GASTRIC EMPTYING, APPETITE, ENERGY INTAKE AND EXERCISE IN MALES

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Abstract

The main aim of this thesis is to expand the existing body of knowledge on the influence of physical activity on gastric emptying (GE) and appetite control. Through a series of three complementary research studies interactions between exercise, appetite, energy intake (EI), eating behaviour and GE were investigated in males. The relationships with body composition and energy expenditure (EE) were also addressed.

Exercise has many health benefits, including weight maintenance, and should be an effective weight loss strategy by increasing EE. However, the efficacy of exercise for weight loss is variable and will depend on changes in other components of energy balance including EI. Although it is intuitive that exercise drives an increase in appetite and EI, this relationship is more complex. Understanding the underlying mechanisms associated with EI and exercise-induced changes in EI - by examining appetite – is particularly relevant considering the increasing prevalence of obesity and levels of physical inactivity associated with modern lifestyles.

While there are many factors that influence EI including environmental and psychological factors, one physiological mechanism involves the gut and GE. The gastrointestinal (GI) tract has a critical role in the control of food intake and GE has been implicated to have a role in increased food intakes and obesity. However, studies comparing GE in lean and obese individuals have shown inconsistent findings, perhaps because of the influence of additional factors, such as participants' habitual physical activity levels. Further, the absolute amount (or alternatively the ratio) of fat and fat free mass, and the total amount of EE could be moderating factors. If these factors do influence GE, then it is relevant that they are measured and controlled for in studies examining GE. In addition, an increased understanding of the mechanisms involved in appetite control, and how exercise and diet interventions affect GE may help to explain appetite and EI responses. In turn, this could help to improve the effectiveness of weight management strategies.

The core research theme of this thesis is to explore the influence of habitual physical activity level and a short-term exercise intervention on GE, appetite, food intake and other aspects of eating behaviour. Methodological issues are also considered. This thesis consists of three studies. The first is methodological, the second is cross-sectional and the third is a longitudinal study. Collectively, they build on existing knowledge on GE and the influence of physical activity. The studies included the assessment of body composition, metabolism, EE, habitual EI, appetite sensations, eating behavior traits, food preferences, processes of food reward and GE.

To determine whether changes in GE and associated measures of appetite, and food intake can be detected and play a role in the effectiveness of interventions, it is necessary to identify their variability under normal conditions. However, little information exists on the variability of these parameters in overweight and obese individuals. The first methodological study aimed to determine the day-to-day variability of GE and associated measures in overweight and obese males. Fifteen overweight and obese males (age: 34.9 ± 10.6 y, BMI: 30.3 ± 4.9 kg/m^{-2}) completed two identical GE tests seven days apart. Data are expressed as mean ± standard deviation throughout. GE was assessed by ^{13}C-octanoic acid breath test over 5 hours following a standard pancake breakfast. GE half time ($t_{1/2}$), the time taken for 50% of the ^{13}C dose to be excreted in the breath, and lag time (t_{lag}), the time taken to maximal $^{13}CO_2$ excretion, were calculated. Appetite sensations and EI were assessed using computerised visual analogue scales and an *ad libitum* lunch meal. Food preferences and processes of food reward ('liking' and 'wanting') were assessed using the Leeds Food Preference Questionnaire (LFPQ).

The intra-individual coefficient of variations (CV) for $t_{1/2}$ and t_{lag} were 7.9% and 7.5% respectively, indicating that GE is reproducible. Sample size calculations based on the day-to-day variations revealed that a minimum of ten participants is sufficient to detect a 10% change in both GE $t_{1/2}$ and $t_{lag,}$ in a paired design study. Appetite sensations showed a consistent fluctuating pattern and were more reproducible for postprandial mean and AUC ratings than fasting ratings. With regard to EI at the *ad libitum* test meal, a strong correlation between test days (r =

0.76, $p < 0.001$) and a CV of 17.4 and 11.5%**,** with and without one outlier respectively was demonstrated. Further, strong test-retest correlations indicate that the LFPQ is a sufficiently reliable tool for assessing food preferences and processes of food reward in this population. Despite no significant mean differences between test days for all outcome measures, evidence that some individuals vary considerably from day to day is relevant when considering individual changes in clinical settings. Collectively, the findings provide valuable information for the design of future studies in this population and support the use of these methods to evaluate changes in GE and associated measures in overweight and obese males in research and clinical settings.

The second cross-sectional study aimed to determine associations amongst habitual exercise, body composition, EE, GE, appetite and EI. Although limited evidence suggests GE is faster in marathon runners, whether GE is altered in habitually active individuals and the associations with body composition, EE, appetite and EI remains to be established. This study addresses some of these gaps by examining GE in a larger cohort of habitual exercisers (participating in a range of activities) and sedentary individuals, and by characterising a number of factors that may account for potential differences in GE and eating behaviour. Forty-four males (Sedentary: n = 22, Age, 30.4 ± 8.5 y, BMI, 27.4 ± 4.2 kg/m^{-2}; Active: n = 22, Age, 29.4 ± 7.8 y, BMI, 24.5 ± 2.6 kg/m^{-2}) participated in the study. Sedentary was defined as participating in one structured exercise session or less per week and not engaged in strenuous work. Active was defined as participating in four or more structured exercise sessions per week. A series of measurements were undertaken including air displacement plethysmography to assess body composition, indirect calorimetry to determine resting metabolic rate, the Three Factor Eating Questionnaire for eating behaviour tests, three day diet recall for habitual diet, and accelerometry for habitual activity energy expenditure (AEE). GE, subjective appetite sensations, *ad libitum* test meal EI, food preferences and 'liking' and 'wanting' were assessed using identical methods to those described in the first study.

GE was faster in active compared to sedentary males (GE t_{lag}: active: 95±13 and sedentary: 110±16 min, $p < 0.001$; GE $t_{1/2}$: active: 157±18 and sedentary, 179±21 min, $p < 0.001$). These data demonstrate that habitual exercisers participating in a range of

activities are characterised by faster GE. Subjective appetite ratings did not differ but the *ad libitum* test meal EI was higher in the active compared to sedentary group (active, 4946 ± 1254 kJ; sedentary, 3241 ± 885 kJ, $p = 0.04$). In addition, active individuals had a higher preference for low fat savoury foods and were characterised by a higher level of dietary restraint compared to the sedentary group (TFEQ Restraint Score: active, 10.1 ± 3.5; sedentary, 6.1 ± 3.7, $p < 0.01$). Therefore, dietary restraint and food preferences appear to exert an effect on the control of EI in active individuals. When the group data were pooled in a correlation analysis ($n = 44$), higher AEE was associated with faster GE half time ($r = -.46$, $p < 0.01$). In addition, GE was significantly associated with body composition. However, after controlling for habitual activity status (active or sedentary), the latter associations disappeared. The only correlations to remain significant were age, AEE and total EE with GE. These findings indicate a link between AEE and GE and highlight that associations between body composition and GE may be a result of differences in physical activity. In addition, in multiple regression analysis the only factors to account for any of the variance in GE $t_{1/2}$ were habitual activity (sedentary or active) and AEE (model adjusted R^2, .34, $p < 0.001$; activity: β, -.45, $p < 0.01$; AEE: β, -.28, $p = 0.05$). In a simple correlation analysis within groups, GE was associated with postprandial fullness (5-hour area under the curve) in the active group, but no associations were demonstrated between GE and fullness in the sedentary group. These data could suggest that appetite in active individuals is better regulated in response to physiological signals. One hypothesis is this is a result of a faster GE and hence an earlier or enhanced release of GI signals from the intestine. However, the cross-sectional design means that a causal relationship between GE, appetite and EI cannot be inferred from this study. Whether faster GE is a consequence of a higher EI, hormonal differences or higher EE in active individuals remains to be established. Overall, the findings highlight the importance of controlling for habitual physical activity level and AEE when examining the role of GE (and parameters that may be affected by GE) in obesity, other conditions, and in response to interventions.

Although the previous cross sectional study indicates GE is faster in active males, the temporal pattern of changes in GE, appetite and EI cannot be determined.

There is limited information on the effects of short-term exercise training on GE in previously sedentary individuals. The final study aimed to determine the effects of a 4-week exercise intervention on changes in GE and associated measures in sedentary overweight and obese males. Fifteen overweight and obese sedentary males (Age: 31.1 ± 8.4 y, BMI: 29.7 ± 3.3 kg/m^{-2}) completed this study. Post testing took place between 48 and 96 hours after the last exercise session. The exercise intervention consisted of five exercise sessions per week for four weeks (20 sessions in total) and all were supervised in the laboratory. Exercise sessions consisted of cycling on a cycle ergometer and alternated between continuous cycling at 50% VO_2max and high intensity interval exercise at 100% VO_2max. All participants completed a minimum of 90% of the prescribed exercise sessions (18 of 20).

GE was unchanged following four weeks of supervised exercise training (GE $t_{1/2}$: pre, 175 ± 22 and post, 179 ± 21 min, p = 0.25; GE t_{lag}: pre, 111 ± 17 and post, 110 ± 18 min, p = 0.71). Although habitual EI, subjective appetite ratings, eating behaviour, food preferences and processes of food reward were unchanged, there was a small but significant increase in *ad libitum* EI at the lunch test meal. Despite no changes in GE or in appetite ratings, significant improvements were observed in a number of health markers following the 4-week intervention. Albeit small, body weight (-0.9 ± 1.1 kg), waist circumference (-2.3 ± 3.5 cm) and percent body fat (-0.9 ± 1.1 %) were significantly lower, while fat free mass was maintained. In addition, both systolic (-6.2 ± 8.4 mmHg) and diastolic (-5.8 ± 2.2 mmHg) blood pressure were significantly reduced and there was a significant increase in VO_2max (+4.4 ± 2.1 ml/kg/min). These findings indicate that, at least in the short-term, GE is not altered by a significant increase in fitness. GE may only adapt to a higher AEE, longer duration of physical activity or changes in other characteristics associated with regular exercise. Other factors must contribute to changes in food intake and should be addressed when individuals commence a physical activity program for weight management.

Overall, the results of this thesis contribute to improving the understanding of the reliability of measuring GE and factors that influence variability in GE. The data provide a foundation for further research by showing that GE and associated measures are reproducible in overweight and obese males, and that GE is faster in

active compared to sedentary individuals and is related to AEE. Further, the results show that in the absence of acute exercise effects, a significant improvement in fitness can be achieved in response to short term exercise training without altering GE. There is a growing interest in targeting the GI tract to reduce energy intake and promote weight loss. However, it would be premature to recommend that increasing or decreasing GE is an appropriate strategy for weight loss or maintenance. The findings from this thesis highlight the importance of controlling for physical activity level and AEE in future studies examining the role of GE in obesity and provide insight into processes potentially contributing to the regulation of energy balance. Further studies measuring gut peptides are needed to explore the hypothesis that faster GE may have a role in regulating food intake in active individuals and to determine the temporal pattern of changes in GE with exercise and the implications for appetite and EI. As obesity levels continue to rise, a better understanding of the mechanisms associated with the findings in this thesis becomes increasingly and urgently relevant.

Table of Contents

Keywords i

ABSTRACT II

Table of Contents viii

List of Figures xii

List of Tables xv

List of Abbreviations xvii

Acknowledgements xx

CHAPTER 1: INTRODUCTION 1

CHAPTER 2: LITERATURE REVIEW 6

2.1 Introduction to appetite control 6

2.2 Focus on the gut in appetite control 8

2.3 Energy homeostasis: tonic and episodic processes 9

2.4 Gastric emptying 11
- 2.4.1 Gastric Emptying and appetite control 12
- 2.4.2 Gastric Emptying in Obesity 14

2.5 The efficacy of bariatric surgery in weight loss: what is the role of the emptying rate? 16

2.6 Additional surgical and pharmacological weight loss strategies: contrasting effects on gastric emptying 21

2.7 Summary of effects of surgical and pharmacological weight loss strategies on gastric emptying 22

2.8 Lifestyle Interventions, gastric emptying and gut peptides 23
- 2.8.1 Energy restriction 27
- 2.8.2 Exercise 28
- 2.8.3 Combining energy restriction and exercise 33

2.9 Methodological Issues: comparing the effects of different strategies 34

2.10 Future Directions: GI targets of appetite control for weight loss 35
- 2.10.1 GI targets of appetite control: part of an integrated process 37

2.11 Conclusions 38

CHAPTER 3: REPRODUCIBILITY OF GASTRIC EMPTYING, APPETITE AND ENERGY INTAKE IN OVERWEIGHT AND OBESE MALES 39

3.1 Background 39

3.2 Methodology 46
- 3.2.1 Participants 46
- 3.2.2 Protocol 46
- 3.2.3 Baseline Assessment Measurements 47
- 3.2.4 Gastric Emptying Test Day Measurements (Day 0 and Day 7) 48
- 3.2.5 Statistical Analysis 54

3.3 Results 55
- 3.3.1 Gastric Emptying 55
- 3.3.2 Subjective Appetite Sensations 58
- 3.3.3 Ad libitum EI 61
- 3.3.4 Palatability of Test Meals, Food Preferences, 'Liking' and 'Wanting' 62
- 3.3.5 Relationships between Variables 65

3.4 Discussion....67
3.4.1 Reproducibility of GE....67
3.4.2 Reproducibility of Subjective Appetite Sensations....69
3.4.3 Reproducibility of *ad libitum* EI....70
3.4.4 Reproducibility of food preferences, 'liking' and 'wanting'....72
3.4.5 Relationships between variables....74
3.4.6 Methodological Considerations....76
3.4.7 Summary....77

CHAPTER 4: THE EFFECT OF HABITUAL PHYSICAL ACTIVITY, ENERGY EXPENDITURE AND BODY COMPOSITION ON GASTRIC EMPTYING, APPETITE AND ENERGY INTAKE....79

4.1 Background....79

4.2 Methodology....84
4.2.1 Participants....84
4.2.2 Design....84
4.2.3 Baseline Measurements....85
4.2.4 Gastric Emptying Test Day Measurements....88
4.2.5 Statistical Analysis....90

4.3 Results....91
4.3.1 Participant Characteristics....91
4.3.2 Gastric Emptying....93
4.3.3 VAS Ratings....97
4.3.4 Ad libitum EI....99
4.3.5 Food Preferences, 'Liking' and 'Wanting'....99
4.3.6 Analysis of Groups by Quartiles....101
4.3.7 Relationships between Variables and determinants of GE....102
4.3.7.1 Simple Correlation Analysis between Variables....102
4.3.7.2 Partial correlations controlling for activity....106
4.3.7.3 Multiple regression analysis....107

4.4 Discussion....109
4.4.1 Physical Activity and GE....109
4.4.2 Body Composition and GE....111
4.4.3 Energy Expenditure and GE....113
4.4.4 Associations between Body Composition, EE, Eating Behaviour, EI and GE....114
4.4.5 Appetite sensations....116
4.4.6 *Ad Libitum* Test Meal EI....118
4.4.7 'Liking' and 'Wanting'....120
4.4.8 Methodological Considerations....121
4.4.9 Future Directions....124

CHAPTER 5: THE EFFECT OF A 4-WEEK EXERCISE INTERVENTION ON GASTRIC EMPTYING, APPETITE AND ENERGY INTAKE IN OVERWEIGHT AND OBESE MALES 127

5.1 Background....127

5.2 Methodology....130
5.2.1 Participants....130
5.2.2 Design....130
5.2.2.1 Exercise Intervention....131
5.2.3 Probe Measurements....132
5.2.4 GE Test Day Measurements....135
5.2.5 Statistical Analysis....137

5.3 Results....138
5.3.1 Exercise Compliance....138
5.3.2 Anthropometry, Body Composition, Fitness and Blood Pressure....138
5.3.3 Participant Habitual Energy Intake and Physical Activity Characteristics....140
5.3.4 Gastric Emptying....141
5.3.5 VAS Ratings....142
5.3.6 Eating Behaviour....144

5.3.7 *Ad Libitum* Test Meal EI 144
5.3.8 Food Preferences, 'Liking' and 'Wanting' 144
5.3.9 Relationships among anthropometric, AEE, GE and *ad libitum* EI changes 146

5.4 Discussion 148
5.4.1 Gastric Emptying 148
5.4.2 Appetite Ratings and *Ad Libitum* Test Meal EI 150
5.4.3 Food Preferences, 'Liking', 'Wanting' and Eating Behaviour 152
5.4.4 Body Composition, Blood Pressure and Fitness 153
5.4.5 Relationships between Variables 154
5.4.6 Methodological Considerations 155
5.4.7 Summary and Future Directions 158

CHAPTER 6: GENERAL DISCUSSION, FUTURE DIRECTIONS AND CONCLUSIONS ... 159

6.1 Summary of Literature Review and Experimental Study Findings 159

6.2 Perspectives and Implications 162

6.3 Methodological Issues 167

6.4 Future Directions 170
6.4.1 Is there a role for GE in obesity? 172
6.4.2 GE, appetite and EI in weight maintenance 172
6.4.3 GE, appetite and EI in weight loss 174
6.4.4 Temporal relationship of changes in GE and food intake with exercise 176
6.4.5 Minimising compensatory responses to exercise training 176
6.4.6 Underlying Mechanisms 177
6.4.7 Other Roles of GE in Health 177
6.4.8 Appetite control in females and other populations 178

6.5 Conclusions 179

6.6 Wider Contexts 179

REFERENCES 181

APPENDICES 215

Appendix A: Overview of Gastric Emptying Reproducibility Studies 215

Appendix B: Review of Gastric Emptying Methods 218
B.1 The ^{13}C-octanoic acid breath test 220

Appendix C: Measurement and Validation of Mass Spectrometry Methods 221
C.1 Accuracy of measured $^{13}CO_2$ abundance 221
C.2 Reproducibility of procedure for filling reference gas samples 222
C.3 Precision of breath $^{13}CO_2$ analysis and inter-laboratory check 222
C.4 Summary 223

Appendix D: Chapter 3 Correlation coefficients (r) of VAS ratings versus lunch energy intake (EI) and gastric emptying (GE) parameters. (n = 15) 224

Appendix E: Chapter 4 Spread of a) BMI, b) Fat Free Mass, c) Body Fat, d) RMR, e) AEE and f) Restraint in Active (n = 22) and Sedentary (n = 22) groups 225

Appendix F: Chapter 4 Palatability Ratings and VAS Alertness Ratings 227

Appendix G: Chapter 4 Comparison of quartiles for GE $t_{1/2}$ 228

Appendix H: Chapter 4 Comparison of quartiles for AEE 229

List of Publications

Horner, K.M., Byrne, N.M., Cleghorn, G.J., Näslund, E, King, N. A. (2011). The effects of weight loss strategies on gastric emptying and appetite control. *Obesity Reviews,* 12(11):935-51.

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List of Figures

Figure 2.1 Satiety cascade showing the relation between appetite control and some mediating psychological and physiological processes. From: Blundell (2010) p. 54 [48], adapted by D.J. Mela from Blundell et al. (1987) [49]. 7

Figure 2.2 A hypothetical model of the relative contributions of gastric distension, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin (all episodic signals influenced by gastric emptying) to satiation and satiety 14

Figure 2.3 Correlation ($y = -0.2391x + 157.9$; $R^2 = 0.95$) between ghrelin and GE half time ($t_{1/2}$) in lean individuals (n = 16). In the same study, no association between ghrelin and GE was demonstrated in obese individuals. From Valera Mora et al (2005) p. 741 [159]. 15

Figure 2.4 Mean (±SEM) immediate and subsequent satiety quotients (SQ) in response to a fixed breakfast at weeks 0 and 12 in A) responders (R; individuals whose actual weight loss was equal to or greater than the expected weight loss after the 12 week exercise intervention) and B) non responders (NR; individuals whose actual weight loss was less than their predicted weight loss). Data from King et al. [99]. 29

Figure 2.5 A model to describe how chronic physical activity could potentially impact on gastric emptying, appetite regulation and energy balance 33

Figure 3.1 Schematic overview of study protocol 46

Figure 3.2 Schematic Representation of Gastric Emptying Study Day Protocol. 48

Figure 3.3 Breath samples for the ^{13}C-octanoic acid breath test are collected by exhaling into a glass exetainer tube using a straw. The tube is then immediately capped. 49

Figure 3.4 Representation of GE parameters calculated from one breath test. These parameters are defined in Tables 3.2 and 3.3. 51

Figure 3.5 Example of Visual Analogue Scale used for Hunger Ratings. 52

Figure 3.6 Bland–Altman plots showing the difference between visits 1 and 2 (y axis) plotted against the mean for the two visits (x axis) for a) t_{lag}, lag time, b) $t_{1/2}$, half time, c) t_{lat}, latency time and d) t_{asc}, ascension time. *n = 15* 56

Figure 3.7 Scatter plots of the relation between the change - from visit 1 to visit 2 - in (a) lag time (t_{lag}) and half time ($t_{1/2}$) ($r = 0.81$, $p < 0.001$), and b) latency time (t_{lat}) and ascension time (t_{asc}) ($r = -0.01$, $p = 0.97$). n = 15. 57

Figure 3.8 Minimum number of participants needed to detect significant differences in a paired design in a) latency time (t_{lat}), b) ascension time (t_{asc}), c) half time ($t_{1/2}$),and d) lag time (t_{lag}), assuming a power of 80% and $\alpha = 0.05$. 58

Figure 3.9 Mean (± SEM) subjective appetite scores on the two test days (Visit 1: black filled circles; Visit 2: grey open circles). 59

Figure 3.10 Bland–Altman plot showing the difference between visits 1 and 2 (y axis) plotted against the mean for the two visits (x axis) for energy intake (EI) at the *ad libitum* lunch meal. *n = 15* 61

Figure 3.11 Energy intake at visit 2 plotted against energy intake at visit 1 at the ad libitum lunch meal. $r = 0.76$, $P < 0.001$. $n = 15$. 62

Figure 3.12 Palatability ratings at Visits 1 and 2 for the breakfast meal. There were no significant differences between visits for any of the parameters ($p > .05$). 63

Figure 3.13 Palatability ratings at Visits 1 and 2 for the lunch meal. There were no significant differences between visits for any of the parameters ($p > .05$). 63

Figure 3.14 Correlation of desire to eat breakfast SQ and GE $t_{1/2}$ ($r = 0.76$, $p = < 0.001$). 66

Figure 4.1 Schematic overview of Study 2 protocol 85

Figure 4.2 Gastric emptying half time ($t_{1/2}$) in individual participants in the active (n = 22) and sedentary (n = 22) groups. 94

Figure 4.3 A comparison of GE $t_{1/2}$ in groups divided according to the median of BMI ($25kg/m^2$), activity level category (active or sedentary), the median of percentage body fat (BF) (20%) and the median of fat free mass (67kg). n = 22 per group for all categories. Error bars indicate SD. 96

Figure 4.4 Mean (± SEM) subjective ratings for a) hunger, b) desire to eat and c) fullness in active and sedentary groups over the course of the GE test morning. n = 22 per group 98

Figure 4.5 A comparison between active (n = 22) and sedentary (n = 22) groups of mean food preferences pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Food preferences were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. 99

Figure 4.6 Mean 'liking' for different foods in active (n = 22) and sedentary (n = 22) groups assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. 100

Figure 4.7 Mean 'wanting' for different foods in active (n = 22) and sedentary (n = 22) groups assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. 100

Figure 4.8 Plot of GE $t_{1/2}$ in individuals divided according to quartiles of GE $t_{1/2}$. n = 11 per quartile. 101

Figure 4.9 Scatter plot of the relation between gastric emptying half time ($t_{1/2}$) and 5h fullness AUC (Area Under Curve) ratings in active males ($r = .55$, $p < 0.01$) indicating a slower GE is associated with greater postprandial fullness. n = 22. 104

Figure 4.10 Scatter plot of the relation between gastric emptying half time ($t_{1/2}$) and 5h fullness AUC (Area Under Curve) ratings in sedentary males ($r = .20$, $p = 0.38$), indicating no association between GE and postprandial fullness. n = 22. 105

Figure 4.11 Scatter plot of the relation between the activity energy expenditure (AEE) and gastric emptying half time ($t_{1/2}$) ($r = -.46$, $p < 0.01$). n = 41. 106

Figure 5.1 Schematic overview of Study 3 protocol. Baseline testing was conducted in the week prior to the exercise intervention. Post intervention testing was conducted ≥ 48h after the last exercise session. 131

Figure 5.2 Variability in individual changes in body weight (kg) after the 4 week exercise intervention (n = 15). 139

Figure 5.3 Variability in individual changes in fat and fat free mass (kg) after the 4 week exercise intervention (n = 15). 140

Figure 5.4 GE half time ($t_{1/2}$) pre and post the 4-week exercise intervention (n = 15). Each participant is represented by a solid line. 141

Figure 5.5 Mean (± SEM) subjective ratings for a) hunger, b) fullness and c) desire to eat over the course of the GE test morning pre and post the 4-week exercise intervention. n = 15. 143

Figure 5.6 Dietary Restraint, Disinhibition and Hunger as assessed by the Three Factor Eating Questionnaire (TFEQ) Pre and Post the 4-week exercise intervention. (n = 15). 144

Figure 5.7 A comparison between pre and post exercise intervention of mean food preferences pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Food preferences were divided according to frequency of choice for high fat savoury (HFSA), low fat

savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. n = 15. 145

Figure 5.8 A comparison between pre and post exercise intervention of mean 'liking' for different foods assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. n = 15. 145

Figure 5.9 A comparison between pre and post exercise intervention of mean 'wanting' for different foods assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. n = 15. 146

Figure 5.10 Correlation between change in AEE with change in t_{asc} after the 4 week exercise intervention. 147

Figure 5.11 Correlation between change in absolute FFM (kg) and change in *ad libitum* EI at the lunch test meal after the 4-week exercise intervention. 147

Figure 6.1 Summary of specific findings from the three thesis studies 161

Figure 6.2 The relationship between energy expended in different occupations and food intake in calories per day in a study of 213 workers in West Bengal as found by Mayer et al. (1956) [10]. This figure is taken from Blundell (2011) [449] and adapted to show an interpretation of Mayer's data in light of more recent evidence on the impact of actively changing physical activity on accurately measured food intake 164

Figure 6.3 A summary of the methodological considerations from the studies 167

Figure 6.4 Summary of recommendations for further research 171

Figure A1 Sequential metabolic steps after ingestion of a ^{13}C-labelled test meal. The rate limiting step of breath $^{13}CO_2$ excretion is represented by the gastric emptying of the meal. Adapted from Deane et al. (2010) [271] 222

Figure A2 Precision of replicate analyses of breath $^{13}CO_2$ enrichment. Three replicates were analysed at QUT (shown as QUT 1, 2 and 3 in the legend) and one at Isoanalytical, Crewe, UK (shown as ISOAN in the legend). 223

Figure A3 Subjective ratings for alertness in active and sedentary groups over the course of the GE test morning. n = 22 per group. 227

List of Tables

Table 2.1 A summary of studies examining the effects of bariatric surgeries on gastric emptying....19

Table 2.2 A summary of studies examining the effects of lifestyle interventions on gastric emptying....24

Table 2.3 A summary of studies examining the effects of lifestyle interventions on fasting and postprandial appetite gut peptides....25

Table 3.1 Summary of studies examining the reproducibility of gastric emptying measured by scintigraphy and breath test. For individual details of the studies, see Appendix A. 40

Table 3.2 Conventional Gastric Emptying Breath Test Time Based Parameters proposed by Ghoos et al. (1993) [297]....50

Table 3.3 Gastric Emptying Time Based Parameters proposed by Schommartz et al. (1998) [312] 51

Table 3.4 Photographic food stimuli used in the food preference and 'liking' and 'wanting' computer task (grouped by food category). 53

Table 3.5 Participant characteristics (n = 15). 55

Table 3.6 Gastric Emptying Time Based Parameters at visits 1 and 2 (n = 15) 55

Table 3.7 Reproducibility of Appetite Ratings at Visits 1 and 2 (n = 15) 60

Table 3.8 Reproducibility of Fasting (Pre-Breakfast) Preferences, explicit liking and explicit wanting at Visits 1 and 2 (n = 15)....64

Table 3.9 Reproducibility of Fed (Post-Breakfast) Preferences, explicit liking and explicit wanting at Visits 1 and 2 (n = 14)....65

Table 4.1 Summary of studies examining solid gastric emptying in lean and obese individuals measured by scintigraphy and breath test. 80

Table 4.2 Participant anthropometric, body composition, resting metabolism and eating behaviour characteristics....91

Table 4.3 Participant energy intake and physical activity characteristics....92

Table 4.4 Gastric Emptying Parameters in Active and Sedentary groups (n = 22 per group). 94

Table 4.5 Body Composition and GE characteristics of 4 subgroups categorised as lean (BMI: 18 – 25kg/m^2) and overweight/obese (BMI: 27 – 34 kg/m^2) sedentary and active....95

Table 4.6 Partial correlations of age, body composition, resting metabolism variables and dietary restraint with GE t_{lag} and $t_{1/2}$ after controlling for activity group (sedentary or active)....107

Table 4.7 Regression model for the relation between GE $t_{1/2}$ and age, percent body fat, activity and respiratory quotient. n = 44. 108

Table 4.8 Regression model for the relation between GE t_{lag} and age, activity and AEE. n = 44....108

Table 5.1 Participant anthropometric, body composition, fitness and blood pressure characteristics pre and post 4 week exercise intervention (n = 15). 139

Table 5.2 Participant energy intake and physical activity characteristics Pre and Post the 4 week exercise intervention (n = 15) 140

Table 5.3 GE Time Based Parameters Pre and Post 4 week exercise intervention (n = 15) 141

Table A1 Overview of studies examining the reproducibility of gastric emptying measured by scintigraphy and breath test. 215

Table A2 Summary of strengths and limitations of gastric emptying methods.....218

Table A3 Palatability ratings from 100mm visual analogue scales for breakfast and lunch meals in active and sedentary groups.....227

Table A4 Age, body composition, resting metabolism and physical activity characteristics of the lower and upper quartiles of gastric emptying half time. 228

Table A5 Age, body composition, resting metabolism and physical activity characteristics of the lower and upper quartiles of activity energy expenditure measured by accelerometery.....229

List of Abbreviations

AEE Activity Energy Expenditure

AgRP Agouti-related peptide

AUC Area Under the Curve

BMI Body Mass Index

BF Body Fat

CCK Cholecystokinin

CHO Carbohydrate

CR Coefficient of Repeatability

CV Coefficient of Variation

EE Energy Expenditure

EI Energy Intake

FFM Fat Free Mass

GE Gastric Emptying

GLP-1 Glucagon-Like Peptide-1

HIIE High Intensity Interval Exercise

HR_{max} Maximum Heart Rate

IBW Ideal Body Weight

NPY Neuropeptide Y

OCTT Orocecal Transit Time

PA Physical Activity

POMC Pro-opiomelanocortin

PP Pancreatic Polypeptide

PRO Protein

PYY Peptide YY

RMR Resting Metabolic Rate

t_{asc} Ascension Time

t_{lag} Lag Time

t_{lat} Latency Time

$t_{1/2}$ Half Time

VAS Visual Analogue Scale

VO_2max Maximal Oxygen Uptake

VCO_2 CO_2 Production Rate

^{13}C-OBT ^{13}C Octanoic Acid Breath Test.

