energy diet (Wright et al 1983). When obese subjects are placed on a energy-restricted diet the rate of gastric emptying is slowed (Corvilain et al 1995). In rats, supplementing the diet with protein, accelerates gastric emptying of meals containing protein (Shi et al 1997). Similarly, in normal healthy adult humans dietary glucose (Cunningham et al 1991c, Horowitz et al 1996) and fat (Cunningham et al 1991b) supplementation increase the rate of emptying of glucose and high fat test meals, respectively.

Andrews et al (1998a) also recently reported that the increase in basal pyloric tone observed in response to intraduodenal glucose infusion was attenuated after dietary glucose supplementation (400 g (3346 kJ) daily for 7 days) in young healthy men. They also reported that after dietary glucose supplementation the effects of intraduodenal lipid on subjective ratings of desire to eat and fullness were diminished compared to before glucose supplementation (Andrews et al 1998a). These findings are consistent with those by French et al (1995) who reported that when exposed to a high-fat diet (19.17 MJ/d; 58% energy from fat) for 2 weeks, ad libitum energy intake (kJ/day) was greater than during a standard diet (10.25 ± 0.49 MJ vs 9.59 ± 0.62 MJ; P= 0.05), with corresponding trends of increasing feelings of hunger and declining fullness over the high-fat diet study period in young healthy subjects (French et al 1995). Furthermore, following the high-fat diet compared to the standard diet, subjects tended to eat more energy at a standard meal (6919 ± 615 vs 6405 ± 540; P= 0.10) and had a greater plasma CCK response to the meal (1285 ± 153 pM/min vs 897 ± 78 pM/min; P= 0.10), suggesting that the increase in food intake may be related to a down-regulation in putative CCK receptors responsible for food intake (French et al 1995) (see section 3.6.1). The potential mechanisms for the adaptation of gastric motility in response to dietary modification have not been fully elucidated, but may include a down-regulation of the number of small intestinal nutrient receptors, a decrease in receptor sensitivity, or a reduction in gut hormone secretion (Shi et al 1997), a reduction in the length of intestine exposed to nutrient, or a change in the central response to a given satiety signal are all possible mechanisms (Andrews et al 1998a). The effects of modifications in dietary composition on gastric emptying and

appetite in the elderly have not been evaluated. If nutrient supplementation is associated with acceleration of gastric emptying, this could favour an increase in food intake. This issue is investigated in the study described in Chapter 13.

3.5.2.2 Patterns of gastric emptying

Gastric emptying is predominantly pulsatile rather than continuous, with both antegrade and retrograde flow between the stomach and duodenum (Malbert & Mathis 1994). In general, gastric emptying of nutrients involves an initial lag phase which is short (< 5 min) for nutrient liquids (Brener et al 1983), and much more prolonged (~20-60 min) for solids (Collins et al 1983). Gastric emptying, particularly of low-nutrient liquids, is influenced by intragastric volume and posture (Horowitz et al 1993), whereas emptying of nutrient liquids and solids is more dependent on the energy content (Hunt 1975, Maerz et al 1994, Vist & Maughan 1995), so that the rate of delivery of nutrients to the small intestine is optimal for efficient digestion and absorption. (Figure 3.4 illustrates the emptying of orally ingested solid and liquid nutrient meal). The lag phase is due to (i) antral grinding of food into smaller particles and (ii) intragastric distribution from the proximal to distal stomach. The duration of the lag phase for solids is dependent on the size of the ingested particles (Urbain et al 1989, Siegel et al 1989). For example, Urbain et al (1989) showed that the lag phase was shorter after ingestion of a homogenised egg meal when compared to isoenergetic test meals of 2.5 mm or 5.0 mm cubed egg particles. The lag phase is then followed by a slower rate of gastric emptying which, at least for the majority of the emptying phase, approximates a linear pattern (Collins et al 1983) (Figure 3.4).

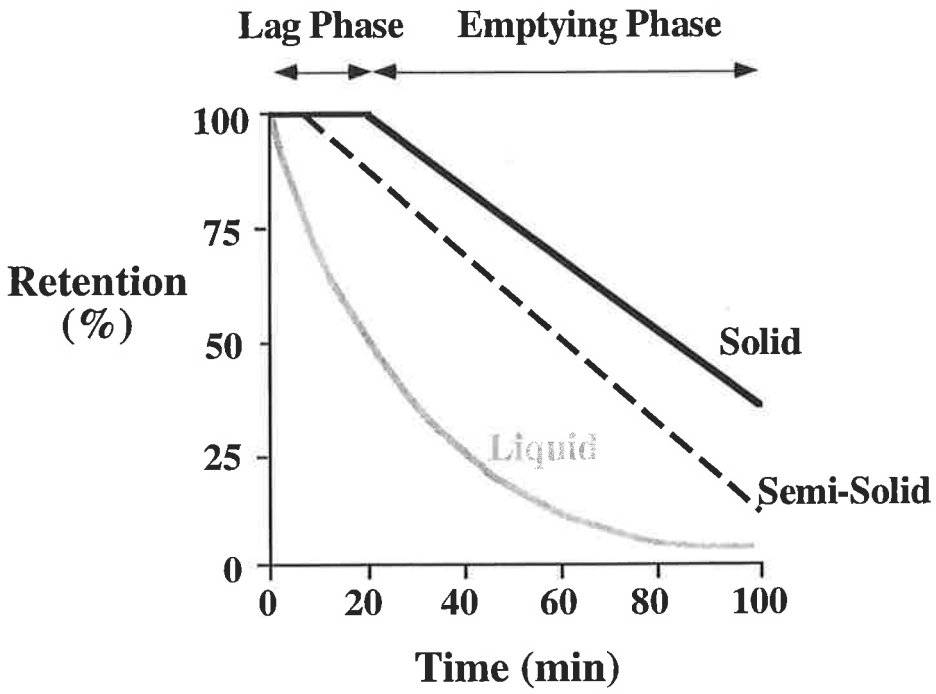

Figure 3.4: Gastric emptying curves for solid (pancake), semi-solid (porridge) and liquid (10% dextrose) represent percent retention in the total stomach over time. The overall pattern of gastric emptying of both solids and semi-solids is linear, following a lag phase which is longer for solids. In contrast, emptying of the low nutrient liquid is mono-exponential with minimal lag phase.

Altering the physical and chemical properties of a meal alters both the pattern and rate of gastric emptying. For example, a low pH and temperature, as well as high osmolality, viscosity, fibre content and energy concentration slow gastric emptying (Hunt 1975, McHugh & Moran 1979, Brener et al 1983, Fisher et al 1987, Lin et al 1990, Maerz et al 1994, Vist & Maughan 1995, Sun et al 1995). In addition, ingestion of a large volume of food increases the rate of gastric emptying (Hunt 1975). Doran et al (1998) recently demonstrated in young healthy subjects that increasing the volume of a solid meal (from 217 g to 650g) decreases the lag phase and increases the post-lag emptying rate. Increasing the energy density of a meal also increases the rate of gastric emptying. For example, Calbet et al (1997) reported that among four different 600 ml test meals (glucose, 0.42 kJ/ml, pea and whey peptide hydrolysates, both 0.84 kJ/ml, and milk protein, 2.93 kJ/ml) the glucose solution was emptied the fastest and the milk protein emptied the slowest.

Changes in the pattern and rate of gastric emptying as a result of modifications in the physical and chemical composition of a meal may affect satiation. Consistent with this is that addition of fibre (Bolton et al 1981, Hulshof et al 1993) or frying (Benini et al 1994) of foods prolongs subjective sensations of satiety and fullness, concordant with the slowing of gastric emptying.

The nature of the energy incorporated in a meal may also influence the rate of gastric emptying. The emptying of fat is different to that of other nutrients. Fat empties in general much like solid food ie a prolonged initial lag phase followed by a slow linear emptying phase (Cortot et al 1982). When fat is consumed as a solid-liquid (Jian et al 1982, Houghton et al 1990, Edelbroek et al 1992b), oil-soup (Edelbroek et al 1992b, Horowitz et al 1993, Carney et al 1995) or oil-water (Boulby et al 1997) meal its emptying rate is much slower than the rest of the meal, including the solid component (Jian et al 1982, Houghton et al 1990, Edelbroek et al 1992b). An intragastric layering of fat above the rest of the meal is observed in these studies which may, in part, account for the slower emptying rate of fat (Jian et al 1982, Houghton et al 1990, Edelbroek et al 1992b, Horowitz et al 1993, Carney et al 1995, Boulby et al 1997).

Several studies have attempted to compare the gastric emptying rates of fat and carbohydrate. For example, Sidery et al (1994) showed that after oral consumption a high-fat meal emptied from the stomach at a slower rate than an isoenergetic high-carbohydrate meal in humans. Other factors, however, such as total volume (energy

density) and osmolality were not controlled for in that study. In this study, however, no attempt was made to selectively label any single component of the meals, and it was assumed that the labelled particles would be distributed throughout the stomach contents once ingested. It was, therefore, not known whether the fat in the high-fat meal emptied with the rest of the meal or whether it separated (ie layered on top of the rest of the meal) and emptied later. Conversely, Maerz et al (1994) showed in the rat that the rate of gastric emptying was constant when isoenergetic infusions of glucose, casien hydrolysate and intralipid were administered intragastrically. Consistent with this is the findings of Cecil et al (1998) who showed that isoenergetic intragastric infusions of fat and carbohydrate emptied at a similar rate. This may account for the similar satiating properties of the intragastric fat and glucose observed in that study. These findings suggest that similar to the affects on energy intake of fat and carbohydrate, differences in the mode of delivery of nutrients ie oral vs intragastric affect their rate of gastric emptying, although this concept remains to be confirmed.

3.5.2.3 The role of gastric emptying in blood glucose homeostasis

The change in blood glucose concentration following ingestion of a carbohydrate meal is potentially dependent on a number of factors including the rate of entry of nutrients into the small intestine, the rate of digestion and absorption of glucose, and the rate of insulin driven hepatic metabolism (Horowitz et al 1993). In both normal healthy subjects (Horowitz et al 1993) and patients with type 2 diabetes mellitus (Horowitz et al 1994, Jones et al 1996) the rate of gastric emptying exerts major effects on the postprandial glycaemic response by controlling the delivery of carbohydrate to the small intestinal epithelium (Moyses et al 1996, Schirra et al 1996a, Horowitz et al 1993). The observation that co-ingestion with food of substances that slow gastric emptying such as viscous polysaccharides by rats (Meyer et al 1986), and guar gum by humans (Blackburn et al 1984), results in lower postprandial blood glucose concentrations, further supports the importance of gastric emptying rate in glucose homeostasis. Moreover, intraduodenal infusion of lipid, which delays gastric emptying, also reduces postprandial blood glucose and insulin concentrations after ingestion of a semi-solid meal (Welch et al 1987).

The incretin hormones gastric inhibitory polypeptide (GIP) (Thor et al 1987)(see section 3.6.4) and glucagon-like peptide 1 (GLP-1)(see section 3.6.3) (Wettergren et al 1993, Schirra et al 1996b) as well as cholecystokinin (CCK) (Liddle et al 1988) (see section 3.6.1) may be involved in the regulation of gastric emptying of carbohydrates

and postprandial insulin release. Exogenous administration of CCK-8 slows gastric emptying and, as a result, reduces plasma glucose and insulin concentrations following an oral glucose load in both normal subjects (Liddle et al 1988) and patients with early onset type 2 diabetes mellitus (Phillips et al 1993). Ishii et al (1994) also showed that total insulin requirement (as measured by the insulin infusion rate during a 4-h feedback control with an artificial endocrine pancreas) during the first 120 min after a standard test-meal was less in type 1 diabetes mellitus patients with gastroparesis than in diabetic patients without gastroparesis. These results suggest that slowing the delivery of nutrients to the small intestine, attenuates the postprandial rise in blood glucose so that the insulin response or requirement is reduced.

Studies in humans have indicated that dietary modification that alter the rate of gastric emptying also affect the glycaemic response to an oral carbohydrate load (Cunningham et al 1991c, Horowitz et al 1996). Horowitz et al (1996) showed that dietary glucose supplementation (440g/day of glucose) for 4-7 days accelerated gastric emptying of both an oral glucose and fructose load (75 g dissolved in 350 ml water) (Figure 3.5). Plasma glucose (at 60 and 75 min) concentrations were lower and plasma insulin and GIP concentrations, higher after ingestion of the glucose load on the glucose-supplemented diet than the non-supplemented diet (Horowitz et al 1996)(Figure 3.6). These findings suggest that the acceleration of gastric emptying of glucose by dietary glucose supplementation induced a greater initial insulin response; the latter was probably at least, in part, a reflection of the higher GIP concentrations observed in this study. Ageing is associated with an increased prevalence of glucose intolerance and diabetes mellitus, and, at least in one report, an increased GIP and GLP-1 response to carbohydrate (Ranganath et al 1998). Accordingly, dietary glucose supplementation may potentially alter the glycaemic response to an oral glucose load in the elderly. This is a subject of further investigation in the study described in Chapter 13. The incretin response to intraduodenal glucose in the healthy elderly has also not been evaluated, this issue is examined in Chapter 9.

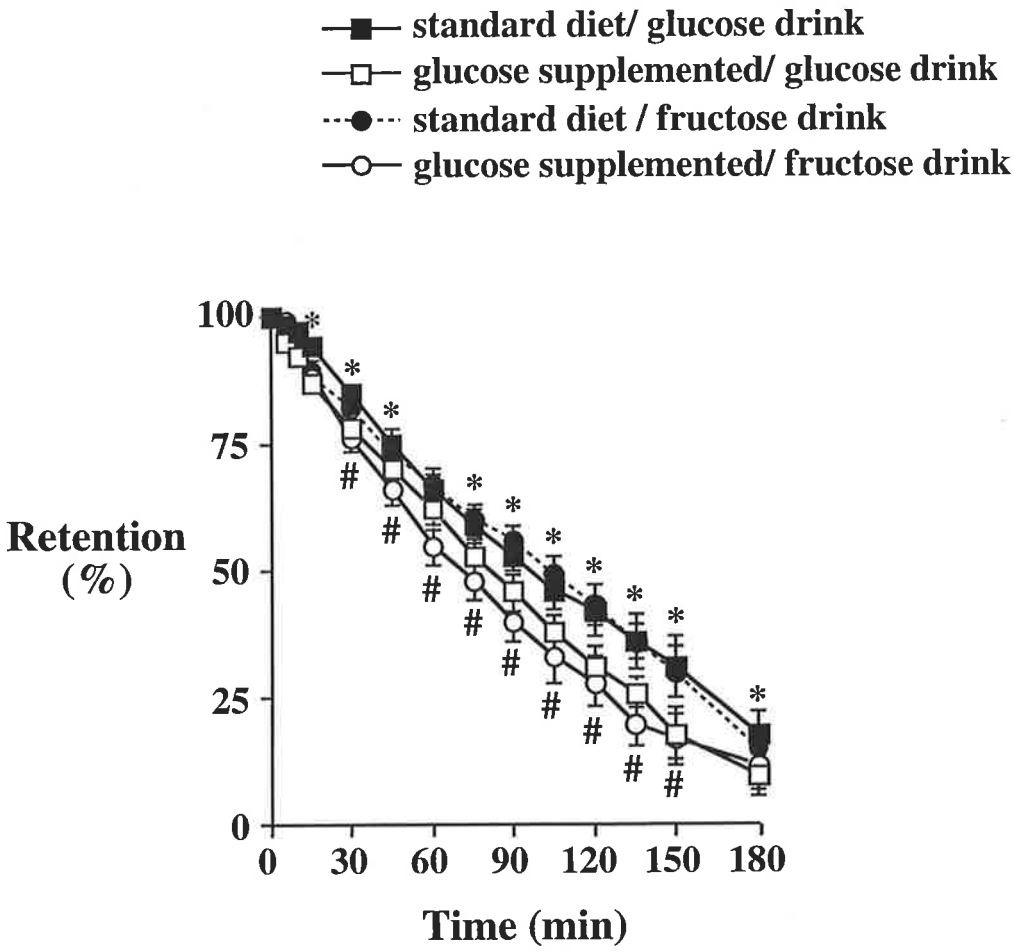

Figure 3.5: Gastric emptying of 350 ml water containing 75 g of glucose or fructose measured on a 'standard' diet and during dietary supplementation with 440 g glucose/day. Gastric emptying of glucose and fructose were both faster ($P< 0.05$) on the glucose-supplemented diet. *$P< 0.05$ glucose vs glucose; #$P< 0.05$ fructose vs fructose (Horowitz et al 1996; redrawn with permission from Diabetalogia).

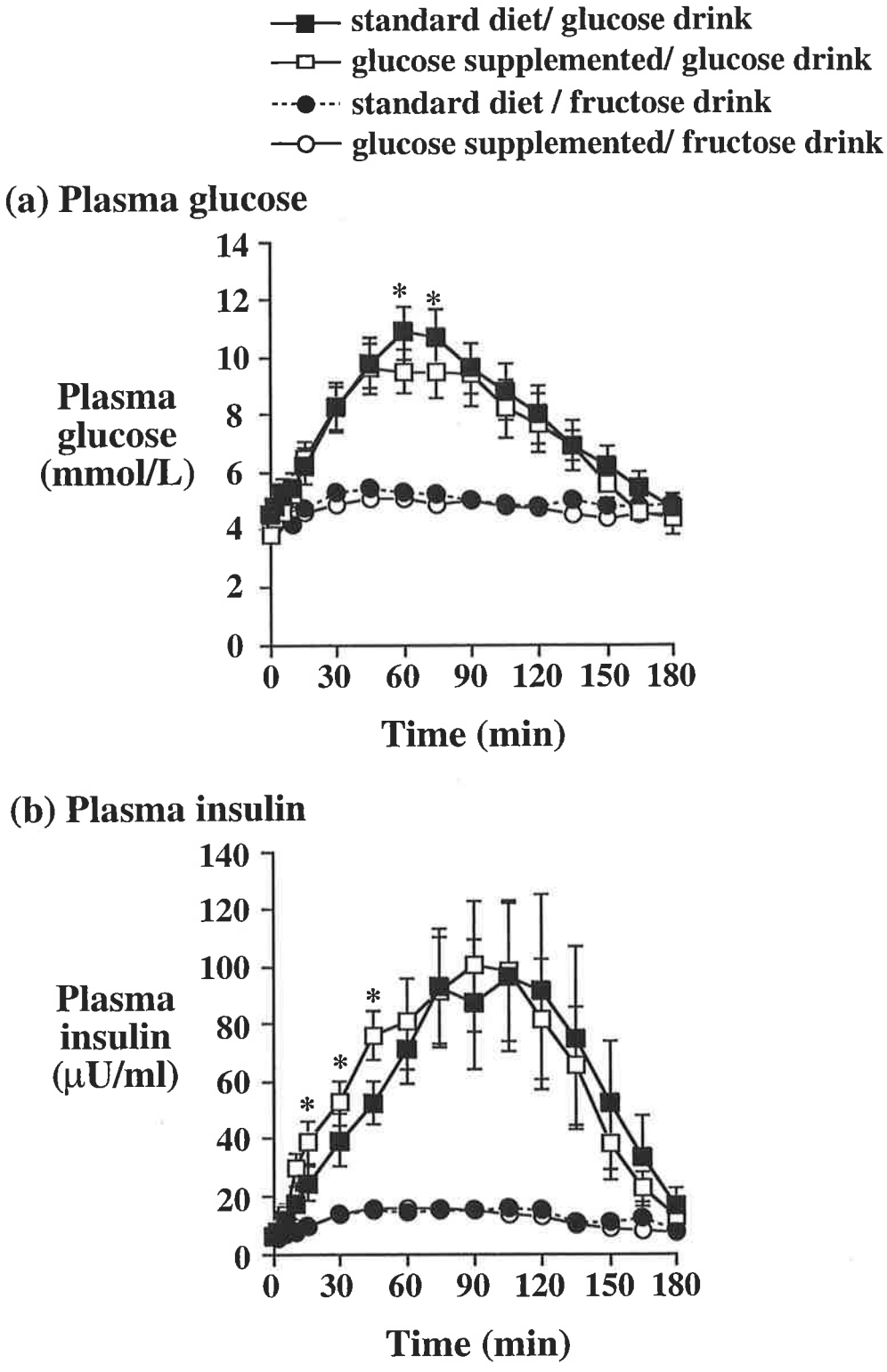

Figure 3.5: Plasma glucose (a) and insulin (b) concentrations after ingestion of 350 ml water containing 75 g of glucose or fructose measured on a 'standard' diet and during dietary supplementation with 440 g glucose/day. Gastric emptying of glucose and fructose were both faster ($P< 0.05$) on the glucose-supplemented diet. *$P< 0.05$ glucose vs glucose (Horowitz et al 1996; redrawn with permission from Diabetalogia).

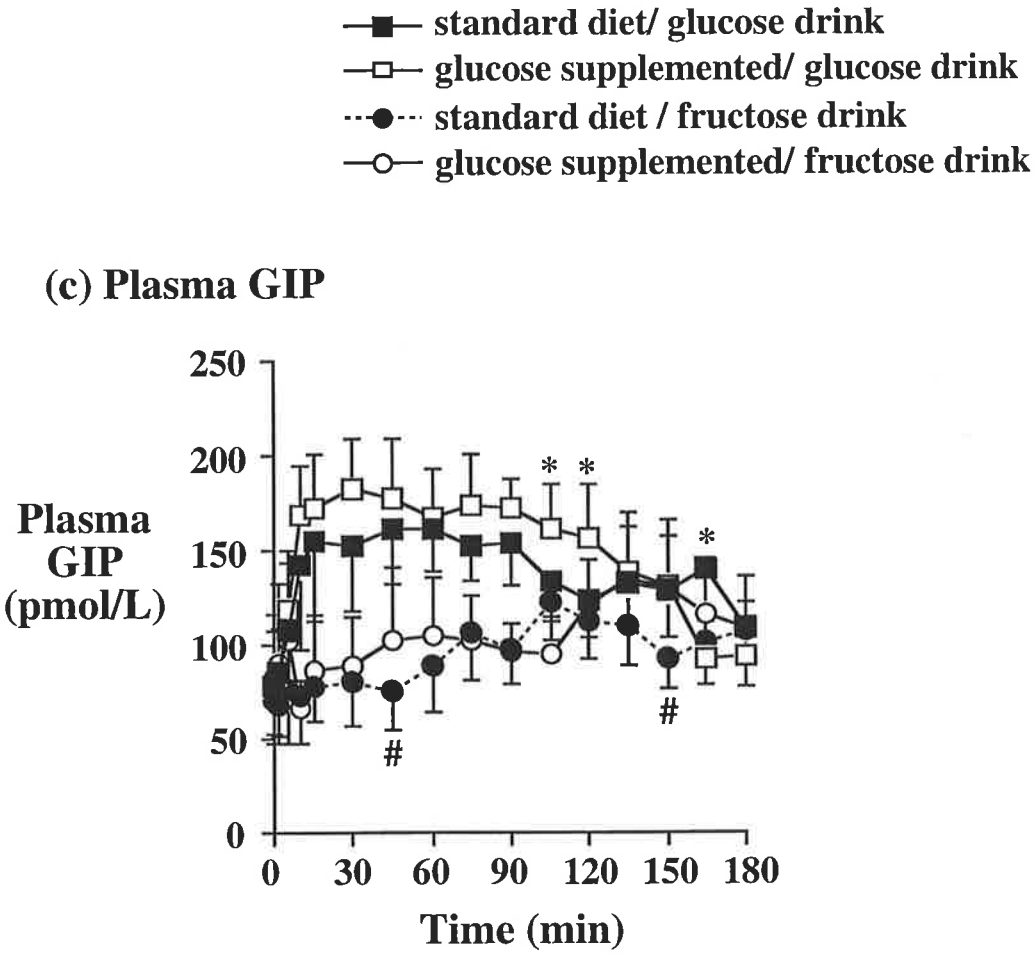

Figure 3.6 cont.: Plasma GIP concentrations after ingestion of 350 ml water containing 75 g of glucose or fructose measured on a 'standard' diet and during dietary supplementation with 440 g glucose/day. Gastric emptying of glucose and fructose were both faster ($P< 0.05$) on the glucose-supplemented diet. *$P< 0.05$ glucose vs glucose; #$P< 0.05$ fructose vs fructose (Horowitz et al 1996; redrawn with permission from Diabetalogia).

3.6 GUT PEPTIDES

Numerous studies provide evidence for neural and humoral mediation of the effects of small intestinal nutrient exposure on satiation (Morley 1990). A major emphasis of these studies has been on definition of the role of gastrointestinal hormones which are released during the passage of a meal through the gastrointestinal tract (Read et al 1994). Much of the evidence relating to the satiating effects of gut peptides has come from animal studies. The absence of specific antagonists for most of these hormones has been a major limitation to clarifying their physiological roles. It is, however, clear that gastrointestinal hormones have a major role in mediating the suppression of appetite resulting from the presence of nutrients in the small intestine. The role of cholecystokinin (CCK)(3.6.1), insulin (3.6.2) glucagon-like peptide-1 (GLP-1)(3.6.3), glucose-dependent insulinotrophic polypeptide (GIP)(3.6.4), peptide YY (PYY) (3.6.5), amylin (3.6.6) and ghrelin (3.6.7) in the regulation of satiation are discussed, as most of these peptides are the subject of further investigation in Chapters 8, 9, 10 and 12-14.

3.6.1 *Cholecystokinin (CCK)*

CCK is released from the lumen of the intestine in response to nutrients, particularly fat and protein, in the proximal gut (Peikin 1989). Fatty acids with a chain length greater than 12 carbons are a particularly potent stimulus for CCK release (McLaughlin et al 1999). CCK is also present in large quantities in the cortex, olfactory bulb, hypothalamus and the midbrain. Concentrations of CCK in the brain tend to vary in fed vs fasted conditions (Peikin 1989). CCK occurs in a number of forms, ie CCK-4, -8, -21, -33, -38, -54 and 58 (Pirke et al 1994), although not all are equipotent. The sulphated, N terminal octapeptide form of CCK (CCK-8) is thought to contain the most biological activity (Morley 1982), and is found in both the brain and gut. There are two types of CCK receptors: CCK_A, which is predominantly found in the gastrointestinal tract (Silver & Morley 1991), and CCK_B, which is distributed in the brain (Silver & Morley 1991). CCK causes contraction of the gallbladder and simultaneous relaxation of the sphincter of Oddi, thus releasing bile into the duodenum. It stimulates pancreatic enzyme secretion (Morley 1989) and slows gastric emptying (Moran & McHugh 1988, McHugh & Moran 1986, Liddle et al 1986, Kleibeuker et al 1988). CCK is under the inhibitory feedback control by bile acids and to a lesser extent pancreatic proteases (Green 1994, Schmidt et al 1991). Somatostatin is also thought to cause feedback regulation of CCK release (Jebbink et al 1992). There is also some suggestion that the gastrointestinal CCK 'receptors' responsible for feedback inhibition of CCK release

may be down-regulated following exposure to a high fat diet in humans. French et al (1995) reported that when exposed to a high fat diet (58% fat) compared to a standard diet, CCK release following a standard meal is increased by 30.2% in young healthy sibjects. There is little knowledge of whether exogenous CCK affects endogenous CCK release in humans. This issue is investigated further in Chapter 10.

There is evidence that CCK released in response to nutrients in the gut is involved in meal termination. Both central and peripheral administration of CCK decrease food intake in a range of animals, including rats and wolves [for review see (Morley 1989)], with most studies indicating that CCK is more potent when administered peripherally than centrally (Morley 1989). Peripheral administration of CCK also reduces food intake in lean and obese young humans by up to 49% (Kissileff et al 1981, Pi-Sunyer et al 1982, Stacher et al 1982, Shaw et al 1985, Muurahainen et al 1988, Lieverse et al 1994b). A few studies, however, have failed to show any suppressive effect of exogenous CCK on food intake (Muurahainen et al 1991, Lieverse et al 1995). Table 3.1 summarises the protocol and outcome of studies evaluating the effect of exogenous CCK infusion in young subjects. Some studies which showed a significant suppression of food intake during exogenous CCK infusion did not report plasma CCK concentrations. It is possible that plasma CCK concentrations may have been supraphysiological in these early studies that demonstrated suppression of food intake (Kissileff et al 1981, Stacher et al 1982, Pi-Sunyer et al 1982, Shaw et al 1985). A few studies, however, have shown that suppression of food intake occurs with doses sufficiently low to produce plasma CCK concentrations within the postprandial physiological range (Muurahainen et al 1991, Lieverse et al 1994b), ie 5-15 pmol/L (Schick et al 1991). Ingestion of a low energy preload appears to be necessary for such concentrations to inhibit food intake (Muurahainen et al 1991, Lieverse et al 1995), since no effect of infusion of CCK in such doses was observed without an adequate preload (Lieverse et al 1993) (see Figure 3.7). It appears, therefore, that a degree of gastric distension is required for physiological concentrations of CCK to suppress appetite in humans.

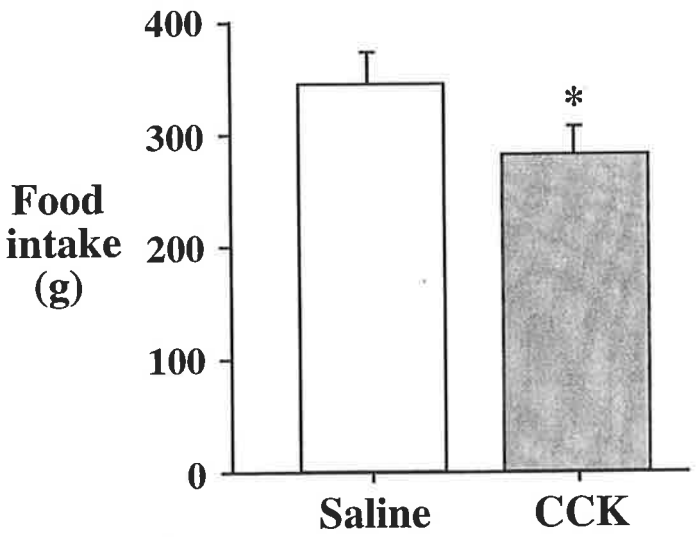

Figure 3.7: Mean food intake (g) at a test meal during iv CCK-33 (1 IDU/kg ideal weight/height for 195 min) and equi-volumetric saline (control) infusion, 15 min after a 'banana shake' preload (552 kJ), in 18 healthy (8 obese and 10 lean) young women; *P< 0.05, (Lieverse et al 1995).

Table 3.1: Studies evaluating the effects of exogenous CCK on food intake in humans

Author	Sample size/ gender	Pre-load	CCK/ Dosage	Outcome
Kissileff et al (1981)	12 lean males	appetiser (900 kJ)	(CCK-8) 4 ng/kg/ min for ~12 min.	19% ↓ in food (g) consumed after CCK vs Control ($P<0.05$).
Pi-Sunyer et al (1982)	8 obese males	no preload	(CCK-8) 4 ng/kg/ min for ~12 min.	13% ↓ in food (g) consumed after CCK vs Control ($P<0.01$).
Stacher et al (1982)	16 lean (8 male/ 8 female)	no preload	(CCK-8) 4.6 ng/kg/min (n=8) 9.2 ng/kg/min (n=8) for 15 min.	15% ↓ in energy (kJ) consumed after $CCK_{4.6}$ vs Control ($P<0.05$) 50% ↓ in energy (kJ) consumed after $CCK_{9.2}$ vs Control ($P<0.01$)
Shaw et al (1985)	14 (9 normal/ 5 vago-tomised men).	no preload	(CCK-8) 2 μg bolus + 5 μg infusion for 45 min.	11% ↓ in energy (kJ) consumed after CCK vs Control in normal subjects ($P<0.05$) Energy (kJ) consumed after CCK vs Control in vagotomised subjects (NS)
Muurahainen et al (1988)	12 lean males	500g soup (795 kJ)	(CCK-8) 2.25 mg/ml/min for 10 min	39% ↓ in energy (kJ) consumed after CCK vs Control ($P<0.01$)
Muurahainen et al (1991)	12 lean males	500g or 100g soup	(CCK-8) 225 ng/kg/min for 10 min	Food (g) consumed CCK vs Control following 100g preload (NS). 32% ↓ in food (g) consumed after CCK vs Control following 500g preload ($P<0.05$).

Table 3.1 cont.:

Author	Sample size/ gender	Pre-load	CCK/ Dosage	Outcome
Muurahainen et al (1991)	12 lean males	500g or 100g soup	(CCK-8) 225 ng/kg/min for 10 min	Food (g) consumed after CCK vs Control following 100g preload (NS). 32% ↓ in food (g) consumed after CCK vs Control following 500g preload P<0.05).
Lieverse et al (1993)	18 (9 lean/ 9 obese) females	no preload	(CCK-33) 1 IDU/kg/ ideal weight/height for 165 min	12% ↓ in food (g) consumed after CCK vs Control (NS).
Lieverse et al (1995)	18 (8 lean/ 10 obese) females	banana 'shake' (552 kJ)	(CCK-33) 1 IDU/kg/ ideal weight/height for 165 min	19% ↓ in food (g) consumed after CCK vs Control (P<0.05).
Greenough et al (1998)	15 lean males	250g soup preload (397 kJ)	(CCK-8) 4 ng/kg/ min for 20 min	49% ↓ in energy (kJ) consumed after CCK vs Control (P<0.01)

NS = not significantly different

The majority of evidence, therefore, suggests that CCK is an endogenous satiety hormone. Intraperitoneal administration of CCK antagonists increases food intake in animals (Brenner & Ritter 1995, Ebenezer et al 1990, Rayner & Miller 1993). Studies in humans have shown a trend for increased food intake after administration of the CCK antagonist, loxiglumide, although these increases have not been statistically significant, most likely due to relatively small subject numbers (Drewe et al 1992, Lieverse RJ, 1994a). Matzinger et al (1999), recently demonstrated that intravenous infusion of loxiglumide (10 mg/kg/min) abolished the intraduodenal (corn oil; 0.376 ml/min for 120 min; ~1530 kJ) fat-induced reduction in energy intake (Figure 3.8). Theoretically, increased circulating concentrations of, or sensitivity to, CCK would inhibit food intake, and if either occurred as a consequence of ageing this could be a cause of the 'anorexia of ageing'. The effects of age on CCK is discussed in more detail in Chapter 4.5.1. The effects of exogenous CCK infusion on food intake in young and older subjects is the subject of further investigation in Chapter 10.

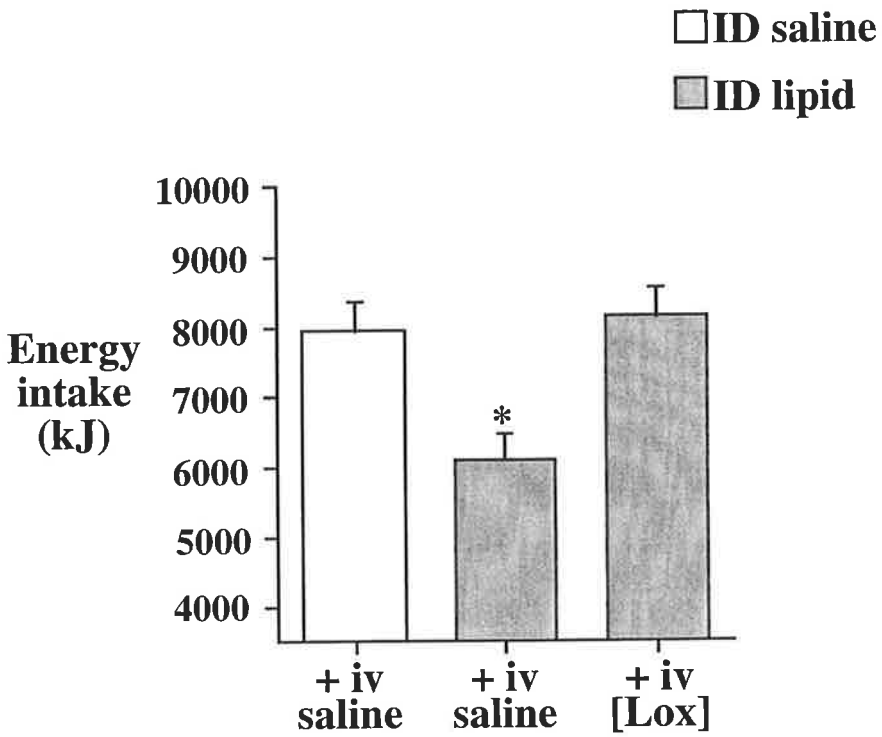

Figure 3.8: Effect of intraduodenal (ID) saline (control) or corn oil (0.375 ml/min for 120 min; ~1530kJ) infusions together with a preload of 'banana shake' and an intravenous (iv) infusion of saline or Loxiglumide (10 mg/kg/hr) on energy intake in 12 young healthy men (Matzinger et al 1999); *P<0.05 vs control.

The peripheral effects of CCK on food intake are mediated, in part, by activating CCK-A receptors on ascending vagal fibers, thus relaying signals to the nucleus solitarius and the paraventricular nucleus of the hypothalamus (Peikin 1989). Vagotomy abolishes the satiating effects of CCK in rats (Garlicki et al 1990). Consistent with this are the observations that the suppressive effects of exogenous CCK-8 on food intake are reduced in vagotomised (6-20 months following vagotomy and pyloroplasty procedure) when compared with healthy lean men (Shaw et al 1985).

There is evidence suggesting that CCK also suppresses food intake, in part, by its effects on gastrointestinal motility. Exogenous CCK administration slows gastric emptying in both animals (Moran & McHugh 1988, McHugh & Moran 1986) and humans (Liddle et al 1986, Kleibeuker et al 1988). Intravenous administration of CCK-8 in doses (0.5 and 1.0 IDU/kg/h) which result in plasma CCK levels within the physiological postprandial range, reduces proximal gastric tone and increase proximal gastric compliance during both isobaric and isovolumetric distensions in young healthy subjects (Straathof et al 1998). Infusion of loxiglumide also accelerates gastric emptying of a liquid meal in animals and humans (Fried et al 1991, Borovicka et al 1996, Schwizer et al 1997). A previous study in rats has suggested that changes in gastric motility are not necessary for CCK-induced satiety (Conover et al 1989). When CCK was administered 15 min before a test meal, it still suppressed food intake even though gastric emptying was not inhibited. More recently, however, Fraser et al (1993) showed that CCK-8 stimulates isolated pyloric pressure waves (IPPW's) and pyloric tone, and suppresses both antral and proximal duodenal contractions in fasted healthy humans. This pattern of pyloric stimulation and antroduodenal suppression induced by CCK-8 is identical to that observed in response to intraduodenal nutrient infusions, which also retard gastric emptying (Fraser et al 1993). Studies involving the use of CCK antagonists, such as loxiglumide, have also shown that the effects of intestinal lipid infusion on intragastric pressure gastric relaxation (Mesquita et al 1997) and antral, pyloric and duodenal contractions (Katschinski et al 1994) are reversed in healthy humans. Melton et al (1992) showed that healthy young women reported more fullness and less hunger with gastric balloon distension, as well as a smaller rise in gastric pressure during CCK-8 (112 ng/kg/min) infusion than during a saline infusion. This suggests that CCK may act to increase the sensitivity to gastric distension (Melton et al 1992). Consistent with this, Feinle et al (1996) recently showed in young healthy subjects that intravenous infusion of loxiglumide partially restored the reduction in gastric tonic and phasic pressure and reduced sensations of a meal-like fullness induced

by intraduodenal lipid (Feinle et al 1996). The relationship between endogenous CCK release in response to intraduodenal lipid infusion and tonic and phasic pyloric pressures is examined further in Chapter 8.

3.6.2 *Insulin*

Insulin is normally released from the pancreatic islet cells in response to the postprandial elevation in blood glucose concentration. The gut plays an important role in mediating the release of insulin. Insulin release is more marked when glucose is administered into the small intestine than directly into the circulation due to the release of insulin secretagogue gastrointestinal hormones- the so called incretin effect (Lavin et al 1997, Lavin et al 1998). The incretin hormones, identified so far are, GLP-1 (section 3.6.3) and GIP (section 3.6.4).

It has been proposed that insulin is a satiety factor though this concept is controversial (Schwartz et al 1992) since patients with type 2 diabetes mellitus prescribed insulin or oral insulin secretagogue treatment often gain weight. The evidence for a satiating role of insulin is limited. Both central and peripheral administration of insulin in pharmacological doses suppress food intake in animals (Woods et al 1984, Brief & Davis 1984), provided hypoglycaemia is prevented. Short term, peripheral, euglycaemic insulin infusions have no effect on appetite or food intake in humans (Chapman et al 1998), although it is possible it has not been administered this way in high enough doses. While, is seems unlikely that insulin, alone, suppresses food intake, it may act in conjunction with other peptides (see below) and/or increased blood glucose (see section 3.7) to influence appetite. Ageing is associated with an alteration in insulin release and insulin resistance (Ranganath et al 1998). This issue is discussed in more detail in Chapter 4.5.2.

3.6.3 *Glucagon-like peptide-1 (GLP-1)*

Glucagon-like peptide-1 (7-36 amide) (GLP-1) is released by endocrine L-cells within the lumen of the distal small bowel in response to nutrients, particularly carbohydrate, in the gut. Fatty acids and amino acids also stimulate GLP-1 release in higher concentrations. GLP-1 acts to stimulate insulin secretion and, together with gastric inhibitory polypeptide (GIP)(3.6.4), is one of the incretin hormones (Schirra et al 1996a). GLP-1 also acts to slow gastric emptying (Naslund et al 1999b), probably by stimulating pyloric motility and inhibiting antral contractions (Young et al 1996, Schirra

et al 1997, Schirra et al 2000). Exogenous GLP-1 also inhibits insulin, glucagon, and peptide YY (PYY) release in humans (Naslund et al 1999b).

There is increasing evidence for a role of GLP-1 in the regulation of satiation. Peripheral administration of GLP-1 suppresses food intake in rats (Turton et al 1996) and intraperitoneal administration of the specific GLP-1 antagonist, exendin, stimulates feeding in satiated (Meeran et al 1999), but not fasted, rats (Turton et al 1996). The central effects of GLP-1 on food intake may be mediated by the inhibition of neuropeptide Y (NPY) (see Chapter 2.3.3) in the hypothalamus (Furuse et al 1997). Evidence for an effect of GLP-1 on food intake in humans, however, has been less consistent. Previous studies have shown that intravenous infusion of GLP-1 suppresses food intake in young normal weight men (Flint et al 1998) and enhances feelings of fullness and satiety in normal weight (Flint et al 1998) and obese (Naslund et al 1998) subjects and patients with non-insulin dependent diabetes mellitus (Gutzwiller et al 1999). Lavin et al (1997) also reported that when the release of GLP-1 and GIP was blocked by intravenous administration of the long-acting somatostatin analogue, octreotide, the satiating effects of an intraduodenal glucose were abolished. In contrast, Long et al (1999) found that GLP-1 infusion (1.2 pmol/kg/min) for 1 hr delayed gastric emptying of a 400 ml water load, but had no significant effect on food intake and satiety. Another study by Naslund et al (1999a) showed that intravenous GLP-1 infusion (0.75 pmol/kg/min) for 8 hr, delayed gastric emptying of a standard breakfast (2.4 MJ), and also suppressed hunger and mean ad libitum energy intake at lunch and dinner in 8 obese men. The difference in the length of time of the iv infusions in these studies (ie 1 hr vs 8 hr) is most likely responsible for the contrasting effects of GLP-1 on satiation. No studies have examined the effects of the GLP-1 antagonist, exendin, on gastric emptying and appetite in humans. Most evidence, however, favours GLP-1 as a satiety hormone. The influence of ageing on GLP-1 release is discussed in Chapter 4.5.2.

Dietary glucose supplementation increases gastric emptying of an oral glucose load and subsequently reduces the glycaemic response in young healthy subjects (Horowitz et al 1996)(see section 3.5.2 and 3.5.2.1). It is not, however, known whether this alteration in the glycaemic response is due to an alteration in GLP-1 release. This issue is addressed in Chapter 13.

3.6.4 *Glucose-dependent insulinotrophic polypeptide (GIP)*

GIP is released from the K-cells of the proximal small intestine in response to ingestion of fat (Falko et al 1975) or glucose (Cataland et al 1973). A number of studies have demonstrated that GIP acts as an incretin - exogenous GIP administration stimulates insulin release only in the presence of hyperglyceamia, not in the normoglycaemic state (Dupre et al 1973). When intravenous GIP and glucose are administered simultaneously, they augment insulin secretion and improve glucose tolerance in young healthy men (Dupre et al 1973). Furthermore, subcutaneous glucagon-like peptide-1 combined with insulin normalises postcibal glycaemic excursions in patients with insulin dependent diabetes mellitus (IDDM) (Dupre et al 1997). To date, however, there is no clear evidence for a satiating effect of GIP. Schirra et al (1996a) showed that GIP release is similar regardless of whether a glucose load is ingested orally or administered directly into the small intestine, suggesting that GIP release is not controlled by the rate of gastric emptying. Abnormalities in the release of both GIP and GLP-1 may result in impaired insulin secretion, similar to that observed in the elderly. This issue is discussed in further detail in Chapters 4.5.2, 9 and 13.

3.6.5 *Peptide YY (PYY)*

PYY is a 36 amino acid peptide (McFadden et al 1989) present in the brain and also released from the distal gut in response to infusions of fat (McFadden et al 1989, Pedersen-Bjergaard et al 1996) and carbohydrate (Pedersen-Bjergaard et al 1996) into the small intestine. PYY is involved in a number of physiological processes including memory, pain, blood pressure, appetite and anxiety (McFadden et al 1989). It acts centrally to stimulate food intake (Corp et al 1990), but its peripheral effects on feeding are less clear. In animals, feeding is increased by central PYY administration (Corp et al 1990), but there is no evidence for any effect on feeding by intraperitoneal administration, although peripherally administered PYY slows gastric emptying (Savage et al 1987, Allen et al 1984, Greeley et al 1989). Ileal perfusion with fat causes an elevation of PYY in the dog (Greeley et al 1989) and in young healthy humans (Vu et al 1999). Further studies in humans are required to determine the role, if any, of PYY in the regulation of satiation. Little is known about the effect of ageing on PYY release in response to small intestinal nutrients. This is discussed in more detail in Chapter 4.5.3 and the effects of intraduodenal lipid and glucose on plasma PYY concentrations and the relationship between PYY and pyloric motility are evaluated in Chapter 8.

3.6.6 *Amylin*

Amylin is a 37 amino acid peptide hormone manufactured, stored and released with insulin from the beta cells of the pancreatic islets. Like insulin, it is secreted in response to a meal and is deficient in people with type 1 diabetes mellitus (Westermark et al 1992, Koda et al 1992). Amylin slows gastric emptying in both animals and humans (Young et al 1995, Kong et al 1997); administration of the amylin analogue, pramlintide, blunts the postprandial hyperglycaemic response to a meal in people with type 1 diabetes mellitus (Kolterman et al 1995).

Peripheral administration of amylin decreases food intake in both diabetic and non-diabetic rodents (Morley et al 1993, Morley et al 1994b), via an effect on the nucleus tractus solitarus. Amylin knockout mice are hyperphagic and overweight. Amylin may to exert its satiating effects via a synergistic interaction with cholecystokinin (Bhavsar et al 1998). Although the effect of amylin on appetite and food intake in humans has not been studied formally to our knowledge, administration of the amylin analogue pramlintide for 4 weeks to people with type 2 diabetes in a recent study was associated with a non-significant 0.7-0.8 kg weight loss compared to the control group (Thompson et al 1998). This suggests that amylin may also reduce food intake in humans. The effects of ageing on amylin secretion is discussed in more detail in Chapter 4.5.4.

3.6.7 *Ghrelin*

The recently characterised peptide hormone, ghrelin, is thought to primarily regulate pituitary growth hormone (GH) (Kojima et al 1999). The recent discovery, however, that ghrelin is predominantly synthesised in the stomach, not the hypothalamus (McKee et al 1997m Howard et al 1996), and thus may provide an endocrine connection between the stomach and hypothalamus and pituitary, has led to further investigations of other physiological actions of ghrelin, ie. a role in the regulation of feeding and energy balance (Tschop et al 2000).

Tschop et al (2000) recently conducted a series of studies to investigate the effects of exogenous administration of ghrelin on food intake and body weight in both mice and rats. They reported that daily subcutaneous injection of ghrelin (2.4 μmol/kg) for 2 weeks in male wild-type mice resulted in a 13.9% increase in body weight, compared to 5.6% increase in the vehicle-injected control group of animals. Ghrelin administration did not alter food intake in the mice, but measurement of body

composition, using dual-energy X-ray absorptiometry (DXA), revealed that the mice treated with ghrelin had 41% more body fat than the control mice. The authors also reported an increase in the respiratory quotient (ie increased utilisation of carbohydrate and reduced utilisation of fat), measured using indirect calorimetry, in ghrelin-treated mice, compared to control animals, which was consistent with the observed increase in body fat. They also demonstrated that the effects of ghrelin on body weight and composition in these mice were independent of the effects of GH release on body weight, further supported by the previous finding that subcutaneous administration of ghrelin (4.5 μmol/kg/d) for one week results in a significant increase in body weight as well as a tendency to overeat in GH-deficient dwarf female rats (Charlton et al 1988). Further studies by Tschop et al (2000) suggested that ghrelin-induced adiposity was mediated centrally since continuous intracerebroventricular administration of ghrelin in normal male Sprague-Dawley rats, at doses of 1.2 nmol/kg/day and 12 nmol/kg/day, for one week increased body weight by ~68% and 87%, enhanced food intake by ~21% and 27% and stimulated mean 24 hr respiratory quotient by ~6% and ~8%, respectively, compared to control animals. They also reported that serum ghrelin concentrations were significantly increased by ~2.9 ng/ml following 48 hr fasting conditions and reduced by ~1.3 ng/ml during ad libitum feeding in normal male Sprague -Dawley rats. Furthermore the finding that oral gavage of 50% dextrose in 5 ml water, significantly reduced serum ghrelin levels whereas oral administration of 5 ml of water alone had no affect on ghrelin concentrations suggested that circulating ghrelin levels were regulated by nutrient intake, rather than gastric filling in these animals (Tschop et al 2000).

This series of studies suggest that, in addition to its regulatory role for GH secretion, ghrelin also acts to signal to the hypothalamus via the stomach when an increase in metabolic efficiency is required. There is currently no information about the effects of ageing on ghrelin activity, however, given that growth hormone secretion progressively declines with age (Johannsson et al 2000), it is likely that ghrelin concentrations may also be altered in the elderly. Theoretically, a reduction in secretion or sensitivity to the effects of ghrelin may contribute to reduced appetite and weight loss, such as that observed in the elderly. Studies involving measurement of serum ghrelin in healthy as well as malnourished elderly subjects would, therefore, be of substantial interest.

3.7 ABSORBED NUTRIENTS AND APPETITE

There is evidence that blood glucose levels may be involved in the modulation of appetite. Transient declines in blood glucose concentrations are associated with increased hunger and meal requests in time-blinded healthy individuals (Melanson et al 1999, Campfield et al 1996). Intraluminal digestion of carbohydrates results in the release of glucose, which is rapidly absorbed resulting in an increase in blood glucose levels. There is some evidence that the postprandial elevation in blood glucose may be indirectly involved in the regulation of satiety. A number of studies have shown that gastric motor and sensory function are affected by acute changes in the blood glucose concentration (Barnett & Owyang 1988, Fraser et al 1991, Hebbard et al 1996, Jones et al 1996, Jones et al 1997b, Andrews et al 1998b). For example, patients with insulin dependent diabetes mellitus report increased feelings of fullness, compared to young healthy subjects, that are associated with increased blood glucose concentrations, both before and after ingestion of a solid-liquid test meal. (Jones et al 1997b). Acute elevation of blood glucose concentration (12-16 mmol/L) also increases the perception of gastric distension and increases the intensity of fullness and nausea sensations during intraduodenal lipid infusion in young healthy subjects (Hebbard et al 1996). Andrews et al (1998b) reported that elevations in the blood glucose within the normal postprandial range also increase the intensity of fullness in young normal subjects. Elevated blood glucose concentrations (12-16 mmol/L) also affect gastroduodenal motor function in both normal subjects (Barnett et al 1988, Fraser et al 1991) and patients with diabetes (Rayner et al 2000), and this may in turn influence appetite. Specifically, an acute elevation in the blood glucose concentration to ~15 mmol/L (Hasler et al 1995) slows gastric emptying (Fraser et al 1990), inhibits antral motility (Barnett et al 1988, Fraser et al 1991), stimulates pyloric motility (Fraser et al 1991) and increases gastric compliance (Hebbard et al 1996). Moreover, a modest in blood glucose concentration (8-11 mmol/L) slows gastric emptying (Schvarcz et al 1997), and suppresses antral pressure waves (Hasler et al 1995, Andrews et al 1998b), but does not affect fasting gastric compliance in young normal subjects (Verhagen et al 1999a). Insulin-induced hypoglycaemia also accelerates gastric emptying in normal healthy subjects (Schvarcz et al 1995) as well as patients with type 1 diabetes mellitus (Schvarcz et al 1993).

There is, however, little evidence to support the direct role of the postprandial rise in blood glucose levels in producing satiation. For example, Lavin et al (1997) showed that infusion of glucose into the small intestine suppressed food intake by 17% more

than an intravenous glucose infusion which produced similar plasma glucose concentrations in young healthy males, suggesting that plasma glucose levels are unlikely to be of substantial importance in the regulation of satiation at least in the short-term. Increases in blood glucose, however, may potentially interact with gastric (ie perception of distension) and small intestinal (ie insulin or incretin hormones) mechanisms associated with satiation.

As discussed, small intestinal nutrient infusion results in a greater suppression of food intake than direct infusion of nutrients into the circulation, at least in the short-term (1-2 hr) (Welch et al 1985, Lavin et al 1997). There is, however, some evidence that intravenous nutrients suppress food intake in both animals (Woods et al 1984, Walls & Koopmans 1989) and humans (Gil et al 1990, Gielkens et al 1999) when given over a longer period of time ie. up to 21 days in animals (Woods et al 1984) and up to 19 days in humans (Gil et al 1990). For example, in rats, Walls & Koopmans (1989) showed that oral food intake was suppressed by up to 70% during intravenous infusion of Travasal (25% d-glucose and 4.25% amino acids) at a rate of 217.5 kJ/day over 4 consecutive days and by 43 % during intravenous infusion of Nutralipid (20% fat emulsion) at a rate of 167.3 kJ/day for 6 days. Gil et al (1990) showed that within 48 hours of receiving parenteral nutrition (combination of glucose, fat and amino acids) for a period of 17-19 days, young healthy men reduced their voluntary oral food intake by approximately 80% of the infused calories. There was, however, no control infusion to compare the suppression of food intake by PPN in this study. Conversely, Geilkens et al (Gielkens et al 1999) recently reported that although intravenous infusions of amino acids at doses of 125 mg protein/kg/hr and 250 mg protein/kg/hr for 6 hours increased sensations of fullness, and decreased preprandial preference for high-fat foods, oral food intake was not reduced compared to an iv saline infusion. Other studies have established that nutrients (mixture of glucose, fat and amino acids) in the circulatory phase influence inhibit gastric emptying and gastric secretion (MacGregor et al 1979, Bursztein-De Myttenaere et al 1994) [see for review (Masclee et al 1996)], which in turn may contribute to the relative intolerance to oral food by patients on PPN. These findings suggest that circulating nutrients (mixture of carbohydrate, fat and amino acids) appear to play a role in the regulation of long-term energy intake, but this may only be under acute conditions and secondary to the inhibitory effects of small intestinal nutrients on short-term satiation. The mechanisms responsible for the effects of absorbed nutrients on appetite may include direct stimulation of receptors in the vagus or within the central nervous system (CNS) which are involved in the regulation of

gastrointestinal motility, or a direct effect on smooth muscle. Alternatively, long-term elevation of plasma nutrient levels may stimulate the release of peptides (eg CCK) which relay satiety signals to the CNS.

3.8 CONCLUSIONS

The peripheral mechanisms involved in the regulation of satiation are complex and interrelated. Signals arising from the interaction of nutrients with the gastrointestinal tract play a major role in the mediation of satiation. A number of alterations in these mechanisms may potentially contribute to the age-related reduction in appetite and food intake. Current knowledge about the possible factors involved in the anorexia of ageing is discussed in Chapter 4, as a prelude to the evaluation of the role of some of the potential mechanisms in the studies presented in Chapter 8-15 in this thesis.

CHAPTER 4

Potential Mechanisms Involved in the Anorexia of Ageing

4.1 INTRODUCTION

Little is known about the mechanisms responsible for the physiological anorexia of ageing. While it has been suggested that there are a number of age-related disturbances in the central and peripheral factors involved in the regulation of appetite, much of the evidence, particularly that relating to central factors has been derived from studies

conducted in animals and therefore, may not be directly applicable to humans. These physiological disturbances, together with the influence of social, psychological, physical or medical factors (see Chapter 1) commonly experienced by the elderly predispose to pathological anorexia and malnutrition. The purpose of this chapter is to review current knowledge of the age-related central, olfactory, gastrointestinal and humoral changes which may potentially contribute to the anorexia of ageing with particular focus on the opioid feeding drive (section 4.2.1), gastrointestinal sensory and motor function (section 4.3), and humoral factors (section 4.4), thus providing a background for the objectives of the studies presented in the following Chapters of this thesis (Chapters 8-14).

4.2 EFFECTS OF AGEING ON CENTRAL MECHANISMS WHICH REGULATE APPETITE

A number of changes in the central mechanisms involved in the regulation of feeding are likely to contribute to the reduction in appetite and food intake observed in the elderly, as summarised in the following discussion; the latter focuses on the effects of ageing on endogenous opioid modulation of feeding (section 4.2.1) with lesser emphasis given to age-related changes in some of the other centrally acting neuropeptides, including neuropeptide Y, nitric oxide and leptin (section 4.2.2).

4.2.1 *Age-related changes in endogenous opioids*

Endogenous opioids play a major role in mediating the short-term sensory reward response to food (see Chapter 2.3.1). There is some evidence, mostly from animal studies, that ageing is associated with a reduced opioid feeding drive. Overall these studies, suggest that ageing is associated with a reduction in endogenous opioid activity within the central nervous system. There is an age-related reduction in the number of opioid receptors (Gosnell et al 1983, Silver & Morley 1992) and this is associated with a decrease in the concentrations of endogenous opioids in major brain regions in older animals. For example, hypothalamic concentrations of enkephalins (Dupont et al 1981, Gambert 1981) and ß-endorphin (Kowalski et al 1992, Forman et al 1981) are lower in older when compared to young male rats. Several studies have shown no changes with ageing in the concentrations of endogenous opioids associated with feeding. For example, a number of studies have reported (Lau & Tang 1995, Gambert 1980, Missale et al 1983, Tang et al 1984) that hypothalamic concentrations of met-enkephalin and ß-endorphin are comparable in 3-mo, 8-mo and 23-mo old male Sprague-Dawley

rats. Wang et al (1993) reported that there was no difference in concentrations of β-endorphin, leucine, (leu)-enkephalin or met-enkephalin in the hypothalamus of 3-, 12- and 22-mo old Sprague Dawley rats during the night or day time, with the exception that met-enkephalin concentrations were less in the 22-mo old rats during the day time. These findings suggest age-related reductions in opioid peptides within the brain may be specific to particular peptides, and that these changes may differ according to diurnal rhythms. The binding of ^{3}H-dihydromorphine is less in the thalamus and midbrain in older (8-mo old) female F344 rats compared to young (26-mo old) rats, suggesting a concomitant decrease in opioid receptors in these areas (Messing et al 1980). A reduction in opioid agonist binding was not, however, demonstrated in a subsequent study in F344 older male rats (Messing et al 1981). These results suggest that the processes which underlie opioid binding affinities with age may be different in males and females. In older rats (14-, 19- and 24-mo old rats) there is a significant decrease in morphine-induced analgesia after peripheral injection compared to younger (aged 4- and 9-mo old) rats (Kramer & Bodnar 1986). The reduction in shock-induced analgesia in old (22-24-mo old) compared to young (5-7-mo old) rats, is also inhibited less following peripheral administration of the opioid antagonist, naloxone, in old young rats (Hamm & Knisely 1985). In relation to the opioid modulation of feeding, Kavaliers & Hirst (1995) reported that intraperitoneal injection of morphine increased food intake in young (1-2-mo old) mice, whereas no stimulatory effect was observed in old (24-30-mo old) animals. Furthermore, the older mice exhibited a reduced sensitivity to the suppressive effects of the opioid antagonist naloxone on cumulative 2 hour food intake during both day and night time, following 24 hr food-deprivation (Kavaliers & Hirst 1995) (see Figure 4.1). Gosnell et al (1983) reported that 1, 2 and 4 hour food intake was decreased in 2- and 12-mo old Fisher-344 rats, but not in 22- and 28-mo old animals, following intraperitoneal injection of low doses (0.1 and 1.0 mg/kg) of the opioid antagonist naloxone (see Figure 4.1). Silver at al (1991) also demonstrated that fluid intake was reduced in young (3-12-mo old), but not old (25-mo old), mice following intraperitoneal administration of naloxone (Silver et al 1991). This evidence suggests that there is an age-related alteration in opioid activity which may contribute to the anorexia of ageing.

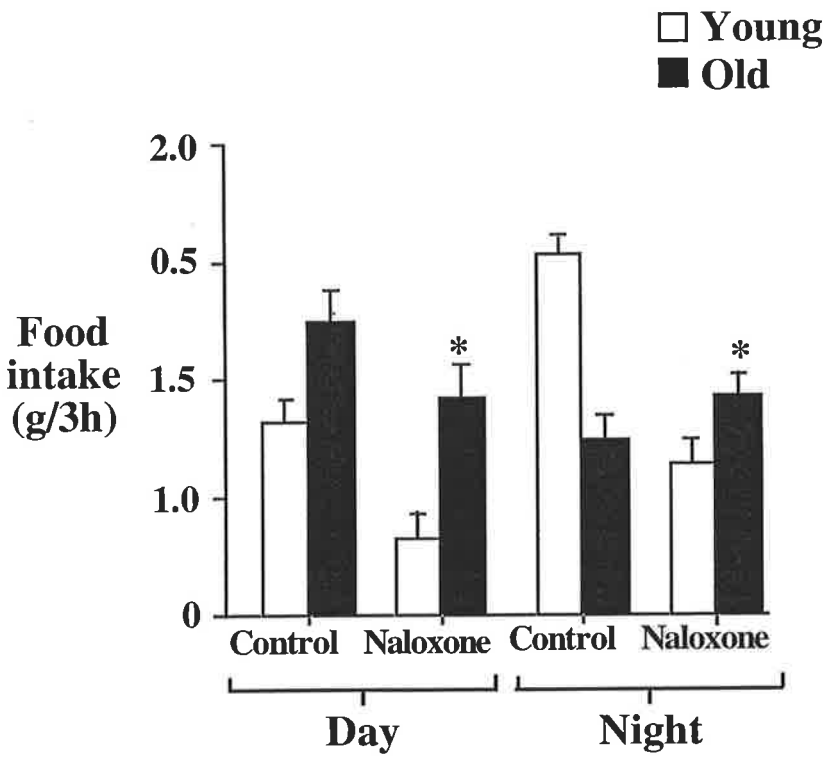

Figure 4.1: Effects of peripheral injection of naloxone (10 mg/kg) during the day and night on 3 hr food intake in fasted young (1-2-mo old) and old (24-30-mo old) mice (Kavaliers et al 1985). *P <0.05 vs control within age group and time period.

Although studies in humans have been more limited these have also provided some evidence for changes in endogenous opioid activity with ageing. For example, Martinez et al (Martinez et al 1993) reported, in 14 elderly (70-86 yr) patients suffering from anorexia-related malnutrition, plasma and cerebrospinal fluid concentrations of ß-endorphin are reduced when compared with 10 normal weight, aged-matched control subjects. Treatment of five of the anorectic patients with megestrol acetate (480 mg daily for 6 months), however, reversed the decrease in cerebrospinal fluid ß-endorphin levels, but failed to normalise body weight or body fat mass (Martinez et al 1993), suggesting that malnutrition and/or weight loss may not be secondary to reduced ß-endorphin levels, but rather due to a reduction in sensitivity to endogenous opioids. Reduced levels of endogenous opioids may simply perpetuate anorexia in the underweight elderly. Conversely, Forman et al (1987) reported no difference in plasma ß-endorphin levels between young (aged 18-21 yr) and older (aged 55-62 yr) healthy men. The possibility that decreased renal clearance of ß-endorphin in the older subjects may obscure the release of ß-endorphin from the pituitary into the plasma cannot be excluded in that study. Although it appears that healthy human ageing is not associated clearly with reduced circulating endogenous opioid levels, it is possible that it may be related to a reduction in sensitivity to the actions of endogenous opioids. There is evidence in humans, that opioid modulation of thirst is altered with ageing. Silver & Morley (1992) evaluated the effects of an intravenous bolus of naloxone (100 µg/kg) after an overnight fluid deprivation in 8 healthy older men aged 69-75 yr and 8 young (aged 23-39 yr) men and found that the inhibitory effect of a intravenous injection of naloxone on fluid intake was about 30% less in the older than the young men.

While naloxone has been shown to inhibit energy intake in young healthy subjects (see Chapter 2.3.1), it is not known whether these effects are modified by ageing. The effects of intravenous administration of naloxone on appetite and food intake in healthy elderly and young subjects are reported in Chapter 12.

4.2.2 *Age-related changes in neuropeptide Y, nitric oxide and leptin*

The roles of nitric oxide (NO), neuropeptide Y (NPY), and leptin in the central regulation of feeding are discussed in detail in Chapter 2.3.2, 2.3.3 and 2.3.4, respectively.

4.2.2.1 Nitric oxide (NO)

Endogenous opioids may stimulate food intake (see Chapter 2.3.1). It has been suggested that alterations in nitric oxide activity may play a role in the anorexia of ageing (Morley 1997). Although not all studies are in agreement, there is evidence in rats that nitric oxide synthase NOS levels and activity increase with ageing in many areas, including the brain (Chalimoniuk & Strosznajder 1998, Yamada & Nabeshima 1998, Morley et al 1996b), kidney (Reckelhoff et al 1999) and skeletal muscle (Capanni et al 1998). Consistent with this is the observation that the inhibitory effects of L-arginine analogues on NO-mediated processes in the kidneys in rats (Tan et al 1998) and the inhibition of food intake in mice (Morley et al 1996b) are enhanced with ageing. These results suggest that ageing in rodents may be associated with an increase in endogenous NO activity which stimulates feeding in compensation for other anorectic effects of ageing, rather than declining nitric oxide activity being a cause of the anorexia of ageing.

It has been postulated that alterations in gut nitric oxide synthase activity lead to the impaired gastric receptive relaxation of the stomach (see Chapter 4.3.2). According to Takahashi et al (1997) the NO-mediated relaxation of gastric muscle preparations from normal rats in response to transmural stimulation, is reduced in preparations from spontaneously diabetic (biobreeding/Worchester, BB/W) rats. Consistent with this is the observation that sublingual glyceryl trinitrate (GT)(a donor of exogenous NO) prior to a meal decreases the antral area and improves intragastric distribution after ingestion of a 500 ml soup meal in patients with type 1 diabetes mellitus. The symptoms of early fullness, nausea and pain which are related to impaired gastric accomodation in these subjects do not, however, improve following GT administration (Undeland et al 1998). NO synthase activity in the gastric corpus is reduced in 52 week old compared to 7 week old rats (Ichikawa et al 1999). These results suggest that a decrease in fundic nitric oxide may contribute to earlier satiation in the elderly.

The situation may not be the same in humans, in whom brain NOS levels do not appear to be affected by ageing (Blum et al 1999). In the only study which evaluated the role of NOS mechanisms on feeding in humans, peripheral administration of the NOS inhibitors L-NAME and L-NMMA had no effect on short term hunger, fullness or food intake in healthy young adults, despite cardiovascular effects indicative of NOS inhibition (Vozzo et al 1999). Both central and peripheral NO may be important in the regulation of food intake (Squadrito et al 1993, Choi et al 1994), and suppression of

central NO production may not have been achieved by peripheral administration of the NOS inhibitors in the study by Vozzo et al (1999). Accordingly, while a role for altered NO activity as a cause of or compensation for the anorexia of ageing may be unlikely, it has not been excluded.

4.2.2.2 Neuropeptide Y (NPY)

There is preliminary evidence from animal studies that ageing is associated with a reduction in neuropeptide Y (NPY) synthesis and/or reduced sensitivity to its stimulatory effects on feeding; these effects may be more marked in males than females. In the arcuate nucleus levels of prepro NPY mRNA are less in old than young rats, both during fasting and ad libitum feeding (Gruenewald et al 1996). There is a reduction in hypothalamic NPY levels between 4 and 26 (senescence) months of age in male (Kowalski et al 1992), but not female (McShane et al 1999) rats. In humans, cerebrospinal fluid levels of NPY-like immunoreactivity actually increase with ageing in women, but not men (Taniguchi et al 1994). In rats the feeding response to NPY injections into the paraventricular nucleus is diminished by ageing (Pich et al 1992), whereas in mice the stimulation of feeding caused by intracerebroventricular NPY administration does not diminish with age (Morley et al 1987). Given the that there are marked discrepancies between observations from both animals and humans, a distinct relationship between a loss in NPY function and anorexia with advancing age is yet to be established.

4.2.2.3 Leptin

Hypothetically, an increase in the activity of the satiety hormone leptin, either due to increased levels, or an enhanced sensitivity to its effects, could play a role in the anorexia of ageing. Adipose tissue leptin RNA expression has been reported to increase with age in rats (Wolden et al 1999) and mice (Mizuno et al 1996), but studies in rats (Wolden et al 1999) and pigs (Qian et al 1999) have not found an increase in serum leptin with ageing. In the former studies, however, differences in fat mass between the older and young animals were not taken into account. Absolute plasma leptin concentrations in humans often increase with ageing, to a large extent attributable to the increased fat mass that also accompanies ageing (Chapter 1.4). Most studies show that after adjustment for fat mass there is no effect of ageing on plasma leptin (Baumgartner et al 1999a, Castracane et al 1998, Ryan & Elahi 1996, Li et al 1998). This is clearly the case in women, but in men some, (Baumgartner et al 1999a) but not all (Li et al 1998), studies suggest that ageing is associated with an increase in circulating leptin

levels, even after allowing for fat mass. This rise in leptin levels with ageing in men may potentially be mediated by the fall in circulating testosterone concentrations which also accompanies normal male ageing (Baumgartner et al 1999a, Luukkaa et al 1998). After adjusting for fat mass, plasma leptin levels in men are inversely related to plasma testosterone (Baumgartner et al 1999a, Luukkaa et al 1998), while testosterone therapy reduces, and inhibition of testosterone production increases, circulating leptin levels (Hislop et al 1999).

There is little information about whether the sensitivity to the satiating effects of leptin is modified by age. In young animals, suppression of plasma leptin concentrations during fasting, stimulates hyperphagia (Himms-Hagen 1999). Reduced suppression of leptin levels by fasting has been reported in ageing rats (Li et al 1998). In humans, the relationship between plasma leptin and body fat stores may be weaker in older than young adults (Moller et al 1998), consistent with the age-related impairment in the activity of a number of homeostatic mechanisms. No studies have examined the effects of ageing on plasma leptin concentrations during fasting in humans.

Although the suppressive of effect of leptin on food intake in humans is probably fairly weak (Heymsfield et al 1999), leptin may play some part in mediating the physiologic anorexia of ageing in men, possibly due to an increase in leptin levels as a consequence of an age-related decline in plasma testosterone, but not women.

4.3 EFFECTS OF AGEING ON OLFACTORY FUNCTION

The role of olfactory function in the regulation of appetite is discussed in Chapter 3.2. Although observations have been somewhat inconsistent, a number of studies have demonstrated that the sense of taste deteriorates with age (Kamath 1982, Gilmore & Murphy 1989, Murphy 1992, Stevens et al 1995, Drewnowski 1997), though probably at a variable rate. Kamath (1982) reported that taste bud regeneration slows with age, but more recent studies have shown that the density and structure of taste buds are maintained in the healthy elderly (Miller 1988). The decline in taste perception with ageing was reported by Stevens et al (1995). They found that healthy older persons exhibited a clear increase in taste thresholds for sucrose compared to young subjects. Some studies also suggest that ageing may be associated with an impaired ability to discriminate between different tastes which may influence their food choice. For example, the impairment of bitter when compared to sweet taste perception was greater

in the healthy older, when compared to young, subjects (Gilmore & Murphy 1989). Drewnowski (1997) also reported that healthy elderly exhibit a decreased preference for salty foods compared to young subjects

It is clear that the sense of smell declines progressively with age (Doty et al 1984, Duffy et al 1995). Doty et al (1984) assessed olfactory function in 1955 persons aged 5-99 yr, using a 40-odourant forced-choice test, and found that performance on the test markedly declined after the age of 50 yr, so that in more than 60% of subjects aged 65-80 yr, and > 80% aged 80 yr or older, there was evidence of major olfactory impairment compared to a prevalence of < 10% in those under 50 yr (Doty et al 1984). Recent studies suggest that the decline in sense of smell contributes to decreased food intake in the elderly and may also influence the type of food eaten. For example, Griep et al (1996) reported that elderly persons (aged 60-90 yr) who exhibited poor odour detection thresholds for isoamylacetate had lower energy intakes, as assessed by 7-day diet diaries, than those with normal odour detection thresholds. Duffy et al (1995) found that in elderly women an impaired sense of smell was associated with reductions in the interest in food and preference for sour/bitter and pungent tastes, and a higher intake of sweet foods. Consistent with this apparent effect of ageing on the types of food eaten is the observation that ageing is associated with a less varied, more monotonous diet (Fanelli & Stevenhagen 1985).

A decline in taste and smell acuity with advancing age may also contribute to a decline in sensory-specific satiety. Rolls & McDermott (1991) examined the effects of age on "sensory-specific satiety" (see Chapter 3.2) and reported that older (>65 yr) subjects failed to develop a sensory-specific satiety to a 300-g test meal of strawberry yoghurt, in contrast to young subjects. This decline in sensory-specific satiety may also contribute to the consumption of a less varied diet in the elderly (Rolls & McDermott 1991). It is, therefore, conceivable that these changes in taste and smell, which result in a decreased recognition of different tasting foods lead to a decrease in energy consumption.

4.4 EFFECTS OF AGEING ON GASTROINTESTINAL MECHANISMS WHICH REGULATE APPETITE

Ageing affects a number of the gastrointestinal mechanisms which regulate feeding, particularly, and gastrointestinal sensory function (section 4.3.1), gastrointestinal motor function (section 4.3.2) and the release of gut peptides (section 4.3.3).

4.4.1 *Age-related changes in gastrointestinal sensory function*

Sensory perception may be reduced in the elderly. A recent study by Aviv (1997) assessed laryngopharyngeal sensory function (by stimulation of the anterior wall of the pyriform sinus with an endoscopically delivered air pulse) in 80 healthy men and women grouped into ages 20-40 yr, 41-60 yr and 61 yr and over. The sensory discrimination threshold (mmHg air pulse pressure) in the subjects aged 61 yr and over was, on average, 31% and 18% higher than in the 20-40 yr and 41-60 yr age groups, respectively. It was suggested that this diminution in laryngopharyngeal sensitivity with increasing age may contribute to the increase prevalence of dysphagia and aspiration in the elderly (Aviv 1997).

A reduction in sensory perception is evident in other areas of the gastrointestinal tract. Ageing is associated with a reduced sensitivity to gastrointestinal tract distension (Bannister et al 1987, Lagier et al 1999, Lasch et al 1997, Weusten et al 1994). For example, Lasch et al (1997) showed that the threshold volume for perception of pain during oesophageal balloon distension was higher in 17 older (>65 yr) men and women than in 10 young healthy subjects, (27 ± 1.4 ml of air vs 17 ± 0.8 ml of air)(Figure 4.2). Furthermore, Weusten et al (1994) reported that the amplitude of viscerosensory cerebral evoked potentials in response to rapid oesophageal balloon distension decreased, whereas the latencies increased, with age, indicative of a defect in afferent sensory pathways. The threshold for perception of rectal distension is also increased in older people (Bannister et al 1987, Ryhammer et al 1997, Lagier et al 1999). An alteration in the perception of gastric distension with ageing is potentially important as it may influence appetite sensations and subsequent food intake in the elderly. This has not, however, been investigated in humans. Chapter 11 evaluates the effects of proximal gastric distension on sensations of fullness, bloating and pain in elderly compared to young healthy men.

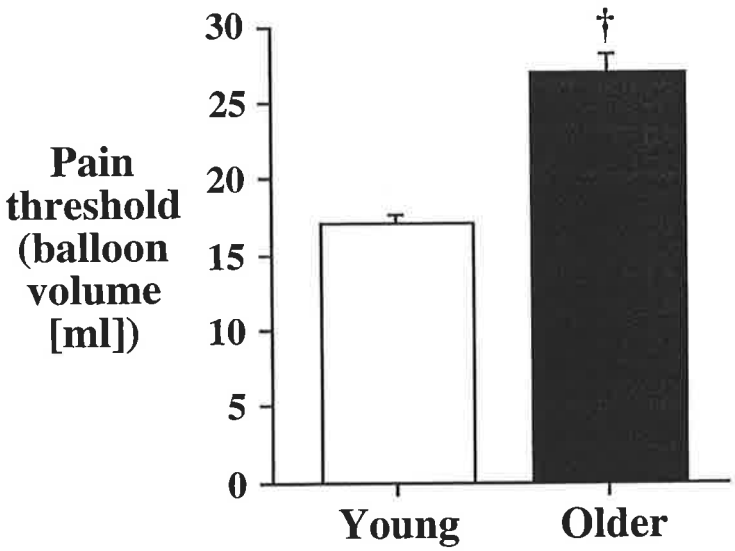

Figure 4.2: Mean (± SEM) pain threshold, expressed as the volume of air in the oesophageal balloon when pain is first registered, during oesophageal balloon distension in 10 young (18-57 yr) and 17 older (65-87 yr) healthy subjects (Lasch et al 1997). †P < 0.01 vs young.

As discussed in Chapter 3.5.1, sensations arising from the small intestine play an important role in the termination of a meal. In young, healthy subjects, intraduodenal nutrient infusion provokes an increase in sensations of fullness and satiety and a decrease in sensations of hunger. Little is known about the effects of age on small intestinal sensory function. A recent study by Seno et al (1996) suggests that ageing is associated with an impairment of afferent nerve function in the rat small intestine. The maximum increase in superior mesenteric artery blood flow during intraduodenal administration of capsaicin (160 μM) or 0.1N hydrochloric acid was less in older (12-mo old) than young (2-mo old) rats.

The age-associated decline in gastrointestinal perception may be due to a reduction in afferent neuronal conduction within the gut. In the myenteric plexus in the guinea pig intestine, ageing is associated with a reduction in the number of neurons (Gabella 1989). Similarly, in humans the number of oesophageal myenteric neurons decreases by up to 62% between the ages 20-40 and 70 yr (Meciano Filho et al 1995). There is also a decline in conduction velocity (ie the time taken (msec) for cerebral evoked potentials to reach their peak after the onset of intragastric balloon distension) within visceral neurones with age in humans (Weusten et al 1994).

Given the observed changes in both small intestinal afferent nerve function and the perception of sensations arising from other regions of the gastrointestinal tract with ageing, it is likely that the effects of small intestinal nutrients on appetite may be abnormal in the elderly, and may contribute to the anorexia of ageing. In the study described in Appendix A, the effects of intraduodenal lipid and glucose infusion on appetite sensations, food intake and pyloric motility were compared in elderly and young healthy men. The results of this study suggest that the stimulation of fullness and satiation and possibly the inhibition of food intake by intraduodenal lipid infusion may be diminished in healthy older subjects. The reason for differences in perception of appetite and food intake between the glucose and lipid infusions in the subjects in this study could not be characterised as a control infusion was not included. The effects of intraduodenal saline (control), glucose and lipid infusions on appetite and food intake at a buffet meal were subsequently investigated in the study described in Chapter 9.

4.4.2 *Age-related changes in gastrointestinal motor function*

Impaired gastric accomodation, such as that observed in patients with functional dyspepsia (Mearin et al 1991, Tack et al 1998) or diabetes mellitus (Samsom et al 1995,

Samsom et al 1998), may contribute to increased perceptions of fullness, bloating and nausea in response to gastric distension. There is indirect evidence that ageing may be associated with an impaired receptive relaxation of the gastric fundus and accomodation. If so, this could contribute, in part, to the early satiation and increased inter-meal interval observed in the elderly (Horowitz et al 1984). For example, Kupfer et al (1985) assessed the rate of gastric emptying of a 500 ml diluted cordial in 14 healthy elderly (aged 66-86 yr) and 14 young (aged 21-28 yr) subjects using ultrasound, and reported that the amount of liquid in the proximal stomach at five minutes, which is likely to be dependent on the adaptive relaxation response of the fundus (see Chapter 3.3.1), was less in the elderly subjects. Similarly, Rashid et al (1990) reported that the intragastric volume 5 min after ingestion of a 500 ml orange drink was less in 7 healthy elderly (mean age 70 ± 1.6 yr) compared to young (mean age 23 ± 1.3 yr), 260 ± 17.9 ml vs 166.7 ± ml, respectively, suggesting that the 'early' phase of gastric emptying may be more rapid in the elderly. In contrast, Horowitz et al (1984) reported an approximately 12% greater retention of liquid in the stomach 10 min after ingestion of a 150 ml 10% D-glucose solution in 13 healthy elderly (aged 70-84 yr) compared to 22 young (aged 21-62 yr) subjects. The effects of ageing on fasting gastric compliance and the accomodation response to meal have not been evaluated. This is the subject of further investigation in the study described in Chapter 11.

Evidence for age-related changes in the rate of gastric emptying is conflicting (see Table 4.1). Early studies reported no difference in gastric emptying in healthy elderly when compared with younger subjects, using the relative insensitive technique of barium fluoroscopy (Van Liere & Northup 1941). Other studies which reported that gastric emptying was markedly slower with ageing included older subjects who had a significant medical disease, or were taking medication known to effect gastric motility (Evans et al 1981, Kupfer et al 1985, Johnson 1995). For example, Evans et al (1981) reported in 11 elderly (mean age 77 yr) compared to 7 young (mean age 26 yr) subjects, that mean emptying times (T50) of a drink were 132 min vs 50 min, respectively, but there was a high incidence of neurological disease in the older subjects.

Table 4.1: Published studies evaluating the effect of ageing on gastric emptying (GE).

Author	Young (sample size/ mean age/ gender)	Elderly (sample size/ mean age/ gender)	Meal ingested	Method to assess GE	Outcome for rate of gastric emptying
(Van Liere &Northup 1941)	N/A	12 healthy, 71 yr, M only	solid	Barium Fluoroscopy.	Elderly (E) = Young (Y)
(Evans & Campbell 1981)	7 healthy, 26 yr, 4M/ 3F	11 patients, 77 yr old, 10M/ 1F (8 with neurological disease)	nutrient liquid	Modified sequential scinti-scanning technique 99mTc-DTPA	T_{50} E < Y, (P<0.001)
(Moore et al 1983)	10 healthy, 31 yr old, M only	10 healthy 76 yr old, M only	solid/ liquid meal	Dual isotope	T_{50} E = Y_{solid} T_{50} E < Y_{liquid} (P<0.05)
(Wright et al 1983)	46 obese, 39.5 yr (range 20-65 yr) 31 age-, sex-matched nonobese, 40 yr (range 22-59 yr)		solid/ liquid meal	Dual isotope	T_{50} $E_{(>40\ yr)}$ = $Y_{(<40\ yr)}$
(Horowitz et al 1984)	22 healthy, 34 yr, 14M/ 8F	13 healthy, 77 yr 6M/ 7F	solid/ liquid meal	Dual isotope	T_{50} E < Y (P< 0.05)
(Kupfer et al 1985)	14 healthy, 24 yr, 12M/ 2F	14 patients, 79 yr, 7M/ 7F (11 neuro. disease)	liquid	Ultrasound	$T_{5\ min}$ E > Y (P< 0.05) GE_{total} NS.
(Wegener et al 1988)	21 healthy, 34 yr, 11M/10F	25 healthy, 82 yr, 10M/ 15F	solid/liquid meal	Lone isotope (imprecise technique)	T_{50} E < Y (P< 0.001)
(Wedman et al 1991)	30 healthy < 50 yr, 15M/ 15F	30 healthy > 50 yr, 15M/ 15F	high-fat liquid	Ultrasound	T_{50} Y_{female} < Y_{male} / E_{female} and E_{male}

Table 4.1 cont.

Author	Young (sample size/ mean age gender/)	Elderly (sample size/ mean age/ gender)	Meal ingested	Method to assess GE	Outcome for GE
(Madsen 1992)	17 healthy, ~ 24 yr, 9M/ 8F	16 healthy, 55-74 yr, 8M/ 8F	semi-solid-cellulose fiber / 2-3 mm plastic particles.	Dual isotope	Mean GE time E = Y
(Brogna et al 1999)	22 healthy, 30 yr, M only	18 healthy, 75 yr, M only	solid/ liquid meal	Ultrasound	GE_{total} E < Y ($P< 0.001$)
(Nakae et al 1999)	5 healthy, 23 yr, M only	6 healthy, 73 yr, M only	high-fat/ non-fat liquid meal	Electrical impedance tomography	GE_{total} E < Y for high-fat liquid only ($P< 0.05$)

M = male, F = female; Y = Young, E = elderly; T_{50} = 50% emptying time.

In most, but not all studies, gastric emptying has been reported to slow slightly, but not always significantly, with increasing age (Clarkston et al 1997, Horowitz et al 1984, Kupfer et al 1985, Madsen 1992, Wegener et al 1988, Nakae et al 1999, Brogna et al 1999). For example, Horowitz et al (1984) found that the T50 for solid meal, measured scintigraphically, was approximately 32% greater in healthy elderly (mean age 77 yr) than young (mean age 34 yr) subjects (Figure 4.3). Similarly, Wegener et al (1988) reported that the time taken for 50% of a mixed liquid/solid meal to empty from the stomach was 136 ± 13 min in elderly (mean age 82 yr) compared with 81 ± 4 min in younger (mean age 34 yr) subjects ($P<0.05$). In a recent study (Nakae et al 1999) gastric emptying of a 400 ml lipid soup, assessed using electrical impedance, was greater in 6 healthy elderly (mean age 73.3 ± 1.6 yr) than 5 young (mean age 23.0 ± 0.6 yr) men. Clarkston et al (1997) reported in healthy older (9 female, 5 male aged 70-84 yr) subjects who were less hungry and more satiated after a meal, than young (9 female, 10 male aged 23-50 yr) subjects, that postprandial hunger was inversely related to the rate of solid gastric emptying.