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: QUT Verified Signature

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Chapter 1: Introduction

Physical inactivity and obesity are two of the biggest public health problems of the 21st century. A substantial portion of disability and mortality arising from diabetes, cardiovascular disease and some cancers is directly attributable to low levels of cardiorespiratory fitness and physical activity and to obesity [1-6]. While the drivers of obesity are complex and multi-factorial and the relative contributions of each are constantly debated, increased energy intakes and decreased levels of physical activity are notable contributors to its increased prevalence in westernised societies [7].

Exercise has many health benefits and should be an effective weight loss strategy by increasing energy expenditure (EE). However, the success of exercise in producing and sustaining weight loss will also be influenced by changes in energy intake (EI), among other factors (see [8] for a detailed review). Studies consistently indicate exercise is crucial for weight maintenance [9] and one hypothesis based on the work of Mayer [10] is that exercise sensitises the physiological mechanisms involved in appetite control [11]. However, the degree of weight loss with prescribed exercise can be quite modest and can be highly variable between individuals [12]. While it is intuitive that exercise drives an increase in appetite and EI - a view that may deter some individuals from participating in physical activity [10] - this relationship appears to be more complex. A wide range of social, cultural, psychological, genetic and physiological factors contribute to the control of EI [13]. Factors such as learned behaviours, reward pathways, stress and eating behaviour related traits (e.g. dietary restraint) can exert a strong influence on food intake. In addition, a number of physiological signals from the gastrointestinal (GI) tract and brain influence appetite and EI. A better understanding of the mechanisms involved in appetite control and responses to diet or exercise is vital if lifestyle strategies are to be more effectively used in weight management.

One such mechanism could involve the gut, gut peptides and gastric emptying (GE, the rate at which food empties from the stomach into the small intestine). Altered GE has long been implicated in increased energy intakes and hence the pathogenesis of obesity [14-20]. The GI tract plays a critical role in the

short-term physiological control of food intake. As food enters the stomach and subsequently empties into the small intestine, a variety of factors - including gastric distension, nutrient stimulation of intestinal mechanoreceptors and chemoreceptors (see [21, 22]) and several gut peptides released from the GI tract - contribute to satiation (control of meal size) and satiety (post-meal inhibition of eating). In 1975, Hunt and colleagues [20] postulated that rapid GE is a predisposing factor in the pathogenesis of increased food intake and hence obesity. However, accelerated [16-18], similar [23-26] and delayed [27-29] emptying rates have since been reported in obese compared to lean individuals, conflicting outcomes that indicate that the role of GE in obesity is still unclear. One hypothesis may be that such inconclusive findings are due to the influence of additional factors which tend not to be measured or controlled for, for example physical activity and associated differences in body composition and EE. Given the growing interest in targeting the GI tract for the treatment of obesity [14, 30-34], and the resources being put into developing such strategies, it is pertinent that a better understanding of factors influencing GE and the implications for appetite control is established.

The core research theme of this thesis is to determine the influence of habitual physical activity level and a short-term exercise intervention on GE and how this relates to appetite, EI and other aspects of eating behaviour. Methodological issues are considered and the associations amongst EE, body composition and GE are also examined. The thesis consists of five subsequent chapters; a literature review, three experimental studies and a discussion chapter.

In **Chapter 2**, the role of GE in appetite control is discussed and literature concerning the effects of surgical, pharmacological, diet and exercise interventions on GE and related gut peptides is reviewed. Little information exists on the effects of exercise on gastric function and appetite control. Therefore, the effects of different weight loss strategies on GE and gut peptides are discussed with a view to explaining how GI mechanisms may contribute to changes in appetite and EI and why particular strategies (e.g. surgery) may be more effective in sustaining long-term reductions in food intake.

The first experimental study consists of a methodological study, which aims to determine the day-to-day variability of GE, appetite and EI in overweight and obese males (**Chapter 3**). To gain a clearer perspective of factors influencing GE and changes in response to treatments, it is necessary that studies are appropriately designed and have sufficient statistical power. Despite being implicated to have a potential role in the pathogenesis of obesity [14-16, 18, 20, 29], and measured in response to numerous interventions [35], the variability of GE, appetite and EI in overweight and obese individuals is poorly documented. If these parameters vary considerably day to day, then much larger sample sizes will be needed to detect differences between groups (e.g. between lean and obese) or in response to treatments. This knowledge provides information on appropriate sample sizes and will assist to determine whether any difference or change in the outcome measure (e.g. in response to exercise) is detectable and clinically meaningful.

The second experimental study (**Chapter 4)** consists of a cross-sectional study, which aims to determine the associations amongst habitual exercise, GE, appetite and EI. Although GE has often been compared in obese and non-obese individuals, the influence of habitual physical activity level and associated differences in body composition and EE on GE is still largely unknown. Previous studies [16-18, 23-25, 27-29] have categorised obese and non-obese individuals on the basis of BMI or ideal body weight. Recently, a series of publications has highlighted the role of resting EE and fat free mass (FFM) (but not BMI) in appetite control [36-39]. However, little attention has been given to the influence of physical activity level, body composition (fat mass (FM) and FFM) and EE on GE. Although some evidence suggests that GE is faster in marathon runners compared to sedentary individuals [40], this study had a small sample size (n = 10 per group), gave limited descriptors of body composition, and did not report EI, appetite and other eating behaviour characteristics. Marathon running is a very specific activity and it therefore remains to be established whether GE is altered in individuals involved in a wider range of physical activities and how this relates to differences in subjective appetite sensations, EI, habitual diet, food preferences, resting and activity EE, processes of food reward, eating behaviour related traits (e.g. dietary restraint) and body composition.

The cross-sectional study in this chapter seeks to address some of these gaps by examining GE across a larger cohort of habitual exercisers (participating in a range of activities) and sedentary individuals (n = 22 per group), and by characterising a number of factors that may account for potential differences in GE and eating behaviour. If these factors have a significant influence on GE, then they should be measured and controlled for in future studies examining changes in GE (and parameters that may be affected by GE) in the pathophysiology of obesity or other conditions and in response to treatments.

Although cross-sectional studies can provide important information, they do not allow for a causal relationship between exercise, GE and appetite control to be determined. Potential differences in GE between active and sedentary individuals could be attributed to differences in body composition, habitual diet or eating behaviours. Furthermore, gut adaptations may only have occurred after a long period of training at high intensity and volume. In the shorter term evidence suggests exercise-induced EE and EI are only weakly coupled (i.e. EI is not matched to increased EE) [11, 41]. It is possible therefore that a period of transition or uncoupling of EE and EI occurs before a steady state (coupling of EE and EI) is achieved [42]. Consequently it could be expected that changes in GE and other mechanisms of appetite control may differ over time as exercise programs progress. GE could be one mechanism that may impede weight loss by contributing to changes in appetite and EI with exercise. However, it has yet to be established whether GE is altered. To investigate this knowledge gap, **Chapter 5** aims to determine the effects of a short-term (4-week) supervised exercise intervention on GE, appetite and EI in previously sedentary overweight and obese males.

The final chapter (**Chapter 6**) of this thesis aims to draw together the findings from the experimental studies, to discuss various methodological aspects and potential implications of the studies, and to discuss areas that warrant future investigation.

In summary, this thesis aims to develop a better understanding of GI processes of appetite control, and in particular GE, by examining (i) the variability of GE that

occurs from day to day in overweight and obese individuals, (ii) the influence of habitual physical activity level and associated differences in body composition and EE on GE, appetite and EI, and (iii) changes in GE, appetite and EI as overweight and obese individuals progress from a sedentary to a physically active lifestyle. The overall purpose is to further explore the processes involved in appetite control, weight regulation and responses to exercise.

Chapter 2: Literature Review

Modified from: Horner, K.M., Byrne, N.M., Cleghorn, G.J., Näslund, E, King, N. A. (2011). The effects of weight loss strategies on gastric emptying and appetite control. *Obesity Reviews,* 12(11):935-51.

2.1 INTRODUCTION TO APPETITE CONTROL

The main focus of this review and thesis is concerned with developing a better understanding of the effects of exercise on GI processes of appetite control, and in particular gastric emptying (GE). However, it is important to acknowledge that a wide range of genetic, environmental, psychological, and physiological factors contribute to the short and long term control of food intake [13]. Some individuals are more prone to increased perceptions of hunger and energy intake than others, which can be explained at least in part by a potent genetic element [43-45]. In addition, a number of environmental aspects including large portion sizes and the ready availability of highly processed foods influence food intake.

The expression of appetite therefore results from complex interactions between biology and the environment. Blundell (1991) conceptualised appetite control to consist of three levels of events and processes [46] which interact to form part of a 'psychobiological system' controlling appetite [47]. These include (i) psychological events (hunger perceptions, cravings, hedonic sensations) and behaviour (meals, snacks, energy and macronutrient intakes), (ii) peripheral physiology and metabolic events, and (iii) neurotransmitter and metabolic interactions in the brain [46]. The relation between these three levels of operations and the sensory, cognitive, post-ingestive and post-absorptive processes which contribute to satiation (control of meal size) and satiety (post-meal inhibition of eating) are shown in **Figure 2.1**.

Image removed for copyright reasons (Blundell, J., *Making claims: functional foods for managing appetite and weight*. Nature Reviews. Endocrinology, 2010. **6**(1): p. 53-56.)

Figure 2.1 Satiety cascade showing the relation between appetite control and some mediating psychological and physiological processes. All of these signals operate through neural sites – particularly in the hypothalamus and brainstem. Satiation refers to the control of meal size, satiety to the post-meal inhibition of eating. From: Blundell (2010) p. 54 [48], adapted by D.J. Mela from Blundell et al. (1987) [49].

Before food is ingested, sensory mechanisms induced by the thought, sight or smell of food can trigger a number of physiological signals, which constitute the cephalic phase of appetite control [46]. In addition, sensory characteristics such as taste or temperature determine the palatability of food that can have a strong influence on food intake [50]. Cognitive dimensions involved in appetite control include the beliefs or expectations regarding the 'expected satiety' from foods [51] and levels of dietary restraint (whereby individuals cognitively attempt to limit food intake) [52]. When food enters the GI tract and subsequently empties from the stomach into the small intestine, a variety of factors including gastric distension, nutrient stimulation of intestinal mechanoreceptors and chemoreceptors (see [21, 22]) and the release of several gut peptides contribute to satiation and satiety. In the post absorptive phase, these GI factors continue to contribute to satiety along with additional mechanisms arising from the metabolism of nutrients. Rising levels of blood glucose as a result of carbohydrate digestion, the thermogenic effect of protein and the assimilation of fatty acids all contribute to the post absorptive phase of satiety [53]. Further, when nutrients enter the bloodstream, hormones such as insulin and leptin are secreted and implicated in satiety [34]. Therefore, while the focus of this thesis and review concerns primarily the role of the GI tract and in particular GE in appetite responses, it is important to recognise that the gut operates amongst a complex integration of a wide range of processes to control food intake.

2.2 FOCUS ON THE GUT IN APPETITE CONTROL

Hunger and satiety have long been associated with gastric motor and sensory functions and the role of the stomach in appetite control has been of interest for at least a century [54]. Altered satiety signalling primarily originating from the GI tract has been implicated in the development of both obesity and type 2 diabetes [14]. Consequently there is a growing interest in targeting the GI tract for the treatment of obesity [14, 30-34]. Bariatric surgery is the only obesity treatment to date associated with a persistent reduction of energy intake and sustained weight loss [55] of which changes in gut physiology may have a fundamental role. Some procedures appear to reduce weight by changing the profile of circulating gut peptides implicated in appetite control [56-58], which may be due in part to alterations in the emptying rate of the gastric remnant [59-65]. Given the central role of the gut in appetite control, alterations in gut physiology may be a key mechanism contributing to changes in appetite and food intake with other weight loss strategies including exercise. However, limited research has examined the effects of exercise on gastric function and appetite control. This chapter therefore reviews the literature concerning the effects of surgical, pharmacological, diet and exercise interventions on GI targets of appetite control with a view to explaining how GI mechanisms may contribute to changes in appetite and EI and why particular strategies (e.g. surgery) may be more effective in sustaining long-term reductions in food intake. This knowledge may assist both scientifically - to help explain factors influencing appetite control - and practically to facilitate the more effective use of non-surgical strategies such as exercise in weight management.

The following sections briefly discuss the peripheral physiological signals (including tonic (i.e., long term) and episodic (i.e., short term) signals) along with the neuronal circuits involved in appetite control. Episodic signalling, including the role of GE in appetite control and alterations in obesity are the focus of the next sections. This is followed by a review of the effects of different weight loss strategies on GE and gut peptides, with a particular emphasis on exercise. Methodological issues, future directions and the evidence to date are then summarised.

2.3 ENERGY HOMEOSTASIS: TONIC AND EPISODIC PROCESSES

It is widely recognised that energy homeostasis is governed by a complex neuroendocrine feedback system between the periphery and the central nervous system which involves the interaction of tonic and episodic signals with a network of hypothalamic, mesolimbic, and hindbrain circuits [66]. To understand why appetite and energy intake might vary in response to different weight loss strategies the various peripheral and neural signals controlling food intake should be considered. Changes in central circuits may mediate alterations in gut function, while tonic signals can also influence the sensitivity to signals from the GI tract.

The hypothalamus plays a key role in appetite control; in particular the arcuate nucleus receives and processes signals not only from other areas of the brain but also from the periphery. Briefly, the arcuate nucleus houses 2 sets of neuronal circuits: an appetite inhibiting circuit and an appetite stimulating circuit. A group of neurons co expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) are part of the appetite stimulating circuit, while pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript neurons are part of the appetite inhibiting circuit [67]. The balance between the two circuits is critical for maintaining energy balance. Ultimately, there is input from these circuits or vagal afferents to the brainstem, particularly the nucleus tractus solitarius, which relays to the vagal motor nuclei to alter gastrointestinal function [66]. While knowledge of the neurocircuitries governing metabolic homeostasis and neuroendocrine changes are also instrumental for developing more effective antiobesity strategies (for detailed reviews see [68, 69]), the focus of this literature review concerns the responses of peripheral signals to weight loss. Peripheral feedback signals are often categorised as short term or long term but the connotation episodic and tonic is functionally more appropriate [70]. These two sets of signals appear to have different roles in the control of appetite [71], and are therefore discussed separately in this section. However, it is important to recognise that tonic signals can influence episodic signals [72, 73] (for example, sensitivity to short-term signals are affected by leptin [74]).

Tonic signals such as leptin are constantly released, mainly by adipose tissue in proportion to the amount of lipid stores, therefore signalling chronic nutritional state [75]. Insulin, released by pancreatic β cells also shares many properties with leptin as a tonic signal regulating energy homeostasis. Centrally, insulin and leptin act in concert, both stimulating POMC and inhibiting NPY to bring about satiety.

However, observations that leptin levels are elevated in the vast majority of obese individuals have led to the hypothesis that most obese subjects are resistant to the actions of leptin [76] and hyperinsulinemia, often indicative of insulin resistance may be also associated with a reduced inhibition of food intake. In response to weight loss, insulin and leptin appear to respond similarly regardless of the type of weight loss intervention. Following an energy deficit induced by bariatric surgery [77-79], diet [78, 80-82] and/or exercise [83-87], leptin and insulin levels decrease, which would be expected to stimulate appetite. This increase in appetite can be reduced by exogenous administration of leptin [88]. These findings, together with evidence of tonic signals responding to changes in energy availability [89, 90] suggest that tonic signals respond primarily to changes in energy balance and body weight, and that their primary role may be to resist energy deficits.

Episodic signals including gastric distension, and orexigenic (ghrelin) and anorexigenic peptides, arise largely from the GI tract and oscillate periodically with the act of eating [71]. While some of these peptides involved in the episodic control of appetite are also produced and secreted in the brain and hypothalamus [91] it is likely that the peripheral and central nervous system production and actions represent parallel pathways in the modulation of feeding behaviour [92]. Ghrelin, unlike other gut peptides detailed in this review stimulates appetite and rises before meals suggesting a role in meal initiation [93]. Some evidence suggests that it is the state of energy balance (i.e,. in energy balance or in negative energy balance) that influences fasting ghrelin levels [77, 89, 90]. Fasting levels of ghrelin increase in response to diet- and exercise-induced energy deficits [86, 94-96]. Following bariatric surgery, changes in ghrelin levels are equivocal [97]. However, in one study increased fasting ghrelin levels were observed in individuals who were in negative energy balance and not in those in energy balance [77], suggesting the energy balance state could explain some of the ambiguity in findings. Other evidence suggests that fasting appetite sensations respond to changes in energy availability and body weight. Data collated from several weight loss intervention studies shows that fasting appetite sensations are strongly associated with body weight changes [98]. However, meal-induced appetite responses are not strongly associated with energy availability or body weight [89, 90, 98, 99]. In essence these are two different measures. The fasting state reflects the homeostatic energy state after a period of reduced body energy (exercise-induced energy expenditure or food deprivation) and the postprandial state reflects

the interactions between the physiologic system and the physiologic action of food on satiety signalling [71, 99]. Changes in postprandial appetite sensations may therefore occur in response to the direct effects of the weight loss intervention *per se* and could help to explain why changes in energy intake vary with different weight loss strategies. The changes in episodic signals implicated to have a role in meal induced appetite responses are discussed further.

Episodic signals are typified by mechanical factors such as gastric distension and gut peptides such as cholecystokinin (CCK) [67] both of which may be influenced by the rate of GE. See Delzenne et al. (2010) [100] for a comprehensive overview of GI targets of appetite control in humans. Numerous reviews have considered the effects of weight loss strategies on appetite related gut peptides (see [79, 101-103]). This review will concentrate on the overlooked issue of GE; gut peptides (although not measured in this thesis) will also be discussed due to their integrative relationship in appetite control.

2.4 GASTRIC EMPTYING

GE is a complex process determined by the coordinated motor activity of the stomach and the proximal intestine [104] and myogenic, neural and hormonal factors. GE of solids has a biphasic pattern. After a lag time during which solid foods are broken down to small particles of 2-mm diameter, GE occurs in a linear pattern similar to that of high calorie or viscosity liquid meals [54]. The parameters reported are therefore essential to take into account when considering the kinetic and temporal pattern of GE in relation to appetite control. For example, GE half time (which is generally defined as the time taken for 50% of the meal to empty) does not reflect the early pattern of GE. Therefore, some studies additionally report lag time, which is generally considered to reflect the initial rate of emptying.

Blood glucose control is also important in the regulation of GE [105] although the underlying mechanisms are unknown. For example, low blood glucose levels induced by insulin infusion have been associated with faster GE in healthy individuals [105]. With nutrient-containing meals, GE is likely regulated by the balance between gastric propulsion, a function of intragastric volume, and negative feedback arising from duodenal receptors [104]. Meal properties have a strong influence on GE. For example, GE is slowed by an increase in meal energy [106] and

CHO content and by an increase in osmolality [107, 108]. In contrast, an increase in the volume ingested has been shown to accelerate GE [109]. In addition, GE is influenced by a variety of individual and test characteristics such as age [110], sex [111], position of the subject [112], drug intake [113], and time of the day [114].

2.4.1 Gastric Emptying and appetite control

Persuasive evidence suggests that GE is associated with appetite and energy intake [115-118]. The GI tract responds to a meal in three integrated and overlapping phases: cephalic, gastric, and intestinal phases [119]. As food enters the stomach and subsequently empties into the small intestine, a variety of factors including gastric distension, nutrient stimulation of intestinal mechanoreceptors and chemoreceptors (see [21, 22]) and several gut peptides released from the GI tract contribute to satiation (control of meal size) and satiety (post-meal inhibition of eating). Although it is intuitive that a faster GE leads to increased appetite, the role of GE in appetite control is complex. Gastric distension influences appetite by triggering stretch and tension mechanoreceptors which relay information to the brain (see [120, 121]). There is a close relationship between the sensation of fullness and antral distension [122, 123]. Furthermore, food intake at a buffet meal has been shown to be inversely related to the volume of gastric contents remaining from a previous meal [124]. These findings suggest that an accelerated rate of GE and hence reduced gastric distension might predispose to overeating.

However, in addition to gastric distension, the presence of nutrients in the small intestine is critical to satiation and satiety [125]. A number of gut peptides are released in response to intestinal nutrients and act by entering the bloodstream and indirectly via the vagus nerve to inhibit appetite [126, 127]. After food intake, CCK is released into the circulation from endocrine I-cells of the duodenum and the jejunum [128] whereas glucagon-like peptide-1 (GLP-1) and polypeptide YY (PYY) are released from L cells located mainly in the distal small intestine. Both GLP-1 and PYY show a biphasic response to meal ingestion [127]. It is most likely duodenal nutrients initiate a neural and/or humoral signal to the distal gut contributing to their early release [129-132], which is then followed by direct nutrient stimulation of L cells in the ileum [127, 133]. In the early postprandial period, a more rapid GE is directly correlated with increases in plasma levels of CCK [134, 135], GLP-1 [61,

130] and PYY [25, 135]. Further evidence suggests a threshold rate of GE exists which must be exceeded to stimulate GLP-1 release [130]. Upon release GLP-1, PYY and CCK in turn act to inhibit GE [136] but the inhibition of GE is not necessary for the control of food intake by intestinal stimulation [22, 137] and a direct effect on satiety centres is also very likely [22, 33, 137]. In contrast, ghrelin acts to accelerate GE [138]. However, a more rapid GE rate is correlated with lower postprandial ghrelin concentrations [139, 140], which in turn are associated with reduced appetite [139]. Taken together these findings suggest that a slowing of the emptying rate might increase and prolong gastric distension but also result in a delayed or reduced release of CCK, PYY and GLP-1 and reduced ghrelin suppression. Therefore, satiety due to gastric distension would be increased but intestinal satiety signalling to the brain diminished [141].

Collectively, the release and maintenance of episodic signals arising from the GI tract likely have an additive effect in satiation, satiety and the ability to compensate accurately for prior energy intake [22, 142-144]. Factors such as the time interval between meals (see **Figure 2.2**) will influence the relative contributions of gastric and intestinal signals to appetite control. Gastric distension by food may play a major role in the sensation of fullness whereas the reduction of hunger feelings after a meal result from an interaction of nutrients with receptors in the small intestine [22]. Differences in the initial versus subsequent rate of GE [145] may explain why accuracy in compensation for prior energy intake diminishes as the time interval to the next meal increases [146] - thus highlighting the importance of considering the kinetic and temporal pattern of GE in relation to appetite control. The characteristics of the meal [120, 147] (see **Figure 2.2**) will also have a role. In the absence of nutrients for example gastric distension appears to be a major factor in the return of hunger [147]. Overall, there appears to be an important integrative relationship between GE and gut peptides in appetite control.