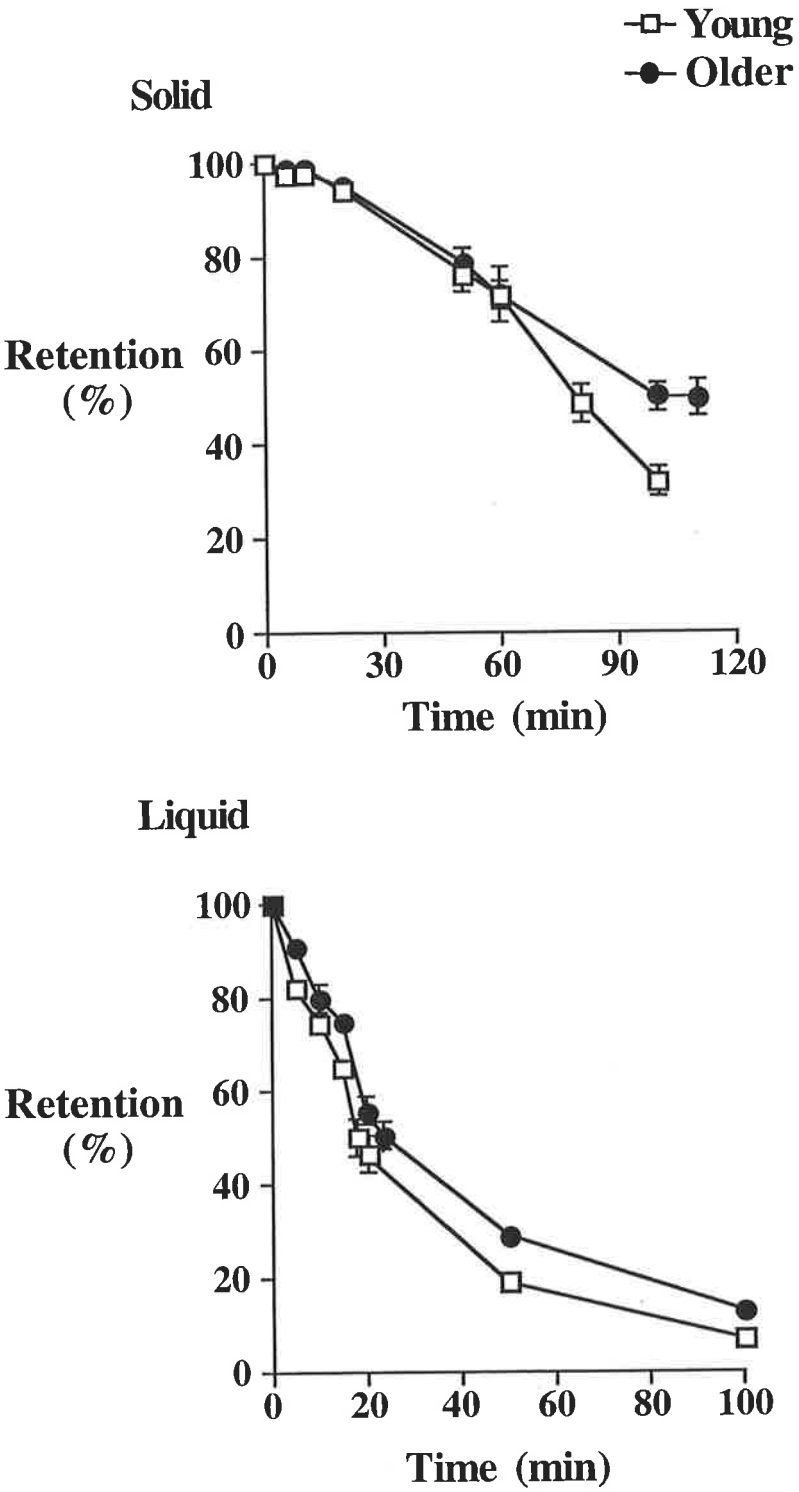

Figure 4.3: Gastric emptying curves for solid (100 g of ground beef) and liquid (150 ml 10% dextrose) in young (21-62 yr) and older (70-84 yr) healthy subjects (Horowitz et al 1984; redrawn with permission from Clinical Science). P <0.01 young vs older (solid); P< 0.05 young vs older (liquid).

The slowing of gastric emptying in the elderly may be a consequence of enhanced small intestinal nutrient-mediated feedback on gastropyloroduodenal motility. In the study reported in Appendix A, the stimulation of phasic and tonic pyloric motility in response to intraduodenal lipid infusion is enhanced in healthy elderly (aged 65-75 yr) compared to young (aged 20-34 yr) men. This enhanced pyloric motor response, may be mediated by changes in the release of gastrointestinal hormones which are known to retard gastric emptying (see Chapter 3.6 and section 4.3). Chapter 8 evaluates the effects of ageing on plasma cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) concentrations in response to intraduodenal lipid and glucose infusion, using blood samples collected during the study described in Appendix A. The relationships of both pyloric motility and sensations of fullness and hunger with plasma gastrointestinal hormone concentrations during intraduodenal nutrient infusion in young and older subjects are also evaluated in Chapter 8.

As discussed in Chapter 3.6.2, dietary modification affects the rate of gastric emptying, with chronic reduction in energy intake tending to slow gastric emptying. The slower rate of gastric emptying in the elderly may, therefore, be the consequence of the lower amounts of food consumed in their usual diet. The decreased food intake that occurs with ageing may be associated with a "vicious" cycle, whereby it slows gastric emptying which in turn leads to a further decrease in appetite and food intake as a result of increased duration of small intestinal nutrient exposure. Thus, increasing dietary energy intake in the elderly could potentially interrupt this cycle. The effects of dietary glucose supplementation on gastric emptying, gastrointestinal hormone release and appetite is evaluated in the study described in Chapter 13.

The age-related slowing of gastric emptying may be associated with changes in gastric pacemaker function, responsible for the rhythmicity of gastric motor activity both at fasting and in response to meal ingestion. Previous studies have attempted to evaluate gastric pacemaker function, using electrogastrography, in the elderly and suggest that there are only minor changes in gastric electrical activity with ageing (Riezzo et al 1991, Nishimura et al 1995, Pfaffenbach et al 1995, Parkman et al 1996). Riezzo et al (1991) found that fasting EGG frequency was similar, but power was lower in 30 elderly (aged 60-78 yr) compared to 30 young (aged 20-49 yr) healthy subjects. Nishimura et al (1995) reported that the frequency of the EGG during fasting in 12 healthy "older" (aged > 60 yr) was slightly, but significantly, greater compared to 13 "young" (aged < 60 yr) subjects; 3.3 cycles/min vs 3.0 cycles/min, respectively. Parkman et al (1996)

assessed both fasting and postprandial gastric myoelectrical activity in 83 subjects aged 20-79 yr. They reported that the postprandial increase in the dominant slow wave frequency was less in men aged ≥ 60 yr than in those < 60 yr. In contrast, Pfaffenbach et al (1995) reported no significant difference in either fasting or postprandial frequency or power of the EGG between older (10 men, 10 women, median ages 69 and 67 yr, respectively) and young (10 men, 10 women, median ages 28 and 25 yr, respectively) healthy subjects. Variations in the methods of analysis of EGG data (ie computerised analysis vs visual inspection) and EGG parameters (eg: frequency vs frequency ratio) used in these studies may have limited the overall interpretation of the EGG as subtle differences between older and young subjects may have been overlooked. As discussed in Chapter 3.5.2 small intestinal feedback modulates the EGG. No studies have assessed the effects of small intestinal nutrient infusion on gastric myoelectrical activity (frequency and power ratio) in healthy older persons. This is the subject of further investigation in Chapter 9.

As discussed earlier, dyspeptic symptoms (Pound & Heading 1998), increased postprandial fullness (Clarkston et al 1997) and early satiation (Rolls 1995) occur frequently in the elderly and may be associated with abnormalities of the EGG. The relationships between symptoms of fullness and satiation and food intake and the EGG frequency and power ratio during intraduodenal nutrient infusion are evaluated in Chapter 9.

Healthy ageing has little, if any, effect on small intestinal (Husebye & Engedal 1992, Fich et al 1989) or colonic (Clarkston et al 1997) motor function. Both orocecal and whole gut transit time are similar in healthy elderly and young subjects (Clarkston et al 1997, Wegener et al 1988). For example, Fich et al (1989) assessed both fasting and postprandial proximal gastrointestinal motility in 13 older (aged 40-65 yr) and 23 young (aged 18-39 yr) men and women who had presented with gastrointestinal symptoms in the absence of any identifiable disease. They found no difference in the periodicity, propagation velocity, maximal number of contractions in phase III of the MMC, or postprandial antral and jejunal motility between the two groups. This study, however, lacked a control group of healthy subjects, so the relevance of these to healthy ageing is unknown. Husebye & Engedal (1992) performed ambulatory small intestinal motility in 15 healthy elderly (aged 81-91 yr) and 19 healthy young (aged 26-35 yr), and reported that the migration velocity of the phase III was less in older than

young subjects (6.5 ± 0.8 cm/min vs 10.8 ± 1.2 cm/min) and older subjects also had more frequent small intestinal "propagated contractions".

4.5 EFFECTS OF AGEING ON HUMORAL FACTORS

Alterations in humoral factors may play a role in the reduction in appetite and food intake observed in the elderly, although this has not been proven. In this section what is known about the effects of age on cholecystokinin (CCK), peptide YY (PYY), amylin, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) activity is reviewed.

4.5.1 *Age-related changes in cholecystokinin (CCK)*

There is evidence that ageing affects CCK activity in both the central nervous system and the gut (see Chapter 3.6.1 for discussion of the role of CCK in appetite). The majority of evidence suggests that there is an increase in CCK release, at least in the periphery, in older animals, which may potentially contribute to the reduced appetite and food intake observed with ageing. Intestinal CCK concentrations are increased in old (3 yr) compared to young (1 yr) guinea pigs (Poston et al 1988); these elevated levels are accompanied by reduced pancreatic and gallbladder CCK receptor sensitivity. Ohta et al (1995) reported a significant increase in CCK concentration, but reduced CCK mRNA levels, in the cerebral cortex of old (24-29-mo old) compared to young (3-8-mo old) male Wistar rats. Miyasaka et al (1997) recently assessed the effects of the potent CCK stimulating agents, KCl and neuromedin C, on CCK release from central (cerebral cortex) and peripheral (duodenojejunum) tissues in older (25-29-mo old) and young (5-8-mo old) male and female Wistar rats. They reported that central CCK release in response to KCl and peripheral tissue CCK concentrations in response to neuromedin C were reduced in older compared to young male rats, whereas no such ageing effect was observed in the female rats.

Immuno-histochemical studies, in humans, have reported increased numbers of CCK immunoreactive cells in the duodenum of 60-69 yr old subjects compared to 20-29 yr old subjects (Sandstrom et al 1999a). There is evidence in humans that CCK release is also increased with ageing. Martinez et al (1993) found that fasting plasma concentrations of CCK-8, but not CCK-33, were higher in elderly (65-86 yr old) men and women with idiopathic senile anorexia (ie without any recognisable organic or

mental disease) compared to age-matched healthy control subjects (mean ± SD; 5.7 ± 2.6 pmol/L vs 3.1 ± 1.6 pmol/L; P< 0.05). There was also a trend for increased CCK concentrations in the cerebrospinal fluid in the anorectic patients (mean ± SD; 5.1 ± 3.2 pmol/L) compared to the control subjects (4.3 ± 1.9 pmol/L). Most studies involving healthy older humans have shown that their postprandial plasma CCK concentrations are higher than those of young adult subjects (Khalil et al 1985, Masclee et al 1988). For example, Khalil et al (1985) found that plasma CCK concentrations were significantly higher both at baseline (19.4 ± 1.7 pg/ml vs 12.0 ± 1.5 pg/ml) and following oral fat (1.5 ml/kg corn oil; ie ~3574 kJ for a 70 kg person) ingestion, in 15 older (60-84 yr) compared to 14 young (22-45 yr) healthy subjects (see Figure 4.4). Gallbladder contraction was similar in both groups. Similarly, Masclee et al (1988) reported similar fasting CCK concentrations (1.2 ± 0.2 pmol/L vs 1.5 ± 0.3 pmol/L), but an enhanced CCK response (214 ± 21 pmol/L/hr vs 153 ± 16 pmol/L/hr) to intraduodenal corn oil (60 ml within 2 min; ~2042 kJ) administration in 13 older (40-78 yr) compared to 26 young (21-39 yr) healthy men and women. In contrast, Berthelemy et al (1995) found that CCK levels were only increased in 7 older patients with malnutrition (mean age 85 yr) and not 7 healthy (mean age 80 yr) older compared to 7 healthy young (mean age 29 yr) subjects after oral administration of a 200 ml (1171 kJ; 40% CHO, 50% fat, 10% protein) preload. The discrepancy in the CCK response in healthy older subjects between the study by Berthelemy et al (1995) and the former two (Khalil et al 1985, Masclee et al 1988) studies, might be due to the much lower energy content (a difference of 870 or more kJ) and the mixed macronutrient vs 100% fat content of the preload that was administered in that study. The effect of a 2 hour intraduodenal lipid and glucose infusion on plasma CCK concentrations in older and healthy young subjects is investigated in Chapter 8.

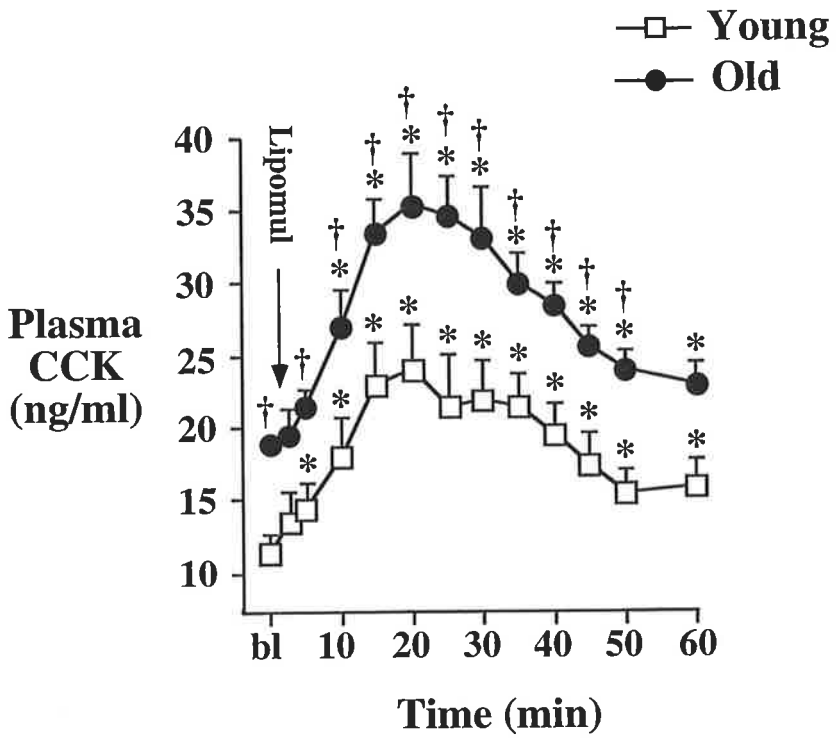

Figure 4.4: Plasma concentrations of CCK in response to oral corn oil (Lipomul; 1.5 ml/kg) in 14 young (22-42 yr) and 15 older (60-84 yr) subjects (Khalil et al 1985; redrawn with permission from Surgery). *P< 0.05 vs baseline (bl); †P< 0.05 older > young.

There is also evidence that the sensitivity to the satiating effects of CCK may increase with age. Voigt et al (1996) examined the effects of intraperitoneal injections of CCK-8 (8 and 40 μg/kg) on food intake under conditions of food-deprivation and fixed (meal) feeding conditions in young (2-mo old) and older (23-mo old) male Wistar rats. They reported that the suppression of food intake, following 16 hour food deprivation, was similar in young and older mice. A greater suppression of food intake by CCK (40 μg/kg) was, however, observed in the older than young rats under the fixed feeding regimen (Voigt et al 1996). It was suggested that the apparent age-related increase in sensitivity to CCK observed following the fixed feeding schedule reflected a decline in memory and learning in older rats, resulting in a reduced ability to adapt to the fixed feeding conditions. Silver et al (1988) also found that intraperitoneal CCK-8 injection (0, 5, 10, 20 and 40 μg/kg) suppressed 1 and 2 hr cumulative food intake in a dose-dependent manner and to a greater extent in old (25-mo old) than young (8-mo old) C57BL/6 Nnia mice (medium life span 27-mo) after 12 hour food deprivation (Silver et al 1988) (Figure 4.5). Plasma levels of CCK were not measured in either of these studies, so that the possibility of a decline in clearance of CCK with ageing may have contributed to the greater suppressive effects of exogenous CCK on food intake in older animals. The effects of exogenous CCK on food intake have not been examined in healthy elderly humans. The effects of intravenous infusion of CCK-8 on appetite and food intake in healthy older and young adult humans is examined in Chapter 10.

As discussed in Chapter 2.3.4, there is evidence that the interaction of leptin with cholecystokinin is important in the inhibition of food intake. The effects of exogenous CCK on plasma leptin concentrations in young and older subjects are also evaluated in Chapter 10.

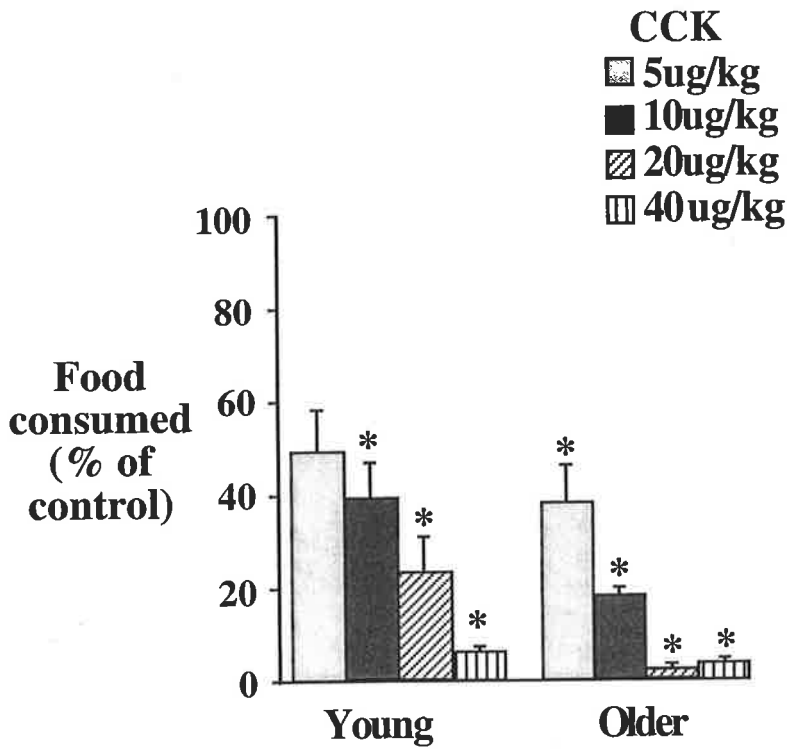

Figure 4.5: Cumulative food intake (% compared to control) during the first 60 min after intraperitoneal injection of CCK (5, 10, 20 and 40 ug/kg) in young (8-mo old) and older (25-mo old) mice (Silver et al 1988). *P <0.05 vs control.

4.5.2 *Age-related changes in insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) and blood glucose homeostasis*

Human ageing is associated with disturbances in insulin metabolism. There is evidence that there is an age-related increase in fasting and post-prandial circulating insulin concentrations (Fraze et al 1987, Colman et al 1995). As discussed in Chapter 3.6.6, insulin may play a role in regulating satiation in conjunction with other peptides or hyperglycaemia. The age-associated increases in insulin concentrations are, however, mainly a reflection of insulin resistance, at least in part resulting from increased total body and central adiposity, and only to a small extent to ageing itself (Colman et al 1995). It, therefore, seems unlikely that insulin contributes substantially, if at all, to the anorexia of ageing.

Decreased insulin secretion as well as increased insulin resistance may contribute to the high prevalence of impaired glucose tolerance (hyperglycaemia) and type 2 diabetes mellitus associated with ageing (Muller et al 1996). Aberrations in both early and late phase insulin responses to intravenous glucose are evident in older compared to young subjects (Bourey et al 1993). The reduction in insulin secretion and activity in the elderly has been attributed to decreased responsiveness of the pancreatic β- cell to glucose (Scheen et al 1996). Reduced insulin activity in the elderly which may lead to hyperglycaemia, may in turn, influence appetite (see Chapter 3.7), although this has not been evaluated.

The impairment in blood glucose homeostasis with ageing may potentially reflect changes in the release and/or sensitivity to the incretin hormones, glucagon-like peptide-1 (GLP-1) (see Chapter 3.6.2) and glucose-dependent insulinotrophic polypeptide (GIP) (see Chapter 3.6.3). Little is known about the effects ageing on the activity of GLP-1 and GIP. Ranganath et al (1998) have reported that fasting and total integrated plasma GLP-1 and GIP concentrations in response to a 100 g oral carbohydrate load were significantly higher in 6 postmenopausal (mean age 67 yr) than 6 premenopausal (mean age 23 yr) women (Ranganath et al 1998). Meneilly et al (1998) also showed that at a blood glucose concentration ~5.4 mmol/L, the stimulation of insulin secretion by intravenous infusion of GIP was less in 16 older (aged 67-79 yr) than 15 young (aged 16-25 yr) subjects. The blood glucose and plasma insulin responses to exogenous GLP-1 infusion have not been assessed in the elderly. Alterations in GIP, and particularly, GLP-1 may potentially influence appetite indirectly

by slowing gastric emptying (see Chapter 3.6.2 and 3.6.3) in the elderly, although there is no evidence for this concept at present. The effects of ageing on plasma insulin, GLP-1, GIP and blood glucose concentrations during intraduodenal infusions of glucose and lipid are evaluated in Chapter 9.

In healthy young subjects the glycaemic response to an oral glucose load is modified by dietary glucose supplementation (see Chapter 3.5.2.1), concordant with adaptive acceleration of gastric emptying of glucose (Horowitz et al 1996). Dietary supplementation may potentially modify the rate of gastric emptying and the glycaemic response to an oral glucose load in healthy elderly subjects. This is a subject of further investigation in Chapter 13.

4.5.3 *Peptide YY (PYY)*

The role, if any, of PYY in the regulation of appetite is uncertain (see Chapter 3.6.4). Little is known about the effects of ageing on PYY. An increase in PYY-containing colonic cells with ageing has been reported in male rats (Sweet et al 1996). Similarly, Sandstrom et al (Sandstrom et al 1999c) observed an increase in number of immunoreactive PYY cells measured in the tissue samples from the colon of 12- and 24-mo old mice than in 3-mo old mice, but there was no difference in the cell secretory index (μm^3 per cell) between the different age groups. In contrast, in a similar study in humans, performed by the same group, there was no difference in number of PYY cells or cell secretory index (μm^3 per cell) measured in tissue samples from the colon between 20-29, 40-49 and 60-69 yr old subjects (Sandstrom et al 1999). These findings suggest that age does not affect PYY endocrine cells of the human large intestine. There are, however, no studies in humans investigating the influence of ageing on postprandial plasma PYY activity *in vivo*. The effect of intraduodenal lipid on PYY release, and the relationships between its release and pyloric motility and food intake in elderly and young healthy subjects are examined in Chapter 8.

4.5.4 *Amylin*

Morley et al (1993) have reported that mice retain their sensitivity to the satiating effects of amylin across the life span (Morley et al 1993) and that plasma amylin concentrations, both fasting and in response to an oral glucose load, increase between middle and old age in humans (Edwards et al 1996a). Other studies have confirmed that basal and glucose-stimulated plasma amylin concentrations are no different between young and elderly adults (Mitsukawa et al 1992), and may be even lower in older

people (Dechenes et al 1998). These findings suggest that increasing amylin activity may not contribute to the anorexia of ageing. There is, however, currently no information relating to the effects of exogenous administration of amylin on food intake in elderly humans, therefore an age-related increase in the sensitivity to the suppressive effects of amylin cannot be excluded.

4.6 CONCLUSIONS

There is now convincing evidence to support the concept of a "physiological anorexia of ageing". While the underlying mechanisms are poorly defined, there is evidence that reduction in the endogenous opioid feeding drive, and neuropeptide Y (NPY) and nitric oxide (NO) activity and subtle alterations in oropharyngeal and gastric sensation, gastric emptying and the release of gut peptides which regulate appetite may be involved. Much of this evidence has, however, been derived from animal studies, and human data is both limited and inconsistent. In this thesis some of the possible mechanisms, proposed on the basis of animal data, which are likely to play a role in the physiological 'anorexia of ageing' are evaluated in humans. Specifically, the aim was to evaluate the effects of healthy ageing on;

(1) the stimulation of CCK, GLP-1 and PYY by intraduodenal lipid and glucose infusion and relationships between appetite, food intake and pyloric motility with the plasma concentrations of these hormones (Chapter 8).

(2) appetite, gastrointestinal hormone release and gastric electrical activity in response to intraduodenal saline, glucose and lipid infusions (Chapter 9).

(3) appetite and food intake in response to intravenous infusion of CCK-8 (Chapter 10).

(4) gastric sensations during gastric distension, fasting gastric compliance, and gastric accomodation in response to a meal (Chapter 11).

(5) appetite and food intake in response to intravenous infusion of the opioid antagonist naloxone (Chapter 12).

(6) dietary energy intake, appetite, gastric emptying and postprandial blood glucose homeostasis in response to dietary glucose supplementation (Chapter 13).

CHAPTER 5

Postprandial Hypotension in the Elderly.

5.1 INTRODUCTION

Postprandial hypotension, defined as a decrease in systolic blood pressure of 20 mmHg or more within 2 hours of the start of a meal (Jansen & Hoefnagels 1991, Jansen & Lipsitz 1995, Mathias et al 1989, Mathias 1991), is now recognised as an important clinical problem, since it can lead to syncope and falls in affected individuals. Although symptoms can be exacerbated by postural change, postprandial hypotension, in contrast to postural or orthostatic hypotension, also occurs in the supine position. Those particularly at risk include the elderly and patients with autonomic dysfunction (the latter is most commonly a result of diabetes mellitus). The mechanisms responsible are poorly defined, but impaired regulation of splanchnic blood flow and the release of gastrointestinal hormones are likely to be important. The purpose of this chapter is to briefly review current knowledge of the epidemiology, clinical significance, pathophysiology and treatment of postprandial hypotension.

5.2 EPIDEMIOLOGY AND CLINICAL SIGNIFICANCE OF POSTPRANDIAL HYPOTENSION

A significant reduction in blood pressure after ingestion of food was first described by Gladstone et al in 1935 in a hypertensive patient. However, it was not until 1977, when Seyer-Hansen (1977) reported a patient with Parkinson's disease who had symptoms of postprandial hypotension, that it was recognised as a clinical problem. Since then, a number of studies have shown that postprandial hypotension is a common disorder, more so than orthostatic hypotension, in the elderly. For example, Fagan et al (1990) measured blood pressure and heart rate for 1 hour before, and 2 hours after, a meal (3304 kJ; 52% carbohydrate, 29% fat, 19% protein) in 82 normotensive and hypertensive individuals aged 19-79 yr, and reported a significant relationship between the reductions in systolic blood pressure and age. Vaitkevicius et al (1991) reported a mean reduction in systolic blood pressure of 17.9 ± 15.5 mmHg within 75 min of a meal (2718 kJ; 65% carbohydrate, 20% fat, 15% protein) in 109 elderly (aged 65-96 yr) nursing home residents in Baltimore, USA; in 41 (38%) of these the magnitude of the reduction was greater than 20 mmHg. In a larger, more recent, cohort study, Aronow & Ahn (1994) evaluated the mean maximal decrease in systolic and diastolic blood pressure following their usual lunch-time meal in 499 elderly (aged 62-100 yr, 145 men and 354 women) ambulatory (68%) and wheelchair-bound (32%) residents of a long term care facility. They reported that in 118 (24%), the maximal decrease in postprandial systolic blood pressure was ≥ 20 mmHg. In former two studies, the meals administered to the subjects were standardised for energy and macronutrient content, whereas in the study by Aronow & Ahn (1994), patients ate their usual lunch-time meal and so the energy and macronutrient (in particular, carbohydrate) content, although not evaluated, would have varied between individuals. It is not known, therefore, whether this may have influenced (ie possibly under-estimated) the prevalence of postprandial hypotension in the patients in this study.

A number of age-related disorders appear to be associated with postprandial hypotension, including hypertension, renal failure treated with haemodialysis and in particular, autonomic dysfunction, which may or may not be associated with diabetes mellitus or Parkinson's disease [for review see (Jansen & Lipsitz 1995)]. In young and middle-aged persons, hypertension may also be associated with postprandial hypotension (Fagan et al 1986, Jansen & Hoefnagels 1989). Healthy ageing is also associated with an increased propensity for a decline in blood pressure following a meal (Lipsitz & Fullerton 1986, Pietzman & Berger 1989, Potter et al 1989). For example,

Pietzman & Berger (1989) who assessed seated and standing blood pressure and heart rate before and after both a standard breakfast and before and after ingestion of water (control) in 16 active community-dwelling older (mean age 82 yr) and 8 healthy young (mean age 35.5 yr) subjects, reported that in the elderly, but not the young, there was a significant fall in systolic and diastolic blood pressure after the meal.

The decline in blood pressure after a meal together with other factors, such as medications, performance of the Valsalva manoeuvre during voiding or straining at stool, or postural change, can predispose the elderly to syncope, falls, nausea, ischaemic cardiac pain, vision changes, and disturbed speech [for review see (Jansen & Lipsitz 1995)]. Jonsson et al (1990) reported that meal-induced falls in blood pressure are significantly greater in elderly institutionalised patients who had experienced falls than in those who had not. The clinical importance of postprandial hypotension in the elderly was supported by the results of a prospective study of 499 long term care residents, over a period of 1-36 months in which the postprandial decrease in systolic blood pressure was an independent risk factor for falls, syncope, new coronary events, new stroke and total mortality (Aronow & Ahn 1997) (Table 5.1).

Table 5.1: Mean maximal fall in postprandial systolic blood pressure (BP) in 499 older persons with and without new falls, new coronary events, new stroke and total mortality at 29-month follow-up.

Type of event	Fall in BP in persons with new event (mmHg)	Fall in BP in persons without new event (mmHg)	*P* value
Falls	20 ± 5	12 ± 4	< 0.001
Syncope	23 ± 5	14 ± 5	< 0.001
Coronary Events	18 ± 6	14 ± 5	< 0.001
Stroke	21 ± 6	15 ± 5	< 0.001
Total Mortality	17 ± 6	15 ± 5	< 0.001

5.3 FACTORS INFLUENCING THE MAGNITUDE OF THE POSTPRANDIAL FALL IN BLOOD PRESSURE.

Several factors influence the magnitude of fall in blood pressure after a meal. The position of the body during meal ingestion appears to be important; while postprandial hypotension can occur in both the sitting and supine positions, prolonged sitting seems to have the greatest effect on postprandial blood pressure (Jansen & Lipsitz 1995), probably as this position induces the greatest increase in splanchnic pooling (Jansen & Lipsitz 1995). The magnitude of postprandial hypotension is also dependent on the meal composition; carbohydrate, particularly glucose has the greatest effect (Potter et al 1989, Jansen et al 1990, Waaler & Eriksen 1992, Sidery et al 1991), since carbohydrates stimulate the greatest postprandial increase in vasodilator hormones, insulin and incretin hormones compared to fat and protein (Jansen & Lipsitz 1995). For example, Jansen et al (1990) reported that ingestion of an oral glucose load (75 g in 300 ml water; ~1227 kJ) by 10 hypertensive elderly subjects (aged 70-80 yr) resulted in a

decrease in mean arterial pressure of 14 ± 2 mmHg ($P < 0.001$ vs control), whereas isovolumetric, isoenergetic solutions of fat and protein had no significant effect on postprandial blood pressure compared to 300 ml of water (control) (Figure 5.1). Furthermore, it has been demonstrated in young healthy subjects that a large meal induces a greater fall in mean arterial blood pressure and peripheral vascular resistance and a greater increase in cardiac output than a smaller meal. This is most likely due to a greater increase in splanchnic blood flow after the large meal (Waaler & Eriksen 1991). Meal temperature may also effect the blood pressure response, although the mechanisms responsible for this effect have not been established. Kuipers et al (1991) reported in 15 healthy elderly subjects (mean age 74 yr) that blood pressure was not affected by ingestion of a chilled glucose solution, whereas it fell significantly after ingestion of glucose solutions which were either at, or greater than, room temperature. It was speculated that a visceral circulatory vasoconstriction response mediated by cold receptors may have prevented the fall in blood pressure gut after ingestion of the chilled glucose solution (Kuipers et al 1991). Conversely, the possibility that ingestion of a warm glucose solution, or glucose at room temperature, activated visceral receptors for warm fluids and induced splanchnic vasodilation could not be excluded (Kuipers et al 1991).

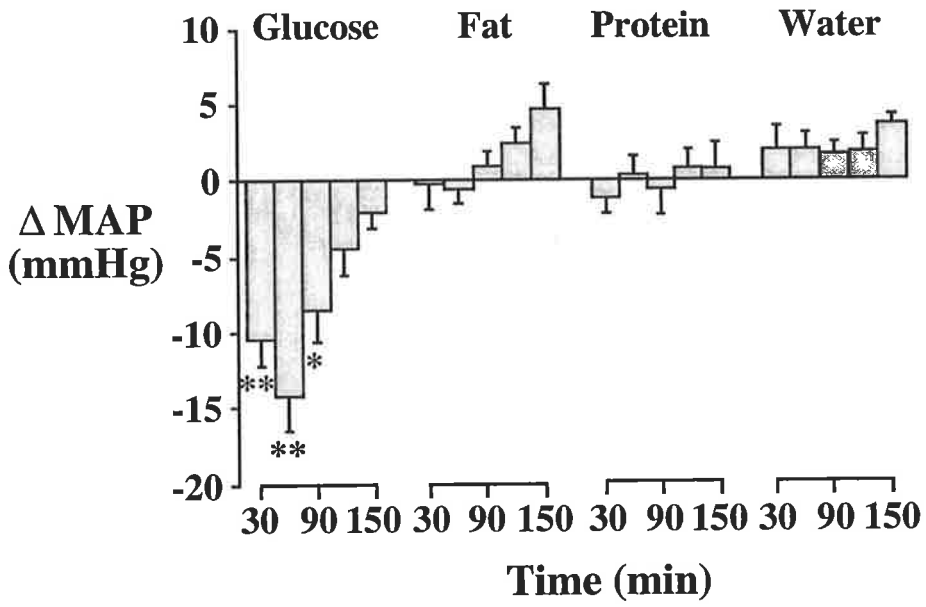

Figure 5.1: Changes of mean arterial pressure (MAP) over a 150 min time period after ingestion of four test solutions ie. glucose, fat, protein and water. *P<0.01 and **P<0.001 when compared with baseline values (Jansen et al 1990).

5.4 NORMAL HORMONAL, NEURAL AND CARDIOVASCULAR RESPONSES TO FOOD INGESTION.

In healthy persons meal ingestion is associated with a number of hormonal, neural and cardiovascular effects. Blood is shunted from the periphery to the gut so that there is a marked increase in splanchnic, and a doubling of superior mesenteric artery blood flow [for review see (Mathias 1991, Jansen & Lipsitz 1995)]. This reduction in intravascular blood volume causes a decrease in systemic blood pressure. There is also secretion of pancreatic and gastrointestinal hormones, some of which (particularly, insulin, gastrin, substance P, neurotensin, calcitonin gene-related peptide (CGRP) and cholecystokinin (CCK)) have vasodilatory effects. Administration of the somatostatin analogue, octreotide, which inhibits the release of these gastrointestinal peptides and modifies splanchnic blood flow, attenuates the postprandial fall in blood pressure in normo- and hypertensive elderly subjects (Jansen et al 1989). Normally systemic blood pressure remains virtually unchanged after a meal despite these changes, as a result of a compensatory increase in heart rate, stroke volume and cardiac output via activation of vascular baroreceptors (Jansen & Lipsitz 1995) as well as an increase in peripheral vascular resistance due to vasoconstriction, mainly mediated by an increase in muscle sympathetic nerve activity (Berne et al 1989). There is an increase in both plasma noradrenaline and renin in response to a meal, indicative of the increase in sympathetic nerve activity (with no change in plasma adrenaline concentrations) (Jansen et al 1995). These compensatory changes, referred to as the "baroreflex response", maintain normal systemic blood pressure after a meal.

5.5 MECHANISMS INVOLVED IN POSTPRANDIAL HYPOTENSION

While the mechanisms responsible for postprandial hypotension are poorly defined, several factors are likely to play a role [for review see (Mathias 1991, Jansen & Lipsitz 1995)]. An impairment in the baroreflex-mediated cardiovascular response to meal ingestion probably contributes to postprandial hypotension in the elderly. The technique of spectral analysis has shown that healthy ageing is associated with impairment in both baroreflex and parasympathetic vagal modulation of heart rate, with relatively greater loss of the parasympathetic component (Lipsitz et al 1990). An inadequate sympathetic response to meal-induced splanchnic vasodilation may also contribute to postprandial hypotension. For example, Haigh et al (1991) reported that the rise in plasma noradrenaline after a meal was less in elderly patients with postprandial hypotension

than in 8 fit, untreated, elderly hypertensive subjects. The increase in muscle sympathetic nerve activity in response to oral glucose ingestion is also attenuated in the elderly, particularly in those with insulin resistance (Fagius et al 1996). In patients with autonomic failure administration of the noradrenaline precursor, 3,4-DL-threo-dihydroxyphenylserine (DL-DOPS), attenuates the postprandial fall in blood pressure and increases both plasma noradrenaline and forearm vascular resistance (Freeman et al 1996).

A role for insulin in postprandial hypotension has been suggested, particularly in view of the relatively greater effects of oral glucose than other macronutrients on postprandial blood pressure (Potter et al 1989, Jansen et al 1990, Waaler et al 1992, Sidery et al 1991). In diabetic patients with autonomic neuropathy intravenous infusion of insulin decreases blood pressure, sometimes resulting in syncope (Brown et al 1986, Mathias et al 1987). It has been suggested that the vasodilatory effects of insulin may be responsible for this effect, since insulin infusion in the, absence of hyperglycaemia, stimulates sympathetic nervous activity and produces forearm vasodilation in young normal subjects (Anderson et al 1991, Lembo et al 1993). Studies in the elderly and in patients with autonomic dysfunction, however, have failed to show a relationship between the increase in plasma insulin concentration and the decrease in blood pressure after oral glucose ingestion (Jansen & Hoefnagels 1987, Lipsitz & Fullerton 1986, Jansen & Hoefnagels 1989). Compared to oral glucose ingestion, intravenous glucose infusion has relatively little effect on blood pressure in hypertensive, but not in elderly subjects without hypertension (probably due to an impairment in the activation of the sympathetic nervous system) which argues against a role for insulin (Verza et al 1988). The role of insulin is therefore, unlikely, but it has not been excluded.

Other vasoactive gastrointestinal peptides have been implicated in the hypotensive response to a meal. As discussed, this concept is supported by a study which showed that intravenous infusion of the long-acting somatostatin analogue, octreotide, which inhibits the release of vasodilatory peptides and decreases splanchnic blood flow attenuates the fall in blood pressure after oral glucose ingestion in hypertensive elderly patients (Jansen & Hoefnagels 1989) and in patients with autonomic dysfunction (Hoeldtke et al 1986, Mathias et al 1989). Observations that plasma concentrations of vasoactive intestinal polypeptide (VIP), substance P, gastrin, motilin and cholecystokinin are not affected by ingestion of glucose in the elderly and patients with autonomic failure, despite significant decreases in blood pressure (Jansen et al 1990,

Hoeldtke et al 1986, Mathias et al 1989) argues against a role for these hormones. In contrast studies have shown that plasma levels of neurotensin, which exert potent hypotensive actions when infused intravenously, possibly as a result of an increase in superior mesenteric artery blood flow, are affected by ageing (Mathias et al 1989). For example, the increase in plasma neurotensin concentrations after a meal is greater in elderly patients with autonomic failure and postprandial hypotension than young healthy subjects (Mathias et al 1989). A more recent study by Edwards et al (1996b) assessed plasma calcitonin gene-related peptide (CGRP) (which is released in response to carbohydrate loading) concentrations, blood pressure and heart rate during a standard oral glucose tolerance test in 29 community-dwelling individuals aged 20-83 years. They reported that 5 (4 of whom were > 60 yr) subjects exhibited a postprandial fall in blood pressure greater than 15 mmHg and there was a positive relationship between the changes in CGRP levels and the fall in blood pressure ($r = 0.39$, $P = 0.037$) in these subjects. The relationship of CGRP and blood pressure change was not present, however, in young or middle aged individuals (Edwards et al 1996b). These results suggest that CGRP may play a role in the pathogenesis of postprandial hypotension.

Blood pressure falls almost immediately after a meal containing carbohydrate, with the maximum response at 30-60 min, which approximates the postprandial peak in blood glucose. In both healthy subjects and patients with diabetes the rate of gastric emptying has a major effect on the blood glucose and insulin response to an oral glucose load (Horowitz et al 1993, Horowitz et al 1996, Jones et al 1996). For example, the more rapid gastric emptying of glucose induced by dietary glucose supplementation in young, healthy volunteers is also associated with significant changes in the glycaemic response to oral glucose (Horowitz et al 1996) (see Chapter 3.6.2 for detailed discussion). These observations suggest that there may be a relationship between the postprandial fall in blood pressure and the rate of gastric emptying.

It is well recognised that disordered gastric emptying occurs frequently in conditions associated with an increased prevalence of postprandial hypotension, including diabetes mellitus (Horowitz et al 1994), autonomic failure (Horowitz et al 1991), and in unwell older persons (Andrews & Horowitz 1996). Jones et al (1998) recently reported in a cross-sectional study that there is a significant relationship between the magnitude of the fall in systolic blood pressure after ingestion of 75 g glucose and the rate of gastric emptying in patients with recently diagnosed type 2 diabetes mellitus, but not in young healthy subjects (possibly because the fall in blood pressure was less in young

subjects); ie the faster the stomach emptied the greater the fall in blood pressure in the diabetic patients (Figure 5.2). This observation suggests that the rate of gastric emptying may also be a determinant of the magnitude of the meal-induced fall in blood pressure in healthy elderly persons. Slowing gastric emptying and thus slowing the rate of absorption of glucose from the small intestine, by dietary or pharmacological means, may potentially reduce postprandial hypotension.

Guar gum is a naturally occurring, gel-forming carbohydrate of vegetable origin (Blackburn et al 1984) which has been shown to slow both gastric emptying and small intestinal glucose absorption in humans (Blackburn et al 1984). Chapter 14 evaluates the effect of guar gum on the postprandial glucose-induced fall in blood pressure in healthy elderly subjects and the relationships between the rate of gastric emptying, small intestinal glucose absorption, blood glucose and plasma insulin concentrations and the postprandial fall in blood pressure.

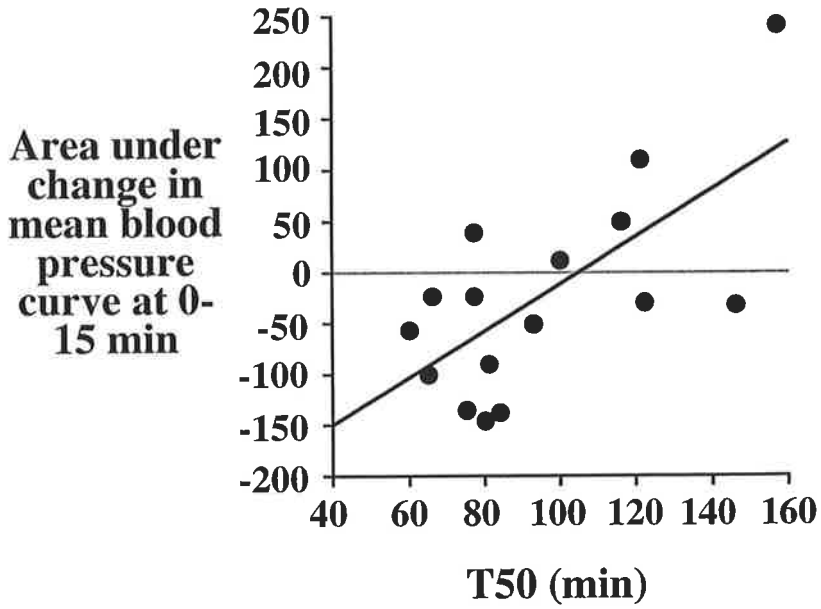

Figure 5.2: Relationship between the area under change in mean blood pressure curve at 0 and 15 min and the 50% emptying time (T50) for gastric emptying of 75 g glucose in patients with non-insulin-dependent diabetes mellitus (NIDDM) ($r=0.67$, $P< 0.005$) (Jones et al 1998; redrawn with permission from Clinical Science).

5.6 POSSIBLE TREATMENTS FOR POSTPRANDIAL HYPOTENSION

Current treatment options for symptomatic postprandial hypotension are limited and less than optimal [for review see (Jansen & Lipsitz 1995)]. A number of behavioural changes have been recommended to prevent or reduce symptoms of postprandial hypotension in the elderly. Reducing the size of meals may help. Since carbohydrate appears to have the greatest effects on postprandial blood pressure, individuals with symptomatic postprandial hypotension may benefit from consumption of low carbohydrate, high protein meals (Jansen & Lipsitz 1995). Since body position affects the magnitude of the fall in blood pressure, resting in a supine position after a meal may be beneficial (Jonsson et al 1990). Dehydration may predispose to postprandial hypotension and should be corrected (Jansen & Lipsitz 1995).

A number of pharmacological agents have been used to treat postprandial hypotension, with varied success. There is some evidence that treatment of hypertension, with nitrendipine (calcium channel blocker) or hydrochlorothiazide (thiazide diuretic) diminishes the fall in blood pressure after glucose ingestion in elderly hypertensive patients (Jansen et al 1988). Furthermore, the meal-induced fall in blood pressure was attenuated in elderly patients after treatment with the calcium channel blocker, nicardipine (Connelly et al 1995). The patients in both these studies were, however, asymptomatic. It is not known whether antihypertensive therapy results in improvement in patients with symptomatic postprandial hypotension.

As discussed, the somatostatin analogue, octreotide, has been shown to inhibit the postprandial fall in blood pressure in hypertensive elderly persons and patients with autonomic failure, although no studies have investigated its effects on blood pressure in elderly patients with symptomatic postprandial hypotension [for review see (Jansen & Lipsitz 1995)]. Treatment with, octreotide, is however expensive, requires frequent, uncomfortable subcutaneous injections and its use can be associated with adverse effects, such as diarrhoea and the formation of gall stones. Long-acting orally administered preparations of octreotide are now available, but these agents have not been used to treat postprandial hypotension.

Other pharmacological agents such as indomethacin, diphenhydramine, cimetidine, dihydroergotamine, denopamine, midodrine and vasopressin have been evaluated for the treatment of postprandial hypotension, with varying results [for review see (Jansen

& Lipsitz 1995)]. Interpretation of the results of these studies is limited by small patient numbers. For example, the meal-induced fall in blood pressure in 6 patients with severe autonomic failure was attenuated by administration of indomethacin, but not diphenhydramine or cimetidine (Robertson et al 1981). Dihydroergotamine had no effect on postprandial hypotension in patients with autonomic failure (Hoeldtke et al 1991). Denopamine (β_1-adrenergic agonist) and midrodine (α_1-adrenergic agonist) have been shown to reduce the postprandial fall in blood pressure in patients with autonomic failure when administered in combination, but not when each was given alone (Hirayama et al 1993).

The use of caffeine for the treatment of postprandial hypotension is often recommended, since it is widely available, convenient, inexpensive and relatively non-toxic. The results of studies evaluating the effects of caffeine of postprandial blood pressure have been inconsistent. A number of studies have reported that caffeine attenuates the fall in blood pressure following a meal in the elderly and in patients with autonomic failure who are asymptomatic [for review see (Jansen & Lipsitz 1995)], whereas others have shown no effect of caffeine administration in patients with symptomatic postprandial hypotension (Kamata et al 1994, Armstrong et al 1990). The discrepancy in these findings may be attributed to the different subject groups studied ie. asymptomatic vs symptomatic elderly. Caffeine administration may only prevent the fall in blood pressure after meal ingestion in persons with 'mild', asymptomatic postprandial hypotension.

5.7 CONCLUSIONS

Postprandial hypotension is an important clinical problem affecting the elderly and, particularly, patients with autonomic failure. Possible pathophysiological mechanisms involved in postprandial hypotension include impairments in regulation of sympathetic nervous activity or baroreflex response, splanchnic blood flow and increased release of gastrointestinal vasodilatory hormones. There is recent evidence that the rate of gastric emptying may be an important determinant of the postprandial fall in blood pressure. The effect of guar gum on the rate of gastric emptying, plasma insulin, small intestinal glucose absorption and blood pressure is examined in healthy older subjects in Chapter 14.

CHAPTER 6

Assessment of Appetite, Energy Intake and Nutritional Status

6.1 INTRODUCTION

A number of techniques can be used to evaluate feeding behaviour and nutritional status in humans. In this Chapter the benefits and limitations of some of these methods, including those used by the author in the studies described in Chapters 8-15, are addressed. These methods can be divided into four categories: (i) assessment of dietary intake, (ii) assessment of appetite and energy intake, (iii) psychological/behavioural questionnaires, and (vi) assessment of nutritional status

6.2 ASSESSMENT OF DIETARY INTAKE

The four methods most commonly used to assess dietary food intake are dietary recall (6.2.1) including 24-hr recall, diet history, food frequency questionnaires, and dietary food records (6.2.2). None of these methods is ideal, because of the inherent potential for underreporting, inaccuracy, subject awareness of the quantification of food intake, and the burden on participants during usual daily activities (Omran & Morley 2000a). An efficient and reliable method of dietary intake assessment was required to compare dietary energy intake in young and older healthy subjects in the studies presented in Chapters 8-14. For reasons discussed below the diet food record was considered the most appropriate and convenient method.

6.2.1 *Dietary recall methods*

There are currently three main types of dietary recall methods that may be used to assess food intake; so called 24 hr recall, diet history, and food frequency questionnaire [review see (Omran & Morley 2000a)].

6.2.1.1 24 hr recall

The 24 hr recall method requires participants to recall the type and amount of foods consumed during the preceding 24 hours. This method is commonly used in professional, dietetic practise, since participants are not burdened by the need to record food intake during daily activities. The accuracy of 24 hr recall, however, is limited, since it is largely reliant on the subjects' memory. Day-to-day variations in the amount and choice of foods are also not taken into account. Validation studies have shown the in contrast to the weighed food record method (see below), estimated intake of nitrogen (N), potassium (P) and beta-carotene by the 24-hr recall method does not relate closely to 24-hr urine N, K and beta-carotene (Bingham 1994). The use of 24-hr recall has also

likely to be less reliable in older persons, as short-term memory tends to decline with age (Caliendo 1981, Omran & Morley 2000a), and cannot be used in individuals with dementia. For these reasons the technique was not used by the author.

6.2.1.2 Diet history

The evaluation of diet history involves a comprehensive review of dietary habits, including food preferences, the number and frequency of meals, portions and sizes of foods, ability to prepare food, place of preparation and consumption, and the number of other people present during food consumption during the preceding 7 days (Omran & Morley 2000a). This method is time consuming, critically dependent on the memory of the participant and necessitates the involvement of a highly trained professional to interview the participants. Diet history can, however be used as a standard criterion to validate other methods of dietary assessment (Omran & Morley 2000a).

6.2.1.3 Food Frequency Questionnaire (FFQ)

The food frequency questionnaire (FFQ) was devised to determine dietary intake over an extended period of time (eg. 1 yr). The original food frequency questionnaire, which takes ~ 1 hour to complete, includes about 100 questions relating to specific food items and asks subjects to recall 'average use of items' in a year (Hu et al 1999, Block et al 1986). Revised versions focus on key nutrients, thus limiting the number of questions to 20, and are reportedly as sensitive as the longer form [for review see (Omran & Morley 2000a)]. Food intake is estimated by computer analysis [for review see (Omran & Morley 2000a)]. This method is useful in establishing eating patterns, and food choices, but tends to provide little information about either the amount of food consumed or the variation in daily food intake (Omran & Morley 2000a). Larkin et al (1989) showed, in 228 men and women aged 24-51 yr, that mean food and nutrient intake estimated from the FFQ was consistently (and significantly) higher than the intake estimated from 16 days of dietary recall and records collected over the course of one year. The use of the FFQ to compare dietary intake between older and young subjects, as in the studies reported in Chapters 8-13, is also limited by the reliance on participant memory.

6.2.2 *Dietary food record*

Subjects are required to record the type and amount, as estimated by weight, of all food and beverages consumed during a set period of time. Since there are often large variations in a food intake between days, especially between Monday-Friday and

Saturday and Sunday (weekend), recording food intake for a period of 7-days is optimal; failing that the time period should include days both between Monday-Friday and either Saturday or Sunday. The record of food intake is less accurate in the later days of a 7-day recording period, when compared to records collected in the first few days (Gersovitz et al 1978). Food records of less than 7 days (ie 3-5 days), provide a comparable estimate of dietary intake (particularly if at least one weekend day is included) and there is less chance of inaccuracy due to declining subject compliance (Gersovitz et al 1978). A major advantage of the diet food record is that it is less reliant on the memory of participants, as the amount and type of food is recorded close to the time at which it is consumed. Some studies have indicated that diet diaries may underestimate food intake in particular groups, such as restrained eaters (Bathalon et al 2000, Lafay et al 1997) and obese (Mertz et al 1991, Lafay et al 1997) and female subjects (Johnson et al 1994)[for review see (Omran & Morley 2000a)]. Nevertheless, diet diaries are considered to be the most accurate method for evaluating short-term dietary intake.

The validity of different dietary assessment methods has been assessed in elderly people [for review see (Omran & Morley 2000)]. Bingham (1994) showed that in a group of 160 women aged 50-65 yr, correlations between 24 hour urine nitrogen excretion (the 'gold standard') and dietary nitrogen estimated from 24 hr recall and food frequency questionnaires were in the order of 0.01 and 0.5, respectively, whereas the correlation between 24 hr urine excretion and dietary nitrogen estimated from 7 day weighed food records was 0.87 (Bingham 1994).

Three and five day diet diaries were used in the studies reported in Chapters 8-13 to estimate dietary intake in healthy older and young subjects. Subjects were asked to record, in a diary that was provided, the details (including weight) of all food and beverage items consumed over 3 or 5 days. When it impractical to weigh the foods or beverages, subjects were asked to measure the items using metric cup or spoon measures. Subjects were also asked to record recipes used for meals as well as the method of cooking of foods in the diary. Individual food items recorded in the diet diaries were entered and the dietary energy intake (kJ) and macronutrient content (% carbohydrate, % fat and % protein) calculated using the DIET 4 Nutrient Calculation software (Xyris Software, Australia, Pty Ltd). The DIET 4 Nutrient Calculation software consists of a database of several thousand different food and beverage items that are available in Australia. Details of each food and beverage item ie. the exact

energy (kJ and kcal), macronutrient (g) and micronutrient (mg or μg), fibre (g) and water content (g)) are recorded in the database, so that when food items from the subject's diet diary are entered, the average energy, % macronutrient content etc. can be calculated.

Basal metabolic rate and the estimated range of daily energy requirement, based on the subject's age, body mass index, gender and physical activity level were calculated using the DIET 4 Nutrient Calculation software [a method based on Schofield's equation (Schofield 1985)]. Physical activity was classified as bed-rest, very sedentary, sedentary, light, light-moderate, moderate, heavy, or very heavy, depending on the number of hours per week the individual participated in either recreational activity or exercise, according to the NHMRC Recommended Dietary Intakes for use in Australia, 1991 (web-address:http://www.nhmrc.health.gov.au/publicat/fullhtml/n6-p4.htm). For example, no recreational activity/ home duties was considered sedentary activity, whereas moderate exercise three times/week, and moderate job activity were considered moderate activity. Subjects' whose intake according to the their food record was less than the calculated minimal energy requirement for their BMI, age, gender and activity level were excluded from the studies, in order to exclude possible under-reporters. The food record method was considered to be the most accurate and convenient method of assessing energy intake for the purpose of these studies.

6.3 ASSESSMENT OF APPETITE AND FOOD INTAKE

6.3.1 *Visual Analogue Questionnaires (VAS)*

Visual analogue questionnaires are currently the easiest to use and most widely used means of assessing subjective appetite ratings in human research studies. Visual analogue questionnaires consist of a number of different sensations or symptoms (each usually with a 10 cm) scale, with the extreme of each sensation at either end, eg. hungry/ not hungry. Subjects are asked to make a vertical mark on each scale to indicate their current feelings. The distance measured in cm of this mark from the end quantifies the response. The choice of sensations or symptoms may be adapted according to the type of study intervention and the expected effects.

There are few validation studies of visual analogue questionnaires. A recent study by Flint et al (2000), assessed the reproducibility and validity of visual analogue scales for assessment of appetite sensations, with and without diet standardisation prior to test

days. The study evaluated appetite sensations (hunger, satiety, fullness, prospective food consumption, desire to eat something fatty, salty, sweet or savoury and the palatability of a meal), before a standard breakfast and every 30 min during a 4.5 hour postprandial period in 55 healthy men (mean age 26 yr). Energy intake was then assessed at an ad libitum lunch. They reported that the highest correlations were between the mean VAS of the appetite parameters during the 4.5 hour post-breakfast period and subsequent energy intake at lunch ($r= 0.50$-0.53; $P<0.001$), and that the reliability of VAS ratings was not influenced by standardisation of the previous diet. There is also evidence for a relationship between gastric emptying and appetite ratings. For example, Sepple & Read (1989), reported in 10 healthy men (aged 19-23 yr) a significant correlation between the time taken for 90% of a standard 286.4 kJ semi-solid meal to empty from the stomach and the postprandial increase in hunger ratings (assessed using 10 cm visual analogue questionnaires) ($r= 0.75$; $P< 0.02$).

Linear regression analysis using Statview 4.1, on VAS and energy intake data obtained in the studies reported in Chapters 9, 10 and 12 in this thesis was performed to assess the validity of the visual analogue questionnaires used in these studies. The relationships between energy intake at the buffet meal and VAS ratings (the mean of absolute VAS ratings at the two timepoints immediately preceding the buffet meal) for hunger and fullness in all subjects ($n= 74$) during all study days were analysed. There was a significant relationship between energy (kJ) intake at the buffet meal and mean hunger ratings ($r= 0.37$; $P< 0.0001$) (Figure 6.1A) and an inverse relationship between energy intake and fullness ratings ($r= -0.28$; $P< 0.0001$) (Figure 6.1B) in all subjects. These observations indicate that the more hungry, and less full, subjects felt immediately before the meal, the more energy they consumed at the buffet meal, and vice versa. When the data were analysed separately for young (18-32 yr, $n= 37$) and older (65-84 yr, $n= 37$) subjects; there was a positive relationship between hunger ratings and energy (kJ) intake at the buffet meal that was significant in the older ($r= 0.48$; $P< 0.0001$) (Figure 6.2B), but not quite significant in the young ($r= 0.17$; $P= 0.072$) (Figure 6.2A) subjects. There was a significant inverse relationship between energy (kJ) intake at the buffet meal and fullness for older ($r= -0.25$; $P< 0.01$) (Figure 6.3B) and young ($r= -0.27$; $P< 0.01$) (Figure 6.3A) subjects when examined separately. Overall, these relationships are relatively poor (with r values less than 0.5), but they are statistically significant and consistent with those of Flint et al (2000), suggesting that the 10 cm visual analogue questionnaires are a valid method for assessment of subjective appetite ratings in older and young subjects. Visual analogue

questionnaires thus provide a marker of both food intake at a subsequent meal and postprandial gastric emptying.

Appetite and other sensations were assessed using visual analogue questionnaires in the studies described in Chapters 8-14. At the commencement of a study subjects were familiarised with the questionnaires and instructed in how to complete them. The questionnaires were administered at baseline and at various times throughout the study (eg. during intraduodenal or intravenous infusions), as stated in the "methods" section of each Chapter. The first few time points before initiation of an intervention were averaged to provide a baseline. As mean hunger ratings at baseline were consistently less in older than young subjects, in Chapters 9, 10 and 12, the use of absolute ratings was considered to be the most appropriate approach to compare appetite ratings between the two age groups. As baseline hunger ratings were not significantly different between the older and young subjects in Chapter 11, the change in subjective ratings from baseline was used to compare ratings between the two groups in this study. As only older subjects were included in the studies reported in Chapters 13 and 14, the change in ratings from baseline was considered to be most appropriate for evaluating the effects of the study intervention (ie glucose supplementation and ingestion of a glucose drink with and without guar gum) on subjective appetite ratings. Comparisons of the change in appetite ratings from baseline (ie in Chapters 11, 13 and 14) were a more sensitive, than absolute ratings, in detecting variations between study interventions. Ratings of hunger, fullness and nausea were of particular interest in the studies described Chapters 8-14. Ratings of drowsiness were also reported in the study presented in Chapter 12, as intravenous infusions of naloxone increased ratings of drowsiness in both young and older subjects. Other questions relating to ratings of dizziness, indigestion, headache, thirst, efficiency and friendliness etc. were included primarily to distract subjects from the sensations which were of particular interest to the investigator. The visual analogue scales illustrated in Appendix IA and B were used in the studies reported in Chapters 8-10 and 12-14, to assess sensations of hunger, fullness, desire to eat, nausea and drowsiness. These questionnaires were based on those used by Sepple & Read (1989). The visual analogue questionnaire used in Chapter 11 shown in Appendix II, was based on that used in previous studies (Hebbard et al 1996, Hebbard et al 1995, Verhagen et al 1999a). This questionnaire was used to assess pain, nausea and discomfort during gastric distension as these symptoms were of particular interest in this study.

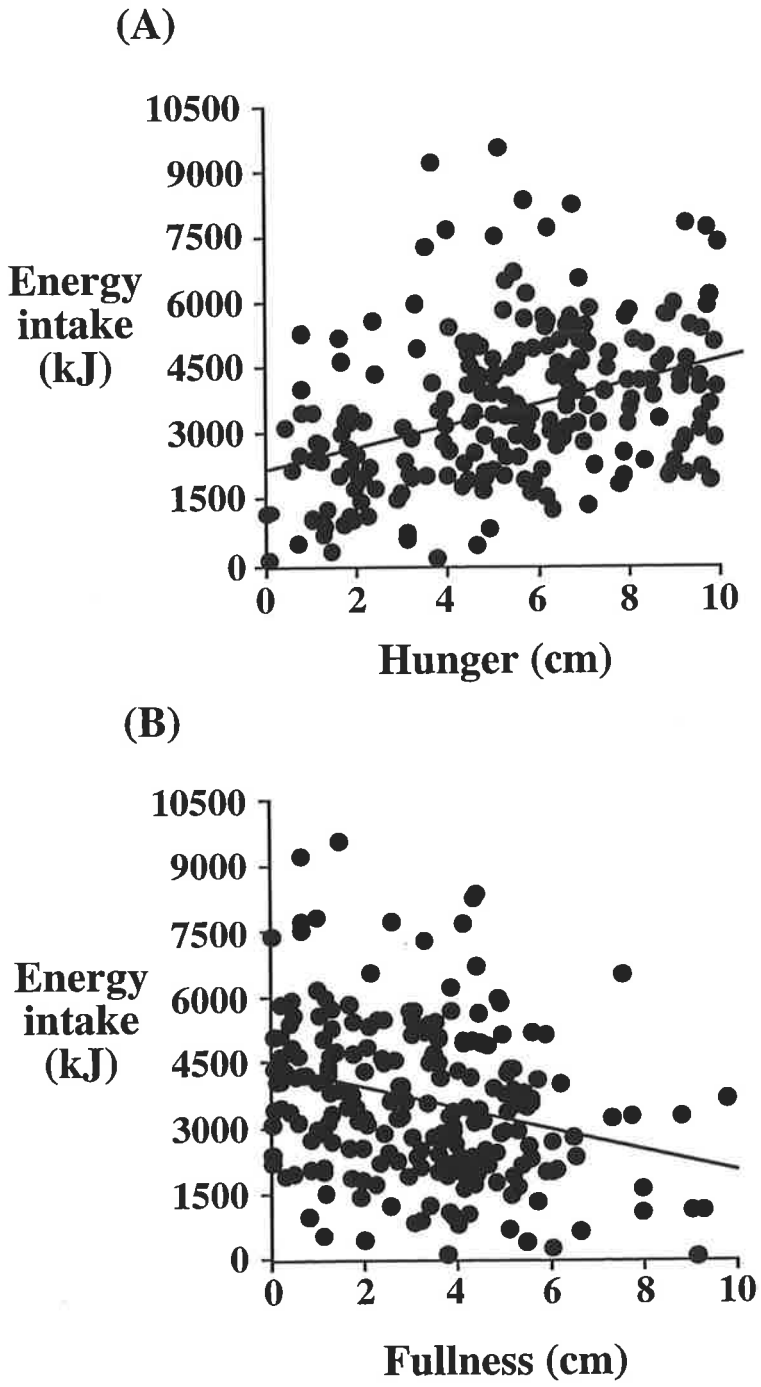

Figure 6.1: Relationship between energy intake at the buffet meal and (A) absolute hunger (mean of two final timepoints immediately before meal)(r= 0.37; P< 0.0001) and (B) fullness (r= - 0.28; P< 0.0001) ratings in young (18-32) yr (n= 37) and older (65-84 yr)(n= 37) subjects during all study days from the studies described in Chapters 9, 10 and 12.

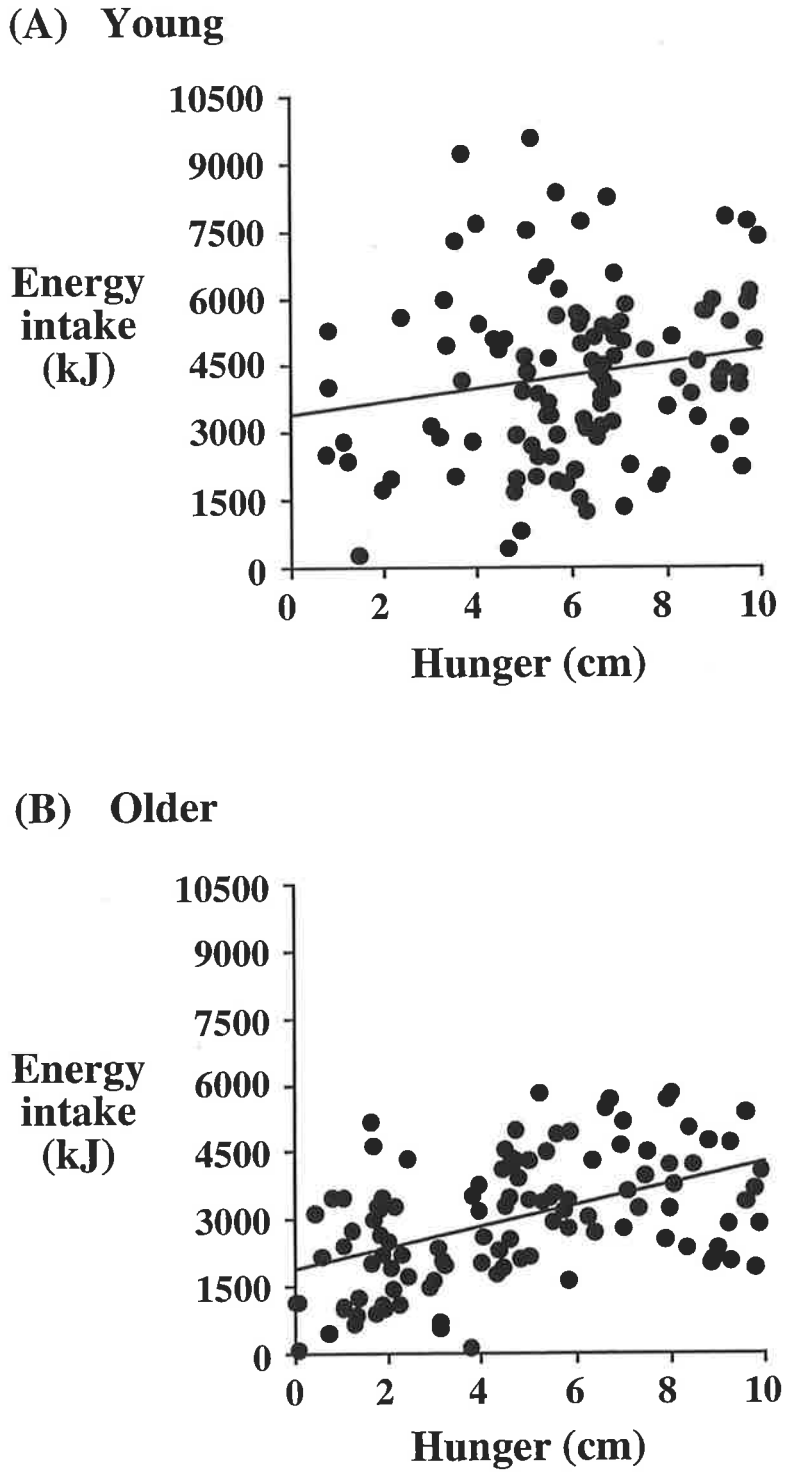

Figure 6.2: Relationship between energy intake at the buffet meal and absolute hunger ratings (mean of two final timepoints immediately before meal) in (A) young (n= 37) (r= 0.17; P= 0.07) and (B) older subjects (n = 37) (r= 0.48; P< 0.0001) during all study days from the studies described in Chapters 9, 10 and 12.

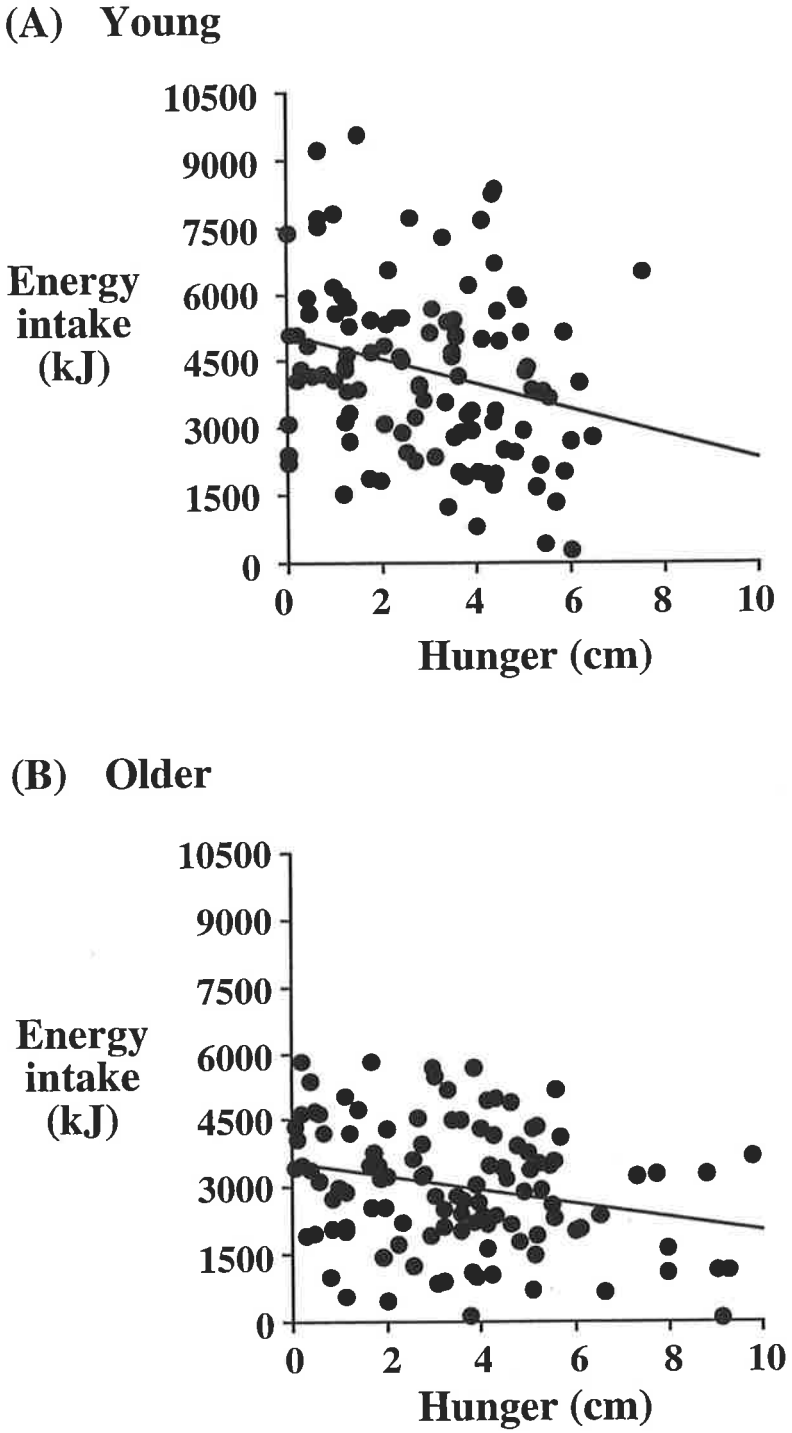

Figure 6.3: Relationship between energy intake at the buffet meal and absolute fullness ratings (mean of two final timepoints immediately before meal) in (A) young (n= 37) (r= -0.27; P< 0.01) and (B) older subjects (n = 37) (r= -0.25; P< 0.01) during all study days from the studies described in Chapters 9, 10 and 12.

6.3.2 *Food Ranking Questionnaire*

As there is some evidence that intravenous naloxone may modify the palatability and choice of food consumed (for discussion see Chapter 2.3.1), a food ranking questionnaire (see Appendix III) was used to assess food preference before and during intravenous naloxone infusion in young and older subjects in the study described in Chapter 12.

6.3.3 *Food intake at buffet meal*

The specific items in the 'buffet meals' used in each of the studies are described in Chapters 9.3.2, 10.3.2, 11.3.5, 12.3.2, 13.3.4 and Appendix A.3.2. Food intake at the buffet meal was calculated by subtracting the weight of any food remaining after the meal from the baseline weight of each specific item of the buffet meal. As discussed, energy intake (kJ) and macronutrient content (% carbohydrate, % fat and % protein) were calculated using the DIET 4 Nutrient Calculation software (Xyris Software, Australia, Pty Ltd).

6.4 BEHAVIOURAL/PSYCHOLOGICAL/HEALTH ASSESSMENT TOOLS

In this section, the Three-Factor Eating Questionnaire used in Chapters 9-13 to assess dietary restraint is discussed. Dietary restraint was assessed in all subjects prior to participation in a study to exclude possible restrained eaters or dieters, as it was crucial to measure true ad libitum energy intake in these studies.

The instruments used in Chapter 15 (ie the Geriatric Depression Scale (GDS), the standardised Mini-Mental State Examination (SMMSE), and the SF-36 Health Survey (SF-36) are also briefly discussed. Using these questionnaires, the degree of depression and cognitive impairment and health status, which represent important 'risk factors' for malnutrition in the elderly, were determined in a sample of 250 elderly persons receiving Domiciliary Care in South Australia. Relationships between nutritional status and the degree of depression, cognitive impairment and physical and mental health status as well as other factors (eg: living alone, number of days in hospital in the preceding 12 months) were evaluated in this study.

6.4.1 *The Three-Factor Eating Questionnaire*

The Three-factor Eating Questionnaire (TFEQ) devised by Stunkard & Messick (1985) (see Appendix VI) is a simple and widely used method to assess dietary restraint in humans. The concept of "eating restraint" - the tendency of some individuals to restrict their food intake in order to control their body weight, was first described by Herman & Mack (1975), who constructed the 10-item "Restraint Scale". Restrained eating was assessed using this scale to predict food intake in response to three kinds of stimuli; preloads of food, ingestion of alcohol and dysphoric emotions [for review see (Stunkard & Messick 1985)]. Restrained eaters exhibited counter-regulation, ie ate more at a test meal following a 'milkshake' preload (Herman & Mack 1975), as well as after alcohol ingestion (Polivy & Herman 1976), whereas unrestrained eaters ate less following both stimuli. Furthermore, when anxious or depressed, restrained eaters ate more or gained weight, whereas unrestrained eaters ate less and lost weight (Polivy & Herman 1976, Herman & Mack 1975). In Chapters 9-13 the TFEQ was used to assess the 'dietary restraint' of potential subjects because it was necessary to include only 'unrestrained' subjects who would vary their food intake in response to an intervention.

The Three-Factor Eating Questionnaire (TFEQ) was based on the 10-item Restraint Scale (Stunkard & Messick 1985) as well as the "Latent Obesity Questionnaire" by Pudel et al (1975). The Three-Factor Eating Questionnaire consists of 51-items relating to eating and dietary behaviour, and measures three factors ie Factor 1 'cognitive restraint of eating', Factor 2 'disinhibition' and Factor 3 'hunger'. Mean ± SD scores for Factor 1, 2 and 3 for restrained eaters (dieters, n= 53)(14.3 ± 3.6, 13.8 ± 4.2 and 7.2 ± 3.9, respectively) and unrestrained eaters (free-eaters, n= 45)(6.0 ± 5.5, 5.6 ± 4.3 and 7.0 ± 4.3, respectively) were defined by studies in which the TFEQ was administered to several populations selected to include persons who exhibited the spectrum from extreme dietary restraint to extreme lack of dietary restraint (for review see (Stunkard & Messick 1985). The construct validity of the TFEQ has been assessed against self-reported mean energy intake per day and to other measures associated with disordered eating and figure consciousness (Laessle et al 1989) and it has been established in 60 women (aged 18-26 yr) that high scores on the TFEQ for Factor I are significantly related to overall energy restriction in everyday life in 60 women (aged 18-26 yr). The reliability of the (TFEQ) has been assessed in a number of studies [for review see (Stunkard & Messick 1985)]. For example, in a cohort of 358 men and women (mean age 42 yr), who were paid to maintain an accurate 7-day record of their dietary intake, higher restraint scores (Factor I scores of 14-20) were associated with

lower and less variable overall intake, especially of fat (r= -0.37; P< 0.05) and carbohydrate (r= -0.37; P< 0.05) (de Castro 1995).

In Chapters 9, 10, 12 and 13, subjects who scored >10 for Factor 1 on the TFEQ were considered 'restrained' eaters and were excluded from participation in the studies, since it was necessary to evaluate true ad libitum food intake. This score was based on the cut-off scores used in previous studies of appetite in humans (Shide et al 1995b, Rolls et al 1994, Rolls 1995, Rolls et al 1995). In Chapter 11, only subjects who scored >13 for Factor 1 were excluded, as dietary restraint was considered not as essential in this study as in the other studies.

6.4.2 *The Geriatric Depression Scale*

The Geriatric Depression Scale (GDS) (See Appendix V) (Yesavage 1988) was used to assess depression in 250 elderly Domiciliary Care recipients as part of the data presented in Chapter 15. The GDS is currently one of the most widely used depression self-reports. The 30-item scale was developed specifically to assess depression in the elderly [for review see (Montorio & Izal 1996)] and has been validated against two other assessment instruments of depression ie the Zung Self Rating Scale for Depression (SDS) (Zung 1965) and the Hamilton Depression Rating Scale (HAMD) (Hamilton 1967) with significant correlations between the classification criteria ("no depression", "mild depression", and "severe depression" and each of the three scales; ie r= 0.82, r= 0.69, and r = 0.83, for the GDS, SDS and HAMD scales, respectively.

The sensitivity and specificity of the GDS was evaluated in samples of subjects from those involved in the study by Yesavage (1988) (ie 60 depressed elderly subjects (patients complaining of depression) and 40 normal elderly subjects (without any history of mental illness) - a score of ≥11 out of 30 correctly classified 84% of depressed elderly (sensitivity) and 95% of those not affected by depression (specificity). At a cut-off score of ≥14 the sensitivity of the scale was 80%, and specificity was 100%. A score of 0 to 10 is considered normal and 11 or more as a possible indicator of depression. Other studies in elderly subjects from day centers, nursing homes, general psychiatric wards as well as elderly persons living in the community have confirmed the high level of sensitivity and specificity of the GDS [for review see (Montorio & Izal 1996)].

6.4.3 *The Standardised Mini-Mental State Examination*

The SMMSE was used to assess cognitive state in 250 elderly Domiciliary Care recipients as part of the data presented in Chapter 15. The Mini-Mental State Examination (MMSE) was developed by Folstein & McHugh (1975) for cognitive grading of patients in the clinical setting [for review see (Tombaugh & McIntyre 1992)]. It is a widely used screening tool for cognitive impairment or dementia in older adults. The tasks set out in the MMSE focus on broad cognitive aspects of mental functioning (ie orientation, registration, short-term memory, attention, etc), rather than emotional problems or disordered forms of thinking (Burns et al 1998). Folstein & McHugh (1975) initially assessed the validity and reliability of the MMSE in heterogenous group of 206 patients with a variety of disorders (dementia, depression, pseudodementia, mania, schizophrenia and personality disorders) and in 63 normal subjects. The validity of the MMSE, assessed by correlating the subjects' scores on the MMSE scores with their scores on the Wechsler Adult Intelligence Scale (WAIS), was relatively high with a correlation coefficient (Pearson r) of 0.78 for verbal IQ, and 0.66 for performance IQ between the two assessment methods. Twenty-four hour test-retest reliability of the MMSE produced a r value of 0.89 with the same examiner, and r= 0.83 between examiners. Furthermore, when the reliability of the MMSE was measured over 28 days in subjects who were diagnosed clinically stable, the r value was 0.98. A number of factors influence the total score on the MMSE including education, cultural environment and sensory impairments [for review see (Burns et al 1998)], the correlation between clinical scales and categorised MMSE was moderate to fair (Juva et al 1994). The MMSE has been modified by different groups, by either extending or reducing the number of questions [for review see (Burns et al 1998)]. Molloy et al (1991) developed a standardised version ie the Standardised Mini-Mental State Examination or SMMSE which was used in Chapter 15 (See Appendix IV) imposing strict guidelines for administration and scoring to improve the reliability of the tool. In addition to its improved reliability, the SMMSE takes less time to administer than the MMSE. 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 et al 1991).

6.4.4 *The SF-36 Health Survey*

The SF-36 Health Survey was used to assess health status in 250 community-dwelling elderly Domiciliary Care recipients as part of the data presented in Chapter 15. Scoring of the SF-Health survey for this study was conducted through the SF-36 Health Survey

website (http://www.qmetric.com/cgi-bin/sf36_demo.cgi). The 36-item short-form (SF-36)(See Appendix IIV) was developed to assess health status in persons aged 14 yr or older and designed for use in clinical practise, research, health policy evaluations and general population surveys (Ware & Sherbourne 1992). The SF-36 consists of a multi-item scale which assesses 8 health concepts (or scales 0-100); 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological stress and well being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. From the 8 health concepts an overall score for physical (0-100) and mental (0-100) health status can be calculated [for review see (Ware & Sherbourne 1992)].

Validation studies have demonstrated that the physical functioning and mental health score obtained using the SF-36 are sensitive to clinical manifestations of medical and psychiatric conditions [for review see (McHorney et al 1993)]. McHorney et al (1994) evaluated the reliability and validity of the 8 scales and the SF-36 Health survey from data obtained from the Medical Outcomes Study conducted in Boston, Chicago and Los Angeles, USA in 1986. Analyses were conducted among 3445 patients, aged 18-98 yr (with chronic medical and psychiatric conditions), and were separated across 24 subgroups differing in sociodemographic characteristics, diagnosis, and disease severity. Across patient groups, all of the scales passed tests for item-internal consistency (97% passed) and item-discriminant validity (92% passed). The reliability coefficients ranged from 0.65 to 0.94 across the 8 scales (median= 0.85), but varied somewhat between patient groups. It was concluded from this study that the SF-36 is a useful measure of both physical and mental health status across a variety of different population subgroups (McHorney et al 1994). Although other studies have confirmed the high reliability and validity of the SF-36 for its use in a wide range of groups (Brazier et al 1992, Anderson et al 1993, Jenkinson et al 1994), Andresen et al (1999), however, recently reported that the SF-36 may of limited use in 'typical' nursing home patients, due to the high prevalence of dementia and inconsistent scores across repeated tests.

6.5 ASSESSMENT OF NUTRITIONAL STATUS

Malnutrition may be difficult to diagnose in the elderly. While marked malnutrition, for example cancer-related cachexia, is easily recognised by inspection, in less malnourished patients this method lacks sensitivity and specificity. Some undernourished patients who are protein depleted do not appear thin or wasted, particularly those who have experienced weight loss, but still have a BMI within the normal range. Patients may also appear well nourished in the presence of hypoproteinaemic oedema.

The methods used to assess nutritional status in the elderly include anthropometry, biochemical markers, clinical evaluation and screening tools or questionnaires. No single method is ideal, and thorough nutritional assessment dictates the use of a combination of methods. In the study presented in Chapter 15 in order to assess the nutritional status of 250 Domiciliary Care recipients over the age of 65 yr, the Mini-Nutritional Assessment (MNA)(section 6.5.3.3) was considered the most efficient and practical method.

6.5.1 *Anthropometric data*

Anthropometric measures are used to assess body fat mass (skin fold thicknesses, BMI) and muscle mass (measurements of mid-arm and calf circumference). These techniques are inexpensive, easy to perform, and non-invasive. The use of anthropometry as a single method for assessment of nutritional status, however, is limited, particularly in the elderly, because there are no normative data available for people aged 75 yr or older (Sullivan et al 1989)[for review see (Omran & Morley 2000a)].

6.5.2 *Biochemical Parameters*

Circulating concentrations of transport proteins (albumin, prealbumin, transferrin, transthyretin and retinol binding protein) are decreased in protein-energy malnutrition (PEM), and are often measured to assess nutritional status [for review see (Omran & Morley 2000b)]. Albumin is a particularly good marker of PEM, probably because of its longer half life and greater volume of distribution than other proteins. There is substantial evidence that severe hypoalbuminaemia is a strong predictor of morbidity and mortality in hospitalised patients (Sullivan et al 1999, Constans et al 1992, Ferguson et al 1993). As markers of malnutrition, however, levels of carrier proteins have limitations. Circulating concentrations fall acutely due to infection or inflammation

and chronically with many malignancies, even in the absence of malnutrition (Yeh & Schuster 1999), making low concentrations of these proteins a sensitive, but non-specific, marker of PEM.

When specific micronutrient deficiencies are suspected, levels of circulating vitamins (particularly B group, C, D and A) and trace elements, such as zinc and selenium (often associated with loss of appetite) can also be measured.

Other methods of detecting malnutrition include assessing the delayed hypersensitivity response to antigens, such as tuberculin, mumps, candida albicans and streptokinase, and total lymphocyte count and T and B cell function (Morley 1997, Yeh & Schuster 1999). These methods, however, are of limited clinical use because they are not readily available and lack sensitivity. For example, impaired immune responses occur with severe trauma, malignancy or chronic sepsis (Yeh & Schuster 1999).

6.5.3 *Clinical Evaluation and Screening tools*

A number of screening tools have been developed for assessing nutritional status in humans. In this section, three of the most common tools are described ie. the Subjective Global Assessment (SGA), the DETERMINE your Nutritional Health questionnaire, and Mini-Nutritional Nutritional Assessment (MNA)

6.4.3.1 The Subjective Global Assessment (SGA)

Several studies have demonstrated the high predictive value of malnutrition for in-hospital mortality when the former is diagnosed using a structured clinical assessment technique (Covinsky et al 1999, Hirsch et al 1992, Volkert et al 1992). The Subjective Global Assessment (SGA) is a standardised clinical examination for the assessment of nutritional status (Detsky et al 1987). It comprises five features of medical history (weight change, dietary intake, gastrointestinal symptoms, and functional impairment) and four of physical examination [evidence of loss of subcutaneous fat, muscle wasting, oedema and ascites (in renal patients only)] (Detsky et al 1987). The SGA does not include any anthropometric measurements or laboratory tests. Individuals are rated as either well-nourished, mildly-moderately malnourished, or severely malnourished.

A number of studies have assessed the validity and reliability of the SGA [for review see (Omran & Morley 2000a)]. For example, in a study by Baker et al (1982), the SGA

rating and objective measurements as assessed by two independent expert examiners were compared in 59 patients (aged 17-76 yr). There was concordance between the two examiners on the SGA assessment of 48 of the 59 patients. Mean values for serum albumin, creatinine-height index, percentage of weight loss, total body potassium and delayed hypersensitivity were significantly lower in malnourished than in well-nourished patients. Furthermore, the mean number of days spent in hospital following surgery (n=36) was 18 in well-nourished, 25 in mildly-moderately malnourished and 49 in severely malnourished patients in this study (Baker et al 1982). The SGA is also a good predictor of complications in patients undergoing liver transplant (Pikul et al 1994) and in patients with dialysis (Enia et al 1993). The nutritional status of 90 elderly patients admitted to a Geriatric clinic in Sweden was assessed subjectively by two independent observers with the SGA form and objectively with a combination of anthropometry and serum protein analysis (Ek et al 1996). The agreement level between the two observers was 78%; one observer had an SGA sensitivity of 85%, whereas the other had only 67%. The necessity of being well trained in order to manage nutritional status assessment, is emphasised in this study (Ek et al 1996). Because of this reason and that the SGA has not been validated in non-hospitalised persons, it is not as practical, or convenient, as the Mini-Nutritional Assessment (MNA)(see section 6.4.4.3) to assess the nutritional status in the study presented in Chapter 15.

6.5.3.2 The DETERMINE Your Nutritional Health Checklist

The 'DETERMINE Your Nutritional Health' Checklist is used frequently as a screening tool for the detection of protein energy malnutrition in the elderly [for review see (Omran ML, 2000)]. The DETERMINE checklist, consists of 10 simple questions relating to dietary, general and social assessment. A cumulative score of 6 or more points (of a possible 21) is indicative of a high risk of malnutrition. The DETERMINE checklist is capable of identifying groups of elderly persons who are at risk of malnutrition in a particular population (Beck et al 1999, de Groot et al 1998), but does not have the capacity to distinguish between severe and low-moderate malnutrition.

The validity of the DETERMINE checklist was evaluated by White et al (1992) in 750 community-dwelling patients aged 70 yr or older by comparing their checklist scores with an objective assessment of their nutrient intake; the checklist identified only 36.2% of people in whom intake was less than 75% of the recommended dietary allowance (in three or more nutrients) when a score of 6 was used as a cut-off. Beck et al (1999) recently reported that a low score on the checklist was not a significant predictor of

mortality in a follow-up study of 115 subjects aged 70-75 yr involved in the Danish part of the Survey in Europe of Nutrition in the elderly, a Concerted Action (SENECA) study. Given this information it is likely that the DETERMINE Your Nutritional Health Checklist is of limited use as a method for assessment of nutritional status, particularly in isolation.

6.5.3.3 The Mini-Nutritional Assessment

The MNA was developed in the USA and validated as a tool for assessment of risk of malnutrition in the elderly. It is composed of 18 simple and rapid measurements and questions (anthropometric assessment, general assessment, dietary assessment and subjective assessment) about the individual (See Appendix IIIV), which can be completed in less than 20 minutes. No blood tests are required in this assessment. It can, accordingly be readily used in population studies, such as that reported in Chapter 15. The total score categorises elderly patients as either normal, at risk of malnutrition or malnourished. Since the MNA is capable of detecting persons both at high, and low-moderate risk, it is likely to be useful in identifying individuals at risk of malnutrition, and thereby facilitating early nutritional intervention (Guigoz et al 1996). The MNA was validated by three consecutive studies [for review see (Vellas et al 1999)]. An initial study by Guigoz et al (1994), showed that in 105 frail elderly patients (mean age 79 yr) from a Geriatric facility and 50 healthy aged-matched elderly persons in Toulouse, France, the 'nutritional status' based on MNA scores matched that based on the assessment of two independent physicians and derived from a comprehensive nutrition assessment that included anthropometrics, food records and biochemical markers, in 92% and 98% of individuals, respectively. In a validation study of 120 frail older people (mean age 79 yr) in Toulouse, France, the MNA correctly classified the nutritional status as defined by a detailed physical assessment including blood tests in 88% of cases (Guigoz et al 1994). Furthermore, there was no added diagnostic benefit in including the biochemical measures with the MNA (Guigoz et al 1994). Using discriminate analysis equations and the nutritional status according to clinical examination as a reference standard, the classification potential of the MNA was assessed by cross-classification of scores for the patients from the two initial studies. 78% of the patients were classified correctly using the MNA (Guigoz et al 1996). According to the cross-validation of results, using albumin as the independent variable, the threshold scores for MNA were selected: a score of ≥24 indicates normal or well-nourished, 17-23.5 indicates at risk of malnutrition and <17 indicates malnourished (Guigoz et al 1994).

Other studies have confirmed the predictive value of the MNA. In a recent study in Switzerland, involving 166 patients aged 70 yr or older admitted consecutively to a Geriatric Ward, a MNA score < 17 (undernourished) was associated with higher cost of care and a longer hospital stay than a score > 17 (Quadri et al 1999). Beck et al (1999) conducted a follow-up study of 147 subjects from the Danish part of the Survey in Europe of Nutrition in the Elderly, a Concerted Action (SENECA). They reported that in subjects with a high MNA score (≥ 24) mortality was lower when compared to subjects with a low MNA score (≤ 24) (17% vs 49%, respectively $P<0.01$).

It should be recognised that some studies have suggested that the MNA may be of limited value in assessing nutritional status in the elderly. For example, Azad et al (1999) compared the MNA, Nutrition Screening Initiative (NSI) and the Chandra screening tool with the results of a detailed nutritional assessment in 160 (86 women) hospitalised patients in Ottawa, Canada. Although they found that the sensitivity of the MNA was relatively low (57%) in identifying the prevalence of malnutrition, this was greater than with the other two methods used and its specificity was moderately high at 69% (Azad et al 1999).

In view of the majority of evidence, the MNA appears to be the best tool to detect malnutrition in older persons short of performing a detailed biochemical and clinical evaluation. The MNA was used in the study described in Chapter 15 for the purpose of assessing nutritional status in a sample of 250 Domiciliary Care recipients.

6.6 CONCLUSIONS

There are number of well-established validated methods to assess of dietary food intake, appetite, feeding behaviour and health/ nutritional status in humans. For the purpose of the studies reported in Chapters 8-13 it was necessary to include subjects who were unrestrained eaters, with a dietary energy intake that was within the recommended daily energy range. Dietary restraint was assessed using the Three Factor Eating Restraint Questionnaire in these studies. Dietary energy intake was evaluated using three and five day food records. Using these food records a comparison of dietary energy intake between the age groups was also possible.

During the specific study interventions (see Chapters 8-14 for details), validated-visual analogue scales were used to assess subjective appetite and gut sensations; and food intake was assessed using a buffet meal.

Chapter 15 reports an assessment of the mental and physical health and nutritional status of 250 Domiciliary Care recipients. For the purpose of assessing the depression, cognitive impairment, health status, the Geriatric Depression Scale (GDS), the standardised Mini-Mental State Examination (SMMSE), and the SF-36 Health Survey (SF-36), respectively, were used. This allowed an evaluation of the relationships between MNA scores and scores on the GDS, SMMSE, SF-36 as well as other factors (eg: living alone, number of days in hospital in the preceding 12 months) to determine what were the most important factors contributing to low MNA scores in this particular sample of elderly people (see Chapter 15 for details).

CHAPTER 7

Assessment of Gastropyloroduodenal Sensory and Motor Function and Small Intestinal Nutrient Absorption

7.1 INTRODUCTION

Techniques used to assess gastric motor and function can be broadly divided into the following categories: measurement of (i) gastric electrical activity, (ii) intraluminal pressures or contractions and (iii) gastric emptying. Small intestinal nutrient absorption can also be assessed by various techniques. The purpose of this chapter is to describe the methods that are most frequently used to measure gastric motor and absorptive function in humans. Particular emphasis is given to surface electrogastrography,

scintigraphy, manometry and electronic barostat techniques, as these methods were employed in the studies described in Chapters 8, 9, 11, 13 and 14.

7.2 MEASUREMENT OF GASTRIC ELECTRICAL ACTIVITY-ELECTROGASTROGRAPHY (EGG)

As discussed in Chapter 3.3.2, the distal stomach (antrum) exhibits phasic contractile activity which is controlled by a pacesetter potential or gastric slow wave, generated in the greater curvature of the stomach (Hasler 1999). The pacemaker usually discharges at a rate of approximately 3 per minute, and determines the maximum frequency of phasic contractions. Gastric electrical activity can be measured using serosal (internal) or cutaneous (external) electrogastrography (EGG) (for reviews see (Verhagen et al 1999b, Camilleri et al 1998, Wiley et al 1999).

Serosal EGG involves the placement of electrodes directly onto the mucosal layer of the stomach wall. This technique is invasive and its use is generally limited to animal studies.

Cutaneous electrogastrography (EGG) is a simple, non-invasive technique for assessing gastric myoelectrical activity in humans (Verhagen et al 1999b). For optimal recording of the EGG signal, subjects need to be in a standardised position, for example reclined at a 45° angle, or supine. The abdominal skin is prepared by removing any hair and gently abrading the skin with fine sandpaper before application of the electrodes. Pre-gelled silver-silver-chloride electrodes (a electrode that is built in a small basin filled with an electrolyte gel-containing sponge), commonly used for electrocardiographic monitoring, are also suitable for use in electrogastrographic studies. Electrodes that include an adhesive disk optimise signal acquisition. Alternatively, an electrode cream may be used. The most common method involves the placement of electrodes close to the antral region, along the antral axis of the stomach to maximise the signal-noise ratio. (Verhagen et al 1999b, Camilleri et al 1998). The technique used in the study reported in Chapter 9 is represented in Figure 7.1.

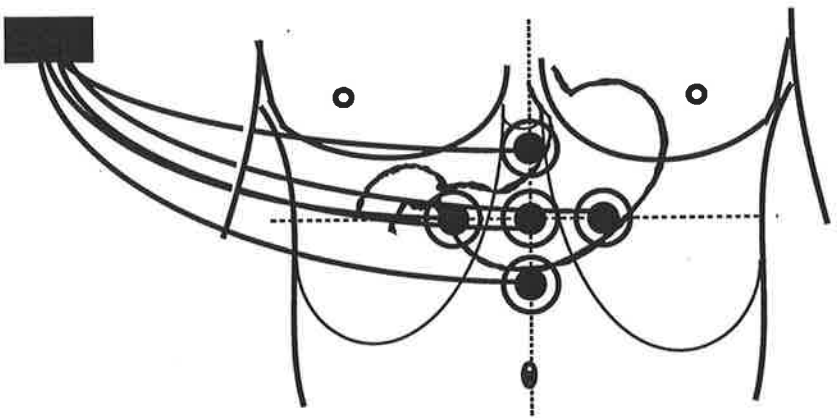

Figure 7.1: Schematic diagram the show the position of the electrodes used in electrogastrographic recordings

Due to their relatively low amplitude (200-500 mV), EGG signals are usually amplified using low-pass and high pass filters to eliminate signals slower than 1 cpm (0.016 Hz), or faster than 16 cpm (0.3 Hz) (Camilleri et al 1998). This filtering eliminates electrical signals from other sites, as well as respiratory and movement artefacts and electrical noise (Camilleri et al 1998). EGG records may be recorded either on magnetic tape or digitised and stored on the hard drive of a computer.

Computer analysis of EGG recordings includes the application of Fast Fourier transformation (FFT) to obtain individual spectra, and thus the power and frequencies contained in the EGG signal. A "flat-top" or Hamming window is used to allow optimal definition of the frequency and amplitude of the EGG signal. A running spectral analysis is then obtained by adding 1 minute of the new EGG signal to the previous 3-4 min of EGG signal (Verhagen et al 1999b). These methods eliminate errors in interpreting the complex and changing waveform, and allow the frequencies to be determined for time periods less than 3 min, so that transient abnormal signals may be detected (Camilleri et al 1998). Of the power spectra, frequencies between 0 and 16 cpm can be visualised.

The frequency of the EGG is identical to the slow waves signal recorded with mucosal or serosal electrodes (ie. 3 cpm) (Hamilton et al 1986). Those frequencies which are 2.6-3.7 cpm are, therefore, defined as normal (Verhagen et al 1999b); frequencies less than 2 cpm for more than 2 minutes, without a definite peak in the normal range in any other recording channels, are defined as bradygastrias, while frequencies between 3.7 (0.062 Hz) and 11 (0.18 Hz) cpm for more than 2 minutes, without a definite peak in the normal range in any other recording channels are defined as tachygastrias (Camilleri et al 1998). Higher harmonics of the gastric fundamental frequency (ie peaks at multiples of the fundamental frequency, such as 6 or 9 cpm may be observed, particularly in the postprandial state. Such harmonics may be inadvertently be classified as dysrhythmias, and therefore need to be excluded. As discussed in Chapter 3.3.2, gastric dysrhythmias have been associated with a number of conditions including gastroparesis, nausea associated with pregnancy, motion sickness and dyspepsia (Hasler 1999).

The EGG power at a given frequency represents the absolute amplitude of the dominant frequency signal relative to other frequencies contained in the EGG signal. The EGG amplitude normally increases after a meal, therefore, a postprandial to fasting power

ratio value can be calculated for each minute which is usually greater than 1. The power ratio is thought to represent the postprandial increase in gastric contractile activity, although it may also be the result of the increase in gastric distension following a meal (Verhagen et al 1999). Some authors have suggested that a power ratio of less than 1 is indicative of a diminished gastric contractile or distension response to a meal (Parkman et al 1995, Chen et al 1994). Other studies have provided evidence that both attenuation of the power signal after meal ingestion and postprandial gastric dysrhythmias are related to delayed gastric emptying (Chen et al 1996). The methods used by the author for recording, filtering and analysis of the EGG are detailed in Chapter 9.3.4.

EGG has limitations as a research and clinical tool for assessing gastric motor function. EGG recording equipment is expensive and the analysis of EGG recordings requires an experienced investigator. The significance of gastric dysrhythmias and the amplitude of the signal derived from EGG recordings has not yet been fully elucidated (Camilleri M, 1998). Even with optimum filtering techniques, movement needs to be restricted and, as a result of this, ambulatory EGG recordings are unreliable. A number of studies have attempted to validate EGG by conducting EGG recordings at the same time as using other methods to measure gastric motor function, such as scintigraphy, manometry, and ultrasound, with varying results [for review see (Camilleri et al 1998)]. For example, Sun et al (1995b) reported in healthy subjects that EGG frequency was related to antral pressure wave activity measured by manometry during intravenous erythromycin but not during phase II of the fasting migrating motor complex and to the number of isolated pyloric pressure waves during intraduodenal lipid infusion (Sun et al 1995b). These findings suggest the capacity of EGG to assess spatial patterns of gastric (antral and pyloric) pressure waves may be limited, particularly when assessing fasting gastric motility. Electrogastrography (EGG) is, however, a simple, non-invasive technique which provides a novel approach to evaluate the effects of intraduodenal saline, glucose and lipid infusion on gastrointestinal function in healthy older compared to young subjects in Chapter 9.

7.3 MEASUREMENT OF GASTRIC EMPTYING

There are a number of methods available for the measurement of gastric emptying in humans. Table 7.1 lists the most common methodologies for assessment of gastric

emptying. The following discussion focuses on scintigraphy, with a brief outline of some of the other methods used to measure gastric emptying.

Table 7.1

Methods to Assess Gastric Emptying
- Scintigraphy
- Radioisotopic breath tests
- Ultrasound
- Paracetamol absorption
- Radiological techniques
- Magnetic Resonance Imaging
- Applied potential tomography / epigastric impedance
- Intubation/ aspiration of gastric contents

7.3.1 *Scintigraphy*

Scintigraphy is probably the most sensitive and clinically applicable method for the assessment of gastric emptying in humans and this technique was used in the studies reported in Chapters 13 and 14. A radiolabeled meal is ingested, either as a liquid, solid or a mixture of both and its emptying from the stomach monitored using a gamma camera. A region of interest (ROI) can be drawn around the stomach on the computer (Figure 7.2) (Collins et al 1983, Collins et al 1985), and this can be divided into the proximal and distal regions to evaluate the intragastric meal distribution. Images are usually acquired relatively frequently (~ every 1-3 min) in the first 30 min, so that the precise time of the lag phase (the time taken for any solid or liquid to enter the proximal small intestine) can be quantified (Collins et al 1983, Collins et al 1985, Jones et al 1996). Gastric emptying measurements usually take place over a period of 2-4 hours

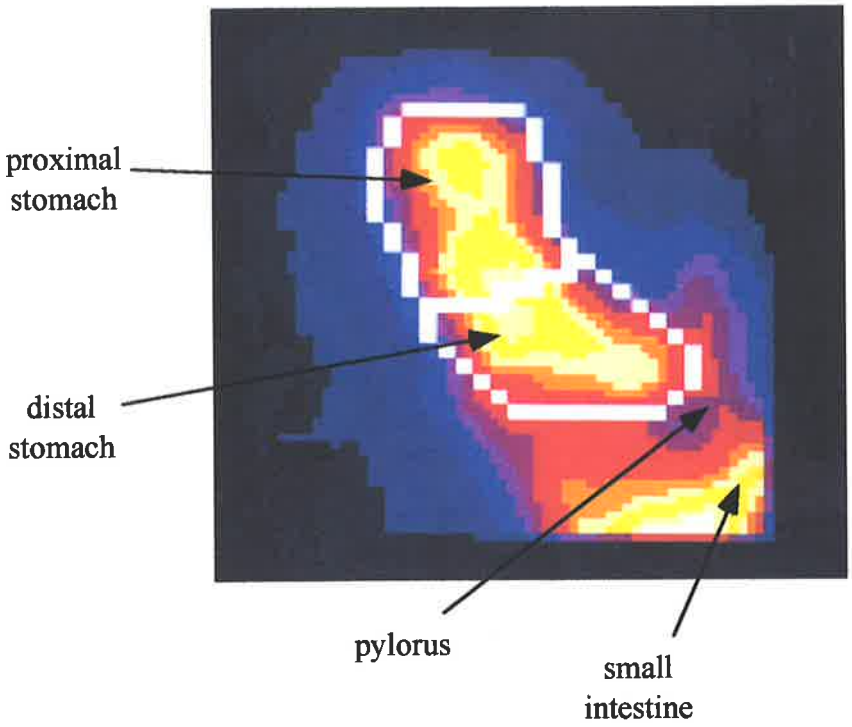

Figure 7.2: Scintigraphic image showing the intragastric distribution of a solid meal in a healthy subject, ~60 min after meal ingestion. The total stomach region of interest (ROI) is divided into proximal and distal regions. The number of counts in the proximal, distal and total stomach may be monitored continously.

depending on the type of meal given (Collins et al 1983). Parameters used to evaluate gastric emptying data include the duration of the lag phase, the intragastric retention at particular time points (eg: 90 min) and the 50% emptying time (T50) (Horowitz et al 1993).

As discussed in Chapter 3.5.2, the rate of gastric emptying is influenced by a number of factors, including the size, temperature, energy and macronutrient content of a meal, as well as the posture of the subject. Both the test meal and the technique used to evaluate gastric emptying should, accordingly, be standardised (Camilleri et al 1998).

The counts detected by the gamma camera must be corrected for radioisotope decay, as well as tissue attenuation (ie. changes in the detected activity related to tissue-depth). A lateral image technique (Collins et al 1985) was used to correct for tissue attenuation in the studies described in Chapter 13 and 14.

The dose and half life of the radioisotopes used in gastric emptying studies should take into account the resulting radiation burden. Technetium (^{99m}Tc) is the most frequently used radioisotope as it has a half life of 6 hours and total body radiation exposure is approximately 1-2 mSv (often less than that obtained from an abdominal x-ray). Furthermore, ^{99m}Tc is relatively inexpensive and widely available. When gastric emptying of more than one meal component is to be measured (eg. solid and liquid, or oil and glucose) a second isotope such as ^{111}In or ^{67}Ga may be used in conjunction with ^{99m}Tc. ^{111}In and ^{67}Ga have half lives of 67 hour and 78 hours, respectively. There are, additional technical problems associated with the performance of dual-isotope studies, including downscatter from the higher energy isotope (usually ^{111}In) into the lower energy window (usually ^{99m}Tc), and scatter of counts from the stomach outside the stomach region of interest (ROI). Corrections for both these factors must be applied in such studies (Camilleri et al 1998).

Radioisotopes are now available to label all the components of a normal meal, ie digestible solid, nondigestible solid, liquid and oil (Horowitz et al 1991, Cunningham et al 1991a Cunningham et al 1991c, Camilleri et al 1998). In the study described in Chapter 13, a dual-isotope technique was used to label the oil and aqueous (glucose) components of a drink; the oil was labeled with ^{99m}Tc(V)-thiocyanate by direct extraction from acidic thiocyanate solution (Cunningham et al 1991a), while the glucose was labeled with ^{67}Ga- EDTA (Bellen et al 1995). In the study described in Chapter 14,

the glucose drink was labeled with ^{99m}Tc-sulphur-colloid (Hveem et al 1996, Horowitz et al 1991).

Antral contractile activity (frequency and amplitude) can also be measured using scintigraphy (Akkermans et al 1980, Urbain et al 1993, Urbain et al 1990, Jones et al 1995). Following ingestion of a radiolabeled test meal, regions-of-interest are drawn around the antrum and the amount of radioactivity in these regions is determined to generate 'antral curves' (Jones et al 1995). Since data must be acquired at frequent intervals of between 1 and 2 seconds, during these studies larger doses of radioactivity, (ie ~3-5 times that of standard gastric emptying studies are required (Urbain et al 1990). This technique has only been used to examine antral curves after ingestion of solid, or semi-solid meals (Akkermans et al 1980, Urbain et al 1993, Urbain et al 1990, Jones et al 1995). While the 'antral curve' scintigraphic technique allows simultaneous evaluation of antral contractile activity and gastric emptying, a distinction can not be made with confidence between those contractions which result in lumen occlusion and those that do not (Jones et al 1995).

Scintigraphy has several minor disadvantages. Radiation exposure limits the number of gastric emptying studies in any subject. There is a progressive dilution of the solid and liquid markers by an unknown quantity of gastric secretion. Furthermore, with the use of solid markers (and in increasing quantities), some isotope empties as a liquid (Camilleri et al 1998).

7.3.2 *Radioisotopic breath tests*

Stable isotope substrates (eg. octanoate or glycine) can be labeled with stable isotopes (eg. ^{14}C or ^{13}C) and mixed with solid or liquid foods. After oral ingestion, the label passes with the food into the small intestine where it is absorbed and excreted in the breath as $^{13}CO_2$ or $^{14}CO_2$. Sequential measurement of labeled CO_2 for up to 6 hrs after a meal thus provides a non-invasive measure of gastric emptying of solids or liquids in humans (Ghoos et al 1993, Maes et al 1994). Studies in healthy subjects have shown that the accuracy of this technique is similar to that of scintigraphy (Maes et al 1998, Maes et al 1994). There is, however, some controversy about the accuracy of the different mathematical techniques used to evaluate the rate of gastric emptying from breath samples, ie the Mayo (Maes et al 1998) vs Leuven (Camilleri et al 1998) technique.

A disadvantage of the radioisotopic breath test technique is that it assumes that gastric emptying is the rate limiting step in the ultimate delivery of CO^2 to the breath; therefore the test may, accordingly, be unsuitable for use in patients in intensive care, during physical exercise or in patients with changes in liver, pancreatic or visceral haemodynamic function as a result of disease (Camilleri et al 1998). Furthermore, this technique has not been validated in patients with gastroparesis (Camilleri et al 1998). Information about intragastric distribution of a meal cannot be obtained using this technique. Radioisotopic breath tests have a number of advantages over scintigraphy in that they may be performed outside the laboratory and breath samples can be stored in sealed containers and sent for analysis, rather than requiring expensive equipment in the laboratory. Lee et al (2000) recently described an office- based non-radioactive breath test using ^{13}C Spirulina platensis as the label for measurement of gastric emptying of solids and reported comparable results with scintigraphy. This method has the advantage of avoiding radiation exposure.

7.3.3 *Ultrasound*

Ultrasound is a non-invasive, readily available technique which can be used for measurement of antral area and transpyloric flow (Hausken et al 1994). The two types of ultrasound techniques are the Doppler with or without 3-D. Transpyloric flow or the postprandial change in diameter of the antrum can be assessed in relation to defined anatomic landmarks eg. the plane of the superior mesenteric vein and aorta (Bolondi et al 1985). After ingestion of a drink the time for the distal stomach content, measured scintigraphically, to decrease to 50% of the maximum correlates closely with the ultrasound T50 (time taken for antral area to decrease to half its maximum), but there was no significant relation between the distal stomach content, expressed as the percentage of the maximum content in the total stomach, and the ultrasound T50 (Hveem et al 1996). Although, some studies have successfully imaged both the antrum and fundus, cross-sectional images of the antrum are more easily obtained (Gilja et al 1996).

Recent studies have shown that ultrasound may be a useful technique to assess antral contractile activity. For example, Hveem et al (1995) reported significant correlations between measurements of antral motility by intraluminal manometry and ultrasound in young healthy subjects (Hveem et al 1995). As with the "antral curve" technique ultrasound cannot distinguish those contractions that do and do not occlude the lumen (Hveem et al 1995).

Ultrasound is not a suitable method for assessing gastric emptying of solids and is impractical for quantifying gastric emptying or GI transit over extended periods of time. Evaluation of gastric emptying or transpyloric flow in persons who are overweight or obese using ultrasound is technically suboptimal. The use of ultrasound also requires a trained investigator. Compared to scintigraphy or breath tests, the use of ultrasound to assess gastric emptying also avoids exposure of subjects or patients to radiation.

7.3.4 *Paracetamol absorption*

Measurement of the absorption kinetics of paracetamol is a simple, in-expensive and non-invasive tool for assessing gastric emptying (Heading et al 1973, Horowitz et al 1989, Medhus et al 1999). Paracetamol (1.5g) is usually given orally with a standard amount of water or liquid, after an overnight fast. After ingestion, the paracetamol passes into the small intestine where it is absorbed. Blood samples are then collected at predetermined time intervals (usually 30 min) and plasma paracetamol levels measured over 4-8 hours. A number of measures have been shown to correlate with scintigraphic assessment of gastric emptying, including time to reach maximum plasma paracetamol concentration ($r = 0.76$; $P < 0.05$), maximum plasma concentration ($r= 0.77$; $P<0.05$) and plasma paracetamol at 30 min ($r= 0.72$; $P<0.05$) (Heading et al 1973). The advantages of this technique are its simplicity, non-invasive nature and the widespread availability of assays to measure blood or salivary concentrations of paracetamol. The paracetamol absorption technique, however, is only an indirect measurement of gastric emptying and, does not allow assessment of intragastric distribution, or measurement of emptying of solid meals (Medhus et al 1999).

7.3.5 *Radiological techniques*

Radiological techniques, including fluoroscopy have been used to assess motility of the gastrointestinal (GI) tract [for review see (Corazziari & Torsoli 1993)]. Following administration of radiological marker (usually plastic tubing), indirect monitoring of GI wall contraction and displacement of contents can be assessed by X-ray (Feldman et al 1984). Fluoroscopic images can be converted and recorded on video enabling immediate playback. The use of radiological techniques for the evaluation of gastric emptying is limited by the radiation exposure (limiting the time period of imaging). Imaging of gastrointestinal motor events following ingestion of radiopaque markers may not be 'physiological' or representative of gastric emptying of a meal (Corazziari et al 1993). For example, Chang et al (1996) reported that there was no significant

relationship between the rate of gastric emptying of a solid meal measured by scintigraphy and radiological measurement of radiopaque.

7.3.6 *Magnetic Resonance Imaging (MRI)*

Magnetic Resonance Imaging (MRI) produces high quality images of the volume of gastric contents over a specified time period as well as information about gastric motility and transpyloric flow (Schwizer et al 1995, Faas et al 2000)[(for review see (Outwater & Mitchell 1999)]. Images of the proximal and distal stomach may be obtained on a second by second basis, by coronal scans providing data about both the frequency and amplitude of lumen and non-lumen occlusive contractions (Schwizer et al 1994, Faas et al 2000).

The advantages of MRI is that it non-invasive and does not involve radiation exposure. Several factors limit the use of MRI in gastric emptying studies, ie. it is extremely expensive, is not readily available and requires relatively prolonged observations to assess gastrointestinal motor function (Schwizer et al 1994).

7.4 MEASUREMENT OF INTRALUMINAL PRESSURES AND CONTRACTIONS

Several techniques have been developed to specifically measure changes in pressure and contractile activity within the gastrointestinal tract are outlined in Table 7.2. In this section, manometry and barostat are discussed in detail as these technique are employed in the studies described in Chapters 8A and 11, respectively.

Table 7.2

Methods to Assess Intraluminal Pressures and Contractions
- Manometry (7.4.1)
- Barostat (7.4.2)
- Scintigraphy (7.3.1)
- Ultrasound (7.3.3)
- Radiological techniques (7.3.5)
- Magnetic Resonance Imaging (7.3.6)

7.4.1 *Manometry*

The technique of manometry allows the measurement of pressures within the gastrointestinal tract (Heddle et al 1988b, Dent 1990, Dent 1976)[for review see (Wiley et al 1999)]. Either solid or water-perfused, flexible, fine-bore catheters may be used. The technique used by the author in Appendix A involves a water-perfused, silicone rubber manometric assembly (4 mm outer diameter) which is inserted into the stomach via an anaesthetised nostril (see Appendix A.3.3 and 9.3.1). The tube may be positioned using fluoroscopy but this is both impractical and inaccurate. A subcutaneous reference electrode placed in the subject's forearm can be used to monitor the transmucosal potential difference (TMPD) between the stomach and duodenum allowing continuous monitoring of catheter position (Heddle et al 1989, Sun et al 1996). A sleeve sensor (~4.5 cm long) used to measure pyloric motility can be positioned precisely, using this technique (Figure 7.3) (Dent 1976). The stomach has a much lower pH, and therefore a more negative electrical potential (≤ -20 mV) than the duodenum (≥ -10mV), so that as the duodenal sensor passes into the duodenum the electrical potential becomes more positive (Dent 1976). The correct position of the tube can be maintained throughout a study using TMPD ie. when the difference in electrical potential between the two sleeve sensors is ~ 20 mV (Heddle et al 1988b). The incorporation of a number of antral and duodenal sideholes in the catheter allows a detailed assessment of antropyloroduodenal pressures (Figure 7.3) (Heddle et al 1988b).

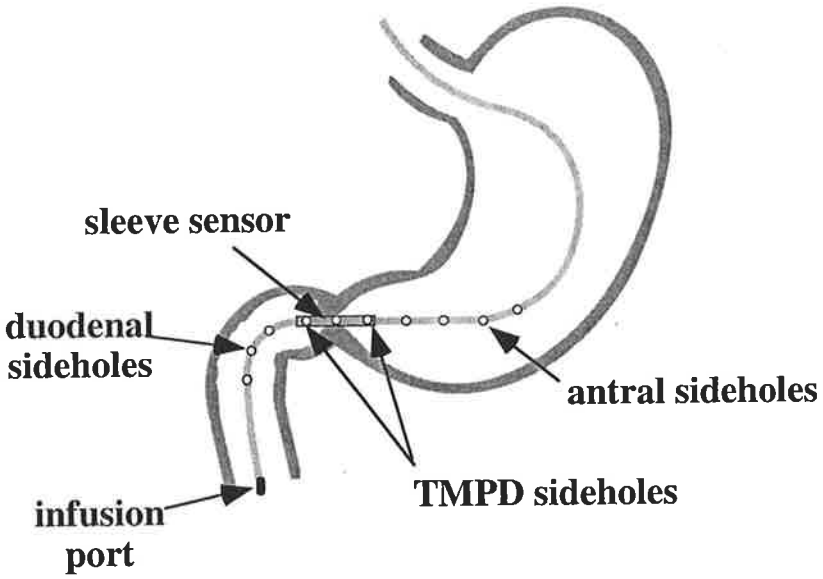

Figure 7.3: Schematic representation of manometric catheter incorporating 5 antral and 4 duodenal sideholes spaced 1.5 cm apart, a sleeve sensor and a duodenal infusion port.

The capacity of the sleeve sensor to accurately measure both phasic and tonic pyloric pressures is a substantial asset; such contractions are of particular interest as they are usually associated with the inhibition of transpyloric flow. In general manometry cannot detect those contractions or pressure waves that do not result in occlusion of the lumen. The combination of other methods, such as barostat or ultrasound, with manometry can provide additional information about gastric wall function. The manometric catheter may also include a port at the distal end to allow intraduodenal infusion (Heddle et al 1988b).

Optimal recording of manometric data requires a computer-based recording system with specialised software. Manometric pressures are usually digitised, using a data acquisition board, and recorded directly to a disk and stored for later analysis (Andrews et al 1998a, Fraser et al 1991).

Manometric variables that can be analysed the amplitude, frequency and organisation of phasic and tonic pressure waves [for reviews see (Dent 1990, Camilleri et al 1998)]. The criteria of manometric variables used in the study reported in Appendix A are described detail in Appendix A.3.3.

In Appendix A, manometry was used to evaluate antropyloroduodenal motility during intraduodenal lipid infusion in young and older subjects.

7.4.2 *Barostat*

The barostat is a technique designed for measurement of intraluminal pressure that reflects phasic contractile activity of the gut, often in the absence of lumen occlusion [for review (Wiley et al 1999)]. A barostat was used in the study reported in Chapter 11, to assess fasting gastric compliance, perception of gastric distension and postprandial gastric relaxation in healthy older and young subjects. In general, the gastric barostat technique involves the insertion of a catheter incorporating a polyethylene bag at the distal end via an anaesthetised nostril and positioning of the bag in the proximal stomach. The barostat maintains a constant intragastric pressure by withdrawing air from the bag during gastric contraction and injecting air into the bag during gastric relaxation. Gastric compliance or tone can be indirectly assessed by the barostat through measuring the volume of air entering or leaving the bag during relaxation (Azpiroz & Malagelada 1985)(Figure 7.4).

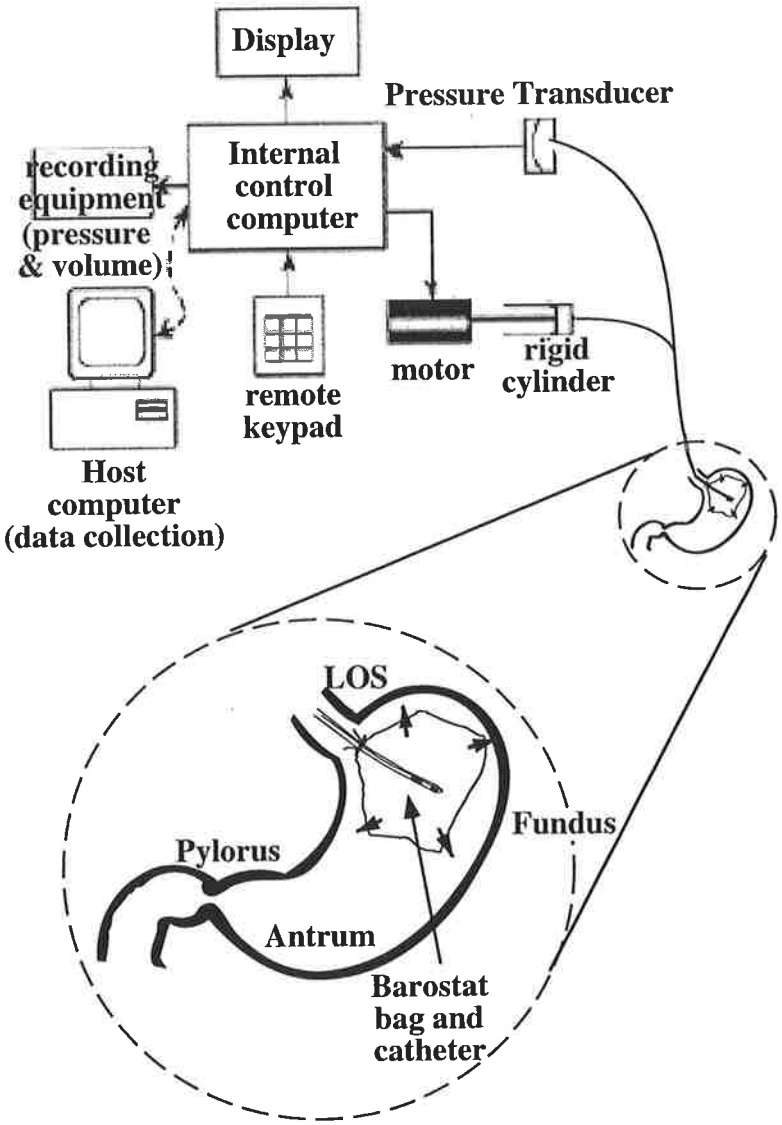

Figure 7.4: Schematic representation of the barostat bag and recording equipment used for assessment of proximal gastric tone and postprandial gastric relaxation.

Initially, the minimal distending pressure needs to be determined, by slowly inflating with air in increments of increasing pressure until the pressure at which continuous respiratory fluctuations are first evident with an intra-bag volume of at least 30 ml (Hebbard et al 1996, Verhagen et al 1999a). Fasting gastric compliance (defined as the pressure-volume relationship at several points) can be measured by two types of distensions; either at steps of fixed pressure (isobaric distensions), or using steps of fixed volume (isovolumetric distensions). Distensions are terminated when the pressure or volume within the bag reaches a predetermined level, ie. within safety limits and below the maximum capacity of the bag, or earlier if the subject reports marked discomfort (Verhagen et al 1999a). Postprandial gastric relaxation can be assessed maintaining intra-bag pressure at a fixed level above the minimal distending pressure and monitoring intra-bag volume of the bag after a meal.

The position of the subject is important during barostat studies, as the weight of adipose tissue overlying the stomach and the tone of the abdominal wall may directly influence barostat recordings (Hebbard et al 1995). Subjects are usually seated upright in a comfortable position with their arms resting on a table or bench (Heddle et al 1988b, Verhagen et al 1998). When studies involve more than one day it is necessary to obtain baseline data on each day, since gastric compliance can vary markedly between study days in some individuals.

The gastric barostat technique also enables the assessment of gastric sensations, such as the perceptions of fullness, bloating and abdominal pain during isobaric or isovolumetric distension of the bag, usually measured with visual analogue questionnaires. As discussed in Chapter 3.4 perceptions of gastric distension may be increased in patients with insulin dependent diabetes mellitus (IDDM), functional dyspepsia; gastro-oesophageal reflux disease and gastroparesis (Feinle et al 1997, Penagini et al 1998, Rayner et al 2000).

The barostat is an invasive and technically demanding technique; factors which limit its use. The presence of the balloon in the proximal stomach has also been shown to influence (ie. increase) the rate of gastric emptying and intragastric meal distribution (Moragas et al 1993).

7.5 MEASUREMENT OF ORAL GLUCOSE ABSORPTION

The technique used by the author to measure small intestinal glucose absorption is well established. This involves oral administration of the non-metabolised glucose analogue 3-O-methylglucose (3-OMG) (Fleming et al 1993). After ingestion of 3-OMG blood or urine samples are collected at pre-determined intervals, usually every 5-15 minutes. The serum or urine concentration of 3-OMG is quantified using either enzymatic analysis, thin-layer chromatography, gas chromatography or high performance (anion exchange) liquid chromatography (HPLC) (Fleming et al 1993). In Chapter 14, serum 3-OMG concentrations were determined using the HPLC method described by Fleming et al (1993).

7.5 CONCLUSIONS

There are now many useful, and complimentary, techniques available for the measurement of gastric motor, sensory and absorptive function; the choice of technique (s) is dictated by a number of factors. In Chapters 8A, 9, 11, 13 and 14, four different methods are used to assess the effect of ageing on proximal gastric function. As manometry, using a sleeve sensor, provides the optimum measurement of pyloric motility, this technique was used to assess the effect of ageing on antropyloroduodenal motility in response to intraduodenal lipid infusion (Appendix A). As discussed, electrogastrography (EGG) is a simple, non-invasive method for assessment of gastric myoelectrical activity and this technique was used to evaluate the gastric myoelectrical response to intraduodenal saline, glucose and lipid infusion in healthy older and young subjects (Chapter 9). The barostat is the best method to assess the effect of ageing on fasting gastric compliance, perception of gastric distension and postprandial gastric relaxation (Chapter 11). Since scintigraphy is the most accurate technique available for measurement of gastric emptying this method was used in Chapter 13 to assess the effect of oral glucose supplementation on gastric emptying in healthy older subjects, and in Chapter 14, to evaluate the effect of guar gum on the gastric emptying of glucose in the healthy older persons.

CHAPTER 8

Effects of ID Nutrients on Plasma CCK, GLP-1 and PYY Concentrations and their relation to Appetite and Pyloric Motility in the Healthy Elderly

8.1 SUMMARY

Ageing is associated with a decrease in appetite and slowing of gastric emptying. The gastrointestinal hormones CCK, GLP-1 and PYY may mediate these changes. The aim of this study was to determine whether ageing influences the secretion of CCK, GLP-1 and PYY and their effect on appetite and pyloric motility.

Eight healthy older (65-80 yr) and 7 young (20-34 yr) males received isoenergetic (12.1 kJ/min) intraduodenal (ID) infusions of lipid and glucose, for 120 minutes on separate days. Plasma CCK, GLP-1 and PYY concentrations were measured.

Plasma CCK concentrations were higher in older than young subjects (P= 0.004), due to higher baseline values (4.7 ± 0.2 vs 3.2 ± 0.2 pmol/L, P< 0.0001), and a greater rise during the lipid infusion (increase from baseline 7.1 ± 0.5 vs 5.3 ± 0.6 pmol/L, P= 0.048). Plasma GLP-1 and PYY concentrations were not different between the age groups. The decrease in hunger during ID lipid was inversely related to the increase in CCK, GLP-1 and PYY in young, but not older, subjects. During ID lipid infusion the increase in isolated pyloric pressure waves (IPPW) frequency was positively related to GLP-1 and PYY and the increase in IPPW amplitude was positively related to CCK in the older but not young subjects, while the increase in IPPW amplitude and pyloric tone was negatively related to GLP-1 and PYY in the young subjects.

Human ageing is associated with increased CCK concentrations which may contribute to slowing of gastric emptying, mediated by increased pyloric motility. The role of increased plasma CCK concentrations in mediating the age-related decrease in appetite remains to be established.

8.2 INTRODUCTION

We have shown that isoenergetic intraduodenal (ID) infusions of glucose and lipid exert nutrient- and age-specific effects on appetite and gastric motility (see Appendix A). Both ID glucose and lipid infusion suppress hunger in young males, whereas neither does in older males, and lipid is a more potent suppressor of hunger and food intake than glucose, in young but not older subjects. Intraduodenal lipid infusion stimulates phasic pyloric pressure waves in both young and older subjects and this response is

greater in older subjects, suggesting that enhanced pyloric pressure responses to ID fat may contribute to the slowing of gastric emptying in older people (see Appendix A).
The present study was performed to determine the effects of ageing on plasma concentrations of CCK, GLP-1 and PYY. All three slow gastric emptying, CCK and GLP-1 also suppress appetite and food intake (Muurahainen et al 1988, Lieverse et al 1994, Flint et al 1998, Turton et al 1996) (see Chapter 3.6.3, 3.6.4 and 3.6.5). We hypothesised that reduced hunger, increased satiety and slower gastric emptying in the elderly are due, in part, to greater release of these satiety hormones in older than young people. We have assessed this by measuring plasma CCK, GLP-1 and PYY concentrations in response to ID lipid and glucose infusions and correlated these results with measures of appetite, food intake and pyloric motility, reported in Appendix A. We were particularly interested in assessing the CCK response to ID lipid infusions, as lipid is a more potent stimulus of endogenous CCK release than glucose (Raybould & Lloyd 1994) and there are conflicting reports about the effect of age on increases in plasma CCK concentrations produced by ingestion of lipid (Masclee et al 1988) or a mixed meal (Berthelemy et al 1995)(see Chapter 3.6.1). We hypothesised that ageing enhances the lipid-induced increase in CCK concentrations.

8.3 SUBJECTS AND METHODS

Subjects are described in Appendix A.3.1.

8.3.1 *Experimental Protocol*

The experimental protocol is described in Appendix A.3.1.

Samples collected during the study described in Appendix A were assayed to determine plasma CCK, GLP-1 and PYY.

The assay techniques used are well established.

8.3.2 *Measurement of plasma CCK*

Plasma CCK was measured by a radioimmunoassay technique (Jansen et al 1983) using antibody T_{204} which binds to all biologically active CCK peptides containing the sulfated tyrosine region with almost equal affinity. The detection limit of the assay was between 0.5 and 1 pmol/L in plasma. The intra-assay precision ranged from 4.6 to

11.5% in the steep part of the standard curve. All samples were assayed in the same run.

8.3.3 *Measurement of plasma GLP-1*

Plasma GLP-1 7-36 was measured, after ethanol extraction of plasma samples, using a radioimmmunoassay method similar to that used by Orskov et al (1994). The antibody used in the method was provided by Professor SR Bloom (Hammersmith Hospital, London), which had been raised in a rabbit immunised with the GLP-1 (7-36) conjugated to bovine serum albumin (BSA) by carbodi-imide. The antibody had 100% cross-reactivity with synthetic entire GLP-1 (7-36) amide (from Peninsula), but did not cross-react with GLP-1 (7-37) amide, glucagon, GIP, or other gut or pancreatic peptides. The minimal detectable limit for the GLP-1 radioimmunoassay was ~2 fmol/L. The interassay coefficient of variation was 18 %.

8.3.4 *Measurement of plasma PYY*

Plasma levels of PYY were measured by a radioimmunoassay technique (Chen & Rogers 1997) using antiserum raised in rabbits immunised to the purified porcine or bovine peptide YY. The antiserum showed minimal to no cross reactivity to bovine or porcine pancreatic polypeptide, NPY or neurotensin. The minimal detectable limit for the PYY radioimmunoassay was 0.3 pmol/L with intra-assay coefficients of variation of 6-11% in the working range of the assay.

8.3.5 *Statistical Analyses*

Results are given as mean ± SEM. Comparisons between the young and older groups in the macronutrient content of the previous diet, fasting plasma albumin and basal hormone (CCK, GLP-1, PYY) concentrations, BMI, and baseline scores for hunger, and fullness were performed using Student's unpaired *t*-test, as these data were normally distributed. The effects of the ID nutrient infusions on scores for hunger, and fullness were analysed using repeated-measures mixed-model analysis of variance (ANOVA). The effects of nutrient infusions on absolute plasma hormone concentrations were analysed using a repeated measures three-way ANOVA with time, age (young vs older) and treatment (glucose vs lipid) as the factors, (plasma GLP-1 and PYY concentrations were log transformed before this analysis since this data was not normally distributed). To test the hypothesis that ID lipid produced a greater CCK response in the older than young subjects, CCK data during the ID lipid infusion were expressed as the change in CCK concentrations from baseline and analysed using a

repeated measures two-way ANOVA, with time and age as the factors. This ANOVA was performed using SigmaStat Statistical Software for Windows Version 1 (Jandel Corporation, San Rafael, CA). Relationships between the changes in plasma hormone concentrations and i) changes in hunger, fullness, and pyloric tone; ii) the number of isolated pyloric pressure waves (IPPW's) and amplitude of IPPW's were evaluated by linear regression with robust variance estimation via Mixed Model analysis to allow for repeated values in each subject (White 1980). A generalised R-square for each relationship was determined in this analysis using 'ordinary' maximum likelihood (Cox & Snell 1989). Relationships between basal and changes in plasma hormone concentrations and i) body weight (kg); ii) BMI (kg/m^2) and iii) previous energy intake (kJ) were analysed using linear regression. Differences between means of regression lines were analysed by Student's *t*-test for unpaired observations. Differences between slopes of regression lines were analysed by F-test. A P value < 0.05 was considered significant in all analyses.

8.4 RESULTS

Energy and macronutrient content of diet in young and older subjects is reported in Appendix A.4.

8.4.1 *Appetite*

Ratings of hunger and fullness during ID glucose and lipid infusion in young and older subjects are reported in Appendix A.4.1.

Energy intake at the buffet meal following ID nutrient infusions in young and older subjects is reported in Appendix A.4.1.

8.4.2 *Antropyloric Pressures*

Phasic and tonic pyloric pressure waves during intraduodenal lipid infusion in young and older subjects are reported in Appendix A.4.2.

8.4.3 *Plasma CCK*

Before ID glucose and lipid infusions, baseline fasting plasma CCK concentrations were higher in the older than in the young subjects (mean of both study days; 4.7 ± 0.3 vs 3.2 ± 0.2 pmol/L, $P < 0.001$), (Figure 8.1.1). The three-way ANOVA revealed a significant effect of age, indicating higher CCK concentrations in the older than young

subjects throughout the nutrient infusions (mean 0-120 min 7.8 ± 0.5 vs 5.7 ± 0.4, P< 0.01). There was a significant effect of treatment (P< 0.001), indicating a greater CCK response to lipid than glucose, and time (P< 0.001). The age × time interaction was not significant (P= 0.27), however there was a significant treatment × time effect (P< 0.001). There was no significant age × treatment × time interaction (P= 0.35), indicating that the timing of the increase in CCK concentrations was not significantly different between older and young subjects. The age × treatment interaction did not quite reach statistical significance (P= 0.078). However, analysis of the plasma CCK response to ID lipid infusion alone, by two-way ANOVA, indicated a significant effect of age due to a greater increase in CCK concentrations in older than young subjects (mean increase from baseline, 10-120 min. 7.1 ± 0.5 vs 5.3 ± 0.6 pmol/L; P= 0.048, Figure 8.1.2). Neither the basal CCK concentrations, nor the response to ID nutrients, were related to body weight, BMI or previous energy intake in either young or older subjects (data not shown).

8.4.4 *Plasma GLP-1*

Baseline plasma GLP-1 concentrations were not significantly different between the older and young subjects (9.2 ± 1.3 vs 6.9 ± 0.9 pmol/L; P= 0.17, Figure 8.2). By three-way ANOVA, there was no significant effect of age (P= 0.39), however there was a significant effect of treatment (P< 0.001), due to higher GLP-1 concentrations during ID lipid than glucose infusion, and time (P< 0.001). Age × treatment and age × time interactions were not significant (P= 0.69 and P= 0.80, respectively), however there was a significant treatment × time effect (P< 0.05), with the plasma GLP-1 concentrations peaking at 60 min during the lipid infusion but rising throughout the glucose infusion. There was no significant age × treatment × time interaction (P= 0.79), indicating that the timing of the increase in GLP-1 concentrations was not significantly different between the older and young subjects. Basal GLP-1 concentrations were not related to body weight, BMI or previous energy intake in either young or older subjects (data not shown). The mean change in GLP-1 concentration during the ID glucose infusion was inversely related to BMI (kg/m^2) (P= 0.046) and weight (kg) (P= 0.014) in the young subjects.

8.4.5 *Plasma PYY*

Before ID glucose and lipid infusions, there was no significant difference in fasting plasma PYY concentrations between older and young subjects (11.3 ± 0.6 vs 10.5 ± 0.6 pmol/L, P= 0.13, Figure 8.3). By three-way ANOVA, there was no significant

effect of age (P= 0.94), however there was a significant effect of treatment (P< 0.001), due to greater plasma PYY concentrations during the ID lipid than glucose infusion, and time (P< 0.001). Age × treatment and age × time interactions were not significant (P= 0.74 and P= 0.41, respectively), but there was a significant treatment × time effect (P< 0.001), with the plasma PYY concentrations peaking earlier during the ID lipid than glucose infusion. There was no significant age × treatment × time interaction (P= 0.793), indicating that the timing of the increase in PYY concentrations was not significantly different between older and young subjects. Neither the basal PYY concentrations, nor the response to ID nutrients, were related to body weight, BMI or previous energy intake in either young or older subjects (data not shown).

8.4.6 *Relationships between appetite and CCK, GLP-1 and PYY plasma concentrations in young and older subjects.*

8.4.6.1 Appetite / CCK

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

8.4.6.2 Appetite / GLP-1

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

8.4.5.3 Appetite / PYY

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

8.4.7 *Relationships between pyloric pressures and plasma CCK, GLP-1 and PYY concentrations in young and older subjects.*

8.4.7.1 Pyloric Motility / CCK

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

8.4.7.2 Pyloric Motility / GLP-1

There was a positive relationship between the increase in the frequency of isolated pyloric pressure waves (IPPW's) and the increase in plasma GLP-1 during the ID lipid infusion in the older (r^2= 0.087; P= 0.009), but not in the young (P= 0.60), subjects. In contrast, there was a significant 'inverse' relationship between the increase in amplitude of IPPW's and the increase in plasma GLP-1 concentrations in the young (r^2= 0.14; P= 0.0001), but not in the older (P= 0.28), subjects. There was a significant

'inverse' relationship between the increase in pyloric tone and the increase in plasma GLP-1 concentrations in young subjects (r^2= 0.044; P= 0.023) but not in the older subjects (P= 0.093).

8.4.7.3 Pyloric Motility / PYY

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

None of the intercepts or slopes of the lines for these regression calculations of plasma CCK, GLP-1 and PYY vs appetite or pyloric motility were different between young and older subjects.

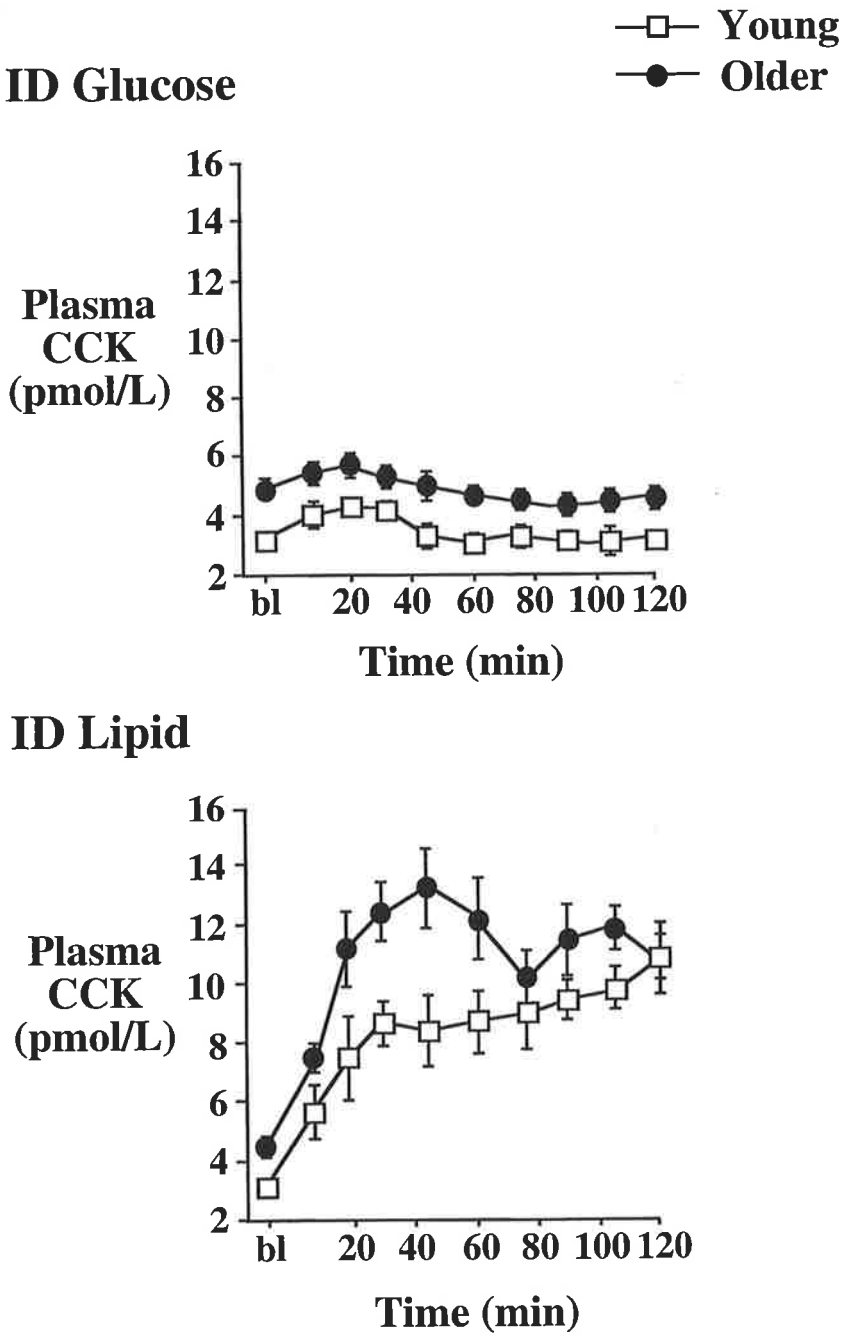

Figure 8.1.1: Plasma CCK concentrations in young and older subjects at baseline (bl) and during intraduodenal (ID) infusions of glucose and lipid. Data are mean ± SEM. Three-way ANOVA; significant effect of treatment (lipid > glucose, $P<0.01$), time ($P<0.001$) and treatment x time ($P<0.05$). Significant effect of age; (older > younger, $P<0.01$).

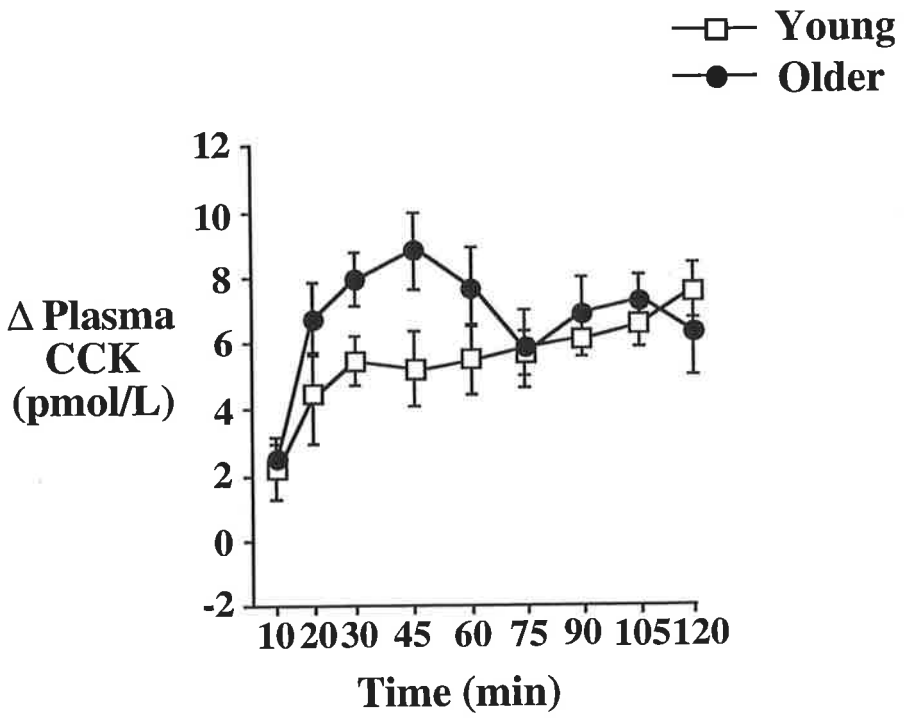

Figure 8.1.2: Mean (± SEM) plasma CCK concentrations in response to intraduodenal (ID) lipid infusion, expressed as the change in plasma concentrations from baseline (-15 min) in 7 young and 8 older healthy subjects. Two-way ANOVA; significant effect of age; (older > younger, $P<0.05$) and time ($P< 0.001$).

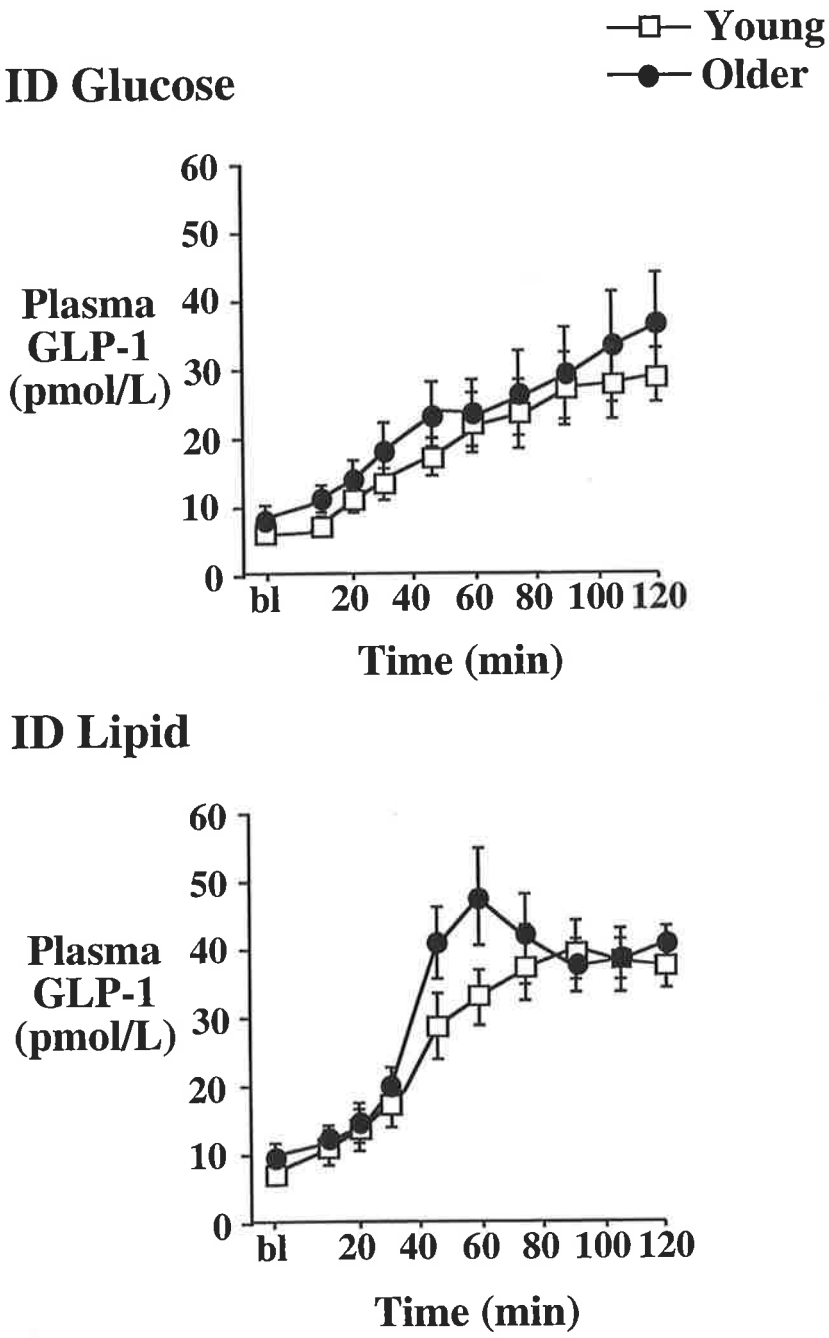

Figure 8.2: Plasma GLP-1 concentrations in young and older subjects at baseline (bl) and during intraduodenal (ID) infusions of glucose and lipid. Data are mean ± SEM. Three-way ANOVA; significant effect of treatment (lipid > glucose, $P<0.01$), time ($P<0.001$) and treatment x time ($P<0.05$).

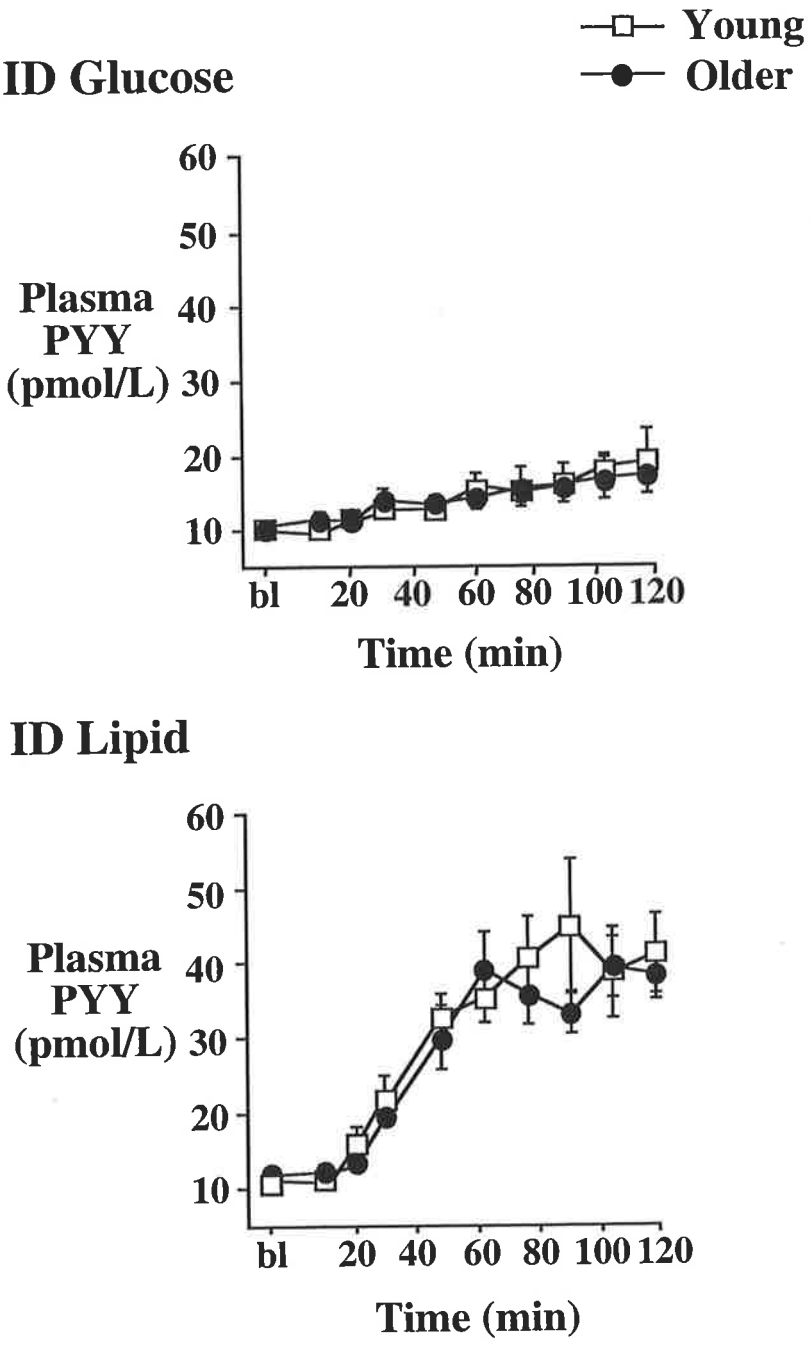

Figure 8.3: Plasma PYY concentrations in young and older subjects at baseline (bl) and during intraduodenal (ID) infusions of glucose and lipid. Data are mean ± SEM. Three-way ANOVA; significant effect of treatment (lipid > glucose, $P<0.01$), time ($P<0.001$) and treatment x time ($P<0.05$).

8.5 DISCUSSION

This study examined the effects of human ageing on plasma concentrations of the gastrointestinal hormones cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), fasting and during isoenergetic, intraduodenal (ID) infusions of lipid and glucose. In both young and older men, lipid and glucose infusions increased CCK, GLP-1 and PYY concentrations, and lipid increased CCK and PYY concentrations more than glucose. The major findings were that (i) older men had higher fasting CCK concentrations and greater increases in CCK concentrations during lipid infusions than younger men, (ii) There was no significant difference in plasma GLP-1 concentrations between young and older men, either fasting or during ID nutrient infusions, and (iii) there was no effect of ageing on plasma PYY concentrations. Nutrient infusions were administered intraduodenally, bypassing a number of gastric mechanisms potentially affecting gastrointestinal hormone secretion including variations in the rate of gastric emptying. Therefore, it cannot be assumed, that plasma CCK concentrations are higher in older than young subjects after oral or intragastric nutrient ingestion. Similarly, our results were obtained in men and may not apply to women.

Previous studies of the effect of ageing on plasma CCK concentrations have produced conflicting results. Masclee et al (1988) found higher CCK concentrations in older than young subjects following ID fat infusion, but no difference in fasting CCK concentrations between the two age groups. Berthelemy et al (1995) reported no difference in CCK concentrations, either fasting or following a liquid meal, between well nourished young and older people, but that postprandial CCK concentrations were higher in malnourished older subjects. This is consistent with evidence that suboptimal energy intake and/or reduced body weight may be associated with increased plasma CCK concentrations (Pirke et al 1994; Naslund et al 1997, Tamai et al 1993)(see Chapter 4.5.1).

Differences in body weight were not responsible for the increased CCK concentrations in our older subjects, as the mean body mass indices of the age groups did not differ. Qualitative and quantitative differences in previous diet are also unlikely to have contributed to the differences in CCK concentrations since (i) the diet of the older subjects had only a slightly lower carbohydrate composition (43.4 ± 1.4% older vs 48.3 ± 1.8% young), while the percentage fat and protein were similar in both age groups (ii) no subjects were malnourished according to the criteria of Berthelemy et al

(1995), ie energy intake less than 4184 kJ/day and body weight more than 20% below ideal and/or plasma albumin concentration less than 20 g/L and (iii) although the average total daily energy intake of the older subjects, as assessed by their food diaries, was less than that of the young subjects, there was no relation between plasma CCK concentrations and daily energy intake.

There is circumstantial evidence that CCK is a physiological satiety factor (see Chapter 3.6.1). Our finding that plasma CCK concentrations are higher in the elderly, therefore suggests that increased endogenous CCK activity may be a cause of the anorexia of ageing. For this to be so, sensitivity to the satiating effects of CCK must be maintained or increase with advancing age. Of note, food intake is suppressed more in older than young rodents by intraperitoneal CCK administration (Silver et al 1988). Although our study did not directly address sensitivity to the satiating effects of CCK, the suppression of hunger ratings and the significant inverse correlation between plasma CCK and hunger ratings in the young (consistent with that of French et al (1993), but not older men during ID lipid infusion provide indirect evidence that human ageing may be associated with reduced sensitivity to the satiating effects of CCK.

Glucagon-like peptide 1 (GLP-1) may also function as a satiety factor (see Chapter 3.6.3). In contrast to previous findings (Layer et al 1995), GLP-1 concentrations increased more during lipid than glucose infusions in both the young and older subjects. The lack of a significant difference between plasma GLP-1 concentrations in young and older subjects under any study condition makes it unlikely that changes in circulating GLP-1 concentrations contribute to the reduced appetite accompanying normal ageing.

The role, if any, of PYY in the control of human feeding is unknown (see Chapter 3.6.5). The effects of ageing on plasma PYY concentrations have, hitherto, not been reported. Plasma PYY concentrations, did not differ between older and young subjects in the present study, indicating that changes in circulating PYY concentrations are unlikely to contribute to the anorexia of ageing.

Previous studies have demonstrated that CCK (Muurahainen et al 1988, Scarpignato et al 1981), GLP-1 (Young et al 1996, Schirra et al 1997) and PYY (Allen et al 1984, Savage et al 1987) all slow gastric emptying (see Chapter 3.6.1, 3.6.3 and 3.6.5). During lipid infusion there was a greater increase plasma concentrations of all three

hormones in both the young and older subjects than during glucose infusion. This finding is consistent with that of Raybould et al (1994) for CCK, but contrasts with previous reports that plasma GLP-1 (Layer et al 1995) and PYY (Pedersen-Bjergaard et al 1996) concentrations increase equally in healthy men after ingestion of a high-carbohydrate and high-fat meal. This discrepancy could reflect oro-sensory or gastric mechanisms involved in the release of PYY. The greater release of these hormones during ID lipid than glucose infusion may contribute to the more powerful stimulatory effect of ID lipid on pyloric motility, previously observed in young adults (Andrews et al 1998a).

Intraduodenal lipid stimulated IPPW's and abolished all antral contractions. The increase in frequency and amplitude of IPPW's during lipid infusion was greater in older than young subjects. The increases in plasma CCK-concentrations were positively related to the increases in amplitude of IPPW's (or phasic response) in the older subjects, although this relationship did not quite achieve statistical significance. In contrast, there was a non-significant inverse relationship in the young subjects. There was also a significant positive relationship between lipid-induced plasma GLP-1 and PYY concentrations and frequency of IPPW's in older subjects but a significant inverse relationship between these hormones and amplitude of IPPW's as well as pyloric tone in the young subjects. The relatively small subject number of subjects in our study makes it difficult to draw strong conclusions from these relationships and it is not known whether the tonic or the phasic pressure response is more important in slowing gastric emptying (Tougas et al 1992). Nevertheless, these results suggest that the enhanced stimulation of pyloric motility induced in the older subjects by ID lipid, may be due to increased secretion of CCK and/or increased sensitivity to the stimulating effects of CCK, GLP-1 and PYY and may in turn be a cause of the slower gastric emptying observed in older people.

In summary, human ageing is associated with increased fasting and lipid-induced plasma CCK concentrations. We have found no clear evidence, however, that increased secretion of this putative satiety hormone is a cause of the reduced appetite and food intake that accompanies normal ageing. Indeed, resistance to the appetite-suppressant effects of CCK may be a feature of ageing. In contrast we found indirect evidence that delayed gastric emptying in old people may be due to enhanced stimulatory effects of CCK, GLP-1 and PYY on pyloric motility.

CHAPTER 9

Effect of Intraduodenal Nutrient Infusion on Appetite, GI Hormone Release and Gastric Myoelectrical Activity in the Healthy Elderly.

9.1 SUMMARY

The mechanisms responsible for the reduction in appetite and slowing of gastric emptying in older persons are poorly understood. The aims of this study were to

evaluate the effects of ageing on small intestinal regulation of appetite, gastrointestinal hormone release and gastric myoelectrical activity.

Thirteen older (65-84 yr) and 13 young (18-32 yr) healthy men received isovolumetric, intraduodenal (ID) infusions of saline (control), lipid and glucose for 120 min, on separate days. The energy content of the lipid and glucose infusions was identical at 12.1 kJ/min. Immediately following the ID infusions each subject was offered a buffet meal and ad libitum food intake was quantified. Blood glucose and plasma insulin, glucagon like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were measured. Gastric myoelectrical activity was measured by surface electrogastrography (EGG).

ID lipid suppressed food intake in both the young and older men ($P< 0.05$), whereas ID glucose suppressed food intake only in the older men ($P< 0.05$). The blood glucose ($P< 0.01$) and insulin ($P< 0.05$) responses to ID glucose were greater in older, than young men, but there were no differences in GLP-1 or GIP responses to any of the infusions. There was a greater increase in the EGG power ratio both during and following ID glucose infusion in the young ($P< 0.05$) than the older men, and an attenuation of EGG frequency by nutrient infusions in older, but not young, men.

Our findings indicate that ageing is associated with nutrient-specific changes in appetite, hormonal and gastric myoelectrical (EGG) responses to ID nutrients. An enhanced satiating effect of small intestinal carbohydrates may potentially contribute to the anorexia of ageing.

9.2 INTRODUCTION

In a previous study we administered isoenergetic fat and glucose ID infusions to healthy young and older men (Appendix A). In contrast to the greater suppression of food intake by fat than glucose in the young men, there was no difference between the effects of the two nutrients in older men. The absence of a control (saline) infusion in that study prevented us from determining whether this age-related difference was due to an increased satiating effect of ID glucose or a reduction in the satiating effect of fat in older compared to young men.

Gastric pacemaker activity, which determines the frequency of gastric contractions, is modulated by feedback signals from the small intestine and can be measured using cutaneous electrodes - so-called electrogastrography (EGG) (see Chapter 7.2) Dyspeptic symptoms (Pound & Heading 1998), increased pre-prandial fullness (Clarkston et al 1997) and early satiation (Rolls et al 1995) occur frequently in the elderly, but it is not known whether these sensations are associated with EGG abnormalities in this group. The effects of small intestinal nutrient infusion on gastric myoelectrical activity have not been investigated in the elderly.

The aims of this study were to determine whether the effects of small intestinal nutrient infusion on appetite, gastrointestinal hormone release and gastric myoelectrical activity are modified by healthy ageing. Based on our previous study (Chapter 8), the broad hypotheses addressed were that: (1) the appetite suppressant effect of ID glucose is greater in older than young adults, and (2) the release of gastrointestinal hormones which suppress appetite and/or slow gastric emptying will be greater in older than young subjects during ID glucose infusion.

9.3 SUBJECTS AND METHODS

Thirteen healthy older men, mean age 72.1 years (range 65-84 yr) and 13 healthy young men, mean age 23.7 years (range 18-32 yr) were recruited by advertisement. Body mass index (BMI) was not different between young and older subjects (young; 23.9 ± 0.6 kg/m^2 vs older; 23.5 ± 1.0 kg/m^2 ; P= 0.13) and all subjects had a 'dietary restraint' score (Factor 1) of < 11 on the Three-Factor Eating Restraint Questionnaire (Stunkard & Messick 1985), indicating that they were unrestrained eaters (see Chapter 6.4.1). Prior to the study all subjects completed a five day diet diary. All subjects were non-smokers and none had a history of gastrointestinal disease, gastrointestinal surgery, nor was taking medication known to influence gastrointestinal motility. Seven of the older subjects were taking medications for the treatment of hypertension, including atenolol, calcium channel blockers and ACE inhibitors. Three subjects were taking simvastatin for hyperlipidaemia. The study protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital and each subject gave written, informed consent. The food intake results for 12 of the 13 young subjects have been included and reported previously in a comparison of lean and obese young men (Chapman et al 1999).

9.3.1 *Experimental Protocol*

Each subject underwent three studies in a single-blind randomised manner on separate days, during which intraduodenal (ID) infusions of saline (control), glucose or lipid were administered. The studies were separated by at least 3 days.

On each study day subjects attended the laboratory at 0800 hr after a 12 hour overnight fast from all intake except water. Upon arrival, a silicone rubber tube (4 mm outer diameter) was inserted into the stomach via an anaesthetised nostril. The tip of the tube was allowed to pass into the duodenum by peristalsis and this took 20-90 min. The positioning of the tube was confirmed by measurement of transmucosal potential difference (TMPD), as described in Chapter 7.4.1. An intravenous cannula was placed in an antecubital vein for blood sampling.

Recordings of gastric myoelectrical activity were made from four pregelled silver-silver chloride ECG electrodes (3M, Australia), using the method of Smout et al, which is described in Chapter 7.2.

Visual analogue scales (VAS) for the assessment of appetite were administered at t= 0 min, then every 20 min to t=140 min, at t=170 min and t=200 min. Venous blood (~15 ml) was collected at identical timepoints for subsequent measurement of blood glucose and gastrointestinal hormones. Plasma was separated within 30 minutes of collection and stored at -70°C for later analysis. Immediately following administration of the VAS and blood sample at t= 20 min, an infusion of either (1) 0.9% sodium chloride (control) at 3 ml/min, (2) 25% glucose (3989 kJ/L, 1390 mOsmol/kg water, Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) at a rate of 3 ml/min, or (3) 10% Intralipid (triacylglycerol emulsion, 4602 kJ/L, 300 mOsmol/kg water, Kabi Pharmacia AB, Sweden) at a rate of 2.6 ml/minute plus 0.9% sodium chloride at a rate of 0.4 ml/min, was commenced and continued for 120 minutes. Infusions were delivered into the duodenum, approximately 10 cm distal to the pylorus, via a port in the nasoenteric tube (Heddle et al 1988b)(see Chapter 7.4.1). During all three infusions each subject received the same volume (3 ml/min) and, for the glucose and lipid infusions, the same energy load (11.97 kJ/min, total 1436 kJ). At 140 minutes the ID infusion was ceased and the tube removed. Subjects were then offered a standardised cold buffet style meal, prepared in excess of what they would normally eat, and invited to eat as much as they wished for the next 30 min. The rate of ingestion and the total amount of food consumed were quantified.

9.3.2 *Assessment of appetite*

Appetite was assessed using 10 cm linear visual analogue scales (see Chapter 6.3.1). The 0 and 20 min values were averaged to provide a baseline (bl), and absolute ratings during the ID nutrient infusions (t= 40-140 min) quantified (Andrews et al 1998a).

The total amount (g) of food consumed at the buffet meal (300 ml full cream milk, 300 ml unsweetened orange juice, 4 slices wholemeal bread, 4 slices white bread, 4 slices ham, 4 slices processed chicken, 4 slices cheddar cheese, 8 slices of tomato, cucumber and lettuce, 2 sachets of mayonnaise, margarine, butter and tomato sauce, 1 pear, 1 orange, 1 banana, 1 apple, 200 g chocolate custard, 200 g strawberry low-fat yoghurt, 50 g ice cream) was calculated. Food intake from the meal was analysed using the DIET 4 Nutrient Calculation software (Xyris Software, Australia, Pty Ltd) to determine both the energy intake (kJ) and macronutrient composition of the meal (see Chapter 6.3.3). The rate of food intake was also determined (kJ/min).

9.3.3 *Measurement of blood glucose, insulin, GIP and GLP-1*

Whole blood glucose concentrations were measured immediately using a portable blood glucose meter (Companion 2 Blood Glucose System; Medisense Inc., Waltham, MA). The accuracy of this method has been confirmed using the hexokinase technique (Horowitz et al 1991).

Plasma insulin was measured using the Abbott Imx Microparticle Enzyme Immunoassay (Abbott Laboratories, Diagnostic Division, Dainabot, Tokyo, Japan) (Chapman et al 1998). The sensitivity of the assay (concentration at 2 standard deviations from the zero standard) was 1.0 mU/ml. The interassay coefficients of variation were 4.5% at 8.3 mU/ml and 3.4% at 40.4 mU/ml. Plasma insulin concentrations were measured during the ID saline and glucose infusions only, since it is known that ID lipid does not affect plasma insulin concentrations in young subjects (Chapman et al 1999).

Plasma GLP-1 was measured by radioimmunoassay (Wishart et al 1998) after ethanol extraction of plasma samples using an antibody supplied by Prof SR Bloom (Hammersmith Hospital, London). The latter did not cross-react with glucagon, GIP or other gastrointestinal peptides and had been demonstrated by chromatography to measure intact GLP-1 7-36 amide (Kreymann et al 1987). The minimal detection

limit for this assay was ~2 pmol/L and the interassay coefficient of variation was 18 %.

Plasma GIP was measured using a standard GIP and anti-human GIP antisera according to an established method (Wishart et al 1992). The minimal detection limit was 2.4 pmol/L with a sample volume of 500 μL, and the interassay coefficient of variation was 15%.

9.3.4 *Electrogastrography (EGG)*

The EGG signals were amplified using filters with low and high cut-off frequencies of 1.2 and 16.8 cpm (0.02 and 0.28 Hz) respectively (Sun et al 1995b), digitised at 10 Hz and stored on the hard drive of a Macintosh SE personal computer. The EGG records were then converted from 10 Hz to 1 Hz using software (Sun et al 1995b) developed from the Lab View package (National Instruments). The EGG signals were subjected to Fast Fourier transformation (FFT) to obtain individual spectra (Sun et al 1995b). A "flat-top" window for the signal was used because this method allows optimal definition of amplitude. The running spectral analysis was updated every minute from the preceding 4 min with an overlap period of 1 min (Sun et al 1995b). Of the power spectra (0-200 min) the frequencies between 0 and 15 cpm were visualised. The mean frequency of the normal 3 cpm (2.6-3.7) component was calculated over 20 min intervals. For each 20 min interval over the 180 min of the study the mean power of the 3 cpm component of the EGG spectra was divided by the mean power of this component during the baseline (20 min) period to derive a power ratio. Tachygastrias were defined as peaks in the individual spectra >3.7 cpm (0.062 Hz) and <11 cpm (0.18 Hz) for more than 2 minutes, without a definite peak in the normal (2.6-3.7 cpm) range in any of the four channels.