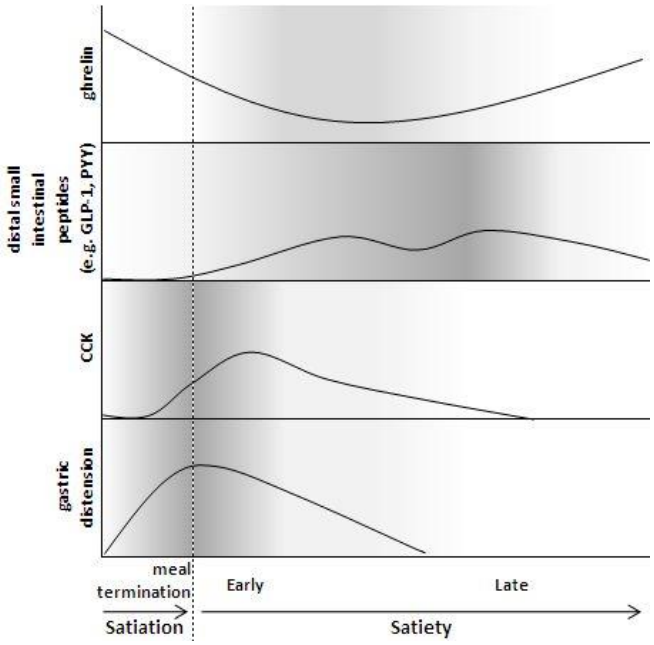


Figure 2.2 A hypothetical model of the relative contributions of gastric distension, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin (all episodic signals influenced by gastric emptying) to satiation and satiety. Darker shading indicates a greater relative contribution. Time (x axis) and magnitude (y axis) are dependent on the meal characteristics. Gut peptides are particularly sensitive to specific macronutrients (e.g. CCK to fat and protein [148]; GLP-1 to carbohydrate and fat [149]; PYY primarily to fat [149]; ghrelin suppression to protein and carbohydrate [150]) and peak at different time points. Ghrelin is the only peptide implicated in meal initiation, rising before a meal and falling approximately 60min after a meal [93]. During a meal and shortly after, gastric distension and CCK (peaking within 15min [151]) are important determinants of satiation. The release of GLP-1 and PYY occurs in a biphasic pattern and can remain elevated for hours after a meal [127, 152]. As the stomach continues to empty, it is likely that the exposure of nutrients to the distal small intestine has an increasingly greater contribution to satiety. These episodic signals may also be modulated by tonic signals such as leptin and insulin which are responsive to energy availability and body adiposity.

2.4.2 Gastric Emptying in Obesity

Although GE has long been implicated in the pathogenesis of obesity, studies investigating the role of GE in obesity have shown inconsistent findings. Faster [16-18], similar [23-25] and slower [27-29] emptying rates have been demonstrated in obese compared to non-obese individuals. Studies that have correlated GE with BMI in non-obese individuals have demonstrated that an increase in body surface area and BMI are associated with slower GE [153-155]. Maddox et al. (1989) similarly demonstrated that increasing BMI was associated with a longer GE half time in a group of 62 obese and non-obese individuals [27]. However, others have shown no

significant correlation [15, 156]. Therefore the association of BMI with GE remains unclear.

A number of hypotheses and differences in control mechanisms have been put forward to try and account for why GE may be altered in obese as opposed to lean individuals. Dysfunction of the autonomic nervous system, decreased CCK sensitivity due to high fat diet [134] and lower plasma concentrations of somatostatin and neurotensin have all been observed in obese compared to normal weight subjects [157]. These alterations in control mechanisms would favour a faster GE. In contrast, a reduction in fundal tone [28], altered sensitivity of stretch receptors [27] and a change in antral area or mixing [158] are proposed mechanisms behind a slower GE in obese individuals [29]. Another hypothesis is that GE may become deregulated at extremes of the body mass spectrum [154]. It is possible that different mechanisms may determine GE in lean and obese individuals. For example, whereas fasting ghrelin is the best determinant of gastric kinetics in healthy controls (see **Figure 2.3**), this action is lost in obesity [159].

Image removed for copyright reasons (Valera Mora, M.E., Scarfone, A., Valenza, V., Calvani, M., Greco, A.V., Gasbarrini, G., and Mingrone, G., *Ghrelin Does Not Influence Gastric Emptying in Obese Subjects*. Obesity, 2005. **13**(4): p. 739-744).

Figure 2.3 Correlation ($y = -0.2391x + 157.9$; $R^2 = 0.95$) between ghrelin and GE half time ($t_{1/2}$) in lean individuals (n = 16). In the same study, no association between ghrelin and GE was demonstrated in obese individuals. From Valera Mora et al (2005) p. 741 [159].

It has also been proposed that a slower GE could have a role in the pathogenesis of obesity through the inactivation of gut peptides signalling satiety from the intestine and a subsequent increase in food intake [29]. Therefore, it is not clear whether an

abnormal GE is a consequence of weight gain or may be a contributing factor to weight gain. Further studies are needed to clarify whether or not the development and maintenance of obesity can be attributed to changes in GE [54].

The contradictory findings from studies to date have generally been attributed to differences in methodologies and some methodological limitations (e.g. not controlling for smoking or menstrual cycle phase (e.g. [27, 160]). In addition, few studies document participants' food intake. Seimon et al. (2013) [26] assessed GE in response to a mixed-nutrient drink in three groups of lean, overweight and obese individuals and also measured prior dietary intakes. No difference in GE or habitual diet were found between the three groups and the authors concluded that in the absence of differences in habitual EI, GE is unaffected in obese individuals [26]. The influence of individual characteristics such as body composition (fat- and fat-free mass), energy expenditure (EE) and physical activity levels on GE have also been poorly documented and could potentially explain some of the inconsistency in findings. To date, BMI or percent of ideal body weight have been the major criteria for distinguishing obese and non-obese groups in studies investigating GE in obesity. However, individuals with similar BMIs could have quite different body compositions. For example, an active individual may have a greater lean mass than a sedentary individual despite a similar BMI. In addition, recent data provide evidence for a fundamental link between EE and EI [39]. It is therefore important to establish whether body composition, EE and physical activity influence GE so that they can be controlled for in studies examining GE in obesity and in other conditions.

2.5 THE EFFICACY OF BARIATRIC SURGERY IN WEIGHT LOSS: WHAT IS THE ROLE OF THE EMPTYING RATE?

The contention that GE has a role in obesity has led researchers to investigate various strategies designed to alter GE with the aim of increasing satiety, reducing energy intake and consequently leading to weight loss (e.g. [161, 162, [163]). Elucidating the mechanisms behind changes in appetite with surgery is important. Such knowledge may facilitate the development and modification of anti-obesity treatments including diet and exercise to achieve at least some of the weight loss of surgery [164]. Early studies indicated that after gastric bypass solids emptied at a slower rate from the small gastric pouch [160, 165, 166]. However, there was no correlation with weight loss [165, 166] suggesting other mechanisms caused weight

loss [165]. These studies were cross sectional and comparisons were made either with controls or other surgeries. Although now an abandoned procedure, with jejunoileal bypass (JIB), GE was slowed 9 months after surgery [17] and unchanged in patients 20 years after JIB compared to controls [167]. Today, the most commonly performed bariatric procedures are Roux-en-Y gastric bypass (RYGB), adjustable gastric banding and sleeve gastrectomy (SG) (see [168] for a detailed description of surgical procedures). RYGB, a procedure that both minimizes gastric capacity and accelerates delivery of nutrients to the distal small intestine, produces greater weight loss and earlier resolution of diabetes than gastric banding, which is a purely restrictive procedure [169].

Various mechanisms have been proposed to contribute to weight loss and post-surgical reductions in appetite after RYGB (see [164] for a more complete review). There is no doubt that gastric restriction plays a role in weight loss [170]. A gastric pouch with a volume of 30ml restricts the amount of food that can be consumed and accommodated following RYGB. However, gastric restriction does not account for the increase in weight loss with RYGB when compared with gastric banding. Evidence suggests that the restrictive and malabsorptive components alone are insufficient to account for the resulting weight loss with RYGB [79]. Reduced ghrelin levels after RYGB have also been implicated [80]. However, increased, decreased and unchanged fasting ghrelin levels after surgery have been reported [97]. Instead, the majority of studies suggest that the efficacy of RYGB in reducing appetite and promoting weight loss may relate predominantly to distal small intestinal effects [170]. After gastric bypass the pyloric 'meter' or brake is absent and the diversionary component allows rapid transit of nutrients to the jejunum [22]. One hypothesis is that the expedited delivery of nutrients to the hindgut may stimulate a faster and enhanced postprandial release of anorexigenic gut peptides [164]. The higher levels and early appearance of PYY and GLP-1 are consistent observations following RYGB [136] but not after gastric banding [171] and could explain the difference in weight loss [172]. A faster emptying rate from the small pouch has been suggested to contribute to the greater anorexigenic neurohormonal response following RYGB [59, 61, 62]. Following RYGB, GLP-1 levels about 5 fold higher than observed after meal ingestion in healthy lean individuals have been reported [62]. In this study similar patterns of decreased hunger, increased fullness and a faster delivery of nutrients to the intestine were observed at 3 days, 2 months and 1

year post RYGB [62]. At 6 weeks after RYGB compared to pre-surgery, Morinigo et al [61] observed an accelerated emptying of the small pouch, an increased postprandial GLP-1 and PYY release, reduced hunger and increased satiety. In contrast, with gastric banding, the emptying rate is unchanged [173-175]. This suggests that a faster emptying rate and increased delivery of nutrients to the distal small intestine [59, 61, 62] could have a role in the enhanced release of PYY and GLP-1 [136], and the greater weight loss that occurs after RYGB compared to gastric banding [169]

An accelerated emptying rate has also been observed after SG, a procedure which does not bypass the duodenum, and may contribute to the increased GLP-1 [176] and PYY [63] secretion after SG. Following RYGB and SG inducing similar weight losses, postprandial PYY levels increased which corresponded to an increase in satiety [63]. Two possible explanations were proposed: 1) SG changes neuronal or humoral signalling that regulate postprandial PYY secretion, and 2) SG produces a decrease in gastric acid secretion and a faster GE; both changes which would lead to a faster delivery of undigested nutrients to the intestine and increase the PYY response [63]. Studies demonstrating an accelerated GE following SG in both the initial [60, 176, 177] and overall emptying rate [60, 64, 176] support the latter explanation. The duodenal switch procedure has also been associated with accelerated GE and an increased PYY response [65]. Although, it should be noted that the non-operated controls were given a larger meal with a higher energy content in this study. Findings from studies investigating the effects of different surgical procedures on GE (or emptying of the small pouch in the case of gastric bypass) are summarised in **Table 2.1**. Collectively, the evidence indicates that in addition to gastric restriction, RYGB and SG reduce weight by changing the profile of circulating gut peptides implicated in appetite control [56-58, 101], which may partially be due to a faster emptying rate and increased delivery of nutrients to the distal small intestine.

Table 2.1 A summary of studies examining the effects of bariatric surgeries on gastric emptying

Procedure	Reference	Weight Loss*	Study design/intervention	Initial GE (outcome)	Overall GE (outcome)
Gastric Bypass	Horowitz et al. (1982) [166]	23 ± 3kg	Cross section of patients (n = 12) 12 months after bypass compared to non-operated controls (n = 11)	faster liquid (t_{lag}, % remaining at 5min, 10min), solid slower except faster in 4 subjects (t_{lag})	faster liquid ($t_{1/2}$). solid slower except faster in 4 subjects ($t_{1/2}$, % remaining at 50, 100min)
Jejunoileal Bypass	Näslund et al. (1997) [167]	44 ± 4 to 31 ± 4 kg/m^2	Cross section of JIB operated subjects 20 +/- 3 yrs ago (n = 7) compared to non-operated obese controls (n = 7)	unchanged (t_{lag})	unchanged ($t_{1/2}$, % remaining at 60, 90, 120 min)
	Näslund et al. (1998) [178]	42 ± 4 to 31 ± 4 kg/m^2	GE measured pre and 9 months post surgery (n = 9)	slower (t_{lag})	slower ($t_{1/2}$)
Roux-en-Y Gastric Bypass	Morignio et al. (2006) [61]	15.4 ± 6.3kg	GE measured pre and 6 weeks post RYGB (n = 9)	faster (AUC_{0-60})	faster (AUC_{0-60})
	Akkary et al. (2008) [59]	50.6kg weight loss in those with faster initial GE compared to 47.3kg in those with slower	Comparison of weight loss in those with slower (n = 116) and faster initial (n = 188) emptying at 1 day postoperative at 1 year follow up	faster initial GE (30min) 1 day postoperatively associated with greater weight loss at 1 year	-
	Falkén et al. (2011) [62]	45.5 ± 1.9 (preoperative) to 38.6 ± 1.8 (at 2 months) to 30.3 ± 1.8 (at 1 year) kg/m^2 .	GE measured pre and 3 days, 2 months and 1 yr post RYGB (n = 12)	Faster (acetaminophen absorption peak time)	Faster (acetaminophen absorption $t_{1/2}$)
Sleeve Gastrectomy	Shah et al. (2010) [64]	-	Cross section of morbidly obese non operated type 2 diabetics (n = 20), morbidly obese type 2 diabetics operated with SG (n = 23), lean controls (n = 24)	not tested	faster ($t_{1/2}$) in operated
	Melissas (2008) [60]	Mean group weight: 140 (preoperative) to 101 (at 6 months) to 89 (at 24 months) kg.	GE measured pre surgery, 6 and 24 months after SG (n = 9)	faster (t_{lag}) at 6 and 24months compared to pre-surgery	faster ($t_{1/2}$, % GE rate) at 6 and 24months compared to pre-surgery
	Braghetto et al (2009) [176]	-	Cross section of obese subjects 3 months after SG (n = 20) compared to normal controls (n = 18)	faster (% remaining at 20, 30, 60 min) in operated	faster ($t_{1/2}$) in operated
	Bernstine et al. (2009) [177]	125 ± 25 to 99 ± 22 kg	GE measured pre surgery and 3 months post SG (n = 21)	faster (% remaining at 30min)	unchanged ($t_{1/2}$, % remaining at 1,2,3,4h)

Gastric Banding	de Jong et al. (2008) [173]	47.8 to 41.7 kg/m^2	GE measured pre surgery and 16 months post gastric banding (n = 16)	unchanged (t_{lag})	unchanged (GE rate (%/h))
	Burton et al. (2010) [174]	143.3 ± 25.5 to 96.3 ± 18.2 kg	Cross over design. 2 GE studies: One with the LAGB at its optimal volume and another with 1 ml within the LAGB (n = 14 patients who had achieved >50% weight loss > 12 months post surgery).	not tested	unchanged ($t_{1/2}$)
	Usinger et al. (2011) [175]	125 ± 8 to 121 ± 8 kg	GE measured pre and 6 weeks post LAGB (n = 8 obese with and without impaired glucose tolerance).	unchanged (acetaminophen absorption peak time)	unchanged (acetaminophen absorption AUC).
BPD-DS	Hedberg et al. (2011) [65]	51.7 to 31.1 kg/m^2	20 patients having undergone BPD-DS in median 3.5 yrs previously compared to previous normal GE data in the same lab	faster (t_{lag}) in operated	faster ($t_{1/2}$) in operated

* Weight Loss in units reported in original paper. AUC: area under curve; BPDS: biliopancreatic diversion with duodenal switch; GE: gastric emptying; LAGB: laparoscopic gastric band; JIB: Jejunoileal bypass; SG: sleeve gastrectomy; $t_{lag:}$ lag time; $t_{1/2:}$ half time.

2.6 ADDITIONAL SURGICAL AND PHARMACOLOGICAL WEIGHT LOSS STRATEGIES: CONTRASTING EFFECTS ON GASTRIC EMPTYING

Other surgical methods such as gastric pacing and gastric electrical stimulation which do not alter normal GI anatomy are also being explored as obesity treatments, but to date have produced inconsistent results with amount of weight loss (see [32] for a review). Their exact mechanism of action has not yet been identified; however, changes in GE have been implicated. Delayed GE in the initial 45 minutes after eating and reduced food intake was demonstrated with a system of stimulation through temporary fundic mucosal electrodes [179]. In contrast, accelerated GE was observed with the TANTALUS system of stimulation [161]; a system found to achieve long term weight loss comparable to gastric banding [180]. This could suggest that modulation of GE may only have a minor role in the effects of these strategies on weight loss. An alternative explanation may be that unlike other gastric electrical stimulation devices, the TANTALUS system is activated only following food ingestion. Sanmiguel et al. [161] postulated that rapid GE may reduce food intake and induce symptoms such as fullness, nausea and bloating, by activating postgastric mechanisms, such as intestinal distension and/or the release of gut peptides that mediate satiation.

Both delayed and accelerated GE have also been implicated in improved appetite control and weight loss in pharmacological studies, further highlighting the lack of consensus on whether accelerated or delayed GE is advantageous. The majority of strategies have been directed towards slowing GE. Initial studies indicated that slower GE was associated with weight loss after 12 weeks of Sibutramine treatment [181]. Recent drug developments have focused on targeting gut peptides such as GLP-1 (exenatide and liraglutide), amylin (pramlintide) and PYY [intranasal PYY (3–36) and AC-162325], which have been demonstrated to slow GE and reduce appetite and food intake [182-190]. In contrast, findings of slowed GE with the cannabinoid dronabinol led to speculation that faster GE and enhanced satiation may be one mechanism behind the appetite and weight reducing effects of Rimonabant; a cannabinoid antagonist [191]. Torra et al (2010) [192] provided the first evidence supporting the hypothesis that pharmacologically accelerating GE could enhance satiation and reduce meal size. Erythromycin

administration in obese individuals exerted a faster GE in the first 15 minutes after initiation of eating which translated into a small reduction in energy intake (135 kcal) compared to individuals who received placebo. However, the most commonly studied prokinetic drugs including erythromycin, metoclopramide, domperidone and cisapride have side effects such as nausea [193-195], making it difficult to separate their effects on appetite. Furthermore, the neurotransmitter systems affected by drugs do not exclusively affect appetite. Another explanation for contradictory findings of both delayed and accelerated GE being implicated in reduced appetite and energy intake may be that some pharmacological approaches can interfere with or override the endogenous release of gut peptides implicated in appetite control such as CCK [196, 197], GLP-1 [197, 198] and PYY [189, 197]. For example delayed GE caused by exendin-4 may cause duodenal nutrient delivery to decrease to such an extent that little endogenous GLP-1 is released [198]. Furthermore, a drug-induced delay in GE results in reduced ghrelin suppression [140]. As a result it is difficult to apply findings from pharmacological studies manipulating GE to strategies such as lifestyle interventions. These effects are different to the normal physiological regulation of food intake whereby the endogenous release of various peptides and suppression of ghrelin play key roles in appetite control.

2.7 SUMMARY OF EFFECTS OF SURGICAL AND PHARMACOLOGICAL WEIGHT LOSS STRATEGIES ON GASTRIC EMPTYING

The majority of recent gastric bypass and SG studies have been found to accelerate the emptying rate, while gastric banding appears not to alter GE. Other surgical interventions and pharmacological strategies targeting appetite control have been demonstrated to both accelerate and delay GE. The ability of these varied strategies to reduce appetite and promote weight loss while having opposite effects of GE could suggest that modulation of GE may only have a minor role in weight loss. However, these contrasting findings could also be attributed to differences between interventions and methodologies and targeting different pathways.

Overall, it appears that both delayed and accelerated GE can modulate food intake. Increasing post-ingestive negative feedback (e.g., anorexigenic gut peptides) has been emphasised as one optimal therapeutic goal in the treatment of obesity [199]. Strategies which accelerate GE represent a reasonable target for preventing

overconsumption by increasing the release of anorexigenic gut peptides. However, an overall faster GE time, despite potentially enhancing the magnitude of satiety sensations from intestinal factors between meals is likely to lead to decreased feelings of fullness arising from the stomach and shorten the interval to the onset of the next meal. Thus meal frequency may be increased and hence net energy intake may not be reduced [192]. Changes in the expression of appetite influenced by GE are expressed behaviourally as changes in meal size, meal frequency and snacking behaviour between meals. For weight loss, the most effective strategy to reduce energy intake would be one which targets satiation, satiety and snacking behaviour. Such a strategy would involve manipulating the emptying rate to maximise both the sensation of fullness from the stomach, and the early onset and prolonged release of gut peptides from the intestine. Overall, the literature reviewed suggests that other strategies which combine a reduced gastric capacity with an accelerated GE and delivery of nutrients to the distal small intestine may stimulate an earlier and enhanced release of anorexigenic gut peptides and improve appetite control.

2.8 LIFESTYLE INTERVENTIONS, GASTRIC EMPTYING AND GUT PEPTIDES

Understanding the effects of energy restriction and exercise-induced energy deficits on GI targets of appetite control may be important for tailoring lifestyle interventions to minimise the impact of weight loss on appetite in future. Studies that have measured the effects of lifestyle interventions on GE and on both fasting and postprandial gut peptide responses are summarised in Tables **2.2** and **2.3** respectively.

Table 2.2 A summary of studies examining the effects of lifestyle interventions on gastric emptying

Lifestyle Factor	Reference	Weight Loss*	Study design/intervention	Initial GE (outcome)	Overall GE (outcome)
Diet	Verdich et al. (2000) [24]	18.8kg, 14.8%	8 wks formula (4.2 MJ p/d), 8 wks restriction (6.3 MJ p/d) followed by 8 wks weight maintenance (n = 19 obese)	slower (t_{lag})	unchanged (total 4h emptying time)
	Tosetti et al. (1996) [200]	10%	parallel study of 4 month energy restriction with or without intragastric balloon (n = 20 obese)	slower	slower (overall emptying rate)
	Corvilain et al. (1995) [201]	-	GE measured after 12hour and 4day fast (n = 12 normal weight, n = 11 obese)	faster (t_{lag})	slower ($t_{1/2,}$ in both groups)
	Hutson et al. (1993) [23]	13.7kg, 8.3%,	3-4 wks of a 100kcal p/d diet. GE tested 2 days after the normal diet had been reinstituted (n = 8 obese)	unchanged (t_{lag})	unchanged ($t_{1/2}$)
Exercise	Shimamoto et al. (2002) [202]	-	cross section of active (n = 7) and inactive (n = 7) elderly individuals	faster in active (time of peak $^{13}CO_2$ concentration)	faster in active (time of peak $^{13}CO_2$ concentration)
	Carrio et al. (1989) [40]	-	cross section of marathon runners (n = 9) and sedentary (n = 9) males	faster in marathon runners (t_{lag})	faster in marathon runners ($t_{1/2}$)
	Horner et al. (2010) [155]	↔	intervention of 3x40 min moderate intensity exercise classes p/wk (n = 9 adolescent girls) compared to a control group (n = 10).	unchanged (t_{lag}, t_{lat})	unchanged ($t_{1/2}$, t_{asc})
Combined Diet and Exercise	Mathus Vliegen et al. (2006) [162]	10.4kg, 9.9%	1 year of daily energy deficit of 2.3MJ (an energy- and fat-restricted diet), exercise (advice on increasing leisure activity and optional aerobics classes), behavioral modification and placebo tablets (n = 9)	unchanged (t_{lag})	slower (emptying rate (%/h)), unchanged ($t_{1/2}$)
	Mathus Vliegen et al. (2006)	3.5kg	2 months of same intervention above (n = 14)	unchanged (t_{lag})	unchanged ($t_{1/2}$), (%/h)
	Mathus Vliegen et al. (2006)	2.3kg	4 weeks of same intervention above (n = 42)	unchanged (t_{lag})	unchanged ($t_{1/2}$), (%/h)
	Wright et al. (1983) [16]	-	undefined intervention - subjects who lost weight during the 18 month study period underwent a second GE test (n = 4 obese)	unchanged (% remaining in the stomach)	unchanged ($t_{1/2,}$ % remaining in the stomach)

* Weight Loss in units reported in original paper. GE: gastric emptying; t_{lat}: latency time; $t_{asc:}$ ascension time $t_{lag:}$ lag time; $t_{1/2:}$ half time.

Table 2.3 A summary of studies examining the effects of lifestyle interventions on fasting and postprandial appetite gut peptides

Lifestyle Factor	Reference	Weight Loss*	Study design/intervention	Fasting gut peptides	Postprandial gut peptides
Diet	Verdich et al. (2001) [203]	18.8kg, 14.8%	pre and post 8 wks formula (4.2 MJ p/d), 8 wks restriction (6.3 MJ p/d) followed by 8 wks weight maintenance (n = 19 obese)	GIP ↓, GLP-1 ↔	GIP ↓, GLP-1 ↑
	Cummings et al. (2002) [80]	17.3kg, 17.4%,	3 months diet-induced weight loss followed by 3 months maintenance at reduced weight (n = 13 obese)	ghrelin ↑	ghrelin ↑
	Doucet et al. (2004) [204]	-	4 day energy restriction of 25% energy intake	PYY ↓	PYY ↓
	Adam et al. (2005) [205]	6.1kg	pre and post 6 wk very low energy diet (n = 32 obese)	GLP-1 ↔ (trend to ↓),	GLP-1 ↓,
	Moran et al. (2007) [206]	4.2 ± 3.9kg	pre and post 8 wks energy restriction (deficit of ~ 30%, intake), (n = 14 with PCOS, n = 14 controls)	ghrelin, PYY, CCK ↔	PYY, CCK, ghrelin ↔
	Lafferere et al. (2008) [81]	9.8kg	pre and post 55 +/- 10 days energy restriction (1000 kcal/d meal replacement plan) (n = 10 obese)	GIP ↔, GLP-1 ↔	GIP ↔, GLP-1 ↔
	Chearskul et al. (2008) [82]	17.9kg, 15%,	pre and post 8wks after restriction of approx 1800kj/d and 1 week of maintenance (n = 12 obese)	CCK ↔	CCK ↓ (30min)
	Olivan et al. (2009) [78]	10kg	pre and post 1000 kcal p/d diet. Post measurements after 10kg weight loss (55± 10d) (n = 10 obese)	ghrelin ↑, PYY ↔	PYY ↔, ghrelin ↔
	Essah et al. (2010) [207]	5.8kg (LF), 1.0kg (LCHO)	Pre and post 8 wks energy restricted (-500kcal p/d) low fat (LF) or low CHO diet (LCHO) (n = 30 obese)	PYY ↓(following both diets)	PYY ↓(following both diets)
	Sumithran et al. (2011) [208]	13.5kg	Pre, post and 1 year post a 10 week very low energy diet (n = 34 overweight/obese)	ghrelin ↑ PYY ↓ GIP ↔ GLP-1↓(wk10) GLP-1↔ (wk62) PP↔ CCK↓(wk10) CCK↔(wk62)Amylin↓	ghrelin ↑ PYY ↓ GIP ↑ GLP-1↔ PP↔ CCK↓(wk10) CCK↔(wk62) Amylin↓
	Sumithran et al. (2013) [209]	13%	Pre, post an 8-wk very low energy ketogenic diet (wk 8), and 2 wks after reintroduction of food (wk 10) (n = 39 obese)	ghrelin ↔ (wk 8)↑ (wk 10), Amylin↓ (wk 8, 10), PYY ↓ (wk 8) ↔ (wk 10), GIP ↔ (wk 8, 10), GLP-1↓(wk 8, 10), CCK ↓ (wk 8, 10) PP ↔ (wk 8, 10)	ghrelin ↔ (wk 8)↑ (wk 10), Amylin↓ (wk 8, 10), CCK ↔ (wk 8) ↓ (wk 10), PYY ↓ (wk 8, 10) GIP ↑ (wk 8, 10), GLP-1↔ (wk8, 10) PP ↑ (wk 8, 10)

	Lips et al. (2013) [210]	6.7kg	Pre and post a 3 wk very low energy diet (600kcal/d) (n = 12 obese with type 2 diabetes)	ghrelin ↔ PYY ↔ GIP ↔ GLP-1↔	GIP ↑ ghrelin ↔ PYY ↔ GIP ↔ GLP-1↔
Exercise	Chanoine et al. (2008) [211]	↔	Pre and post 1h p/d aerobic exercise (65-75%HRmax) for 5 days, (n = 34 normal, overweight adolescent boys)	GLP-1 ↔	GLP-1 ↑ (30 min)
	MacKelvie et al. (2007) [212]	↔	Pre and post 1h p/d aerobic exercise (65-75%HRmax) for 5 days (n = 34 normal, overweight adolescent boys)	total ghrelin ↔, AG ↑, DG ↔	total ghrelin ↔, AG ↑, DG (in normal weight) ↓, DG (in overweight) ↑
	Hurley et al. (1991) [213]	↔	Pre and post 10 wk exercise intervention (20 min at 70% VO_2max 3d p/wk) (n = 7 normal weight sedentary)	PP slight ↑, GIP ↔	PP slight ↑, GIP ↔
	Martins et al. (2010) [86]	3.5kg	Pre and post 12 wk aerobic exercise intervention, (5 d p/wk, 500kcal at 75% HR max) (n = 15 overweight)	AG ↑, total ghrelin, GLP-1, PYY ↔	↑ AG suppression, ↑ GLP-1, PYY ↔
	Kelly et al. (2009) [87]	2.8 ± 0.5 kg.	Pre and post 12 wk exercise (5d p/wk at 75%VO_2max) intervention combined with a eucaloric diet (n = 10)	PYY ↔, GIP ↔	PYY↑ (0-30min), GIP ↔
	Martins et al. (2012) [214]	3.5kg	Pre and post 12 wk aerobic exercise intervention, (5 d p/wk, 500kcal at 75% HR max) (n = 15 overweight)	GIP ↓ CCK ↔ obestatin ↔	GIP ↔ CCK ↔ obestatin ↔
	Guelfi et al. (2012) [215]	2kg(aerobic) 0.4kg gain (resistance)	12wk aerobic training (n = 12 overweight)/obese, 12wk resistance training (n=13 overweight/obese) 3 d/wk	PYY ↔ PP↔ ghrelin ↔	PYY ↔ PP↔ ghrelin ↔
Combined Diet, Exercise	Valderas et al. (2010) [63]	16.6 ± 4%	Pre and post 8wk diet (1300–1800 kcal/d (20–25 kcal/kg of ideal weight), behavioral modification and 180 min/wk aerobic, resistance exercise (n = 8 obese)	PYY ↓	PYY ↓
	Kelly et al. (2009) [87]	8.3 ± 1.1kg	Pre and post 12 wk exercise intervention (5d p/wk at 75%VO2max) combined with hypocaloric diet (~700kcal less daily, reduced fat intake by 5%) (n = 9 obese).	PYY ↔, GIP ↔	PYY↑ (0-30min), GIP ↓
	Leidy et al. (2007) [96]	2.5 ± 0.9kg	Pre and post 13 wk exercise intervention (5 d p/wk at 70-80% HRmax) combined with energy restriction to achieve -30-60% energy deficit, (measured ghrelin profiles over 24h) (n = 8 normal weight)	ghrelin ↑	ghrelin ↓
	Mathus Vliegen et al. (2006) [162]	2.3 (at 1wk) to 10.4 (at 1 year) kg.	Pre and post 4 wks, 8 wks and 1 yr. 1 yr intervention of energy restriction, exercise advice, behavioral modification and placebo tablets (n = 9 obese)	CCK ↔ (at all time points)	CCK ↔ (at all time points but trend to ↓ at 4 weeks)

* Weight Loss in units reported in original paper. AG: acylated ghrelin; DG: deacyl ghrelin; CCK: cholecystokinin; CHO: carbohydrate; GIP: gastric inhibitory polypeptide; GLP-1: glucagon-like peptide-1 PCOS: polycystic ovarian syndrome; PP: pancreatic polypeptide; PYY: peptide YY; HRmax: maximum heart rate; VO_2max: maximal oxygen uptake

2.8.1 Energy restriction

There is some evidence that energy restriction is associated with a slower GE. Patients with anorexia nervosa experience delayed GE [216-219], which returns to typical rates when re-fed [217, 218]. After a 4-day fast, GE of a glucose drink was slower in lean and obese subjects [201]. Postprandial PYY also decreased after 4 days of a 25% energy restriction [204]. The few studies which have examined the effects of energy restriction-induced weight loss on GE have also indicated a slower emptying [24, 162, 200], although some report no change [16, 23]. Four months of energy restriction and marked weight loss resulted in slower GE in 20 morbidly obese individuals [200]. Following 16 weeks of dietary intervention achieving a mean weight loss of 18.8kg and a further 8 weeks of weight stabilisation, GE was slowed during the initial 30 minutes but the overall emptying rate was unaffected in 19 obese subjects [24]. The authors suggested that this slowing of the initial emptying rate might postpone meal-termination and thereby pre-dispose to overconsumption and regain of a weight loss. This contention is supported by evidence that energy restriction-induced weight loss is associated with a blunted postprandial release of PYY and GLP-1 after a solid meal [205, 207]. Following a 10kg weight loss induced by energy restriction or RYGB, postprandial PYY [78] and GLP-1 [81] levels during an oral glucose tolerance test were increased after surgery compared to no change after energy restriction. This could partially explain the relative efficacy of RYGB compared with energy restriction in weight loss. The authors speculated this may be due to a more rapid delivery of nutrients to the intestine after gastric bypass. One explanation for findings of unchanged postprandial PYY and GLP-1 levels after energy restriction in these studies [78, 81] and others [210] is the use of a low-energy liquid meal. With regard to CCK, no significant differences in fasting or postprandial CCK concentrations following diet induced weight loss have been observed [82, 220], although peak values occurred 30 minutes later following 8 weeks of energy restriction, suggesting that pancreatic or gastric and intestinal functions might have changed to cause this shift [82]. Recently, a range of gut peptide responses to weight loss were comprehensively characterised in a study of 50 overweight and obese patients enrolled in a 10 week weight loss program involving a very low energy diet [208]. Circulating levels of leptin, ghrelin, PYY, GIP, GLP-1, amylin, pancreatic polypeptide (PP), CCK, insulin and subjective

ratings of appetite were examined at baseline (before weight loss), at 10 weeks (after program completion), and at 62 weeks. Ghrelin, GIP and PP increased following weight loss, whereas PYY, CCK, and amylin were all reduced and remained lower one year after weight loss. This was mirrored by an increase in hunger both after the weight loss program and when measured 1 year later. The authors concluded that long term strategies to counteract this change may be needed to prevent obesity relapse [208].

While further studies are needed, these findings collectively suggest that an energy deficit induced by energy restriction may delay and/or reduce the release of postprandial gut peptides involved in the episodic control of appetite, possibly mediated by a slower delivery of nutrients to the intestine. These changes could contribute to reduced appetite control and hence be a contributing factor to the relative lack of efficacy of energy restriction in long term weight loss and maintenance.

2.8.2 Exercise

It could be argued that the effects of an exercise-induced energy deficit on mechanisms of appetite control should be the same as a dietary-induced deficit, as both are simply the body responding to reduced adiposity or energy deficit, rather than being a response to the treatment strategy *per se*. In support of this contention, both diet and/or exercise induced energy deficit decrease fasting leptin and insulin concentrations and increase fasting ghrelin concentrations [86, 94-96], all alterations expected to restore energy balance. These changes likely contribute to the increased fasting hunger and drive to eat that has been observed with both caloric restriction [221, 222] and exercise [86, 99]. However, evidence suggests that exercise can also influence appetite independent of weight loss as exercise affects at least 2 processes of appetite control [99]. King et al. (2009) [99] demonstrated that in addition to increasing the overall (orexigenic) drive to eat, exercise also paradoxically increases the satiating efficiency of a fixed meal. Interestingly, in this study similar increases in the satiating efficiency of the meal were observed in both those who lost a significant amount of weight and those who didn't (see **Figure 2.4**), suggesting changes in meal induced appetite responses occurred in response to the exercise intervention independent of weight loss *per se* [99]. Other evidence indicates that

different compensatory appetite responses occur with an exercise compared to diet induced energy deficit (e.g. [223, 224]. Compensation in energy intake is observed when an energy deficit is created by meal omission [223] but not following a deficit induced by an acute bout of exercise [225, 226]. Collectively, these findings suggest that exercise may have different effects to diet on episodic mechanisms of appetite control.

Image removed for copyright reasons (King, N.A., Caudwell, P., Hopkins, M., Stubbs, J.R., Näslund, E., and Blundell, J.E., *Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety*. American Journal of Clinical Nutrition, 2009. **90**(4): p. 921-927).

Figure 2.4 Mean (±SEM) immediate and subsequent satiety quotients (SQ) in response to a fixed breakfast at weeks 0 and 12 in A) responders (R; individuals whose actual weight loss was equal to or greater than the expected weight loss after the 12 week exercise intervention) and B) non responders (NR; individuals whose actual weight loss was less than their predicted weight loss). The SQ, determined by calculating the change in appetite scores relative to the breakfast's energy content, reflects the capacity of the breakfast consumed to modulate postprandial sensations. A higher SQ represents greater satiety. These data demonstrate that the satiating efficiency of the fixed breakfast increased in both responders and non- responders following a 12 week exercise intervention. Post B, immediately post breakfast; Plus1, 1 hour post breakfast; Plus 2, 2 hours post breakfast; Plus3, 3 hours post breakfast; Plus4, 4 hours post breakfast. Data from King et al. [99].

The effects of an exercise-induced energy deficit on GI mechanisms of appetite control and GE in particular have received little investigation. Instead, as GE plays a

major role in the availability of ingested drinks during exercise [227], research has focused on determining the optimal properties of sports drinks to enhance performance. In general, the evidence clearly supports a delay in GE during strenuous exercise [228-234], and although there is some disparity [107, 235], there is an acceleration in GE during mild to moderate exercise [231, 236, 237]. Few studies report appetite or energy intake however. One study recently reported total stomach volume was negatively correlated with hunger sensations following fluid ingestion after acute exercise [238]. Changes in GE therefore could be an important physiological mechanism contributing to changes in appetite and EI with exercise.

Mechanisms proposed which could contribute to exercise-induced alterations in GE include changes in contraction frequencies, antral area [239] and gastric myoelectrical activity [240], [241], hormonal [233, 237, 242] and neural factors (mainly vagal in origin) [236], gut blood flow [231], and the mechanical effects of "bouncing of the gut" [231, 233, 237, 243] during exercise. GE responses during exercise are therefore likely a result of complex interactions of neural, hormonal and mechanical influences. More powerful and stable gastric myoelectrical activity following moderate exercise could contribute to a faster GE observed after moderate exercise [241, 244]. Different GE responses at different intensities could also depend on the relative dominance of parasympathetic or sympathetic tone [236]. In addition, the mode of exercise could influence the GE response to exercise. As the magnitude of accelerations of the body are more than twice as high during running compared to cycling, it could be expected that this would result in a faster GE rate during running [243]. Whereas when the abdominal muscles are more relaxed, GE would be expected to be similar to at rest. Evidence of no change in GE following moderate intensity cycle exercise supports this theory [107, 245]. However, this is not a consistent finding, as others have found GE to be accelerated by varying intensities of cycle exercise [233, 236, 239, 246]. How these changes relate to alterations in gut peptides and appetite are likely to depend on a number of factors such as the timing of measurement of the parameters of interest, test meal and the timing of meal ingestion. For a review of studies examining changes in gut peptides and appetite with acute exercise, see Martins et al., 2008 [86].

From a weight loss and maintenance perspective, it is the effects of repeated exercise bouts on appetite control that are also of importance. A single exercise bout

does not represent the repeated and sustained challenges to energy balance that characterize long term exercise training [247]. Few studies have examined the effects of short-term exercise on GE. Any adaptations in GE that occur due to exercise could influence the compensatory responses in energy intake. One study reported no significant change in GE after a 7 week intervention in adolescent girls compared to a control group [155]. In this study the intervention consisted of 40 minutes moderate intensity exercise 3 days per week. The duration and/or intensity (i.e. volume) of exercise may not have been sufficiently large to significantly alter GE. Studies reporting changes in appetite and gut peptides with exercise interventions have involved larger sample sizes and interventions of supervised exercise of greater intensity, frequency and/or duration (70%HRmax, 5 days per week over 12 weeks) [12, 86, 99, 211]. Furthermore, no significant weight loss was observed with exercise [155]. The effect of exercise-induced weight loss on GE is therefore unknown. Nevertheless, changes in postprandial gut peptide levels (see **Table 2.3**) and appetite [99] have been observed following exercise interventions with and without weight loss suggesting exercise may also influence GE independent of weight loss or energy balance.

In terms of chronic adaptation to regular exercise (and hence potentially an influence on appetite in weight maintenance), two cross sectional studies provide limited evidence that GE is faster in habitually active individuals. Faster GE was reported both initially (at 30 min) and throughout the postprandial period after a 400 kcal meal in marathon runners (n = 10) compared to sedentary individuals (n = 10) [40]. GE was similarly reported to be faster in a small sample of active individuals (n = 7) compared to inactive individuals (n = 7) [202]. Body composition characteristics were not reported, and age was not matched between the active and inactive groups in this study. Furthermore, in both studies energy intake and appetite were not reported. Mechanisms proposed to explain a faster GE in active individuals include a training induced predominant parasympathetic tone [40] and increased gastric electroactivity [202]. Habitual diet must also be considered. For example, absolute energy intake or macronutrient composition could also contribute to differences in gut physiology and GE between active and sedentary individuals. In a cross sectional study of 20 men representing a wide range of daily physical activity levels, energy and nutrient intakes, increasing energy intake was significantly correlated with faster orocecal transit time (OCTT) [248]. Although it was suggested

that the high energy intake associated with chronic exercise may be associated with significant gastrointestinal adaptations (i.e. accelerated OCTT), it could also be speculated that increased physical activity levels may have led to faster OCTT, and thus higher caloric intakes as a result of a shorter satiety period. The causal nature of this relationship may be critical to understanding the long term effects of exercise on energy intake.

Whether altered gut physiology with regular exercise has a mechanistic role in the improved appetite control (ability to compensate for prior energy intake) that has been observed in habitually active individuals [249] also remains to be established. A faster emptying rate particularly in the initial postprandial period could have a beneficial role in appetite control when considered together with the critical role of GE in postprandial ghrelin suppression [139, 140], and evidence of increases in anorexigenic gut peptides in response to faster nutrient delivery to the small intestine [61, 130, 134, 181]. However, as a high-fat diet-induced increase in GE is associated with diminished sensitivity to the appetite suppressing effects of gut peptides (for a review, see [250]), it is possible that faster GE in chronic exercisers may reflect an adaptation to a higher energy intake and be similarly associated with diminished sensitivity to the appetite suppressing effects of episodic gut peptides. Nevertheless given consistent evidence of improved appetite control in chronic exercisers [249, 251], the following hypothetical model outlines one possible mechanism contributing to the efficacy of exercise in weight maintenance (**Figure 2.5**). Further, it identifies one mechanism to explain why activity-induced energy expenditure could mediate the inverse relationship between meal frequency and adiposity, despite a higher energy intake [252].

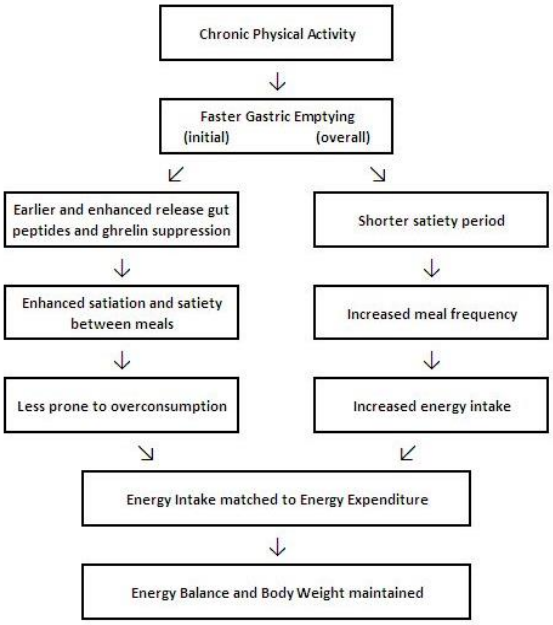


Figure 2.5 A model to describe how chronic physical activity could potentially impact on gastric emptying, appetite regulation and energy balance

Collectively, evidence of faster GE in active individuals is limited to only two cross-sectional studies involving the elderly [202] and marathon runners [40]. Further studies are need to determine whether GE is altered in habitually active individuals participating in a range of activities. In addition, the temporal pattern of changes in GE with exercise programmes and the implications for appetite and EI remains to be established.

2.8.3 Combining energy restriction and exercise

A common method of improving weight loss is to combine energy restriction with exercise training. Findings from studies which have investigated the effects of combined exercise and dietary intervention on gut physiology are not conclusive, mainly due to differences in how the diet was manipulated and the volume of exercise employed. Varying degrees of weight loss have been observed, along with differing effects on appetite and gut peptides (see **Table 2.3**). GE was unchanged after both 4 and 8 weeks of energy restriction combined with exercise advice but the overall emptying rate was delayed after 1 year of the same intervention [162]. Following a combination of a reduced-fat energy-restricted diet and 12 weeks of exercise intervention, the PYY response was increased [87]. In contrast, when energy restriction was combined with 180min/week of exercise, the PYY response

decreased [63]. The effects of different combinations of diet and exercise manipulations on GI mechanisms of appetite control warrant further investigation.

2.9 METHODOLOGICAL ISSUES: COMPARING THE EFFECTS OF DIFFERENT STRATEGIES

The literature reviewed highlights a number of important methodological issues. It is worth noting that the effects of different strategies on GI targets could vary depending on the magnitude of weight loss. Further, the effects may vary temporally – that is, acute changes may differ from chronic changes. It is critical to determine whether alterations in GI function occur in response to weight loss or the treatment strategy *per se* and to distinguish between changes in fasting and postprandial and exogenous and endogenous levels of peptides. While bariatric surgery may be used as a model to understand physiological weight loss [97], the mechanisms influencing the emptying rate after surgery may be different to those that influence GE after other interventions. This review has considered the effects of different strategies on gut peptides as currently measured in the systemic circulation. However, peripheral plasma levels may not necessarily reflect the local effects of gut peptides as they may also exert their action by paracrine or neurocrine routes [253, 254]. As a result the measurement of subjective appetite sensations should not be undervalued as increases or decreases of endogenous plasma levels, particularly of a peptide measured in isolation, may not translate to changes in hunger or fullness. However, subjective appetite sensations are not reported in many studies [23, 59, 63, 65, 78, 80-82, 87, 176, 177, 207, 211], and when they are reported the measures vary. The behavioural expression of appetite being measured (e.g. meal size, meal frequency) should also be considered. Similarly, the GE parameters and methods used need to be taken into account as there may be no relationship between the initial (i.e., lag phase) and overall emptying [255]. In addition to GE, although beyond the scope of the current review, the efficiency of intestinal absorption (determined by factors such as the viscosity and structure of the meal (see [256]) will influence the release of gut peptides. A liquid meal for example, despite a faster GE, may be absorbed entirely in the duodenum and not reach the lower part of the ileum to directly stimulate L cells [81, 257]. Collectively, these methodological issues are important to acknowledge as they may have different implications for appetite control.

Knowledge of the day-to-day variability of measurements is also essential to understand whether any change in the outcome measure is clinically meaningful. This is important when interpreting findings regarding changes in GE and appetite in response to interventions. Surprisingly, despite being implicated in the pathogenesis of obesity [29], and measured in response to numerous interventions - as evident in this review - information regarding the reproducibility of GE and appetite in overweight and obese individuals specifically is lacking. The day to day variability of these parameters in an overweight/obese population will therefore be relevant to document in future studies.

2.10 FUTURE DIRECTIONS: GI TARGETS OF APPETITE CONTROL FOR WEIGHT LOSS

As previously discussed, altered GE has long been implicated in the pathogenesis of obesity [20], however, faster [16-18], similar [23-26] and slower [27-29] emptying rates have since been reported in obese compared to lean individuals, conflicting outcomes that indicate that the role of GE in obesity is still unclear. One hypothesis may be that such inconclusive findings are due to the influence of additional factors which tend not to be measured or controlled for, for example physical activity and associated differences in body composition and EE. Given the growing interest in targeting the GI tract for the treatment of obesity [14, 30-34], it is pertinent that a better understanding of factors influencing GE and the implications for appetite control are established.

Knowledge of the effects of weight management strategies on GI targets of appetite control is also important to facilitate their more effective use in future. A large percentage of weight loss with Roux-en-Y gastric bypass could be attributed to the associated neuroendocrine changes [79]. Dietary, pharmacological and surgical strategies attempting to mimic the effects of bariatric surgery on GI responses are likely to be an ongoing area of intense research. The focus of pharmacological strategies may centre on gut peptides and their receptors [258], developing long acting peptides [101] and combination strategies of long acting adiposity signals with short term episodic signals as has recently been shown to be effective with a combination of pramlinitide and metreleptin [66]. Others are working to identify a drug that accelerates GE when administered orally to limit meal size [259]. The

major issue related to dietary manipulations using functional foods is how to minimise the increase in hunger while maintaining an energy deficit [260]. The development of novel foods targeting G protein-coupled receptors [31] and foods designed to reach the distal small intestine earlier and so stimulate an earlier and enhanced postprandial release of PYY and GLP-1 may counteract the blunted response of these gut peptides to energy restriction. Doucet and Cameron (2007) [103] proposed that offering low calorie snacks specifically designed to elicit maximal post snack PYY and GLP-1 levels to coincide with main courses could lead to better dietary control and compliance. However, the same group recently reported the timing of a high protein preload had no effect on PYY, GLP-1 or energy intake [261].

Currently, there is limited evidence concerning the effects of exercise on GI mechanisms of appetite control and weight loss. Exercise is universally available and has the added attraction of many additional health benefits [262] including weight maintenance [263]. One hypothesis by which exercise facilitates weight maintenance, based on the early work of Mayer [10] is that exercise alters sensitivity to episodic hunger and fullness signals [249, 251, 264] through an increase in postprandial satiety signalling driven by changes in gut peptides [86, 99, 247]. Faster GE may have a role in improved appetite control with chronic exercise as hypothesised in this literature review. However, this is based on limited cross sectional evidence. Changes in GE that result from exercise interventions designed to assess the efficacy of exercise for weight loss should also be examined as they may not be identical to chronic adaptations that occur in habitual exercisers over longer periods of time. Short term evidence suggests exercise-induced energy expenditure (EE) and energy intake (EI) are only weakly coupled [11, 41].

A further issue is whether it would be possible to structure a combination of dietary and exercise regimes to favourably alter GE and endogenous levels of peptides in such a way that the effects of weight loss on appetite and other compensatory mechanisms could be minimised. The independent and combined effects of dietary manipulations and exercise are important. For example, a high-fat diet may undermine any potential beneficial effects of exercise on GI mechanisms of appetite control. It is possible that in some people exercise increases the selection of high-fat energy dense foods [8], which could therefore contribute to the large inter-

individual variability observed in weight loss response to exercise [12]. In contrast, individuals successful at losing weight with a 12 week intensive exercise intervention increased their fruit and vegetable intake [265]. Chaput et al (2007) [266] demonstrated that using a combination of exercise with a diet designed to maximise satiation, significant weight loss could be achieved, indicating that the combination of a healthy satiating diet and exercise allows the achievement of metabolic benefits while potentially avoiding body weight relapse. GI mechanisms were not measured in these studies. Future studies investigating the individual effects of different manipulations of diet and exercise on GE and gut peptides along with different temporal combinations will advance the understanding of manipulating these lifestyle factors for better use in both weight loss and maintenance.

2.10.1 GI targets of appetite control: part of an integrated process

The concept that appetite may be consciously overridden in the short-term but this is superseded in the longer term by a biologically determined set-point has been proposed as one mechanism by which lifestyle interventions fail to sustain weight loss [101]. As there is a strong volitional control over eating behaviour, psychological influences should not be undervalued [42]. Reward pathways, stress, social values, diurnal rhythm, learned behaviours and eating behaviour related traits (e.g. dietary restraint) could have a strong influence on food intake. The resulting eating patterns may be influenced by and also contribute to alterations in gut physiology. Following RYGB, the 'supra-normal' nutrient stimulated gut peptide response [62, 171, 172, 267] may override both homeostatic defences of body weight and non-homeostatic factors influencing food intake. Changes in GE and gut peptides induced by lifestyle interventions such as exercise are unlikely to be of the same magnitude as those following surgery. Other factors such as the hedonic response to foods could over-ride signals from the GI tract [75]. While developing a strategy to target the GI tract to maximise satiation and satiety may have a critical role in facilitating weight loss with lifestyle strategies, the greatest weight loss will be achieved by adopting a multidisciplinary approach.

2.11 CONCLUSIONS

A key factor in the long-term success of a weight loss intervention is to minimise the impact of an energy deficit on compensatory responses including energy intake. The complexity of appetite control should not be limited to GE and gut peptides but their measurement is important to understand the mechanisms by which weight loss strategies may act to increase or decrease appetite and food intake and thus influence weight regain. This review shows that different strategies exert diverse effects on GE and gut peptides. Relative to lifestyle interventions, surgical interventions produce greater short- and long-term weight loss which may be explained by changes in appetite. From the evidence so far it is likely that in addition to a reduced gastric capacity, gut peptides play a key role in the improved appetite control experienced by those who undergo surgical procedures such as SG and RYGB. Although findings remain associative, this response may be due in part to an accelerated delivery of nutrients to the distal small intestine. Energy restriction in contrast slows GE and is associated with a blunted release of appetite-related gut peptides. With regard to exercise, limited evidence suggests chronic exercise is associated with a faster GE, which it is hypothesised will impact on appetite control and energy balance. However, the collective evidence is still limited and the study designs vary in the methods used and the parameters reported.

By 2030, some estimates project that over 3 billion people may be overweight and obese [268]. As bariatric surgery remains impractical on a population level due to the large number of patients that qualify for a surgical procedure, additional strategies are needed to improve appetite control. Given the growing interest in targeting the GI tract for the treatment of obesity, it is relevant to establish the influence of factors potentially contributing to variability in GE and appetite such as physical activity level, body composition and EE. In addition, a better understanding of how lifestyle interventions such as diet and exercise affect GI targets of appetite control and the implications for food intake could allow for strategies which counteract compensatory increases in appetite to be designed, and thus facilitate their more effective use in weight management.

Chapter 3: Reproducibility of Gastric Emptying, Appetite and Energy Intake in Overweight and Obese Males

Modified from: Horner, K.M., Byrne, N.M., Cleghorn, G.J., King, N. A. (2013). Reproducibility of gastric emptying in overweight and obese males. *Clinical Nutrition,* In Press.

3.1 BACKGROUND

Gastric emptying (GE), appetite and energy intake (EI) have long been studied in lean and obese individuals to further understand differences in the operation of appetite control. In addition, the effects of various interventions (e.g. food supplements, pharmacological treatments and surgery) targeting appetite and EI have been widely investigated in overweight and obese individuals [35]. The significance of methodological aspects of such studies has recently been highlighted [269] and the need for accurate and reliable measurements emphasised [270].

The reproducibility of measurements reflects both the true biological variation within the individual from day-to-day, as well as the measurement error [271]. Knowledge of the day-to-day variability of measurements is essential in both research and clinical settings to understand whether any change in the outcome measure is detectable and clinically meaningful. This is important when investigating changes in GE and appetite with interventions such as pharmacological treatments, diet or exercise as well as changes in various medical conditions. Surprisingly, despite being implicated in the pathogenesis of obesity [29], and measured in response to numerous interventions [35], information regarding the reproducibility of GE, appetite and EI in overweight and obese individuals specifically is lacking. Given some evidence that gut peptide [159] and appetite responses may vary according to body composition or body mass index, it should not be assumed that outcomes observed in a group of lean individuals will be identical in overweight and obese individuals [269]. The day-to-day variability of GE, appetite and EI might

therefore be different in overweight and obese compared to lean individuals and requires further study in this population.