9.3.5 *Statistical Analysis*

Before ID nutrient infusions, comparisons between the young and older groups in the macronutrient content of the previous diet, as well as ratings of hunger, fullness and nausea were performed using Student's unpaired t-test, as these data were normally distributed. Ratings of hunger, fullness and nausea (t= bl-140 min) and EGG frequency and power ratio were analysed by repeated measures 3-way analysis of variance (ANOVA). When a significant interaction between factors was observed, contrasts were used to test preplanned hypotheses of interest enabling paired comparisons between the three studies. Relationships between hunger, fullness and

nausea ratings and energy intake and EGG parameters were assessed by linear regression analysis using Statview Version 4.1 (Abacus Concepts Inc., California). Differences in energy and macronutrient intake from the buffet meal between the two groups were evaluated by two-way ANOVA. All data, except the regression analysis, were analysed using SuperANOVA Version 1.11 (Abacus Concepts Inc., California). Data are presented as mean values ± SEM. A P value < 0.05 was considered significant in all analyses.

9.4 RESULTS

The study protocol was well tolerated by all subjects.

9.4.1 *Baseline data*

As assessed by diet diary, mean energy intake in the usual diet of older subjects was approximately 10 % less than young subjects. This difference was not significant (8678 ± 468 kJ/day vs 9585 ± 824 kJ/day, P= 0.69). There was also no difference between the two groups in dietary macronutrient composition (data not shown). There was no significant difference in the eating restraint scores between the older and young (older: 6.38 ± 0.99 vs young: 4.67 ± 0.67; P= 0.18) as indicated by Factor 1 of the Eating-restraint questionnaire (Stunkard & Messick 1985).

9.4.2 *Appetite*

Since fullness was stimulated maximally (to 10 cm on VAS scale) and hunger suppressed maximally (to 0 on VAS scale) following the buffet meal in almost all subjects, only appetite ratings during the intraduodenal (ID) saline, glucose and lipid infusions (t= 40-140 min) are presented and analysed.

Hunger ratings were lower in the older than young subjects both at baseline (3.4 ± 0.3 cm vs 6.0 ± 0.2 cm; P< 0.001) and throughout the ID infusions, (effect of age; P< 0.01). (Figure 9.1a-c). Ratings of hunger increased during the ID nutrient infusions; effect of time (P<0.01). This increase was largely confined to the older subjects (3.4 ± 0.3 cm to 5.1 ± 0.5 cm vs 6.0 ± 0.2 cm to 6.1 ± 0.4 cm; interaction of time × age, P< 0.05). Although the older subjects were less hungry at baseline there was no difference between hunger ratings in the older and young men at the end of the infusions (5.1 ± 0.5 cm vs 6.1 ± 0.4 cm; P= 0.10). Hunger ratings were also affected

by the type of nutrient infused; effect of treatment ($P< 0.01$); with hunger ratings lower during the glucose infusion than both the control ($P< 0.001$) and lipid ($P< 0.05$) infusions. There were no significant age × treatment, treatment × time or age × treatment × time interactions for ratings of hunger.

There was no difference ratings of fullness either at baseline (3.6 ± 0.2 cm vs 3.7 ± 0.3 cm, $P= 0.75$) or during the ID infusions between young and older subjects (effect of age; $P= 0.60$) (Figure 9.2a-c). Fullness ratings were neither affected by the type of nutrient infused ($P= 0.15$) nor over time ($P= 0.35$). There were no interactions between age, treatment and time for ratings of fullness.

There was no difference in ratings of nausea either at baseline (0.92 ± 0.16 cm vs 1.38 ± 0.26 cm; $P= 0.31$) or during the ID infusions between the young and older subjects (effect of age; $P= 0.45$) (Data not shown). Nausea ratings were not affected by the type of nutrient infused ($P= 0.38$), but were affected by time ($P< 0.001$); nausea ratings in both age groups increased from 1.14 ± 0.22 cm at baseline to 1.70 ± 0.29 cm by the end of the infusions. There were no interactions between the effects of age, treatment and time, for ratings of nausea.

9.4.3 *Energy intake*

As in their usual diet, assessed by 3 day diet diaries, the older subjects consumed about 10% less than the young subjects on each study day following ID infusions, although this difference was not significant ($P= 0.35$)(Figure 9.3). There was an effect of infusion type on energy intake ($P< 0.01$); subjects ate significantly less following both ID lipid (3243 ± 336 kJ) and glucose (3433 ± 256 kJ) than the control infusion (4032 ± 235 kJ), with no difference between energy intake following the lipid and glucose infusions ($P= 0.36$).

In the older subjects the suppression of energy intake by the glucose infusion, when compared to the control day intake, was greater than in the young subjects (22.2 ± 6.1% vs 4.9 ± 8.2%), whereas the magnitude of the suppression by ID lipid was not significantly different between the two age groups (older; 21.3 ± 8.4% vs young; 26.1 ± 8.2%). This was reflected in the significant interaction of treatment × age ($P< 0.001$). Consistent with this, when the effect of the different treatments was analysed within each age group, the older subjects consumed less energy after ID glucose than saline ($P< 0.01$), whereas the young subjects did not ($P= 0.32$). Conversely, young

subjects consumed less energy after lipid than glucose (P< 0.001), but the older subjects did not (P= 0.53). The macronutrient content of the buffet meal (% protein, % fat and % carbohydrate) was not significantly different between young and older subjects, nor between ID lipid and glucose infusions (data not shown). There was no effect of age or the type of ID nutrient on the rate of eating (data not shown).

9.4.4 *Relationships between appetite and energy intake*

The amount of energy subjects consumed at the buffet meal was weakly but positively related to baseline ratings of hunger (r= 0.28, P< 0.05) and negatively related to baseline ratings of fullness (r= -0.27, P< 0.05). The relationship between baseline hunger scores and the amount of food eaten at the buffet meal was significant in the young (r= 0.38, P< 0.05), but not in the older (r= 0.11, P= 0.52), subjects. In contrast, the inverse relationship between baseline fullness scores and the amount of food eaten at the buffet meal was significant in the older (r= -0.38, P< 0.05) but not the young (r= -0.11, P= 0.49), subjects. Ratings of hunger immediately before the meal (t= 140 min) were positively related (r= 0.31, P< 0.01) and ratings of fullness were negatively related (r= -0.27, P< 0.05) to the amount eaten at the buffet meal in both the young and older subjects (see Chapter 6.3.1).

9.4.5 *Blood glucose and plasma insulin*

Fasting (baseline) blood glucose concentrations were higher in the older than young subjects (mean of three study days; 5.6 ± 0.1 mmol/L vs 5.1 ± 0.1 mmol/L, P< 0.01). ID saline and lipid did not affect blood glucose concentrations. During ID glucose infusion the rise in blood glucose concentrations was greater in the older than young subjects (Figure 9.4a); three way ANOVA revealed a significant effect of age (mean blood glucose from baseline to 140 min; 6.7 ± 0.5 mmol/L vs 5.6 ± 0.4 mmol/L, P< 0.001), treatment (P< 0.001) and time (P< 0.001). There were significant treatment × age (P< 0.001), treatment × time (P< 0.001) and time × age (P< 0.001) interactions for blood glucose concentrations and also a significant age × treatment × time interaction (P< 0.001), indicating that the peak blood glucose response to ID glucose infusion occurred significantly later in the older than young subjects.

Fasting plasma insulin concentrations were not significantly different between the older and young subjects (mean of control and glucose study days; 5.8 ± 0.2 mU/L vs 4.3 ± 0.4 mU/L, P= 0.15). ID saline infusion did not affect plasma insulin concentrations. During ID glucose infusion the rise in plasma insulin concentrations

was greater in older than young subjects (Figure 9.4b); three way ANOVA revealed a significant effect of age (mean plasma insulin from baseline to 140 min; 85.0 ± 9.7 mU/L vs 47.0 ± 4.0 mU/L, P< 0.05), treatment (P< 0.001), and time (P< 0.001). There were significant treatment × age, (P< 0.05), treatment × time (P< 0.001) and time × age (P< 0.001) interactions for plasma insulin concentrations and also a significant age × treatment × time interaction (P< 0.001), indicating that the greatest increase in plasma insulin concentrations during ID glucose infusion occurred significantly later in the older than the young subjects.

9.4.6 *GLP-1 and GIP concentrations during ID nutrient infusions*

Baseline plasma GLP-1 concentrations were not significantly different between the older and young subjects (mean of three study days; 10.9 ± 0.9 pmol/L vs 11.9 ± 0.7 pmol/L respectively, P= 0.36) and levels were not affected by saline infusion (Figure 9.5). There was a significant effect of treatment on plasma GLP-1 concentrations (P< 0.001)(Figure 9.5); GLP-1 concentrations were higher during ID glucose (P< 0.001) and lipid (P< 0.001) infusions compared to control infusion, and during ID lipid infusion (P< 0.05) compared to ID glucose infusion. GLP-1 concentrations increased during the ID infusions in both young and older subjects, effect of time (P<0.001). There was no effect of age on plasma GLP-1 concentrations during ID infusions (P= 0.49) and this was so even when the glucose infusion was analysed alone (effect of age; P= 0.63). There was a significant treatment × time interaction (P<0.001); GLP-1 concentrations increased more over time during the ID glucose and lipid infusions than during the ID control infusion, and during the ID lipid infusion compared to the ID glucose infusion. There were no significant treatment × age, time × age, or treatment × time × age interactions.

Baseline plasma GIP concentrations were not significantly different between the older and young subjects (mean of three study days; 24.3 ± 2.7 pmol/L vs 30.8 ± 2.8 pmol/L respectively, P= 0.10) and the control infusion did not affect plasma GIP concentrations (Figure 9.6). There was a significant effect of treatment on plasma GIP concentrations (P<0.001)(Figure 9.6); GIP concentrations were higher during ID glucose (P<0.001) and lipid (P<0.001) infusions compared to the saline infusion. GIP concentrations were slightly higher during ID glucose, compared to ID lipid infusion, but this difference was not quite significant (P= 0.07). GIP concentrations increased during the ID infusions in both young and older subjects; effect of time (P< 0.001). There was no significant effect of age on plasma GIP concentrations during ID

infusions (P= 0.65), even when the glucose infusion was analysed alone (effect of age; P= 0.53) (Figure 9.6). There was a significant treatment × time interaction (P< 0.001); GIP concentrations increased more over time during the ID glucose and lipid infusions than during the ID saline infusion, and during the ID glucose infusion compared to the ID lipid infusion. There were no significant treatment × age, time × age or treatment × time × age interactions for plasma GIP concentrations.

9.4.7 *Electrogastrography (EGG)*

Technically adequate EGG recordings were only available for analysis in 8 of 13 young and 12 of 13 older subjects due to logistical factors. Analysis of the EGG spectra for individual subjects identified a clearly defined peak in 66 % of the spectral lines. There was no significant difference in the proportion of analysable spectral lines between the young and older subjects.

9.4.7.1 Frequency

EGG frequency during baseline (t= 0-20 min), first hour (t= 40-80 min) and second hour (t= 100-140 min) of infusion and the postprandial (t=170-200 min) period are summarised in Figure 9.7a-c. As evaluated by three-way ANOVA (age, treatment and time as the parameters), EGG frequency was not affected by age (P= 0.28), treatment (P= 0.48) or time (P= 0.76) and there were no interactions between these parameters.

When the EGG frequency was analysed within each age group there was no effect of treatment (P= 0.41), or time (P= 0.54) in the young subjects. In contrast, in the older subjects; postprandial EGG frequency was lower during both the ID glucose (P< 0.05) and the ID lipid (P< 0.05) study days, compared to the control day.

9.4.7.2 Power ratio

Three-way ANOVA indicated a significant difference in EGG power ratio between time segments (P< 0.001), such that the post-prandial power ratio was greater than the power ratio in both the first hour and second hour of the ID infusions (Figure 9.8a-c). There was also a significant effect of treatment, (P< 0.01) so that the power ratio was higher during the ID glucose than both ID saline (P< 0.01) and ID lipid (P< 0.05) infusions. The power ratio tended to be higher in young than older subjects (P< 0.05). When the power ratio of the EGG was analysed within each age group (with treatment type and time as parameters), there was a significant effect of treatment type in the young (P< 0.05); such that the power ratio during the ID glucose (2.02 ±

0.30) infusion day was higher compared to the ID saline (1.10 ± 0.13, F = 12.76, P< 0.05) and ID lipid (1.53 ± 0.19; P< 0.05) infusion days; but not in the older (P= 0.80) subjects.

9.4.7.3 Tachygastrias

By three-way ANOVA, the incidence of tachygastrias was significantly greater in the young than the older subjects (P< 0.05) during the ID saline (0.05 ± 0.05% vs 0.2 ± 0.2%, respectively), ID glucose (1.8 ± 0.7% vs 0.5 ± 0.3%, respectively) and ID lipid (8.0 ± 3.9% vs 1.2 ± 0.5%, respectively) infusions. The incidence of tachygastrias was greater during ID lipid compared to the ID saline (P< 0.05) and ID glucose (P< 0.05) infusion.

9.4.8 *Relationships of appetite and food intake with EGG frequency, power ratio and % tachygastrias*

Mean ratings of hunger, fullness and nausea and mean frequency, power ratio and % tachygastrias were determined for the first and second hours of the ID infusions. There was no significant relationship between the ratings hunger, fullness or nausea and EGG frequency, power ratio and % tachygastrias during either the first or second hour of the ID infusions in the young and older subjects combined.

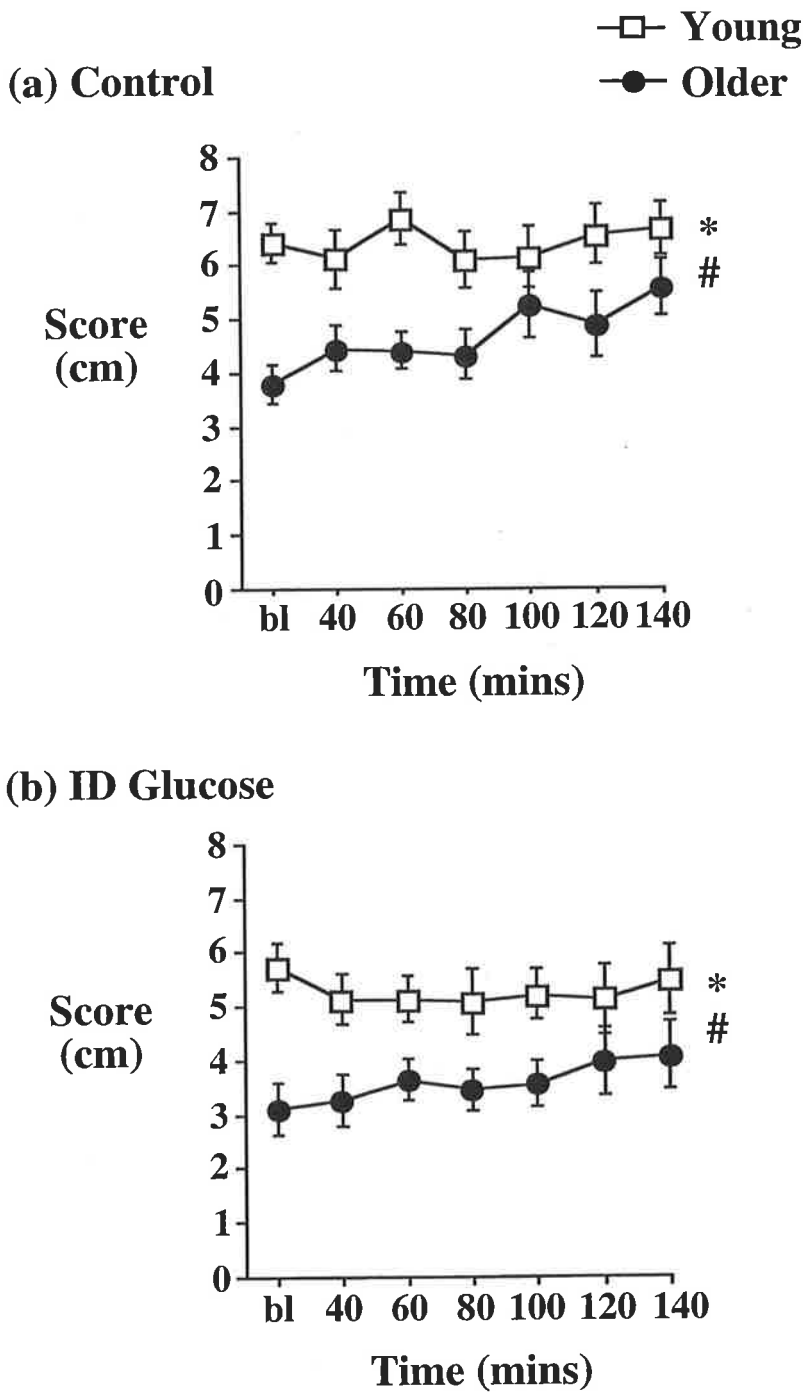

Figure 9.1: Subjective ratings of hunger at baseline (t=0-20 min) and during intraduodenal (ID) (a) saline, (b) glucose and (c) lipid infusions in young and older subjects. Data are mean ± SEM. #P< 0.05 vs baseline, *P< 0.05 young vs older.

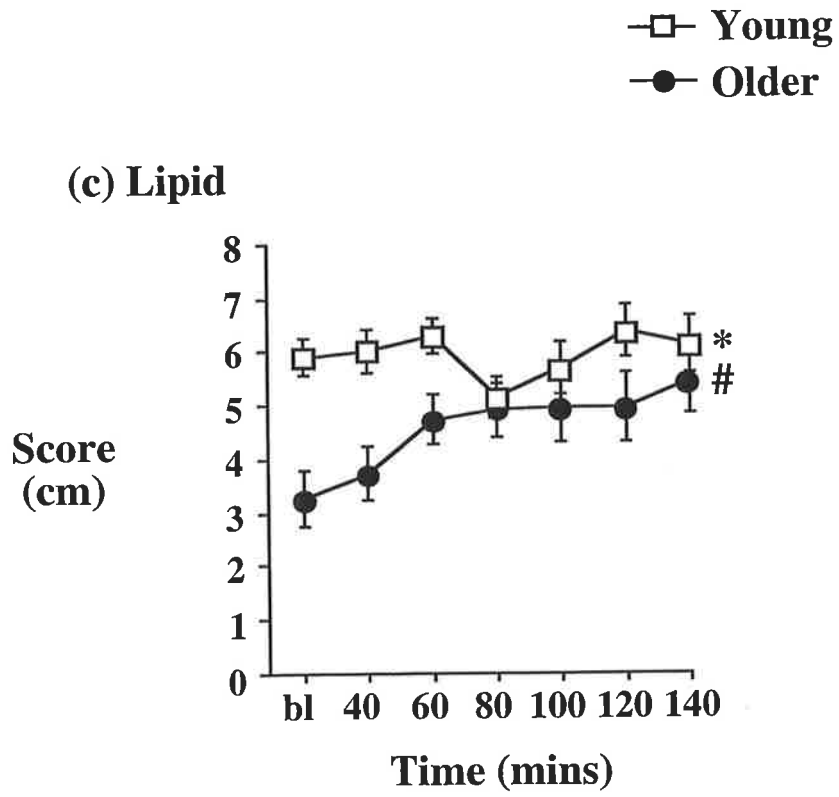

Figure 9.1 cont. : Subjective ratings of hunger at baseline (t=0-20 min) and during intraduodenal (ID) (a) saline, (b) glucose and (c) lipid infusions in young and older subjects. Data are mean ± SEM. #P< 0.05 vs baseline, *P< 0.05 young vs older.

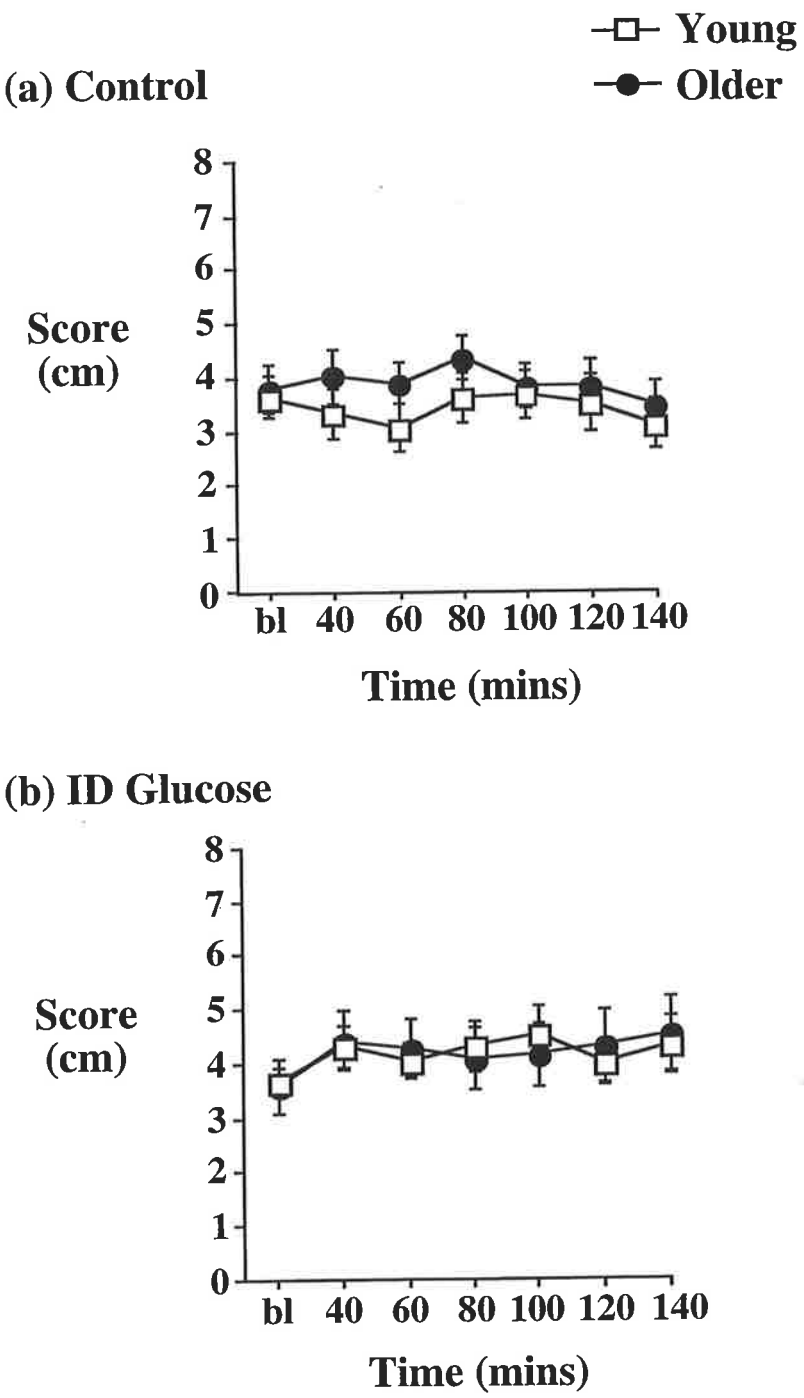

Figure 9.2: Subjective ratings of fullness at baseline (t=0-20 min) and during intraduodenal (ID) (a) saline, (b) glucose and (c) lipid infusions in young and older subjects. Data are mean ± SEM.

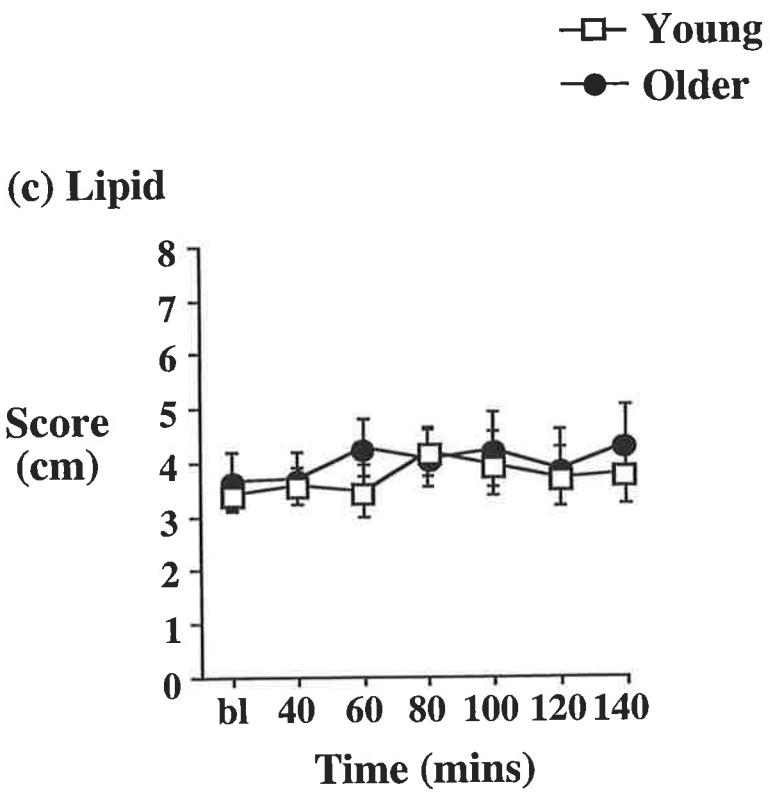

Figure 9.2 cont.: Subjective ratings of fullness at baseline (t=0-20 min) and during intraduodenal (ID) (a) saline, (b) glucose and (c) lipid infusions in young and older subjects. Data are mean ± SEM.

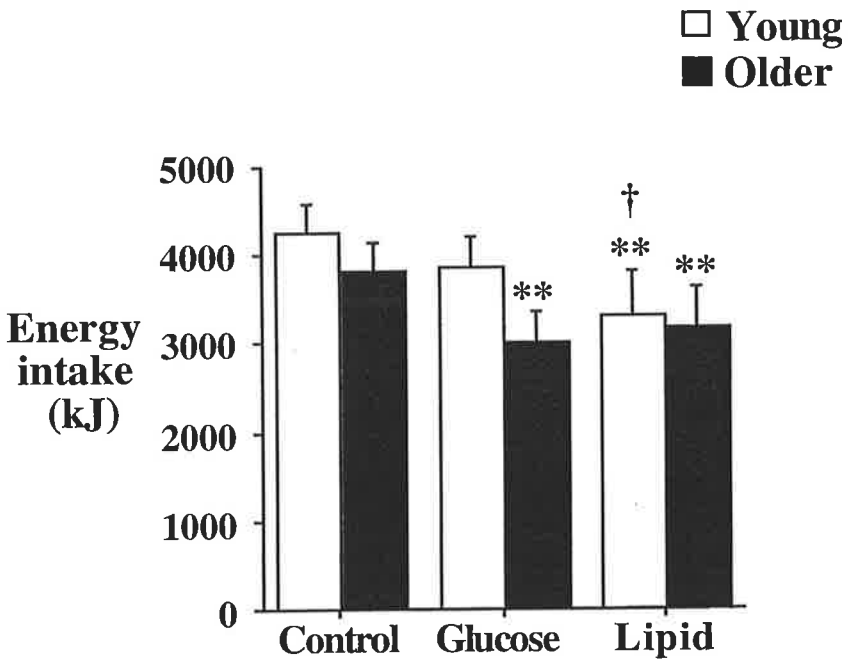

Figure 9.3: Energy content (kJ) of buffet meal consumed after intraduodenal saline (control), glucose and lipid in young and older subjects. **P< 0.05 vs control; †P<0.05 lipid vs glucose. Data are mean ± SEM.

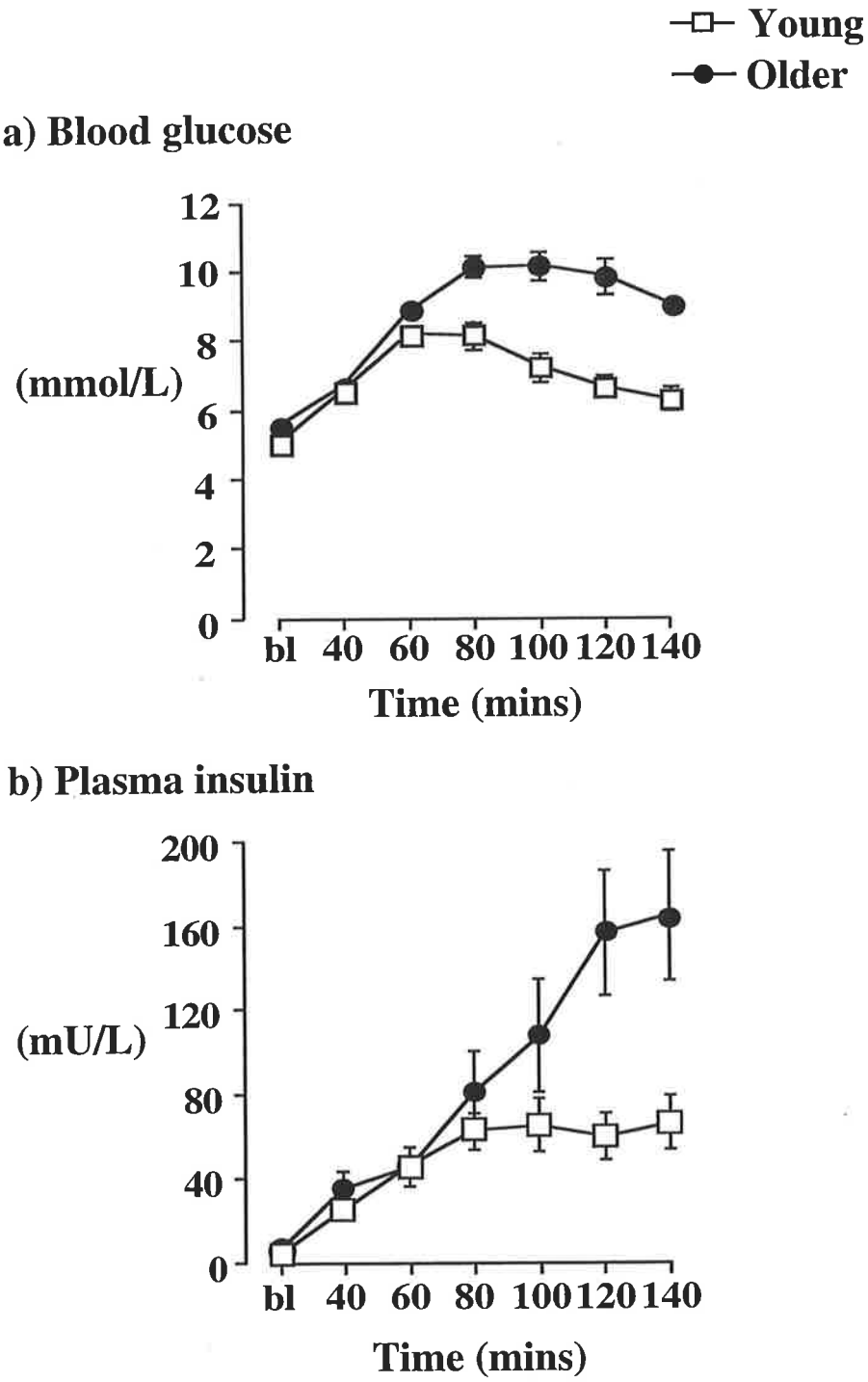

Figure 9.4: Blood glucose (a) and plasma insulin (b) concentrations in young and older subjects at baseline (t=0-20 min) and during intraduodenal infusion (t= 40-140 min) of glucose. Data are mean ± SEM. $P<0.01$; Age x treatment x time interaction for both blood glucose and plasma insulin levels.

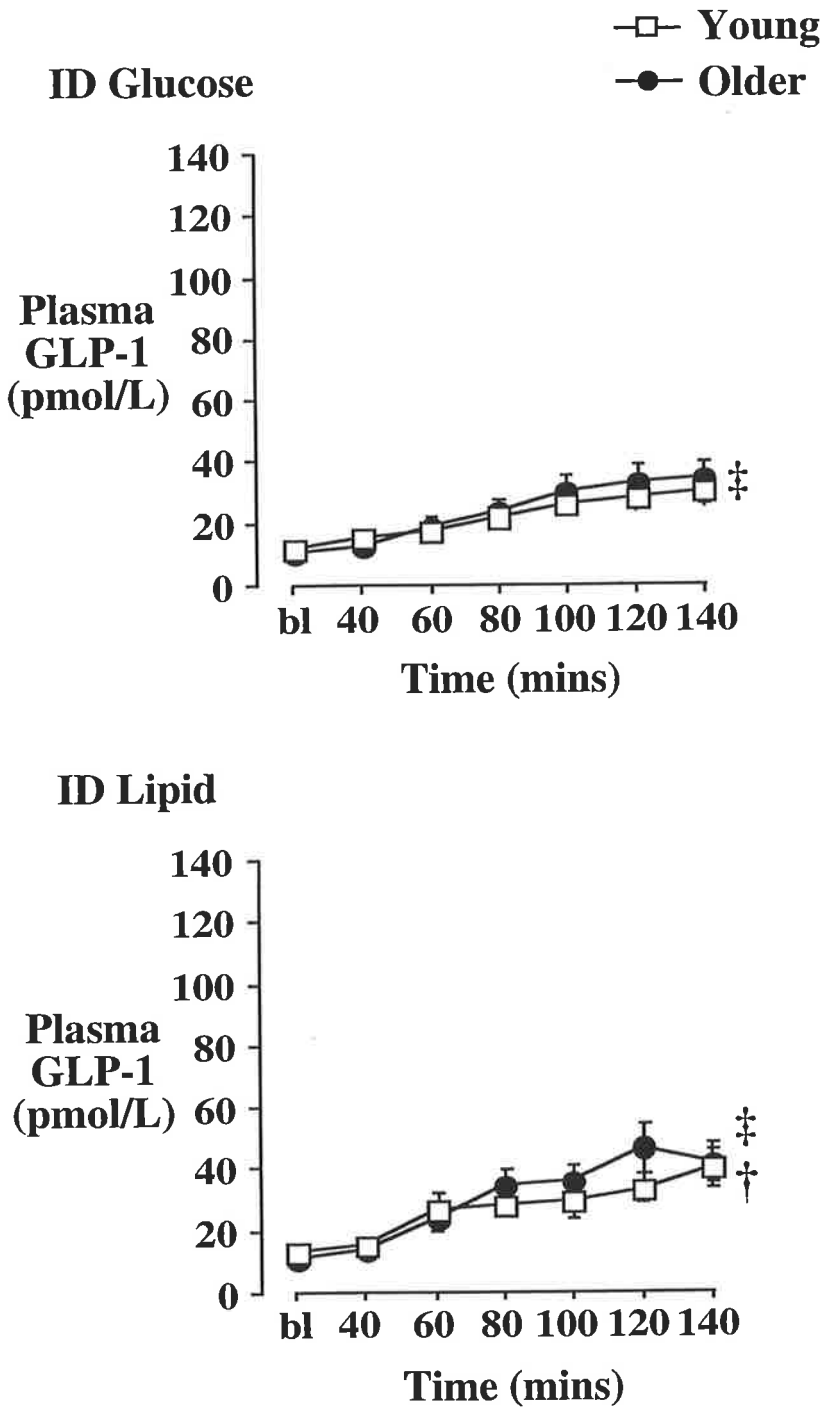

Figure 9.5: Plasma GLP-1 concentrations in young and older subjects at baseline (t=0-20 min) and during intraduodenal (ID) glucose and lipid infusions (t= 40-140 min) . Data are mean ± SEM. ‡P< 0.05; effect of time, †P< 0.05 lipid vs glucose.

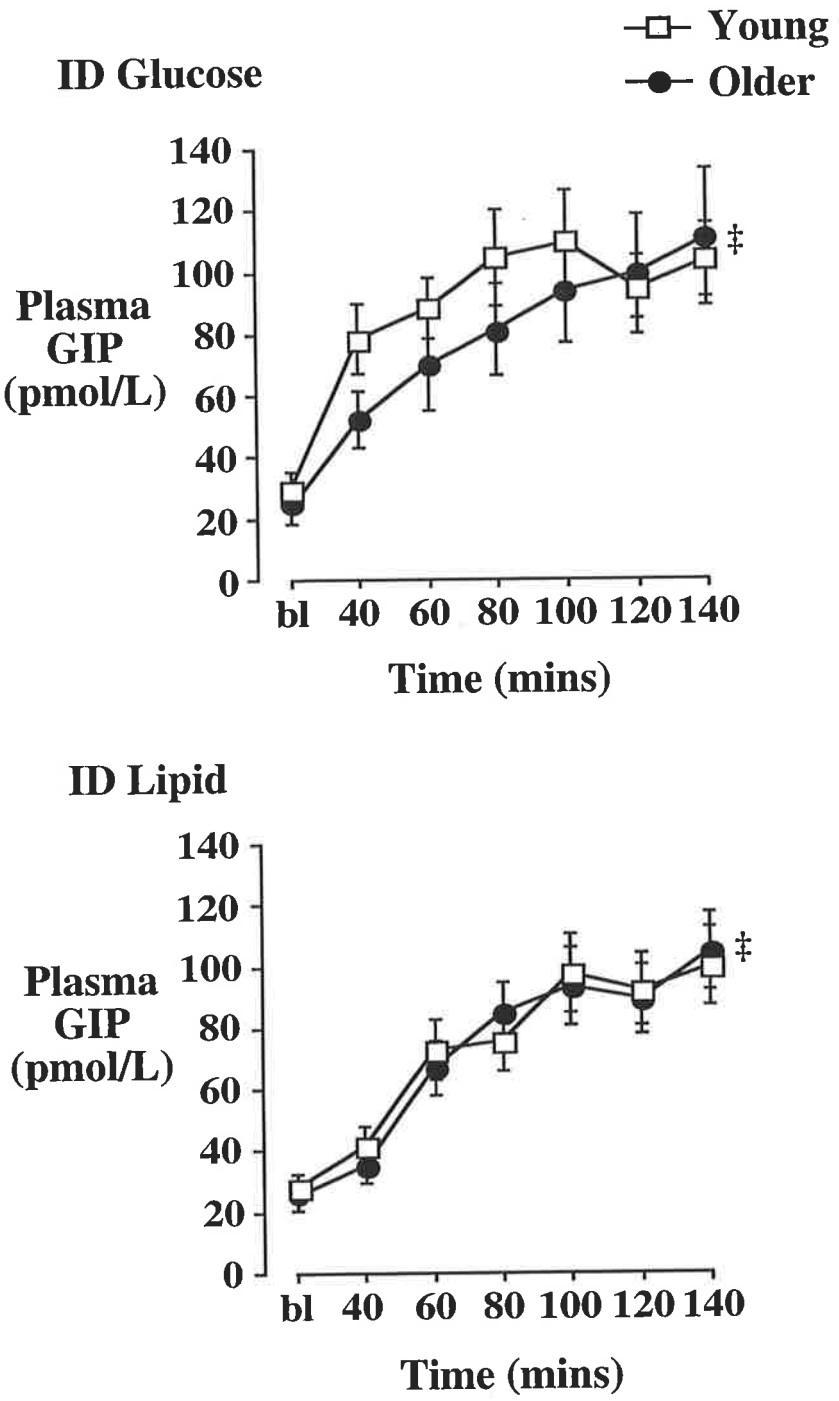

Figure 9.6: Plasma GIP concentrations in young and older subjects at baseline (t=0-20 min) and during intraduodenal (ID) glucose and lipid infusions (t= 40-140 min). Data are mean ± SEM. ‡P< 0.05; effect of time.

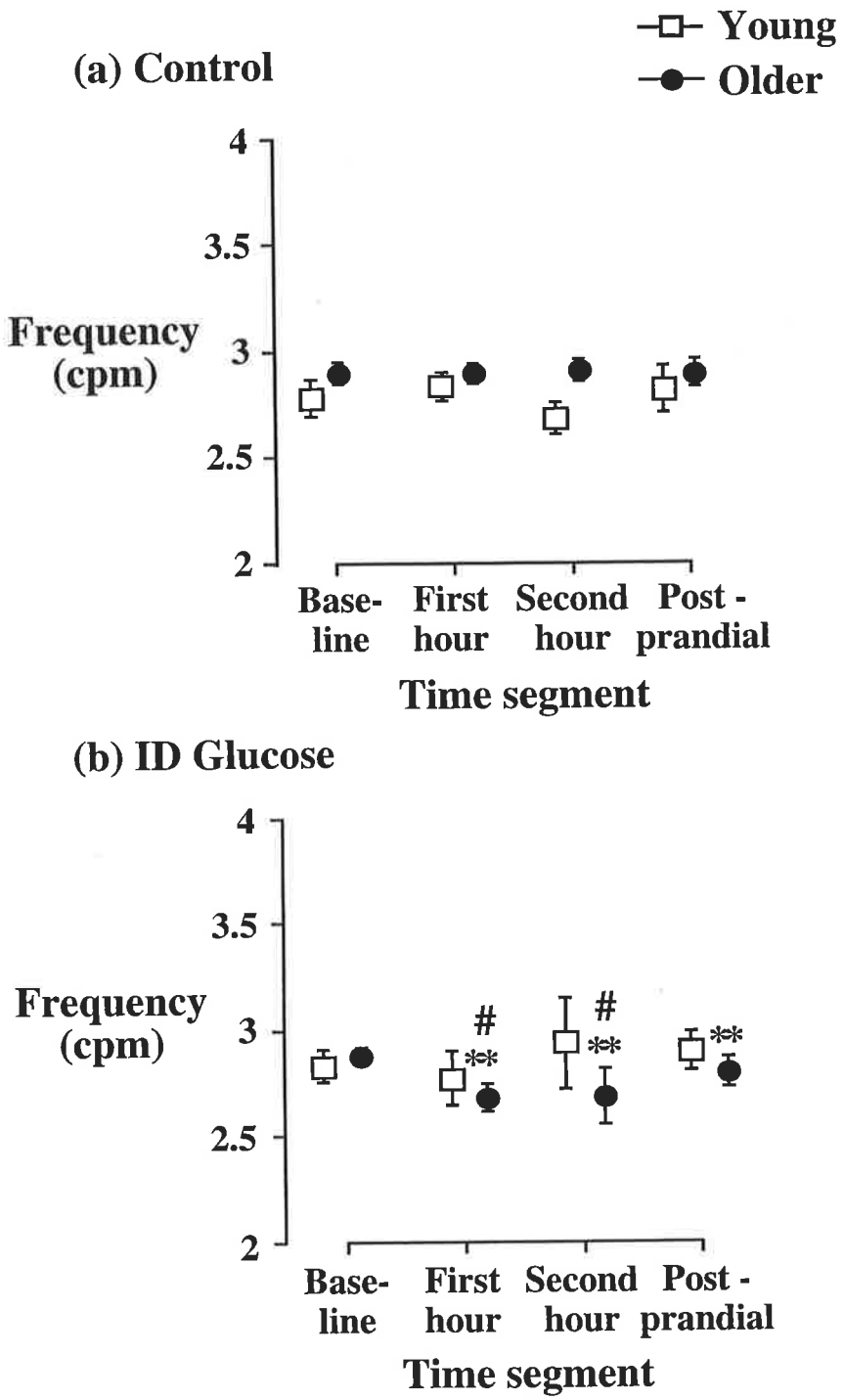

Figure 9.7: Mean frequency of EGG during baseline (t=0-20 min), first (t=40-80 min) and second (t= 100-140 min) hours of the intraduodenal (a) saline, (b) glucose and (c) lipid infusions and postprandial periods (t= 170-200 min) in young and older subjects. Data are mean ± SEM. #P< 0.05 vs baseline, **P< 0.05 vs control, ¥ P<0.05 vs postprandial period.

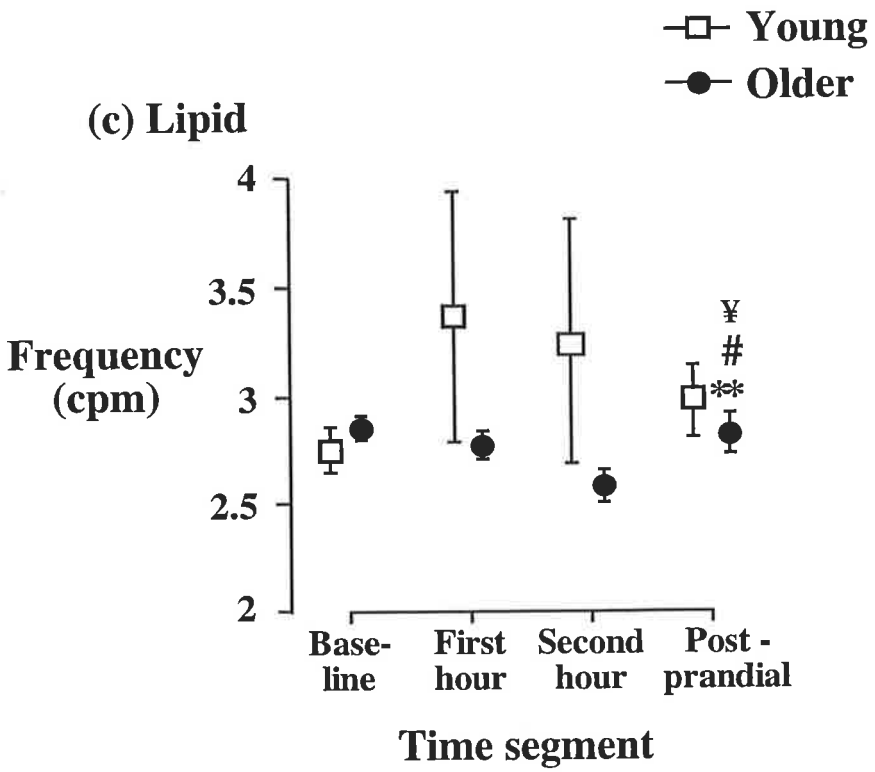

Figure 9.7 cont.: Mean frequency of EGG during baseline (t=0-20 min), first (t=40-80 min) and second (t= 100-140 min) hours of the intraduodenal (a) saline, (b) glucose and (c) lipid infusions and postprandial periods (t= 170-200 min) in young and older subjects. Data are mean ± SEM. #P< 0.05 vs baseline, **P< 0.05 vs control, ¥ P<0.05 vs postprandial period.

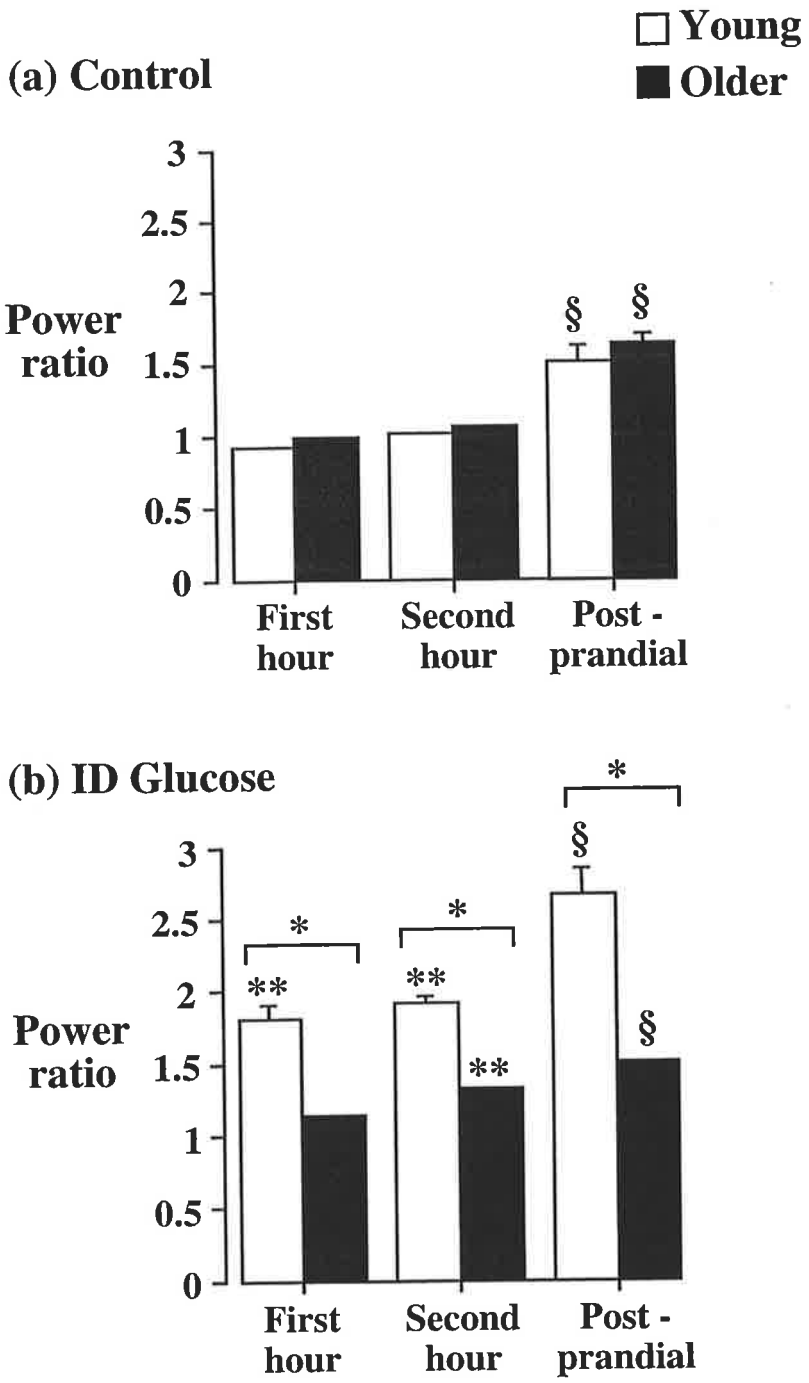

Figure 9.8: Mean EGG power ratio during the first (t=40-80 min) and second (t= 100-140 min) hours of the intraduodenal (a) saline, (b) glucose and (c) lipid infusions and postprandial periods (t= 170-200 min) in young and older subjects. Data are mean ± SEM. *P <0.05 young vs older, **P< 0.05 vs control, § P<0.05 vs first and second hours of the ID infusions.

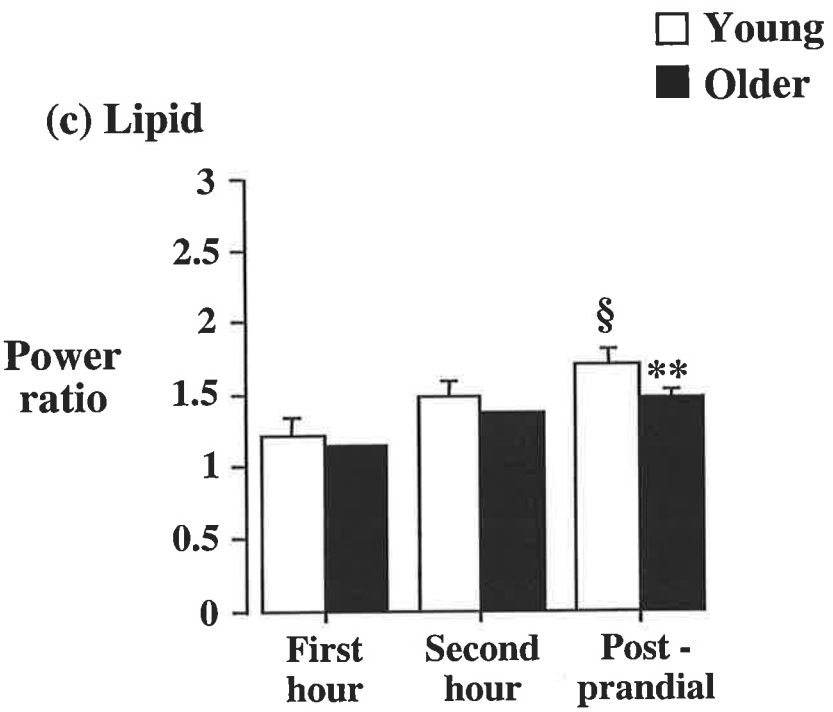

Figure 9.8 cont.: Mean EGG power ratio during the first (t=40-80 min) and second (t= 100-140 min) hours of the intraduodenal (a) saline, (b) glucose and (c) lipid infusions and postprandial periods (t= 170-200 min) in young and older subjects. Data are mean ± SEM. *P <0.05 young vs older, **P< 0.05 vs control, § P<0.05 vs first and second hours of the ID infusions.

9.5 DISCUSSION

The major findings of this study are that (i) intraduodenal (ID) lipid infusion suppresses food intake more than ID saline (control) in both older and young men and (ii) food intake is suppressed by ID glucose in older, but not young, men, and ID lipid suppresses food intake significantly more than did ID glucose in young, but not older, men. These results suggest that an enhanced satiating effect of small intestinal carbohydrate may contribute to the anorexia of ageing.

Several potential limitations of our study should be recognised. The nutrient infusion rate (12.1 kJ/min), which was chosen to match that of our previous studies [see Appendix A and (Chapmen et al 1999)], is at or slightly above the upper end of the physiological normal range for the overall rate of gastric nutrient emptying. Only men were studied, as previous findings suggest that young men have the greatest capacity to regulate energy intake in response to energy manipulation (Rolls 1995) and we have found that women are less tolerant of these infusions than men (Chapman et al 1999); accordingly, our observations cannot necessarily be extrapolated to women. Similarly, we have tested the effects of ID administration of only one form of carbohydrate and fat - our findings may not apply to the effects of ID administration of carbohydrates other than glucose or fats other than a triglyceride emulsion. While, there was a relatively small number of subjects in this study (thirteen per age group), we have now performed three studies, including this one (Appendix A and Chapman et al 1999), using the same ID nutrient infusion protocol (12.1 kJ/min intralipid or glucose for 2 hours), with consistent results. If the subjects from these studies are combined a significant difference in intake after equi-energetic lipid and glucose infusions is evident in the 28 young (P= 0.01), but not 21 older (P= 0.6) men (unpublished data).

The greater increase in ratings during intraduodenal lipid, glucose and saline infusions in older than young subjects is probably attributable to the lower baseline ratings for hunger in the elderly; despite the greater increase in hunger during the infusions in the older subjects, absolute hunger ratings were, non-significantly, less in the older than young men immediately before the buffet meal (t= 140 min)(5.1 ± 0.5 cm vs 6.1 ± 0.4 cm; P= 0.10).

Previous reports about the relative satiating effects of enterally administered fat and carbohydrate are conflicting (see Chapter 3.5.1.3). We have consistently found that ID administration of fat in the form of triglyceride emulsion ("Intralipid") is a more potent suppressor of food intake than equi-energetic and isovolumetric ID infusions of glucose in both lean (Chapman et al 1999, Andrews et al 1998) and obese (Chapman et al 1999) young men. We have now found that this differential effect of the two nutrients is lost in older men due to an enhanced satiating effect of glucose. Even though ageing appears to be accompanied by a reduced activity of some of the homeostatic mechanisms that regulate appetite and body weight (Roberts et al 1994, Rolls 1995), it is perhaps surprising that the satiating effect of lipid is not increased in older men; we have previously reported that plasma concentrations of the putative satiety hormone cholecystokinin (CCK) are higher in older than young men, both fasting and in response to ID lipid infusions (see Chapter 8); moreover in the study reported in Chapter 10 suggests that the sensitivity to the satiating effects of exogenous CCK are at least retained in older compared to young healthy adults.

There are few reports of the relative effects of carbohydrates and fat on satiation in the elderly and none relating to ID administration. Consistent with our findings, Rolls (1995), reported equivalent 23% and 24% suppression of energy intake at lunch 30 minutes after oral ingestion of a high fat and high carbohydrate yoghurt preload, respectively, in a group of older men.

It is unclear why ID glucose is more satiating in the older than young men. Satiety signals from the gut are mediated by the stimulation of afferent vagal activity and gastrointestinal hormones, such as cholecystokinin (CCK), and, possibly, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). ID glucose infusion is not a significant stimulus for cholecystokinin release, and we have found previously that the relatively minor CCK response to this ID glucose infusion does not differ between young and older men (Chapter 8). There is evidence that the incretin peptides, GLP-1 and GIP, particularly the former, may mediate the suppression of appetite by glucose ingestion (see Chapter 3.6) and increased GIP and GLP-1 responses to oral glucose have been reported in older compared to young women (Ranganath et al 1998) (see Chapter 4.5.2). We found no difference between young and older subjects in their plasma GLP-1 and GIP responses to ID glucose, making it unlikely that these hormones contribute to the increased satiating effects of ID glucose in the older subjects in this study, unless sensitivity to any such effects of

these hormones increases with age. There was a significant difference in the osmolality of the 25% glucose and 10% lipid solutions. While there is no clear evidence that the osmolality of nutrients has a significant effect on energy intake in humans. The possibility that older subjects may be more sensitive to hyperosmolar solutions, therefore, cannot be excluded.

Ageing is associated with impaired glucose tolerance and increased fasting and post-prandial insulin concentrations (Colman et al 1995)(see Chapter 4.5.2). Consistent with this, blood glucose and insulin concentrations increased more in the older than young subjects during ID glucose infusion. Previous animal studies have reported that insulin may act as a satiety hormone (see Chapter 3.6.2), and hyperglycaemia may have satiating effects (DeFronzo et al 1983, Campfield et al 1996)(see Chapter 3.7). Hyperglycaemia and/or hyperinsulinaemia may, therefore, be causes of the greater satiating effects of glucose in older subjects in this study. Studies in humans involving euglycaemic insulin infusions have, however, revealed no effects of hyperinsulinaemia on short term appetite or food intake (Chapman et al 1998). Similarly, mild hyperglycaemia in the absence of hyperinsulinaemia appears to have little, if any, satiating effect (Vanderweele 1994, Lavin et al 1998). It is possible that neither hyperglycaemia nor hyperinsulinaemia in isolation is satiating but when they co-exist, as they usually do, they interact to have this effect.

This is the first study to examine the effects of ageing on the electrogastrographic EGG responses to ID nutrients. In general, in young adults, EGG frequency, which is a marker of the frequency of gastric contractions, increases after a meal (Smout et al 1994) and during ID nutrient infusion (Verhagen et al 1998, Sun et al 1995). There are conflicting data on the effects of ageing on EGG frequency (see Chapter 4.4.2). In this study, we found a small, but significant, decrease in the EGG frequency during both ID glucose and lipid infusions in the older subjects, which was more marked during the glucose infusion. In contrast there was no change in frequency during the infusions in the young subjects. The cause of this nutrient-mediated suppression of gastric myoelectrical activity in older men is unknown.

The significance of EGG power ratio is unclear, but it is thought to reflect the strength of gastric contractions (Verhagen et al 1999b, Verhagen et al 1998, Sun et al 1995b)(see Chapters 3.5.2 and 7.2). Verhagen et al (1998) reported that ID glucose administered at 16.7 kJ/min for 25 minutes decreased the EGG power in young

subjects. In contrast, we infused nutrients into the duodenum at a lower, more physiological, rate, for a longer duration and found an increase in the power ratio of the EGG with glucose compared to the other two infusions, consistent with observations in young subjects (Brown et al 1975). ID lipid infusion has no effect on EGG power in either the young or the older subjects, indicating that ID fat and glucose have different effects on the EGG power ratio. The observed glucose-induced increase evident in the young subjects and, to a lesser extent, in the older men is consistent with the study of Parkman et al (1996), who found that the increase in the postprandial-fasting ratio tended to be less in older than young men.

While the combination of altered EGG and satiety responses to ID glucose, but not lipid, in the older men observed in this study may suggest a nutrient-specific effect of ageing on small intestinal responses to nutrients, we found no relationship between symptoms of hunger, fullness and nausea and tachygastrias or total gastric dysrhythmias and, accordingly, do not know if they are in any way connected. Further studies are required to examine this concept.

In summary, we have found that ageing in men is associated with nutrient-specific changes in appetite, hormonal and gastric myoelectrical (EGG) responses to ID nutrients. The appetite-suppressant effect of ID glucose, but not ID lipid, is enhanced in older men, while EGG responses to nutrients, particularly glucose, are attenuated. An enhanced satiating effect of small intestinal carbohydrate may contribute to the anorexia of ageing.

CHAPTER 10

Effects of Intravenous Infusion of Cholecystokinin (CCK) on Appetite and Energy Intake in the Healthy Elderly

10.1 SUMMARY

Healthy ageing is associated with reductions in appetite and food intake, which may predispose to severe weight-loss and malnutrition. We have previously shown that both fasting and nutrient-stimulated plasma CCK concentrations, are higher in healthy older compared to young subjects. The effect of human ageing on the sensitivity to CCK is not known. Little is known about the effect of exogenous CCK-8 on plasma leptin and endogenous CCK release in humans.

Twelve older (6M/6F) (67-83 yr) and 12 young (6M/6F) (18-33 yr) healthy subjects were studied on 3 days in double-blind, randomised order. On each day, after an overnight fast the subjects received an oral preload ('banana milkshake')(t= 93 min) then, intravenous (iv) infusions of saline (C), CCK-8 lower dose (LD) (1 ng/kg/min) or CCK-8 higher dose (HD) (3 ng/kg/min)(t= 100-125 min). At t= 110 min each subject was offered a meal and food intake was quantified. Blood samples were taken at ~10-20 min intervals for measurement of plasma CCK-8 and CCK (>12), and leptin concentrations. Hunger, fullness and nausea were assessed using visual analogue questionnaires.

On all three days, older subjects ate less energy ($P<0.05$) at the test meal than young subjects. In both age groups, energy intake was less ($P<0.05$) during the CCK-8 (HD) infusion than CCK-8 (LD) and (C) infusions. During the CCK-8 (LD) and (HD) infusions food intake was suppressed to a greater extent in the older than young subjects (32 ± 6.0% vs 15.5% ± 6.4%; $P< 0.05$). Plasma CCK-8 concentrations increased more in older than young subjects during CCK-8 infusions and the magnitude of the suppression of energy intake was related to plasma CCK-8 concentrations at 110 min in both age groups ($r= 0.30$; $P< 0.05$). Plasma CCK (>12) concentrations were higher in older than young subjects ($P<0.001$), were not different between treatments ($P= 0.14$), and decreased over time ($P< 0.001$) during the CCK-8, but not control infusions in both age groups. Plasma leptin concentrations decreased over time during all three treatment infusions ($P<0.05$), were comparable in both age groups ($P= 0.60$) and were not affected by exogenous CCK-8 ($P= 0.76$).

In conclusion, exogenous CCK-8 (i) suppresses food intake and increases plasma CCK-concentrations to a greater extent in healthy older subjects, but the relationship between the rise in plasma CCK-8 and suppression of food intake was not different between older and young subjects, and (ii) suppresses endogenous CCK release. Increased CCK activity may play a role in the "anorexia of ageing".

10.2 INTRODUCTION

Cholecystokinin is an important mediator of gastrointestinal satiation signals (Morley 1990). In young adults intravenous infusion of CCK-8 and CCK-33 induces a dose-dependent suppression of food intake, which is mediated by stimulation of vagal afferent activity, slowing of gastric emptying and increasing the 'sensitivity' of the stomach to distension (see Chapter 3.6.1).

The stimulatory and inhibitory regulation of a number of mechanisms modulating endogenous CCK secretion have been identified, including the stimulatory effects of ingested fat and protein and the inhibitory feedback effect of bile and to a lesser extent pancreatic enzymes (see Chapter 3.6.1). There is uncertainty as to whether CCK affects its own release.

There is evidence in humans that CCK release increases with ageing (see Chapter 4.5.1). As reported in the study in Chapter 8, plasma CCK levels are higher in healthy elderly compared to younger adult subjects, both in the fasted state and in response to an intraduodenal infusion of lipid. The diminished food intake in older rodents when compared to younger animals may reflect an age-related increase in the sensitivity to the satiating effects of CCK (see Chapter 4.5.1). It is possible, therefore, that reduced appetite and food intake in elderly humans may, in part, be a consequence of increased fasting and postprandial CCK concentrations combined with enhanced or preserved sensitivity to the satiating effects of CCK.

CCK may act in synergy with other gastrointestinal peptides to exert its satiating effects (see Chapter 3.6.1). A recent study in rats suggests that CCK-induced satiety may be mediated by release of gastric leptin with a consequent rise in plasma leptin concentration (see Chapter 2.3.4). No studies have evaluated the effect of CCK-8

administration on plasma leptin concentrations in humans, nor is it known whether this response is altered with ageing.

The aims of this study were to determine whether the effects of intravenous administration of CCK-8 on appetite and food intake and plasma endogenous CCK and leptin concentrations are affected by healthy ageing. The broad hypotheses addressed were that 1) intravenous CCK-8 infusion suppresses appetite and energy intake in a dose-dependent manner in both young and older subjects; 2) the suppression of appetite and food intake is greater in the older compared to young subjects; 3) plasma leptin concentrations are increased by intravenous CCK-8 administration in both age groups and possibly to a greater extent in older than young subjects.

10.3 SUBJECTS AND METHODS

12 healthy older subjects, mean age 71.2 ± 1.3 yr (range 67-83 yr) and 12 healthy young subjects, mean age 22.6 ± 1.2 yr (range 18-33 yr), 6 males and 6 females in each age group, were recruited by advertisement. Older subjects were selected so that their body mass index (BMI) was "matched" (within 1 kg/m^2) to one of the young subjects; accordingly the BMI of the two age groups was not significantly different (24.1 ± 0.7 kg/m^2 ; older vs 23.5 ± 0.8 kg/m^2 ;young; P= 0.59). In all subjects mean energy intake was >4182 kJ/day (1000 kcal/day) as assessed by a food diary kept for 3 successive days prior to the first study day (see Chapter 6.2.2). All subjects were unrestrained eaters (score< 11 for Factor I ('cognitive restraint') on the Three Factor Eating Questionnaire (see Chapter 6.4.1), non-smokers, and none had suffered any serious illness, had a history of gastrointestinal disease or gastrointestinal surgery, or was any taking medication known to influence appetite or gastrointestinal motility. The study was approved by the Human Ethics Committee of the Royal Adelaide Hospital and each subject gave written, informed, consent.

10.3.1 *Experimental protocol*

Each subject underwent three studies on separate days, in random order. The experimental protocol is summarised in Figure 1. On each study day, the subject arrived at the laboratory at 0830h after ~ 12 hr overnight fast.

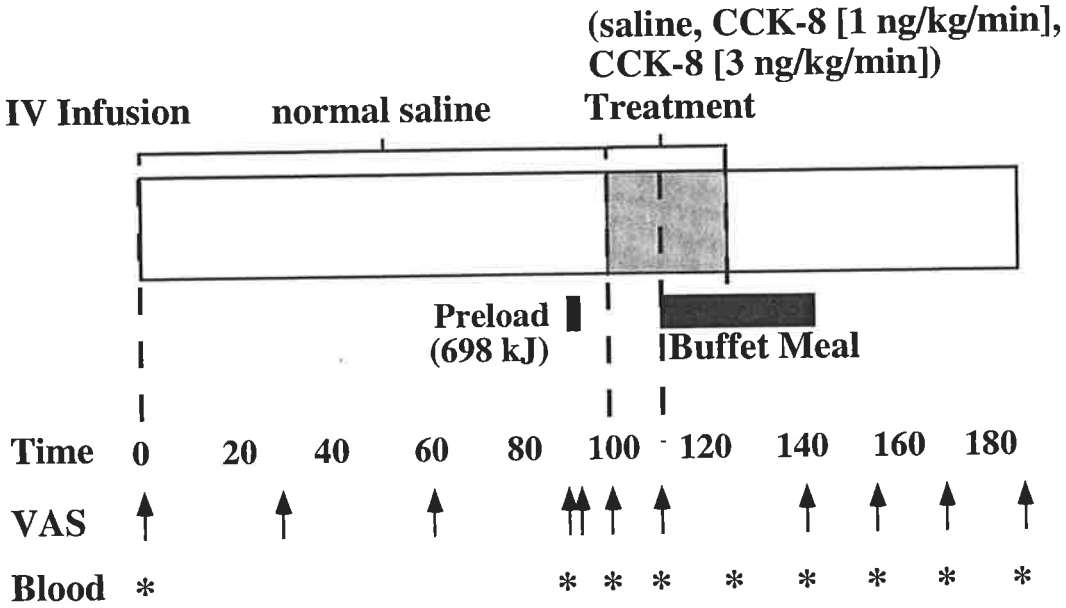

Figure 10.1: Summary of the experimental protocol. Following a 90 min baseline period, each subject received an oral preload, then in random order intravenous (iv) infusions of saline (Control), CCK-8 lower dose (1ng/kg/min; LD), or CCK-8 higher dose (3 ng/kg/min; HD) over 25 minutes on separate days. During the infusions, a buffet meal was offered and energy intake was subsequently quantified. * indicates the timing of venous blood samples for measurement of plasma CCK, leptin and insulin and the arrows the administration of visual analogue scales to assess subjective hunger, desire to eat, fullness and nausea ratings.

On arrival, an intravenous cannula was placed in a left antecubital vein for blood sampling and another in a right antecubital vein for infusion of the treatment solution. Approximately 30 min following insertion of the intravenous cannulae (t= 0 min) isotonic saline (40 ml/hr) was infused intravenously for 90 min. At t= 90 min a "preload" [similar to that used by Lieverse et al (1995)] in the form of a 'shake' consisting of 125g of banana blended with 150 ml of low-fat milk (0.1% fat) and 150 ml of water (698 kJ) was consumed within 3 min. Between t= 100 - 125 min an intravenous infusion of either saline, CCK-8 (Sincalide; Squibb Diagnostics) "lower dose" (LD)(1 ng/kg/min) or CCK-8 "higher dose" (HD)(3 ng/kg/min), was given for 25 min. At t=110 min, each subject was offered a standardised high-carbohydrate, low-fat meal prepared in excess of what they would normally eat, and invited to eat as much as they wished. The rate of ingestion and the total amount of food consumed were quantified (see Chapter 6.3.3). Following the meal, the subjects remained for a further 45 min. Visual analogue scales were administered at t= 0, 30, 60, 90, 93, 100, 110, 125, 140, 155, 170 and 185 min to assess subjective appetite ratings (see Chapter 6.3.1). At t= 0, 90, 100, 110, 125, 140, 155, 170, and 185 min, venous blood was collected in ice-chilled dipotassium EDTA tubes containing 400 KIU aprotinin (Trasylol) per ml of blood for measurement of plasma CCK-8, CCK, insulin and leptin. Plasma was separated by centrifugation within 30 min of collection and stored at -70°C until assay. When discharged from the laboratory, subjects were given a record sheet and asked to record their food intake for the rest of the day until midnight.

10.3.2 *Measurement of appetite*

Appetite was assessed using 10 cm linear visual analogue scales (see Chapter 6.3.1 and Appendix AI). Hunger, fullness and nausea were quantified (other questions related to sensations of dizziness, indigestion, headache and thirst). Subjects were familiarised with these scales at the commencement of each study day and instructed to make a vertical mark on the scale indicating assessment of their current feelings. Since baseline ratings of hunger increased significantly ($P< 0.01$) from 0 to 90 min in both older (from 2.7 ± 0.4 cm to 4.0 ± 0.3 cm) and young (5.5 ± 0.4 cm to 6.1 ± 0.3 cm) subjects, the 90 min values provided a standard baseline, and absolute ratings of appetite during the study were quantified.

The total amount (grams) of food consumed during the meal (375 ml low-fat iced coffee; 300 ml unsweetened orange juice; 100g tomato, onion and garlic pasta sauce

(Dolmio®) mixed with 200g (uncooked weight) white pasta; 1 wholemeal dinner roll; 1 white dinner roll; 2 slices of wholemeal bread; 2 slices of white bread; 1 slice of light cheddar cheese (Coon®); 8 slices of tomato and cucumber; 1 sachet of margarine; 200 g low-fat artificially sweetened strawberry yoghurt; 150g lime flavoured jelly and 400g of tropical fruit salad in heavy syrup) and the meal duration (min) and the rate of food intake (kJ/min) were calculated (see Chapter 6.3.3). Food intake from the meal was analysed using the DIET/4 Nutrient Calculation software (Xyris Software) to determine both energy intake (kJ) and macronutrient composition (% protein, % fat and % carbohydrate) of the meal (see Chapter 6.3.3).

10.3.3 *Measurement of plasma concentrations of peptides*

10.3.3.1 CCK-8

Two different assay techniques were used to measure CCK concentrations from the plasma samples collected during this study.

CCK-8 was measured by radioimmunoassay using an adaption of the method of Santangelo et al (1998). Standards (synthetic sulphated CCK-8, Sigma Chemical, St Louis, MO, USA) were prepared in charcoal-stripped plasma and were extracted in 66% ethanol along with the samples. Extracts were dried down under N_2 and resuspended in assay buffer (50 mM phosphate, 10 mM EDTA, 2 g/L gelatin, pH 7.4). Antibody (C2581, Lot 105H4852, Sigma Chemical) was added at a working dilution of 1/17,500 with 5 kBq sulphated CCK-8 ^{125}I-labeled with Bolton and Hunter reagent (74Tbq/mmol Amersham International, Amersham, Bucks, USA) as tracer incubation was for 72 hr at 4 °C. The antibody bound fraction was separated by the addition of dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal in 30 ml assay buffer) and the radioactivity determined in the supernatants following centrifugation. The intra-assay coefficient of variation at 50 pmol/L was 9.5%.

10.3.3.2 CCK (>12)

A radioimmunoassay technique (Jansen & Lamers 1983; Jansen et al 1992), was used to measure CCK peptides containing > 12 amino acid residues [CCK (>12)]. The antibody (1703), was raised in a rabbit by immunisation with 30% CCK bound to all COOH terminal CCK-peptides containing at least 12 amino acid residues (Jansen & Lamers 1983). The detection limit of the assay was between 0.5 and 1 pmol/L in plasma. The intraassay precision ranged from 4-11%.

10.3.3.3 Leptin

Plasma leptin was measured using the DSL ACTIVE™ Human Leptin Enzyme-Linked Immunosorbent (ELISA) immunoassay kit (Diagnostic Systems Laboratories Inc., Webster, Texas, USA). The sensitivity of the assay (calculated by interpolation of the mean plus 2 standard deviations of 12 replicates of the 0 ng/ml Human Leptin Standard) was 0.05 ng/ml. The intraassay coefficients of variation were 4.4% at 4.8 ng/ml and 1.5% at 46.3 ng/ml. The interassay coefficients of variation were 4.9% at 4.7 ng/ml and 4.2% at 37.9 ng/ml.

10.3.4 *Statistical Analyses*

Results are given as mean ± SEM. Comparisons between the young and older groups in the energy and macronutrient content of the previous diet, restraint score and BMI were performed using Student's unpaired test, as these data were normally distributed. The data were analysed using Statview Version 5.0 (Abacus Concepts Inc., California). Baseline scores for hunger, fullness, and nausea and fasting plasma concentrations of plasma CCK-8 and CCK (>12), insulin and leptin and differences in mean energy intake (kJ), meal duration, rate of eating and macronutrient content, of the buffet meal and energy intake for the remainder of the day were analysed by repeated-measures two-way analysis of variance (ANOVA), with age and treatment as the factors. Baseline appetite ratings (t=0-90 min), the effects of the preload (t=90-100 min), the intravenous infusions of saline (control), CCK-8 "lower dose" (LD) and CCK-8 "higher dose" (HD) (t=100-110 min), and the buffet meal (t=140-185 min) on absolute ratings of hunger, fullness and nausea, plasma concentrations of CCK-8 and CCK (>12), insulin and leptin were analysed using repeated measures three-way ANOVA, with time, age and treatment as the factors. When a significant interaction between factors was observed, contrasts were used to test preplanned hypotheses of interest enabling paired comparisons between the three study days. The ANOVA's were performed using SuperANOVA Version 1.11 (Abacus Concepts Inc., California). Relationships between energy intake (kJ and % suppression) and plasma CCK (>12) and CCK-8 concentrations were assessed by linear regression analysis using Statview Version 5.0 (Abacus Concepts Inc., California). A P value < 0.05 was considered significant in all analyses.

10.4 RESULTS

The study protocol was well tolerated. As assessed by 3-day diet diaries, energy intake from the usual diet was approximately 30% less in older than young subjects (7114 ± 293 kJ/day vs 9819 ± 828 kJ/day; P< 0.01). There was no difference between older and young subjects in proportion of intake as carbohydrate (47.0 ± 2.0 % vs 48.8 ± 2.2 %; P= 0.57), fat (30.8 ± 2.0 % vs 29.8 ± 1.6 %; P= 0.70) or protein (19.3 ± 1.1 % vs 17.4 ± 0.8 %; P= 0.21). There was no significant difference in eating restraint scores between the older and young subjects (6.3 ± 0.8 vs 4.3 ± 0.9; P= 0.11).

10.4.1 *Appetite*

Absolute ratings of hunger, fullness, and drowsiness at baseline (t= 90 min), after the preload (t=93-100 min), during the saline (control) and CCK-8 lower dose (LD) and CCK-8 higher dose (HD) infusions (t= 100-125 min), and after the buffet meal (140-185 min) in young and older subjects, are represented in Figure 10.2, 10.3 and 10.4, respectively. There were no significant treatment × age or treatment × time interactions for any ratings throughout the study days in either age group.

10.4.1.1 Hunger

Hunger ratings were less in older than young subjects during fasting (t= 90 min; 4.0 ± 0.3 cm vs 6.1 ± 0.4 cm; P< 0.01) and throughout the study days (effect of age; P< 0.01) (Figure 10.2). Hunger ratings decreased during the study days (effect of time; P< 0.001). There was no difference in ratings of hunger between the three study days (effect of treatment; P= 0.62) in either age group. There was a significant time × age interaction for hunger ratings during the three study days (P< 0.001); hunger ratings decreased more in young than older subjects. There was a significant treatment × time × age interaction for hunger ratings during the three study days (P< 0.05); following the buffet meal hunger ratings decreased more during the CCK-8 (HD) and CCK-8 (LD) infusions in the young than older subjects.

10.4.1.2 Fullness

Fullness ratings were similar in older and young subjects during fasting (t= 90 min; 0.9 ± 0.2 cm vs 0.9 ± 0.2 cm; P= 0.85) (effect of age; P= 0.60) (Figure 10.3), and increased throughout the study days (effect of time; P< 0.001), but were not different between the three study days (effect of treatment; P= 0.17) in either age

group. There were no significant time × age or treatment × time × age interactions for fullness ratings throughout the study days in young and older subjects (data not shown).

10.4.1.3 Nausea

Ratings of nausea were similar during fasting in older and young subjects (t= 90 min; 0.4 ± 0.1 cm vs 0.4 ± 0.1 cm; P= 0.86). There was no effect of age (P= 0.60), treatment (P= 0.53) or time (P= 0.25) on ratings of nausea throughout the study days (Figure 10.4). There were no significant time × age or treatment × time × age interactions for nausea ratings throughout the study days in young and older subjects (data not shown).

10.4.2 *Food intake*

10.4.2.1 Energy intake at buffet meal

The effect of treatment infusions on energy intake at the buffet meal is shown in Figure 10.5. Older subjects ate approximately ~41% less than young subjects across the three study days (effect of age; P< 0.01). CCK-8 infusion was associated with a dose-dependent suppression of energy intake (effect of treatment; P< 0.001), with CCK-8 (LD) and CCK-8 (HD) infusions associated with a 7.7% and 35.2% suppression of energy intake, compared to the control infusion, respectively. Energy intake was significantly lower during CCK-8 (HD) than both control (P< 0.001) and CCK-8 (LD) (P< 0.001), whereas the reduction in intake during the CCK-8 (LD) compared to the control infusion was not significant (P= 0.19). There was no significant treatment × age interaction (P= 0.51).

When energy intake during the CCK-8 infusions was expressed as a percentage of the control day intake (Figure 10.6), CCK-8 administration suppressed intake more in the older than young subjects (effect of age; P<0.05), with suppression by 16% and 48% (mean 32%) on the (LD) and (HD) days, respectively in the older subjects compared to +3% and 34% (mean 15.5%) in the young subjects. There was no significant treatment × age interaction (P= 0.74). The effects of age and treatment on weight (g) of food consumed were the same as those on energy intake (data not shown).

The duration of the meal (min) tended to be less in older than young subjects (15.3 ± 0.5 min vs 19.3 ± 1.1 min; P= 0.06). There was a significant effect of treatment on the duration of the meal in both age groups (P< 0.05); the duration of meal consumption was less during the CCK-8 (HD)(15.2 ± 1.2 min) compared to the CCK-8 (LD)(18.4 ± 1.2 min, P< 0.01) and control (18.2 ± 1.1 min, P<0.05) infusions, whereas there was no difference in the duration of the meal between the CCK-8 (LD) and control (P= 0.67) infusions. There was no significant treatment × age interaction (P= 0.71) for meal duration.

There was an effect of age on the rate of eating (kJ/min) during the infusions (P< 0.05); older subjects ate the meal at a slower rate than young subjects (134.3 ± 10.0 kJ/min vs 191.7 ± 11.8 kJ/min). There was a significant effect of treatment on the rate of eating in both age groups (P< 0.001); subjects ate slower during the CCK-8 (LD)(164.7 ± 12.5 kJ/min, P< 0.05) and CCK-8 (HD) (139.1 ± 14.7 kJ/min, P< 0.001) infusions compared to the control (185.2 ± 15.3 kJ/min) infusion. The effect of CCK on the rate of eating was dose-dependent in both age groups; subjects ate at a slower rate during the CCK-8 (HD) than the CCK-8 (LD) infusions (P< 0.05). There was no significant treatment × age interaction (P= 0.68).

10.4.2.2 Macronutrient content of buffet meal

There were no differences between young and older subjects in intake of carbohydrate (P= 0.07), fat (P= 0.10) and protein (P= 0.20) as a percentage of the total energy intake at the buffet meal (data not shown). There was no effect of treatment on the percentage of carbohydrate (P= 0.53), fat (P= 0.68) or protein (P= 0.54) consumed at the buffet meal. There were no significant treatment × age interactions for any macronutrients (data not shown).

10.4.2.3 Energy intake for remainder of study day

There was an effect of age on energy intake for the remainder of the study day with older subjects eating approximately 35% less than young subjects (3874 ± 242 kJ vs 5986 ± 586 kJ; P< 0.01). There was no effect of treatment (P= 0.72) and no treatment × age interaction (P= 0.35) for energy intake for the rest of the study day (data not shown); indicating that the suppressive effects of the intravenous CCK-8 infusions on food intake were only temporary.

10.4.3 *Plasma CCK-8 concentrations*

Plasma CCK-8 concentrations were higher in the older than young subjects, both at baseline (mean of three study days; 4.9 ± 1.6 pmol/L vs 1.2 ± 0.1 pmol/L; P< 0.05) and throughout the three study days (effect of age; 5.08, P<0.05; mean of baseline-170 min; 17.3 ± 1.5 pmol/L vs 8.2 ± 0.9 pmol/L), including on the control day when no CCK-8 was infused (9.6 ± 1.3 pmol/L vs 3.7 ± 0.5 pmol/L) (Figure 10.7a-c). There was a small, but significant, rise in CCK-8 concentrations as a result of ingestion of the preload (from t= 90 to t=100 min, 3.1 ± 2.3 pmol/L to 5.8 ± 0.9 pmol/L; P< 0.05). CCK-8 concentrations increased in a dose responsive manner during CCK-8 infusions [effect of treatment (P< 0.001) and time (P< 0.001), and treatment × time interaction (P< 0.001)], CCK-8 concentrations at t=110 and 140 min were higher on both the CCK-8 (LD) and (HD) than control day; and higher on the (HD) than (LD) day (Figure 10.7b and 10.7c; mean peak t= 110 min; (LD) 33.6 ± 4.1 pmol/L vs (HD) 59.7 ± 5.1 pmol/L) and they were higher in the older than young subjects [(time × age interaction (P< 0.001) and treatment × time × interaction (P< 0.01)] with CCK-8 concentrations of 8.3 ± 2.3 pmol/L, 44.7 ± 6.3 pmol/L and 74.1 ± 6.0 pmol/L after 10 min (t= 110 min) of the control, CCK-8 (LD) and CCK-8 (HD) infusions, respectively in the older subjects compared to 3.4 ± 1.1 pmol/L, 22.4 ± 2.4 pmol/L and 45.4 ± 5.7 pmol/L in the young subjects (Figure 10.7a-c).

10.4.4 *Plasma CCK (>12) concentrations*

Plasma CCK (>12) concentrations were higher in the older than young subjects both at baseline (mean of three study days; 3.8 ± 0.1 pmol/L vs 2.3 ± 0.1 pmol/L, P< 0.001) and throughout the three study days (effect of age; P< 0.001; mean of baseline-185 min; 4.1 ± 0.1 pmol/L vs 2.8 ± 0.1 pmol/L) (Figure 10.8a-c). There was a small, but significant, rise in CCK (>12) concentrations as a result of ingestion of the preload, from 3.1 ± 0.1 pmol/L to 3.8 ± 0.2 pmol/L (P<0.05). There was no effect of treatment on CCK (>12) concentrations (P= 0.14), but there was a significant effect of time (P< 0.001), so that concentrations were decreased during the intravenous infusions (t=100 vs 125 min; 3.4 ± 0.1 pmol/L vs 2.9 ± 0.1 pmol/L). There was a significant treatment × time interaction (P< 0.01), so that plasma CCK (>12) concentrations were lower during both CCK-8 infusions (t=125 min) than the control infusion, indicating that infusion of CCK-8 was associated with a suppression of endogenous CCK (>12) concentrations. There were no

significant treatment × age, time × age or treatment × time × age interactions for plasma CCK (>12) concentrations (data not shown).

10.4.5 *Relationship between plasma CCK-8 and extent of suppression of food intake at the buffet meal*

There was an inverse relationship between mean energy intake (kJ) and absolute plasma CCK-8 concentrations at t= 110 min during the intravenous infusions (r= -0.34, P< 0.01) when all 24 subjects were analysed together (Figure 10.9), but not when the young (r= -0.32; P= 0.06) or older (r= -0.20; P= 0.30) subjects were analysed alone. There was a significant positive relationship between the percentage suppression of energy intake by the CCK-8 (LD) and (HD) infusions and the increase in plasma CCK-8 concentrations provided by these infusions (CCK-8 concentrations at 110 min - CCK-8 concentrations at 100 min) (r= 0.30, P< 0.05, Figure 5) when all 24 subjects were analysed together, but not when either the young (r= 0.33; P= 0.12) or older (r= 0.11; P= 0.60) subjects were analysed alone. There was no significant difference between the slopes of these regression lines in young and older subjects (t= 0.89, P= 0.35); suggesting that the extent of suppression of food intake associated with a given rise in plasma CCK-8 concentrations as a result of the CCK-8 infusions was similar in young and older subjects.

10.4.6 *Relationship between the rise in plasma CCK-8 and the suppression of plasma CCK (>12)*

There was an inverse relationship (r= -0.30, P< 0.05) between the change in plasma CCK (>12) concentrations during CCK-8 (LD) and (HD) infusions (t= 100 to t= 125 min) and the change in plasma CCK-8 concentrations during these infusions (t= 100 to t=110 min) (Figure 10.10), but not when either the young (r= -0.39; P= 0.06) or older (r= 0.01; P= 0.96) subjects were analysed alone; indicating that the greater the increase in plasma CCK-8 concentrations, the greater suppression of plasma CCK (>12) concentrations.