3.1.1 Reproducibility of Gastric Emptying

Measurement of GE is essential to understanding mechanisms behind alterations in appetite and EI in various pathologic conditions, as well as changes that occur in response to treatments. For example, GE could play an important role in the aetiology of obesity through processes of satiety and satiation. The day-to-day variability of GE has been studied in various populations, including infants [272, 273], children [274], critically ill patients [275], diabetic patients [276] and healthy lean adult males [117] and females [277] (See **Table 3.1**). However, no prior studies could be found which have investigated the day-to-day variability of GE in overweight and obese individuals.

Table 3.1 Summary of studies examining the reproducibility of gastric emptying measured by scintigraphy and breath test. For individual details of the studies, see Appendix A.

Population	n	Meal Form(s)	Meal Energy Content(s)	GE Method(s)	$t_{1/2}$ $CV_{intra(s)}$	t_{lag} $CV_{intra(s)}$	Reference
Healthy Adults	5-21	S, L	119-638kcal	Sc, BT	7-35%	8-54%	[276, 278-298]
Critically Ill	12	L	106kcal	BT	32%	-	[275]
Diabetes	6-14	S, L	418kcal	Sc, BT	29-67%	-	[287] [276]
Functional Dyspepsia	20	S, L	250kcal	BT	67-73%	-	[299]
Healthy infants	14	L	-	BT	6%	-	[272]
Preterm Infants	16-28	L	20-24kcal	BT	11-24%	-	[273, 300]
Healthy Children	19-30	S, L	105-230kcal	BT	5-13%	6-17%	[274, 301, 302]
Children with GERD	30	L	105-160kcal	BT	5%	6%	[302]

Ranges of values are listed above where more than one study applies.
S = Solid, L = Liquid, Sc = Scintigraphy, BT = Breath Test, GERD = Gastro Esophageal Reflux Disease.

The test conditions (e.g. the test meal used [276]) and the GE parameters reported [281] may also influence the intra-individual variability. Knowledge of the reproducibility of different phases of GE and hence GE parameters is important given the kinetic and temporal nature of GE and relation to appetite control [35]. Although half -time tends to be the primary focus in GE studies - as it is considered the most useful parameter in clinical practice - it does not reflect the complete pattern

of GE. Since the ^{13}C-octanoic acid breath test (^{13}C-OBT) was proposed as a safe, reliable and non-radioactive alternative to scintigraphy for measurement of GE [293], the test has been widely used in a variety of populations including obese individuals [29]. A number of GE parameters have been proposed that reflect the various phases of GE (e.g. Schommartz et al. [303]). However, little information exists on the reproducibility of the different parameters or phases of GE.

3.1.2 Reproducibility of Subjective Appetite Ratings

Given the central role of the gut in appetite control, GE is often studied in combination with ratings of appetite. Visual analogue scales (VAS) are widely used to assess appetite sensations and have been accepted as the standard tool for measuring subjective appetite [270]. VAS for appetite ratings have varying degrees of reproducibility in normal weight adults in both a free living context [304], and in the laboratory following a single fixed [305, 306] or *ad libitum* meal [307, 308]. When subjects were fed to energy balance with fixed meals at fixed times, their ratings of hunger showed a consistent fluctuating, diurnal pattern which was highly reproducible within subjects [309, 310]. In contrast, when tested twice on separate days following a fixed test meal, Raben et al. 1995 [306] reported large coefficients of repeatability (CR) (21 (satiety) – 38 (prospective consumption) mm) for mean subjective ratings in 9 normal weight males. However, the palatability of the meal was rated lower on the second test day in this study, which may explain in part the low reproducibility. In a larger study, appetite ratings before and after a fixed breakfast meal were found to be reliable in normal weight males [305] but the reproducibility varied depending on the parameter reported. Fasting ratings were less reproducible (CR, 23 (hunger) – 30 (satiety) mm) than 4.5-hour mean ratings (CR, 15 (prospective consumption) – 24 (hunger) mm). Overall, it appears that while the reproducibility will vary depending on the parameter reported; on balance VAS exhibit a good degree of within-subject reproducibility (see Stubbs et al. 2000 [310] for a comprehensive review).

Limited evidence exists on the reproducibility of VAS for appetite ratings in overweight and obese individuals however. Previous studies have focused on normal weight males [305, 306]. One study compared the reproducibility of appetite ratings in normal weight and obese individuals [307]. Participants were offered a

homogenous lunch meal *ad libitum* on 5 occasions. The amount of food intake was recorded, along with appetite ratings before and after lunch. While mean ratings of fullness were reproducible in both groups, mean ratings for desire to eat were found to be reproducible in normal weight but not obese men. In contrast, hunger ratings were more reproducible in obese men than normal weight men. This study highlights that the day-to-day variability in appetite ratings may vary depending on the subject population. However, VAS ratings were taken at 2 time points only; pre and post lunch. In contrast, in the majority of GE and appetite studies test meals are consumed in a fasted state, often in the morning using a fixed meal size. In addition, VAS are generally completed before and immediately after a test meal and then subsequently at regular intervals (varying from 15-30min up to hourly) usually for 3 – 5 hours or until the start of the next meal [269]. To assist in the design and interpretation of studies, there is a need for further understanding of the reproducibility of VAS ratings in overweight and obese individuals in response to a fixed meal under standardised conditions.

3.1.3 Reproducibility of Food Preferences, 'Liking' and 'Wanting'

Food intake is influenced by both homeostatic (e.g. hunger) and non-homeostatic (e.g. food reward) systems; therefore to better understand changes in appetite control there is also a need to measure food reward systems driving food choice and preference. It has been hypothesised that the food reward system consists of two functional components – 'liking' (pleasure/palatability) and 'wanting' (appetite/incentive motivation) [311]. Often 'liking' and 'wanting' are coupled: 'we want what we like and like what we want' [311, 312]. However, some individuals such as restrained eaters may habitually select less liked food items to prevent weight gain [313, 314]. The relative contribution of each component must therefore be identified to better understand changes in consumption [315]. Experimental procedures devised to separate components of 'liking' and 'wanting' [315-317] are increasingly being undertaken in conjunction with measurements of gut peptides [318], appetite and energy intake [315, 317-321]. The combined measurements allow for the interaction of hedonic and homeostatic systems to be characterised and thus to better understand food choices, appetite control and food intake.

To identify changes in taste preferences and 'liking' and 'wanting' within individuals and between groups, it is important that the measurement is reproducible. However, little information exists on the reproducibility of these experimental procedures. Using a computer based test showing images of 72 different food items, Lemmens et al. [312] tested the reproducibility of 'liking' and 'wanting' in 73 healthy males and females in the fasted state on 2 separate days. The percentage reproducibility was calculated as the proportion of concordance between the repeated measurements, expressed as percent. The 'liking' and 'wanting' part of the test showed a reproducibility of 62–73%, which was considered sufficient reproducibility, and comparable to a test-retest correlation of 80% demonstrated in a study of 20 subjects measuring the reinforcement value of food [322]. Finlayson et al. [315] developed a computerised tool - the Leeds Food Preference Questionnaire (LFPQ) to measure 'liking' and 'wanting'. Food images were organised according to categories of foods of specific tastes and macronutrient compositions e.g. sweet/savoury - high/low fat. Clear preferences for particular food categories and alterations in preferences after food intake were demonstrated [316]. The procedure has since been applied in various contexts including assessing the effects of exercise on liking, wanting and subsequent EI [320]. However, no information on the reproducibility of the tool was provided in this study. No studies could be found which have examined the reproducibility of 'liking' and 'wanting' in both the fasted and fed state or in overweight and obese individuals specifically.

3.1.4 Reproducibility of ad libitum energy intake

Energy intake (EI) is often measured in the laboratory setting using an *ad libitum* test meal. Participants are given a test meal provided in excess of what would normally be eaten and the amount of food consumed is recorded. Using a buffet-type meal where subjects had access to a variety of foods *ad libitum* on two identical sessions, high correlations were demonstrated between the amount of EI consumed on both days ($r = 0.97$) and a within subject CV of 8.2% reported in healthy males [308]. These data demonstrated the high reproducibility of the ad libitum buffet meal [308] and have since been supported by others [117, 323]. The reproducibility of buffet type meals however may be confounded by variations in macronutrient intake at the meal [324]. Gregersen et al. 2008 [325] tested the reproducibility of an ad

libitum test meal of fixed macronutrient composition to measure EI in two groups of normal weight men; one group with and one without prior diet standardisation. On two identical test days, participants were given a fixed breakfast meal in the laboratory and 4.5 hours later an *ad libitum* homogenous pasta lunch meal. Although there was considerable individual variation in EI at lunch between the 2 test days, no differences in mean EI were found. CVs of 8.9% and 14.5% and CRs of 1.5MJ and 1.8MJ were found for standardised and not standardised groups respectively. Furthermore, no effect of prior standardization was seen on the reproducibility of the EI measurements when the data from both groups were pooled (p = 0.56). The authors concluded that the *ad libitum* meal to measure EI is reproducible and the reproducibility does not seem to be influenced by prior diet standardisation. Further studies are needed to confirm these findings in other groups based on age, gender and BMI [325].

Few studies have investigated the day-to-day variability of *ad libitum* EI in overweight and obese individuals. In one study *ad libitum* EI was found to be highly reproducible in a sample of eight overweight/obese subjects using the preload paradigm [326]. Participants were given a control or whey protein-containing liquid preload and later offered a homogenous *ad libitum* pasta lunch meal. The intrasubject CVs were 4.5% and 11.2% for control and whey protein preloads respectively. The authors concluded the reproducibility of *ad libitum* EI is similar in overweight and obese to normal weight individuals. However, in this study the *ad libitum* meal was offered 90 minutes after the preload, unlike the 4.5hour time interval in the study of Gregersen et al. 2008 [324]. It is possible that the reproducibility of *ad libitum* EI may vary depending on the time interval since the previous meal. Some evidence indicates the accuracy of compensation for prior EI is influenced by the time interval [146, 327]. For example Rolls et al. 1991 [146] examined the influence of three time intervals (30, 90, and 180 min) between preloads and an *ad libitum meal* and demonstrated that as the time interval increased compensation for prior intake was less precise. However, reproducibility was not examined in this study. Further studies are needed to assess the reproducibility of EI with a more typical duration of inter-meal interval (e.g. 3-5hrs) in overweight and obese individuals.

3.1.5 Relationships between variables

By measuring the reproducibility of multiple variables which may influence appetite and food intake simultaneously, relationships between hedonic and homeostatic processes and appetite and EI can be assessed. It is sometimes assumed that changes in physiological measures such as GE translate to changes in appetite or EI but often these parameters are not measured in the same study. Measuring these outcomes simultaneously will facilitate the characterisation of these changes in appetite control, and the interactions of homeostatic and hedonic features.

3.1.6 Aims

The aims of this study were to determine in overweight and obese males:

(i) The reproducibility of GE, subjective appetite ratings, *ad libitum* lunch EI, food preferences and 'liking' and 'wanting';

(ii) Relationships between variables; and

(iii) Minimum sample sizes required to detect a hypothetical treatment effect in GE, appetite, EI, food preferences and 'liking' and 'wanting.

Achievement of these aims is essential for the design of studies that investigate changes in these parameters in the pathogenesis or treatment of obesity.

3.2 METHODOLOGY

3.2.1 Participants

Fifteen overweight and obese men (BMI 30.3 ± 4.9 kg/m^2) participated in the study. Based on previous work [29] a sample size of 15 participants per group was sufficient to detect a mean difference of 11.4 min in mean GE half-time between repeated tests with a power of 90% and a significance level of 0.5%. Height was measured without shoes to the nearest 0.5cm and weight to the nearest 0.01kg. Body composition was measured using air displacement plethysmography (Bodpod, Concord, CA). All participants had no history of gastrointestinal disease or surgery, significant illness nor were taking any medication known to affect gastrointestinal motility or appetite. Ethical approval for the study was granted by Queensland University of Technology Research Ethics Committee. All participants provided written informed consent prior to taking part in the study.

3.2.2 Protocol

Each participant undertook a baseline assessment followed by 2 identical GE tests 7 days apart (**Figure 3.1**). Subjects arrived at the laboratory between 7.00am and 9:00am. Start time was standardised within participants.

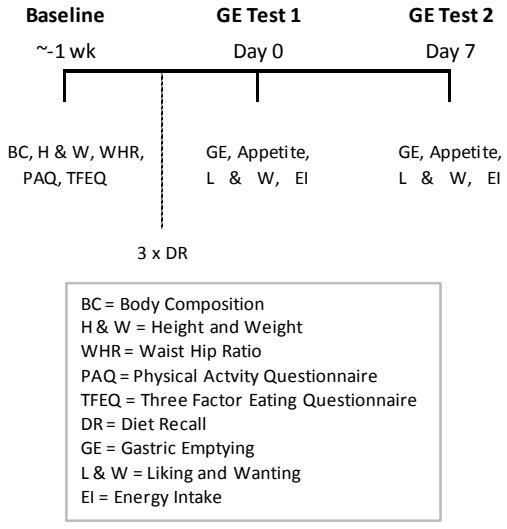


Figure 3.1 Schematic overview of study protocol

3.2.3 Baseline Assessment Measurements

Prior to baseline measurements, participants were requested to fast overnight and avoid strenuous exercise and alcohol for 24 hours. Measurements were performed in the following order:

3.2.3.1 Anthropometry and Body Composition

Participants were asked to wear form fitting bike shorts for anthropometric and body composition measurements. Height was measured without shoes to the nearest 0.5cm using a stadiometer. Weight was measured to the nearest 0.1kg on an electronic scale. Body mass index (BMI) was calculated as weight[kg]/height[m^2).

Body composition (fat mass and fat free mass) was determined by air displacement plethysmography (BodPod™, Concord, CA). Fat free mass is everything except fat and includes muscle, water, bone and internal organs. The BodPod™ uses whole body densitometry to determine body composition. Body mass (weight) was measured using a precise electronic scale. Body volume was determined by monitoring changes in pressure within the closed chamber of the BodPod™. Participants were asked to sit stationary inside the BodPod™ chamber and breathe normally. Two measurements were undertaken to ensure consistent readings. If the two measurements deviated from the acceptable range determined by the BodPod™ software, a third volume measurement was taken and the two closest measured were averaged. If the three measures were inconsistent, the BodPod™ was recalibrated and the participant was re-tested. Air in the lungs was predicted using lung volume prediction equations and body density was then calculated as Density = Mass/Volume, and corrected for lung volume. The relative proportions of fat and fat free mass were then determined using the Siri equation [328]:

$$\% \text{ fat} = ((4.95/\text{body density}) - 4.50) \times 100$$

3.2.3.2 Three Factor Eating Questionnaire

Eating Behaviour related traits have emerged as important dispositions in identifying susceptibility to weight gain and disturbed eating behaviours [329]. Restraint, Disinhibition and Hunger were assessed using the Three Factor Eating Questionnaire (TFEQ) [330]. Disinhibition refers to a tendency towards overeating and opportunistic eating behaviours and Restraint refers to efforts at limiting food intake to control body weight [331]. The factor of Hunger refers to the extent to which

hunger feelings are perceived and the extent to which such feelings evoke food intake. The questionnaire is a 51-item questionnaire separated into two parts, the first 36- items involve a true/false response format, while the remaining 15-items use a 4-point Likert scale response format [331].

3.2.4 Gastric Emptying Test Day Measurements (Day 0 and Day 7)

Each participant undertook 2 identical GE test days 7 days apart. Participants were provided with an evening meal (McCain Beef Lasagne (2447kJ (584 kcal)) to consume at home around 7pm the night before the test, and asked to then fast until coming to the Human Appetite Research Centre the following morning. One glass of water was allowed upon waking. Participants were instructed to refrain from vigorous exercise and alcohol for 24 hours prior to the test day. In addition, as GE was assessed using the ^{13}C-OBT [293], participants were instructed to avoid consumption of naturally ^{13}C-enriched foods (corn or corn products, pineapple, kiwi fruit, cane sugar and exotic fruits) for at least two days prior to the study. An overview of the GE test is shown in **Figure 3.2**.

The test day included the following measurements:

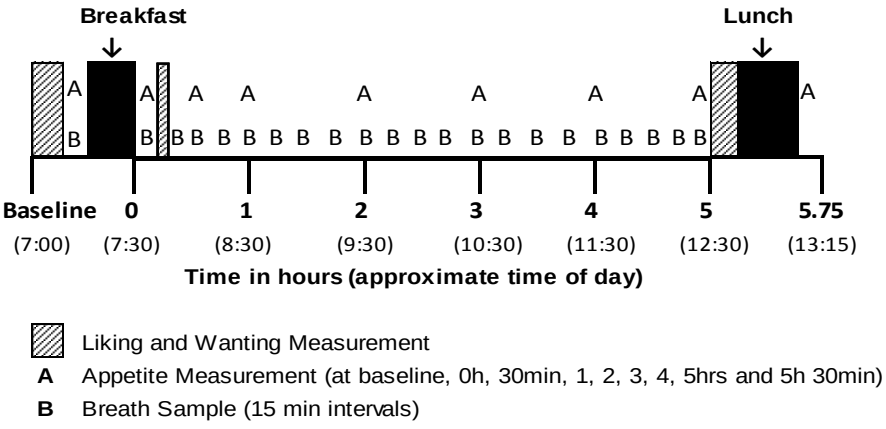


Figure 3.2 Schematic Representation of Gastric Emptying Study Day Protocol.

3.2.4.1 Gastric Emptying

A description for the rationale behind the ^{13}C-OBT is provided in **Appendix B**. The egg yolk of a standardized pancake breakfast meal [1676 kJ (400 kcal); 15g (15%)

PRO, 17g (37%) Fat, 48g (48%) CHO)] was labelled with 100mg ^{13}C-octanoic acid (Cambridge Isotope Laboratories, Andover, USA). Participants consumed the meal together with 250ml of water within 10 minutes. Breath samples were collected in 10ml glass Exetainer tubes (Labco, Buckinghamshire, UK) prior to breakfast, immediately after, and subsequently at 15 minute intervals for 5 hours after breakfast (see **Figure 3.3**). The participant was instructed to place the straw at the end of the tube and blow gently into the tube for a count of 8 to10 seconds or until water vapour appeared on the side of the tube, then slowly draw the straw out of the tube while continuing to exhale, and then cap the tube. Participants remained sedentary throughout.

Figure 3.3 Breath samples for the ^{13}C-octanoic acid breath test are collected by exhaling into a glass exetainer tube using a straw. The tube is then immediately capped.

3.2.4.1.1 ^{13}C isotope analysis

The ^{13}C enrichment of breath samples was subsequently analysed by isotope ratio mass spectrometry (Hydra 20-20) and compared to a reference gas (5% CO_2, 75% N_2, 20% O_2 calibrated with a standard of $^{13}CO_2$). For further details, please refer to **Appendix C**.

3.2.4.1.2 ^{13}C data analysis

The amount of $^{13}CO_2$ present in breath samples was expressed as a delta over baseline ratio which represents the change in the $^{13}CO_2/^{12}CO_2$ (mass 45-44) ratio of breath samples collected before and after ^{13}C-octanoic acid ingestion [298]. To calculate the cumulative percent of ^{13}C dose recovered and the percentage $^{13}CO_2$ recovery per hour, enrichment values were multiplied by the estimated total CO_2 production (VCO_2) for each individual. Resting VCO_2 was predicted from body

surface area [332]. This is the most commonly used method of estimating VCO_2 in ^{13}C breath tests and assumes a constant value of 300 mmol CO_2 h^{-1} m^{-2} body surface area or 5 mmol min^{-1} m^{-2} for all individuals [333]. Body surface area was calculated from height and weight using the formula of Haycock et al. (1978) [334].

Data were fitted to the original GE mathematical model devised by Ghoos et al. (1993) by non-linear regression analysis using Microsoft EXCEL's solver function, applying the formula:

$$y = m\,(1\text{-}e^{-kt})^{\beta}$$

where y is the percentage of cumulative 13C excretion in breath, t is time in hours; and m, k and β are constants, with m being the cumulative ^{13}C recovery when time is infinite [335].

The r^2 coefficient between the modelled and raw data was calculated and accepted if $r^2>0.90$. Once the formula was solved, the constants were then extracted and used to calculate the conventional GE time based parameters proposed by Ghoos et al. (1993) [335] (see **Table 3.2**) and the parameters latency time (t_{lat}) and ascension time (t_{asc}) proposed by Schommartz et al. (1998) [303] (see **Table 3.3**).

The results of GE breath tests correlate well with scintigraphy (the 'gold standard' method) but they are not identical [293, 336], as both methods differ in their approach to describing the emptying process [337]. For scintigraphy, half-time ($t_{1/2}$) refers to the time it takes for half the meal to empty from the stomach and lag time (t_{lag}) is generally defined as the time it takes from meal ingestion to the onset of emptying. As a result, half emptying times and lag times determined by breath test are longer than those determined by scintigraphy, likely due to the time it takes for the absorption and oxidation of octanoic acid subsequent to its emptying from the stomach [293].

Table 3.2 Conventional Gastric Emptying Breath Test Time Based Parameters proposed by Ghoos et al. (1993) [293]

Gastric Emptying Parameter	Definition	Formula
Half time ($t_{1/2}$)	time taken for 50% of the ^{13}C dose to be excreted in the breath	$t_{1/2} = (-1/k) \times \ln (1\text{-}2^{-\beta})$
Lag time (t_{lag})	time taken to maximal $^{13}CO_2$ excretion in the breath	$t_{lag} = (\ln\beta)/k$

Other parameters derived from the same mathematical model have since been proposed. By definition the same part of the $^{13}CO_2$ exhalation curve is used for the calculation of both t_{lag} and $t_{1/2}$. The different phases of GE (e.g. a delayed initial emptying but an accelerated subsequent emptying or vice versa) could therefore be difficult to distinguish using these parameters and direct correlations observed between t_{lag} and $t_{1/2}$ suggest they can largely replace each other [303]. This prompted Schommartz et al (1998) [303] to propose the parameters latency time (t_{lat}) and ascension time (t_{asc}), described in **Table 3.3**. These parameters were found to be uncorrelated in healthy individuals and may more sensitively reflect different phases of GE of solid food than the conventional parameters [303].

Table 3.3 Gastric Emptying Time Based Parameters proposed by Schommartz et al. (1998) [303]

Gastric Emptying Parameter	Definition	Formula
Latency Time (t_{lat})	initial delay in the cumulative ^{13}C curve, calculated as the intersection of the tangent at the inflection point at the x-axis	$t_{lat} = 1/k\ [\ \ln(\beta) + 1/\beta - 1\ ]$
Ascension Time (t_{asc})	time course between the latency phase and half excretion time, representing a period of high ^{13}C excretion rates	$t_{asc} = -1/k\ [\ \ln(1-2^{-1/\beta}) + \ln(\beta) + 1/\beta - 1\ ]$

The parameters t_{lag}, $t_{1/2}$, t_{lat} and t_{asc} defined in **Table 3.2** and Table **3.3** are illustrated in **Figure 3.4** below.

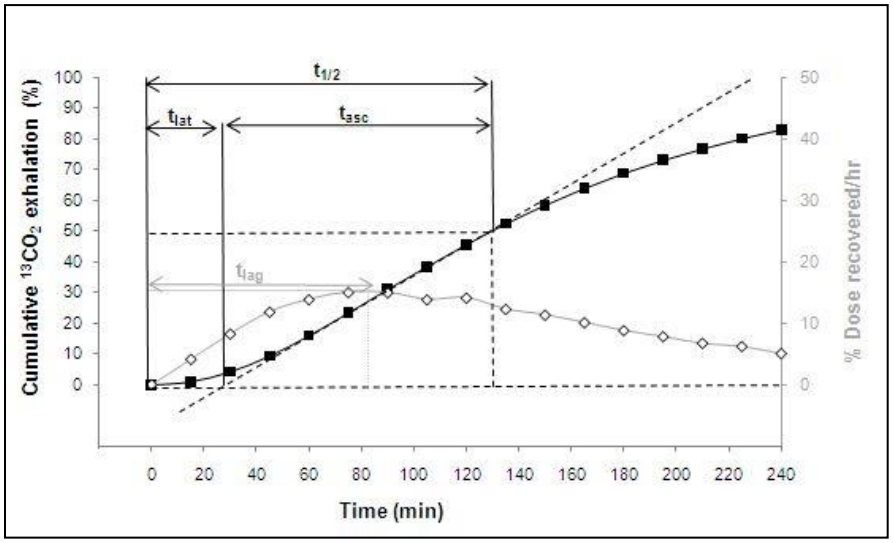


Figure 3.4 Representation of GE parameters calculated from one breath test. These parameters are defined in Tables 3.2 and 3.3.

3.2.4.2 Appetite

Subjective appetite sensations were measured throughout the test day (see **Figure 3.2**) using an electronic appetite rating system [338]. Participants were asked to rate subjective sensations of hunger, fullness and desire to eat on 100 mm visual analogue scales, anchored at each end with the statements "not at all" and "extremely" (see **Figure 3.5**). The participant is asked to put a mark on the line corresponding to their current sensation. The sensation is then quantified by measuring the distance from the left end of the line to the mark.

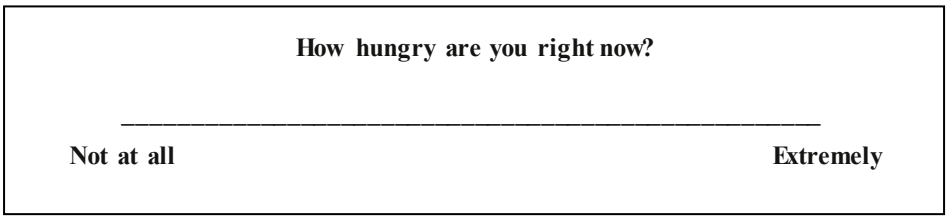


Figure 3.5 Example of Visual Analogue Scale used for Hunger Ratings.

Five hour postprandial area under the curve (AUC) was calculated using the trapezoidal rule. In addition, the satiety quotient (SQ) was calculated. The SQ relates the suppression of hunger, desire to eat or change in fullness to the amount of energy consumed. The SQ was calculated for each sensation at breakfast and lunch using the formula:

SQ (mm/kcal) = (Pre-meal rating (mm) – Post-meal rating (mm)) / Energy consumed (kcal)

The formula has the capacity to provide a quantitative measure of the satiety produced by different foods when used with fixed sized meals [269]. A higher SQ indicates a greater decrease in hunger and desire to eat in response to the meal. Whereas, for fullness, a higher SQ indicates a greater decrease in fullness.

3.2.4.3 Food Preferences, Liking and Wanting

Food preferences and 'liking' and 'wanting' were measured on 3 occasions during the test day (see **Figure 3.2**) using a computer-based procedure - the Leeds Food Preference Questionnaire (LFPQ) (for a detailed description see [339]). The

questionnaire involved an array of 16 photographic food images administered using experiment software (E-prime v.1.2, Psychology Software Tools, ND). The foods were organised into separate categories of high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) (**Table 3.4**).

Table 3.4 Photographic food stimuli used in the food preference and 'liking' and 'wanting' computer task (grouped by food category).

HFSA	LFSA	HFSW	LFSW
Chips (fries)	Tomatoes	Doughnuts	Jelly beans
Pizza	Chicken	Chocolate	Juice
Meat pie	Rice	Milkshake	Mixed fruits
Swiss cheese	Boiled potatoes	Ice-cream	Apple

HFSA, high fat savoury; LFSA, low fat savoury; HFSW, high fat sweet; LFSW, low fat sweet.