10.4.7 *Plasma Leptin concentrations*

Baseline plasma leptin concentrations were similar in the older and young subjects (8.8± 1.7 μmol/L vs 6.8 ± 1.3 μg/L; P< 0.58). There was no significant effect of age (P=0.46), treatment (P=0.55) or time (P= 0.25) on plasma leptin concentrations during the intravenous infusions (Figure 10.11a and b). There were no other

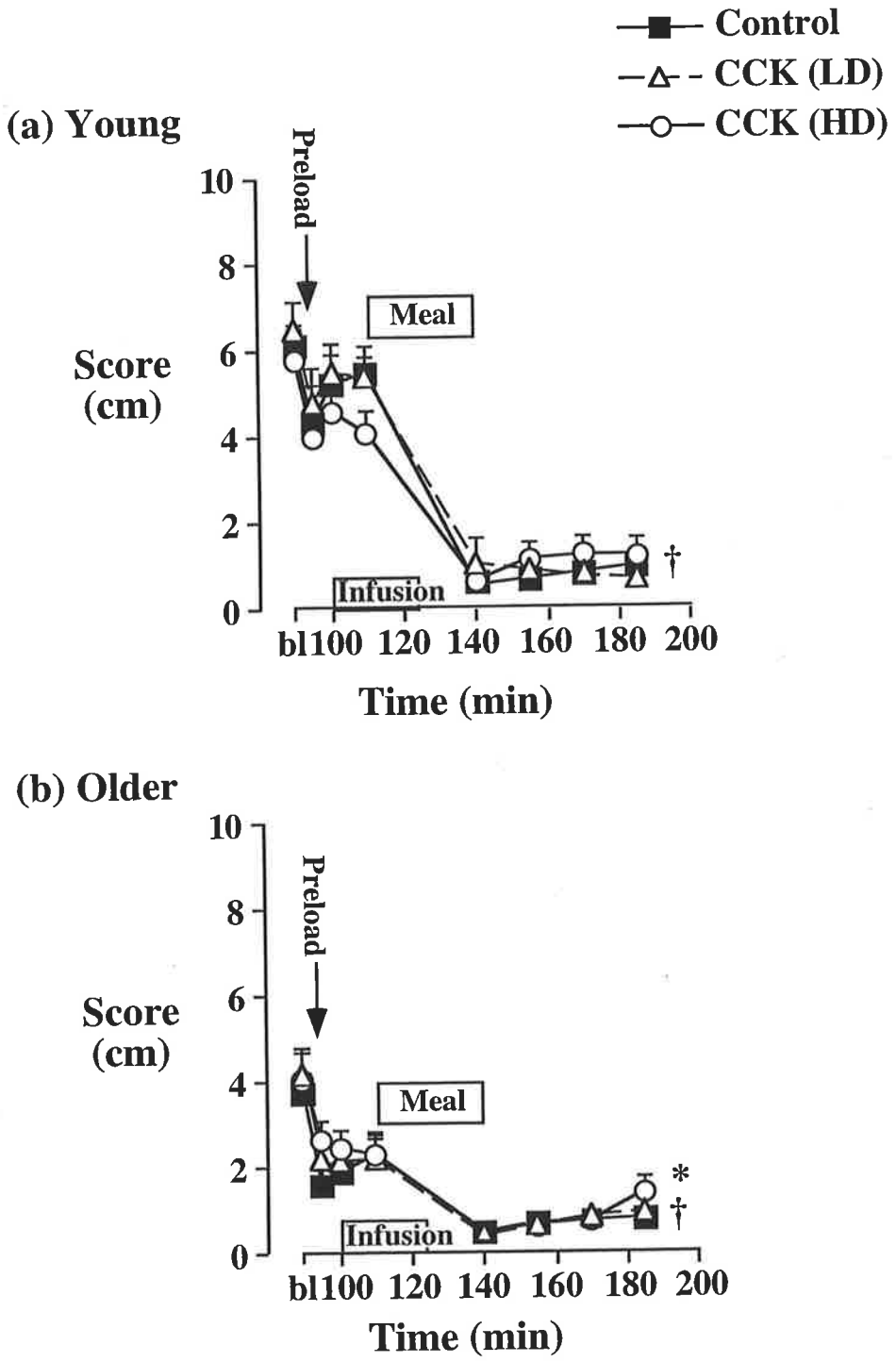

Figure 10.2: Absolute ratings of hunger in (a) 12 young and (b) 12 older subjects at baseline (t= 90 min), after the preload (t= 93-100 min), during intravenous infusions of saline (control), CCK-8 lower dose (1 ng/kg/min; LD) and CCK-8 high dose (3 ng/kg/min; HD) (t= 100-125 min), and following the buffet meal (t= 140-185 min). Data are mean ± SEM. Three-way ANOVA; *P<0.05, effect of age (older < young); †P<0.05, effect of time; P< 0.05, treatment × age, and treatment × time × age interactions for hunger ratings.

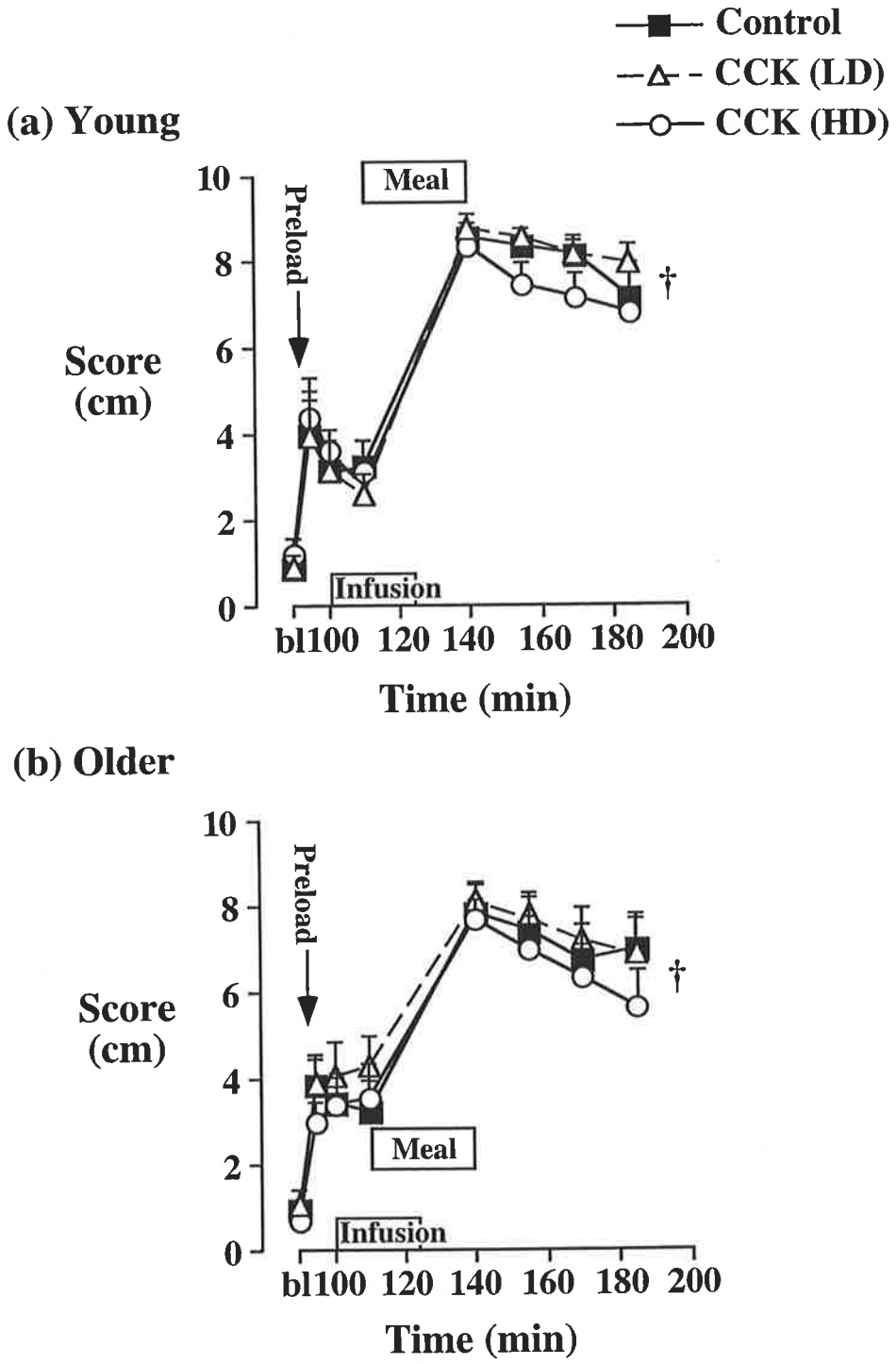

Figure 10.3: Absolute ratings of fullness in young (a) and older (b) subjects at baseline (t= 90 min), after the preload (t=93-100 min), during intravenous infusions of saline (control), CCK-8 lower dose (1 ng/kg/min; LD) and CCK-8 higher dose (3 ng/kg/min; HD)(t= 100-120 min), and following the buffet meal (t= 140-185 min). Data are mean ± SEM. Three-way ANOVA; †P<0.001, effect of time.

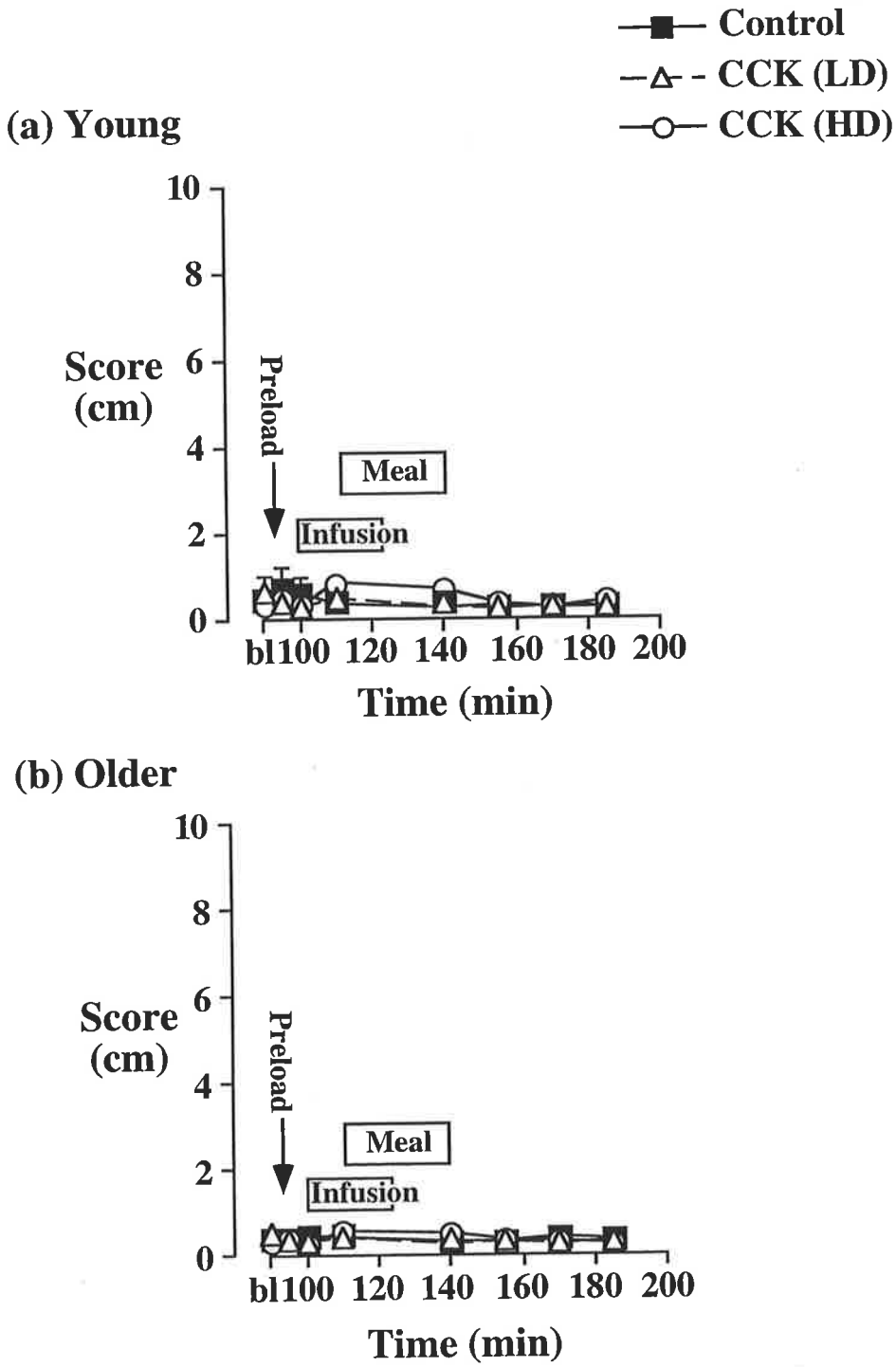

Figure 10.4: Absolute ratings of nausea in (a) 12 young and (b) 12 older subjects at baseline (t= 90 min), after the preload (t=93-100 min), during intravenous infusions of saline (control), CCK-8 lower dose (1 ng/kg/min; LD) and CCK-8 higher dose (3 ng/kg/min; HD)(t= 100-120 min), and following the buffet meal (t= 140-185 min). Data are mean ± SEM.

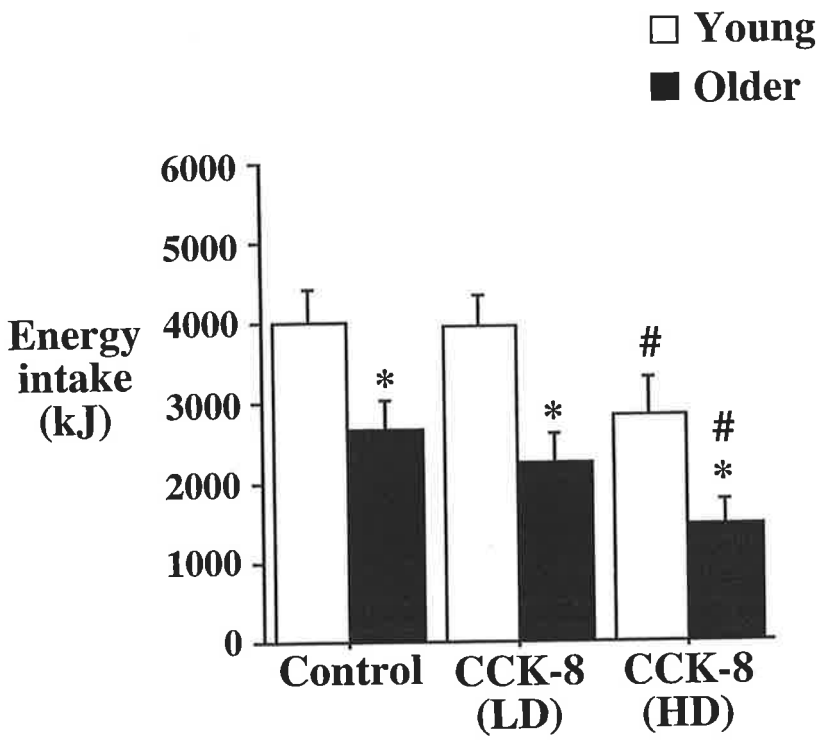

Figure 10.5: Energy intake (kJ) at buffet meal consumed during intravenous infusions of saline (control), CCK-8 lower dose (1 ng/kg/min; LD) and CCK-8 higher dose (3 ng/kg/min; HD) in 12 young and 12 older subjects. Data are mean ± SEM. Two-way ANOVA; Effect of age, *P<0.01 vs young; Effect of treatment, #P<0.001 vs control.

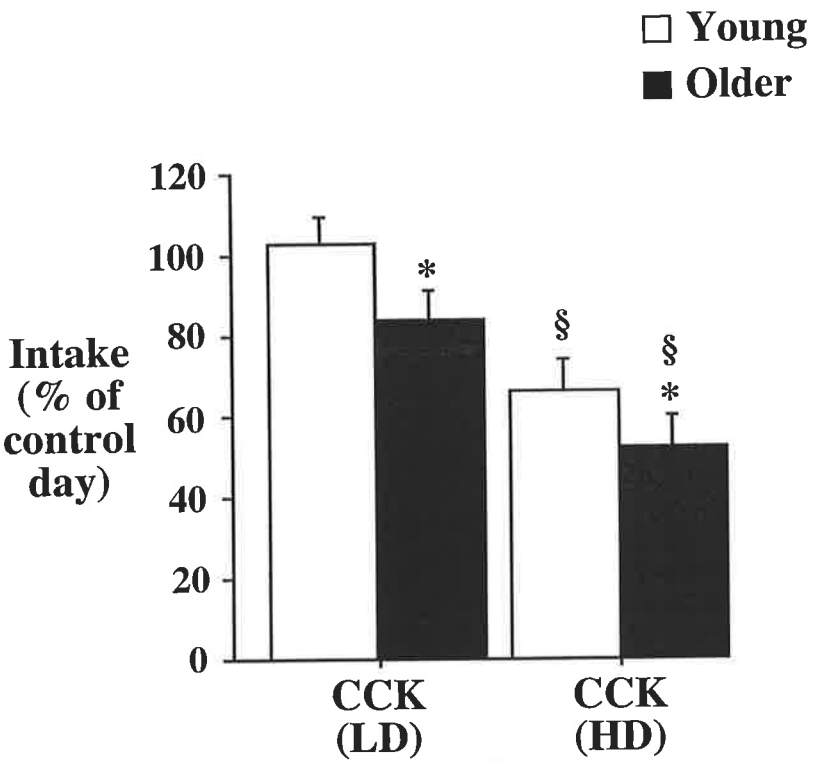

Figure 10.6: Food intake at the buffet meal (expressed as the percentage of the control day) during intravenous infusions of saline (control), CCK-8 lower dose (1 ng/kg/min; LD) and CCK-8 higher dose (3 ng/kg/min; HD) in 12 young and 12 older subjects. Data are mean ± SEM. Two-way ANOVA; Effect of age, *P<0.05 vs young; Effect of treatment, §P<0.001 vs CCK-8 (LD).

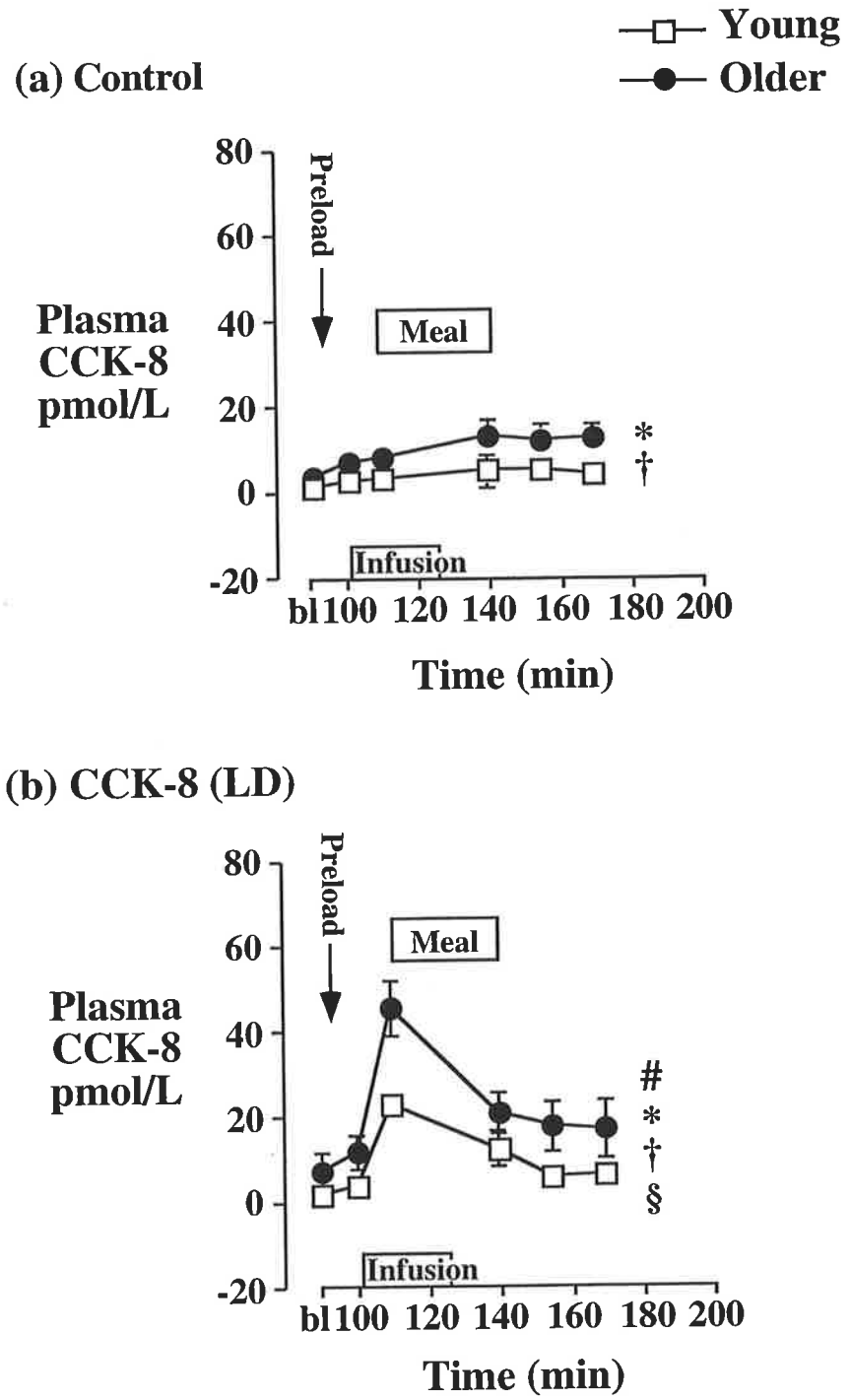

Figure 10.7: Plasma CCK-8 concentrations (pmol/L) at baseline (t= 0 min), after the preload (t= 100 min), during intravenous infusions of (a) saline and (b) CCK-8 lower dose (LD) (1 ng/kg/min) and following the buffet meal (t= 140-170 min) in 12 young and 12 older healthy subjects. Data are mean ± SEM. 3-way ANOVA, Effect of age; *P< 0.05, older > young, Effect of treatment; #P< 0.001 vs control and §P<0.05 HD > LD; Effect of time; †P< 0.001; P< 0.001, time × age, treatment × time and treatment × time × age interactions.

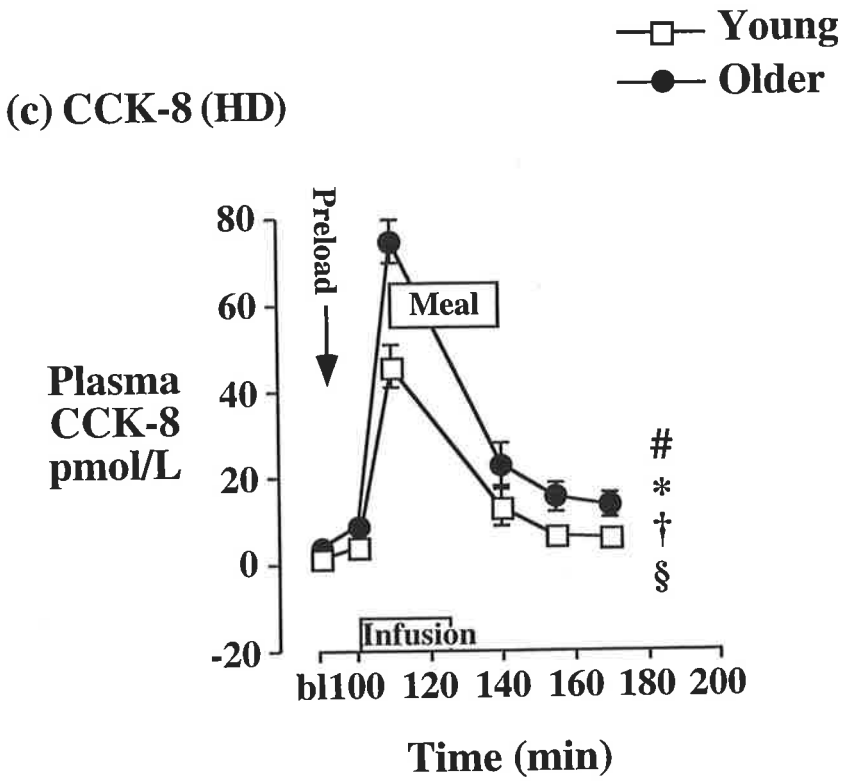

Figure 10.7 cont.: Plasma CCK-8 concentrations at baseline (t= 0 min), after the preload (t= 100 min), during intravenous infusion of CCK-8 higher dose (3ng/kg/min; HD), and following the buffet meal in 12 young and 12 older healthy subjects. Data are mean ± SEM. 3-way ANOVA, Effect of age; *P< 0.05, older > young, Effect of treatment; #P< 0.001 vs control and §P<0.05 LD vs HD; Effect of time; †P< 0.001, Time × age, treatment × time and treatment × time × age interactions; P< 0.001.

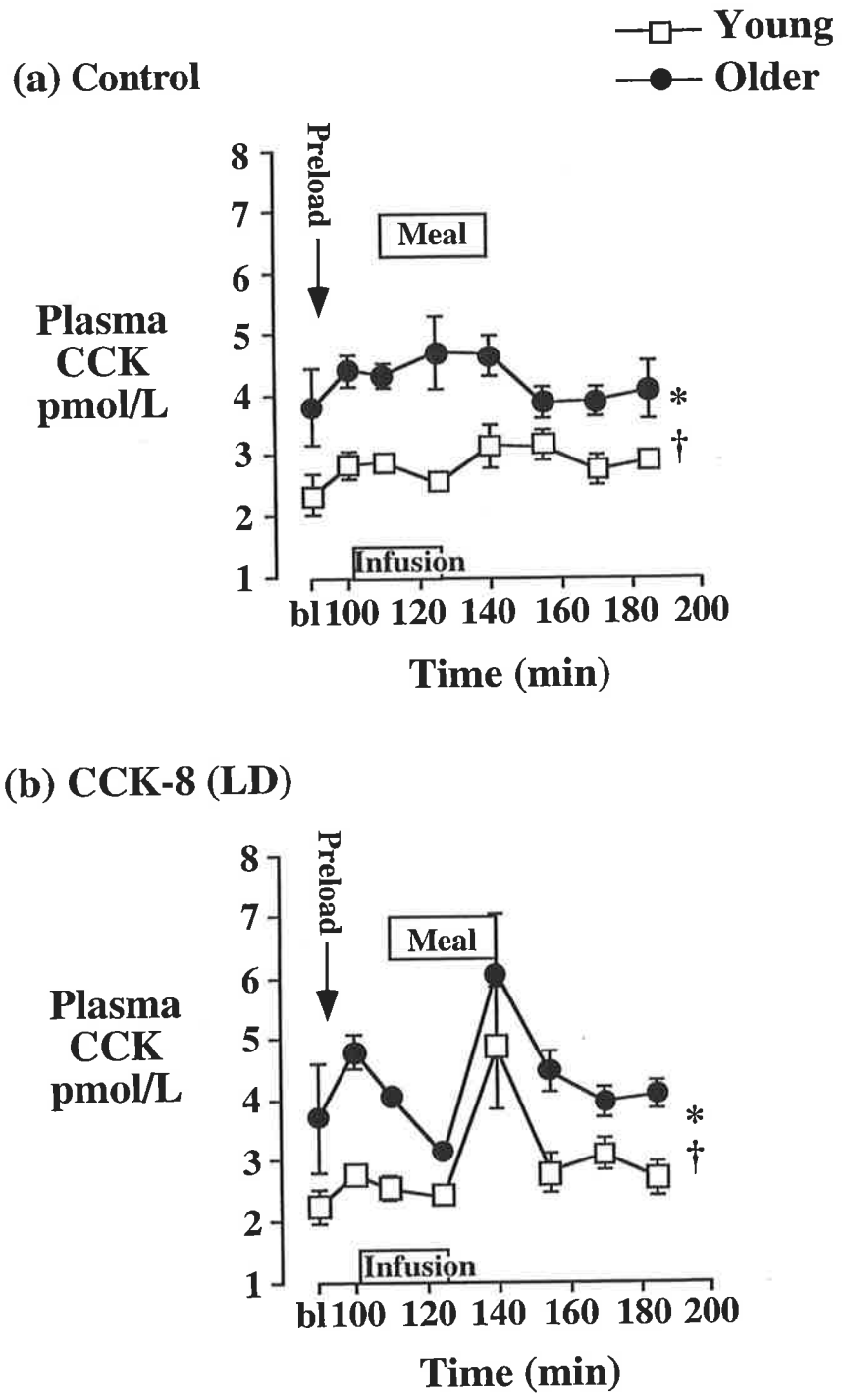

Figure 10.8: Plasma CCK (>12) concentrations at baseline (t= mean of 0 and 90 min), after the preload, during intravenous infusions of (a) saline and (b) CCK-8 lower dose (1ng/kg/min; LD) and following the buffet meal (t= 140-185 min) in 12 young and 12 older healthy subjects. Data are mean ± SEM. Three-way ANOVA; Effect of age; *P< 0.001, Effect of time †P< 0.001. P< 0.01, treatment × time interaction.

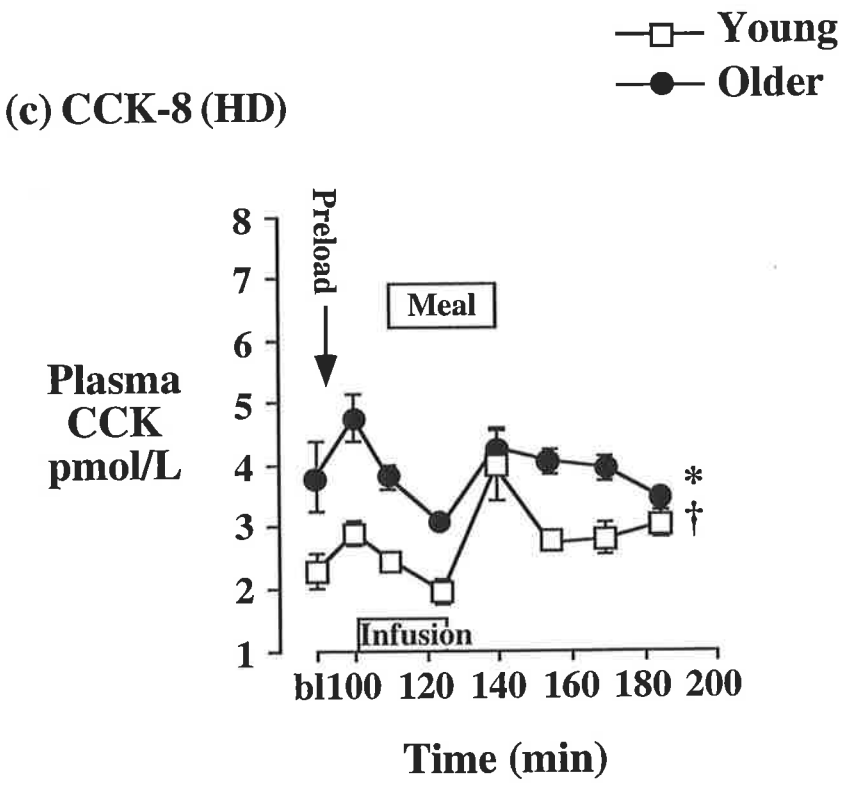

Figure 10.8 cont.: Plasma CCK (>12) concentrations at baseline (t= mean of 0 and 90 min), after the preload, during intravenous infusions of CCK-8 higher dose (3 ng/kg/min; HD) and following the buffet meal (t= 140-185 min) in 12 young and 12 older healthy subjects. Data are mean ± SEM. Three-way ANOVA; Effect of age; *P< 0.001, Effect of time †P< 0.001. P< 0.01, treatment × time interaction.

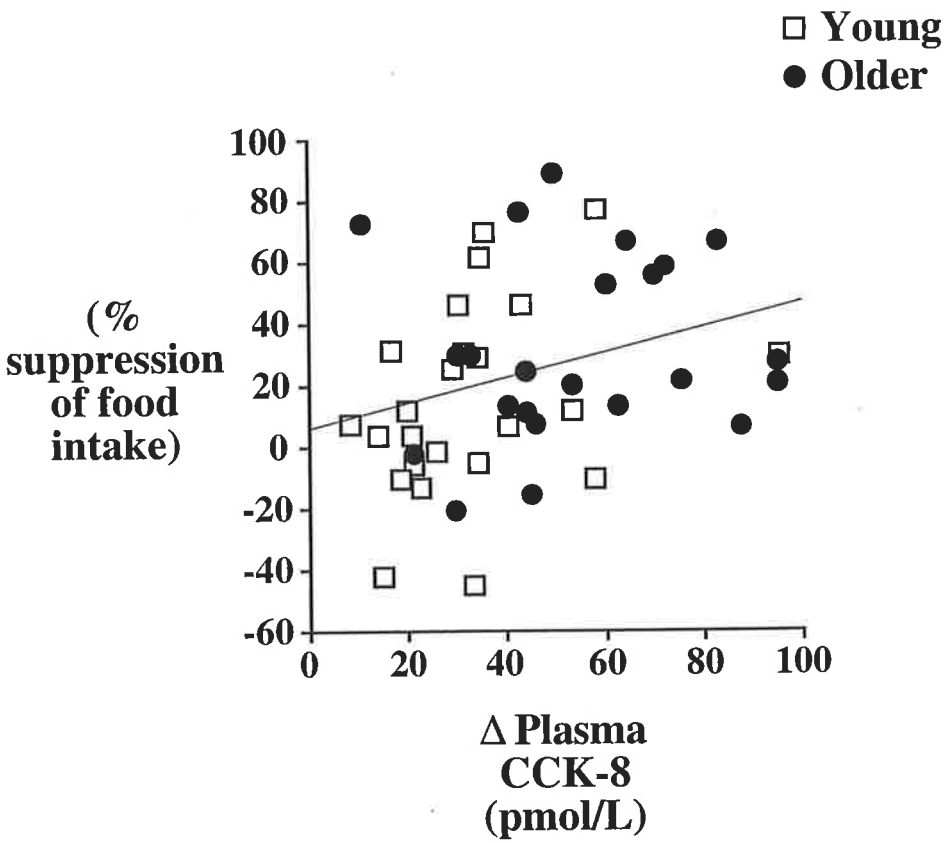

Figure 10.9: Relationship between mean plasma CCK-8 concentrations at 110 min and the percentage suppression of energy intake at the buffet meal during the lower dose and higher dose CCK-8 infusions in 12 young and 12 older subjects. (% Suppression = 100 - {[control day (kJ)/(LD) or (HD)(kJ) x 100}) ($r = 0.30$; $P < 0.05$).

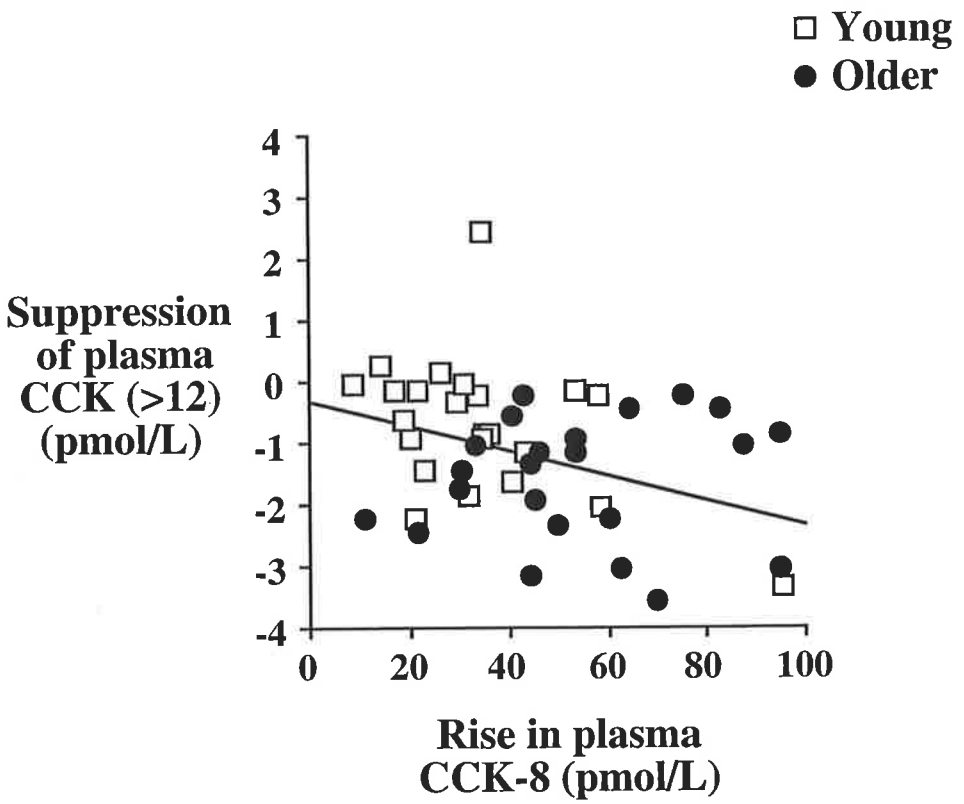

Figure 10.10: Relationship between the rise in plasma CCK-8 concentrations [CCK-8 concentrations at 110 min - CCK-8 concentrations at 100 min] and the suppression of plasma CCK (>12) concentrations [CCK (>12) concentrations at 125 min - CCK (>12) concentrations at 100 min] during intravenous infusions of CCK-8 lower dose (1 ng/kg/min; LD) and CCK-8 higher dose (3 ng/kg/min; HD) in young and older subjects ($r= -0.30$, $P< 0.05$).

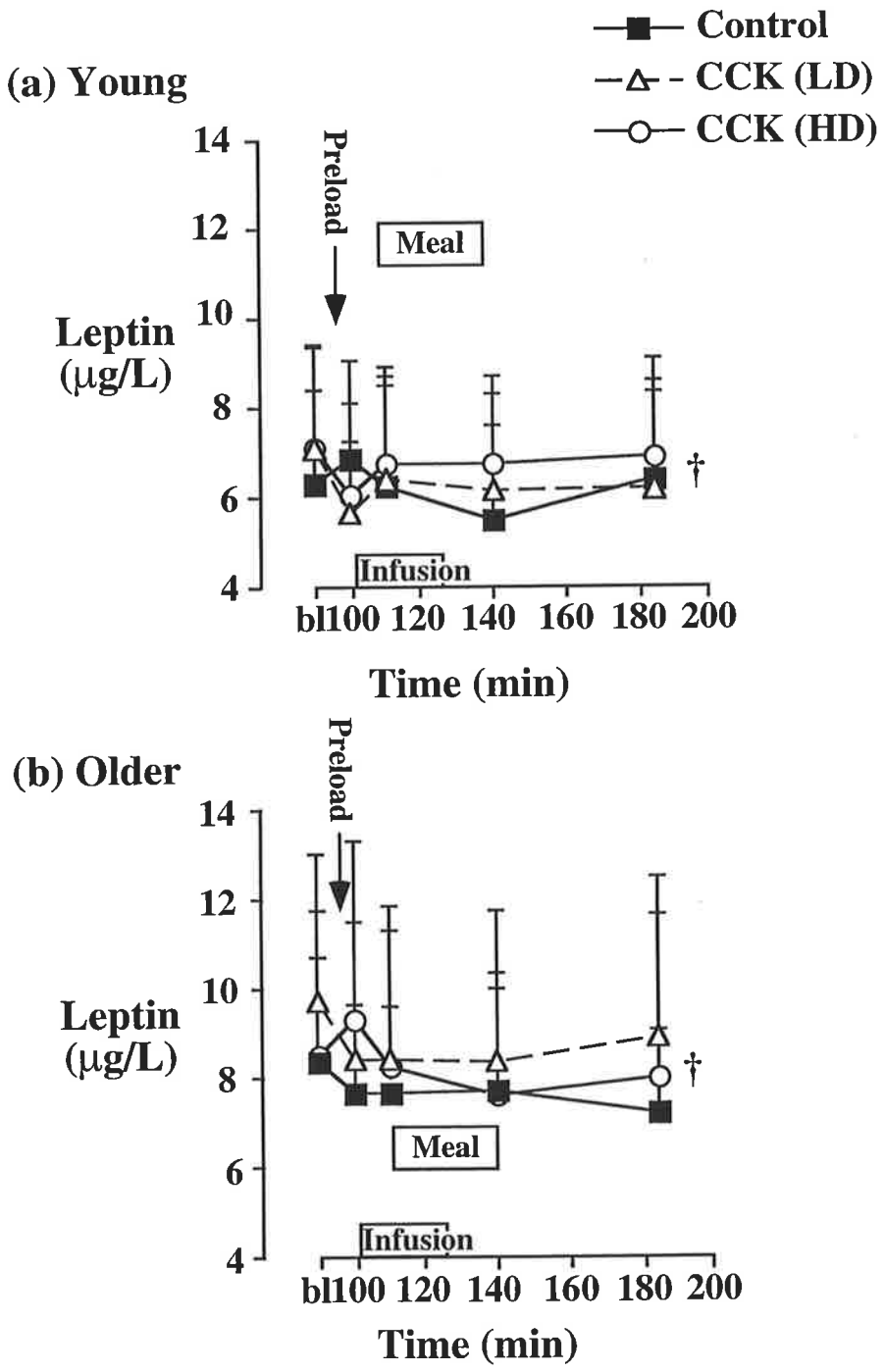

Figure 10.11: Plasma leptin concentrations (μg/L) in (a) 12 young and (b) 12 older healthy subjects at baseline (t= 0 min), after the preload (t= 100 min), during intravenous infusions of either saline (control), CCK-8 lower dose(1 ng/kg/min; LD), or CCK-8 higher dose (3 ng/kg/min; HD) (t= 100-125 min), and following the buffet meal (t= 140-185 min). Data are mean ± SEM. Three-way ANOVA; †P< 0.05 effect of time.

10.5 DISCUSSION

This study is the first to evaluate the effects of intravenous infusion of CCK-8 on appetite, energy intake, and plasma CCK, insulin and leptin concentrations in healthy older humans when compared to young adults. The major observations are that: (i) food (energy) intake was less in the older than young subjects both in their usual diet and during the control infusion. This is consistent with previous observations by ourselves (see Chapters 8,9 and 12 and Clarkston et al 1997) and others (Rolls 1995, Wurtman et al 1988, Morley 1997) (see Chapter 1.5); ii) energy intake was suppressed by CCK-8 infusion in a dose-dependent manner both in young and older subjects; (iii) the suppression of food intake by CCK-8 infusion was greater in the older than young subjects and occurred as a result of a reduction both in the duration and rate of eating; (iv) plasma CCK-8 concentrations were higher in older than young subjects at baseline and during the control and CCK infusions. The magnitude of the suppression of food intake was related to the plasma CCK-8 concentration, however, the sensitivity to CCK-8 did not appear to differ between the two groups; (v) plasma concentrations of endogenous CCK (>12) were suppressed by CCK-8 infusion, and (vi) plasma leptin concentrations were not affected by CCK-8 infusion.

Previous studies in healthy young subjects have demonstrated that peripheral administration of CCK-8 and -33; acutely decreases food intake by 15-50% (see Chapter 3.6.1) and exogenous administration of CCK antagonists increases food intake in animals (Brenner & Ritter 1995, Ebenezer et al 1990, Rayner & Miller 1993) and abolishes the intraduodenal fat-induced reduction in energy intake in young healthy humans (Matzinger et al 2000). Accordingly, this is strong evidence that CCK is a physiological satiety hormone in humans (see Chapter 3.6.1). The doses of CCK-8 used in this study were chosen on the basis of previous findings (Lieverse et al 1995, Muurahainen et al 1991, Pi-Sunyer et al 1982) and the CCK-8 lower-dose (LD) (1 ng/kg/min) infusion in the present study resulted in plasma CCK concentrations in young subjects similar to normally observed after a meal, but higher in older subjects. The CCK-8 high-dose (HD) (3 ng/kg/min) produced plasma CCK levels approximately double physiological postprandial concentrations in both older and young subjects.

Exogenous CCK-8 suppressed food intake approximately twice as much in the older compared to the young subjects (32% vs 15.5% compared to control). This is consistent with a previous report that older mice are more sensitive than young mice to the satiating effects of exogenous CCK-8 (Silver et al 1988) (see Chapter 4.5.1). Plasma CCK concentrations increased more during CCK-8 infusions in the older than young subjects, which may explain the greater suppression of energy intake by CCK-8 infusions in the former group. The extent of suppression of energy intake at the buffet meal was significantly related to the increase in plasma CCK-8 during CCK-8 infusion, with no difference between the older and young subjects in the extent of the suppression of food intake associated with a given rise in CCK-8 level. These results suggest that the sensitivity to the suppressive effects of exogenous CCK-8 is retained in the healthy elderly. Together with the higher plasma CCK concentrations in older than young subjects demonstrated in this and in previous studies (Khalil et al 1985, Masclee et al 1988 and Chapter 8), it is reasonable to postulate that increased endogenous CCK activity contributes to the physiological reduction in appetite and food intake observed with ageing.

It is uncertain why the increases in plasma CCK-8 concentrations during the CCK-8 infusions were greater in older than young subjects. The effects of ageing on the volume of distribution and clearance of exogenously administered CCK have not been reported. CCK-8 is rapidly cleared (elimination half-life ~ 5 min in young healthy subjects (Jebbink et al 1990) by the liver, and the hepatic uptake of CCK depends on its carboxyl-terminal tetrapeptide (trp-Met-Asp-PheNH_2) (Hunter et al 1990). For lipophilic compounds, like CCK-8, volume of distribution correlates better with total body weight (TBW) than with lean body mass (LBM) (Morgan et al 1994). Since there was no significant difference in TBW between older and young subjects in our study, it is unlikely that the higher plasma CCK-8 concentrations in the older subjects during CCK-8 infusions reflects a decrease in the volume of distribution. For many drugs eliminated predominantly by the liver, there is a positive correlation between systemic clearance and lean body mass (Morgan et al 1994). As lean body weight declines as a percentage of total body weight with advancing age (Morgan et al 1994), the systemic clearance of CCK-8 may be reduced in older subjects compared to young subjects of the same weight but greater lean body mass. By administering CCK-8 on a per-kilogram-total-body-weight basis, as done in this study might, in effect, delivered a higher dose of CCK-8 to the older subjects.

It has been suggested that CCK inhibits food intake by inducing nausea (Greenough et al 1998). There is no evidence for this in the present study, as nausea ratings were not affected by CCK-8. Two of the older women felt nauseous on presentation of the meal during the higher dose CCK infusion, but these feelings soon subsided and did not prevent food intake. In particular, none of the older subjects felt sick during the CCK-8 (LD) infusion which caused a 15.9% suppression of food intake.

The mechanisms by which CCK suppresses food intake are summarised in Chapter 3.6.1. One of these mechanisms is via the interaction with other gastrointestinal hormones released by the gut in response to a meal (see Chapter 3.6.1). A recent study in rats suggests that gastric stores of the satiety hormone leptin may also be involved in CCK-mediated suppression of food intake (Bado et al 1998) (see Chapter 2.3.4); intraperitoneal CCK-8 administration decreases the leptin content of the gastric fundus and increases in plasma leptin concentration within 15 min (Bado et al 1998). *In vitro* studies in rats also suggest that leptin and CCK may act synergistically via direct stimulation of gastric vagal afferents to inhibit food intake (Wang et al 1997). In this study plasma leptin concentrations did not differ between the treatment infusions or following the buffet meal in either age group. It is possible that plasma leptin may have increased between 110 -140 min (and so was not detected) and that meal-induced leptin concentrations may have been lower during the CCK-8 infusions since overall food intake at the buffet meal was less than during the control infusion. Plasma leptin concentrations did not, however, increase after the meal during the control infusion in either age group, which contrasts with the finding in rats that plasma leptin increases following 18 hr food deprivation (Bado et al 1998). This observation is most likely related to the smaller time period of fasting of subjects in this study. Nevertheless, these results suggest that the effect of CCK infusions on food intake in both age groups was not related to an increase in plasma leptin concentrations.

In young subjects, exogenous administration of CCK-8 slows the rate of gastric emptying (see Chapter 3.6.1), and this is associated with an increase in pyloric contractions, suppression of antral and duodenal motility and increase in sensitivity to gastric distension (Murphy et al 1987, Fraser et al 1993, Melton et al 1992). Furthermore, studies in humans have shown that CCK-antagonists accelerate gastric emptying (see Chapter 3.6.1). Ageing is associated with a modest slowing of gastric emptying (see Chapter 4.4.2), which may potentially contribute to increased

satiation, by prolonging the duration of gastric distension and small intestinal nutrient exposure (Clarkston et al 1997). We have reported that the stimulation of isolated pyloric pressure waves and endogenous release of CCK in response to intraduodenal lipid infusion is greater in healthy older when compared to young subjects (see Appendix A and Chapter 8). Although gastric emptying and pyloric motility were not evaluated in the present study, we speculate that since the elderly retain their sensitivity to the satiating effects of CCK-8, CCK may be a factor responsible for the slowing of gastric emptying accompanying healthy ageing. Furthermore, CCK-8 administration has been shown to amplify the satiety signals induced by balloon distension of the stomach in young women (Melton et al 1992). Satiety signals induced by gastric distension may, therefore, be enhanced in the presence of increased plasma CCK concentrations in the elderly. The effects of ageing on the CCK-induced slowing of gastric emptying, or sensitivity to gastric distension have yet to be examined in humans.

To our knowledge, the present study is the first to demonstrate that CCK inhibits its own release; the exogenous CCK-8 infusion was associated with a significant suppression of the plasma concentrations of CCK fragments greater than 12 amino acids in length [CCK (>12)] and the increase in plasma CCK-8 concentrations in response to CCK-8 infusion was significantly related to the fall in CCK (>12). CCK fragments with >12 amino acid residues are the most abundant circulating biologically active forms (Jansen et al 1992) (see Chapter 3.6.1). Our finding that CCK-8 inhibits its own release is in contrast to that of Jebbink et al (1992) who found no effect of intravenous CCK-8 infusion (70 pmol/kg/hr [1.33 ng/kg/min] for 60 min) on plasma CCK >12 after a high fat meal in healthy young subjects. This discrepancy suggests that the auto-inhibitory effects of CCK only be manifested under certain circumstances ie. CCK-8 can overcome the secretory drive to CCK >12 release produced by a low fat, low energy food (such as the preload in the present study) but not a strong stimulus to endogenous CCK release such as a high fat meal. Alternatively, differences in the method of administration of CCK-8 in these two studies may have contributed to the contrasting effect of exogenous CCK-8 on endogenous CCK release; in the study by Jebbink et al (1992), CCK-8 was administered over a longer duration (ie 60 min vs 25 min), but at a rate that was comparable to the CCK-8 low-dose administered in this study. Further studies are required to investigate this negative feedback control of CCK in relation to appetite and gastric emptying. Fatty acids and amino acids are potent stimulators

(McLaughlin et al 1999, Shi et al 1999, Peikin 1989) and bile acids and pancreatic proteases are potent inhibitors (Green 1994, Schmidt et al 1991) of CCK release in humans (see Chapter 3.6.1). Exposure to a high-protein diet in rats (Shi et al 1997) and a high-fat diet in humans (French et al 1995) is also thought to down-regulate the CCK receptors responsible for feedback inhibition of CCK release (see Chapter 3.6.1).

In summary, we have found that exogenous CCK-8 is more satiating in older than young adults. Older subjects have higher plasma endogenous CCK than young subjects, fasting and in response to a low energy preload. Despite higher circulating CCK concentrations older people retain their sensitivity to the satiating effects of exogenous CCK. This suggests that enhanced endogenous CCK activity may contribute to the anorexia of ageing and identifies a possible therapeutic role for CCK antagonists as a means of increasing food intake in the anorexic and/or malnourished elderly. This may warrant further investigation.

CHAPTER 11

Proximal Gastric Motor and Sensory Function in the Healthy Elderly

11.1 SUMMARY

Healthy ageing is associated with a reduction in appetite and food intake, which may predispose to pathological weight loss and malnutrition. Changes in intragastric mechanisms mediating satiation in the elderly have not been studied. The aim of this study was to evaluate the effects of ageing on (i) fasting gastric compliance and the perception of gastric distension, and (ii) food intake and gastric accommodation to a meal.

Five healthy older (aged 68-73 yr) and 5 healthy young (aged 22-27 yr) men, matched for body mass index, were each studied on three occasions following an overnight fast. On one day ("barostat day"), isovolumetric and isobaric distensions of the proximal stomach were performed and meal-induced changes in intrabag volume were measured

using an electronic barostat. On another day ("tube-only day") subjects were intubated with a nasogastric tube without an intragastric bag before the meal. On the third day ("control day") subjects were given the meal without intubation. Energy intake from the buffet meal was quantified and perceptions assessed using visual analogue questionnaires.

During both isobaric and isovolumetric distensions, the pressure-volume relationship did not differ significantly between older and young subjects. During gastric distensions perceptions of fullness ($P< 0.01$), abdominal discomfort ($P< 0.05$) and bloating ($P< 0.05$) were less in older than young subjects, whereas the perception of hunger ($P< 0.05$) was less in the young compared to older subjects. There was no difference in energy intake ($P= 0.44$) between young and older subjects. Food intake was less on the "barostat day" ($P< 0.01$) and the "tube-only day" ($P< 0.01$) than on the "control day" in young subjects, but was not affected by the different study conditions in the older subjects. Following the meal, the maximum intrabag volume occurred later in the older compared with the young subjects (105 ± 4 min vs 36 ± 8 min, $P< 0.05$) and the intrabag volume change was greater ($P= 0.05$) in the older than the young later in the postprandial period.

Healthy ageing is associated with decreased perception of gastric distension without any change in fasting gastric compliance, and reduced gastric tone late in the postprandial period when compared to the young. Control of food intake is less sensitive to external stimuli in older than in young subjects.

11.2 INTRODUCTION

The causes of the anorexia of ageing are likely to be multifactorial (Fischer & Johnson 1990)(see Chapter 1.2.3). The contribution of disordered stomach function has not been specifically investigated.

Studies in gut regions other than the stomach indicate diminished perception of oesophageal and rectal distension in older subjects (see Chapter 4.4.1), and a diminished effect of intraduodenal glucose or lipid infusions on appetite ratings, compared to young volunteers (see Chapters 8 and 9, and Appendix A).

The relatively few studies that have examined the effect of ageing on the stomach show a modest slowing of gastric emptying (Clarkston et al 1997, Horowitz et al 1984) compared to the young (see Chapter 4.4.2). The effect of ageing on the proximal region of the stomach has never been studied, but is potentially important; by acting as a reservoir for the solid component of a meal, the proximal stomach influences the overall rate of gastric emptying (see Chapter 3.3.1), and abnormalities of this region, in sensitivity to distension, or accommodation to a meal, have been implicated in the origin of symptoms in conditions such as diabetes mellitus (Samsom et al 1995, Samsom et al 1998) and functional dyspepsia (Tack et al 1998, Mearin et al 1991) (see Chapter 3.3.1).

The aims of this study were to evaluate, using an electronic barostat, the effects of ageing on fasting compliance and the perception of distension of the proximal stomach, and on energy intake in response to fundic distension as well as meal-induced gastric accommodation.

11.3 SUBJECTS AND METHODS

Five healthy older men (median age 71 yr, range 68-73 yr; median body mass index 25.6 kg/m^2, range 22.4 - 30.7) and 5 healthy young men (median age 23 yr, range 22-27 yr; median body mass index 24.4 kg/m^2, range 20.7-31.2) were studied. No subject had a history of gastrointestinal or significant systemic disease, nor was taking medication known to influence gastrointestinal motility or appetite. Two older subjects were taking simvastatin for the treatment of hyperlipidaemia and one older subject was taking sotalol hydrochloride for the treatment of a cardiac arrhythmia. No subject was a restrained eater, as assessed by the Three-factor Eating Restraint Questionnaire (Stunkard & Messick 1985) (see Chapter 6.4.1). All subjects had participated in studies involving nasogastric intubation previously. The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and was in accordance with the Declaration of Helsinki.

11.3.1 *Experimental Protocol*

Each subject underwent three studies, on separate days. These studies involved either (i) proximal gastric distension with a barostat "barostat day" (ii) nasogastric intubation without gastric distension "tube-only day" or (iii) no intubation "control day". The order of the first two studies was randomised and the "control day" was last. Each study was separated by at least 3 days. On all study day subjects attended the

laboratory between 0900 hours and 1100 hours, following an overnight fast of at least 12 hours.

On the "barostat day", a catheter incorporating a polyethylene bag at the distal end was inserted through an anaesthetised nostril and positioned with the bag in the proximal stomach (see Chapter 7.4.2). The bag was unfolded by introducing 500 ml of air, which was subsequently withdrawn. The subject was then seated in a semi-kneeling position on a specially designed chair, with the body upright and the arms resting on a table adjusted to the height of the elbows (Hebbard et al 1996, Verhagen et al 1999a). The bag was then inflated with air in increments of 1 mmHg, one minute at a time, to determine the minimal distending pressure, which was defined as the pressure at which continuous respiratory fluctuations were first evident in the pressure and volume tracings, together with a volume of at least 30 ml in the bag (Hebbard et al 1996, Verhagen et al 1999a). Two series of proximal gastric distensions were then performed; one at steps of fixed pressure (isobaric distensions) with 1 mmHg increments, and another using steps of fixed volume (isovolumetric distensions) with 100 ml increments. Distensions were terminated when either the pressure in the bag was $\geq$ 20 mmHg, the volume was $\geq$ 800 ml, or the subject reported marked discomfort (Verhagen et al 1999a). The duration of each distension step was 2 minutes; each series was separated by at least 15 minutes, and the order of distensions (isobaric or isovolumetric) was randomised. After completion of the second distension there was a "rest" period of 15 minutes. Following this, the bag was inflated with air to maintain the intrabag pressure at 2 mmHg above the previously determined minimal distending pressure. After 30 minutes a semi-solid meal was offered, from which subjects ate ad libitum for 30 minutes while the bag remained inflated. The volume in the bag was then recorded for a further 120 minutes.

On the "tube-only day", a similar catheter was inserted through an anaesthetised nostril with its tip in the stomach, this time without the polyethylene bag at the distal end. After a period of 60 minutes, which was similar to the time taken to perform fasting gastric distensions on the other study day, an identical buffet meal was offered, from which subjects again ate ad libitum for 30 minutes.

On the "control day", subjects attended the laboratory following an overnight fast. Nasogastric intubation was not performed; subjects were offered an identical meal,

from which they ate ad libitum for 30 minutes. On each of the three study days the meal was consumed at about 1130 hours.

11.3.2 *Performance of gastric distensions*

An electronic barostat (Distender Series II, G & J Electronics, Ontario, Canada) was used to introduce or withdraw air from the polyethylene intragastric bag, via a silicone rubber catheter assembly (1570 mm long, internal luminal dimensions 1.9 x 2.4 mm) (Dentsleeve, SA, Australia) (see Chapter 7.4.2 and Figure 7.4). The pressure in the bag was measured by the barostat via a separate channel in the catheter (internal diameter 0.6 mm). Changes in the volume of air in the bag were made at a rate of 33 ml/s. The intragastric bag had a high compliance (>4000 ml/mmHg) in the volume range used in the study (0-800 ml), and was approximately spherical at its maximum volume of about 1000 ml. The catheter assembly also incorporated a sleeve sensor 60 mm proximal to the bag; the assembly was positioned with the sleeve located in the lower oesophageal sphincter and the bag in the proximal stomach.

A Powermac computer (Apple Computer, CA, USA) was used to program the distensions and acquire pressure and volume data from the barostat (sampling rate 1 Hz), using custom-written software (DAD, written by Dr GS Hebbard using Labview, National Instruments Corporation, TX, USA). The volume in the bag was corrected on-line for air compression using an experimentally determined correction factor (1.848 ml/mmHg) (Whitehead & Delvaux 1997, Samsom et al 1995). The mean pressure and volume during the last minute of each distension step were determined after importing the data into a display and analysis program (AcqKnowledge, Biopac Systems, CA, USA). In the comparison of isobaric distensions, pressures are given relative to the minimal distending pressure. In the comparison of isovolumetric distensions, pressures are given relative to the mean pressure during inflation to 30 ml for 1 minute; the latter was performed as an initial step before each isovolumetric series. The pressure-volume relationship in each distension series was used as a measure of gastric compliance (Hebbard et al 1996, Verhagen et al 1999a).

The pressure in the intragastric bag was maintained at 2 mmHg above minimal distending pressure for 30 minutes before and during the meal, and for 120 minutes after the meal and intrabag volume was measured in the same way and used as an index of proximal gastric tone (Tack et al 1998, Hebbard et al 1996). The maximum volume reached during the postprandial period was determined, and expressed as the maximum

change in volume from the mean value during 30 minutes of fasting; the time from the end of the meal period at which this occurred was also measured. The mean change in volume from the fasting value was also calculated for each 10 minute period after the meal in each subject.

11.3.4 *Measurement of perception*

Perceptions of fullness, nausea, abdominal discomfort, bloating, hunger, and desire to eat were measured twice before each series of distensions and averaged to provide a baseline, and then in the last minute of each distension step using 100 mm visual analog scales (Hebbard et al 1996, Verhagen et al 1999a)(see Chapter 6.3.1 and Appendix BII). Perceptions during gastric distension are expressed as the change from the baseline score.

11.3.5 *Measurement of energy intake*

Intake (g) at the buffet meal (350 ml flavoured milk, 300 ml unsweetened orange juice, 350 ml water , cooked pasta (200 g dry weight) with 100 g tomato/onion/garlic pasta sauce, 10 g parmesan cheese, 400 g canned potato salad, 200 g coleslaw with 60 g mayonnaise, 400 g tropical fruit salad in natural juice, 150 g raspberry jelly, 200 g chocolate custard, 200 g strawberry low-fat yoghurt) was calculated, and using the DIET 4 Nutrient Calculation software (Xyris Software, Australia, Pty Ltd), energy intake (kJ) determined (see Chapter 6.3.3).

11.3.6 *Statistical analysis*

Pressure, volume, energy intake from the buffet meal, baseline perception scores (between study days) as well as the change in perception scores from baseline were compared using repeated measures analysis of variance (SuperANOVA 1.11, Abacus Concepts Inc., California). The Wilcoxon signed rank test was used to compare minimal distending pressures, mean volumes during the 30 minute fasting period, and maximum volume change after the meal and its timing (StatView 5, SAS Institute Inc., NC, USA). P- values < 0.05 were considered significant. Data are presented as means $\pm$ SEM.

11.4 RESULTS

The study was tolerated well by all subjects.

11.4.1 *Perception of gastric distension*

There was no difference in baseline scores for perception in any category between young and older subjects (data not shown).

During isobaric distensions, perceptions of fullness, abdominal discomfort, and bloating increased from baseline in both age groups ($P< 0.05$ for all). Perceptions of desire to eat, hunger, and nausea did not change from baseline in either age group.

In the young subjects, perceptions of fullness (Figure 11.1), abdominal discomfort (Figure 11.2), bloating (Figure 11.3), and nausea increased, and perceptions of desire to eat and hunger (Figure 11.4) decreased from baseline ($P\leq 0.05$ for each) during isovolumetric distensions. In contrast, only abdominal discomfort ($P< 0.05$) changed significantly from baseline in the older subjects as volume increased.

During both isobaric and isovolumetric distensions, perceptions of fullness (Figure 11.1) ($P< 0.01$ and $P< 0.001$, respectively); abdominal discomfort (Figure 11.2) ($P= 0.59$ and $P< 0.05$), and bloating (Figure 11.3)($P< 0.01$ and $P< 0.05$) increased more in the young than older subjects (ANOVA, age × pressure interaction), whereas hunger (Figure 11.4) was suppressed to a greater extent during isovolumetric ($P< 0.05$) distensions in the young compared to older subjects (ANOVA, age × pressure interaction). Perceptions of nausea and desire to eat were not significantly affected by age (data not shown).

11.4.2 *Fasting pressure-volume relationships*

Both the minimal distending pressure (7.4 ± 0.9 mmHg vs 4.4 ± 0.5 mmHg; $P< 0.05$) and the pressure at 30 ml inflation (used as the basal pressure for comparison of isovolumetric distensions) (7.8 ± 0.7 mmHg vs 5.5 ± 0.8 mmHg, $P< 0.05$) were greater in the older than the young subjects. During both isobaric ($P= 0.47$) and isovolumetric ($P= 0.79$) distensions, the pressure-volume relationship did not differ significantly between older and young subjects (Figure 11.5).

11.4.3 *Energy intake at the meal*

There was no effect of age on mean energy intake (kJ) at the buffet meal on the three study days (P= 0.44). In the young subjects, energy intake at the meal differed between the three study days (P= 0.01); subjects ate ~22% less on the "barostat day" (P= 0.006) and ~17% less on the "tube-only day" (P= 0.02) than during the "control day"; the difference between the former two days was not significant (P= 0.44). In contrast, there was no effect of the different study conditions on energy intake in the older subjects (P= 0.29), and they ate similar amounts on the "tube-only day" and the "control day". The effects of age and study condition on the weight (g) of food consumed was the same as those on energy intake (Figure 11.6).

11.4.4 *Proximal gastric tone before and after the meal*

Mean intrabag volume in the 30 minutes before the meal did not differ (P= 0.89) between older (142 ± 12 ml) and young (142 ± 24 ml) subjects. There was no difference in the volume of the bag during the meal (65.4 ± 33.7 ml vs 37.5 ± 15.5 ml respectively, P= 0.35) or the magnitude of the peak volume change after the meal (361 ± 70 ml vs 248 ± 51 ml respectively, P= 0.35) between the older and young subjects. The maximum volume occurred at 105 ± 4 minutes in the older, compared with 36 ± 8 minutes in the young subjects (P< 0.05) and the mean intrabag volume change was greater in the older than the young, later in the postprandial period (P= 0.05 by ANOVA, age × time interaction), so that the mean volume change was greater at 60-70, 90-100, 100-110, and 110-120 minutes (P< 0.05) in older than young subjects (Figure 11.7).

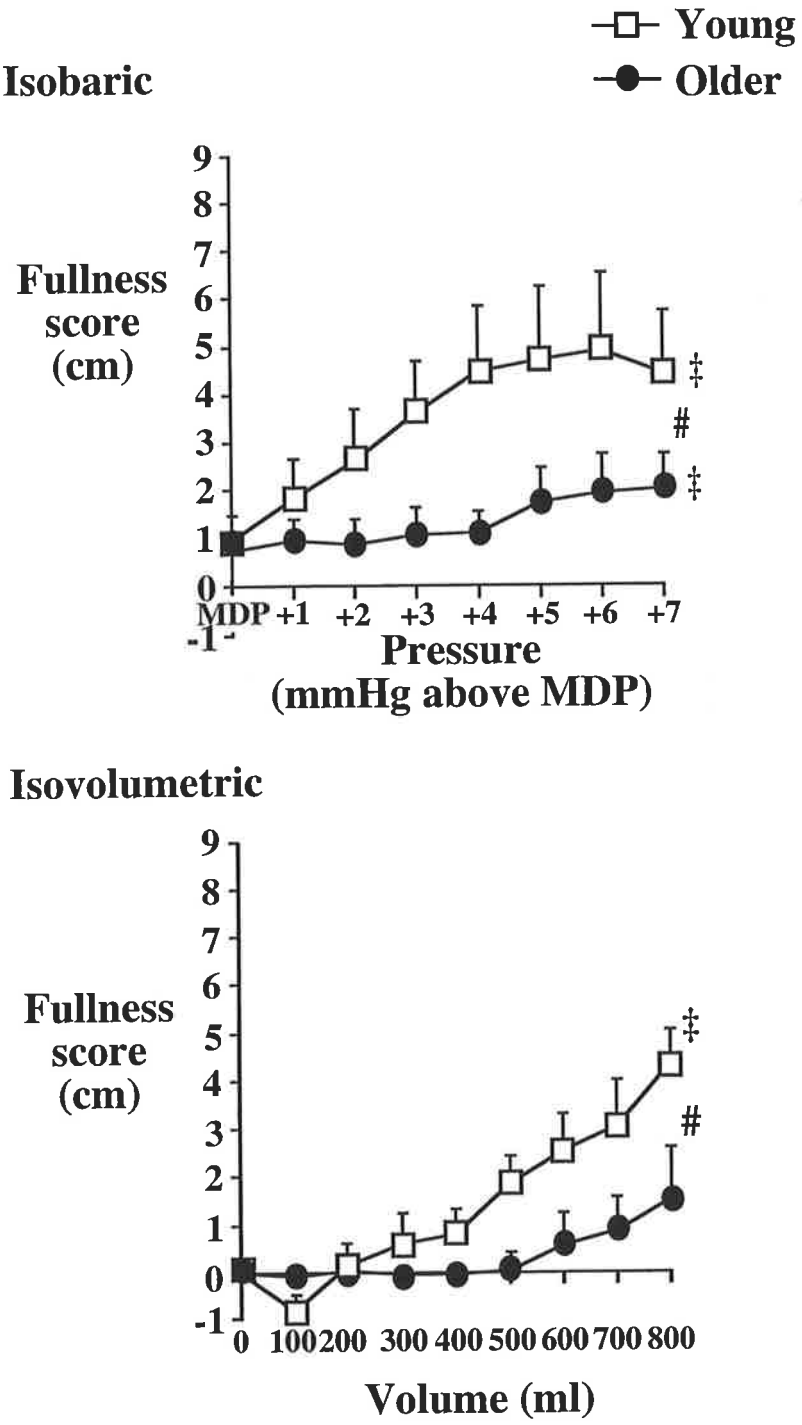

Figure 11.1: Change in sensations of fullness from baseline in young and older subjects during isobaric and isovolumetric gastric distensions. Data are mean ± SEM. Two way ANOVA; *P< 0.05 and #P< 0.01 age x time interaction, One-way ANOVA; ‡ P< 0.05 change from baseline.

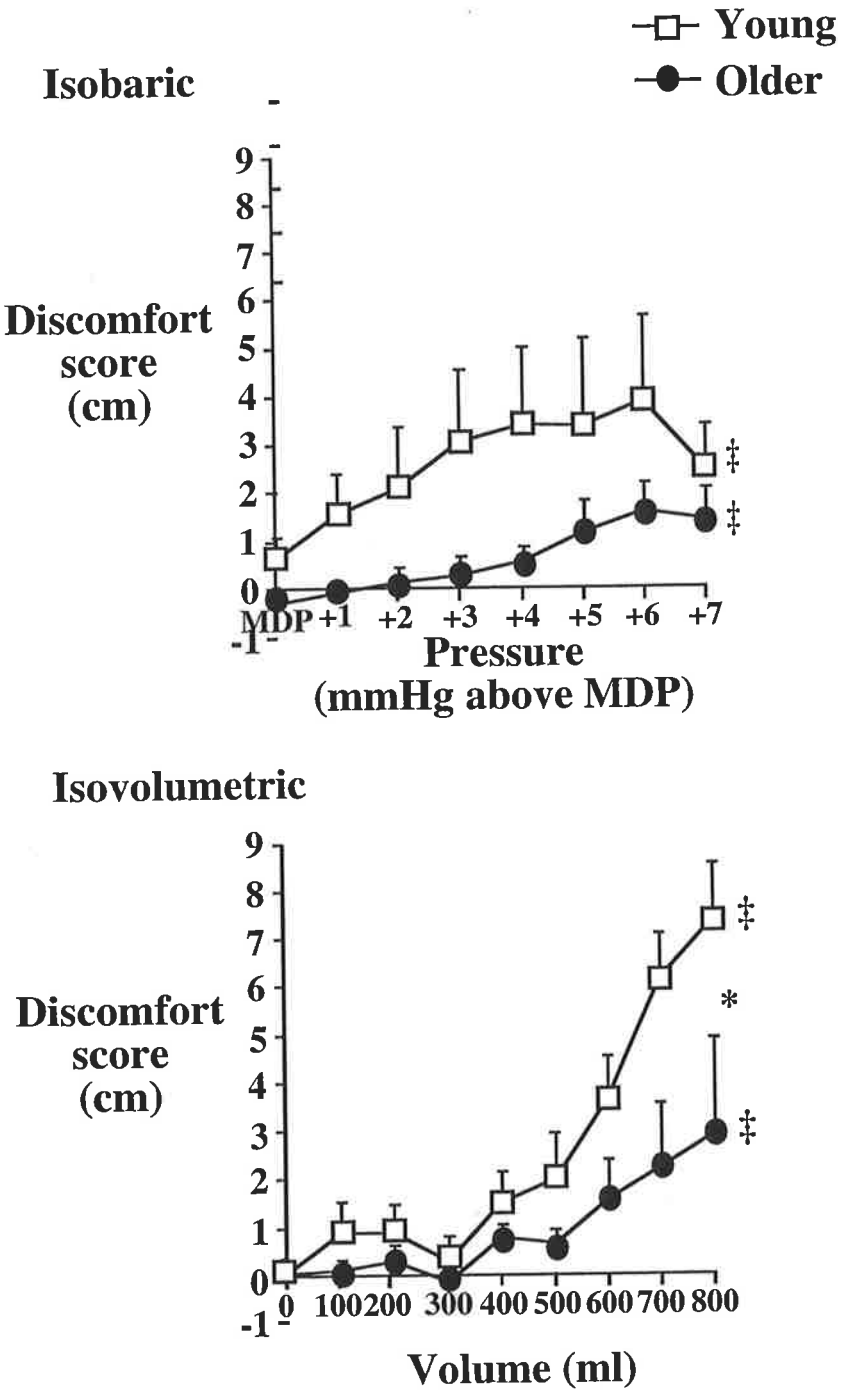

Figure 11.2: Change in sensations of abdominal discomfort from baseline in young and older subjects during isobaric and isovolumetric gastric distensions. Data are mean ± SEM. Two way ANOVA; *P< 0.05 age x time interaction, One-way ANOVA; ‡ P< 0.05 change from baseline.

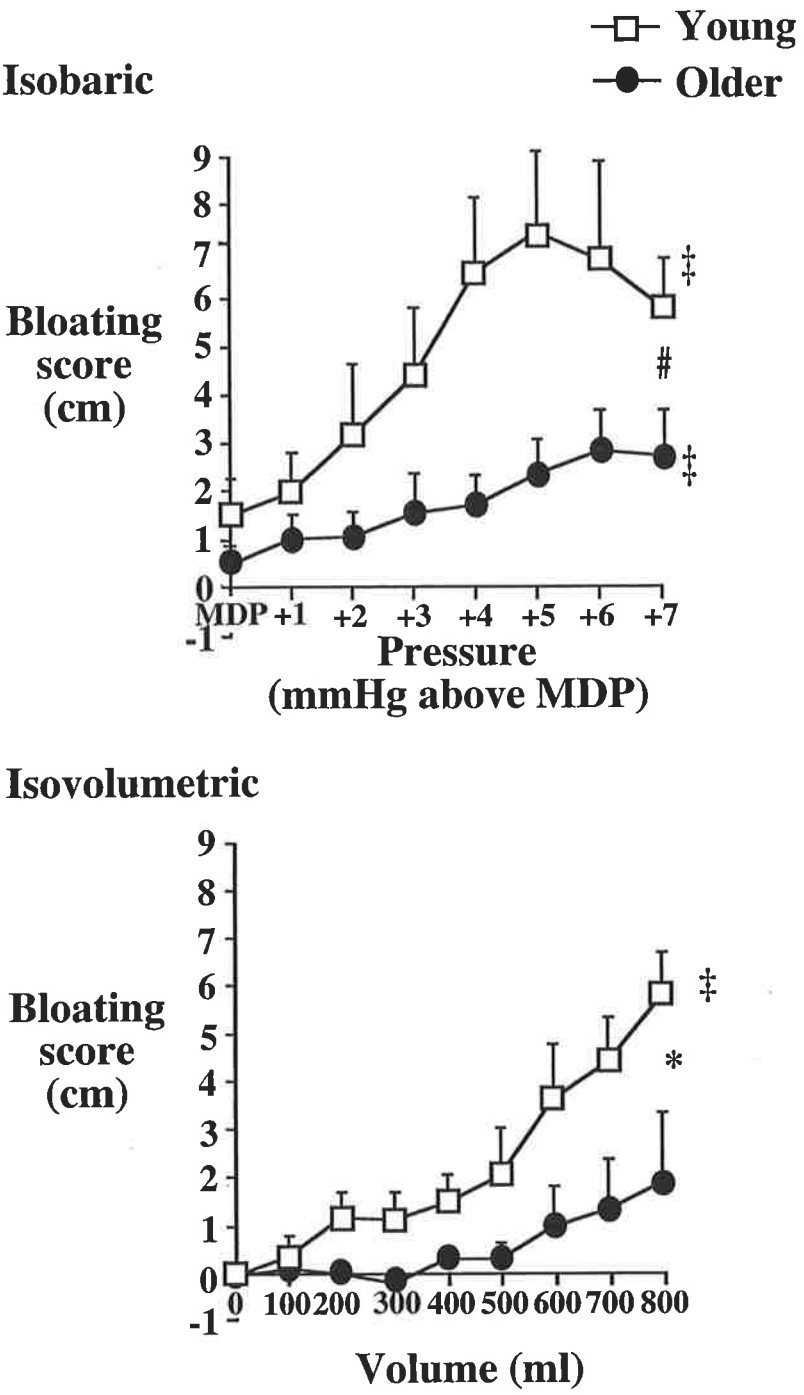

Figure 11.3: Change in sensations of bloating from baseline in young and older subjects during isobaric and isovolumetric gastric distensions. Data are mean ± SEM. Two way ANOVA; *P< 0.05 and #P< 0.01 age x time interaction, One-way ANOVA; ‡ P< 0.05 change from baseline.

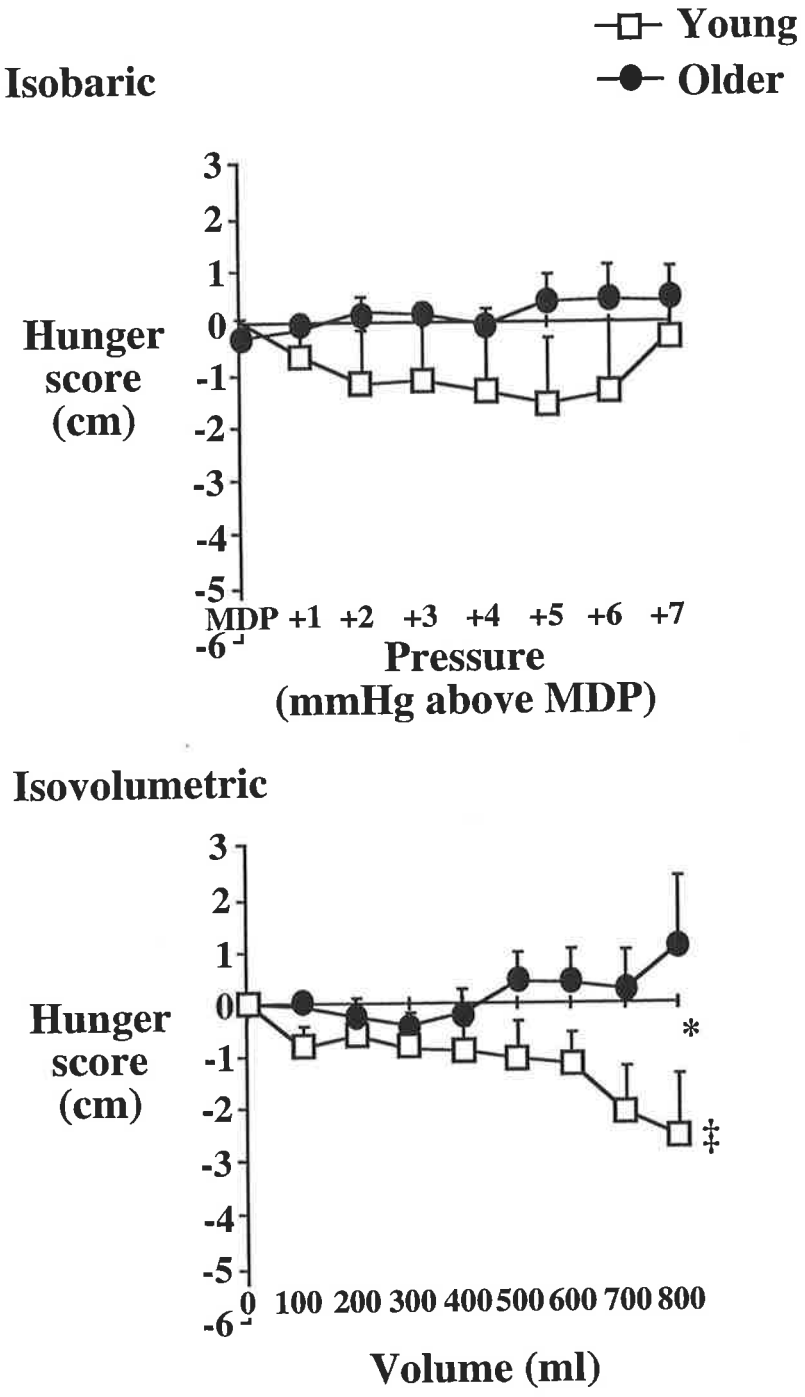

Figure 11.4: Change in sensations of hunger from baseline in young and older subjects during isobaric and isovolumetric gastric distensions. Data are mean ± SEM. Two way ANOVA; *P< 0.05 age x time interaction, One-way ANOVA; ‡ P< 0.05 change from baseline.

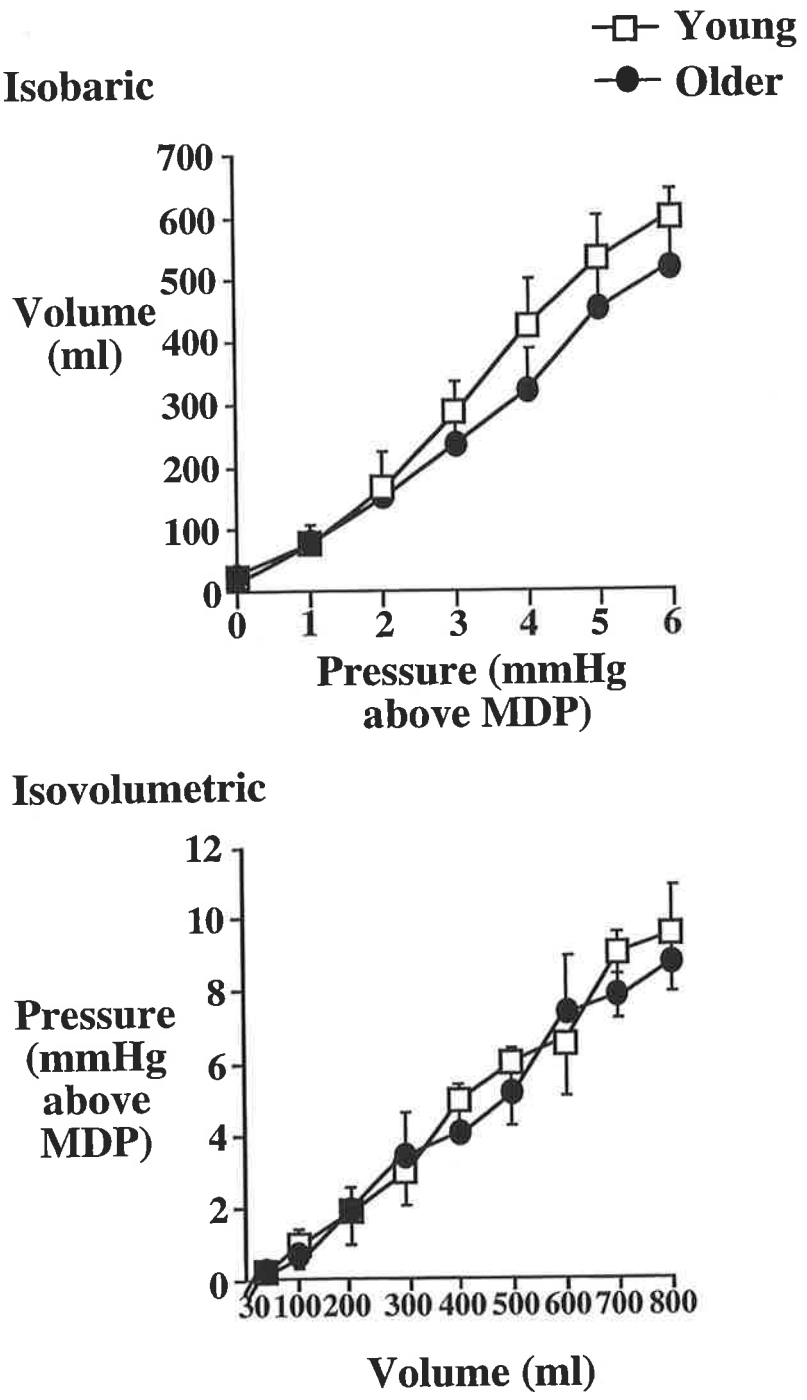

Figure 11.5: Pressure-volume relationships during isobaric and isovolumetric gastric distensions in young and older subjects. Data are mean ± SEM.