Liking (the conscious feeling of pleasure expected from tasting each food [339]) was measured by presenting each food image one at a time on the computer screen. Participants were asked to rate their perceived pleasantness of that food on a 100mm visual analogue scale, anchored at each end with 'not at all' and 'extremely'. Mean ratings for each category were calculated. Explicit wanting (the conscious desire for each food [339]) was measured using a similar visual analogue scale format whereby participants were presented with a single food image on the screen and asked to respond to the question "How much do you want some of this food now?". Food preference was measured by presenting the food images in pairs and participants were instructed to select the food "they would most like to eat right now". Each food item was paired with an alternative from the array of images and consisted of 96 pairings and food preferences for particular categories were assessed by the frequency of choice.

3.2.4.4 Ad Libitum Energy Intake

At the end of the GE test, participants were provided with an *ad libitum* pasta lunch meal and water and told to consume as much as they wished until comfortably full. The ingredients in the pasta lunch consisted of penne pasta (Woolworths Select, Woolworths, Australia), grated cheese (Tasty Cheese, Woolworths, Australia) and pasta sauce (Chunky Pasta Sauce, Woolworths, Australia) and were mixed in a saucepan before baking in the oven in a single dish (47% CHO, 35% FAT, and 18% PRO, and an energy content of 7.6kJ/g). The amount (g) of food consumed from the

ad libitum meal was determined by weighing the meal before and after consumption and energy intake (kJ) was calculated.

3.2.5 Statistical Analysis

Data are expressed as mean ± standard deviation (SD), unless otherwise stated. Differences between the two test days were compared by paired *t* test. Intraindividual variability was expressed as the coefficient of intrasubject variation (CV_{intra}) ($CV_{intra} = SD_d/(m\sqrt{2})$ where SDd is the standard deviation of the differences between the repeated tests and *m* is the mean of the repeated tests [275, 276, 340]). To assess reproducibility of the GE results within individuals, the difference between results on the 2 separate days was plotted against the mean of the results for each subject, according to Bland and Altman [340]. The coefficient of repeatability (CR = 2 x SD) for the mean differences between visits 1 and 2 was calculated. The CR indicates the absolute variability of the method whereas the CV measures the relative variability [324]. Pearson correlations were used to determine test-retest correlations for appetite sensations, food preferences, 'liking' and 'wanting' and relationships between GE, energy intake and appetite. Based on day-to-day variability observed in these parameters, minimum effect sizes required to detect a hypothetical treatment effect with 80% power were calculated. Minimum differences that would be detected by a sample of fifteen subjects were also calculated. Statistical analysis was performed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL) and Graph Pad Prism version 5.0 for Mac (GraphPad Software, San Diego, CA, USA). Sample size calculations and minimum detectable differences were calculated using Graph Pad StatMate version 2.0 for Mac (GraphPad Software, San Diego, CA, USA). Sample size calculations for unpaired designs were undertaken by selecting the "compare two means (unpaired t test)" option, entering the mean SD observed and the level of significance of 0.05. For paired design sample size calculations, a similar process was undertaken except the "compare two paired means (paired t-test)" option was selected and the observed SD of the difference between pairs was entered. Statistical significance was set at $P < .05$ unless otherwise stated.

3.3 RESULTS

All participants completed the study. Participant characteristics and eating behaviour (assessed by TFEQ) characteristics are shown in **Table 3.5**.

Table 3.5 Participant characteristics (n = 15).

	Mean	SD	Range
Age (years)	34.9	10.6	24-52
Weight (kg)	92.5	18.6	76-139
BMI (kg/m^2)	30.3	4.9	26-40
Body composition			
% Body Fat	32.1	8.0	20-48
FFM (kg)	62.6	8.5	43-72
TFEQ			
Restraint	7.5	2.9	4-12
Disinhibition	7.8	3.0	2-13
Hunger	5.6	3.3	1-12

FFM, fat-free mass; TFEQ, Three Factor Eating Questionnaire.

3.3.1 Gastric Emptying

The mean CV_{intra} varied depending on the parameter reported from a minimum of 7.5% (t_{lag}) to a maximum of 11.4% (t_{asc}) (**Table 3.6**). For all GE parameters, no significant difference was found between the two test days (**Table 3.6**).

Table 3.6 Gastric Emptying Time Based Parameters at visits 1 and 2 (n = 15)

	Visit 1 (min)	Visit 2 (min)	*P*-value	CV_{intra} (%)
t_{lag}	108 ± 8 (99-124)	104 ± 14 (87-136)	0.23	7.5
$t_{1/2}$	179 ± 15 (160-204)	176 ± 19 (149-210)	0.56	7.9
t_{lat}	34 ± 5 (25-44)	32 ± 8 (22-49)	0.19	11.4
t_{asc}	145 ± 17 (124-174)	144 ± 17 (121-170)	0.84	9.4

Data are means ± SD. Range is indicated in brackets.
$t_{1/2}$, half time; t_{lag}, lag time; $t_{1/2s}$, t_{asc}, ascension time; t_{lat}, latency time.

Bland Altman plots for GE time based parameters are shown in **Figure 3.6**.

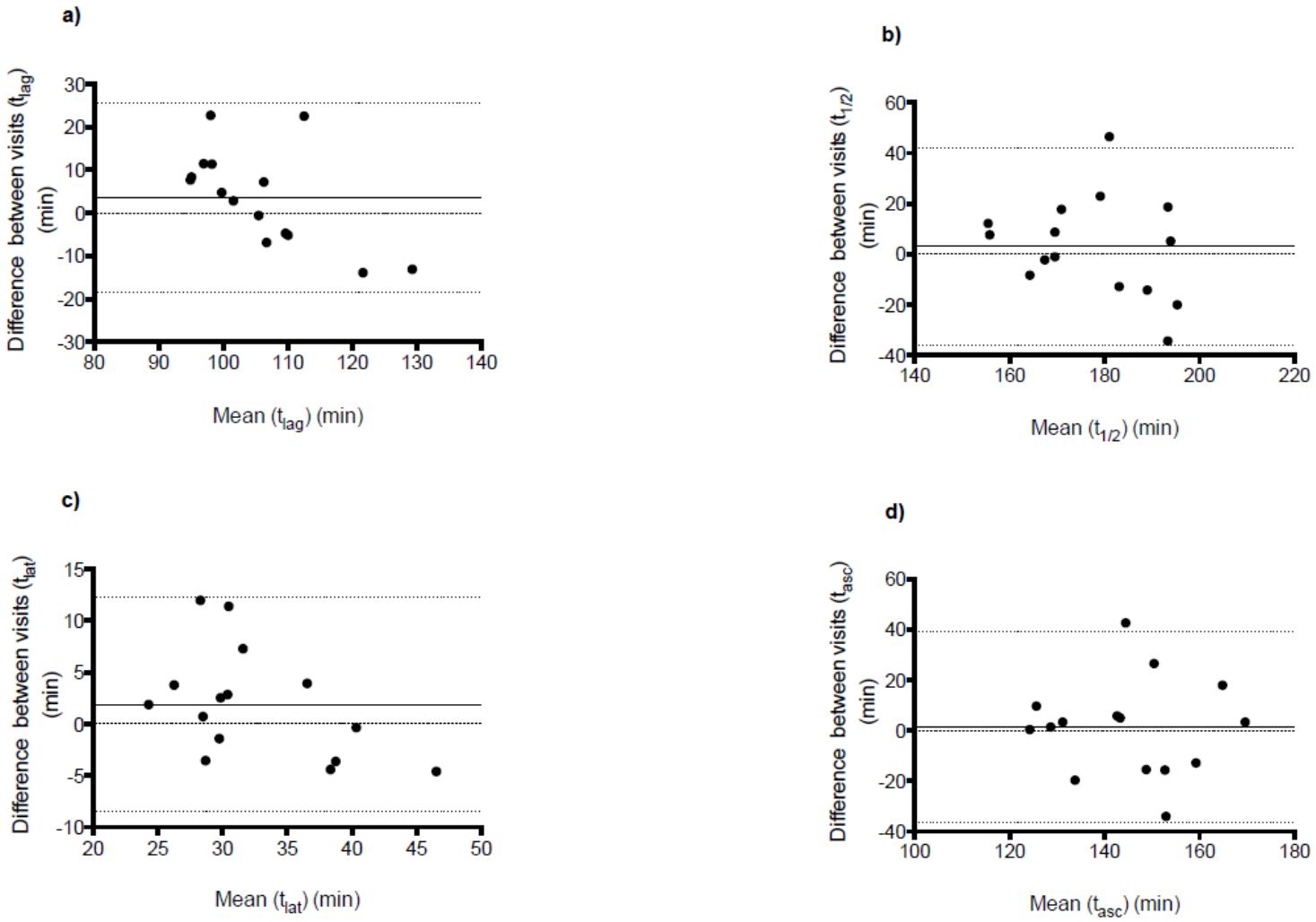


Figure 3.6 Bland–Altman plots showing the difference between visits 1 and 2 (y axis) plotted against the mean for the two visits (x axis) for a) t_{lag}, lag time, b) $t_{1/2}$, half time, c) t_{lat}, latency time and d) t_{asc}, ascension time. Solid line indicates mean bias. Dashed lines indicate *95% limits of agreement. n = 15*

The mean difference in GE between test days was small for all parameters. However the 95% limits of agreement were quite large ranging from -18.3 min to 25.6 min for t_{lag} and from -35.9 min to 42.1 min for $t_{1/2}$. As shown in **Figure 3.6,** one participant was outside the 95% limits of agreement for $t_{1/2}$, with a mean difference between test days of 46 minutes. There was no obvious reason for excluding this participant as they reported to adhere to all study protocol instructions. Therefore, this change may represent the extreme of intra-individual variability.

3.3.1.1 Relationships between GE Variables

Change in t_{lag} from visit 1 to 2 was significantly correlated with change in $t_{1/2}$ ($r = 0.81$, $p < 0.001$). Changes in all parameters between visits 1 and 2 were significantly correlated ($p < 0.05$), except for t_{lat}. Change in t_{lat} was significantly correlated with change in t_{lag} ($r = 0.77$, $p = 0.001$). However, there was no significant correlation between change in t_{lat} with change in $t_{1/2}$ ($r = 0.25$, $p = 0.36$) or t_{asc} ($r = -0.011$, $p = 0.97$, **Figure 3.7**).

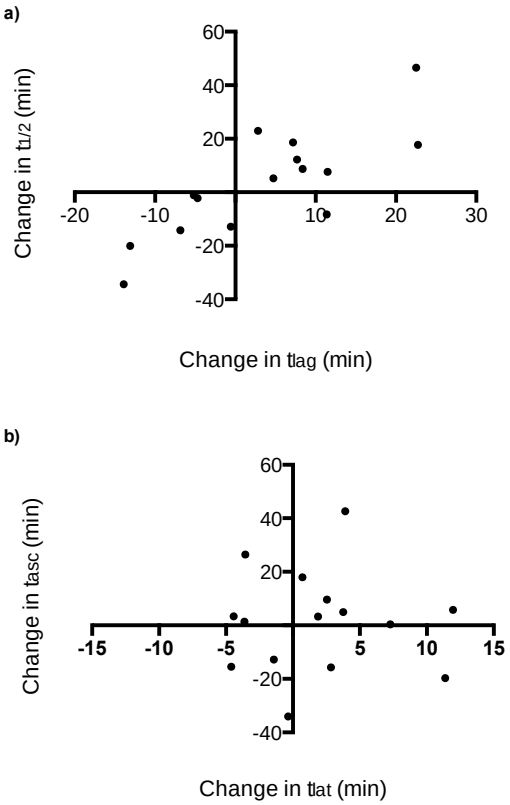


Figure 3.7 Scatter plots of the relation between the change - from visit 1 to visit 2 - in (a) lag time (t_{lag}) and half time ($t_{1/2}$) ($r = 0.81$, $p < 0.001$), and b) latency time (t_{lat}) and ascension time (t_{asc}) ($r = -0.01$, $p = 0.97$). $n = 15$.

3.3.1.2 Calculation of sample sizes for GE parameters

Based on the day-to-day variations observed, calculations revealed that in order to detect a treatment effect, in a paired design with a power of 80% and $\alpha = 0.05$, minimum mean effect sizes for GE $t_{1/2}$ would need to be $\geq$ 14.40min, $t_{lag} \geq 8.1$ min, $t_{asc} \geq 13.9$ min and $t_{lat} \geq 3.8$ min. An estimate of the minimum number of participants needed to detect significant differences in a paired design study assuming $\alpha = 0.05$ and a power of 80% was calculated and used to construct a nomogram (**Figure 3.8**).

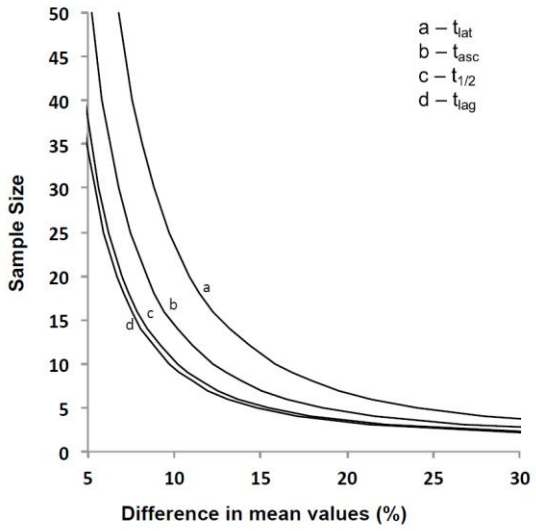


Figure 3.8 Minimum number of participants needed to detect significant differences in a paired design in a) latency time (t_{lat}), b) ascension time (t_{asc}), c) half time ($t_{1/2}$),and d) lag time (t_{lag}), assuming a power of 80% and $\alpha = 0.05$.

To detect a 10% change in a paired design study the minimum number of participants needed for t_{lag} and $t_{1/2}$ would be 10, t_{lat} 25 and t_{asc} 14. To detect a 10% difference between groups in an unpaired design study, the minimum number of subjects needed for t_{lag} would be 9, $t_{1/2}$ 12, t_{lat} 43 and t_{asc} 22.

3.3.2 Subjective Appetite Sensations

Subjective appetite scores over the test morning for hunger, fullness and desire to eat ratings are shown in **Figure 3.9**. The response curves were similar after both visits.

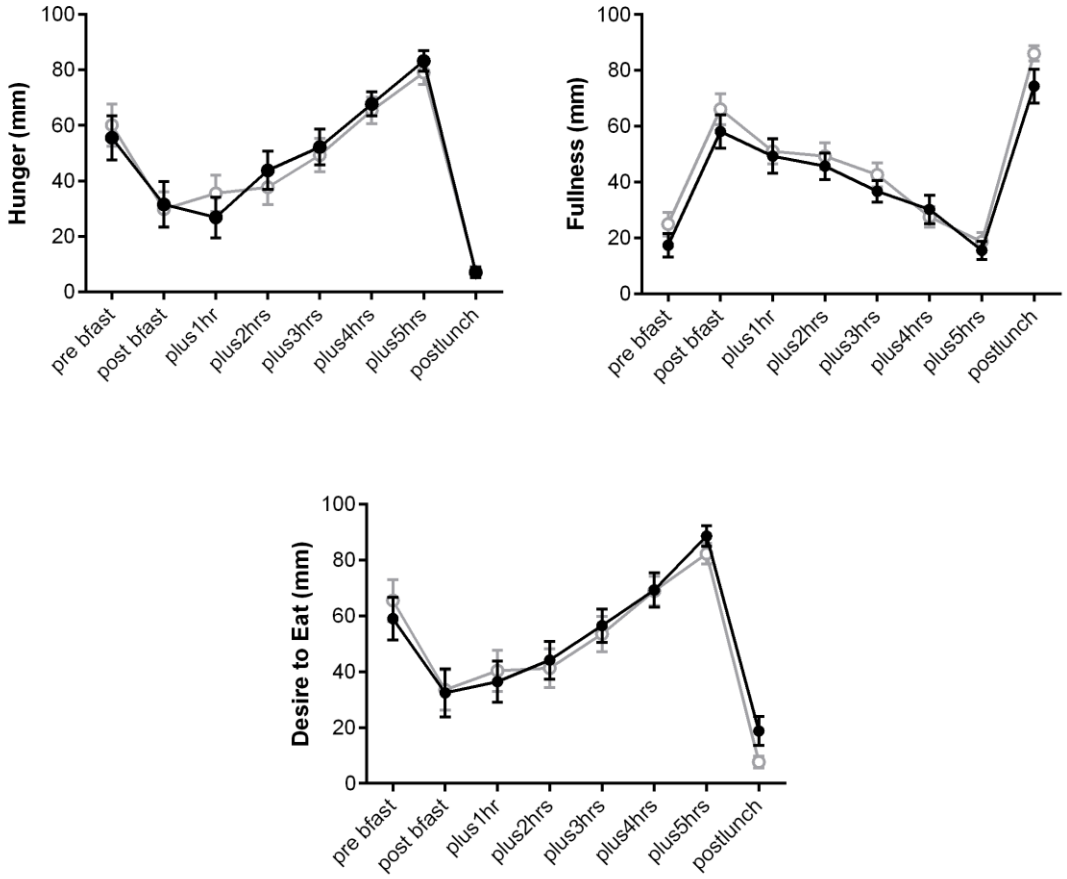


Figure 3.9 Mean (± SEM) subjective appetite scores on the two test days (Visit 1: black filled circles; Visit 2: grey open circles). Pre bfast: pre breakfast; post bfast: post breakfast; plus1hr: 1 h post breakfast; plus2hrs: 2 h post breakfast; plus 3hrs: 3h post breakfast; plus 4hrs: 4hpost breakfast; plus 5hrs: 5h post breakfast; post lunch: rating immediately after *ad libitum* lunch meal. $n=15$.

The reproducibility of fasting values, breakfast and lunch satiety quotients, 5 hour postprandial mean ratings and 5 hour AUC values at Visits 1 and 2 for hunger, fullness and desire to eat are shown in **Table 3.7**. There were no significant differences in any of the parameters between Visits 1 and 2 as indicated by paired t-test ($p > 0.05$), except for the satiety quotient (SQ) for hunger at lunch. The SQ for hunger at lunch indicated a stronger suppression of hunger relative to the amount of energy intake at lunch at Visit 1 compared to Visit 2 ($p = 0.02$).

Correlations between ratings on the first and second visit were strongest for mean 5h ratings and 5h AUC ratings and were weakest for fasting ratings (**Table 3.7**). Similarly, CRs were larger for fasting values (range 35-65mm) than mean 5h

ratings (range 13-20mm). Coefficients of variation were largest for fasting ratings (34-68%) and lowest for mean 5h ratings (11-20%) and 5hAUC (12-20%).

Table 3.7 Reproducibility of Appetite Ratings at Visits 1 and 2 (n = 15)

Variable	Visit 1	Visit 2	*P*-value	*CV (%)*	*CR*	*r*
Fasting VAS Ratings (mm)						
Hunger	55.5 ± 30.9	60.1 ± 29.3	0.54	35.0	57.2	**0.55***
Fullness	17.4 ± 16.2	24.9 ± 16.7	0.21	68.1	40.7	0.18
Desire to Eat	59.0 ± 29.5	65.5 ± 29.1	0.42	34.4	60.5	0.47
Breakfast Satiety Quotient (mm/kcal)						
Hunger	5.7 ± 5.4	7.6 ± 7.2	0.21	59.0	11.1	**0.65****
Fullness	-10.5 ± 6.4	-10.7 ± 5.3	0.88	40.8	12.2	0.44
Desire to Eat	6.6 ± 6.6	8.6 ± 1.1	0.18	48.0	14.8	**0.54***
Mean 5h VAS Ratings (mm)						
Hunger	50.9 ± 20.5	49.5 ± 19.8	0.51	11.4	16.16	**0.92*****
Fullness	39.3 ± 14.3	42.5 ± 14.1	0.30	20.3	23.5	**0.66****
Desire to Eat	54.6 ± 21.8	53.7 ± 21.3	0.72	12.9	19.7	**0.90*****
Postprandial 5h AUC VAS Ratings (mm.min)						
Hunger	14878 ± 6235	14544 ± 6130	0.60	11.6	4814.0	**0.92*****
Fullness	11922 ± 4314	12774 ± 4224	0.36	19.9	6938.9	**0.67****
Desire to Eat	16024 ± 6648	15782 ± 6668	0.77	13.7	6155.3	**0.88*****
Lunch Satiety Quotient (mm/kcal)						
Hunger	8.6 ± 3.3	6.9 ± 2.0	**0.02***	22.7	5.0	**0.66****
Fullness	-6.8 ± 4.2	-6.6 ± 2.3	0.85	35.4	6.7	**0.60***
Desire to Eat	7.8 ± 2.7	7.4 ± 2.3	0.37	18.2	4.0	**0.70****
Ad Libitum EI (kJ)						
EI	4095 ± 1068	4572 ± 1639	0.1	17.4	2129	**0.76*****

Values are Means ± SD. VAS, Visual Analogue Scale; EI, energy intake. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Based on the standard deviations observed in the present study, sample size calculations showed that in a paired design study a minimum of 65 and 35 participants would be needed to detect a 10mm difference in fasting hunger and fullness ratings respectively. In contrast, a smaller sample size of 6 and 12 is sufficient to detect a 10mm change in 5h mean hunger and fullness ratings respectively. In an unpaired design a minimum of 42 participants per group are

needed to detect a 10mm difference between groups in fasting fullness ratings and >100 per group are needed to detect a difference in hunger ratings. Whereas for 5h mean ratings, a minimum sample size of 66 for hunger and 33 for fullness ratings are required per group to detect a 10mm difference.

3.3.3 *Ad libitum* EI

There were no significant differences in EI at the *ad libitum* lunch meal between the two visits as indicated by paired t test (Visit 1: 4095 ± 1068 kJ and Visit 2: 4572 ± 1639 kJ, p = 0.10). To illustrate the reproducibility, a Bland Altman plot is shown in **Figure 3.10** below.

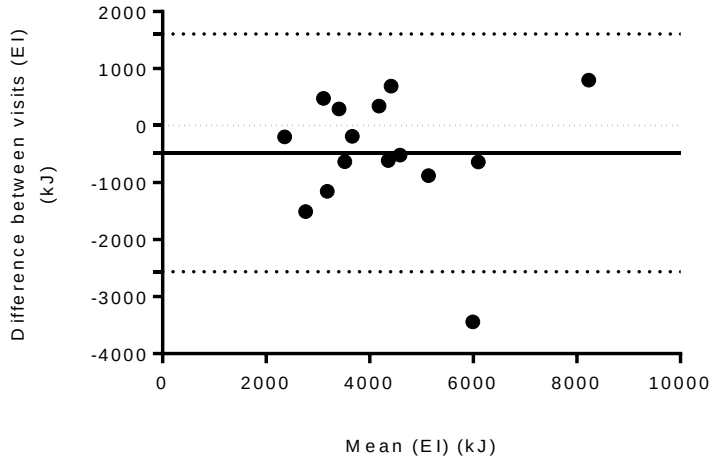


Figure 3.10 Bland–Altman plot showing the difference between visits 1 and 2 (y axis) plotted against the mean for the two visits (x axis) for energy intake (EI) at the *ad libitum* lunch meal. Solid line indicates mean bias. Dashed lines indicate *95% limits of agreement. n = 15*

The mean bias is represented in **Figure 3.10** by the solid dark line and indicates a mean bias of -477 kJ. The coefficient of reproducibility (CR) from this is ± 2129 kJ and the intra-individual CV 17.4%. The outlier had a mean difference in EI of 3442kJ between visits. As the outlier was 2.8 SD away from the mean, values are reported both with and without the outlier. When the outlier is removed, the mean bias decreases to -265kJ, the CR to ± 1408 kJ and the intra-individual CV is reduced to 11.5%. The correlation between EI at test days 1 and 2 was r = 0.76 (R^2 = 0.62, p

< 0.001, **Figure 3.11**). With the outlier removed the correlation coefficient increased to r = 0.9 (R^2 = 0.82, p < 0.0001).

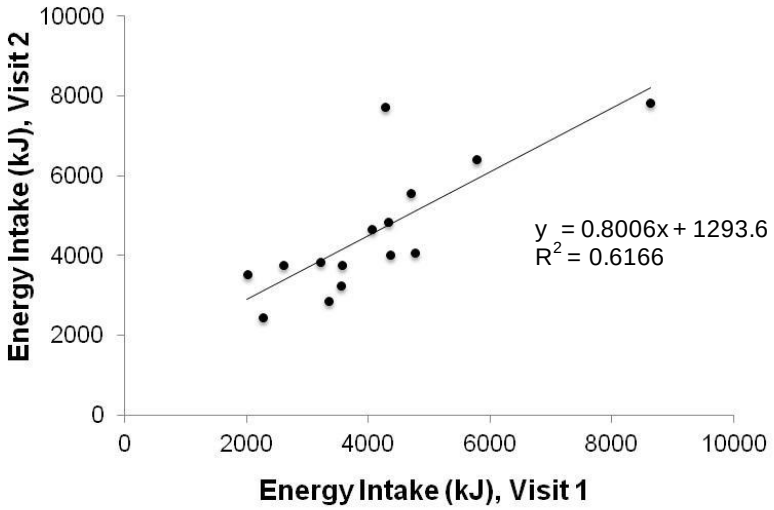


Figure 3.11 Energy intake at visit 2 plotted against energy intake at visit 1 at the ad libitum lunch meal. r = 0.76, P < 0.001. n = 15.

3.3.4.1 Calculation of sample sizes for ad libitum EI

To detect a 500kJ difference in a paired design study, a minimum sample size of 36 is needed, whereas a sample size of 10 is sufficient to detect a 1000kJ difference. Excluding the outlier in calculations, the minimum sample size required to detect a 500kJ and 1000kJ difference in a paired design would be 17 and 6 respectively.

In an unpaired design, to detect a 1000kJ difference between groups, a sample size of 42 is sufficient, whereas to detect a 500kJ difference between groups a sample size >100 is needed. Sample size calculations for the unpaired design were unaffected by outliers.

3.3.4 Palatability of Test Meals, Food Preferences, 'Liking' and 'Wanting'

3.3.4.1 Palatability

Palatability ratings for 'sweet', 'savoury', 'tasty', 'pleasant', 'filling' and 'satisfying' for breakfast (**Figure 3.12**) and lunch (**Figure 3.13**) meals were similar between test days. The mean ratings for 'taste', 'pleasant', 'filling' and 'satisfying' were on the positive end of the scale for both meals.

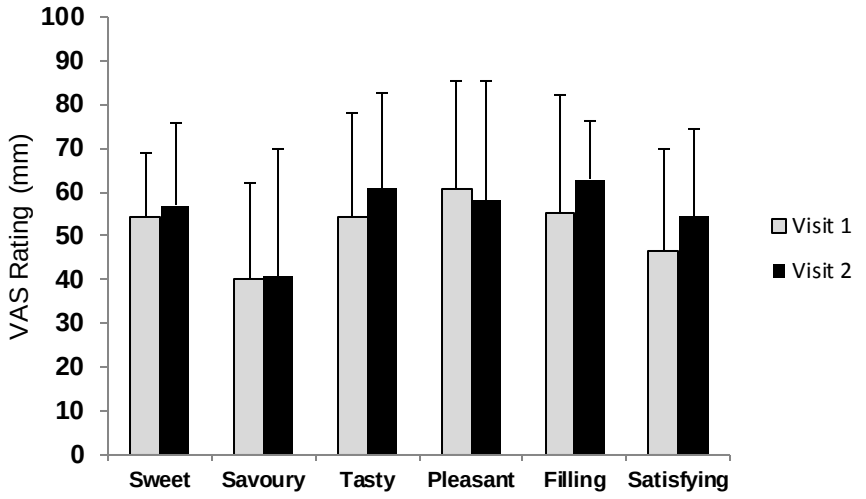


Figure 3.12 Palatability ratings at Visits 1 and 2 for the breakfast meal. There were no significant differences between visits for any of the parameters ($p > .05$).

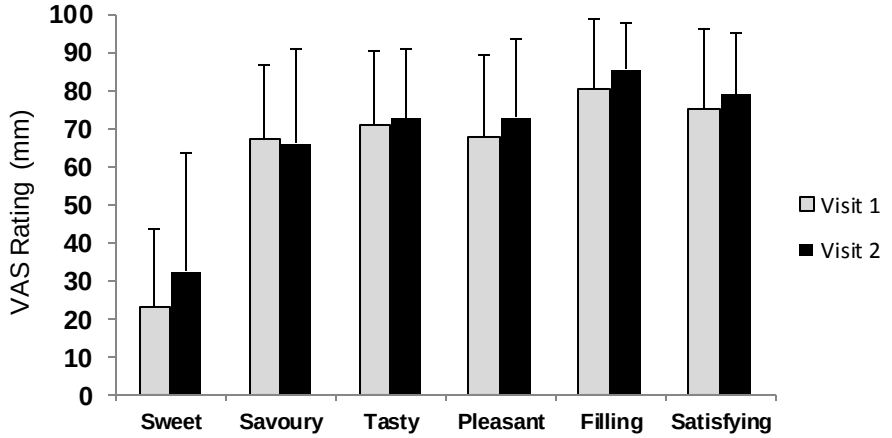


Figure 3.13 Palatability ratings at Visits 1 and 2 for the lunch meal. There were no significant differences between visits for any of the parameters ($p > .05$).

Paired t-tests comparing mean ratings for breakfast and lunch meals indicated participants found the lunch meal more savoury ($p < 0.01$), more filling ($p < 0.001$), more satisfying ($p < 0.001$) and less sweet ($p < 0.001$), compared to the breakfast meal. There was a trend towards a higher rating of 'tasty' for the lunch meal ($p = 0.06$) compared to the breakfast meal and no significant difference in 'pleasant' ratings ($p = 0.19$) between breakfast and lunch meals.

3.3.4.2 Food Preferences, 'Liking' and 'Wanting'

Results at Visits 1 and 2, CVs, CRs, and correlation coefficients for pre and post breakfast ratings are shown in **Table 3.8** and **Table 3.9** respectively. Due to human error, one participant did not complete the LFPQ post breakfast. Therefore data post breakfast are for n = 14. There were no significant differences between visits 1 and 2 for any of the parameters. Strong and significant correlations were seen between test days in both the fasted (**Table 3.8**) and fed (**Table 3.9**) state in all cases except for explicit liking for the low fat sweet category prior to breakfast (**Table 3.8**). CVs and CRs were higher in the fed compared to fasted state.

Table 3.8 Reproducibility of Fasting (Pre-Breakfast) Preferences, explicit liking and explicit wanting at Visits 1 and 2 (n = 15)

Variable	Visit 1	Visit 2	*P*-value	*CV (%)*	*CR*	*r*
Preference (freq)						
HFSA	23.9 ± 8.6	25.7 ± 8.8	0.18	14.2	10.0	**0.85*** **
LFSA	23.0 ± 10.7	22.3 ± 11.1	0.85	16.9	10.8	**0.85*** **
HFSW	22.7 ± 10.5	22.1 ± 9.2	0.90	13.3	8.4	**0.90*** **
LFSW	26.4 ± 8.5	25.9 ± 8.4	0.64	10.4	7.7	**0.90*** **
Explicit Liking (mm)						
HFSA	44.3 ± 26.3	48.0 ± 25.4	0.17	15.5	20.3	**0.93*** **
LFSA	41.9 ± 27.0	46.5 ± 27.0	0.08	15.8	19.7	**0.94*** **
HFSW	47.6 ± 28.5	48.7 ± 28.0	0.75	20.4	27.8	**0.87*****
LFSW	50.3 ± 19.1	49.3 ± 17.9	0.86	29.5	41.5	0.40
Explicit Wanting (mm)						
HFSA	43.0 ± 26.6	45.9 ± 26.3	0.42	22.7	28.6	**0.86*****
LFSA	40.2 ± 27.7	45.7 ± 26.6	0.12	19.1	23.2	**0.90*** **
HFSW	45.7 ± 29.2	45.1 ± 30.4	0.90	24.9	32.0	**0.84*****
LFSW	49.9 ± 18.2	48.7 ± 19.9	0.80	26.3	36.7	**0.56***

Values are Means ± SD. HFSA, high fat savoury; LFSA, low fat savoury; HFSW, high fat sweet; LFSW, low fat sweet. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 3.9 Reproducibility of Fed (Post-Breakfast) Preferences, explicit liking and explicit wanting at Visits 1 and 2 (n = 14)

Variable	Visit 1	Visit 2	*P*-value	*CV (%)*	*CR*	*r*
Preference (freq)						
HFSA	24.9 ± 10.6	23.7 ± 9.0	0.55	25.4	17.3	**0.63****
LFSA	19.2 ± 10.9	21.1 ± 10.4	0.4	21.8	12.3	**0.84*****
HFSW	27.3 ± 8.0	24.1 ± 9.4	0.17	19.8	14.5	**0.68****
LFSW	24.6 ± 9.8	27.1 ± 7.8	0.14	18.2	13.4	**0.74*****
Explicit Liking (mm)						
HFSA	37.8 ± 28.8	44.8 ± 28.5	0.17	27.8	32.1	**0.85*****
LFSA	32.4 ± 23.8	36.7 ± 19.6	0.19	20.7	20.1	**0.91******
HFSW	45.8 ± 30.9	49.4 ± 28.5	0.65	39.6	53.2	**0.62****
LFSW	43.9 ± 20.9	46.9 ± 19.6	0.65	28.2	35.8	**0.62****
Explicit Wanting (mm)						
HFSA	33.3 ± 27.5	41.0 ± 29.2	0.06	26.6	27.9	**0.89*****
LFSA	30.5 ± 20.7	37.4 ± 20.6	0.18	33.5	31.7	**0.71*****
HFSW	40.6 ± 31.2	47.7 ± 30.1	0.24	37.0	46.5	**0.72*****
LFSW	36.5 ± 18.3	46.1 ± 20.9	0.06	30.0	35.0	**0.63****

Values are Means ± SD. HFSA, high fat savoury; LFSA, low fat savoury; HFSW, high fat sweet; LFSW, low fat sweet. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.3.5 Relationships between Variables

Correlation coefficients of all subjective appetite ratings with GE parameters are shown in **Appendix D**. To correct for multiple comparisons when examining relationships amongst the different variables, only results with statistical significance at $p < 0.01$ are discussed. The breakfast satiety quotient (SQ) was the only parameter significantly related to GE. Mean breakfast SQ for fullness (representing the mean of the 2 visits) was negatively correlated with mean GE $t_{1/2}$ ($r = -0.69$, $p = 0.004$) and mean GE t_{asc} ($r = -0.66$, $p = 0.007$). This indicates a greater increase in fullness immediately post breakfast was associated with a longer GE $t_{1/2}$. In contrast, mean breakfast SQ for desire to eat was positively correlated with mean GE $t_{1/2}$ ($r = 0.76$, $p = 0.0009$, **Figure 3.14**) and mean GE t_{asc} ($r = 0.65$, $p = 0.009$). This indicates a greater suppression of the desire to eat after breakfast was also associated with longer GE $t_{1/2}$. These correlations were only significant for the means of the 2 visits and were not significant for the separate test days.

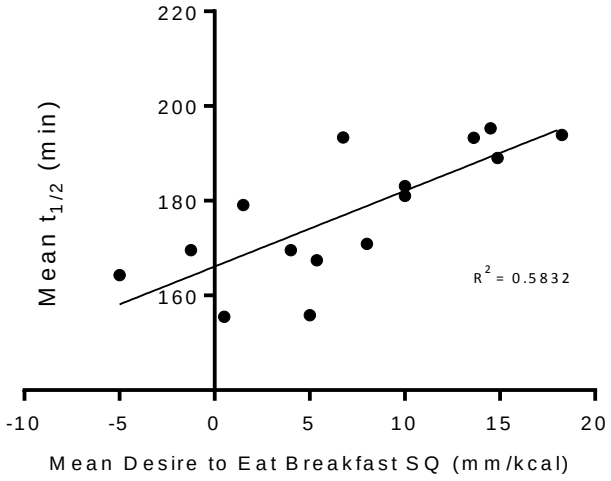


Figure 3.14 Correlation of desire to eat breakfast SQ and GE $t_{1/2}$ (r = 0.76, p = < 0.001).

There were no significant associations between changes in GE, appetite or EI from visit 1 to visit 2 (**Appendix D**). In addition there were no significant associations between any of the GE parameters and EI at lunch. The Lunch SQ was the only appetite parameter associated with EI at lunch. A greater suppression of hunger and desire to eat after the lunch meal relative to the amount of EI was associated with lower EI on individual test days and when the means of both test days were correlated (**Appendix D**).

3.4 DISCUSSION

Methodological issues such as reliability and sensitivity are often overlooked in the design of studies. This study provides evidence that GE and associated measures are reproducible in overweight and obese males. No significant differences in any of the outcome measures (GE, subjective appetite sensations, *ad libitum* EI, food preferences and 'liking' and 'wanting) were found between two identical test days. However the degree of variability within individuals varied for different outcome measures. Collectively, the findings provide valuable information for the design of future studies in this population and support the use of these methods to evaluate changes in GE and associated measures in overweight and obese males in research and clinical settings.

3.4.1 Reproducibility of GE

Some evidence indicates that the release of gut peptides and appetite may be disturbed in obese individuals [341, 342]. For example, ghrelin is associated with GE in lean but not obese individuals [159]. The hypothesis in the present study was that the day-to-day variability in GE might be different. However, the present findings demonstrating a mean intra-individual coefficient of variation of ~ 8% for GE half time in healthy overweight and obese males, are comparable to studies in lean individuals using test meals similar in energy content [276, 280, 283-285, 288, 294]. In healthy lean adults, an intra-individual coefficient of variation ranging from 7% [294] to 30% [297] has been reported for half time of solid meals, with the majority of studies indicating an intra-individual variability of between approximately 11-15% [279-281, 283-285, 288, 290, 291, 294]. Although others have reported a higher intra-individual variability in GE of between 20-30% in healthy adults [278, 293, 297, 298, 343], differences in study methodologies may account for this inconsistency. The low energy content of the test meals (ranging from 200 – 250 kcal) in the latter studies [278, 293, 297, 298, 343] may contribute to the lower reproducibility [344]. It is also possible that the true variability in GE may go undetected if the test meal does not challenge motility [345], therefore in the current study a test meal which reflects a more typical size of meal (400 kcal) was used. Collectively, these data indicate that unlike other populations where the variability of

GE has been shown to be higher (e.g. diabetic patients [276, 287], patients with functional dyspepsia [299], preterm infants [273] and critically ill patients [275]), the reproducibility of GE in healthy overweight and obese males is similar to that reported in healthy lean adults.

The findings also indicate that the parameters used to characterise GE differ with regard to their reproducibility. The intra-individual variability was similarly lowest for t_{lag} and $t_{1/2}$ derived from the original ^{13}C-OBT mathematical model [293], than for other GE parameters. This finding contrasts with others who have shown the lag phase or initial emptying to be less reproducible than subsequent emptying parameters [281, 288, 346]. Cremonini et al. (2002) [281] demonstrated that the variability was considerably lower for GE end points, reporting an intra-subject coefficient of variation of 20% for GE at 1 hour compared to 4% at 4 hours. Similarly, Loo et al. (1984) [346] found the initial emptying to be less reproducible than the subsequent emptying parameters calculated. One explanation may be that GE was measured in these studies by scintigraphy and lag time derived by scintigraphy is known to be difficult to quantify [345]. Chey et al. [345], examined the reproducibility of GE parameters measured by both ^{13}C breath test and scintigraphy and reported that while for scintigraphy half time was considerably more reproducible, for the breath test lag time was more reproducible. The current findings also suggest that in contrast to some scintigraphic studies, lag time derived by breath test is a reproducible GE parameter.

In addition to lag and half times, other parameters attempting to more accurately reflect the biphasic nature of GE have been proposed. By definition the same part of the $^{13}CO_2$ exhalation curve is used for the calculation of both t_{lag} and $t_{1/2}$ and both parameters have been shown to be highly correlated [303]. As a result the different phases of GE (e.g. a delayed initial emptying but accelerated subsequent emptying or vice versa) could be difficult to distinguish. This prompted Schommartz et al (1998) to propose the parameters latency time and ascension time, described in **Table 3.3**. Although, little information exists on the reproducibility of these parameters, intra-individual coefficient of variations of ~ 11% and 9% for latency and ascension times respectively were reported here, indicating that both parameters are reproducible in overweight and obese males. Interestingly, the current findings also suggest that while changes in lag time and half time from visits 1 to 2 were

highly correlated, changes in latency and ascension times were not. These findings suggest that these additional parameters may be more sensitive to detecting changes in different phases of GE, and would be useful to determine in addition to the conventional parameters in repeated measures studies.

Information regarding the day-to-day variability of GE is necessary to determine appropriate sample sizes when designing studies. For example, Lartigue et al. (1994) [276] calculated that to detect a 20% change in GE half time in a paired design study, 7 healthy subjects would be required whereas 18 diabetics would be required. In the present study, it was found that a 20% change in all parameters (lag, half, latency and ascension times) could be detected in overweight and obese males with a sample size of 7 participants. These results demonstrate that only a small number of participants are needed to detect clinically relevant changes in GE. As GE studies are often carried out in small numbers e.g. measured pre and post surgical procedure, these findings illustrate the potential efficiency of undertaking smaller studies before larger studies are undertaken.

3.4.2 Reproducibility of Subjective Appetite Sensations

With regard to the reproducibility of subjective appetite sensations, these data in overweight and obese males showed a consistent 'peak and trough' pattern, similar to previous studies [309]. In addition, CVs for mean 5h postprandial ratings and 5hAUC ratings ranged between 11 and 20%. These findings are consistent with previous studies in normal weight adults reporting a CV of between 7-24% for 4.5h mean and AUC ratings [305]. Therefore, despite previous evidence of altered appetite sensations in overweight and obese individuals [341] and our hypothesis that the day to day variability of appetite ratings might therefore be different, these findings suggest the reproducibility of postprandial appetite ratings is similar in overweight and obese and normal weight adults.

The findings also demonstrate that the reproducibility of appetite ratings varies depending on the appetite parameter of interest, being most reproducible for postprandial mean and AUC ratings and least reproducible for fasting ratings. The CVs and CRs were much higher for fasting ratings compared to other parameters in the current study. CRs for fasting ratings were large (40 – 60 mm) but similar to

those reported by Raben et al. 1995 [306] (29-52mm) in a sample of 9 lean males. Correlations between appetite ratings on the 2 test days were also strongest for mean 5h VAS and AUC ratings (r = 0.66-0.92) and weakest for fasting ratings (hunger: r = 0.55, fullness 0.18, desire to eat 0.47). These findings are consistent with Flint et al. (2000) [305] who reported similar findings in 55 normal weight adults. As highlighted by the authors, the high degree of reproducibility of mean values is not surprising as the role of a single erroneous rating is reduced [305]. The higher variability of fasting ratings would also explain the higher variability of the breakfast SQs in the present study as the SQ takes into account the pre-post meal changes. In contrast, the SQ at lunch was more reproducible which could in part be explained by the anticipation of the next meal influencing the ratings to converge toward one end of the appetite scale as lunch time approached [305]. Overall, the data highlight that VAS are reproducible when considering the group mean data but the underlying data shows differing degrees of intra-individual variability depending on the parameter of interest.

Based on the standard deviations observed in the current data, minimum sample sizes that would be needed to detect a 10mm difference in appetite ratings were calculated. For fasting ratings much larger sample sizes of at least 65 are required to detect a 10mm change. In contrast, for mean 5 hour hunger, desire to eat and fullness ratings a sample size of 11, 17, and 23 respectively would be sufficient to detect a 10mm change with 80% power. The present data in overweight and obese males supports anecdotal evidence which suggests 20-25 subjects are generally sufficient to capture a 10% difference in the mean or AUC appetite ratings in a paired design [269]. These findings are important to consider when both designing and interpreting studies in this population depending on the appetite variable of interest.

3.4.3 Reproducibility of *ad libitum* EI

Similar to GE and appetite ratings, there were no significant differences in EI between test days as indicated by paired t-test. However, as indicated by a Bland Altman plot there was considerable variation within some individuals from day to day - the extreme difference being an increase in EI of 3444kJ in one participant at the second visit. Interestingly this was the same individual who was also outside the

95% confidence interval for GE $t_{1/2}$ with a GE $t_{1/2}$ that was 46 minutes shorter at the second visit. This may illustrate the extreme of intra-individual variability in these parameters. Including this participant in the analysis led to a mean bias of -477kJ and would suggest the ad libitum meal is biased towards a greater EI at the second visit. However, excluding the outlier the bias is reduced to -265kJ (-63 kcal) and the CR to ± 1.4 MJ. This compares favourably to the CRs of ± 1.5 and ± 1.8MJ reported in a previous study of diet standardised and non-standardised groups of normal weight males [324]. A recent study [347] in 12 overweight and obese males and females analysed the reproducibility of ad libitum EI from a computerised vending machine and similarly showed that the CR was significantly influenced by an outlier, being ± 6.2 MJ and ± 3.4MJ with and without the outlier respectively. The fact that EI was assessed over 3 days is the most likely explanation for the greater CRs in their findings compared to the single meal in the present study. Taken together, these findings highlight that while there may be no significant differences in mean group data for ad libitum EI, some individuals are likely to have considerable variation from day to day.

The intra-individual CVs of 17.4 and 11.5% in the present study and correlation coefficients of 0.76 and 0.9 with and without the outlier respectively however suggest the *ad libitum* meal is a suitable method for assessing EI in overweight and obese males. Furthermore, consistent with others[324] the variability did not appear to depend on the size of *ad libitum* EI. Gregersen et al. (2008) [324] reported a CV of 14.5% and r = 0.65 for EI at an *ad libitum* meal served 4.5h after a fixed breakfast in normal weight males. Others have reported a lower CV of 8.2% in normal weight males [308]. In a small sample of 8 overweight and obese subjects, Lara et al. (2010) [326] examined the reproducibility of *ad libitum* EI of a pasta lunch meal served 90 minutes after a control or whey protein preload. The CVs were 4.5 and 11.2%, intraclass correlations 0.97 and 0.72 and mean differences between the two identical visits -50kJ and -142kJ, for control and whey protein preloads respectively. The authors concluded the preload paradigm was highly reproducible in overweight and obese subjects. Although the values in the present study are slightly higher, one likely explanation is the longer time interval of 5 hours between the breakfast and lunch meal in the present study. The ability to compensate accurately for prior energy intake has been shown to become less precise as the time interval increases [146]. The present study therefore adds to the previous work of Lara et al.

(2010), by demonstrating that with a more typical inter-meal interval of 5 hours, an ad libitum pasta lunch meal is a suitable method of measuring *ad libitum* EI in the laboratory in overweight and obese males. However, it highlights that there may be considerable variations within some individuals from day to day.

3.4.4 Reproducibility of food preferences, 'liking' and 'wanting'

In addition to investigating homeostatic processes, one objective of the current study was to investigate the reproducibility of non-homeostatic processes implicated in appetite control in overweight and obese males. Using the Leeds Food Preference Questionnaire (LFPQ), strong correlations between days for food preferences and 'liking' and 'wanting' were found in the fasted state for all foods ($r = 0.84$ to $r = 0.94$) except for 'liking' for the low fat sweet (LFSW) category.

Previous studies that have assessed food preferences and 'liking' and 'wanting' have similarly demonstrated strong and significant correlations between test days in all cases except the desire to eat something sweet in the fasted state [305]. Using visual analogue scales, Flint et al. (2000) [305] assessed the reproducibility of the desire to eat something 'salty', 'sweet', 'fatty' or 'savoury' and demonstrated strong and significant correlations between fasting ratings for all parameters in normal weight males, except for desire to eat something sweet ($r = .15$). There is no obvious explanation why the reliability of 'liking' for LFSW in the fasted state was low in the present study compared to other food categories. One possibility may be that other factors such as social pressure to prefer a taste that is less sweet [348] may have influenced participants choices and ratings. However this would not explain the strong correlations of the food preferences for LFSW or 'liking' for the HFSW categories. Others have recently demonstrated a similar computer based procedure for 'liking' and 'wanting' to be sufficiently valid (reliable and sensitive) [312]. Lemmens et al. (2010)[312] reported a reproducibility of 62-73% for 'liking' and 'wanting' in the fasting state in 73 males and females (BMI: 19-31kg/m^2), and demonstrated that the test was sensitive to detect differences between pre-to post consumption of 2 different test foods. Although, sensitivity was not tested in the present study, the LFPQ has previously been shown to be sensitive to detect preferences for particular foods that were modulated by food intake [315]. The present findings therefore add to previous work by demonstrating the LFPQ is a

reliable tool for assessing food preferences, 'liking' and 'wanting' in the fasted state in overweight and obese males.

Knowledge of the reliability of the LFPQ in the fed state is also important as food preferences, 'liking' and 'wanting' are often measured in both the fasting and postprandial state in appetite research. In the fed state (immediately post breakfast) correlations between test days were significant for all food categories in the current study. This finding is similarly consistent with the findings of Flint et al. (2000) [305] who demonstrated strong and significant correlations between test days for 4.5 hr mean postprandial ratings of desire to eat all food categories. In the present study, correlations between test days were generally slightly weaker in the fed state (r= 0.62 to 0.91) compared to the fasted state (r = 0.84 – 0.94, excluding LFSW categories). This was mirrored by higher CVs and CRs in the fed state compared to fasted state, illustrating a greater intra-individual variability in food preferences, 'liking' and 'wanting' when assessed immediately post food intake. This is an important finding to consider in the design of studies as it infers that a considerably larger number of subjects will be needed to detect relevant changes in these outcomes if the interest is in both fasting and postprandial differences.

When comparing the reproducibility of food preferences and 'liking' and 'wanting', food preferences appear to be the most reproducible measure in the fasting state as demonstrated by generally lower CVs (10-17%) compared to 'liking' (15-29%) and 'wanting' (22 – 26%). Similarly in the fed state, CVs were lowest for food preferences. It is not possible to compare these findings to previous studies as CVs and CRs were not reported [312] and no studies could be found which have compared the reproducibility of 'liking', 'wanting' and food preferences in both fasted and fed states. Previously a reproducibility of 60% (calculated as the proportion of concordance between repeated measurements) has been considered sufficiently reliable [312]. Despite strong correlations between the two test days in the present study, large CVs and CRs in some cases indicate considerable variation is present for some parameters in the underlying data. While acknowledging this individual variability, strong correlations between identical tests for the majority of parameters, consistent with previous work [312], indicates the LFPQ is a sufficiently reliable tool for assessing food preferences, and 'liking' and 'wanting' in both fasted and fed states in overweight and obese males.

3.4.5 Relationships between variables

In the present study, the relationships between GE of a solid meal, appetite and EI in overweight and obese males were assessed. Previous studies have shown reproducible patterns of GE to be associated with reproducible patterns of EI at an *ad libitum* lunch in healthy lean males [117] and females [277]. In these studies, GE of a liquid meal was measured in lean individuals. Findings may therefore not be applicable to the emptying of a solid meal and cannot be generalised to overweight individuals. In the present study no significant associations between GE and EI were observed for any of the GE parameters. One explanation may be differences in the timing of the *ad libitum* meal. The meal was served at 5 hours after breakfast in the present study, in contrast to the previous studies of lean individuals [117, 277]. In these studies, the amount of EI was related to the amount of gastric content remaining at 90min after the meal [117, 277]. In contrast, 5 hours after breakfast in the present study, the amount of breakfast meal remaining in the stomach would have been minimal. Similar to hunger ratings very late in the post meal period (e.g. 4-6h)[269] it is likely that differences in GE when most of the previous meal has emptied, may have much less association to EI than differences earlier in the post meal period. Although, the current study suggests that in contrast to lean males, GE is not related to EI at an *ad libitum* meal in overweight and obese males, further studies involving larger sample sizes and measuring EI over different time intervals are needed to draw firm conclusions.

When comparing the associations between GE and appetite sensations, our results demonstrated that the breakfast SQ was the only appetite parameter associated with GE. A longer GE $t_{1/2}$ was associated with a greater suppression of the desire to eat and increase in fullness after breakfast. This relationship was only significant when mean values of the two test days were pooled, but not for the individual test days. This would support the contention that patterns of physiological responses instead of single markers may be needed to demonstrate relationships between physiology and behaviour [349]. While there was a trend towards the SQ for hunger to be positively associated with GE $t_{1/2}$ in the present study, it did not reach statistical significance. Others have similarly shown relationships between GE and fullness, but not hunger [122, 350]. Although hunger and fullness are often interpreted as opposites, they are mediated by different mechanisms. Fullness may be more related

to a sensation of fullness in the stomach [269], whereas reduced feelings of hunger after a meal may be more related to postabsorptive signals [351]. The macronutrient composition of the meal may also influence relationships between GE and appetite. Cecil et al. [350] reported that following a high carbohydrate soup fullness was correlated with remaining gastric content but hunger was not. On the other hand, following a high fat soup fullness was significantly correlated with gastric content and hunger was inversely correlated. However, others have shown no association between GE and self-reported appetite [352]. Overall, it appears that a possible association between VAS and physiological measures such as GE remain a matter of debate [353]. As GE was tested twice under identical conditions in the present study, further studies are needed to assess associations between changes in GE and appetite by manipulating GE in overweight and obese individuals.

It is reasonable to expect that when other factors are carefully controlled subjective appetite sensations should be associated with the subsequent onset or amount of food intake [269]. This is supported by many laboratory studies which show that hunger ratings are related to food intake [269]. In the present study, the lunch SQ was the only appetite variable associated with EI at the ad libitum meal. Lunch SQs indicated that a greater reduction in hunger and desire to eat post meal relative to food intake was associated with a lower food intake at the meal. Given that the lunch meal was consumed 5 hours after breakfast when hunger ratings converged towards the upper end of the scale and subjects were instructed to eat the *ad libitum* meal until comfortably full, this finding is not surprising. In contrast to others, we found no significant associations between other appetite outcome measures and food intake. Flint et al. (2000) found pre-lunch VAS values, the difference between pre and post lunch values and the 4.5h mean of postprandial values after breakfast were all correlated with subsequent lunch EI in 55 normal weight males [305]. This is consistent with several studies which have demonstrated relationships between hunger ratings and food intake [269]. One explanation for the lack of significant relationship in the present study may be that the relationship of VAS ratings to food intake is highly dependent on the timing of the meal. Relatively large differences in hunger especially in the middle of the scale are most likely to relate to EI [269]. A larger number of participants compared to the 15 in the present study may also be needed. Furthermore, a variety of factors such as the reward value of food, 'social desirability' or cognitive factors such as disinhibition may have been

more likely to influence appetite ratings and EI in overweight and obese individuals [307, 341]. Each variable contributes to a better understanding of an individuals appetite control. Therefore the greater the number of variables which can be measured simultaneously in studies will lead to a better ability to characterise factors influencing changes in appetite and EI in overweight and obese individuals.