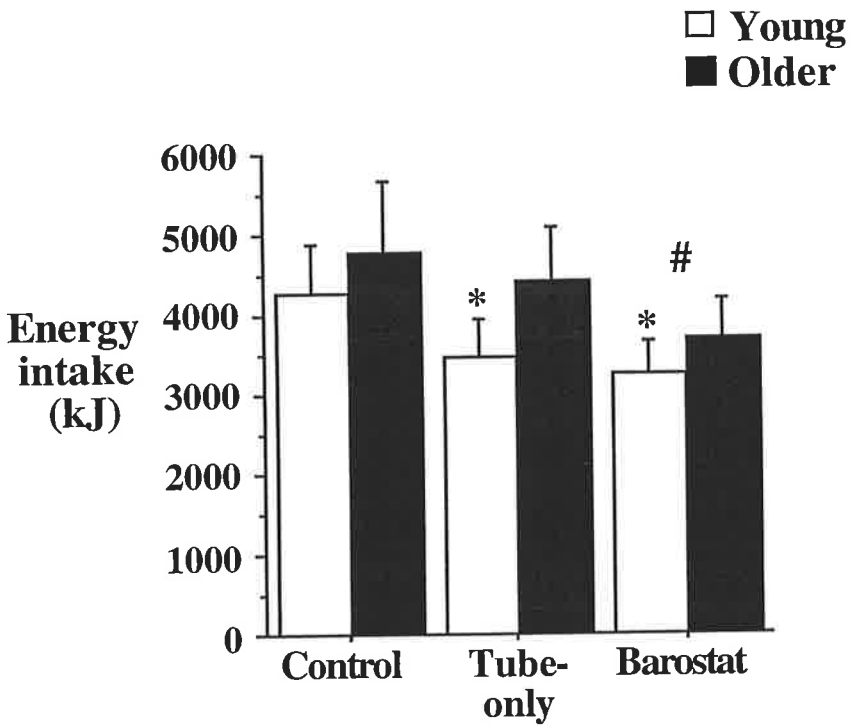

Figure 11.6: Energy content (kJ) of buffet meal consumed during the "'control day", "tube-only day" and "barostat day" in young and older subjects. Data are mean ± SEM. Two-way ANOVA; #P< 0.05 vs control in young and older subjects. One-way ANOVA; *P<0.05 vs control in young subjects.

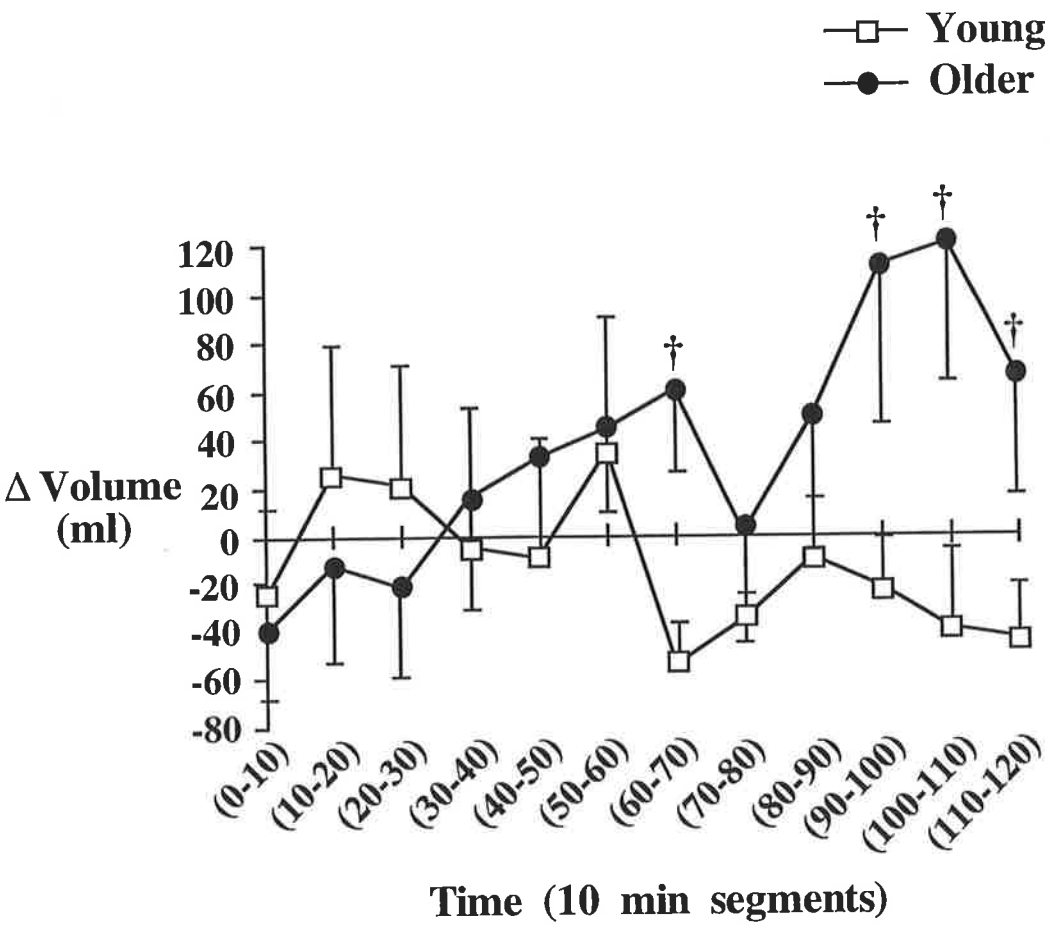

Figure 11.7: Change in intrabag volume following the meal on the "barostat day" in young and older subjects, expressed as the mean over 10 minute time segments. Data are mean ± SEM. Two-way ANOVA, P= 0.05, †P <0.05 young vs older.

11.5 DISCUSSION

Our observations indicate that healthy ageing is associated with decreased perception of gastric distension, without changes in fasting gastric compliance. In addition, the gastric accommodation response appears to be delayed in older subjects, with lower gastric tone late in the postprandial period compared to the young. Nasogastric intubation reduces food intake in young, but not older subjects.

This study is the first to evaluate the effect of ageing on proximal gastric function. We restricted our study to the healthy elderly; subjects with gastrointestinal disorders and those taking medication that may have altered gastric motility or appetite were excluded. Previous studies (see Chapters 8-10 and Appendix A) and others (Clarkston et al 1997, Rolls 1995, Wurtman et al 1988) (see Chapter 1.4) have established that healthy ageing is associated with a reduction in appetite and food intake, otherwise known as "the anorexia of ageing" (Morley 1996). The absence of any difference in food intake between the two groups in the current study is likely to reflect a type 2 error. The relatively small number of subjects in each group is a limitation of the study; nevertheless, the differences in perception between the groups were marked, with only modest intersubject variability within each group. Postprandial volume responses were more variable, and accordingly, interpretation of these data should be circumspect. The failure to demonstrate any difference in pressure-volume relationships, fasting tone, or the magnitude of the maximum postprandial volume change between the two groups potentially represents a type 2 error; however, the P values did not approach significance; if a difference exists it is likely to be small. All subjects had experienced nasogastric intubation prior to the study, but it was clearly not feasible for subjects to be blinded as to the nature of each study day, although the order of the two studies involving intubation was randomised. Only male subjects were studied to avoid the potential effects of hormonal changes at the menopause. While it should be recognised that there may be gender differences in the perception of gastrointestinal sensation (Nguyen et al 1995), our observations are likely also to apply to females.

The perception of gastric distension was markedly diminished in the older subjects compared to the young. This observation is consistent with previous studies indicating that ageing increases pain thresholds in response to balloon distension of both the rectum (Lagier et al 1999, Bannister et al 1987) and oesophagus (Lasch et al 1997, Weusten et al 1994) (see Chapter 4.4.1). As is the case with the rectum, these observations are not attributable to alterations in compliance, ie. a difference in gastric

wall tension at the same distending pressure or volume (Lagier et al 1999). Rather, our findings suggest an impairment in the function of gastric mechanoreceptors, afferent pathways, or central processing of visceral signals in older subjects (see Chapter 4.4.1). It is well established that other sensory modalities are affected by ageing - a decline in visual sensory pathways probably contributes to changes in visual function with age (Tobimatsu 1995), while reductions in taste and smell suggest a defect in olfactory sensory pathways (de Graaf et al 1994)(see Chapter 4.3).

While our observations appear to rule out hypersensitivity of the proximal stomach as a predisposing factor to the "anorexia of ageing" in the healthy elderly, it is possible that diminished sensation may predispose to reduced appetite. Taste and smell are known to influence the hedonic qualities of food, and impairment of them is associated with a loss of appetite (Schiffman & Warwick 1993) (see Chapter 4.3). In the same way, perceptions arising from gastric distension, such as fullness, may be considered pleasurable up to a point (Feinle et al 1998); impairment of these visceral sensations could remove a positive stimulus for eating. We also observed that the presence of a nasogastric tube inhibited food intake in young, but not older subjects. Our findings suggest that in the elderly, food intake is insensitive to gastrointestinal stimuli, a concept that is compatible with an impaired capacity to modify energy intake following periods of overeating or undereating in this group (Roberts et al 1994) (see Chapter 1.4).

During gastric distensions, the minimal distending pressure was higher in the older when compared to the young subjects. The minimal distending pressure represents the pressure required to overcome intra-abdominal pressure, and may have been higher in the older subjects because of increased intra-abdominal fat, even though the groups were matched for body mass index (Cefalu et al 1995, Klatt et al 1997). We observed no difference in either gastric compliance, or the fasting tone of the proximal stomach (assessed as the mean volume in the intragastric bag while the pressure was maintained at a constant value before the meal), between the older and younger subjects. Though based on a small number of subjects, our observations are consistent with those of Lagier et al (1999) who found no effect of healthy ageing on rectal compliance. Following the meal, which was not significantly different (grams or energy content) between the two groups, intrabag volume increased, indicative of proximal gastric relaxation. This response occurred in all subjects, even though it is masked in Figure 11.7 which shows only the mean volume change over 10 minute periods. The peak

volume of this "accommodation response" occurred later in the older subjects, and in the second hour after the meal proximal gastric tone was less in the older than in the young. These phenomena could result from, as well as contribute to, the slower gastric emptying previously noted in older compared to young subjects (Clarkston et al 1997, Horowitz et al 1984, Wegener et al 1988) (see Chapter 4.4.2); delayed emptying of nutrients into the small intestine may delay the enterogastric accommodation response, while lower proximal gastric tone late in the postprandial period may cause retention of food in this region, thus slowing gastric emptying. The result may be more sustained exposure of the small intestine to nutrients. Although food intake did not differ between the groups following a prolonged overnight fast, it would be of interest to examine whether intake was inhibited at a subsequent meal in the older subjects.

The isobaric distension stimulus at 2 mmHg above minimal distending pressure ("barostat day") had no effect on food intake in either the young or the older subjects compared to the "tube-only day". Somewhat greater distension stimuli appear to be required to inhibit food intake; in pigs, isobaric distension to a pressure of at least 11 mmHg is required to suppress intake (Lepionka et al 1997), while a distension of at least 400 ml in volume appears necessary in humans (Geliebter 1988). Thus our study design was not optimal to examine the relative effects of gastric distension on food intake, but it appears unlikely that intake in the older subjects would be impaired by distension more than in the young, on the basis of our observations during fasting distensions.

CHAPTER 12

Effect of Intravenous Infusion of Naloxone on Appetite in the Healthy Elderly

12.1 SUMMARY

Ageing is associated with a reduction in appetite and food intake, the so-called "anorexia of ageing". Endogenous opioid activity stimulates eating behaviour, and there

is evidence from studies in animals that a reduced stimulation of feeding by endogenous opioids may be important in the pathogenesis of the "anorexia of ageing".

To determine if this is the case in humans, 12 older (5M/7F)(65-84 yr) and 12 young (5M/7F)(20-26 yr) healthy subjects received, in double-blinded random order, intravenous (iv) bolus (10 min) and then continuous (140 min) infusions of saline (C), naloxone low dose (LD)(bolus 27 μg/kg; continuous 50 μg/kg/hr) or naloxone high dose (HD)(bolus 54.5 μg/kg; continuous 100 μg/kg/hr). After 120 min, each subject was offered a buffet meal and energy intake quantified. Hunger, fullness, nausea and drowsiness were assessed using visual analogue scales.

The naloxone low dose and high dose infusions had no significant effect on ratings of hunger, fullness or nausea, but increased drowsiness ($P< 0.01$) compared to the control infusion in both age groups. Older subjects ate less ($P< 0.001$) at the buffet meal than young subjects during all three infusions. Naloxone infusions reduced energy intake compared to control ($P< 0.001$), low dose by $13.2 \pm 5.0\%$ and high dose by $10.7 \pm 5.0\%$, with no difference between the doses ($P= 0.71$). Overall, naloxone suppressed energy intake in both young and older subjects ($P< 0.01$). This suppression was slightly, but not significantly, greater in the young than older subjects (mean of LD and HD $16.4 \pm 4.9\%$ vs $7.5 \pm 4.9\%$, $P= 0.42$), due to a trend to reduced suppression in older women.

We conclude that healthy older adults retain their sensitivity to the suppressive effects of naloxone on food intake. Possible gender differences in this sensitivity warrants further investigation. A decline in opioid activity is unlikely to contribute substantially to the physiological "anorexia of ageing" observed in older people.

12.2 INTRODUCTION

There is persuasive evidence that the endogenous opioid system plays a role in modulating feeding and food choice in both animals and humans (see Chapter 2.3.1).

Ageing is associated with a reduction in opioid activity and some opioid-mediated responses (see Chapter 4.2.1). The effect of ageing on opioid-mediated feeding has, however, not been studied in humans.

The aims of this study were to determine whether the effects of intravenous administration of naloxone on appetite and food intake are altered by healthy ageing. The broad hypotheses addressed were that 1) intravenous naloxone infusion would suppress food intake in both young and older subjects and 2) the suppression of appetite and food intake by naloxone would be less in older than young adults.

12.3 SUBJECTS AND METHODS

Twelve healthy older subjects, mean age, 72 yr (range; 65-84 yr) and 12 healthy young subjects, mean age, 23 yr (range: 20-26 yr), 5 men and 7 women in each age group, were recruited by advertisement. Older subjects were selected so that their body mass index (BMI; in kg/m^2) was matched (within 1 kg/m^2) to that of one of the younger subjects; accordingly, the BMI's of the two age groups were not significantly different [25.0 ± 0.5 kg/m^2 (range: 21.7-27.2 kg/m^2) in the older subjects compared with 24.7 ± 0.7 kg/m^2 (range: 20.5-27.9 kg/m^2) in the young subjects; P= 0.77]. Percentage body fat was assessed by bioelectrical impedance (Bodystat 1500, Bodystat Ltd, Isle of Man) in all subjects. Energy intake was > 4182 kJ/day as assessed by a food diary kept for three successive days before the first study day (see Chapter 6.2.2). All subjects were unrestrained eaters (score < 10 for Factor 1 (cognitive restraint) on the Three-Factor Eating Restraint Questionnaire (Stunkard & Messick 1985) (see Chapter 6.4.1), non-smokers, and none suffered from serious illness, had a history of gastrointestinal disease or gastrointestinal surgery, or was any taking medication known to influence appetite. Two of the older women were taking oestrogen replacement therapy. The study was approved by the Human Ethics Committee of the Royal Adelaide Hospital and each subject gave written informed consent.

12.3.1 *Experimental Protocol*

Each subject underwent three studies on separate days, in randomised order, and double-blind fashion. The experimental protocol is summarised in Figure 12.1. On each of the study days, subjects arrived at the laboratory at 8:30 AM after a 10-hr overnight fast. On arrival, an intravenous cannula was placed in a left antecubital vein for infusion of the treatment solution. Approximately 30 min later, at t= 0 min, an intravenous isotonic (0.9%) saline infusion (30 ml/hr) was commenced and continued for 150 min. At 30 min, an intravenous treatment infusion of 0.9% saline (control), low dose naloxone (LD) or high dose naloxone (HD), was commenced. All infusions

were given as an initial bolus of 0.81 ml/kg body weight/hr for 10 min, followed by a continuous infusion at 0.25 ml/kg body weight/hr for 140 min. Naloxone (Boots Inc., North Rock, NSW) stock solution (400 μg/l 0.9% saline) was diluted in 0.9% saline to provide LD [27 μg/kg bolus, 50 μg/kg/hr continuous (total 144 μg/kg)] and HD [54.5 μg/kg bolus, 100 μg/kg/hr continuous (total 288 μg/kg)] infusions. At 120 min, subjects were offered a standardised, buffet meal including a range of palatable high fat, high carbohydrate, foods prepared in excess of what they would normally eat, and told that they had 60 min in which to eat as much as they wanted and could ask for up to one extra serving of any particular food if they wished. The total amount of food consumed was quantified (see Chapter 6.3.3). Following the meal, the subjects remained in the laboratory for a further 30 minutes. At -10, 0, 30, 40, 60, 80, 100, 120, 150, 180 and 210 min, visual analogue scales were used to measure subjective ratings of appetite (see Chapter 6.3.1). Subjects were told that the specific purpose of the study was to assess the effects of naloxone on palatability of foods and were not informed that the assessment of energy intake was an aim of the study.

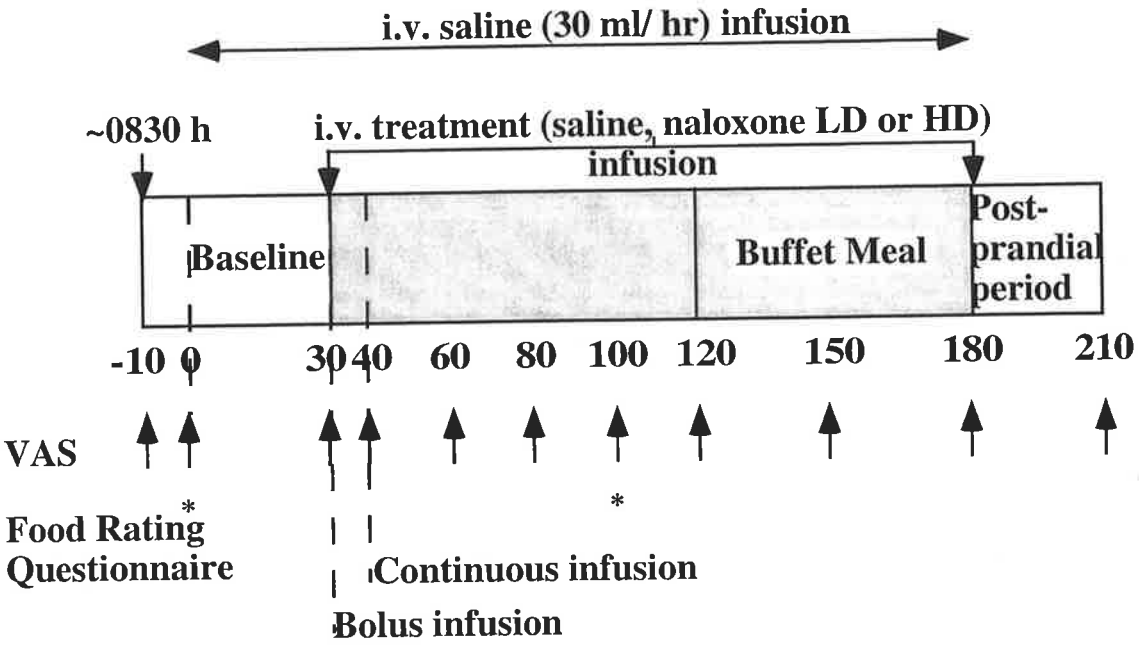

Figure 12.1: Summary of the experimental protocol. Following a 30 min baseline period, each subject received in random order intravenous (iv) infusions of 0.9% saline (control)(C), naloxone low dose (LD) (bolus 27 μg/kg over 10 min; then continuous 50 μg/kg/hr for 140 min) or naloxone high dose (HD) (bolus 54.5 μg/kg over 10 min, then continuous 100 μg/kg/hr for 140 min), on separate days. At 120 min, a buffet meal was offered and energy intake quantified. The arrows indicate the timing of administration of visual analogue scales to assess subjective ratings of hunger, fullness, drowsiness and nausea.

12.3.2 *Assessment of appetite*

Appetite was assessed using 10 cm linear visual analogue scales (see Chapter 6.3.1, Appendix BI.2). Absolute ratings of hunger, fullness, drowsiness and nausea at baseline (mean of 0 and 30 min) and during intravenous infusions (40-120 min) were quantified.

12.3.4 *Energy intake*

The total amount (g) of food consumed during the meal (food provided was 375 ml flavoured milk; 300 ml unsweetened orange juice; 350 ml of spring water; 4 slices of wholemeal bread; 4 slices of white bread; 4 slices of ham; 4 slices of processed chicken; 4 slices of cheddar cheese; 8 slices of tomato and cucumber; 2 sachets of margarine and mayonnaise; 200 g chocolate custard; 6 shortbread biscuits; 50 g plain potato crisps; 6 snack size milk chocolate; 3 cheddar cheese and cracker portions and 70 g jelly beans) was calculated. Both energy intake (kJ) and macronutrient composition (% protein, % fat and % carbohydrate) of the buffet meal were determined (see Chapter 6.3.3).

12.3.5 *Palatability*

At 0 and 90 min subjects were asked to fill out a questionnaire, ranking in order of preference, nine items from the buffet meal, with 1 being the most palatable and 9 the least. The scores at 90 min for individual food items were subtracted from the 0 min scores to derive a change in preference score for each food item during each of the treatment infusions. Differences in these scores between the three study days and between age groups were determined (see Chapter 6.3.2 and Appendix BIII).

12.3.6 *Statistical Analysis*

Results are given as mean ± SEM. Comparisons between the young and older groups in the energy and macronutrient content of the previous diet, restraint score, BMI, and % body fat were performed with Students' unpaired *t* test using Statview (version 5.0; Abacus Concepts Inc., California), because these data were normally distributed. Baseline scores for hunger, fullness, drowsiness and nausea were analysed by repeated-measures three-way analysis of variance (ANOVA) with age, gender, and treatment as the factors. The effects of the intravenous infusions of saline, naloxone low dose and high dose on perceptions (ie, hunger, fullness, drowsiness and nausea) were analysed by repeated-measures four-way ANOVA with age, gender, treatment and time as the factors. When a significant interaction between factors was observed,

contrasts were used to test preplanned hypotheses of interest, enabling paired comparisons between the control and naloxone infusions. Differences in the mean preference scores for each food item according to the ranking questionnaire and mean energy intakes at the buffet meal during the intravenous infusions were analysed by repeated-measures two-way ANOVA. ANOVA's were performed using SuperANOVA (version 1.11; Abacus Concepts Inc., California). A P value < 0.05 was considered significant in all analyses.

12.4 RESULTS

The study protocol was well tolerated by all subjects. Older subjects had a higher percentage of body fat than young subjects (33.4 ± 2.6 % vs 20.9 ± 2.5 %; P< 0.01). As assessed by 3 day food diaries, energy intake from the usual diet was approximately 30% less in the older than young (8295 ± 634 kJ/day vs 11442 ± 1253 kJ/day, P< 0.05) subjects. There was no difference between the older and young subjects in the proportion of intake as carbohydrate (44.8 ± 1.4 % vs 46.3± 3.0 %; P= 0.66), fat (34.2 ± 1.7 % vs 32.8 ± 2.5 %; P= 0.65) or protein (18.6 ± 1.1 % vs 16.0 ± 0.84 %; P = 0.07). There was no significant difference in eating restraint scores between the older and young subjects (5.3 ± 0.7 vs 4.5 ± 0.8; P= 0.46).

12.4.1 *Appetite*

Absolute ratings of hunger, fullness, and drowsiness at baseline (mean of 0 and 30 min) and during the saline (C) and naloxone (LD) and (HD) infusions, before the buffet meal (40-120 min), are represented in Figures 12.2, 12.3 and 12.4. There were no significant treatment × age, treatment × time or treatment × time × age interactions for any sensations during the infusions in either age group.

12.4.1.1 Baseline appetite ratings

Baseline ratings (mean of 0 and 30 min) of hunger tended to be less (5.5 ± 0.4 cm vs 7.0 ± 0.4 cm; P= 0.08) and ratings of fullness greater (3.3 ± 0.4 cm vs 2.3 ± 0.2 cm; P= 0.11) in older than young subjects. There was no difference in baseline ratings of drowsiness (3.5 ± 0.4 cm vs 3.6 ± 0.4 cm, P= 0.90) or nausea (1.0 ± 0.3 cm vs 1.7 ± 0.4 cm, P= 0.34) between older and young subjects. There was no difference in baseline ratings of hunger (P = 0.12), fullness (P= 0.20), drowsiness (P= 0.62) or nausea (P= 0.32) between the three study days.

12.4.1.2 Hunger

There was no effect of age (P= 0.61), or treatment (P= 0.37) on ratings of hunger during the intravenous infusions in older and young subjects (Figure 12.2). Hunger ratings increased with time (P< 0.001) in both age groups, and the increase in hunger was greater in the older than young subjects (time × age interaction; P< 0.05).

12.4.1.3 Fullness

There was no effect of age (P= 0.10) or treatment (P= 0.14) on ratings of fullness during the intravenous infusions in older and young subjects (Figure 12.3). Fullness ratings decreased with time (P< 0.05) in both age groups but there was no significant time × age interaction (P= 0.52).

12.4.1.4 Drowsiness

There was no effect of age (P= 0.32) on ratings of drowsiness during the intravenous infusions in young and older subjects (Figure 12.4). There was a significant effect of treatment on drowsiness ratings (P= 0.05) in both age groups; drowsiness was greater during the naloxone (LD) (P= 0.05) and (HD) (P= 0.05) infusions compared to the control infusion. There was a significant effect of time (P= 0.03) on drowsiness, but no significant time × age interaction (P= 0.07).

12.4.1.5 Nausea

There was no effect of age (P= 0.39), treatment (P= 0.49) or time (P= 0.40), nor any time × age (P= 0.78) interactions on ratings of nausea (data not shown).

12.4.2 *Energy intake*

The effects of naloxone on energy intake at the buffet meal in the different age groups are shown in Figure 12.5a. There was an effect of age on energy intake (P< 0.01), with older subjects eating approximately 35% less than young subjects. There was a significant effect of treatment on energy intake (P< 0.01); naloxone low dose and high dose infusions were associated with a 14.4% and 17.6% suppression of energy intake, respectively, compared to the control infusion. The suppressive effect of naloxone was, however, not dose-dependent; suppression of energy intake was not significantly different between the low- and high-dose naloxone infusions (P= 0.38) in either age group. There was no interaction of treatment × age (P= 0.42), indicating that the suppression of energy intake by naloxone was not significantly different in older compared to young subjects. There was no significant relationship between the amount

eaten at the buffet meal during naloxone infusions and the extent of suppression by naloxone (R= 0.003; P= 0.99). When intake during the naloxone low dose and high dose infusions was expressed as a percentage of the control day (Figure 12.5b) there was no effect of age (P= 0.26) or treatment (P= 0.71), nor was there a significant treatment × age interaction (P= 0.63).

By 3 way-ANOVA there was an effect of gender (P< 0.001) on mean energy intake, with women eating approximately 33% less than men (see Figure 12.6a and b for mean energy intake and % suppression, respectively, in the older and young men and women). There was a significant age × gender interaction (P< 0.01) in that the suppressive effect of ageing (see above) on energy intake was greater in men than women; energy intake in the older men was 46% less than in young men, whereas intake in older women was 23% less than in young women. There were no significant treatment × age (P= 0.47), treatment × gender (P= 0.11) or treatment × age × gender (P= 0.41) interactions for mean energy intake (Figure 12.6a). There were no significant effects of gender or any interactions between age, treatment and gender on the percentage suppression of energy intake during the low dose and high dose naloxone infusions compared to the control infusion (Figure 12.6b).

10.4.3 *Macronutrient content*

Older subjects ate less carbohydrate (P< 0.01) and more fat (P< 0.05) as a percentage of the total energy intake at the buffet meal during both the naloxone and control infusions when compared to the young subjects (Table 12.1), with no difference in protein intake between the two groups (P= 0.09). There was no effect of treatment on the percentage of carbohydrate (P= 0.60), fat (P= 0.65) or protein (P= 0.82) consumed at the buffet meal. There were no significant treatment × age interactions for any macronutrients.

Table 12.1: Macronutrient content of buffet meal [% protein, % Fat, and % carbohydrate (CHO)] during intravenous infusions of control, and naloxone low dose (LD) and high dose (HD) in young and older subjects.

	Treatment	Young	Older
% Protein	Control	14.1 ± 0.8	15.3 ± 1.1
	Naloxone LD	13.2 ± 0.5	15.7 ± 1.0
	Naloxone HD	14.2 ± 0.7	15.3 ± 0.8
% Fat	Control	38.5 ± 1.2	43.4 ± 2.1**
	Naloxone LD	36.7 ± 2.2	43.1 ± 1.5**
	Naloxone HD	38.5 ± 1.3	41.7 ± 1.5**
% Carbohydrate	Control	47.7 ± 0.9	41.3 ± 1.7*
	Naloxone LD	50.0 ± 2.4	41.3 ± 1.0*
	Naloxone HD	47.3 ± 1.3	43.2 ± 1.5*

Data are mean ± SEM. *P<0.001 older < young; **P<0.05 older > young.

10.4.4 *Palatability*

There was no significant difference in the preference scores for any of the food items (data not shown) during the three treatment infusions between the older and young subjects except for the jelly beans (P= 0.49); young subjects preference for jelly beans was less than the older subjects. For example, orange juice was rated as the most palatable and jelly beans the least palatable by both age groups. There was a significant effect of treatment on preference for 2 of the 9 foods. The preference for crackers increased during the naloxone (0.17 ± 0.20) compared to the control (-0.79 ± 0.34)(P< 0.01) infusion, whereas the preference for potato chips decreased during the naloxone

(0.19 ± 0.22) compared to control (0.63 ± 0.23)($P < 0.05$) infusions. There were no significant treatment × age interactions, indicating that these changes in palatability occurred in both age groups regardless of gender.

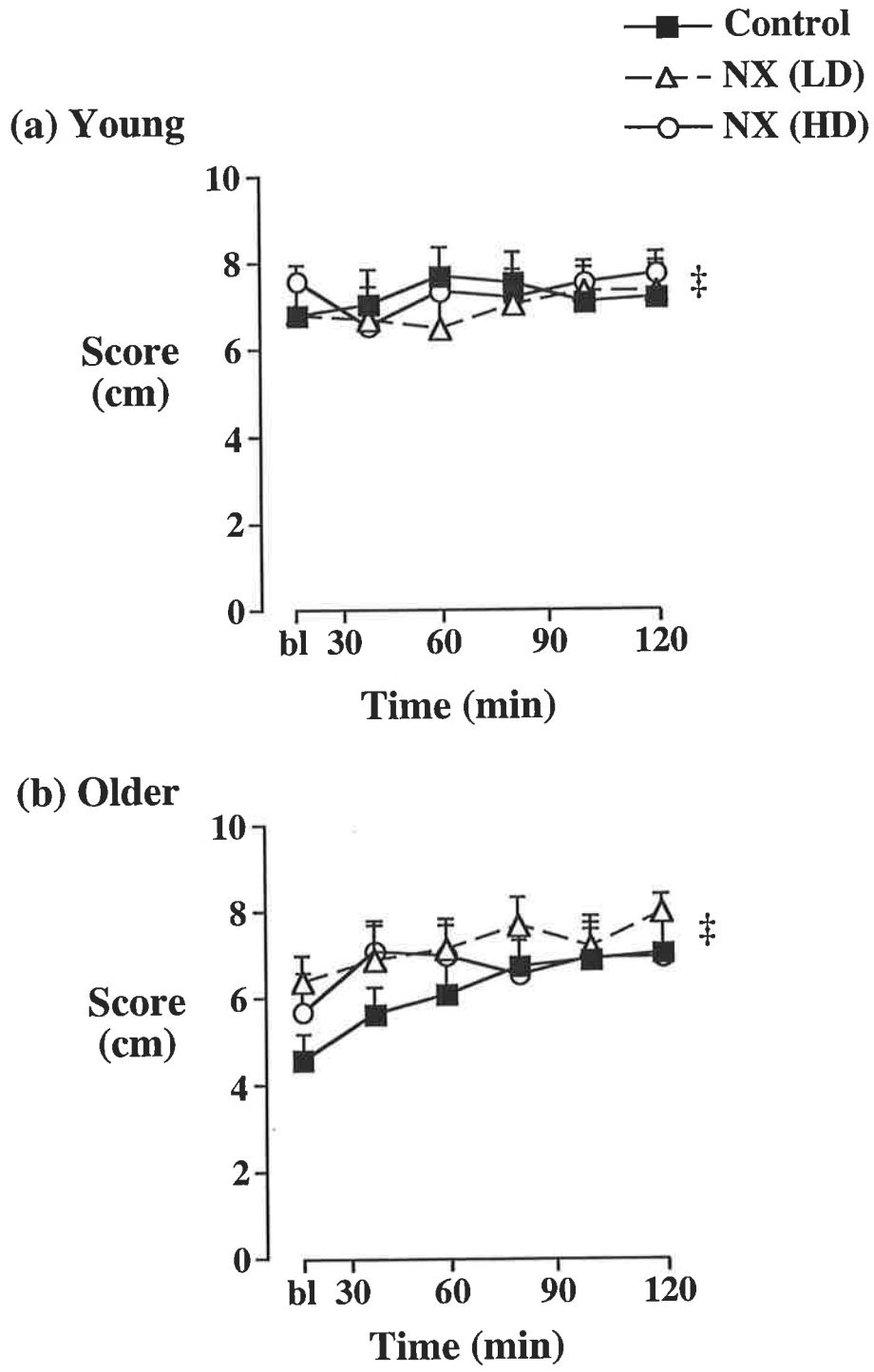

Figure 12.2: Absolute ratings of hunger at baseline (bl) and during intravenous infusion of either saline (control), Naloxone (NX) low-dose (LD), or NX high-dose (HD) infusion in 12 young (a) and 12 older (b) healthy subjects. Data are mean ± SEM. Three-way ANOVA; Effect of time; ‡ $P< 0.001$ hunger increased with time. Time × age interaction $P< 0.05$, the increase in hunger was greater in older than young subjects.

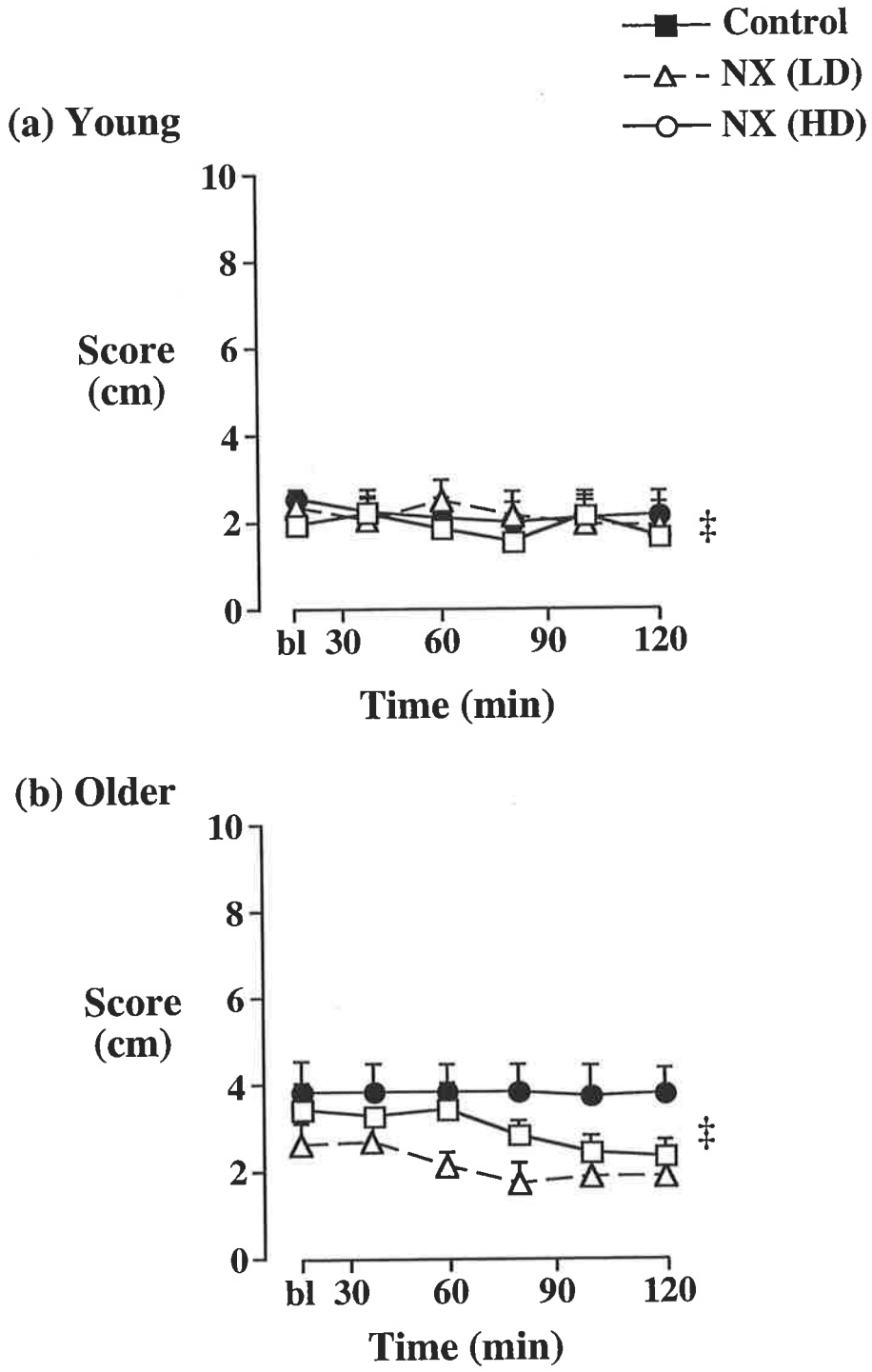

Figure 12.3: Absolute ratings of fullness at baseline (bl) and during intravenous infusion of either saline (control), Naloxone (NX) low-dose (LD), or NX high-dose (HD) infusion in 12 young (a) and 12 older (b) healthy subjects. Data are mean ± SEM. Three-way ANOVA; Effect of time ‡P<0.05, fullness decreased with time.

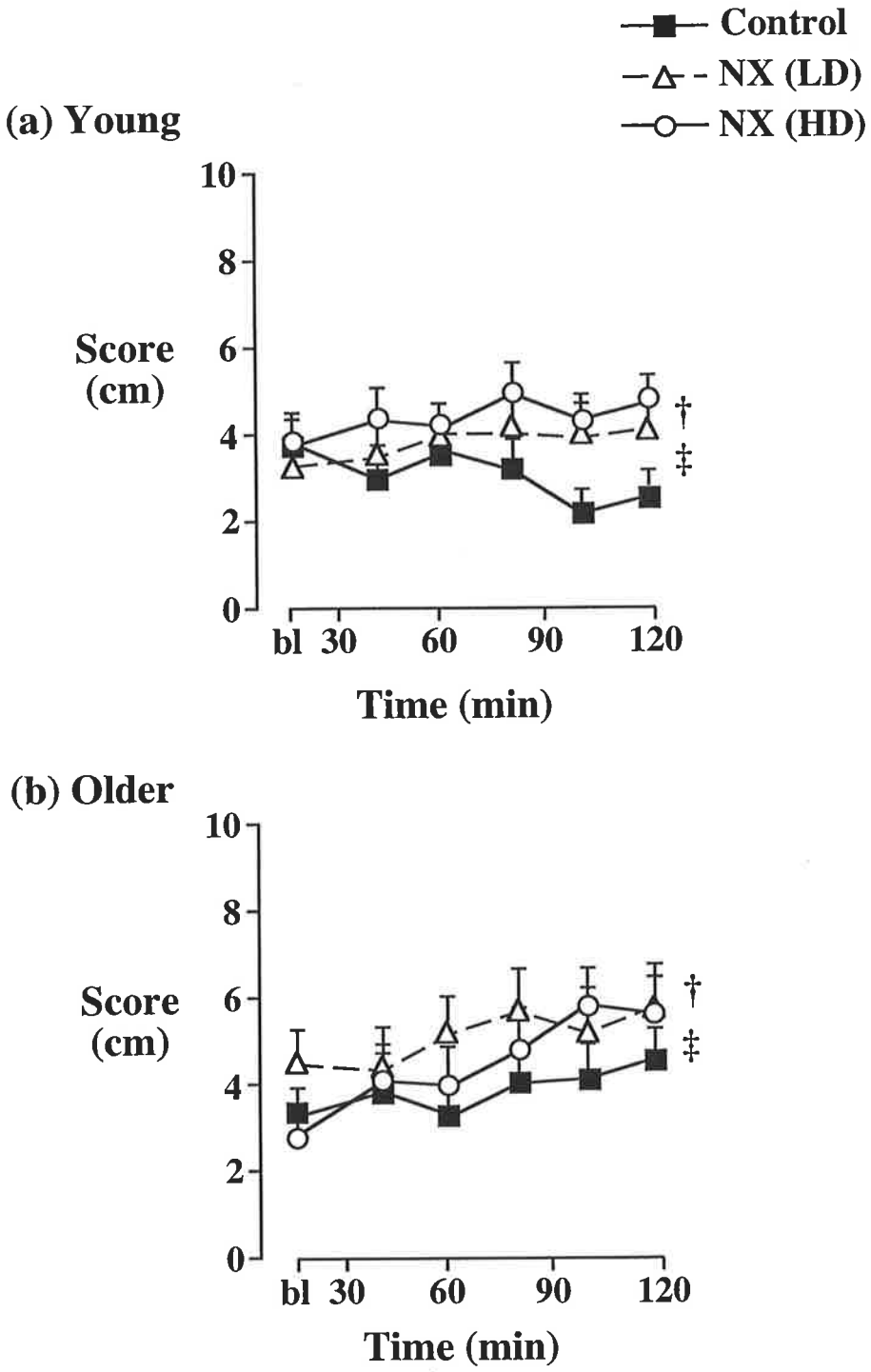

Figure 12.4: Absolute ratings of drowsiness at baseline (bl) and during intravenous infusion of either saline (control), Naloxone (NX) low-dose (LD), or NX high-dose (HD) infusion in 12 young (a) and 12 older (b) healthy subjects. Data are mean ± SEM. Three-way ANOVA; Effect of treatment; †P< 0.05, drowsiness increases with naloxone. Effect of time; ‡P<0.05 drowsiness increases with time.

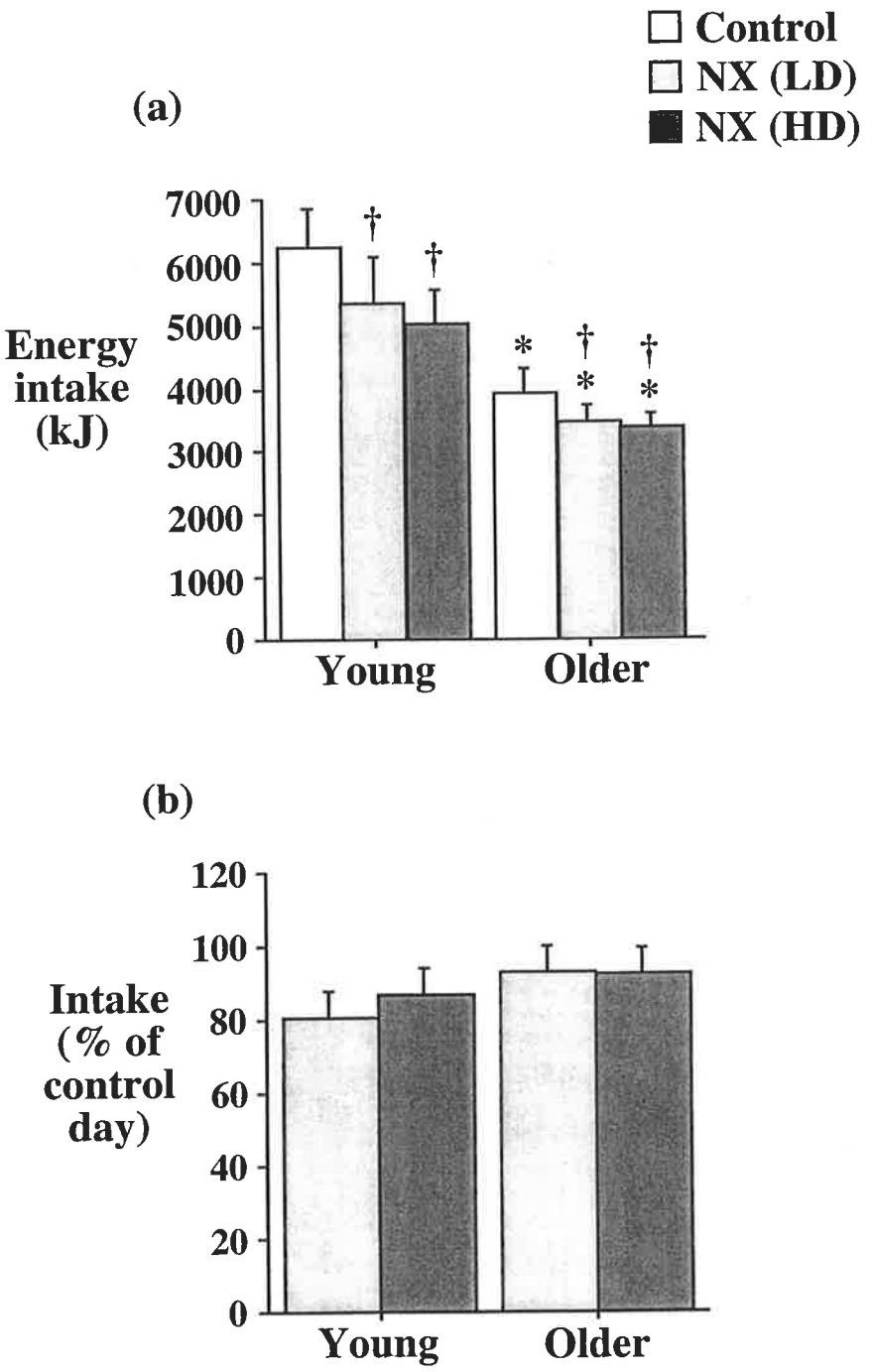

Figure 12.5: (a) Energy intake (kJ) at buffet meal during intravenous (iv) infusions of saline (control), Naloxone (NX) low-dose (LD) and NX high-dose (HD) in young and older subjects. (b) Energy intake expressed as the percentage of the control day during the iv NX (LD) and NX (HD) infusions in young and older subjects. Data are mean ± SEM. Two-way ANOVA; Effect of age, *P<0.001 vs young; Effect of treatment, †P<0.001 vs control.

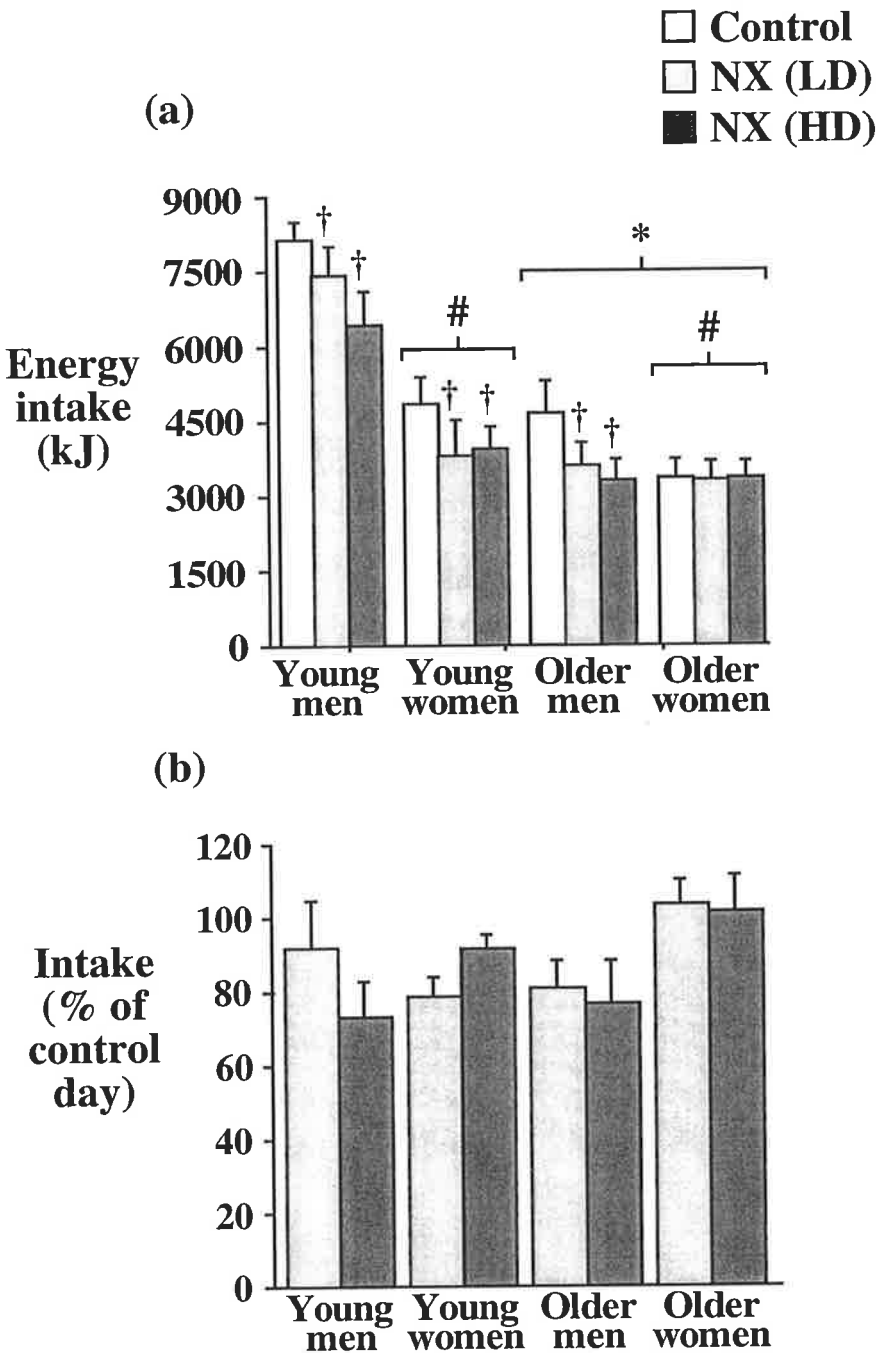

Figure 12.6: (a) Energy intake (kJ) at buffet meal during intravenous (iv) infusions of saline (control), Naloxone (NX) low-dose (LD) and NX high-dose (HD) in young and older men and women. (b) Energy intake expressed as the percentage of the control day during the iv NX (LD) and NX (HD) infusions in young and older men and women. Data are mean ± SEM. P< 0.01 Age × gender interaction. Three-way ANOVA; Effect of age, *P<0.001 vs young; Effect of treatment, †P<0.001 vs control; Effect of gender #P<0.001 vs males; P<0.01 age × gender interation for mean energy intake.

12.5 DISCUSSION

This study is the first to examine the effects of naloxone on appetite and food intake in healthy older subjects and to compare them to those in young adult subjects. The major findings were that: (i) mean energy intake from the buffet meal was reduced during both the low dose and high dose naloxone infusions compared to the control infusion, with no difference between the two doses (iii) naloxone suppressed food intake in both young and older subjects, with no significant difference between the age groups and (iv) naloxone increased drowsiness, but had no effect on perceptions of hunger, fullness or nausea in either age group.

Healthy ageing is associated with a decrease in appetite and food intake which may predispose to severe weight-loss and malnutrition. Our finding that healthy elderly subjects eat less on average in their usual diet than healthy young adults is consistent with previous observations by ourselves (see Chapters 8-10) and others (see Chapter 1.4) and is compatible with the concept of a physiological 'anorexia of ageing'.

Studies using both opioid agonists and antagonists have established a role for endogenous opioids in the regulation of energy intake (see Chapter 2.3.1).

Evidence from studies in rodents suggests that ageing is associated with a decline in opioid-mediated responses (see Chapter 4.2.1). Furthermore, Silver et al (1991) reported that older humans are less sensitive to the suppressive effects of naloxone on fluid intake, following overnight water deprivation than young subjects. We therefore hypothesised that the suppressive effects of naloxone on feeding would be reduced in older people.

The doses of naloxone used in this study were based on those shown to suppress food intake in previous studies (Cohen et al 1985, Trenchard & Silverstone 1983, Mitchell et al 1986) (see Chapter 2.3.1). Total doses of 1.6 mg (Trenchard & Silverstone 1983), 18 mg (Mitchell et al 1986), and ~150 mg (2 mg/kg) (Cohen et al 1985) have been associated with reduced food intake in young adults, although in another study (Atkinson 1982), 15 mg did not suppress food intake in young, lean subjects. The total dose of naloxone administered during low and high dose infusions, in our study, was approximately 11 and 22 mg respectively (75 kg subject). We used two doses of naloxone, since we hypothesised that the older subjects would be less sensitive than the young and, therefore, the low dose might not have an effect on energy intake in the

older subjects. The finding of similar suppressive effects on energy intake by both low and high dose naloxone in both age groups suggests, however, that there may be a threshold dose of naloxone which antagonises endogenous opioid effects on feeding, such that above this there is little, if any, additional suppressive effect on energy intake.

Naloxone administration was associated with a statistically significant reduction in food intake of approximately 12% compared to control in this study. This finding in a group of twenty four young and older subjects is consistent with previous studies in which peripheral naloxone administration reduced short-term food intake by 10-30% (see Chapter 2.3.1). The extent of the suppression of food intake by naloxone was not statistically different between older (7.5 ± 4.9 %) and young (16.4 ± 4.9%) subjects, with P values of 0.42 and 0.62 for the interactive effect of treatment and age on absolute energy intake and energy intake relative to the control day, respectively. This observation argues against the concept that the suppressive effect of naloxone on food intake is affected by ageing as suggested by results of studies in rats (Gosnell et al 1983) and mice (Kavaliers & Hirst 1995), in which older animals were less sensitive than young animals to the suppression of feeding by naloxone (see Chapter 4.2.1). It should, however, be recognised that a Type 2 statistical error cannot be excluded. Our power calculations suggest that we would have had to study an additional 80 subjects for the observed difference, if maintained, to be statistically significant. The possible reduced suppression of energy intake in the older subjects may also reflect a 'floor effect', that is there may be a certain energy intake at which naloxone does not suppress intake any further. If present, in our study this 'floor' was apparently an intake of about 3,300 kJ at the buffet meal (Figure 12.7b). At present it seems reasonable to conclude that any effect of ageing on the response to naloxone is small and unlikely to be of biologically significance; certainly far less than the difference in energy intake at baseline between young and older subjects.

Naloxone administration (low and high dose) reduced energy intake by a mean of 14.9% (8 and 21%), 17.5% (27 and 8%) and 21.4% (19 and 23%) in young men, young women and older men, respectively, whereas naloxone (low and high dose) increased energy intake by a mean of 2.5% (1 and 3%) in the older women. Although this reduction of suppression in the older women was not statistically significant (ie age × treatment × gender, P= 0.31). This tendency for a reduced suppression of energy intake in older women, suggests that the age-related changes in the opioid modulation of feeding may be gender-specific. There is some evidence from animal studies to

support this concept. Messing et al (1980) have shown that the declines in mu-opioid binding affinity in the thalamus, midbrain and cortex with ageing are greater in female than male rats. The capacity of naloxone to suppress food intake in rats, reportedly declines with increasing age in ovariectomised, but not sham operated, female animals (Islam et al 1993). The latter observation suggests that oestrogen deficiency may have been responsible for the reduction in the sensitivity to naloxone in older female animals. It could be speculated that oestrogen deficiency may contribute to the reduced sensitivity to naloxone in the older women in this study. Indeed, only two of the seven older women in this study were taking oestrogen replacement therapy and although one of these had an increase in food intake during naloxone infusion, the other had the greatest suppression (18% compared to control) of any of the older women. There is abundant evidence for interactions between gonadal steroids and endogenous opioids in the control of other factors including thermoregulation, pain perception, thirst, and reproductive (Wade & Gray 1979, Wade 1976). While ovariectomy decreases endogenous dynorphin levels in the cortex in rats (Morley et al 1984), Morley et al (1984) also report that ovariectomised female rats are more sensitive to the inhibitory effects of naloxone on food intake than oestradiol-treated ovariectomised rats. Further studies in humans are required to examine the possible gender-specific changes in opioid modulation of feeding with age.

Consistent with previous reports in both normal and obese young adult subjects (see Chapter 2.3.1), hunger and fullness ratings were not affected by naloxone in either age group, supporting the concept that naloxone decreases food intake without affecting subjective symptoms of appetite. The suppressive effect of naloxone on food intake was unlikely to be a result of side effects. Cohen et al (1985) reported nausea, weakness and stomach ache following a bolus iv. dose of naloxone of 2 mg/kg, whereas other studies using lower doses reported no significant adverse effects (see Chapter 2.3.1). We used a dose of naloxone (high dose) which was less than one sixth of the total dose used by Cohen et al (1985), and infused it over 150 min rather than as a bolus. Perhaps as a consequence this naloxone dose did not cause nausea or abdominal discomfort. There was, however, a significant increase in drowsiness during the naloxone low dose and high dose infusions in both age groups, consistent with the study of Kyriakides et al (1980), who reported an increase in drowsiness in subjects with Prader-Willi Syndrome following subcutaneous administration of naloxone in single doses of 0.8 and 1.6 mg/kg. While statistically significant, the mean increase (ie. 1.5 cm on the 10 cm scale) during the naloxone infusion compared to

control infusion, is unlikely to be of biological significance, since drowsiness was not evident to the blinded observer during any treatment infusion, and no subject fell asleep or complained of tiredness or drowsiness.

Opioid peptides have also been implicated in the modulation of food choice and the hedonic responses involved in food intake (see Chapter 2.3.1). Previous reports examining the effects of opioid antagonists on food choice in humans have been inconsistent (see Chapter 2.3.1). Yeomans & Gray (1997) found that oral naltrexone (50 mg) reduced food intake, predominantly by reducing the pleasantness of the taste of the food offered. Drewnowski et al (1995) reported that preferences for high fat/high sugar foods were decreased during naloxone infusion in both binge-eaters and non binge-eaters, but the carbohydrate, protein and fat content of food eaten during the test meal was reduced only in the binge-eaters. In contrast, Cohen et al (1985) reported that naloxone reduced intake of foods high in fat and protein, but not carbohydrate, in young subjects. In that study (Cohen et al 1985), the intake of each macronutrient was, however, expressed in grams, not as a percentage of total energy intake. This did not account for the suppression of energy intake by naloxone and the suppressive effect of naloxone on each macronutrient may have accordingly been over-estimated. In our study, we assessed the macronutrient content as a percentage of the total energy intake and found that while older subjects ate a higher percentage of fat than young subjects, there was no significant effect of naloxone on any of the macronutrients consumed. In regard to individuals food preferences, we found that naloxone administration was associated with an increased preference for crackers, a food low in fat, and decreased preference for potato chips, a food high in fat, in both age groups. This may suggest some suppression of the preference for high fat foods by naloxone. These results contrast with those of Arbisi et al (1999) who reported that oral naltrexone (50 mg) decreased the liking of sweet, but not salty, bitter or sour tastes in healthy women, suggesting that the effect of opioid antagonism on food preference may be dependent on the type of antagonist administered. Overall, our results do not suggest an important role for naloxone in modulating the intake of and preference for particular macronutrients.

In summary, intravenous naloxone suppressed short term food intake similarly in healthy older and young subjects. This suggests that human ageing is not associated with a significantly reduced endogenous opioid feeding drive. Other factors seem likely to play a more important role in the physiological anorexia of ageing.

CHAPTER 13

Effects of Glucose Supplementation on Gastric Emptying, Postprandial Blood Glucose Homeostasis and Appetite in the Healthy Elderly

13.1 SUMMARY

The aims of this study were to evaluate the effects of dietary glucose supplementation on gastric emptying (GE) of both glucose and fat, postprandial blood glucose homeostasis and appetite in 8 older subjects (4M, 4F, age 65-84 yr).

GE of a drink (15 ml olive oil and 33g glucose dissolved in 185 ml water), blood glucose, insulin, glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) and appetite (diet diaries, visual analog scales and food intake at a buffet meal consumed following the GE study) were evaluated twice; after 10 days on a standard or a glucose-supplemented diet (70g glucose three times a day).

Glucose supplementation accelerated GE of glucose ($P< 0.05$), but not oil; there was a trend for an increase in GIP ($P= 0.06$), no change in GLP-1, an earlier insulin peak ($P< 0.01$) and a subsequent reduction in blood glucose ($P< 0.01$). Glucose supplementation had no effect on food intake during each diet so that energy intake was greater ($P< 0.001$) during the glucose supplemented diet. Appetite ratings and energy intake at the buffet meal were not different.

We conclude, that in older subjects glucose supplementation (i) accelerates GE of glucose, but not fat, (ii) modifies postprandial blood glucose homeostasis and (iii) increases energy intake.

13.2 INTRODUCTION

In young subjects, gastric emptying is modified by recent nutrient intake, presumably as a result of changes in the magnitude of small intestinal feedback (see Chapter 3.5.2.1). Ageing is associated with a slight but significant slowing of gastric emptying (see Chapter 4.4.2). The effects of dietary glucose supplementation on gastric emptying in older subjects have not been evaluated. This is an important issue to explore as any acceleration of gastric emptying of nutrients may potentially favour an increase in food intake.

The more rapid gastric emptying of glucose induced by dietary glucose supplementation in young adults, is also associated with significant changes in the glycaemic response to oral glucose; ie the increased insulin response to oral glucose as a result of dietary

glucose supplementation in young subjects (Horowitz et al 1996), may be attributable to higher levels of glucose-dependent insulinotrophic polypeptide (GIP), but plasma concentrations of the other important incretin hormone, glucagon-like peptide-1 (GLP-1) (Nauck 1999) have not been evaluated (see Chapter 3.5.2.3). Ageing is associated with an increased prevalence of glucose intolerance and diabetes mellitus, and an increased GIP and GLP-1 response to carbohydrate (Ranganath et al 1998) (see Chapter 4.5.2).

The aims of this study were to determine in healthy older subjects the effects of dietary glucose supplementation on: (i) gastric emptying of both glucose and fat, (ii) blood glucose and plasma insulin, GIP and GLP-1 concentrations and (iii) food intake.

13.3 SUBJECTS AND METHODS

Eight healthy, older subjects (4 male, 4 female) mean age 70.6 ± 2 yrs (range 65-84 yr) with a body mass index (BMI) of 26.2 ± 1.9 kg/m^2 (range 23.7-29.2 kg/m^2) were recruited by advertisement. No subject had a history of gastrointestinal disease or surgery, significant illness (including diabetes mellitus), or was taking medication known to affect gastrointestinal motility or appetite. All subjects were non-smokers; well nourished [score > 24 on the Geriatric Mini-Nutritional Assessment (Guigoz et al 1996)] (see Chapter 6.5.3.3); unrestrained eaters [score <10 on the Three-Factor Eating Questionnaire (Stunkard & Messick 1985)] (see Chapter 6.4.1); and were not depressed [score > 15 on Geriatric Depression Questionnaire (Yesavage 1988)] (see Chapter 6.4.2 and Appendix BVI). Before the commencement of the study, energy intake was assessed by a 3-day diet diary to ensure that all subjects had an average energy intake > 4182 kJ/day (see Chapter 6.2.2). The study protocol was approved by the Ethics Committee of the Royal Adelaide Hospital and each subject gave written, informed, consent.

13.3.1 *Experimental Protocol*

Each subject completed two different diets, each for a period of 10 days in randomised order (Figure 13.1a). The diets were: (i) a 'standard' diet consisting of the subject's usual diet plus 350 ml of low-joule lemon cordial [(Country Gold™, Villawood, Australia) diluted 72 ml cordial in 278 ml water (33.5 kJ)], consumed three times daily immediately after each main meal (total energy 100.5 kJ/day) and (ii) a 'glucose-supplement' diet, consisting of the subject's usual diet supplemented with 210g of

glucose per day. This was given as one sachet of 70g glucose added to 350 ml of the low-joule cordial [47 ml cordial in 303 ml water (22.2 kJ)] three times daily (total energy 3513 kJ/day). The two dietary periods were separated by a 'washout' period of at least 10 days during which each subject ate ad libitum. Compliance was assessed by weighing the unused glucose sachets returned following the glucose supplemented diet. All subjects were weighed at baseline, and following each of the dietary periods.

On the day immediately following each 10 day dietary period, subjects attended the Department of Nuclear Medicine after an overnight fast (14 h from solids and 12 h from liquids). A cannula was placed in a left antecubital vein for blood sampling. After a 15 min recovery period subjects ingested an oil/glucose drink. Gastric emptying of the drink and appetite were then measured and blood samples taken for subsequent measurement of gastrointestinal hormones. Immediately following completion of the gastric emptying study (t=210 min), each subject was offered a standard buffet style meal and invited to eat as much as they wished over 30 minutes (see Chapter 6.3.3) (Figure 13.1b).

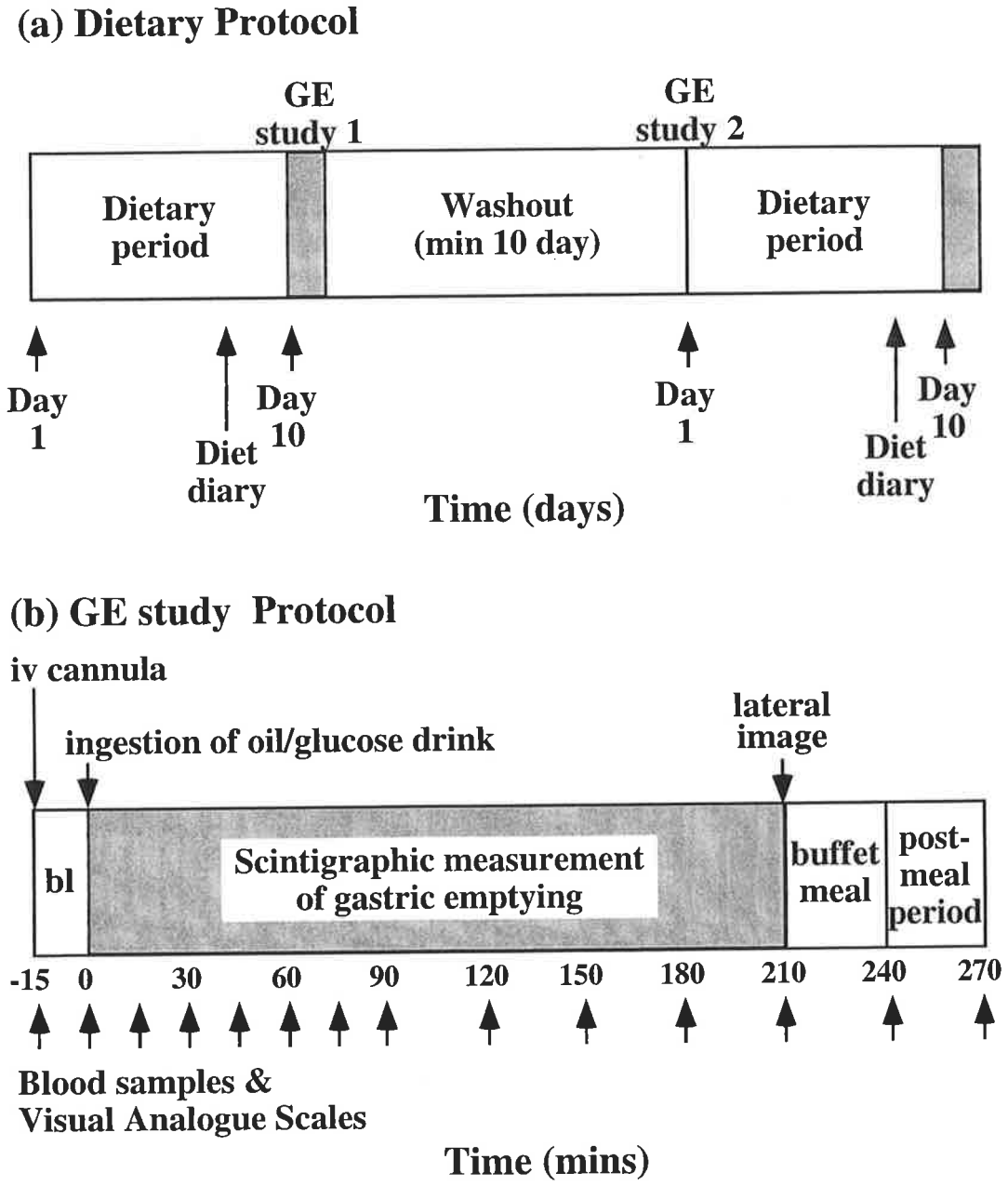

Figure 13.1: (a) Summary of experimental protocol. Each subject completed two 10 day dietary (either a 'standard' diet or a 'glucose-supplement' diet 210g of glucose per day) periods in random order separated by a wash-out period of at least 10 days. Three day diet diaries were completed during the final three days of each dietary period. At the end of each dietary period subjects underwent a study to measure gastric emptying and appetite. (b) Summary of gastric emptying study protocol. Each subjects completed two studies. Subjects ingested an oral oil (15 ml olive oil (527 kJ) labeled with 20MBq 99mTc-(V)-thiocyanate and 33 g glucose (519 kJ) dissolved in 185 ml water, labeled with 8MBq 67Gallium-EDTA) drink at t= 0 min, and gastric emptying was measured for 210 min. The timing of venous blood samples and administration of visual analogue scales to assess hunger and fullness is indicated by arrows.

13.3.2 *Measurement of gastric emptying*

Gastric emptying was measured for 210 minutes, starting immediately after ingestion of the test drink, which comprised 15 ml olive oil (100% fat (10% poly-unsaturated, 76% mono-unsaturated and 15% saturated), 527 kJ) labeled with 20 MBq ^{99m}Tc-(V)-thiocyanate (Cunningham et al 1991a) and 33 g glucose (519 kJ) dissolved in 185 ml water, labeled with 8 MBq ^{67}Gallium-EDTA (Bellen et al 1995), - total volume of 200 ml. Radioisotopic data were collected in 30 second frames for the first 30 minutes, followed by 3 minute frames for the remaining 180 minutes (where t= 0 represents the time of completion of the drink). Data were corrected for subject movement, radionuclide decay, and gamma ray attenuation using established methods (Collins et al 1983) (see Chapter 7.3.1). A region-of-interest was drawn around the total stomach and the percentage retention of the oil and glucose components at t= 0, 15, 30, 45, 60, 90, 120, 150, 180 and 210 min calculated. The duration of the lag phase, calculated as the time point immediately preceding that in which activity was first seen in the proximal small intestine, and the 50% emptying time (T50) were also determined (Collins et al 1983) (see Chapter 7.3.1).

13.3.3 *Blood glucose and gastrointestinal hormones*

Blood samples (~10 ml) were taken, immediately before (-2 min) ingestion of the test drink and then at 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240 and 270 min for measurement of blood glucose, and plasma insulin, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (Lavin et al 1998).

Blood glucose concentrations were determined immediately, using a portable blood glucose meter (MediSense Companion 2 meter, MediSense Inc., Waltham, MA). The accuracy of this method has been confirmed using the hexokinase technique (Horowitz et al 1991).

Plasma insulin was measured using the Abbott Imx Microparticle Enzyme Immunoassay (Abbott Laboratories, Diagnostic Division, Dainabot, Tokyo, Japan) (Chapman et al 1998). The sensitivity of the assay (concentration at 2 S.D. from the zero standard) was 1.0 mU/ml. The interassay coefficients of variation were 4.5% at 8.3 mU/ml and 3.4% at 40.4 mU/ml. The time to peak plasma insulin was also calculated.

Plasma GIP was measured by radioimmunoassay using anti-human GIP antisera according to an established method (Horowitz et al 1996). The minimum detectable limit for this assay was ~5 pmol/L and the interassay coefficient of variation was 15%.

Plasma GLP-1 was measured by radioimmunoassay after ethanol extraction using antibody supplied by Professor SR Bloom (Hammersmith Hospital, London), which did not cross-react with glucagon, GIP or other gut peptides and had been demonstrated by chromatography to measure intact GLP-1 7-36 amide (Kreymann et al 1987, Wishart et al 1998). The minimum detectable limit for this assay was ~ 2 pmol/L and the interassay coefficient of variation was 18%.

13.3.4 *Appetite and food intake*

Energy intake during the dietary periods was assessed using a 3-day diet diary (see Chapter 6.2.2) maintained on the last 3 days of each diet.

Immediately before and after ingestion of the test meal, hunger and fullness were assessed using 10 cm visual analog scales, at -5, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240 and 270 min (see Chapter 6.3.1 and Appendix BI.1). The t=-5 value was used as the baseline (fasting) rating.

The buffet style meal contained food prepared in excess of what would normally be eaten (275 ml iced coffee; 300 ml unsweetened orange juice; 4 slices whole meal bread; 4 slices white bread; 4 slices cheddar cheese; 4 slices processed chicken; 4 slices processed ham; 4 slices of tomato, cucumber and lettuce; 2 sachets of mayonnaise; 4 sachets margarine; 1 pear; 1 apple; 1 banana; 200g chocolate custard; 200g strawberry yogurt; 50g ice-cream). Subjects were invited to eat as much as they wished for 30 min and the total amount (grams and kJ), as well as the macronutrient content of food consumed were calculated using the DIET 4 Nutrient Calculation Software (Xyris Software, Queensland, Australia) (see Chapter 6.3.3).

13.3.5 *Statistical analysis*

Data were analysed using a two-way repeated measures analysis of variance (ANOVA) (SuperANOVA Abacus concepts, Version 1.11). Contrasts were performed to test preplanned hypotheses of interest, enabling paired comparisons at particular time points (Horowitz et al 1996). The relationships between variables were evaluated using linear

regression analysis. Data are presented as mean values ± SEM. A P value of < 0.05 was considered significant.

13.4 RESULTS

The study was well tolerated by all subjects. Four of the 8 subjects received the standard diet first. Compliance with the glucose supplement was excellent with 7 subjects returning all 30 glucose sachets empty. One subject returned 3 sachets of glucose which were not consumed during the first five days of the glucose supplement diet.

13.4.1 *Gastric emptying*

13.4.1.1 Oil

Gastric emptying of oil approximated a linear pattern after an initial lag phase (glucose supplement: 58.4 ± 9.5 min vs standard diet: 72.8 ± 15.5 min; P= 0.15). There was no difference in gastric emptying between the two diets (T50 glucose supplement: 122.1 ± 14.7 min vs standard diet: 117.1 ± 14.2 min; P= 0.57). At t=210 min, ie immediately before consumption of the buffet meal most of the oil had emptied from the stomach (glucose supplement: 16.6 ± 1.9 % remaining vs standard diet: 20.0 ± 4.9 % remaining; P= 0.35) (Figure 13.2).

13.4.1.2 Glucose

Gastric emptying of glucose approximated a monoexponential pattern after a short lag phase (glucose supplement: 0.6 ± 0.1 min vs standard diet: 2.1 ± 1.3 min; P= 0.28) (Figure 13.2). The glucose emptied faster than the oil component of the drink on both diets (P< 0.001) due to a longer lag phase for oil (glucose supplement: lag phase oil: 58.4 ± 9.5 min vs glucose: 0.6 ± 0.1 min; P< 0.0005 and standard diet: oil: 72.8 ± 15.5 min vs glucose: 2.1 ± 1.3 min; P< 0.005). Gastric emptying of glucose was faster following the glucose supplement compared to the standard diet (eg. T50: 48.6 ± 5.9 min vs 62.0 ± 6.1 min; P= 0.04). At t= 210 min, most of the glucose had emptied from the stomach (glucose supplement: 12.8 ± 1.6 % remaining vs standard diet: 11.5 ± 1.8 % remaining; P= 0.18).

13.4.1.3 Oil and glucose

There was no difference in the total number of kilojoules emptied between the two diets (data not shown).

13.4.2 *Blood glucose and gastrointestinal hormones*

Fasting blood glucose (glucose supplement: 5.4 ± 0.2 mmol/L vs standard diet: 5.4 ± 1.2 mmol/L; P= 0.94), plasma insulin (glucose supplement: 4.7 ± 0.7 mU/L vs standard diet: 4.1 ± 0.7 mU/L; P= 0.56), GIP (glucose supplement: 27.0 ± 4.2 pmol/L vs standard diet: 26.6 ± 4.5 pmol/L; P= 0.93) and GLP-1 (glucose supplement: 7.9 ± 1.2 pmol/L vs standard diet: 8.6 ± 1.7 pmol/L; P= 0.71) were not significantly different between the two diets.

13.4.2.1 Blood glucose

On both days blood glucose increased following the test drink (P< 0.001), returning to baseline levels at ~150 min. Blood glucose concentrations at 75 min and 90 min were less following the glucose supplemented diet (eg. at 75 min: 8.3 ± 0.5 mmol/L vs 9.4 ±0.3 mmol/L; P< 0.01) (Figure 13.3a).

13.4.2.2 Plasma insulin

Plasma insulin increased after ingestion of the test drink and declined to baseline levels at ~180 min (P< 0.001) on both days. The time to peak insulin concentration was less (31.9 ± 6.0 min vs 73.1 ± 8.2 min; P< 0.01) following the glucose supplemented diet when compared to the standard diet and plasma insulin at 30 min tended to be greater (P= 0.054), with a subsequent reduction at 60 min (P< 0.01) and 120 min (P< 0.05) (Figure 13.3b).

13.4.2.3 Plasma GIP and GLP-1

Plasma GIP (Figure 13.3c) and GLP-1 (Figure 13.3d) increased after ingestion of the test drink on both days (P< 0.001). There was a trend for plasma GIP concentrations to be greater immediately after ingestion of the test drink (eg at t= 15 min P= 0.06) following the glucose supplemented diet when compared to the standard diet. There was no difference in plasma GLP-1 concentrations between the two diets.

13.4.3 *Appetite*

13.4.3.1 Baseline energy intake and body weight

The energy intake of the diet before entry into the study was 7661 ± 640 kJ/ day [43% (carbohydrate) CHO, 36% fat and 18% protein]. There was no change in body weight following either the glucose supplemented (68.9 ± 2.7 kg) or the standard (68.6 ± 2.9 kg) diets when compared to baseline body weight (68.2 ± 2.8 kg).

13.4.3.2 Energy intake during dietary periods

There was no difference in energy intake during the two diets when the glucose-supplement was excluded (glucose supplement: 6737 ± 581 kJ vs standard diet: 6913 ± 556 kJ; P= 0.79). When the additional energy (3513 kJ) provided by the glucose supplement was included, energy intake was greater (P< 0.001) during the glucose supplemented diet when compared to the standard diet (Figure 13.4). There were no differences in macronutrient content (% carbohydrate, fat or protein) during the glucose supplement diet compared to the standard diet (data not shown).

13.4.3.3 Hunger and Fullness

There were no differences in fasting ratings of hunger (glucose supplement: 7.7 ± 1.1 cm vs standard diet: 8.1 ± 0.3 cm; P= 0.75), and fullness (glucose supplement: 2.7 ± 0.8 cm vs standard diet: 3.0 ± 0.8 cm; P= 0.84) between the two diets (Figure 13.5a and b). After ingestion of the drink there was a reduction in hunger from baseline (P< 0.05) on both diets without any difference between them. In contrast, fullness increased (P< 0.05) from baseline on the standard diet but not the glucose supplemented diet with no significant difference between them (Figure 13.5b).

13.4.3.4 Energy intake at buffet meal

There was no difference between the two diets in either the energy (glucose supplement: 3446 ± 343 kJ vs standard diet: 3559 ± 305 kJ; P= 0.58) or macronutrient content of food eaten at the buffet meal (Figure 13.6). There was an inverse relationship (r=-0.55, P< 0.05) between the kJ content of the food eaten at the buffet meal and the amount of the oil/glucose drink (kJ) remaining in the stomach immediately prior to the buffet meal (glucose supplement: r= -0.77, P< 0.05; standard diet: r= -0.51, P= 0.20) (Figure 13.7).

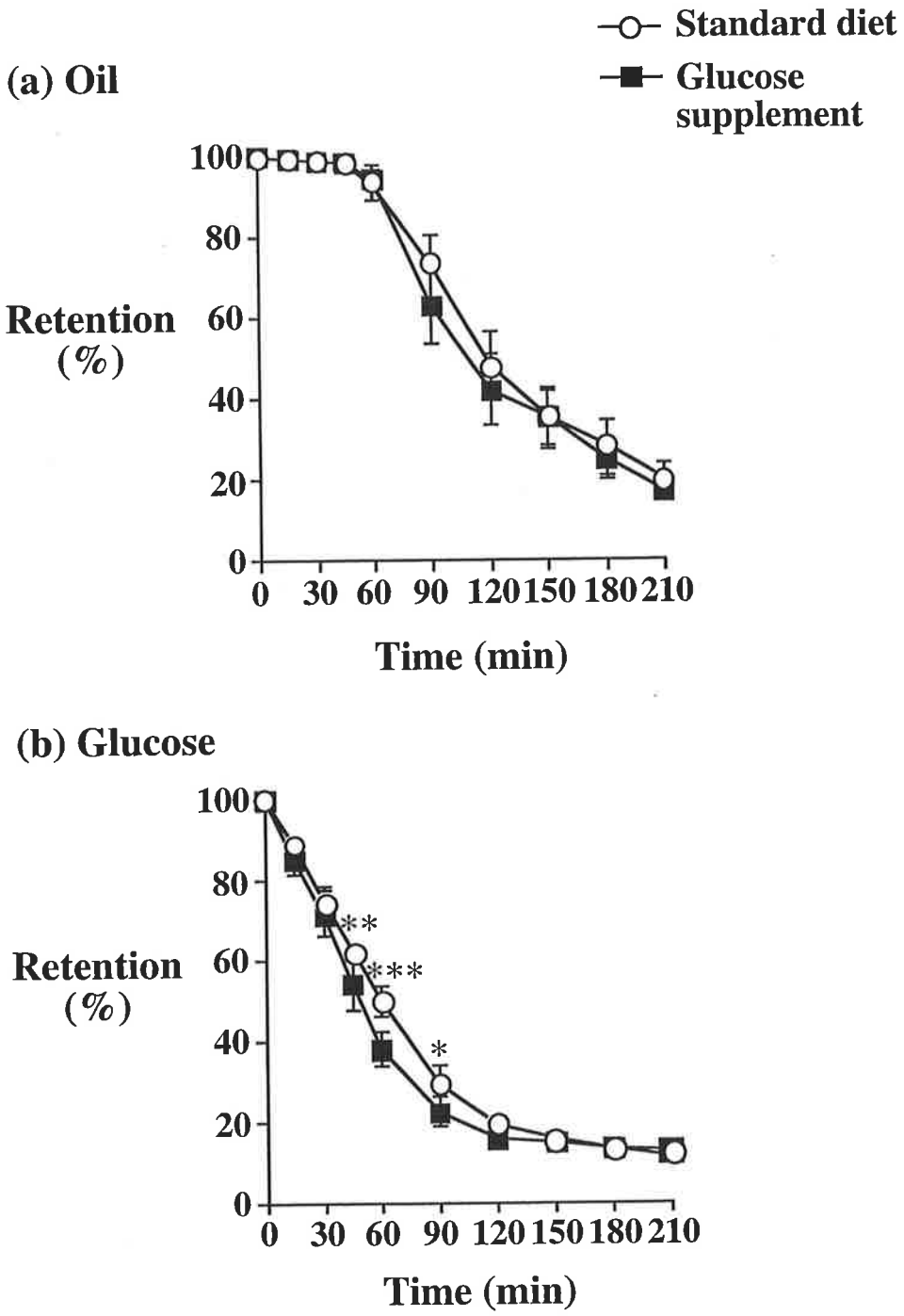

Figure 13.2: Gastric emptying of the test drink containing (a) oil and (b) glucose components following a glucose supplemented and a standard diet in 8 healthy elderly subjects. Data are mean ± SEM. *P< 0.05, **P< 0.01 and ***P< 0.001glucose supplemented vs standard diet.

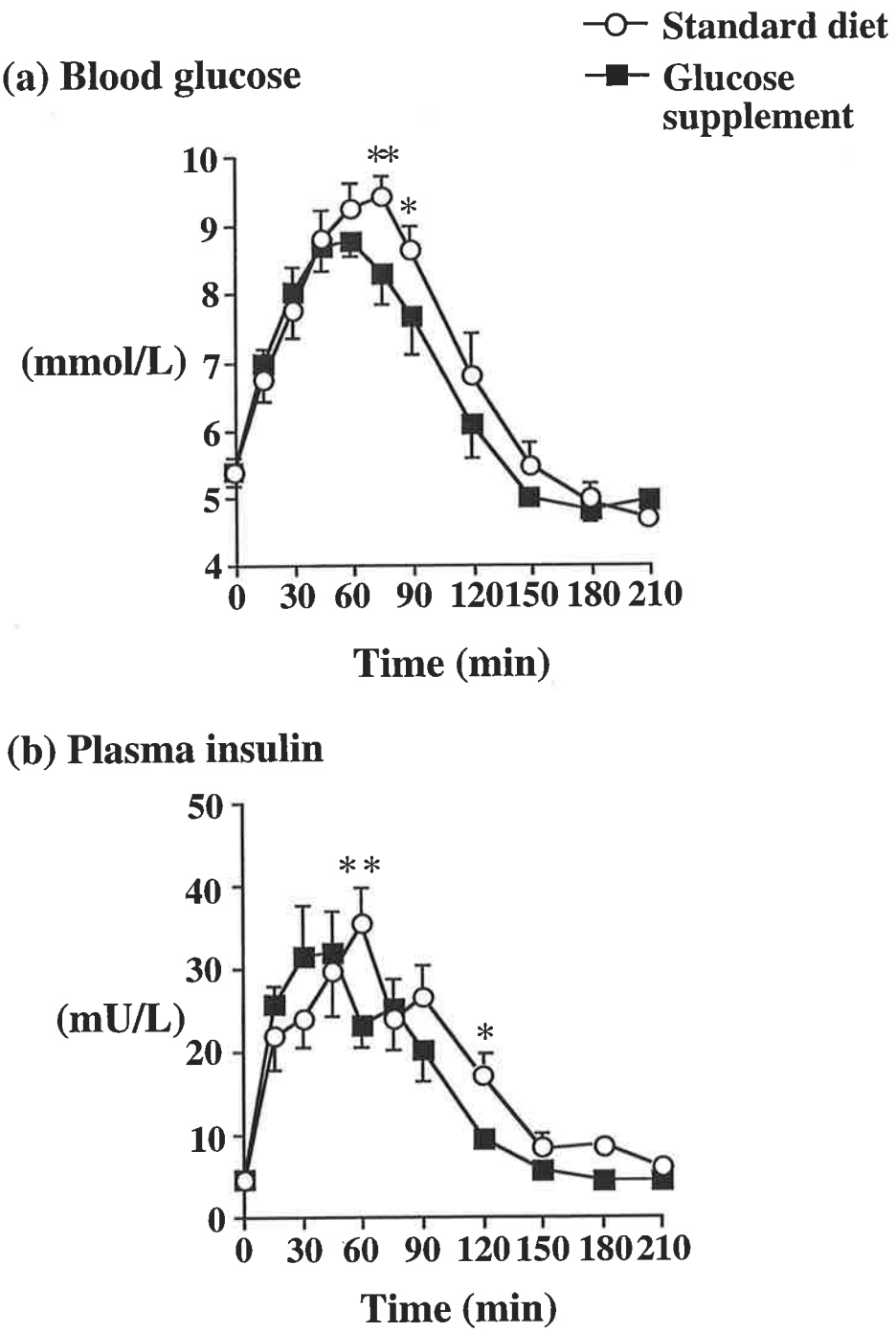

Figure 13.3: Concentrations of (a) blood glucose and (b) plasma insulin after ingestion of an oil/glucose drink following a glucose supplemented and a standard diet in 8 healthy elderly subjects. Data are mean ± SEM. *P< 0.05 and **P< 0.01 glucose supplemented vs standard diet.

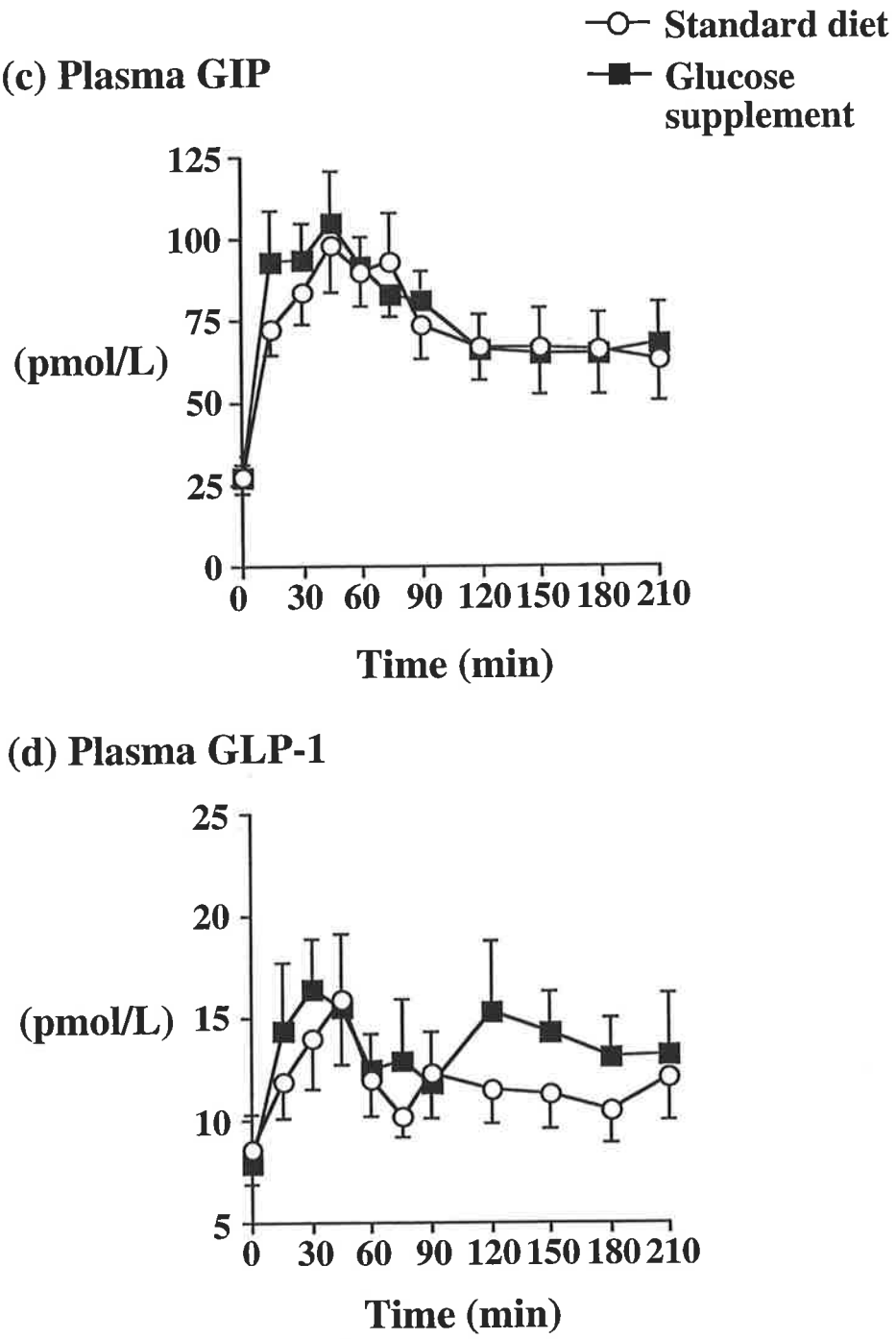

Figure 13.3 cont.: Concentrations of (a) plasma GIP and (b) plasma GLP-1 after ingestion of an oil/glucose drink following a glucose supplemented and a standard diet in 8 healthy elderly subjects. Data are mean ± SEM.

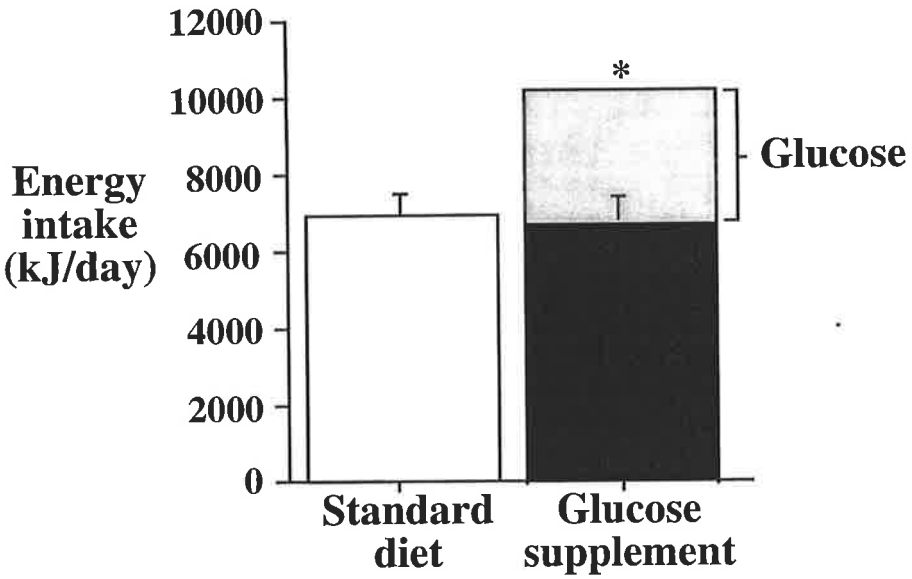

Figure 13.4: Energy intake (kJ/day), measured on the last 3 days of a glucose supplemented and a standard diet in 8 healthy elderly subjects. Data are mean ± SEM. The contribution of the glucose supplemented to total energy intake is shown. *P<0.001 vs standard diet.

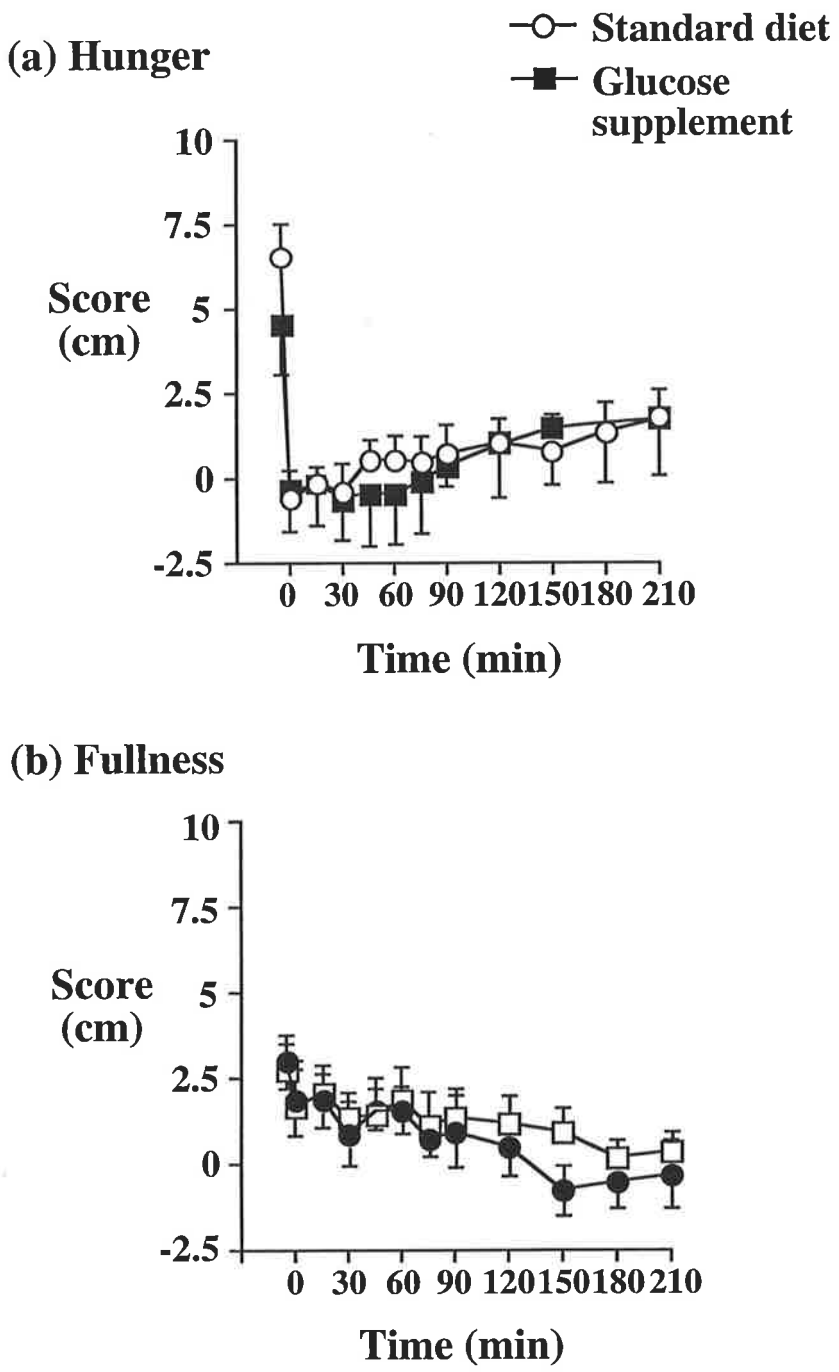

Figure 13.5: Change in ratings of hunger (a) and fullness (b) from baseline after ingestion of a oil/glucose drink following a glucose supplemented and a standard diet in healthy older subjects. Data are mean ± SEM. Two-way ANOVA; $P < 0.01$ and $P < 0.05$ effect of time for hunger and fullness, respectively.

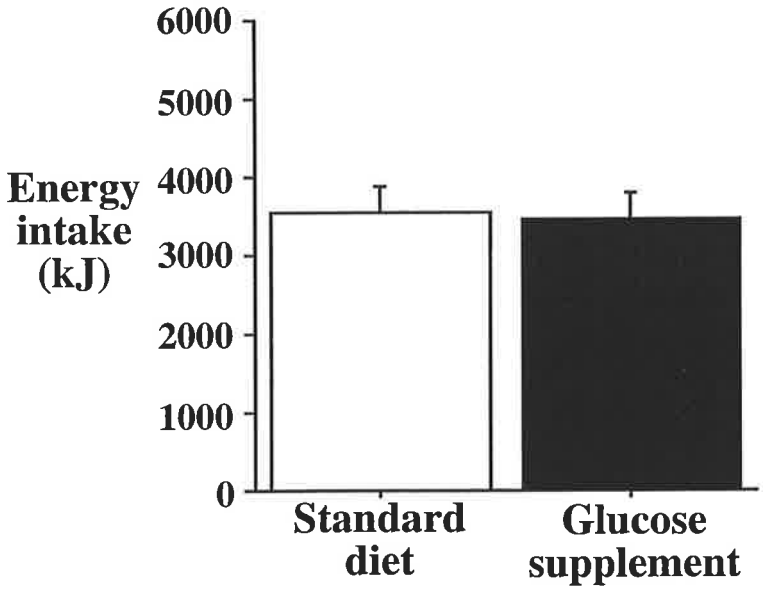

Figure 13.6: Energy intake (kJ) of the buffet meal 3 hrs after ingestion of a oil/glucose drink following a glucose supplemented and a standard diet in 8 healthy elderly subjects. Data are mean ± SEM.

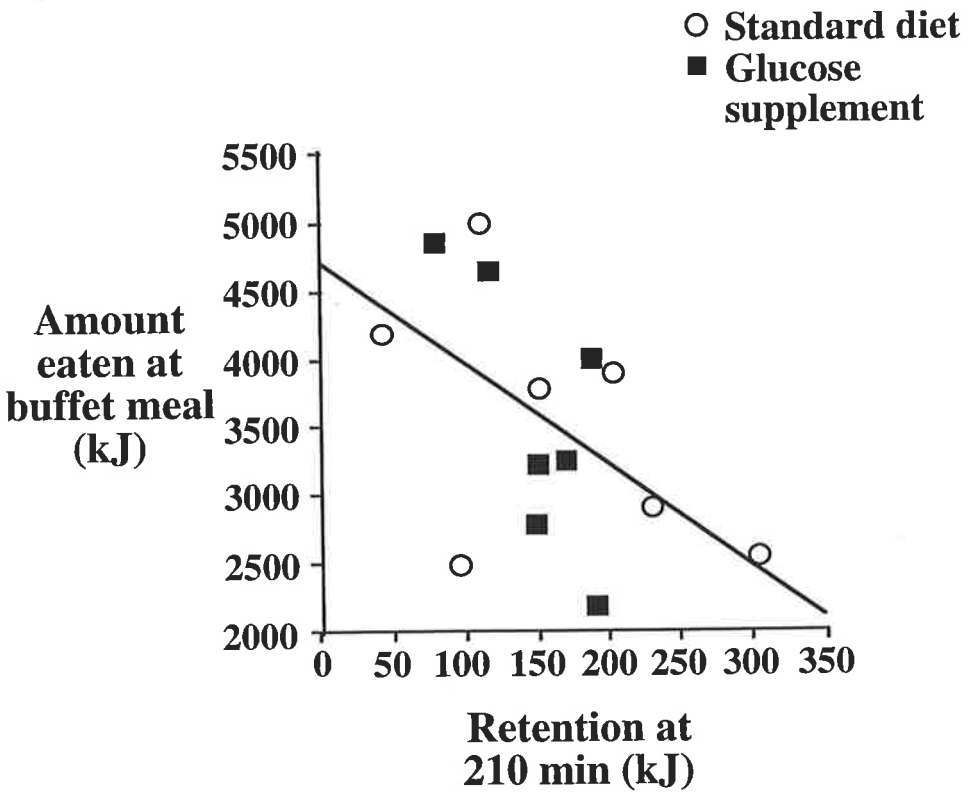

Figure 13.7: Relationship between the amount of food eaten at the buffet meal (kJ) and amount of oil/glucose preload (kJ) remaining in the stomach immediately prior to the buffet meal ($r = -0.55$, $P < 0.05$).

13.5 DISCUSSION

Our study has evaluated the effects of short-term dietary glucose supplementation on gastric emptying, and the glycaemic response to a drink containing glucose and food intake in healthy, older subjects. The results establish that, as in young subjects, dietary glucose supplementation accelerates gastric emptying of glucose (Horowitz et al 1996) and that this is associated with significant changes in blood glucose, plasma insulin and, almost certainly, GIP (Horowitz et al 1996). Novel observations are that: (i) the acceleration of gastric emptying of glucose is not associated with any change in gastric emptying of oil, (ii) the stimulation of insulin secretion is not associated with any change in GLP-1 and (iii) dietary glucose supplementation does not decrease food intake, so that caloric intake was substantially greater while subjects were taking the glucose supplement.

A number of studies have established that dietary modification may influence gastric emptying (Horowitz et al 1996, Corvilain et al 1995, Cunningham et al 1991b, Cunningham et al 1991c) and gastric motor function (Andrews et al 1998a) in healthy young adults (see Chapter 3.5.2.1). The magnitude of the acceleration of gastric emptying of glucose after dietary glucose supplementation in older subjects is comparable to that observed in the young (Horowitz et al 1996, Cunningham et al 1991c). This is perhaps not surprising as the effects of healthy ageing on gastrointestinal function, including gastric emptying, are relatively small (Andrews & Horowitz 1996). In our previous studies in younger subjects (Andrews 1998a, Horowitz et al 1996, Cunningham et al 1991c) the duration of dietary glucose supplementation was comparable, but both the glucose supplement (210g/day vs 440g/day in the study by Horowitz et al (1996) and the amount of glucose in the drink used to measure gastric emptying (33g vs 75g) were less in the current study. Our glucose supplement was intentionally selected to be smaller, as older people eat substantially (30-50%) less than young adults (Rolls 1995, Wurtman et al 1988)(see Chapter 1.4); proportionately, the increase in daily energy intake was comparable to the supplements used in younger subjects (Andrews et al 1998a, Horowitz et al 1996, Cunningham et al 1991c). Gastric emptying of glucose is regulated primarily by feedback from receptors in the lumen of the small intestine (Brener et al 1983, Lin et al 1995a) mediated, at least in part, by the release of gastrointestinal hormones including cholecystokinin (CCK), and possibly, GIP and GLP-1 (Fried et al 1991, Wishart et al 1998) (see Chapter 3.5.2 and 3.6).

Infusion of glucose and other nutrients directly into the small intestine is associated with stimulation of phasic and tonic pressure waves localised to the pylorus (Andrews et al 1998a, Edelbroek et al 1992a), suppression of antral pressure waves (Andrews et al 1998a), a reduction in proximal gastric tone (Azpiroz & Malagelada 1985) and slowing of gastric emptying (see Chapter 3.5.2). We have recently established in young adults that dietary glucose supplementation is associated with attenuation of the tonic pyloric response to intraduodenal glucose (Andrews et al 1998a). This observation provides persuasive evidence to support the concept that the acceleration of gastric emptying of glucose by dietary glucose supplementation reflects diminished small intestinal feedback on the neural/humoral mechanisms which regulate gastric emptying. It remains to be established whether the latter occurs as a result of a decrease in the sensitivity of small intestinal "glucoreceptors", or the recruitment of less receptors as a result of more rapid absorption and, hence reduced nutrient exposure (Horowitz et al 1996).

Dietary glucose supplementation had no effect on gastric emptying of fat (oil), consistent with the observation that dietary glucose supplementation does not modify the pyloric motor response to intraduodenal lipid (Andrews et al 1998a). The slowing of gastric emptying by small intestinal glucose and fat are mediated by different receptors (Lin et al 1995a, Lin et al 1995b). It accordingly, appears that adaptive changes in gastric emptying occurring as a result of dietary nutrient modification may be relatively nutrient-specific.

The effects of dietary glucose supplementation on the glycaemic profile after oral glucose are compatible with the observed acceleration of gastric emptying and more rapid delivery of glucose to the small intestine ie an earlier rise in plasma insulin and subsequent reduction in blood glucose. The increased insulin response is likely to be attributable to the earlier rise in plasma GIP (P= 0.06), as there was no difference in plasma GLP-1. GIP is released as a result of the interaction of nutrients with the proximal small intestine and its release is, accordingly, potentiated when gastric emptying is faster (Horowitz et al 1996). In contrast, GLP-1 is released predominantly from the distal small intestine (Nauck 1999) and plasma concentrations are inversely related to the rate of gastric emptying (Wishart et al 1998).

Our observation that older subjects did not modify their diet to compensate for additional energy provided by the glucose supplement is consistent with the concept of

an age-related impairment in the homeostatic mechanisms which regulate appetite, with a lack of compensation for dietary manipulations (Roberts et al 1994) (see Chapter 1.5). In our study glucose supplementation also had no effect on perceptions of appetite following a glucose/oil "preload" or energy intake at the buffet meal consumed at 210 minutes after this "preload". Accordingly, the use of glucose or other carbohydrate supplements may prove to be effective in increasing total energy intake and preventing weight loss in the elderly. Although at the time when the buffet meal had been consumed the majority of the glucose/oil drink had emptied from the stomach, there was an inverse relationship between the amount (kJ) eaten and the intragastric content at this time. This latter observation suggests that, as in the young (Jones et al 1997a, Lin et al 1995a) gastric distension modifies food intake.

CHAPTER 14

Effect of Guar Gum on Postprandial Blood Pressure, Gastric Emptying, and Blood Glucose Homeostasis in the Healthy Elderly

14.1 SUMMARY

The aims of this study were to determine whether slowing of gastric emptying and glucose absorption with guar gum would reduce the fall in blood pressure after an oral glucose load in older subjects.
Ten healthy subjects, aged 67-78 yr underwent a randomised, experimental, cross-over study, involving simultaneous measurements of gastric emptying, blood pressure, blood glucose, serum insulin and oral glucose absorption [3-O-methyl-D-glucose (3-OMG)] on two occasions after ingestion of 300 ml water containing 50g glucose and 30 ml lemon juice, 3g 3-OMG labeled with 99mTc-sulphur colloid; with or without 9g guar gum. Blood pressure and gastric emptying were monitored for 180 min.

The magnitude of the falls in systolic ($P< 0.05$), diastolic ($P< 0.05$) and mean arterial ($P= 0.05$) blood pressure were less, and gastric emptying slower ($P< 0.05$), after guar. Blood glucose, insulin and 3-OMG concentrations were reduced ($P< 0.001$ for all) by guar. 3-OMG concentrations were inversely related to the intragastric retention of glucose ($r= -0.72$, $P= 0.02$) and blood pressure was inversely related to 3-OMG ($r= -0.64$, $P< 0.05$) after the drink without guar. The blood glucose concentration was related to 3-OMG ($r= 0.64$, $P< 0.05$).

Guar gum reduces the magnitude of the fall in blood pressure after oral glucose. Slowing of gastric emptying and glucose absorption may represent a novel approach to the treatment of postprandial hypotension.

14.2 INTRODUCTION

Postprandial hypotension, defined as a decrease in systolic blood pressure > 20 mmHg, occurring within 2 hours of the end of a meal (see Chapter 5.1), is an important clinical problem in the elderly because it increases the incidence of syncope and falls (Jansen et al 1995) (see Chapter 5.2).

The mechanisms responsible for postprandial hypotension are incompletely understood, but impaired regulation of splanchnic blood flow and the release of gastrointestinal hormones appear to be important (Jansen & Hoefnagels 1991, Jansen & Lipsitz 1995, Mathias et al 1989, Mathias et al 1991) (see Chapter 5.5).

There is recent evidence to suggest that the rate of delivery of carbohydrates (ie gastric emptying and/or small intestinal absorption) to the small intestine may influence the magnitude of the fall in postprandial blood pressure in patients with diabetes mellitus (see Chapter 5.5). This latter observation suggests that slowing of gastric emptying, by dietary or pharmacological means, may reduce postprandial hypotension. Guar gum, a naturally occurring, gel-forming carbohydrate of vegetable origin (French & Read 1994), has been used to slow both gastric emptying and small intestinal absorption in humans (French & Read 1994).

Studies conducted by both ourselves (Horowitz et al 1993, Jones et al 1996) and others (Schwartz et al 1995) indicate that the rate of gastric emptying is a significant determinant of the postprandial rise in blood glucose in both normal subjects (Horowitz et al 1993) and patients with diabetes (Jones et al 1996) (see Chapter 5.5). A deficiency in the majority of these studies is that glucose absorption was not measured directly - the latter can be assessed using the glucose analogue 3-O-methyl-D-glucose (3-OMG) (Kong et al 1997, Fleming et al 1993).

The purpose of this study was to determine whether slowing gastric emptying and small intestinal absorption with guar gum reduces the postprandial fall in blood pressure in older subjects.

14.3 SUBJECTS AND METHODS

Ten healthy older subjects, recruited by advertisement, (5 male and 5 female) median age 70 yr (range 67-78 yr), median body mass index (BMI) 25.3 kg/m^2 (range 19.5-28.9 kg/m^2), were studied. All subjects were non-smokers and none had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory or cardiac disease, chronic alcohol abuse or epilepsy. No subject was known to be hypotensive, nor was taking medication which may influence either blood pressure or gastrointestinal function.

14.3.1 *Experimental Protocol*

Each subject had concurrent measurements of blood pressure, heart rate, gastric emptying, blood glucose concentrations, oral glucose absorption and appetite (hunger and fullness) on two days after ingestion of a drink comprising 50g glucose, 30 ml lemon juice, 3g 3-O-methyl-D-glucose (3-OMG) (Sigma-Aldrich, Steinheim, Germany)

and 20 MBq 99mTc-sulphur colloid, made up to 300 ml with water; with or without 9g guar gum. The two studies were randomised and separated by at least three days. The subjects were not blinded to the test meals due to the greater viscosity of the drink containing guar. Subjects attended the Department of Nuclear Medicine at either 0900h or 1200 h following a fast (14h for solids; 12h for liquids), but at the same time for both studies. A cannula was placed in a right antecubital vein for blood sampling and subjects were seated with their back against a gamma camera, with a blood pressure cuff around their left arm. Cardiovascular autonomic nerve function was evaluated on one of the study days. Each subject gave written, informed consent prior to the commencement of the study and the protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

14.3.2 *Measurements*

14.3.2.1 Blood pressure and heart rate

Blood pressure (systolic (SBP), diastolic (DBP), mean arterial (MAP)) and heart rate were measured using an automated oscillometric blood pressure monitor (DINAMAP; Johnson & Johnson Pty. Ltd.) immediately before (-2 min) ingestion of the drink, at 3 min intervals for the first 60 min and then at 15 min intervals for a further 120 min (Jones et al 1998). Postprandial hypotension was defined as a fall in systolic blood pressure approximately 20 mmHg after the glucose meal that was sustained for at least 30 min (4). Incremental areas under the change in mean blood pressure curve at 0-15 min, 0-30 min, 0-45 min, 0-60 min, 0-90 min, 0-120 min, 0-150 min and 0-180 min were calculated using the trapezoidal rule.

14.3.2.2 Gastric emptying

Subjects consumed the drink within 5 minutes. Radioisotopic data were acquired for 180 min (30 sec frames for the first 30 min, 3 min frames thereafter) (Jones et al 1998). Time zero was defined as the time of completion of the glucose drink. Data were corrected for subject movement, radionuclide decay and γ-ray attenuation (Collins et al 1983). Gastric emptying curves (expressed as the % retention over time) were derived for the total stomach at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. The lag phase was determined visually as the time before any of the radioactivity had entered the proximal small intestine (Collins et al 1983) (see Chapter 7.3.1).

14.3.2.3 Blood glucose, insulin and glucose absorption

Venous blood samples (20 ml) were obtained immediately before (-2 min) ingestion of the drink and then at 15, 30, 45, 60, 120, 150 and 180 min. Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Companion 2 meter, Medisense Inc., Waltham MA, USA) (Jones et al 1998). Serum was stored at -70 °C until analysis of insulin (Abbott Laboratories, Japan [Intra-assay coefficients of variation were 4% at 8.3 μU/ml, 2.9% at 40.4μU/ml and 2.5% at 121.7 μU/ml)] (Horowitz et al 1996). Plasma 3-OMG was measured by high-performance liquid chromatography (intra-assay coefficient of variation 2.7% for plasma at 0.155 mmol/l) (Fleming et al 1993) (see Chapter 7.5).

14.3.2.4 Hunger and Fullness

Hunger and fullness were evaluated using a validated visual analogue questionnaire (Sepple & Read 1989) (see Chapter 6.3.1 and Appendix BI.1). Measurements were obtained immediately before ingestion of the drink and at 15, 30, 45, 60, 90, 120, 150 and 180 min.

14.3.2.5 Cardiovascular autonomic function

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

14.3.3 *Statistical Analysis*

Data were evaluated using repeated measures analysis of variance (ANOVA) and are presented as mean ± SEM, unless stated otherwise. Contrasts were used to examine point by point comparisons to test preplanned hypotheses of interest. Changes in blood pressure from baseline were calculated separately for the first 30 min, as the maximum postprandial fall in blood pressure is known to usually occur during this time (Jansen & Lipsitz 1995). Relationships between blood pressure, gastric emptying, and 3-OMG levels were assessed using linear regression analysis. A P value < 0.05 was considered significant in all analyses.

14.4 RESULTS

The studies were generally well tolerated. Two female subjects experienced mild dizziness and faintness after the glucose meal without guar, which resolved after lying in the supine position for 5 minutes. The median score for autonomic nerve dysfunction was 2.5 (range: 0 - 4) and five of the ten subjects had evidence of subclinical cardiovascular autonomic dysfunction (Ewing & Clarke 1982, Piha 1991). These subjects predominantly had parasympathetic dysfunction, which has been shown to be closely related to ageing (Piha 1991).

14.4.1 *Blood Pressure and heart rate*

There were no significant differences in baseline blood pressure between the two study days (Figure 14.1a-c). There were falls in systolic, diastolic and mean arterial blood pressure after the drink on both days ($P< 0.0001$). The magnitude of the falls in systolic ($P< 0.05$), diastolic ($P< 0.05$) and change in mean arterial ($P< 0.05$) blood pressure were less after guar gum (Figure 14.1a-c). Postprandial hypotension was evident in 3 subjects without guar, and in 1 subject with guar.

There was no change in heart rate following the drinks, with no difference between the two study days ($P= 0.32$) (data not shown).

14.4.2 *Gastric emptying*

Gastric emptying approximated an overall linear pattern after a short lag phase (no-guar: 2.2 ± 0.48 min vs guar: 3.8 ± 0.8; $P= 0.13$) (Figure 14.2). The overall rate of emptying of the drink was slower ($P< 0.05$) after guar (Figure 14.2).

14.4.3 *Blood glucose, insulin and glucose absorption*

On both days there were increases in blood glucose (Figure 14.3) ($P< 0.0001$), serum insulin (Figure 14.3) ($P< 0.0001$) and serum 3-OMG (Figure 14.4) ($P< 0.0001$) after the drinks; the magnitude of these increases was in all cases greater ($P< 0.005$) after the drink without guar.

14.4.4 *Hunger and Fullness*

There were no differences in baseline scores for either hunger (no-guar: 28.8 ± 8.7 mm vs guar: 31.1 ± 11.8 mm; $P= 0.76$) or fullness (no-guar: 17.7 ± 7.3 mm vs guar: 14.6 ± 7.7 mm; $P= 0.57$) between the two study days. Hunger initially decreased after the drink with ($P< 0.05$) but not without guar, but later increased over time ($P< 0.0001$)

after both the drink with and without guar (Figure 14.5). Hunger was less ($P< 0.05$) after ingestion of the drink containing guar than without guar (Figure 14.5). Fullness increased after the drink with guar ($P< 0.05$) and fullness scores were higher during the first 60 min ($P< 0.05$) after the drink containing guar compared to that without guar (Figure 14.5).

14.4.5 *Relationships between blood pressure, gastric emptying, blood glucose, insulin, 3-OMG concentrations and autonomic function*

There were no significant relationships between blood pressure and gastric emptying, although there was a trend for an inverse relationship between the change in mean arterial blood pressure and retention of the drink in the stomach ($r= -0.61$, $P= 0.06$) without, but not with guar gum ($r= 0.13$, $P= 0.69$). The magnitude of the postprandial fall in systolic ($r= -0.64$, $P= 0.05$) (but not diastolic ($r= -0.41$, $P= 0.23$) or mean arterial ($r= -0.61$, $P= 0.06$), blood pressure was inversely related to glucose absorption 15 min after ingestion of the drink without, but not with guar gum (Figure 14.6). The fall in (systolic, diastolic and mean arterial) blood pressure was not related to either blood glucose or insulin concentrations (data not shown). Oral glucose absorption ($r=-0.72$, $P< 0.05$) (Figure 14.7) was related to gastric emptying 15 min after ingestion of the drink without, but not with guar gum. Blood glucose concentrations were related to glucose absorption on both study days eg. at 30 min (guar: $r= 0.69$, $P< 0.05$, no-guar: $r= 0.64$, $P< 0.05$) (Figure 14.8). There were no relationships between the postprandial fall in blood pressure and the score for autonomic function.

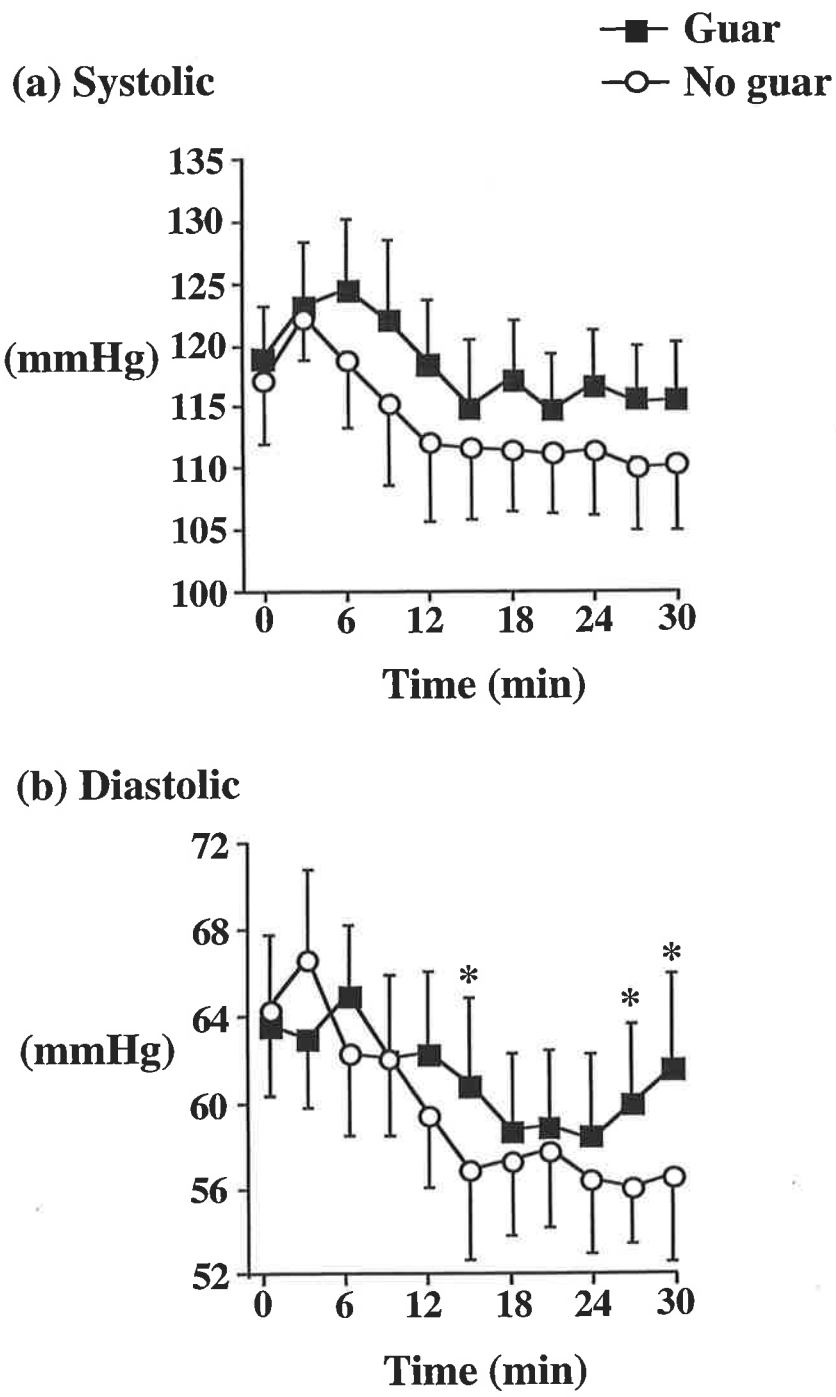

Figure 14.1: Systolic (a) and diastolic (b) blood pressure during the first 30 min after ingestion of 50 g glucose with or with out 9 g of guar gum in healthy older subjects. Data are mean ± SEM. Two way ANOVA; $P < 0.05$ effect of treatment guar < no guar, *$P < 0.05$ guar vs no guar at individual timepoints.

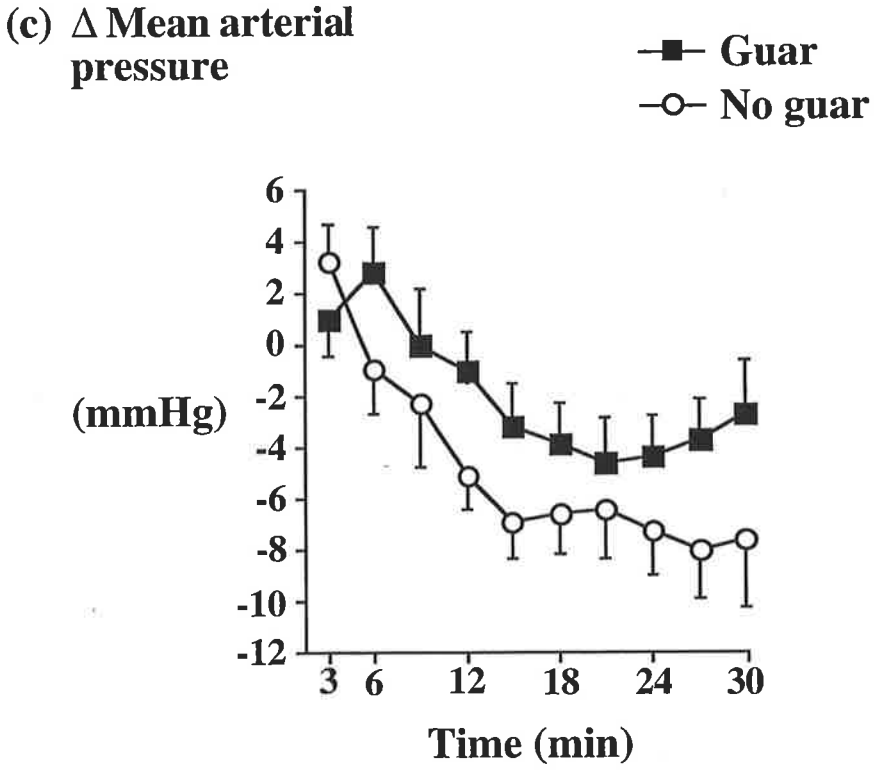

Figure 14.1 cont.: Change in mean arterial blood pressure during the first 30 min after ingestion of 50 g glucose with or with out 9 g of guar gum in healthy older subjects. Data are mean ± SEM. Two-way ANOVA; P= 0.05 guar < no guar.

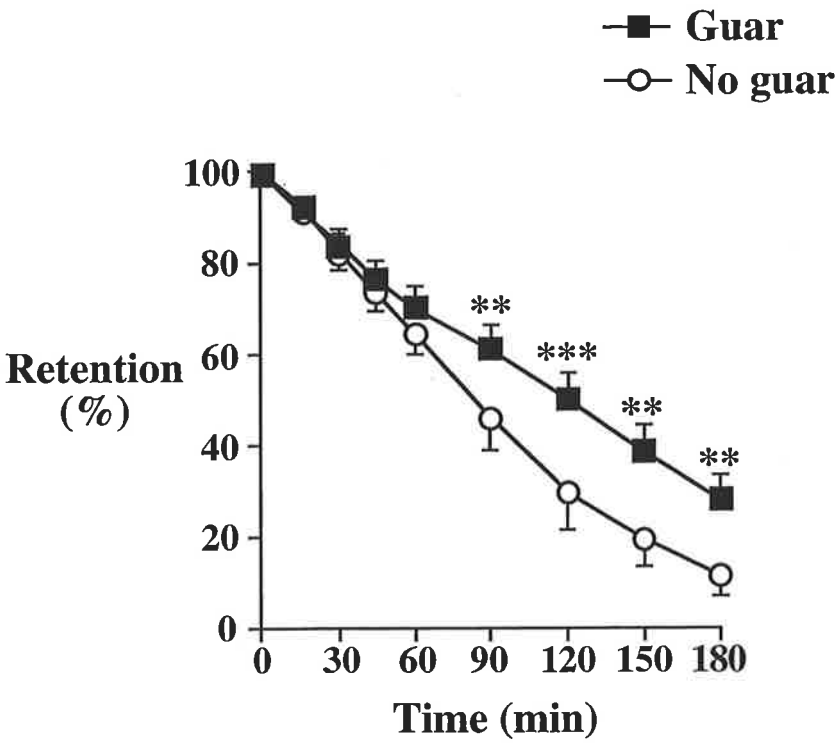

Figure 14.2: Gastric emptying after ingestion of 50 g glucose with or with out 9 g of guar gum in healthy older subjects. Data are mean ± SEM. *P< 0.05, **P< 0.01 and ***P< 0.001 guar vs no guar at individual timepoints.

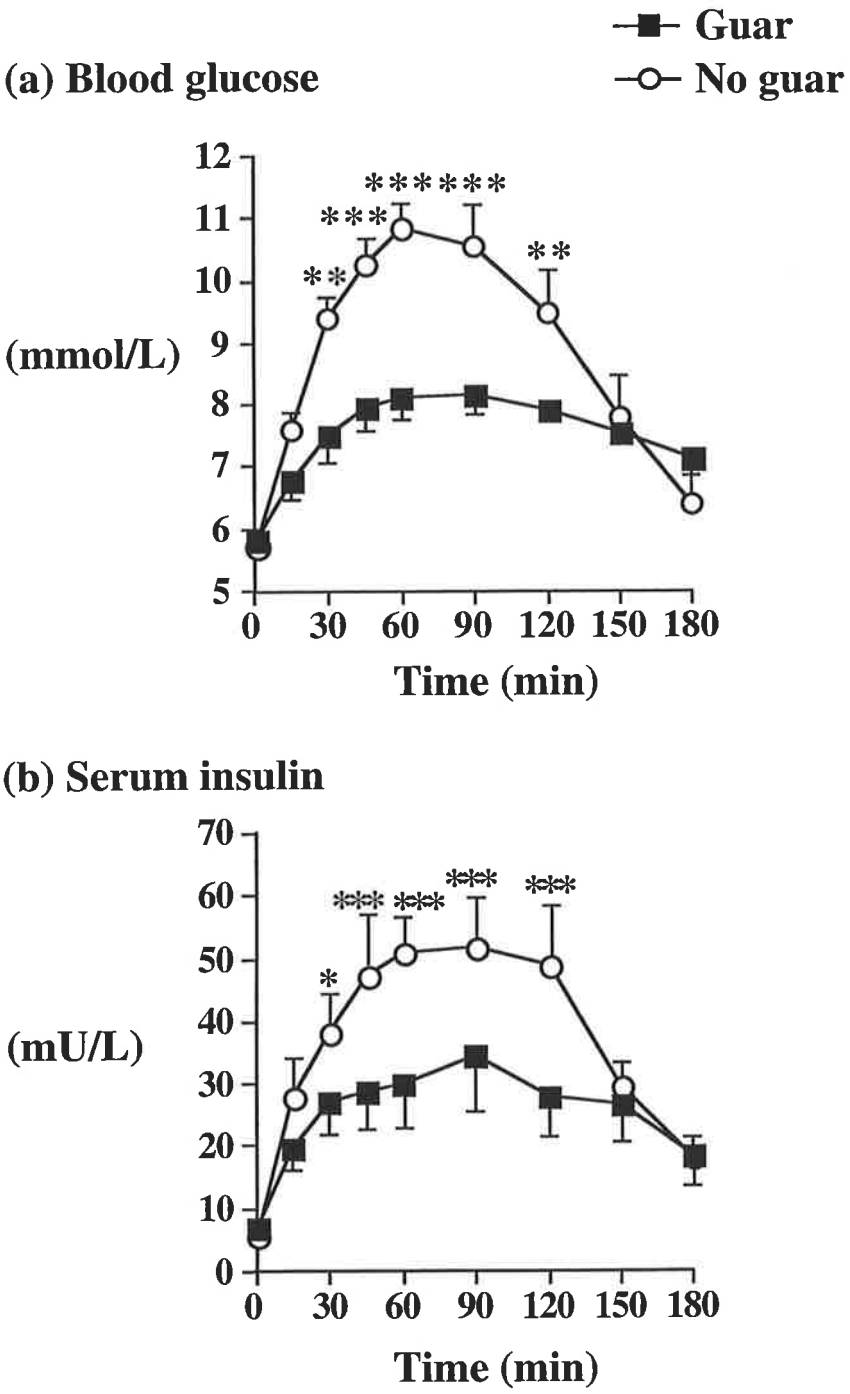

Figure 14.3: Blood glucose (a) and serum insulin (b) concentrations after ingestion of 50 g glucose with or with out 9 g of guar gum in healthy older subjects. Data are mean ± SEM. *P< 0.05, **P< 0.01, ***P< 0.001 guar vs no guar at individual timepoints.

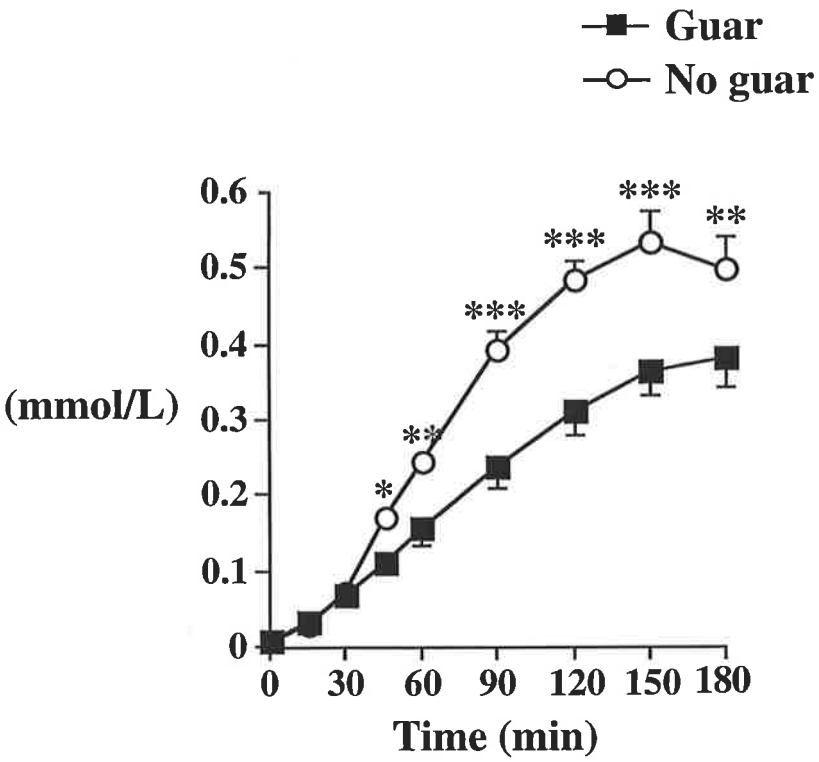

Figure 14.4: Serum 3-OMG concentrations after ingestion of 50 g glucose with or with out 9 g of guar gum in healthy older subjects. Data are mean ± SEM. *P< 0.05, **P< 0.01, ***P< 0.001 guar vs no guar at individual timepoints.

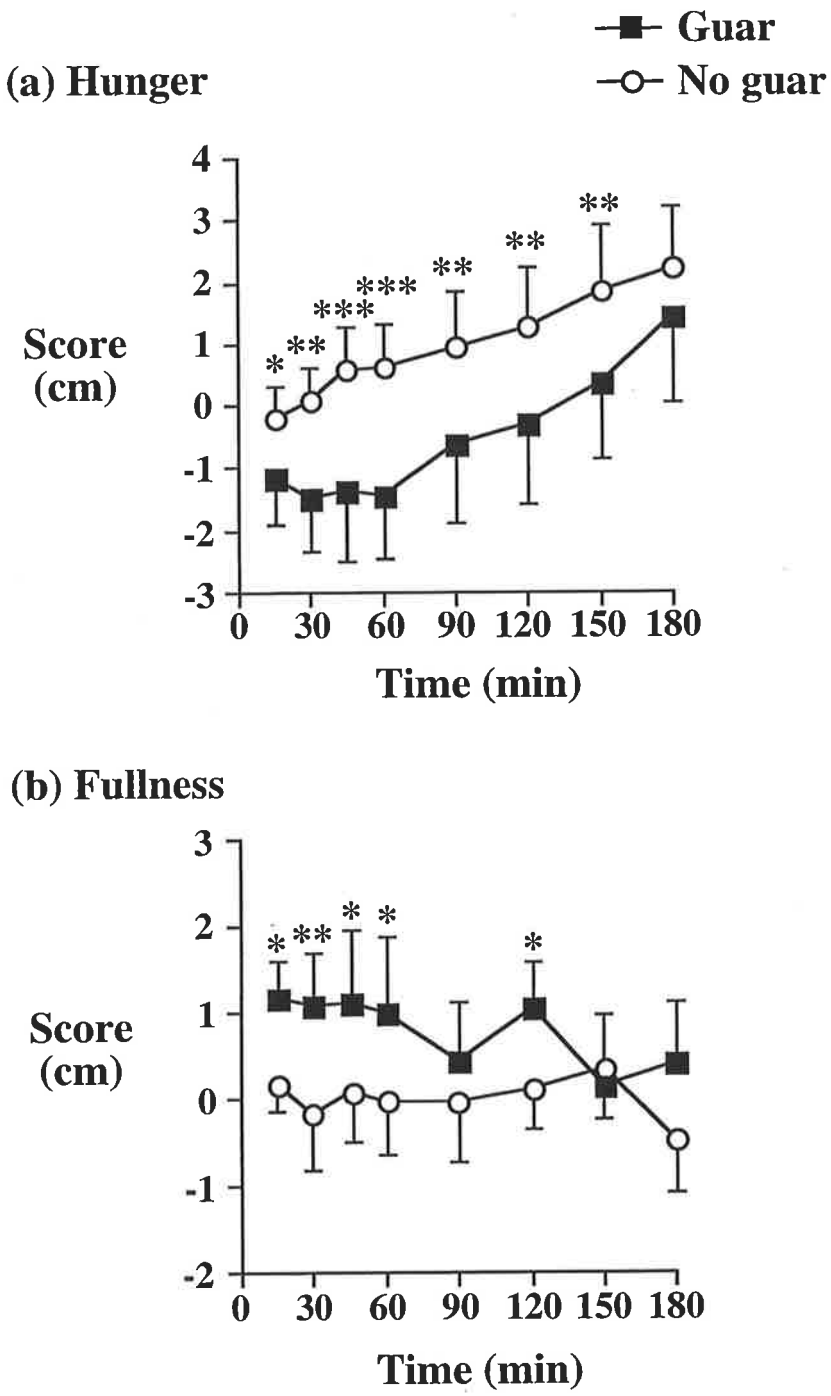

Figure 14.5: Change in ratings of hunger (a) and fullness (b) from baseline during the first 30 min after ingestion of 50 g glucose with or with out 9 g of guar gum in healthy older subjects. Data are mean ± SEM. *P< 0.05, **P< 0.01,***P< 0.001 guar vs no guar at individual timepoints.

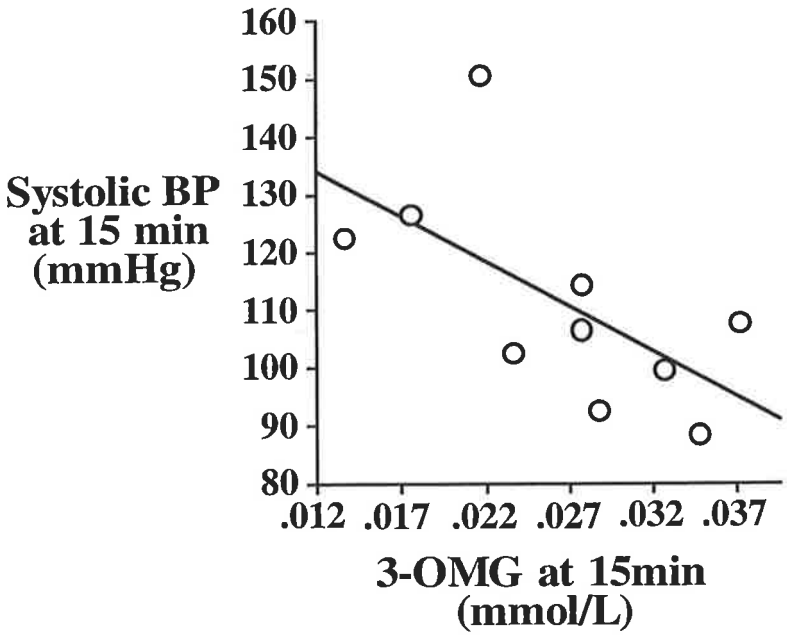

Figure 14.6: Relationship between systolic blood pressure (BP) and oral glucose absorption (3-OMG) 15 minutes after ingestion of 50 g glucose without guar gum. ($r = -0.64$, $P < 0.05$).

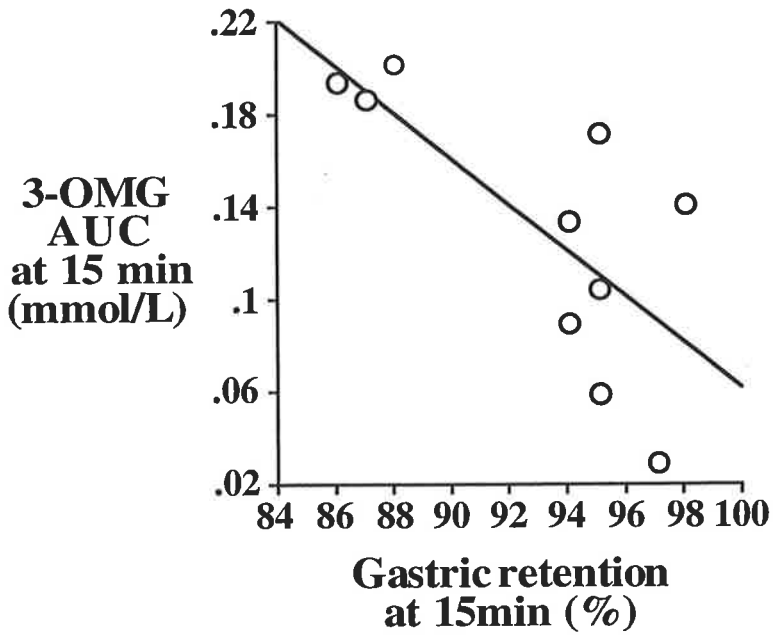

Figure 14.7: Relationship between oral glucose absorption (3-OMG) and gastric emptying 15 minutes after ingestion of 50 g glucose without guar gum. ($r = -0.72$, $P < 0.05$).

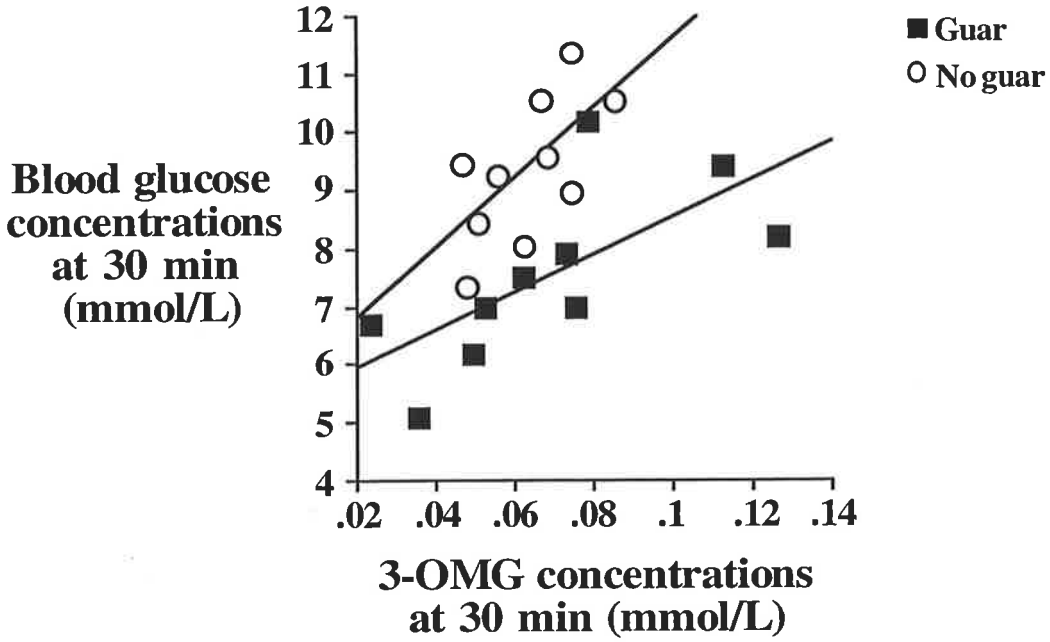

Figure 14.8: Relationship between blood glucose concentrations and oral glucose absorption (3-OMG) 30 minutes after ingestion of 50 g glucose with (r= 0.64, P= 0.05) and without (r= 0.69, P= 0.03) guar gum.

14.5 DISCUSSION

Our study establishes that administration of guar gum slows gastric emptying and glucose absorption and reduces the fall in blood pressure after oral glucose in healthy, older subjects. This latter observation has substantial implications for the management of postprandial hypotension.

Current treatment therapies for symptomatic postprandial hypotension have been shown to be less than optimal (Jansen & Lipsitz 1995). (see Chapter 5.6). The use of guar gum to a meal potentially represents a relatively simple and non-invasive approach to the treatment of postprandial hypotension.

The potential impact of the rate of gastric emptying and small intestinal nutrient absorption on postprandial hypotension had not been examined until a recent study by our group (Jones et al 1998). This confirmed that, following ingestion of 75g glucose, the fall in blood pressure is greater in healthy "older" normal subjects and patients with recently diagnosed type 2 diabetes mellitus managed with diet, when compared to "younger" normal subjects. The fall in blood pressure was shown to be related to the rate of gastric emptying of glucose in patients with type 2 diabetes mellitus (Jones et al 1998) (see Chapter 5.5 and Figure 5.2). The current study provides support for the hypothesis that gastric emptying and small intestinal nutrient absorption are important determinants of postprandial hypotension. While the slowing of gastric emptying by guar was associated with a reduction in the rate of small intestinal absorption of glucose, there was little, if any, difference in gastric emptying rate between the two drinks in the first 30 minutes, when the maximum fall in blood pressure was evident. In contrast, blood glucose and plasma insulin were significantly less after guar in the first 30 minutes and differences in oral glucose absorption were evident at 45 minutes. These observations suggest that the effect of guar on blood pressure, at least in this model, is related primarily to a reduction in the rate of glucose absorption from the lumen of the small intestine, rather than a slower rate of glucose delivery to the small intestine. The slowing of small intestinal absorption by guar may be attributable to increased viscosity of the luminal content (Blackburn et al 1984, Lavin et al 1995), or delayed access of glucose to the intestinal epithelium, thereby increasing the time that the nutrient remains in the small intestine to interact with receptors. An initial reduction in the length of small intestine exposed to the nutrient may also play an important role (Lin et al 1995a).

Our observations establish a role for the rate of nutrient absorption as a determinant of the postprandial fall in blood pressure in older subjects; there was a significant correlation between the fall in systolic blood pressure and serum 3-OMG levels after ingestion of the drink without guar.

The beneficial effects of guar on blood pressure may also potentially relate to changes in the secretion of insulin or other gastrointestinal hormones. While we cannot exclude this possibility, previous studies suggest that any role for insulin is likely to be minor (Jansen & Hoefnagels 1987)(see Chapter 5.5).

Our study also provides novel information about the determinants of postprandial blood glucose concentrations; the latter potentially include the rate of gastric emptying, small intestinal absorption and hepatic glucose metabolism. Studies in both normal subjects (Horowitz et al 1993) and those with type 2 diabetes (Jones et al 1996) indicate that the rate of gastric emptying accounts for about 36% of the variance in blood glucose concentrations after a 75g glucose load. The observation that slowing of gastric emptying improves glycaemic control in type 2 diabetes (Phillips et al 1993), also supports a substantial role for gastric emptying in postprandial blood glucose homeostasis. Other groups (Frank et al 1995) have however, suggested that hepatic glucose release is the most important determinant of postprandial blood glucose concentrations (Frank et al 1995). In our previous studies (Horowitz et al 1993, Jones et al 1996) and those of others (Schwartz et al 1995, Phillips et al 1993, Frank et al 1995), oral glucose absorption was not measured. The current study has established that there is a strong relationship between serum 3-OMG and both the rate of gastric emptying and blood glucose concentrations after oral glucose (accounting for 41-48% of the variance in glucose levels). These observations indicate that the rate of gastric emptying is a major determinant of both the rate of glucose absorption and postprandial blood glucose concentrations; at least in the first 60 minutes after glucose.

We studied healthy older individuals, who had no history of autonomic neuropathy, other conditions associated with autonomic neuropathy and were not taking medications that would influence autonomic function. The finding that five of the ten subjects had evidence of autonomic dysfunction using criteria adjusted for age (Piha 1991) is not totally unexpected (Clarkston et al 1997), as it is well recognised that ageing is associated with a reduction in both parasympathetic control of heart rate and maintenance of blood pressure on standing (Tonkin & Wing 1994).

The observation of a decrease in hunger and increase in fullness after guar are consistent with previous studies (Lavin et al 1995). The changes in appetite may be attributable to increased exposure of nutrients with small intestinal chemoreceptors as a result of delayed small intestinal absorption (Lavin et al 1995) (see Chapter 3.5.1).

In conclusion, this study has shown that guar gum reduces the magnitude of the fall in blood pressure after a glucose load in older subjects. These observations suggest that dietary modification may represent a novel, non-invasive, therapeutic approach to the treatment of patients with symptomatic postprandial hypotension. Gastric emptying and the rate of nutrient absorption can be slowed by dietary modification in a number of ways (French & Read 1994, Welch et al 1987). For example, in healthy subjects, the addition of corn oil to a meal of mashed potato slows gastric emptying, thereby reducing postprandial blood glucose and insulin concentrations (Welch et al 1987). Similarly, the rate of alcohol absorption is much slower when an alcohol-containing drink is consumed either with, or after, a solid meal, because gastric emptying is slower (Horowitz et al 1989). Studies designed to evaluate the effects of other dietary modifications on postprandial blood pressure are now indicated.

CHAPTER 15

Prevalence of Malnutrition in a Population of 250 Elderly Domiciliary Care Recipients in South Australia

15.1 SUMMARY

Although malnutrition is a common clinical problem in the elderly, it remains largely unrecognised in community-dwelling persons. The aim of this study was to determine the prevalence of malnutrition in a sample of elderly individuals who receive publicly-funded domiciliary care as assessed by the Mini Nutritional Assessment

(MNA), and those factors associated which are associated with a low MNA score in these subjects.

A total of 250 persons, who had registered with the Eastern Domiciliary Care Service in Adelaide, South Australia, between July 1999 and February 2000, took part in the study (73 men and 177 women; mean age 78.9 ± 0.42 yrs, range 67-99 yrs). The Mini-Nutritional Assessment (MNA) was used to evaluate the risk of malnutrition. Quality of life was determined by the SF-36 Health Survey®(SF-36). Cognitive ability was assessed using the Standardised Mini-Mental State Examination (SMMSE). The Geriatric Depression Scale (GDS) was used to assess depression. Potential 'risk factors' for impaired nutritional status that were evaluated included living status (ie alone/spouse or other), the amount of domiciliary care/ formal care (hr/month) received, medical illness, number and type of medications, and recent hospital admissions (number of days within the last 12 months) were also recorded. Associations between scores on the MNA, the SF-36, SMMSE, GDS as well as the other 'risk' listed above were determined.

Of the 250 subjects, 142 (56.8%) were assessed by the MNA as being well nourished, 96 (38.4%) were at risk for malnutrition and 12 (4.8%) were malnourished. Previous diagnosis of respiratory disorder ($P< 0.001$), receipt of 'Meals on Wheels' ($P< 0.05$), a greater number of days spent in hospital in the past 12 months ($P< 0.05$), role limitation due to emotional problems [SF-36 (RE)] ($P< 0.01$), impaired physical functioning [SF-36 (PF)] ($P< 0.001$) and a lower perception of general health [SF-36 (GH)]($P< 0.01$) and of mental health [SF-36 (MH)] ($P< 0.0001$), were all independent predictors of poor nutritional status.

The prevalence of being 'at risk' of malnutrition was high in this population, therefore the 'functionally dependent' community-dwelling elderly may represent a subset of the elderly population who may benefit from routine screening for prevention and/or treatment of malnutrition.

15.2 INTRODUCTION

Protein-energy malnutrition (PEM) is a common problem in the elderly, with up to 15% of community-dwelling older persons, 5-12% of home-bound patients, 20-65% of hospitalised patients and 5-85% of nursing home residents in the USA reportedly

suffering from the condition according to one review of multiple studies (Mion et al 1994) (see Chapter 1.7). In Australia, between 15-50% of hospitalised patients are malnourished (see Chapter 1.7). PEM is a major cause of increased hospitalisation and mortality amongst the older population (see Chapter 1.7).

Most surveys of the prevalence of malnutrition, conducted both overseas and in Australia, have focussed on the institutionalised elderly, even though most elderly people are not dependent on institutionalised care (see Chapter 1.7.1).

Domiciliary care services are used to help elderly people who have been afflicted by moderate or severe functional limitations or disabilities (ie 'functionally dependent') remain at home. In South Australia some 10% of persons over the age of 65 yrs receive some kind of publicly funded in-home care (Australian Bureau of Statistics 1997). This includes Domiciliary care (including the provision of equipment, house-cleaning, assistance with shopping and assistance with personal care, medication supervision and meal preparation) and "Meals on Wheels" (ie. pre-cooked, home-delivered meal, usually sufficient for 5 main meals per week) (Australian Society for Geriatric Medicine 1997). There is some evidence suggest that the 'functionally dependent' elderly may be at high-risk for malnutrition (Payette et al 1995), and that an increased dependency in activities of daily living is associated with reductions in dietary quality (Sem et al 1988) and nutrient intake (Bianchetti et al 1990) (see Chapter 1.7.1). If so, it would suggest that this group represents a subset of the elderly population that could be targeted for both prevention or treatment of malnutrition. There is, however, currently little information about the nutritional status of 'community dwelling' elderly persons in Australia.

The Mini-Nutritional Assessment (MNA) is a well-validated screening tool for the detection of malnutrition in older persons short of performing a detailed biochemical and clinical evaluation and considered is the most appropriate method to assess nutritional status in the sample population in this study (see Chapter 6.5.3.3 for summary of the development and validation of the MNA). Scheirlinckx et al (1998) who studied a group of 330 older people in varying states of health, proposed that a MNA score of 27/30 or greater is a reliable reference value for 'successful ageing'.

The aim of this study was to determine the prevalence of malnutrition as defined by the MNA in a sample of elderly individuals who receive domiciliary care.

Associations between MNA scores and potential risk factors for impaired nutritional status including living status, formal care received, chronic disease conditions, number of medications, recent hospital admissions, health status (assessed using the SF-36 Health Survey®), cognitive ability (assessed using the Standardised Mini-Mental State Examination), and depression (assessed using the Geriatric Depression Scale) were also determined.

15.3 SUBJECTS AND METHODS

15.3.1 *Study Population*

A total of 250 recipients of Domiciliary Care participated in the study. This sample population consisted of 173 females and 77 males aged 67-99 yr (mean age 78.9 yr); with a body mass index (BMI) range of 14.0-44.4 kg/m^2 (mean 26.1 kg/m^2); 144 (58%) lived alone, 86 (34%) lived with partners and 20 (8%) lived with other people (eg: son or daughter).

The names and phone numbers of all subjects (n= 939) who had registered with the Eastern Domiciliary Care Service in Adelaide, South Australia, between July 1999 and February 2000, were obtained from the Eastern Domiciliary Care Service (EDCS) database with the permission of the Clinical Programs Committee of the Royal Adelaide Hospital. Those subjects who were under the age of 65 yr, non-English-speaking and without access to an interpreter, or had been clinically diagnosed with dementia were excluded. Of the remaining 618 people, 598 subjects were sent an information sheet, inviting them to participate in the study and were subsequently contacted by telephone to confirm they participation. Of these 598 subjects (43%) elected not to participate, and another 15% either could not be contacted, were hospitalised or in a nursing home or hospice, or were deceased. Those persons who were agreeable (41%) were interviewed in their homes.

Age, body weight, height and current smoking were recorded and nutritional status, health status and quality of life, cognitive state and depression were assessed using validated questionnaires (see below). Subjects responses to specific questions relating to food intake from the MNA were determined [ie number of full meals eaten per day, selected consumption markers for protein intake (ie whether they consumed at least one serving of dairy products per day; two or more servings of legumes or eggs per

week; and meat, fish or poultry every day), consumption of two or more servings of fruits or vegetable per day and consumption of fluids (water, coffee, tea, juice, milk etc.) per day (ie < 3 cups, 3-5 cups or >5 cups)]. The amount of formal care (hr/month), receipt of "Meals on Wheels", number of medications used per day (prescribed and over the counter), and hospital admissions within the last 12 months were recorded. Subjects' medical history was primarily obtained from the subject, supplemented by review of individuals' Eastern Domiciliary Care case-file to determine the prevalence of previous diagnosis of medical disorders known to influence nutritional status in the elderly; they include cardiovascular disorders (including myocardial infarction, atherosclerosis); stroke; gastrointestinal (GI) disorders (including gastro-oesophageal reflux disease, diverticulitis, irritable-bowel syndrome, and GI surgery); cancer (excluding melanoma); osteoporosis; respiratory disorders (including asthma, emphysema and chronic obstructive airway disease); fractured hip; Parkinson's disease and depression.

Subjects were classified as well nourished, at risk of malnutrition or malnourished according to the MNA score. Associations between the subjects MNA score and their scores on the SF-36, SMMSE, GDS as well as history of specific medical disorders and the other 'risk' factors listed above were determined.

15.3.2 *Mini-Nutritional Assessment*

The Mini-Nutritional Assessment (MNA) (Appendix BIV) was used to assess nutritional status (Guigoz et al 1994, Guigoz et al 1996) (see Chapter 6.5.3.3 for a summary of the development and validation of the MNA). The score on the MNA (maximum of 30) were classified as follows: >24 normal or well-nourished, 17-23.5 at risk for malnutrition, and <17 malnourished.

15.3.3 Standardised Mini-Mental State Examination

The Standardised Mini Mental State Examination (SMMSE) (Appendix BVII) (Molloy et al 1991), a standardised version of the Mini-Mental State Examination developed by Folstein & McHugh (1975), was used to assess cognitive impairment or dementia in this study (see Chapter 6.4.3 for summary and validation of the SMMSE). 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 et al 1991).

15.3.4 Geriatric Depression Scale

The Geriatric Depression Scale (GDS) (Appendix BVI) was used to assess self-reported depression (Yesavage 1988) (see Chapter 6.4.2 for summary and validation of the GDS). A score ≥ 15 on the GDS is an indicative of depression (Yesavage 1988).

15.3.5 SF-36 Health Survey®

The 36-item short-form (SF-36) Healthy Survey® (Appendix BVIII) was used to assess health status and quality of life (see Chapter 6.4.4 for summary and validation of the SF-36). The SF-36 consists of a multi-item scale which assesses 8 health concepts; 1) limitations in physical functioning (SF-36-PF) because of health problems; 2) role limitations due to physical problems (SF-36-RP); 3) bodily pain (SF-36-BP); 4) role limitations due to emotional problems (SF-36-RE) 5) general mental health (SF-36-MH) (psychological stress and well being); 6) limitations in social functioning (SF-36-SF) because of health problems; 7) vitality (energy and fatigue)(SF-36-VT); and 8) general health perceptions (SF-36-GH). From the 8 health concepts an overall score for physical (SF-36-PCS) (0-100) and mental (SF-36 MCS) (0-100) health status can be calculated [for review see (Ware et al 1992)].

15.3.6 Statistical Analysis

Mean values ± SEM for subject variables, including age, BMI, 'risk factors' and SF-36 Health Survey® (SF-36) (scores for the 8 health concepts as well as the mean physical and mental component scores), the standardised Mini-Mental State Examination (SMMSE), and Geriatric Depression Scale (GDS) scores in well-nourished, 'at risk of malnutrition' and 'malnourished' were derived using Statview 5.0 (Xyris software). Associations between MNA score and risk factors and SF-36, SMMSE, and GDS scores were assessed by univariate analysis in order to identify subjects characteristics for inclusion in a multivariate analysis. The final statistical model yielded independent predictors of nutritional status. A P value < 0.05 was considered significant in all analyses.

15.4 RESULTS

Data from 250 (77 men and 173 women) out of a total of 618 Eastern Domiciliary Care clients who were eligible to participate in the survey were analysed, representing a 40.5% response rate.

Compared to the total Domiciliary Care population (n= 618) who were eligible to participate in this study, the sample population in this study was of comparable age (80.2 ± 6.9 yr vs 78.9 ± 6.6 yr; P = 0.99); gender (proportion of men and women; 34% and 66% vs 31% and 69%; P = 0.52) and living status (proportion living alone, with a spouse or other; 46%, 42% and 12%, respectively vs 58%, 34% and 8%; P= 0.06).

15.4.1 *Comparison of parameters between men and women*

Table 1 summarises data for MNA scores, socio-demograpic data, body mass index (BMI), and health and functional parameters (current smoking, number of medications, formal care, receipt of Meals on Wheels, hospital admissions in the past 12 months, medical history, SF-36 scores, SMMSE and GDS scores of men and women in the sample population.

There was no significant difference in the mean MNA score between men and women (P= 0.25). Of the men, 48 (62.3%) were well-nourished, 28 (36.4%) were at risk of malnutrition and 1 (1.3%) were malnourished and of the women 94 (54.3%) were well-nourished, 68 (39.3%) were at risk of malnutrition and 11 (6.4%) were malnourished. There was no effect of gender on the MNA category (P= 0.17).

There was no significant difference between the men and women in age (P= 0.28), body mass index (P= 0.91), current smoking (P= 0.99), number of medications (P= 0.12), total number of hours of formal care (P= 0.23), or receipt of 'Meals on Wheels' (P= 0.10). There was trend for an effect of gender on the number of days spent in hospital in the past 12 months (P= 0.09); ie men spent more time in hospital than women.

There was no significant difference between the number of men and women who had a previous diagnosis of cardiovascular (CV) disorder (P= 0.77), diabetes (P= 0.38), Parkinson's disease (P= 1.00), depression P= 0.84), respiratory disorder (P= 0.71), or gastrointestinal (GI) disorder (P= 1.00). Previous diagnosis of stroke (P= 0.009) and cancer (P= 0.046) was more common in men than women, whereas previous diagnosis of osteoporosis (P< 0.0001) and fractured hip (P= 0.006) was more common in women than men.

There was no significant difference in scores on the Standardised Mini-Mental State Examination (SMMSE) between men and women (P= 0.34) and no significant effect of gender on the degree cognitive impairment (P= 1.00).
There was no significant difference in mean GDS score between men and women (P= 0.30) and no effect of gender on those subjects who were depressed or not depressed according to the GDS (P= 1.00).

There was no difference between men and women in the SF-36 domains of general health perception (P= 0.99), social functioning (P= 0.17), role limitation due to emotional problems (P= 0.39), mental health (P= 0.38), or the overall mental component score (P= 0.80). Women scored significantly less than men on the SF-36 domains of physical functioning (P< 0.0002); bodily pain (P= 0.002); role limitation due to physical problems (P= 0.046); and the overall physical component score (P< 0.001) and there was a trend for women to score less than men on the SF-36 domain of vitality (P= 0.07).

Table 1: Comparison of nutritional status and other parameters assessed between men and women (mean ± SEM)

	Men	Women	P value	Group Total
N	77	173	-	250
Age (yr)	23.5 ± 0.3	24.0 ± 0.3	0.28	23.6 ± 0.2
Mean MNA score	78.2 ± 0.4	79.2 ± 0.5	0.25	78.9 ± 0.4
Body mass index (BMI; kg/m^2)	26.1 ± 0.4	26.0 ± 0.4	0.91	26.1 ± 0.5
current smoking (%)	4 (5.2)	3 (1.7)	-	7 (2.8)
Live alone (%) #	33.8	68.2	<0.0001	57.6
Number of medications	4.6 ± 0.3	5.2 ± 0.2	0.12	5.0 ± 0.2
Formal Care (hr/month)	2.6 ± 0.6	2.6 ± 0.5	0.23	2.6 ± 0.4
Receipt of Meals on Wheels (%)	12.0	20.2	0.10	17.6
Hospital admis. (days in last 12 months)	19.6 ± 2.7	14.3 ± 1.7	0.09	15.9 ± 1.4
Previous diagnosis of CV disorder (%)	67.5	67.4	0.77	65.6
Previous diagnosis of stroke (%) #	29.9	15.0	0.009	19.6
Previous diagnosis of GI disorder (%)	20.2	19.5	1.00	20.0
Previous diagnosis of cancer (%) #	24.7	13.9	0.046	17.2
Previous diagnosis of osteoporosis (%) #	5.2	31.8	< 0.0001	23.6
Previous diagnosis of diabetes (%)	20.8	16.2	0.37	17.6

Table 1 cont:

	Men	Women	P value	Group Total
Previous diagnosis of respiratory disorder (%)	22.1	24.3	0.71	23.6
Previous diagnosis of fractured hip (%) #	1.3	11.6	0.006	8.4
Previous diagnosis of Parkinson's disease (%)	5.2	4.6	1.00	4.8
Previous diagnosis of depression (%)	14.3	13.3	0.84	13.6
SF-36 (PF) #	32.8 ± 1.4	27.8 ± 0.6	< 0.001	29.4 ± 0.6
SF-36 (RP) #	40.9 ± 0.8	38.1 ± 1.3	0.046	39.0 ± 0.8
SF-36 (BP) #	46.6 ± 1.5	41.1 ± 1.0	0.002	42.8 ± 0.8
SF-36 (RE)	49.6 ± 1.0	27.8 ± 0.5	0.99	50.3 ± 0.5
SF-36 (MH)	51.3 ± 1.2	50.0 ± 0.9	0.38	50.4 ± 0.7
SF-36 (SF)	45.5 ± 1.5	43.2 ± 0.9	0.17	43.9 ± 0.8
SF-36 (VT)	42.9 ± 1.3	40.3 ± 0.7	0.07	41.1 ± 0.6
SF-36 (GH)	40.8 ± 1.3	40.8 ± 0.8	0.99	40.8 ± 0.7
SF-36 (PCS) #	35.6 ± 1.2	31.0 ± 0.7	<0.001	32.4 ± 0.6
SF-36 (MCS)	53.4 ± 1.0	53.7 ± 0.7	0.80	53.6 ± 0.6
SMMSE score	26.7 ± 0.3	26.9 ± 0.2	0.34	26.8 ± 0.2
GDS score	8.3 ± 0.6	9.1 ± 0.5	0.30	8.8 ± 0.4

#P< 0.05 men vs women

PF = physical functioning; RP = role limitation due to physical problems; BP= bodily pain; RE = role limitation due to emotional problems; MH = mental health; SF = social functioning; VT = vitality; GH = general health; PCS = physical component score; MCS = mental component score.

15.4.2. *Associations between MNA score and MNA category and other parameters assessed*

Table 2 summarises parameters within the three MNA categories (well nourished, at risk of malnutrition and malnourished). The groups were compared in terms of the different potential variables.

In total, 142 (56.8%) were well nourished, 96 (38.4%) were at risk for malnutrition and 12 (4.8%) were malnourished, as assessed by the MNA.

According to the specific responses on the MNA, there was a significant difference in the number of full meals consumed per day between those who were well-nourished, at risk of malnutrition and malnourished (mean 2.25 ± 0.14 vs 1.98 ± 0.07 vs 1.5 ± 0.15 full meals/day, respectively; $P< 0.0002$), and a significant difference between the MNA categories in the number of full meals eaten per day ie 15.1% of well-nourished, 28.1% of subjects at risk of malnutrition and 50% of malnourished subjects ate only 1 full meal per day. There was a significant difference between the MNA categories in two of the three responses for selected consumption markers for protein intake ie 11% of well-nourished, 17.7% of subjects at risk of malnutrition and 25.0% malnourished did not consume at least one serving of dairy products per day ($P< 0.01$), and 19.8% of well-nourished, 28.0% of subjects at risk of malnutrition and 58.3% malnourished did not consume meat, fish or poultry every day ($P< 0.001$); but there was no difference between the MNA categories in the consumption of legumes or eggs ($P= 0.42$), ie 32.2% of well-nourished, 39.6% of subjects at risk of malnutrition and 33.3% malnourished consumed less than two or more servings of legumes or eggs per week. There was a significant difference between the MNA categories in the consumption of fruits and vegetables ($P< 0.001$); ie 4.8% of well-nourished, 13.5% of subjects at risk of malnutrition and 33.3% malnourished consumed less two or more servings of fruits or vegetable per day. There was also a significant difference between the MNA categories in fluid consumption ($P< 0.05$; well-nourished vs at risk and malnourished subjects); ie 23.3% of well-nourished, 36.5% of subjects at risk of malnutrition and 33.3% malnourished consumed less than 5 cups of fluids per day.

Univariate Analysis revealed the following associations between the MNA score and subject characteristics nutritional status (ie MNA category of well nourished, at risk of malnutrition or malnourished).

15.4.2.1 Socio-demographic data

There was no significant association between MNA score (P= 0.60) and age, and there was no significant difference in age of subjects between the MNA categories (P= 0.09; a trend for malnourished subjects to be younger than subjects who were well-nourished and 'at risk' of malnutrition). There was no significant association between the MNA score and those recipients who lived alone, with their partner or with others and MNA score (P= 0.12; a trend for subjects with a lower MNA to live alone), nor any difference in living arrangements among well nourished, at risk, and malnourished recipients (P= 0.12; a trend for subjects at risk of malnutrition to live alone) (Table 2).

15.4.2.2 Body mass index (BMI)

There was a significant positive association between MNA score and body mass index (BMI), ie as BMI increased by 1 kg/m^2, the MNA score increased by 0.22 points (P< 0.0001), as well as a significant difference in (BMI) between recipients who were well nourished, at risk of malnutrition and malnourished (P< 0.0001)(see Table 2).

15.4.2.3 Health and functional parameters

There was an inverse association between MNA score and the number of prescription medications taken per day, so that an increase in the number of medications by 1, was associated with a decrease in the MNA score by 0.43 points (P< 0.0001). There was also a significant difference between the MNA categories in the number of prescription medications subjects took every day (P< 0.0001); at risk and malnourished recipients took more medications per day than well-nourished recipients (Table 2). When the MNA score was corrected for the points attributable to the number of medications (ie > 3/day vs < 3/day), the association between MNA score and subjects the number medications per day remained significant (P< 0.01).

There was no significant association between either the MNA score or the MNA category and previous diagnosis of stroke (P= 0.96 and P= 0.71, respectively) or Parkinson's disease (P= 0.53 and P= 0.30, respectively). While there was no significant association between the MNA score, or the MNA category, and previous diagnosis of cardiovascular disorder (P= 0.56 and P= 0.13, respectively), there was a trend (P= 0.13) for a greater incidence of cardiovascular disorder in the well nourished and at risk than malnourished subjects (Table 2). There was no significant

association between the MNA score, or the MNA category, and previous diagnosis of fractured hip (P= 0.48 and P= 0.48, respectively), or cancer (P= 0.12 and P= 0.20, respectively), but there was a trend for a greater incidence of osteoporosis (P= 0.07 and P= 0.09, respectively), in malnourished subjects than in those who were well nourished and at risk of malnutrition (Table 2). There was no significant association between the MNA score, or the MNA category, and previous diagnosis of depression (P= 0.14 and P= 0.62, respectively), but there was a trend (P= 0.14) for a greater incidence of depression in those subjects who were at risk of malnutrition or malnourished than well nourished subjects (see Table 2). Although there was no significant association between the MNA score and previous diagnosis of diabetes mellitus (P= 0.27), there was a significant association between the MNA category and previous diagnosis of diabetes (P< 0.01); so that those subjects who had been diagnosed with diabetes were more likely to be well nourished. There was a significant association between both the MNA score and the MNA category with previous diagnosis of respiratory (P< 0.001 and P< 0.01, respectively), as well as GI (P< 0.01 and P= 0.05, respectively) disorder; those subjects who had a previous diagnosis of respiratory disorder or GI disorder were more likely to be at risk of malnutrition or malnourished than well nourished.

There was a trend for an association between the MNA score and the total number of hours of formal care received by the subjects (P= 0.06), and a significant difference in the total number of hours of formal care per month between those who were well nourished, at risk of malnutrition and malnourished (P< 0.05) (Table 2).

There was a small, but significant, difference in the mean MNA score between those who received 'Meals on Wheels' and those who did not (ie 22.4 ± 0.5 vs 23.9 ± 0.2; P< 0.05), and a significant association between the MNA category and being in receipt of 'Meals on Wheels'; recipients who were at risk of malnutrition or malnourished were more likely to receive 'Meals on Wheels' than well nourished subjects (P< 0.01).

There was an inverse association between the MNA score and the number of days spent in hospital in the last 12 months; so that an increase in the number of days spent in hospital by 1, was associated with a decrease in the MNA score by 0.02 points (P< 0.05), There was a trend for a significant difference in the number of days spent in

hospital in the last 12 months between subjects who were well-nourished, at risk of malnutrition or malnourished (P= 0.06) (Table 2).

There was no significant association between MNA score and the score on the Standardised Mini-Mental State Examination (SMMSE) (P= 0.33), and there was no significant difference in median SMMSE score between well nourished, at risk and malnourished subjects (P= 0.98). There was also no significant association between the MNA score or MNA category, and the degree of cognitive impairment (P= 0.66 and P= 0.66, respectively).

There was a significant association between the MNA score and the score on the Geriatric Depression Scale (GDS) (P< 0.001) and a significant difference in mean GDS score between the MNA categories; ie. well nourished subjects had a lower GDS score than those at risk and malnourished subjects (P< 0.0001). In those subjects who were depressed [according to the GDS (score ≥ 15)] the MNA score was less than in those subjects who were not depressed (P< 0.0001), and were more likely to be at risk of malnutrition or malnourished (P< 0.0001) (see Table 2). When the MNA score was corrected for the points attributable to depression, the association between MNA score and depression remained highly significant with a mean corrected MNA score of 20.8 ± 0.5 in depressed subjects and 22.7 ± 0.2 in those subjects who were not depressed (P< 0.0001).

There was a significant association between both the MNA score and MNA category and all SF-36 domains of quality of life - except for bodily pain (P= 0.07 and P= 0.23, respectively). These domains comprised physical functioning, role limitation due to physical problems, general health perception, vitality, social functioning, role limitation due to emotional problems, and mental health (P< 0.0001 for both MNA score and category). (Table 2 lists specific comparisons between well nourished, at risk, and malnourished groups).

Table 2: Comparison of parameters between subjects classified as well-nourished, at risk of malnutrition or malnourished.

	MNA ≥ 24 (no risk of malnutrition)	**MNA 17-23.5 (at risk of malnutrition)**	**MNA < 17 (malnourished)**	**P value of statistical result**
N (%)	146 (56.8)	96 (38.4)	12 (4.8)	-
Age (yr)	78.4 ± 0.5	79.9 ± 0.7	76.2 ± 1.9	0.09
BMI (kg/m^2)	27.2 ± 0.4	25.3 ± 0.6	19.2 ± 1.0	< 0.0001
mean MNA score	26.0 ± 0.1	21.1 ± 0.2	15.3 ± 0.4	-
Live alone (%)	52.1	66.7	54.5	0.12
Number of medications*	4.3 ± 0.2	5.9 ± 0.3	6.5 ± 1.2	< 0.0001
Formal Care (hr/month)‡†	1.8 ± 0.3	3.1 ± 0.6	8.0 ± 5.3	< 0.05
Receive Meals on Wheels (%)*	11.6	25.8	27.3	< 0.01
Hospital admissions (days)	13.8 ± 1.8	17.9 ± 2.2	28.1 ± 11.8	0.06
Cardiovascular disorder (%)	69.9	61.3	45.5	0.12
GI disorder (%)*	15.1	26.9	27.3	0.05
Cancer (%)	15.1	18.3	36.4	0.20
Diabetes (%)*	13.7	25.8	0.0	< 0.05
Osteoporosis (%)	21.2	23.7	54.5	0.09
Respiratory disorder (%)*	16.4	33.3	36.4	< 0.01

Table 2 cont:

	MNA ≥ 24 (no risk of malnutrition)	MNA 17-23.5 (at risk of malnutrition)	MNA < 17 (malnourished)	P value of statistical result
Stroke (%)	19.9	20.4	9.1	0.71
Fractured hip (%)	7.5	8.6	18.2	0.48
Parkinson's disease	3.4	6.5	9.1	0.30
Past depression (%)	11.6	16.1	18.2	0.62
SF-36 (PF) *	31.4 ± 0.8	27.1 ± 1.0	20.9 ± 2.0	< 0.0001
SF-36 (RP) *	40.9 ± 0.9	36.9 ± 1.0	30.5 ± 2.7	< 0.01
SF-36 (BP)	44.0 ± 1.1	41.6 ± 1.4	37.0 ± 2.7	0.23
SF-36 (RE)*	51.8 ± 0.6	48.5 ± 0.9	45.3 ± 1.6	< 0.0001
SF-36 (MH)*	53.6 ± 0.7	46.5 ± 1.3	40.9 ± 3.7	< 0.0001
SF-36 (SF)*	47.0 ± 0.9	40.0 ± 1.3	36.5 ± 4.9	< 0.0001
SF-36 (VT)*	43.6 ± 0.9	38.1 ± 1.1	32.2 ± 1.4	< 0.0001
SF-36 (GH)*†	43.1 ± 0.8	38.7 ± 1.0	26.9 ± 2.6	< 0.0001
SF-36 (PCS)‡†	33.9 ± 0.6	31.0 ± 0.9	23.7 ± 1.1	< 0.01
SMMSE score	26.7 ± 0.2	26.9 ± 0.3	27.5 ± 0.5	0.98
GDS score*	7.0 ± 0.4	11.0 ± 0.6	13.7 ± 2.0	< 0.0001

*= at risk and malnourished vs well-nourished, ‡= well nourished and at risk of malnutrition vs malnourished; †= at risk of malnutrition vs malnourished.

PF = physical functioning; RP = role limitation due to physical problems; BP= bodily pain; RE = role limitation due to emotional problems; MH = mental health; SF = social functioning; VT = vitality; GH = general health; PCS = physical component score; MCS = mental component score.

15.4.3 *Independent predictors of nutritional status*

Those parameters which were significantly associated with the MNA score, or category, in the univariate analysis were entered into a multivariate analysis. The number of medications, BMI and depression were not entered into the multivariate analysis, as these factors contributed 2, 3 and 2 points on the MNA, respectively, and were expected to be a determinant of nutritional status in the subject group.

All parameters that were included in the multivariate analysis (ie previous diagnosis of respiratory disorder, receipt of 'Meals on Wheels', increase in number of days spent in hospital in the past 12 months, role limitation due to emotional problems [SF-36 (RE)], physical functioning [SF-36 (PF)], general health perception [SF-36 (GH)], and mental health [SF-36 (MH)]), except for previous diagnosis of GI disorder were independent predictors of the MNA score. Together, these parameters account for 36.3% of the variance in MNA scores.

Table 15.3: Parameters which were significantly associated with the MNA score, or category included in a multivariate analysis to determine the independent predictors of nutritional status

Parameter in multivariate analysis	P value
Previous diagnosis of respiratory disorder	$P < 0.01$
Previous diagnosis of GI disorder	$P = 0.09$ NS
Receipt of 'Meals on Wheels'	$P < 0.05$
Increase in number of days spent in hospital in the past 12 months	$P < 0.05$
Role Limitation due to Emotional Problems [SF-36 (RE)]	$P < 0.01$
Physical Functioning [SF-36 (PF)]	$P < 0.001$
General Health Perception [SF-36 (GH)]	$P < 0.01$
Mental Health [SF-36 (MH)]	$P < 0.0001$

15.5 DISCUSSION

This study provides a preliminary descriptive assessment of the nutritional status and related socio-demographic and health and functional parameters of 250 domiciliary care recipients in Adelaide, South Australia. The major findings were that (i) there was a high percentage of subjects who were at risk of malnutrition (38.4%) and a relatively low percentage of subjects who were malnourished (4.8%) and (ii) the independent predictors of poor nutritional status assessed in this survey were previous diagnosis of respiratory disorder, receipt of 'Meals on Wheels', increase in number of days spent in hospital in the past 12 months, role limitation due to emotional problems [SF-36 (RE)], physical functioning [SF-36 (PF)], general health perception [SF-36 (GH)], and mental health [SF-36 (MH)]).

According to the Mini-Nutritional Assessment, 56.8% of subjects in the sample population of Domiciliary care recipients were well nourished, 38.4% were at risk of malnutrition and 4.8% of recipients were malnourished. These findings are consistent with previous reports conducted in the United Kingdom, Europe and the United States of America in which the prevalence of malnutrition was generally between 2 and 15% in community-living older people (Mion et al 1994, Guigoz et al 1998), although a prevalence as high as 80% has been reported (Lehmann 1989). Compared to the study by Cobiac & Syrette (1995) (see Chapter 1.7.1), who assessed 'risk' of nutritional problems in a randomly selected sample of 940 non-institutionalised people over 70 yr living in Adelaide, using the ANSI checklist, the proportion of subjects at risk (moderate risk of nutritional problems) of malnutrition was lower (20.6% vs 38.4%) and the proportion of malnourished (at high risk of nutritional problems) was higher (30% vs 4.8%) than in our survey. Such inconsistencies may be related to the specific populations studied; ie random selection in that study vs selection of a specific population group in this study and/or to the method of assessing nutritional status, ie differences in the specific items assessed between the MNA and the ANSI checklist. For example, the MNA includes measurements of body mass index, mid-arm and calf anthropometric measurements, and questions relating to suffering severe psychological stress, depression or dementia, and self perception of nutritional status as well as the presence of pressure sores or skin ulcers are not part of the ANSI checklist. Conversley, the ANSI checklist which contained questions relating to excess alcohol consumption, inadequate income to buy food, and loss of independence in relation to shopping and cooking which are not part of the MNA.

Several potential limitations of this study should be recognised. Malnutrition is difficult to diagnose in the elderly, and there is no single method (ie anthropometry, biochemical markers, clinical evaluation and screening tools or questionnaires) which is ideal, or universally accepted (see Chapter 6.5). While the MNA is a well validated screening tool and for the purpose of this study was considered the most efficient and practical method of assessment, a combination of methods may provide a more precise nutritional assessment. There may have been a potential selection bias in the choice of the sample population; 368 of the remaining 618 subjects who were eligible elected not to participate. We specifically excluded those individuals who were non-English speaking, without access to an interpreter (11% of the population, n=939) or had been clinically diagnosed with dementia (12% of the population, n= 939) predominantly for logistical reasons. The prevalence of malnutrition in community-dwelling elderly in non-English speaking countries in Europe (Kuskowska-Wolk & Rossner 1990), is similar to that of the UK, USA and Australia (see Chapter 1.2). There is currently no documentation from either African or Asian non-English speaking countries in relation to the prevalence of malnutrition in the elderly. There is also a high prevalence of malnutrition in elderly persons with dementia (see Chapter 1.6.2). Accordingly, our observations cannot be extrapolated with confidence to these specific subject groups. Moreover, many previous studies, particularly in the USA (Mion et al 1994, Guigoz et al 1998), have included persons from different races whereas the majority of the subjects in this study were caucasian [ie 98.8% (with the rest either of asian, indian or aboriginal descent [0.4% each])]. Our observations cannot, therefore, be extrapolated to people of different races other than caucasian. Furthermore, there may have been a potential bias towards the inclusion of 'more robust' subjects in the survey, as ~16% (53 out of 323 subjects who were still alive or not in a nursing home) of those subjects who did not participate were either too ill, in hospital or could not be contacted and of those who were it is possible that the less rhobust were less inclined to take part in the survey. It is accordingly likely that the prevalence of malnutrition and at risk of malnutrition may have been underestimated. The source of medical information for this study was primarily provided by the subject, supplemented by Eastern Domiciliary Care case-file review. Such information may be incomplete, thus compromising meaningful statistical analysis.

Parameters, other than the MNA, assessed in this study ie age, BMI, medication use, living status, formal care, receipt of Meals on Wheels, hospital admissions in the past 12 months, previous diagnosis of cardiovascular disorders stroke; GI disorders;

cancer; osteoporosis; respiratory disorders; fractured hip; Parkinson's disease and depression (see section 15.3.1 for details), health status, cognitive ability and depression assessed in this survey have all been suggested as risk factors that may contribute to poor nutritional status (see Chapter 1.6).

The use of body mass index (BMI) as a single method for assessment of nutritional status, is limited, particularly in the elderly [for review see (Omran & Morley 2000a)]. This is likely to account for the relatively weak, albeit significant, association between MNA score and BMI after the MNA score the had been corrected for the points (a maximum of 3) attributed to this factor.

Medication use is a recognised risk factor for under-nutrition, both as a general marker of ill- health and because some medications may contribute to undernutrition (see Chapter 1.6.4.1). Medication may adversely affect appetite, swallowing, cognitive function, and nutrient absorption, metabolism and excretion (Omran and Morley 2000). It is not surprising, therefore, that we found a significant association between the MNA score and medication use, even after the MNA score had been corrected for the points (a maximum of 2) attributable to this factor.

Depression is common in the elderly and a well-recognised risk factor for malnutrition (see Chapter 1.6.2). Consistent with this, 15.4% of subjects in this study were classified as depressed [ie scored 15 or more on the Geriatric Depression Scale (GDS)] even after those with a pre-study diagnosis of depression were excluded and there was a strong association between MNA score and the score on the GDS, when the MNA score had been corrected for the points (a maximum of 2) attributable to this factor.

Home-delivered meal programs, such as "Meals on Wheels", are low-cost long-term care services that have potential to delay institutionalisation and maintain self-sufficiency and quality of life among community-dwelling frail elders (Choi 1999). The observation that receipt of "Meals on Wheels" was a independent predictor of poor nutritional status in this study is consistent with a previous study by Coulston et al (1996), who reported that among 230 Meals-on-wheels applicants (aged 65-90 yr) 74% were at risk for poor nutritional status according to anthropometric, dietary, and laboratory data. It is unlikely that Meals on Wheels decreases nutritional status, rather that the combination of other factors such as recent acute illness and admittance to

hospital may contribute to the lower score in these subjects. While the receipt of "Meals on Wheels" was identified as an independent predictor of poor nutritional status, the response may be less than optimal as the duration of Meals on Wheels was not, however, evaluated in this study. Nevertheless, these findings suggest that those individuals receiving Meals on Wheels may need additional nutrition assessment and intervention to remain independent and in good nutritional status.

The prevalence of malnutrition is high among hospitalised elderly people (McWhirter & Pennington, 1994; Mowe et al, 1994; and see Chapter 1.7.1) and there is also evidence that anorexia, weight loss and inadequate dietary intake may continue for prolonged periods following discharge from hospital for illnesses that tend to cause reduced nutritional status (Williams et al, 1990). Accordingly, it is not surprising that the number of hospital admissions in the past 12 months was an independent predictor of MNA score.

Under-nutrition is a well recognised association of chronic dyspnoea (see Chapter 1.6.4). The observation that a previous diagnosis of respiratory disorder was an independent predictor of MNA score is likely to reflect the additional energy requirement for the increased exertion of breathing, the fact that many of these patients may be too breathless to eat and the association with cigarette smoking (Perkins et al 1989). It is however uncertain why previous diagnosis of respiratory disorder was a stronger predictor of nutritional status than any other specific medical risk factor in this study. An association between MNA score and previous diagnosis of GI disorder was present, but this was not an independent predictor of nutritional status, perhaps because they were heterogeneous in presentation. Cancer, osteoporosis, fractured hip relating to falls, Parkinson's disease and depression are all conditions that have been recognised as factors contributing to, or caused by, malnutrition in the elderly (see Chapter 1.6) Although not significant, there were clear trends for associations between MNA score/category and these conditions in the elderly subjects in this study. The lack of significance for these associations was most likely a type 2 error.

Previous investigators have linked quality of life domains, specifically, physical functioning, general health perception, social functioning and mental health assessed using the SF-36 Health survey to nutritional status (Griep et al 2000). This study reports a significant association between physical functioning, general health

perception, role limitation due to emotional problems, mental health and nutritional status, compatible with the notion that health-related quality of life impacts upon nutritional status and, alternatively, that nutritional status is a strong determinant of quality of life.

The seven different parameters that were independent predictors of nutritional status accounted for approximately 36% of the variance, therefore other factors not assessed in this survey would have accounted for a large percentage of the variance in this sample population. This is consistent with the concept the factors contributing to malnutrition in the elderly are multiple (see Chapter 1.6).

A follow-up study is planned to evaluate subject outcome at 12 months, and to assess the value of the MNA in predicting future hospitalisation, institutionalisation, further weight-loss and death among 'functionally dependent' community living older people.

CHAPTER 16

Conclusions

The studies presented in Chapters 8-13 in this thesis provide insights into the potential mechanisms of the age-related 'physiological' reduction in appetite and food intake in humans.

The study described in Appendix A, the effects of intraduodenal (ID) infusion of lipid and glucose on appetite, pyloric motility and subsequent energy intake were evaluated in young and older healthy men. This initial study indicated that healthy elderly subjects were less sensitive to the effects of ID nutrients on appetite than young subjects - consistent with the concept that ageing is associated with an impairment in the homeostatic mechanisms which regulate feeding (Roberts et al 1995). It was demonstrated that energy intake was suppressed more following ID lipid than ID glucose in young subjects, whereas there was no difference between the effect of the two nutrients in the in the older subjects. This study did not, however, allow evaluation of the satiating effects of ID glucose compared to a control infusion. This issue was addressed in the study reported in Chapter 9, which demonstrated that energy intake was suppressed more by ID glucose compared to ID saline in older, but not young, subjects. In considering hormonal mechanisms which may potentially be responsible for the greater suppression of energy intake by ID glucose in older subjects, results of the study argue against a role for either GLP-1 or GIP, although the combination of hyperglycaemia and hyperinsulinaemia may potentially be important. This issue could be addressed by measuring the comparable effects of ID glucose and ID fructose infusion on blood glucose and plasma insulin and subsequent food intake in healthy older subjects, since ID fructose results in a relatively small glycaemic response when compared to ID glucose infusion in young healthy subjects (Rayner et al 2000). Further studies to evaluate the effects of other carbohydrates (and possibly fats of different saturation and chain length fatty acids), to assess how 'specific' are the effects of such nutrients on energy intake in healthy older compared to young subjects would also be of interest.

It should be recognised that the greater suppression of energy intake by glucose in the older subjects may have been due to changes in the release of, or sensitivity to, other gut peptides which were not measured. For example, an alteration (reduction) in the release of ghrelin, a newly discovered gut peptide which has been shown to increase

food intake in animal studies (Kojima et al 1999) (Chapter 3.6.6), in response to ID glucose could contribute to the enhanced satiating effects of ID glucose in the elderly. Investigation of the effects of ageing on the release of ghrelin are warranted. Alternatively, ageing may be associated with an increase in sensitivity to the satiating effects of highly osmolar solutions like the ID glucose used in this study. There is evidence that, when compared to nutrients of low osmolality, oral ingestion of nutrients of high osmolality reduces food intake in animals (Davis et al 2000, Rossi et al 1998). No studies have, however, evaluated this issue in humans or determined the effects of ageing on this sensitivity. Further studies of the effects of ID solutions of varying osmolalitys on appetite and energy intake in humans are indicated. There is no information relating to the satiating effects of protein in the healthy elderly. Evaluation of the effects of ID protein solutions on energy intake in the healthy older compared to young subjects therefore would also be of interest. The effect of chronic exposure to glucose on appetite and food intake was subsequently assessed in the study in Chapter 13.

The enhanced phasic pyloric response to ID lipid in healthy older subjects, observed in the study reported in Appendix A, may potentially be important in the aetiology of the modest slowing of gastric emptying observed in the elderly. An evaluation of the potential hormonal mechanisms responsible for this increased pyloric response was reported in Chapter 8. In this study, plasma CCK concentrations, but not those of GLP-1 or PYY, were higher both at baseline and in response to ID nutrients in older than young subjects. Although these observations are consistent with the concept that CCK may contribute to enhanced phasic pyloric motility, at least in response to ID fat, the possibility of a role for other peptides such as GIP and amylin cannot be excluded. Plasma GIP concentrations in response to ID lipid and glucose infusions in young and older subjects were subsequently measured in Chapter 9. ID glucose and lipid infusions stimulated plasma GIP release in both older and young subjects compared to the ID saline infusion with no significant difference between the nutrients, and the stimulation of GIP concentrations did not differ between the two age groups.

An enhanced release of CCK could contribute to the reduced appetite and food intake in the elderly. For this to be so, the elderly would have to retain their sensitivity, or be more sensitive, to the satiating effects of CCK. The sensitivity to CCK was subsequently evaluated in the study reported in Chapter 10, which indicated that the elderly were at least as sensitive to the suppressive effects of intravenous infusion of

CCK-8 on energy intake as young subjects, ie food intake was suppressed more in the presence of higher CCK-8 plasma concentrations in the older than the young subjects, but the relationship between this suppression and the rise in plasma CCK-8 did not differ between the two age groups. The higher CCK levels in the older subjects during the CCK-8 infusions were most likely related to reduced systemic clearance of CCK-8, associated with a lower percentage of lean body mass (Morgan et al 1992), rather than a reduced volume of distribution in the older compared to young subjects. Investigation of the effects of intravenous infusion of a CCK antagonist, eg. loxiglumide, on appetite and energy intake in young and older subjects is indicated, to both establish a role for CCK in the anorexia of ageing, and explore the potential therapeutic role for the use of CCK antagonists as a means of increasing food intake in the anorexic and/or malnourished elderly. The observation that exogenous CCK-8 suppresses endogenous CCK release is indicative of an autocrine negative feedback mechanism that is involved in the regulation of endogenous CCK secretion. Further evaluation of this feedback mechanism is indicated.

In Chapter 11, the effects of ageing on proximal gastric sensory and motor function were evaluated. This study established that ageing is associated with decreased perception of gastric distension without any change in fasting gastric compliance, and indicated that postprandial relaxation may be increased when compared to the young. It is possible that impaired perception in the elderly may influence the satiating effects of a meal, ie perceptions arising from gastric distension, such as fullness, may be considered pleasurable up to a point (Feinle et al 1998); impairment of these visceral sensations could remove a positive stimulus for eating. An effect of ageing on energy intake was also observed; older subjects ate similar amounts regardless of treatment (barostat vs tube only vs control study day), whereas young subjects ate less on the barostat day and tube only days compared to the control day, consistent with the concept that the control of energy intake is less sensitive to external stimuli in older than young subjects, as indicated by the impaired capacity to modify energy intake following periods of overeating or undereating in this group (Roberts et al 1994) (see Chapter 1.4). The design of this study was less than optimal for evaluating the effect of gastric distension on food intake, ie a bag volume of ≥400 ml is suggested by studies in young subjects (Geliebter et al 1988). Further studies are indicated to explore this issue. Furthermore, postprandial gastric relaxation is assessed optimally using a standard meal (in the author's study the size of the meal was intentionally variable) in order to evaluate the effects of gastric distension on food intake and this should be

assessed, ideally with concurrent measurement of gastric emptying. Evaluation of the effects of intragastrically administered loads of varying volume or energy content on appetite and food intake in the healthy elderly would also be of interest. Similarly, assessment of the effects of ageing on the perception of proximal small intestinal distension and the synergy between "gastric" and "small intestinal" stimuli (Feinle et al 1997) would be of interest.

In the study reported in Chapter 12, the effects of intravenous infusion of naloxone on appetite and energy intake were evaluated in young and older healthy subjects. Overall, naloxone suppressed energy intake in both young and older subjects and the magnitude of this suppression was slightly, albeit not significantly, greater in the young than older subjects, reflecting a trend to reduced suppression in older women. These findings suggest that healthy older adults (particularly older men) retain their sensitivity to the suppressive effects of naloxone on energy intake, indicating that a decline in opioid activity is unlikely to contribute substantially to the physiological "anorexia of ageing". This suggests that, in contrast to animals, human ageing is not associated with a reduced endogenous opioid feeding drive. Other factors such as increased CCK activity appear more likely to play an important role in the anorexia of ageing.

A limitation of this study is that plasma naloxone concentrations were not determined and it is possible that these may have been higher in older than young subjects. The effects of ageing on the volume of distribution and clearance of naloxone have not been reported. For many drugs that are eliminated predominantly by the liver, like naloxone (Goldfrank et al 1986), there is a positive correlation between systemic clearance and lean body mass (Morgan et al 1994). On average, lean body weight declines as a percentage of total body weight with advancing age (Morgan et al 1994). It would accordingly, not be surprising if the systemic clearance of naloxone was lower in the older subjects than weight-matched young adults in the study reported in Chapter 12. Administering naloxone on a per-kilogram-total-body-weight basis, as in this study, might, in effect, deliver a higher dose of naloxone to the older subjects. If plasma naloxone levels were higher in the older subjects, it may be speculated that older subjects, particularly women, may be less sensitive to the suppressive effects of naloxone on food intake. This hypothesis could be pursued by evaluating the effect of an opioid agonist, eg. butorphonal tartrate, on food intake in healthy older and young subjects.

The tendency for a reduced suppression of energy intake by naloxone in older women, suggests that the age-related changes in the opioid modulation of feeding may be gender-specific. This is potentially an important issue as women make up a majority of the elderly population susceptible to weight loss and malnutrition. Furthermore, there is some evidence from animal studies to support this concept; a reduced sensitivity to naloxone in older women may be related to oestrogen deficiency. This hypothesis could be evaluated by comparing the effect of intravenous naloxone infusion on food intake in postmenopausal women with and with out oestrogen replacement.

In Chapter 13, the effects of dietary glucose supplementation on gastric emptying, postprandial blood glucose homeostasis, and appetite after a glucose/oil "preload" were evaluated in healthy older subjects. Glucose supplementation accelerated gastric emptying of glucose, but had no significant effect on gastric emptying of oil; there was a trend for an increase in GIP, no change in GLP-1, an earlier insulin peak and a subsequent reduction in blood glucose. These findings are consistent with those observed in young subjects following dietary glucose supplementation, and indicate that the capacity of feedback mechanisms in the gut to adapt to dietary modification is retained in the healthy elderly. Evaluation of the effects of other forms of dietary supplementation (eg. high protein or mixed nutrient supplements) on gastric emptying and appetite would be of interest, particularly in the malnourished elderly. The effect of glucose supplementation on the postprandial blood glucose and plasma insulin is likely related to the modest increase in GIP concentrations. This issue also warrants further exploration. The elderly reduced ability of the elderly to compensate for the additional energy from the dietary glucose supplement, is consistent with the concept of an impairment of the homeostatic mechanisms involved in the regulation of feeding (Roberts et al 1994). Thus there is a strong rationale for the use of dietary energy supplementation, to prevent weight loss in the elderly. It is not known, however, whether the effects of dietary glucose (or carbohydrate) supplementation on gastric emptying and appetite in the elderly are maintained over longer periods of time, (ie 3-6 months); further studies are required to examine this issue. It would also be of interest to evaluate the effects of supplementation with other macronutrients, particularly as the satiating effect of carbohydrate may potentially be greater than that of protein (Chapter 9).

In considering the studies described in Chapters 8-13 it should be recognised that some of the observations are inconsistent with those obtained in animals, indicating the

requirement for human studies in the evaluation of specific hypotheses. Of the potential mechanisms involved in the physiological anorexia of ageing those supported by these studies include an increase in endogenous CCK activity, increased sensitivity to the suppressive effects of carbohydrate, diminished gastric perception, and possibly a decline in opioid modulation of feeding, particularly in older women. Hence, these studies have provided insights relevant to future studies appetite regulation in the elderly. All of these studies were conducted in healthy elderly subjects. Further, investigation of effect of malnutrition or illness on some of potential mechanisms involved in the regulation of appetite evaluated in the healthy elderly in this thesis is warranted. For example, the effect of a preload on gut hormone release and food intake in malnourished compared to healthy elderly subjects would be of interest.

Postprandial hypotension is associated with falls and increased morbidity in the elderly. In the study reported in Chapter 14, the effects of guar gum on postprandial blood pressure, gastric emptying and small intestinal glucose absorption were evaluated in healthy older subjects. Guar gum reduced the magnitude of the fall in blood pressure after oral glucose, and slowed gastric emptying and glucose absorption. The effect of guar gum on glucose absorption and postprandial glycaemia is more likely to be a "small intestinal" than "gastric" effect, and this could be clarified by further studies eg. investigation of the effect of intraduodenal infusion of glucose, bypassing the influence of gastric emptying on blood pressure in the elderly. Despite these limitations the findings suggest a novel approach to the treatment of postprandial hypotension in the elderly ie. slowing carbohydrate absorption. One possible implication of these observations for the elderly at risk of symptomatic postprandial hypotension may be to consume solids or semi-solid prior to glucose containing liquids during a meal; thereby slowing gastric emptying and/or absorption of nutrients, as has been shown to be the case with alcohol absorption (Horowitz et al 1989). Further investigation of other potential therapies which may influence gastric emptying and/or glucose absorption, for example, evaluating the effect of the addition of oil to a meal, are indicated. Studies to evaluate the effects of guar gum on postprandial blood pressure, gastric emptying and glucose absorption in elderly persons with symptomatic postprandial hypotension are also warranted.

In Chapter 15, the prevalence of malnutrition and factors which may potentially contribute to compromised nutritional status were evaluated in 250 Domiciliary Care recipients. While the prevalence of malnutrition according to the Mini-Nutritional

Assessment was relatively low (4.8%) in this population and comparable with other studies in community-dwelling elderly persons, the percentage of those at risk of malnutrition was high (38.4%). It may be appropriate to perform routine screening of nutritional status in the 'functionally dependent' community-dwelling elderly, such as those in this survey, particularly to identify those persons at risk of malnutrition. In this group nutritional status would be monitored and further deterioration, hopefully, prevented. Such a strategy would clearly have substantial public health implications. This study should be considered preliminary and potential limitations recognised. For example, there may have been a potential bias in the selection of the sample population, since those subjects who had been diagnosed with dementia and those who were non-English speaking and did not have access to an interpreter, were excluded. Moreover, there may have been a potential bias for 'more robust' subjects to take part in the survey, as many of those who refused to participate were too ill, in hospital or uncontactable. It is, accordingly, possible that the observed prevalence of malnutrition represents an underestimate. A history of respiratory disease, receipt of "Meals on Wheels", number of days spent in hospital in the past 12 months, and 4 of the 8 domains (physical functioning, mental health, general health perception and role limitation due to emotional problems) on the SF-36 Health Survey were independent predictors of nutritional status in this sample of elderly persons. A follow-up study at 12 months is planned to document subject outcome, particularly mortality and living status, thus allowing evaluation of the value of the MNA in predicting the risk of hospitalisation, institutionalisation, and death among 'functionally dependent' community living older people. As discussed, there is evidence that nutritional supplementation in malnourished elderly hospital patients and nursing home residents is beneficial (see Chapter 1.7.3.1). Studies are planned to determine whether community-living subjects benefit from such intervention and can be identified. When a subject was classified as 'malnourished' according to the MNA in this study they were offered (and 4 out of the 12 subjects accepted) additional dietary assessment and advice by a qualified dietitian. If those malnourished subjects who received additional advice and/or intervention have a better outcome at follow-up than those who did not, further investigation of the impact of nutritional screening and intervention on health outcomes and health care cost savings in functionally-dependent community dwelling elderly may be warranted.

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APPENDIX A

Effect of Intraduodenal Nutrients on Appetite and Pyloric Motility in the Healthy Elderly

APPENDIX A.1 SUMMARY

The mechanisms responsible for the reduction in appetite and slowing of gastric emptying in older persons are unknown. The aims of this study were to evaluate the effects of ageing on small intestinal regulation of appetite and pyloric motility.

Eight healthy older (age 65-75 yr) and seven healthy young (age 20-34 yr) male subjects received isoenergetic (12.1 kJ/min) intraduodenal infusions of lipid and glucose for 120 min, each on separate days. During the intraduodenal infusions

perceptions of hunger, desire to eat, and fullness were assessed by visual analog scales. Pyloric motility (isolated pyloric pressure waves and tonic pyloric pressure) was measured by manometry during the intraduodenal lipid infusion. On each day after completion of the intraduodenal nutrient infusion the subject was offered a buffet meal and food intake was quantified.

Before intraduodenal nutrient infusions, sensations of hunger ($P< 0.01$) and desire to eat ($P< 0.05$) were less in the older when compared to the young subjects. In the young, intraduodenal lipid suppressed hunger to a greater extent than intraduodenal glucose ($P< 0.05$). In older persons neither intraduodenal nutrient infusion suppressed hunger. Intraduodenal lipid and glucose increased fullness in both age groups ($P< 0.05$ for both), with no significant difference between the two nutrients. There was no significant difference in food intake from the buffet meal between the elderly and young subjects. Intraduodenal lipid infusion stimulated phasic pyloric pressure waves in both age groups ($P< 0.01$ for both) and this response was greater ($P< 0.05$) in older persons. There was an increase ($P< 0.01$) in tonic pyloric pressure during intraduodenal lipid infusion, which was not significantly different between the two age groups.

We conclude that the effect of small intestinal lipid infusion on hunger is attenuated and the stimulation of phasic pyloric pressure waves increased in healthy older persons when compared to healthy young males. Increased feedback from small intestinal nutrients does not appear to be responsible for the physiological anorexia of ageing.

APPENDIX A.2 INTRODUCTION

Small intestinal nutrient-mediated feedback is a major mechanism responsible for regulating the satiation and the rate of gastric emptying (see Chapter 3.5). In young healthy subjects infusion of nutrients into the small intestine suppresses hunger, increases satiation, reduces subsequent meal consumption (see Chapter 3.5.1) and also retards gastric emptying by suppressing antral contractions and stimulating phasic and tonic pyloric pressures (see Chapter 3.5.2). Intraduodenal (ID) infusion of fat suppresses subjective appetite ratings and stimulates pyloric motility to a greater extent than an equi-energetic ID glucose infusion, in young healthy adults (see Chapter 3.5.1.3). The effects of small intestinal nutrient (fat and glucose) exposure on appetite and pyloric motility have not been evaluated in the healthy elderly.

The aims of this study were to determine whether the effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by healthy ageing. The broad hypotheses addressed were that: (i) in both older and young adults intraduodenal infusion of fat suppresses appetite more than an isoenergetic intraduodenal glucose load; (ii) the suppression of appetite by intraduodenal infusion of either fat or glucose would be greater in older than young subjects; and (iii) the stimulation of pyloric motility by intraduodenal lipid infusion would be greater in older than young subjects.

APPENDIX A.3 SUBJECTS AND METHODS

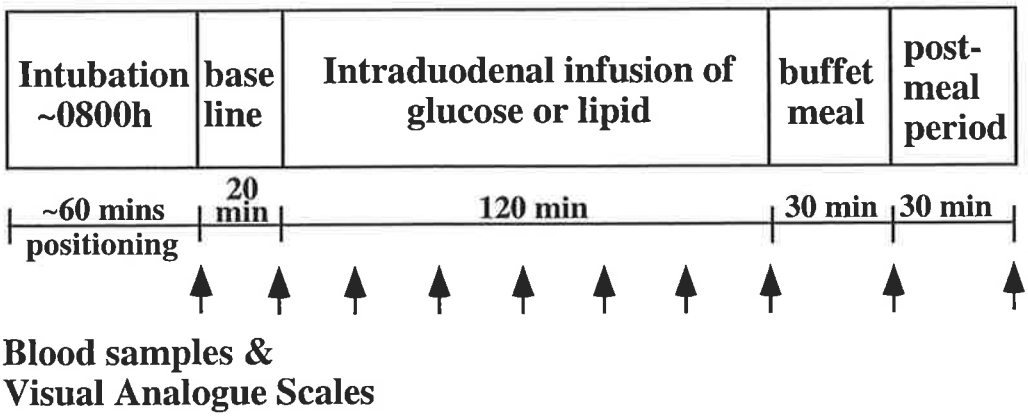
Paired studies were carried out in 8 healthy older males, mean age 70 yr (range 65-75 yr) and mean body mass index (BMI) 25.8 kg/m^2 (range 18.2-30) and 7 healthy "young" males, mean age 27 yr (range 20-34 yr) and mean BMI 26.8 kg/m^2 (range 24.4-31.8), who were recruited by advertisement. The two groups were matched for body mass index and all subjects were assessed as unrestrained eaters with an energy intake > 6273 kJ/day. All subjects were non-smokers and none had a history of gastrointestinal disease nor gastrointestinal surgery, nor was taking medication known to influence gastrointestinal motility. One older subject was taking allopurinol for treatment of gout and another enalapril for hypertension, but otherwise none of the subjects was taking medication regularly at the time of the study. The study protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital and each subject gave written informed consent.

Appendix A.3.1 *Experimental Protocol*

Each subject underwent paired studies on separate days, in random order. The two study days were separated by a mean interval of 10 days (range 5-14). Before the first study day each subject was required to keep a food diary for five successive days (see Chapter 6.2.2).

The experimental protocol is summarised in Appendix A, Figure 1. On each of the two study days subjects attended the laboratory at 0900h after a 12 hour overnight fast. Upon arrival, a silicone rubber manometric assembly (4 mm outer diameter) was inserted into the stomach via an anaesthetised nostril. The tip of the tube was allowed to pass into the duodenum by peristalsis, which took between 20-180 min. A subcutaneous saline-filled reference electrode (20G intravenous cannula) was inserted

into the subject's forearm to enable measurement of transmucosal potential difference (TMPD), so that the position of the manometric assembly could be monitored (see Chapter 7.4.1). Once the tube was positioned correctly across the pylorus an intravenous cannula was placed in a left antecubital vein for blood sampling. At time = 0 an isoenergetic intraduodenal infusion of either 25% glucose (Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) at a rate of 3 ml/minute, or lipid (10% Intralipid, Kabi Pharmacia AB, Sweden) at a rate of 2.6 ml/minute, was commenced and continued for 120 minutes. The intraduodenal infusions were commenced approximately 30 minutes after the manometric catheter had been positioned correctly. Infusions were delivered into the duodenum, approximately 10 cm distal to the pylorus, via a port in the manometric catheter. The rate of delivery of both infusions was ~12 kJ/min. Subjects were asked to record their feelings of hunger, desire to eat and fullness on visual analogue scales which were administered at -15, -5, 0, 10, 20, 30, 45, 60, 75, 90, 105, and 120 minutes (see Chapter 6.3.1). At -15, 10, 20, 30, 45, 60, 75, 90, 105, and 120 minutes, venous blood was collected in ice-chilled dipotassium EDTA tubes containing 400 KIU aprotinin (Trasylol) per ml of blood for measurement of CCK, GLP-1 and PYY. Plasma was separated by centrifugation within 30 minutes of collection and stored at -70°C until assay (see Chapter 8). Antropyloric motility was recorded during the intraduodenal lipid infusion, but not during the intraduodenal glucose infusion (for logistical reasons relating to the availability of recording equipment). We have previously shown in young subjects that intraduodenal lipid is a more potent stimulus of pyloric motility than intraduodenal glucose (Andrews et al 1998a). At 120 minutes the intraduodenal infusion was ceased and the manometric catheter was removed. Subjects were then presented with a standardised cold buffet style meal, prepared in excess of what they would normally eat, and invited to eat as much as they wished. The rate of ingestion and the total amount of food consumed were quantified (Lavin et al 1997).



Appendix A, Figure 1: Summary of the experimental protocol. Each subject received isocaloric intraduodenal infusions of glucose and lipid for 120 minutes on separate days. After completion of the infusions, a buffet meal was offered. The timing of venous blood samples and administration of visual analogue scales to assess desire to eat, hunger and fullness is indicated by arrows.

Appendix A.3.2 *Assessment of appetite*

Appetite was assessed using validated 10 cm linear visual analogue scales (see Chapter 6.3.1). The -15, -5 and 0 values were averaged to provide a baseline, and the change in ratings from baseline, during the intraduodenal nutrient infusions, quantified (Andrews et al 1998a, Lavin et al 1997).

The total amount (g) of food consumed at the buffet meal (300 ml full cream milk, 300 ml unsweetened orange juice, 4 slices wholemeal bread, 4 slices white bread, 4 slices ham, 4 slices processed chicken, 4 slices cheddar cheese, 4 slices of tomato, cucumber and lettuce, 2 sachets of mayonnaise, margarine, butter and tomato sauce, 1 pear, 1 orange, 1 banana, 1 apple, 200 g chocolate custard, 200 g strawberry yoghurt, 50 g ice cream) and the rate of food intake (kJ/min) were calculated (Lavin et al 1997). Food intake from the meal was analysed using the DIET/4 Nutrient Calculation software (Xyris Software, Australia, Pty Ltd) to determine both the energy intake (kJ) and macronutrient composition (% protein, % fat and % carbohydrate) of the meal (see Chapter 6.3.3).

Appendix A.3.3 *Measurement of antropyloric pressures*

The technique used to record antropyloric pressures during the intraduodenal lipid infusion is described in Chapter 7.4.1.

A computer-based recording system (Andrews et al 1998, Fraser et al 1991) was used (Powermac 7100/75, Apple Computer, Cupetino, CA, USA), running software - MAD - written by Dr C Malbert (Unité de Flux Digestifs, INRA, Saint Gilles, France) in Labview 3.0.1 (National Instruments). Manometric pressures were digitised, using a NBM1016H data acquisition board, recorded direct to disk at a frequency of 10 Hz and stored for later analysis. Data were only analysed when the sleeve sensor was positioned correctly across the pylorus, as defined by TMPD criteria (Heddle et al 1989, Sun et al 1996)(see Chapter 7.4.1).

Manometric variables analysed included: (a) isolated pyloric pressure waves (IPPW's). These were defined as a pressure wave ≥10 mmHg in amplitude recorded by the sleeve sensor with or without a concurrent pressure wave in channel 8 or 9, with no antral or duodenal pressure wave detected with an onset within 5 seconds of the onset of the IPPW (Sun et al 1996). The number and amplitude of IPPW's was calculated

(Andrews et al 1998a, Edelbroek et al 1992); (b) pyloric tone (basal pyloric pressure). This was determined by subtracting the mean basal pressure excluding phasic pressures in an antral sidehole (channel 5) 1.5 cm proximal to the sleeve from the mean basal pressure, recorded by the sleeve (Andrews et al 1998a, Edelbroek et al 1992, Sun et al 1996). This was calculated each minute by in-house software (MAD) and the means of five minute blocks calculated; (c) antral pressure waves. The number of these waves was quantified as described elsewhere (Edelbroek et al 1992).

For temporal analysis of IPPW's, recordings were divided into 10 minute periods from time 0, while for analysis of tonic pyloric pressure, recordings were divided into 5 minute periods. For both IPPW's and pyloric tone, responses between 0-40 min ("early") and 40-120 min ("late") were also compared (Fraser et al 1992).

Appendix A.3.4 *Statistical Analysis*

Before intraduodenal nutrient infusions, comparisons between the young and older groups in the macronutrient content of the previous diet, as well as scores for hunger, desire to eat and fullness, were performed using Student's unpaired t-test, as these data were normally distributed. The effects of the intraduodenal nutrient infusions on scores for hunger, desire to eat and fullness were analysed using repeated measures mixed model analysis of variance (ANOVA). Energy and macronutrient intake from the buffet meal, as well as phasic and tonic pyloric pressure waves in the two groups, were also analysed using ANOVA. The number of subjects studied was considered too small to perform meaningful correlation analyses. Data are presented as mean values ± SEM. A P value < 0.05 was considered significant in all analyses.

APPENDIX A.4 RESULTS

The study protocol was generally well tolerated. One of the elderly subjects experienced severe nausea, which had a rapid onset 65 minutes after the commencement of intraduodenal lipid infusion, and on this day only data up to 60 min were included in the analysis. The manometric catheter was positioned correctly across the pylorus 90.8% of the time (90.3% in the young and 91.4% in the older subjects). As assessed by the five-day diet diary, energy intake was less in the older (7377 ± 949 kJ) than the young (10200 ± 623 kJ) subjects ($P< 0.05$). This was predominantly due to the older men eating less during main meals compared with young men. There was no significant

difference in the number of snacks eaten between meals in young compared with older men, (1.7±0.29 vs 1.4±0.32, P= 0.5). The proportion of intake as carbohydrate was less in older (43.3 ± 1.4%) than young (48.3 ± 1.8%) subjects (P< 0.05), while there was no difference in fat (37.0 ± 1.2% vs 34.3 ± 2.5%) or protein (17.1 ± 0.7% vs 15.7% ± 0.8%) between the two groups. In all subjects plasma albumin concentration was within the normal range for healthy adults in our laboratory (34-48 g/L), with no difference between the age groups.

Appendix A.4.1 *Appetite*

Before commencement of the intraduodenal nutrient infusions, mean scores for both hunger (1.9 ± 0.6 cm vs 5.2 ± 0.8 cm; P< 0.01) and desire to eat (2.4± 0.5 cm vs 5.1 ± 0.7 cm; P< 0.05) were less in the older persons when compared to the young, while there was no difference in the baseline sensation of fullness (0.6 ± 0.3 cm vs 0.7 ± 0.4 cm) between the two groups.

In the young subjects there was a significant reduction from baseline in the sensation of desire to eat during intraduodenal lipid infusion (P< 0.05), but not during intraduodenal glucose infusion. In the young subjects hunger also decreased (P< 0.01) during intraduodenal lipid, but not intraduodenal glucose infusion and the suppression of hunger was greater (P< 0.05) with intraduodenal lipid than intraduodenal glucose. During both intraduodenal nutrient infusions the sensation of fullness increased (P< 0.05) with no significant difference between intraduodenal glucose and lipid (Appendix A, Figure 2).

In older persons neither intraduodenal nutrient infusion affected the sensations of hunger nor desire to eat when compared to baseline. In contrast, both intraduodenal glucose (P< 0.01) and intraduodenal lipid (P< 0.01) infusions increased fullness, with no significant difference in the magnitude of the response between the two nutrients (Appendix A, Figure 3).

During intraduodenal glucose infusion the decrease in scores for desire to eat (P< 0.05) and hunger (P< 0.05) were greater in the young than the older subjects, whereas there was no difference in fullness between the two groups. During intraduodenal lipid infusion, the changes in sensations of desire to eat (P< 0.05) and hunger (P< 0.01) were greater in the young than the older subjects, with no difference in fullness between the groups (Appendix A, Figure 4).

Older and young subjects ate similar amounts (weight and energy) after the intraduodenal nutrient infusions. In both age groups intake from the buffet meal was less, following intraduodenal lipid than intraduodenal glucose, although this was only significant ($P< 0.05$) for the young (Appendix A, Figure 5). The macronutrient content of the buffet meal (% protein, % fat and % carbohydrate) was not significantly different between young and older subjects, nor between intraduodenal lipid and glucose infusions (data not shown). There was also no affect of either age or the type of intraduodenal nutrient on the rate of eating (after intraduodenal glucose and lipid respectively: young 199.9 ± 29.7 kJ/min and 216.2 ± 29.7 kJ/min; older 200.3 ± 27.6 kJ/min and 181.9 ± 27.6 kJ/min).

Appendix A.4.2 *Antropyloric motility*

Appendix A.4.2.1 Isolated pyloric pressure waves (IPPW's)

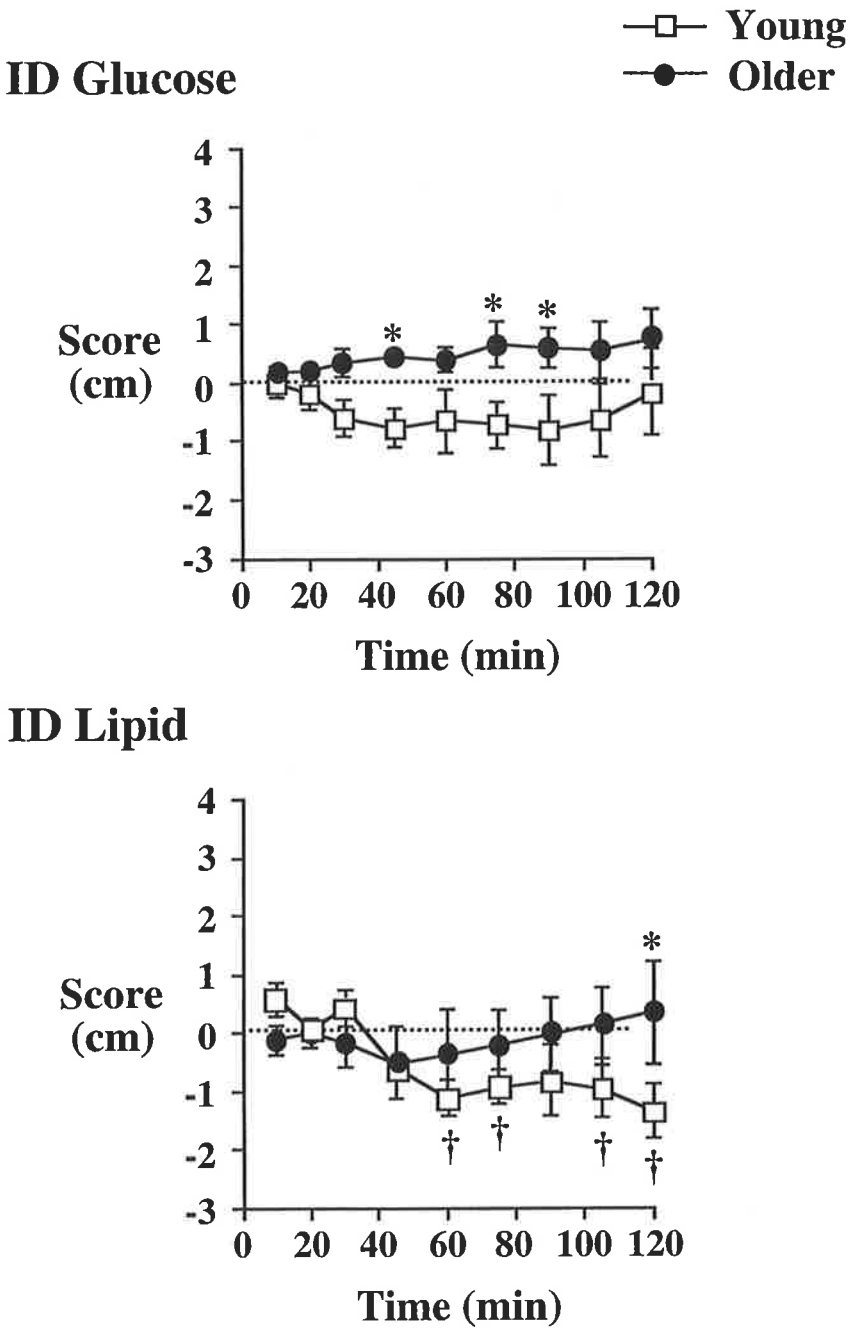
In both groups intraduodenal lipid infusion stimulated an increase in the frequency ($P< 0.01$) and amplitude ($P< 0.03$) of IPPW's. ANOVA for the whole curve showed that the overall frequency of IPPW's was greater ($P< 0.05$) in the older than young subjects (Appendix A, Figure 6), whereas there was no significant difference in the amplitude of IPPW's between the two groups (Appendix A, Figure 7). However, between 60-69 min the amplitude of IPPW's was greater ($P< 0.05$) in the older subjects. In both the young and older subjects, both the number and amplitude of IPPW's decreased with time ($P< 0.05$ for both), with a reduction from the peak response at about 50 min .

Appendix A.4.2.2 Pyloric tone

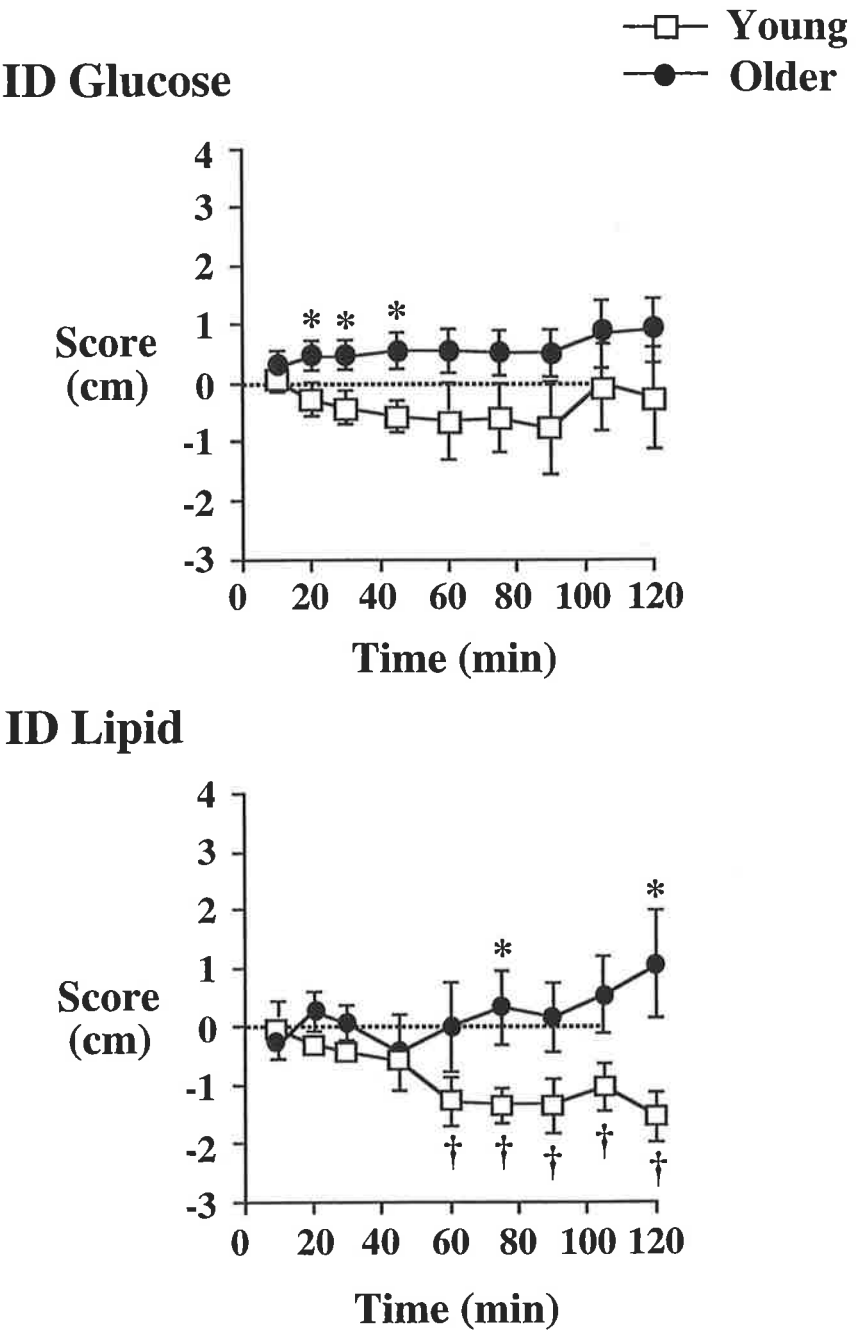
Before intraduodenal lipid infusion there was no difference in pyloric tone between the young and older subjects. In both young and older subjects there was an initial increase ($P< 0.01$ for both) in pyloric tone during intraduodenal lipid infusion, and the overall response was not significantly different between the two groups. In both groups there was attenuation ($P< 0.05$ for both) of the tonic response with time from a maximum at about 40 minutes. Although the mean "late" (40-120 min) response was less in the elderly the young subjects, this difference was not statistically significant ($P= 0.16$)(Appendix A, Figure 8).

Appendix A.4.2.3 Antral pressure waves

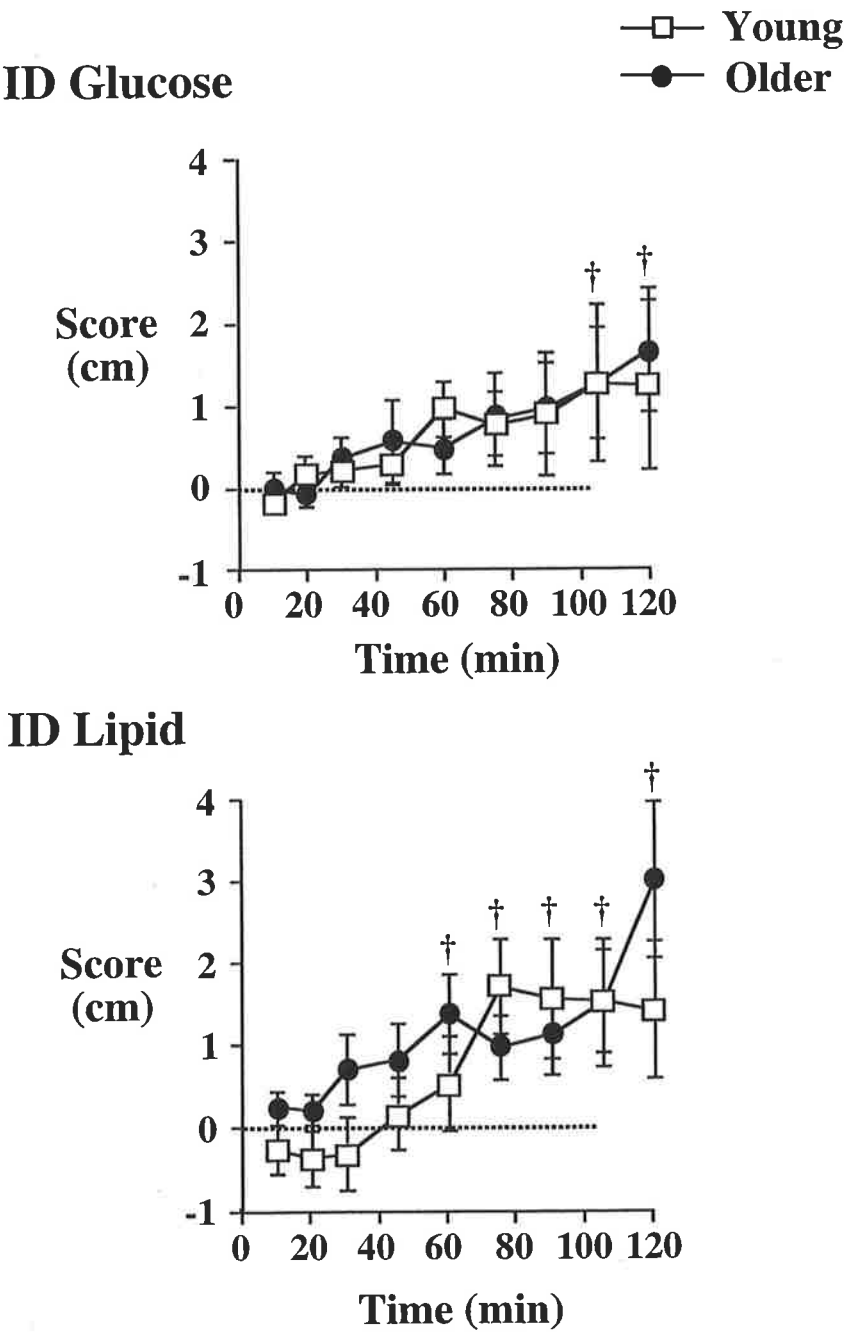
There were no antral pressure waves after the onset of intraduodenal lipid infusion in either young or elderly subjects.



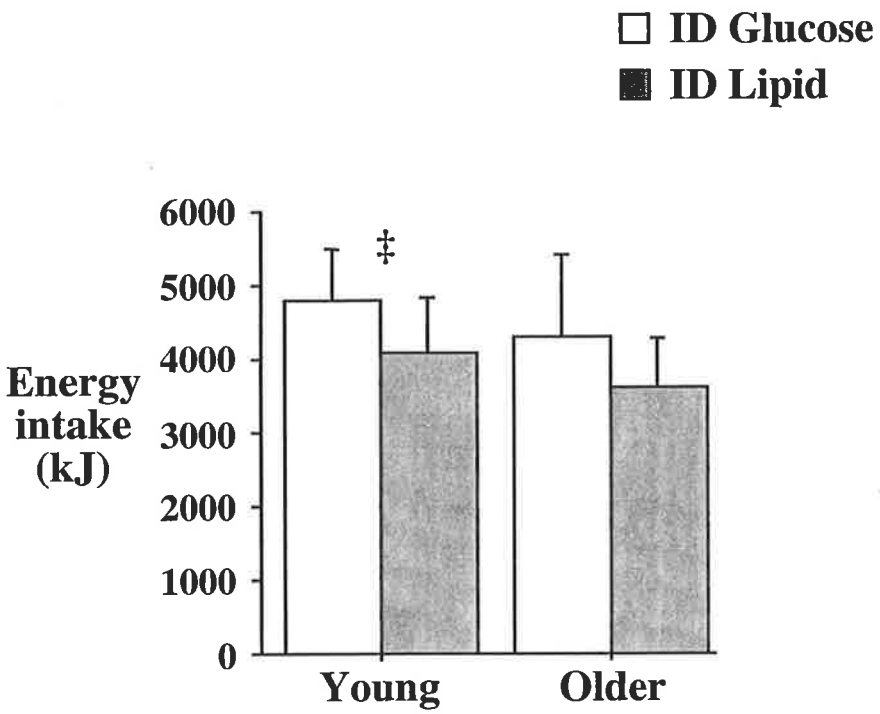
Appendix A, Figure 2: Subject sensations of desire to eat in young and older subjects during intraduodenal (ID) infusions of glucose and lipid. †P <0.05 from baseline; *P <0.05 young vs elderly at individual time points. Data are mean ± SEM.



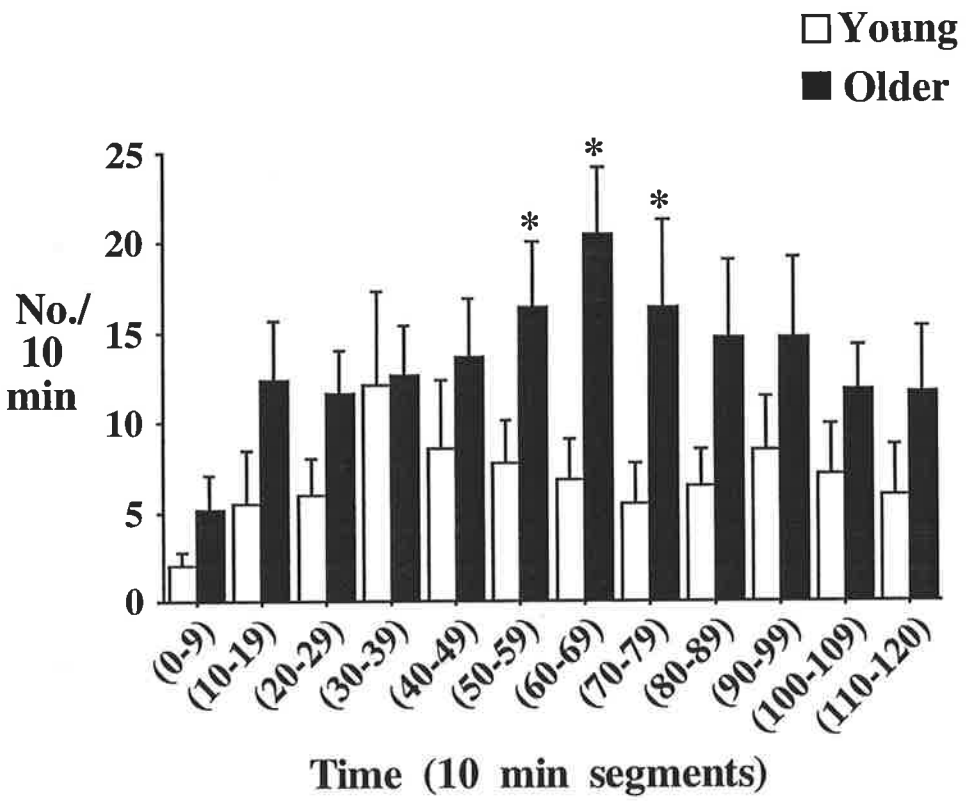
Appendix A, Figure 3: Subject sensations of hunger in young and older subjects during intraduodenal (ID) infusions of glucose and lipid. †P <0.05 from baseline; *P <0.05 young vs elderly at individual time points. Data are mean ± SEM.



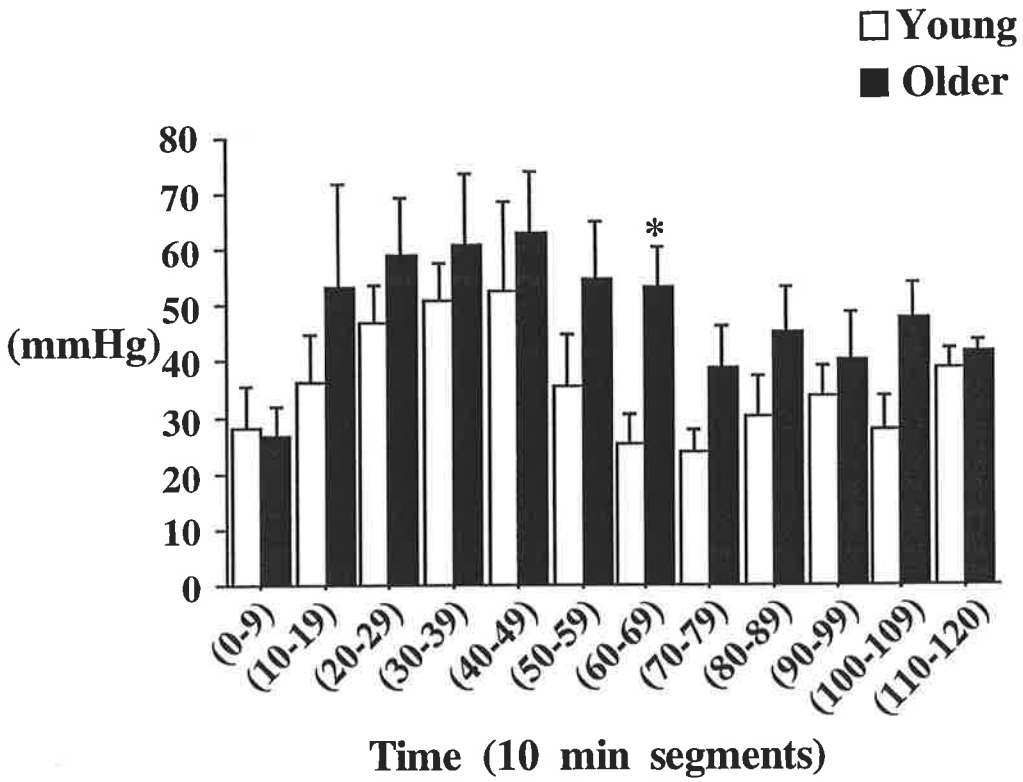
Appendix A, Figure 4: Subject sensations of fullness in young and older subjects during intraduodenal (ID) infusions of glucose and lipid. †P <0.05 from baseline; Data are mean ± SEM.



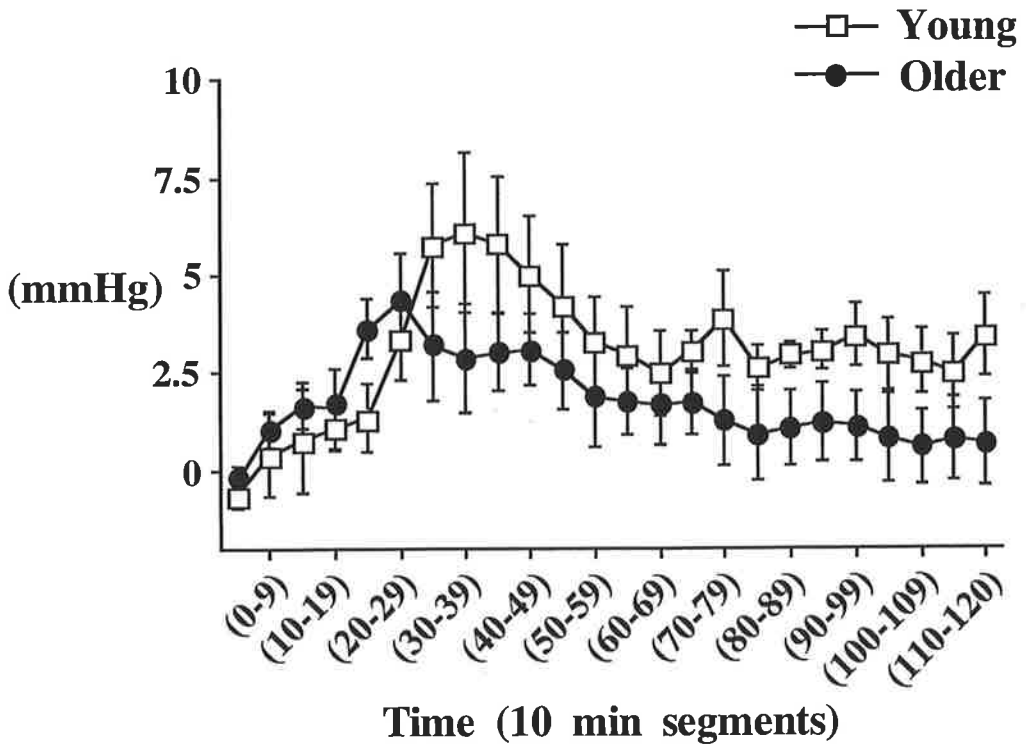
Appendix A, Figure 5: Energy content (kJ) of buffet meal consumed after intraduodenal (ID) infusions of glucose and lipid in young and older subjects. ‡ $P < 0.05$ glucose vs lipid. Data are mean ± SEM.



Appendix A, Figure 6: Frequency of phasic pyloric pressure waves (IPPWs) during intraduodenal lipid infusion in young and elderly subjects. P <0.05 young vs older for whole curve; *P< 0.05 young vs older at individual time points. Data are mean ± SEM.



Appendix A, Figure 7: Amplitude (mmHg) of phasic pyloric pressure waves (IPPWs) during intraduodenal lipid infusion in young and elderly subjects. *P <0.05 young vs older at individual time points. Data are mean ± SEM.



Appendix A, Figure 8: Pyloric tone (basal pyloric pressure)(mmHg) expressed as change from baseline during intraduodenal lipid infusion in young and older subjects. P <0.05 from baseline for both young and older. Data are mean ± SEM.

APPENDIX A.5 DISCUSSION

This study has demonstrated that the effects of intraduodenal lipid and glucose infusions on both appetite and pyloric motility are different in healthy older men when compared with healthy young men. The major findings are that: (i) older men are less hungry and have less desire to eat after an overnight fast, and also spontaneously eat less when compared with young men; (ii) in young men intraduodenal lipid suppresses hunger, desire to eat and subsequent food intake compared to intraduodenal glucose infusion; (iii) this suppression of subjective feelings of hunger by intraduodenal lipid is absent in healthy older males; (iv) the stimulation of phasic, but not tonic, pyloric pressure waves by intraduodenal lipid infusion is greater in older than young men. These observations indicate that suppression of appetite by small intestinal nutrients is impaired in older persons and also suggest that the slower gastric emptying reported in older persons may be the result of increased nutrient-mediated small intestinal feedback on phasic pyloric motility. These observations suggest that the physiological anorexia of ageing is not mediated by the interaction of nutrient with receptors in the small intestine.

The demonstration that both energy intake in their usual diet and hunger before a meal were less in healthy elderly when compared to young subjects is consistent with previous observations (Clarkston et al 1997, Rolls 1995, Wurtman et al 1988) and compatible with the concept that ageing is associated with a physiological anorexia (Morley 1996, Rolls 1995, Rolls et al 1995) (see Chapter 1.4).

There is no previous information about the effects of small intestinal nutrient exposure on appetite in the elderly. The rate of intraduodenal delivery of nutrients used in our study (12.1 kJ/min) is slightly greater than the usually quoted mean rate of gastric emptying (~8.4-10.5 kJ/min) of glucose (Horowitz et al 1991) and lipid (Horowitz et al 1993). The inclusion of a non-nutrient (saline) intraduodenal infusion would have allowed the effects of individual nutrients to be evaluated, but required three study days which we considered was probably not feasible in the older subjects. Moreover we, and others, have shown that intraduodenal fat and glucose reduce appetite in healthy young volunteers in the doses used in this study (Andrews et al 1998a, Lavin et al 1997, Read et al 1994, Welch et al 1985). In these studies a progressive increase in scores for hunger and desire to eat was evident during intraduodenal saline infusion (Lavin et al 1997). Therefore, although no true control (no nutrient) condition exists in this study it seems reasonable to assume that both nutrient infusions would cause a

decrease in hunger in the young subjects. It should also be recognised that only males were studied because this group appears to have the greatest capacity to regulate energy intake in response to energy manipulation (Rolls 1995) and our observations should therefore not be extrapolated to females. The relatively small number of subjects studied also raises the possibility of Type 2 statistical errors eg in older subjects intake from the buffet meal was less after intraduodenal fat than intraduodenal glucose, but this difference was not significant. The effect of intraduodenal lipid infusion on hunger was substantially less in the elderly than the young. This observation may potentially result from the lower baseline scores for both hunger and desire to eat in the elderly before commencement of the intraduodenal infusions. However, this seems unlikely since there was no suggestion of a reduction in hunger during either intraduodenal nutrient infusion in the elderly. In both age groups there was an increase in the sensation of fullness during the intraduodenal nutrient infusions, supporting the concept that perceptions of hunger and fullness are mediated by different mechanisms (Read et al 1994). Clearly, hunger and fullness are not always simply reciprocals of each other.

There is a lack of consensus as to whether fat and carbohydrate exert different effects on appetite (Andrews et al 1998a, Cotton et al 1994, Rolls et al 1995)(see Chapter 3.5.1.3). In this study, we concur that the suppressive effects of intraduodenal fat on hunger and subsequent food intake are greater than those of an isoenergetic intraduodenal glucose load in young males (Andrews et al 1998a). In contrast, a differential effect of the two nutrients on appetite ratings was not evident in older persons, although intake at the buffet meal was similar in the older when compared to the young subjects and tended to be less after intraduodenal lipid. These observations are compatible with the concepts that the homeostatic mechanisms which regulate appetite and body weight are impaired in older persons (Roberts et al 1994, Rolls 1995) (see Chapter 1.5) and that subjective feelings of appetite do not correlate closely with subsequent food consumption (Sepple & Read 1989). Our findings suggest that the presence of small intestinal nutrients alone does not play a major role in the pathophysiology of the anorexia of ageing. A previous study suggests that gastric emptying may be a more important determinant of anorexia in older persons (Clarkston et al 1997).

This study has not addressed the mechanisms by which the presence of nutrients in the small intestine influences appetite (see Chapter 3.5 and 3.6). Evaluation of the effects of intraduodenal nutrients on the secretion of other hormones including glucagon-like

peptide 1 and gastric inhibitory polypeptide in older and young subjects would be of interest (see Chapter 8).

There is considerable controversy as to the effects of healthy ageing on gastrointestinal function (Clarkston et al 1997, Andrews & Horowitz 1996, Lovat 1996, Husebye & Engedal 1992, Wegener M, 1988, Kupfer et al 1985, Horowitz et al 1984, Van Liere & Northup 1941) (see Chapter 4.4.2).

The presence of nutrients within the small intestine retards gastric emptying (Heddle et al 1989) and stimulates increases in phasic (isolated pyloric pressure waves) and tonic pressures localised to the pylorus (Edelbroek et al 1992, Heddle et al 1989) (see Chapter 3.5.2). The demonstration that the phasic component of the pyloric motor response to intraduodenal lipid is greater in older persons provides a possible mechanism for the slower gastric emptying of nutrients observed in this group (Clarkston et al 1997, Horowitz et al 1984, Wegener et al 1988). There was no difference in the initial stimulation of pyloric tone by intraduodenal lipid between the young and elderly subjects. A discordance between the tonic and phasic response of the pylorus has previously been reported in humans, indicating possible mediation by different neural and/or humoral signals (Fraser et al 1993, Fraser et al 1991). The increased stimulation of phasic pyloric pressure waves by intraduodenal lipid in the elderly may reflect a reduction in the activity of inhibitory neural pathways (Lovat 1996). Attenuation of both the phasic and tonic pyloric motor responses during prolonged intraduodenal nutrient infusion has been demonstrated previously in young, normal volunteers (Edelbroek et al 1992, Fraser et al 1992). It should be recognised that there was a non significant trend for the attenuation of the tonic pyloric response to be more marked in the elderly, and as the number of subjects studied was relatively small, this is an issue which should be addressed in future studies. Evaluation of the effects of ageing on other motor mechanisms which may play a role in the regulation of gastric emptying and appetite, particularly fundic motility, would also be of interest.

This study addressed the hypothesis that the effects of intraduodenal nutrients on appetite and pyloric motility would be modified by ageing. While substantial differences between young and elderly subjects were demonstrated it was expected that these motor and sensory changes may be related, particularly as there is evidence of a linkage between the rate of gastric emptying and satiation in the elderly (Clarkston et al 1997). Accordingly, observations that the subjective effect of intraduodenal nutrients

on appetite is reduced in the elderly, while the stimulation of phasic pyloric motility is increased was unexpected. However, on the basis of the former observation it seems unlikely that the physiological anorexia of ageing reflects an increased subjective sensitivity to the presence of nutrients in the small intestine.

APPENDIX A.6 SPECULATION

These data together with our previous study (Clarkston et al 1997) suggest that intragastric mechanisms, rather than the exposure of the small intestine to nutrients is the major determinant of the physiological anorexia of ageing (see Chapter 3.4). Studies by ourselves and others have suggested that nitric oxide may play a role in the regulation of both appetite (Morley 1996b) and gastric motility (Sun et al 1996) (see Chapter 2.3.2). Studies in older animals have suggested that decreased nitric oxide synthase may be responsible for the anorexia of ageing (Morley et al 1996b) (see Chapter 4.2.2.1). The hypothesis that nitric oxide reduces satiation by increasing fundal compliance and reducing antral distension can be tested in humans by measurement of fundal compliance with a barostat and antral diameter by ultrasound (Jones et al 1997a) in the presence and absence of nitric oxide donors and nitric oxide synthase inhibitors.

APPENDIX BI.1:

Visual Analogue Questionnaire

Name: **Time:**

Please answer each question by placing a **vertical mark** on the horizontal line.
Furthest **LEFT** means you **do not have the symptom.**
Furthest **RIGHT** means that you **feel the symptom very much.**

I feel anxious _____

I feel sleepy _____

How strong is your desire to eat?

_____

WEAK STRONG

I feel hungry _____

How much food do you think you could eat?

_____

A small amount A large amount

I feel sick _____

I feel full _____

I feel dizzy _____

I have indigestion

_____

My tummy is rumbling

_____

I have a headache

_____

I feel thirsty _____

If you were given a meal now, would you want to eat it

YES **NO**

APPENDIX BI.2:

Visual Analogue Questionnaire

Please indicate how you are feeling at this moment by placing a **vertical mark** at the appropriate point on each scale below. Please do not make a cross or a sloping mark. Mark all scales.

Not nauseated _____ Nauseated

Drowsy _____ Alert

Calm _____ Anxious

Tired _____ Energetic

Muddled _____ Clearheaded

Withdrawn _____ Sociable

Happy _____ Sad

Strong _____ Feeble

Efficient _____ Inefficient

Antagonistic _____ Friendly

Hungry _____ Not hungry

Satiated _____ Not satiated

Empty _____ Full

How strong is your desire to eat

Weak _____ Strong

How much food do you think you could eat ?

None _____ A large amount

APPENDIX BII:

Visual Analogue Questionnaire

Name:

Step:

Time:

Please answer each question by placing a vertical mark on the horizontal line

I don't feel full at all	———	I feel extremely full
I don't feel sick at all	———	I feel extremely sick
I don't feel any abdominal discomfort	———	I feel extremely uncomfortable in the abdomen
I don't feel any abdominal bloating	———	I feel extreme abdominal bloating
I don't feel hungry at all	———	I feel extremely hungry
I have no desire to eat	———	I want to eat a large amount

APPENDIX BIII:
Food Ranking Questionnaire

Name: **Time:**

Please rank the following food items in order of preference of how much you like to eat each item now. 1 = you would like to eat it the most / 9 = you would like to eat it the least.

Food item	Preference Rating (1-9)
Orange Juice	
Flavoured milk	
Ham	
Crackers	
Shortbread	
Chocolate bar	
Jelly beans	
Potato crisps	
Cheddar cheese	

APPENDIX BIV:

Mini-Nutritional Assessment

I. Anthropometric Assessment

1. Body Mass index (BMI = weight/ height^2, in kg/ m^2)

   Scoring: BMI < 19 = 0 points; 19 ≤ BMI < 21 = 2 points;

   22 ≤ BMI < 23 = 2 points; BMI ≥ 23 = 3 points.

2. Mid-arm circumference (MAC, cm)

   Scoring: MAC < 21 = 0 points; 21 ≤ BMI ≤ 22 = 0.5 points;

   BMI > 22 = 1 point.

3. Calf circumference (CC, cm)

   Scoring: CC < 31 = 0 points; BMI ≥ 31 = 1 point.

4. Weight loss during last 3 months

   Scoring: weight loss > 3 kg = 0 points; unknown = 1 points;
   weight loss between 1 and 3 kg = 2 points;
   no weight loss = 3 points.

II. Global Assessment

5. Does the subject live independently in contrast to a nursing home?

   Scoring: no = 0 points; yes = 1 point.

6. Does the subject take more than 3 prescription drugs per day?

   Scoring: no = 1 points; yes = 0 point.

7. In the past 3 months, has the subject suffered psychological stress or acute disease?

   Scoring: no = 1 points; yes = 0 point.

8. Mobility.
Scoring: bed- or chair-bound = 0 points; able to get out of bed/chair but does not go out = 1 point; goes out = 2 points;

9. Neuropsychological problems.
Scoring: severe dementia or depression = 0 points; mild dementia = 1 points; no problems = 2 points.

10. Pressure sores or skin ulcers
Scoring: no = 1 points; yes = 0 point.

III Dietary Assessment

11. How many full meals does the subject eat daily?
Scoring: 1 meal = 0 points; 2 meals = 1 point, 3 meals = 2 points.

12. Does he/she consume:
At least one serving of dairy products per day ?
Two or more servings of beans of eggs per week ?
Meat, fish or poultry every day ?
Scoring: 0 or 1 yes = 0 points; 2 yes answers = 0.5 points; 3 yes answers = 1 point.

13. Does he/she consume two or more servings of fruits or vegetables per day ?
Scoring: no = 0 points; yes = 1 point.

14. Has the subject's food intake declined over the past 3 months due to loss of appetite, digestive problems, or chewing or swallowing difficulties?
Scoring: severe loss of appetite = 0 points; moderate loss of appetite = 1 point; no loss of appetite = 2 points.

15. How many cups/glasses of beverages does the subject consume per day ?
Scoring: less than 3 glasses = 0 points; 3 to 5 glasses = 1 point; more than 5 glasses = 2 points.

16. Mode of feeding.

Scoring: fed requiring assistance = 0 points; self-fed with some difficulty = 1 point; self-fed without any problem = 2 points.

IV. Subjective Assessment

17. Does the subject think he/she has any nutritional problems ?

Scoring: major malnutrition = 0 points; moderate malnutrition or does not know = 1 point; no nutritional problem = 2 points.

18. In comparison with other people of the same age, how would the subject consider his/her health?

Scoring: not as good = 0 points; does not know = 0.5 points; as good = 1 point; better = 2 points.

Total Score: ≥ 24 points = well-nourished.

17 to 23.5 points = At risk of malnutrition.

< 17 points = malnourished.

(Guigoz et al 1994)

APPENDIX BV

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	2	3	4	
almost never	seldom	at least once a week	almost every day	-3

48. How likely are you to consciously eat less than you want?

1	2	3	4	
unlikely	slightly likely	moderately likely	very likely	+1

49. Do you go on eating binges though you are not hungry?

1	2	3	4	
never	rarely	sometimes	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	2	3	4	
not like me	a little like me	pretty good description of me	describes me perfectly.	+2

APPENDIX BVI:

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
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 BVII:

Standardised Mini-Mental State Examination

	Maximum Score	Score
ORIENTATION		
What is the <year> <season> <date> <day> <month>	5	( )
Where are we: <state> <country> <suburb> <street> <house no.>	5	( )
REGISTRATION		
Name 3 objects: 1 second to say each. Then ask the subject all 3 after you have said them. Give 1 point for each correct answer. Then repeat them until the subject learns all 3. Count the trials and record.	3	( )
Number of trials:_____		
ATTENTION AND CALCULATION		
Serial 7's. 1 point for each correct. Stop at 5 answers. Alternatively spell "world" backwards.	5	( )
RECALL		
Ask for the 3 objects repeated above. Give 1 point for each correct.	3	( )
LANGUAGE		
Name a pencil, and watch.....	2	( )
Repeat the following, "No ifs, ands or buts"	1	( )
Follow a 3-stage command: "take a paper in your right hand, fold it in half, and put it on the floor."	3	( )
Read and obey the following: CLOSE YOUR EYES.....	1	( )
Write a sentence	1	( )
Copy design	1	( )

ASSESS level of consciousness along a continuum:

Alert Drowsy Stupor Coma

(Molloy et al 1994) Total 30 ( )

APPENDIX BVIII:

SF-36© Health Survey

Instructions: This questionnaire asks for your views about your health, how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer the question, please give the best answer you can.

In general, would you say your health is:

excellent	❒ *(Check one box)*
very good	❒
good	❒
fair	❒
poor	❒

Compared to one year ago, how would you rate your health in general now ?

much better now than one year ago	❒ *(Check one box)*
somewhat better now than one year ago	❒
about the same as one year ago	❒
somewhat worse now than one year ago	❒
much worse now than one year ago	❒

The following questions are about activities you might do during a typical day. Does your health now limit you in these activities ? If so, how much ?

Activities	yes, limited a lot	yes, limited a little	no, not limited at all
• **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	❒	❒	❒
• **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, playing golf	❒	❒	❒
• Lifting or carrying groceries	❒	❒	❒
• Climbing **several** flights of stairs	❒	❒	❒
• Climbing **one** flight of stairs	❒	❒	❒
• Bending, kneeling, or stooping	❒	❒	❒
• Walking **more than one kilometre**	❒	❒	❒

- Walking **half a kilometre** ..... ❒ ❒ ❒
- Walking **100 metres** ..... ❒ ❒ ❒
- Bathing or dressing yourself ..... ❒ ❒ ❒

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health ?

Cut down on the **amount of time** you spent on work or other activities
yes ❒
no ❒

Accomplished less than you would like
yes ❒
no ❒

Were limited by the **kind** of work or other activities
yes ❒
no ❒

Had **difficulty** performing the work or other activities (for example, it took extra effort)
yes ❒
no ❒

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

Cut down on the **amount of time** you spent on work or other activities
yes ❒
no ❒

Accomplished less than you would like
yes ❒
no ❒

Didn't do work or other activities as **carefully** as usual
yes ❒
no ❒

During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

not at all ❒ *(Check one box)*
slightly ❒
moderately ❒
quite a bit ❒
extremely ❒

How much bodily pain have you had in the past 4 weeks ?

no bodily pain	❒	*(Check one box)*
very mild	❒	
mild	❒	
moderate	❒	
severe	❒	
very severe	❒	

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework) ?

not at all	❒	*(Check one box)*
slightly	❒	
moderately	❒	
quite a bit	❒	
extremely	❒	

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of your time during the past 4 weeks - *(Check one box on each line)*

	all of, the time	most of the time	a good bit of the time	some of the time	a little of the time	none of the time
• Did you feel full of life? ...	❒	❒	❒	❒	❒	❒
• Have you been a very nervous person?	❒	❒	❒	❒	❒	❒
• Have you felt so down in the dumps that nothing could cheer you up?	❒	❒	❒	❒	❒	❒
• Have you felt calm and peaceful?	❒	❒	❒	❒	❒	❒
• Did you have a lot of energy?	❒	❒	❒	❒	❒	❒
• Have you felt down?	❒	❒	❒	❒	❒	❒
• Did you feel worn out?	❒	❒	❒	❒	❒	❒
• Have you been a happy person?	❒	❒	❒	❒	❒	❒
• Did you feel tired?	❒	❒	❒	❒	❒	❒

During the past 4 weeks, how much of your time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

all of the time	❒ *(Check one box)*
most of the time	❒
some of the time	❒
a little of the time	❒
none of the time	❒

How TRUE or FALSE is each of the following statements for you?*(Check one box on each line)*

	definitely true	mostly true	don't know	mostly false	definitely false
• I seem to get sick a little easier than other people?	❒	❒	❒	❒	❒
• I am as healthy as any body I know?	❒	❒	❒	❒	❒
• I expect my health to get worse?	❒	❒	❒	❒	❒
• My health is excellent?	❒	❒	❒	❒	❒