3.4.6 Methodological Considerations

There are various methodological aspects to this study and GE reproducibility studies in general which deserve further consideration. How best to represent intra-individual variability remains a matter of debate [354]. The discussion has focused on the intra-individual coefficient of variation for GE because it allows comparison across the majority of other studies and hence populations and methods. Although likely to lead to only small differences, there is some discrepancy in how the coefficient of variation is calculated (e.g. CV_{intra} = SD/m, where m is the mean and SD is the intra-subject standard deviation [284]; and $CV_{intra} = SD_d/(m\sqrt{2})$ where m is the mean and SDd is the standard deviation of the differences between tests [275, 276]). In contrast, for psychological tests or questionnaires, the correlation coefficient between 2 sets of responses is most often used to test the test-retest reliability of instruments. Thus for analysing the reproducibility of the LFPQ for assessing food preferences, 'liking' and 'wanting', we have discussed mainly this outcome similar to others [312]. For determining the reproducibility of VAS ratings some studies have compared the reproducibility of tests using paired t-tests or correlations [355, 356]. However, neither of these statistical procedures sufficiently describe the reproducibility of a method [340]. As highlighted by Flint et al. 2000, [305] it is clear that a strong correlation is not necessarily synonymous with a low CR and vice versa, indicating the correlation analysis should not stand alone when assessing reproducibility. Therefore, although we have reported a range of different parameters to assess the reproducibility of the different measures we have focused on discussing primarily those which allow comparison with other studies in different populations.

The parameters used to describe GE measured by ^{13}C-OBT also vary. Some report half and lag times that are corrected to scintigraphy equivalent values [291, 293, 297]. However, variations in regression equations have been used [357] and the

rationale for the correction of values has been questioned as it is possible to obtain negative and physiologically insignificant values [303]. For this reason, the original uncorrected lag and half times proposed by Ghoos et al. [293], have been reported; which is similar to others [272, 273, 275, 279, 290, 292, 298, 301, 302, 345]. It should also be noted that many other parameters in addition to those described in the current study have been proposed for the ^{13}C-OBT [358]. The reproducibility of four of the most commonly used parameters which aim to characterise the biphasic nature of GE was analysed. Similarly, our findings apply to healthy overweight and obese males and therefore future studies in females are warranted.

The current study did not determine the accuracy of the ^{13}C-OBT against the 'gold standard' scintigraphy. However, scintigraphic measurements may be hampered in obese individuals as defining the gastric areas of interest can be difficult and consequently the acceptance of scintigraphy as the 'gold standard' has been considered by some an arbitrary choice [291]. The optimal method of measuring GE in overweight and obese individuals remains unclear. Other non-invasive non-radioactive methods such as the paracetamol absorption test and ultrasound have only been validated for liquid emptying and ultrasound in particular is considered a suboptimal method for measuring GE in overweight or obese individuals. In contrast, the ^{13}C-OBT measures solid meal emptying, has been validated against scintigraphy, has a day-to-day variability comparable to scintigraphy [293], is sensitive enough to detect pharmacological influences on GE [359] and has been successfully used in obese individuals [29]. The present findings further suggest that GE measured by ^{13}C-OBT is reproducible in overweight and obese individuals and therefore the ^{13}C-OBT represents a promising method for measuring changes in GE in this population.

3.4.7 Summary

Food intake methodology is becoming increasingly important in functional food research and anti-obesity drug development [270] as well as in better understanding the effects of different weight loss strategies such as diet and exercise on appetite control [35]. It follows that claims about changes in appetite or the effects of interventions in overweight or obese people should be accompanied by evidence on these specific types of individuals [269]. However, before intervention studies can be undertaken, it is important to first identify the variability that occurs from day to

day without intervention in this population. Knowledge of the day-to-day variability of GE and associated measures in overweight and obese individuals is lacking. This study has demonstrated that in overweight and obese males GE of a solid meal is reproducible. In addition, this study has shown that similar to lean adults; appetite sensations as assessed by VAS are reproducible, being more reproducible for mean and AUC ratings than fasting ratings. With regard to *ad libitum* EI, this study demonstrated that while one or two individuals can vary considerably from day to day, in general high correlations between test days indicated the *ad libitum* meal is a reproducible method of measurement in overweight and obese males. Further, strong test-retest correlations indicate that the LFPQ is a sufficiently reliable tool for assessing food preferences and processes of food reward in this population. However, despite no mean differences between test days for all outcome measures, evidence that some individuals can still vary considerably from day to day is important to take into account when considering individual changes in clinical settings. This knowledge will assist in the interpretation of previous studies and design of future studies that aim to investigate changes in these parameters in the pathogenesis or treatment of obesity.

Chapter 4: The Effect of Habitual Physical Activity, Energy Expenditure and Body Composition on Gastric Emptying, Appetite and Energy Intake

4.1 BACKGROUND

Although gastric emptying (GE) has long been implicated in the pathogenesis of obesity [14-20], the role of altered GE in obesity is still unclear. Both faster GE and a shorter satiety period [20], and slower GE and a delay in the release of satiation signals in response to intestinal nutrients [29], have been proposed to predispose to obesity. Studies have shown accelerated [16-18], similar [23-25] and delayed [27-29] emptying rates in obese compared to lean individuals (see **Table 4.1**). Further, studies that have correlated GE with BMI in non-obese individuals have demonstrated that an increase in body surface area and BMI are associated with slower GE [153-155], while others have shown no significant correlation [15, 156]. This inconsistency in findings has generally been attributed to differences in methodologies (e.g. meal size, age and gender) as well as methodological limitations (e.g. failing to adjust for Compton scatter in scintigraphic studies). Another hypothesis is that GE may become deregulated at extremes of the body mass spectrum [154].

Table 4.1 Summary of studies examining solid gastric emptying in lean and obese individuals measured by scintigraphy and breath test.

Reference	Participant Characteristics		Meal Energy, Form	GE Method	Finding in obesity
	Obese	Controls			
Wright et al. (1983) [16]	41 obese (mean 177% IBW)	36 (non-obese)	191kcal, S	Sc	Faster
Horowitz et al. (1983) [28]	15 (163-282% IBW)	11 (± 10% IBW)	272 kcal, S,L	Sc	Slower
Horowitz et al. (1986) [160]	7 (198-261% IBW)	17 (± 10% IBW)	270kcal, S,L	Sc	Slower
Maddox et al. (1989) [27]	31 (Median BMI, 42.4, 140-274% IBW)	31 (± 20% IBW)	272kcal, S,L	Sc	Slower
Hutson et al. (1993) [23]	30 (125-218% IBW)	23 (87-120% IBW).	379kcal, S,L	Sc	ND
Glasbrenner et al. (1993) [360]	24 (125-216% IBW)	8 (± 10% IBW)	504kcal, S,L		ND
Tosetti et al. (1996) [200]	20 (BMI, 45 -58)	20 (BMI, 20-25)	644kcal, S	Sc	Faster
Gryback et al. (1996) [361]	9 (mean BMI, 42)	21 (mean BMI 24)	360kcal, S	Sc	Faster
Naslund et al. (1998) [17]	9 (mean BMI, 42)	9 (mean BMI, 23)	280kcal, S	Sc	Faster
Verdich et al. (2000)[24]	19 (mean BMI, 39)	12 (mean BMI, 23)	597kcal, S	Sc	ND
Jackson et al. (2004) [29]	16 (mean BMI, 34)	16 (mean BMI, 23)	478kcal, S	BT	Slower
Mathus-Vliegen et al. (2005) [18]	45 (BMI, 28-43)	10 (BMI, 18-25)	410kcal, S,L	Sc	ND
Valera Mora et al. (2005) [159]	20 (BMI, > 30)	16 (BMI < 25)	-, S	Sc	Faster
Vasquez-Roque et al. (2006) [25]	24 obese (mean BMI, 30), 24 overweight (mean BMI, 28)	24 (mean BMI, 23)	296kcal, S,L	Sc	ND
Cardoso-Junior et al. (2007) [362]	14 (mean BMI, 47)	24 (mean BMI, 25)	-, S, SS.	BT	S, Faster. SS, ND
Bucholz et al. (2012) [363]	19 (mean BMI, 45)	20 (mean BMI, 26)	SS	Sc	ND

IBW, ideal body weight, ND = no difference, Sc = scintigraphy, BT = breath test, S = solid, L = liquid, SS = semisolid, - = information not provided in manuscript.

A further hypothesis may be that inconclusive findings are due to the influence of additional factors such as habitual physical activity and associated differences in body composition (fat and fat free mass) and energy expenditure. To date, BMI or percent of ideal body weight have been the major criteria for distinguishing obese and non-obese groups in studies investigating GE in obesity (see **Table 4.1**). However, individuals with similar BMIs could have quite different body compositions. For example, an active individual may have a greater lean mass than a sedentary individual despite a similar BMI. Only 2 studies could be found which have reported the body composition of subjects. Vasquez-Roque et al (2005)

comprehensively characterised gastric functions and hormone profiles in normal weight, overweight and obese individuals (BMI, 18-24.9, 25-29.9 and > 30kg/m^2 respectively) and reported a lean mass of 43, 44 and 50kg in each group respectively [25]. Although no significant differences were found between groups, increased body weight was associated with faster GE. In another cross sectional analysis, body size and composition were found to be the only determinants of GE in 45 males and females entering a weight reduction program [18]. A faster solid emptying was reported in taller subjects with high fat-free mass, and in subjects with more intra-abdominal fat. The findings of these studies suggest a possible relationship between GE and body composition. In the majority of studies showing faster GE in obesity in **Table 4.1**, the mean BMI is > 40kg/m^2 [17, 200, 361, 362]. Faster GE could potentially be related to a higher FFM in individuals with a BMI in this range compared to the overweight category because FFM increases in proportion to weight [37]. Further support to speculate that GE and FFM may be somehow related could be drawn from strong evidence of faster GE in males compared to females [16, 111, 361] and younger compared to older individuals [110]. Based on the collective evidence it could be hypothesised that a relationship exists between body composition (FM and/or FFM) and GE, yet further studies are needed to confirm this. Despite numerous studies examining the role of GE in obesity, body composition (FM and FFM) has received little investigation.

Differences in energy expenditure (EE) may also contribute to differences in GE, appetite and energy intake (EI). However, subjects' habitual physical activity levels (and total daily EE) are rarely considered when comparing GE in lean and obese individuals. Although it is intuitive that a relationship exists between EE and EI the association is complex. In the long term free-living situation a positive relationship between physical activity and EI has been observed [364-366], but below a certain activity level in what has been termed the sedentary range a decrease in activity is not followed by a decrease in food intake [10]. Recently, a series of publications has highlighted the role of resting metabolic rate (RMR; the largest component of daily EE) in appetite control [36-39]. RMR was positively associated with meal size, daily EI and hunger profiles across a day in overweight and obese individuals [38] strongly implying that RMR acts as a "driver for food intake" [37]. As an individual's RMR is quite consistent from day to day [367] the authors proposed that RMR provides a tonic signal for the drive to eat, thereby creating a

relatively constant energetic demand to maintain vital body functions [36]. Activity EE (AEE) on the other hand tends to vary from day to day and may exert a different mechanistic influence on appetite likely through episodic signals arising from the GI tract [37, 86]. As GE occurs in an episodic pattern in response to food intake, it could be speculated that if a mechanistic role for GE exists in the relationship between EE and EI, it is likely influenced by AEE. However, little research has examined the influence of RMR or habitual physical activity (and the associated increased EE) on GE.

Limited evidence suggests that GE may be faster in active compared to sedentary individuals but this relationship has been under examined. Carrio et al. (1989) [40] reported faster GE in 10 marathon runners compared to 10 sedentary individuals. In this study body surface area was the only proxy characteristic of body composition reported. GE was similarly reported to be accelerated in a small sample of 7 active elderly individuals compared to 7 inactive individuals [202]. Descriptors of body composition were not reported and the inactive group were older than the active group in this study. Furthermore, in both studies energy intake and appetite were not reported. Mechanisms proposed to explain a faster GE included a training induced predominant parasympathetic tone [40] and increased gastric electroactivity [202]. Physical activity (hence EE) might not be the only factor to vary between active and sedentary individuals. Dietary habits, including total EI [248], frequency of eating, eating behaviours (e.g. dietary restraint), food preferences, energy and non-energy fluid intake, processes of food reward and macronutrient intake could also vary. Collectively, these factors could affect GE via the quantity, frequency and composition of nutrients that pass through the gut and small intestine [368]. A high-fat/low-carbohydrate diet for example has been shown to attenuate the effects of fat on GE [369, 370]. Furthermore, gut adaptations observed in marathon runners may only have occurred after a long period of training at high intensity and volume or could be specific to running due to an adaptation to the mechanical effects of the "jostling of the gut" during running [231, 233, 237, 243]. Although these studies suggest that chronic exercise may lead to a faster GE, their methodological limitations mean that much remains to be learned about the effects of habitual exercise on GE and the implications for appetite and energy intake.

It is clear that an integrative relationship likely exists between body composition, exercise and EE in the control of EI (See Blundell et al. (2012) [37]). Yet, despite limited evidence suggesting increased physical activity is associated with faster GE [40, 202], information regarding physical activity levels of subjects is rarely provided in body composition studies examining GE [16-18, 23-25, 27-29]. Likewise, studies examining habitual physical activity and GE report limited information on body composition of subjects [40, 202]. A number of cross sectional studies have separately investigated the associations between physical activity and/or body composition (based on BMI) and GE, appetite control and food intake. A faster GE and therefore a shorter satiety period [115] may well represent a plausible mechanism for the increase in EI that has been observed in chronic exercisers [364-366]. However, this has not been investigated. Furthermore, how altered gut physiology with exercise relates to differences in subjective appetite sensations, EI, habitual diet, food preferences, resting and activity EE, processes of food reward, eating behaviour related traits (e.g. dietary restraint) and body composition is unknown. To the best of my knowledge there are no studies that have examined these parameters and measured body composition (fat and fat free mass) and physical activity. In light of this, the present cross-sectional study was undertaken to investigate the association of body composition, physical activity and EE with GE in active and sedentary males.

4.1.1 Aims

The aims of this study were to determine in active and sedentary males:

(i) the effect of habitual physical activity level and EE on GE

(ii) the effect of body composition on GE

(iii) homeostatic and hedonic features of appetite control, and their associations with GE

This knowledge is essential to further understand the mechanisms potentially mediating differences in EI with habitual exercise and to better understand processes involved in appetite control and weight regulation.

4.2 METHODOLOGY

4.2.1 Participants

Forty-four males were recruited to participate in the study through recruitment emails and flyers in the university and local area. All participants completed an initial screening questionnaire to assess their eligibility. Inclusion criteria were as follows: male, aged 18-55, BMI 18-40, weight stable (± 4 kg over last 6 months), no history of GI surgery or disorder, non-diabetic, no medical conditions known to influence any of the outcome measures and not taking any medication known to influence any of the study outcome measures, willing to consume study test meals, not a heavy smoker (<10 per day) and either sedentary (participating in 1 structured exercise session or less per week and not engaged in strenuous work) or active (participating in 4 or more structured exercise sessions per week) over the last 6 months. One exercise session was defined as at least 40 minutes of moderate to high intensity activity [249]. If participants' self reported physical activity levels were between categories they were excluded. Based on the standard deviations of the data presented in Chapter 3, a sample size of 22 participants per group was sufficient to detect at least a 10% difference between groups for three of the four outcome measures (t_{lag}, $t_{1/2}$, and t_{asc}), and a mean difference of 13 minutes in mean GE half time between groups with a power of 80% and a significance level of 5%. Ethical approval for the study was granted by Queensland University of Technology Research Ethics Committee and all participants provided written informed consent prior to taking part.

4.2.2 Design

Participants attended the laboratory on 2 separate test days one week apart (see **Figure 4.1**). At the first testing session, body composition, resting metabolic rate (RMR) and eating behaviour (e.g. dietary restraint) were measured. At the second test session, GE, subjective appetite sensations, food preferences and 'liking' and 'wanting' were assessed. Between the two testing sessions, participants were given an accelerometer to wear for 7 days and a 24-hour diet recall was taken on 3 separate days.

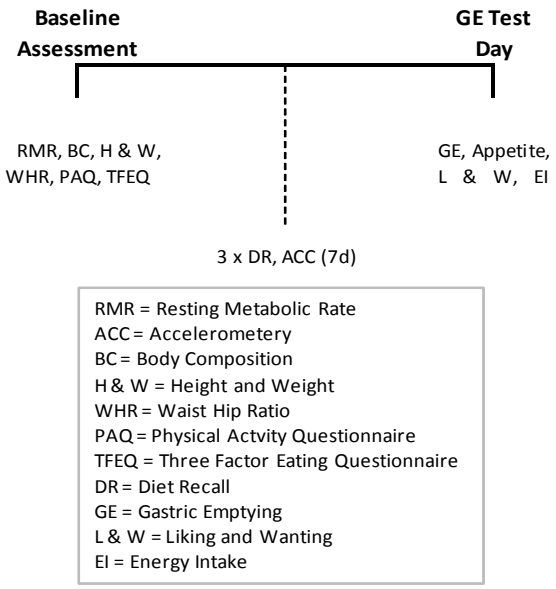


Figure 4.1 Schematic overview of Study 2 protocol

4.2.3 Baseline Measurements

Participants attended the laboratory after a 12-hour overnight fast, and having avoided alcohol and strenuous exercise for 24 hours. Measurements were taken in the following order:

4.2.3.1 Anthropometry and Body Composition

Height was measured without shoes to the nearest 0.5cm and weight to the nearest 0.01kg. Waist and hip circumferences were taken and body composition was measured using air displacement plethysmography (Bodpod, Concord, CA). Procedures were identical to those described in Chapter 3.

4.2.3.2 Three Factor Eating Questionnaire

Restraint, Disinhibition and Hunger were assessed using the Three Factor Eating Questionnaire (TFEQ) [330]. For a detailed description, refer to Chapter 3.

4.2.3.2 Resting Metabolic Rate

RMR was measured by indirect calorimetry using a ventilated hood system (TrueOne 2400 Metabolic Cart, ParvoMedics, Utah, USA). Before each measurement, the

system was calibrated for flow using a standard 3 litre calibration syringe, and for gas concentration using room air and a standardised gas of known concentration according to manufacturer instructions. During the RMR measurement the participant lay supine in a thermoneutral environment and resting oxygen uptake (Vo_2), carbon dioxide production (Vco_2) and the respiratory quotient (RQ; Vco_2/ Vo_2) were measured over 30 minutes. Resting heart rate was measured continuously using a heart rate monitor (Polar Electro Oy, Kempele, Finland). RMR was calculated using the Weir formula [371], as the average resting energy expenditure over the 10 minutes with the lowest coefficient of variation (CV) [367]. Similarly, RQ and resting heart rate were calculated as the average values over the same 10 minutes used to calculate energy expenditure.

4.2.3.3 Physical Activity and Energy Expenditure

Physical activity was monitored using a tri-axial GT3X accelerometer (Actigraph, Fort Walton Beach, FL, USA). Participants were provided with the activity monitor to wear for 7 days between the baseline assessment and the GE test day. This duration is estimated to result in 90% reliability for the measurement of physical activity [372]. In addition, participants were asked to record the time they put the accelerometer on and removed it in an activity diary, as well as any physical activity lasting 10 minutes or more during that day. The accelerometer was attached to an elastic belt and worn on the waist in line with the right hip. Participants were instructed to wear the accelerometer during waking hours and to only take the device off during contact with water (e.g. when showering or swimming). The GT3X is a portable lightweight activity monitor (27g; 3.8cm x 3.7cm x 1.8cm) and records accelerations ranging between 0.05 and 2.5Gs. Accelerations are measured in three individual orthogonal planes (vertical, antero-posterior and medio-lateral) and activity counts are provided as a composite vector magnitude of these three axes (VM3) [373]. The device samples accelerations at a rate of 30Hz, and the resultant analogue signal is digitised by a 12-bit analogue to digital converter. This digital output is then filtered using a band pass filter with a frequency range of 0.25-2.5 Hz (see Sasaki et al. 2011 [373] for a detailed description). Data were downloaded and processed using ActiLife software (version 6.4.5). VM3 counts were summed over 60 second epochs and levels of activity were defined as counts per minute using cut

point values in accordance with validated recommendations: light activity, 0 to 2690 counts·min^{-1}, moderate activity 2691–6166 counts·min^{-1}, vigorous activity 6167–9642 counts·min^{-1}, and very vigorous activity≥9643 counts·min^{-1} [373]. Data were checked for spurious data (counts per minute of >15,000). A non-wear period was defined as at least 90 minutes of consecutive zero counts without interruption [374]. Wear time exceeding 600 minutes was considered a valid day [375] and a valid dataset was considered a combination of at least 3 valid weekdays and 1 weekend day [376, 377]. Mean minutes per day of time spent in moderate and vigorous (combining vigorous and very vigorous) activity were calculated. Activity count data were converted to energy expenditure using the 'Freedson VM3 combination ('11)' option in Actilife software (version 6.4.5). Using this option, when the vector magnitude counts per minute (VMCPM) are less than 2453, the Williams Work-Energy equation:

$$Kcals/min=CPM\times0.0000191\times BM$$

is used and when VMCPM are greater than 2453, the Freedson VM3 ('11) formula:

$$Kcals/min= 0.001064\times VM + 0.087512(BM) - 5.500229$$

Where VM = Vector Magnitude Combination (per minute) of all 3 axes (sqrt((Axis 1)^2+(Axis 2)^2+(Axis 3)^2]), VMCPM = Vector Magnitude Counts per Minute, CPM = Counts per Minute and BM = Body Mass in kg,.

Mean activity energy expenditure (AEE) per valid day was calculated and mean total energy expenditure (TEE) per day was subsequently calculated in Microsoft EXCEL using the following formula:

$$TEE = (AEE + REE) \times 1.11$$

where AEE = activity energy expenditure, REE = resting energy expenditure, and the thermic effect of food is fixed at 10% of TEE [252].

4.2.3.4 Diet Recall

A multiple-pass 24-hour diet recall was conducted on 3 separate occasions including one weekend day during the week prior to the GE test to assess participants' habitual diet. The 24-hour recall aims to provide a complete record of all food and drink eaten on the previous day between midnight and midnight, and consists of three passes including 1) a quick list of food and beverages consumed, 2) a detailed description of

type, amount, cooking method and time of consumption; and 3) review of intake to report any items that may previously have been forgotten, state whether intake was typical and list any dietary supplements used. Foodworks Professional Edition dietary analysis software (Foodworks; Xyris Software, Highgate Hill, Queensland, Australia) was used to quantify total energy intake and macronutrient composition of the diet over the 24 hours prior to the GE test, as well as the average of the 3 separate days.

4.2.4 Gastric Emptying Test Day Measurements

The GE test day took place 7 days after the baseline assessment and followed an identical protocol to Chapter 3 (for an overview see **Figure 3.2** page 48**)**. Participants were instructed to refrain from vigorous exercise and alcohol for 24 hours prior to the test day. In addition, participants were instructed to avoid consumption of naturally ^{13}C-enriched foods (corn or corn products, pineapple, kiwi fruit, cane sugar and exotic fruits) for at least two days prior to the study and to fast for 12 hours overnight until coming to the Human Appetite Research Centre the following morning. One glass of water was allowed upon waking.

4.2.4.1 Gastric Emptying

GE parameters were calculated using the ^{13}C-OBT [293], using an identical procedure to that described in detail in Chapter 3. In brief, the egg yolk of a standardized pancake breakfast meal [1676 kJ (400 kcal); 15g (15%) PRO, 17g (37%) Fat, 48g (48%) CHO)] was labelled with 100mg ^{13}C-octanoic acid (Cambridge Isotope Laboratories, Andover, USA). Participants consumed the meal together with 250ml of water within 10 minutes. Breath samples were collected in 10ml glass Exetainer tubes (Labco, Buckinghamshire, UK) prior to the breakfast, immediately after, and subsequently at 15 minute intervals for 5 hours after breakfast. Participants remained in sedentary activities throughout.

4.2.4.1.2 ^{13}C breath test analysis

Procedures for ^{13}C breath and data analysis were identical to Chapter 3 (see page 49 for a detailed description), except two different procedures for predicting VCO_2 were

used in calculations. In brief, ^{13}C enrichment of breath samples was measured by isotope ratio mass spectrometry (Hydra 20-20) and compared to a reference gas (5% CO_2, 75% N_2, 20% O_2 calibrated with a standard of $^{13}CO_2$). Data were analysed according to Ghoos et al [293] and fitted to the original GE mathematical model by non-linear regression analysis. To calculate the cumulative percent of ^{13}C dose recovered and the percentage $^{13}CO_2$ recovery per hour, enrichment values were multiplied by the estimated total CO_2 production (VCO_2) for each individual. Resting VCO_2 was predicted from body surface area [332]. This is the most commonly used method of estimating VCO_2 in ^{13}C breath tests and assumes a constant value of 300 mmol CO_2 h^{-1} m^{-2} body surface area or 5 mmol min^{-1} m^{-2} for all individuals [333]. Body surface area was calculated from height and weight using the formula of Haycock et al. (1978) [334]. This procedure is identical to the study presented in chapter 3. As it is possible that VCO_2 predictions could influence the outcome, a constant value of directly measured VCO_2 calculated over the 10 minutes with the lowest CV during the RMR measurement was also used in a separate analysis to allow a comparison of GE times using both procedures. For both, the r^2 coefficient between the modelled and raw data was calculated and accepted if $r^2>0.9$. The conventional uncorrected time based parameters (t_{lag} and $t_{1/2}$) proposed by Ghoos et al.[293] and the parameters latency time (t_{lat}) and ascension time (t_{asc}) proposed by Schommartz et al. (1998)[303] were calculated.

4.2.4.2 Subjective Appetite Sensations

Subjective appetite sensations were measured throughout the test day using an electronic appetite rating system [338]. Participants were asked to rate feelings of hunger, fullness and desire to eat on 100 mm visual analogue scales, anchored at each end with the statements "not at all" and "extremely". Five hour postprandial area under the curve (AUC) was calculated using the trapezoidal rule. In addition, the satiety quotient (SQ) was calculated. The SQ relates the suppression of hunger, desire to eat or change in fullness to the amount of energy consumed. For a detailed description, see Chapter 3 page 52.

4.2.4.3 Food Preferences, 'Liking' and 'Wanting'

Food preferences and 'liking' and 'wanting' were measured on 3 occasions (pre-breakfast, post-breakfast and pre-lunch) during the test day using a computer-based

procedure - the Leeds Food Preference Questionnaire (LFPQ) [339]. An identical procedure to that described in detail in chapter 3 (page 53) was followed.

4.2.4.4 Ad libitum Energy Intake

At the end of the GE test, participants were provided with an *ad libitum* pasta lunch meal identical to that is described in chapter 3 (47% CHO, 35% FAT, and 18% PRO, and an energy content of 7.6kJ/g) and water and instructed to consume as much as they wished until comfortably full. The amount (g) of food consumed from the *ad libitum* meal was determined by weighing the meal before and after consumption and energy intake (kJ) was calculated.

4.2.5 Statistical Analysis

Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Differences between active and sedentary groups were assessed by t test and Mann-Whitney U test in the case of non-normally distributed data. To further explore the influence of certain characteristics including BMI, FFM, and percent FM on GE a separate analysis was undertaken by dividing groups according to median values of these variables. In addition, groups were split into quartiles for GE $t_{1/2,}$ t_{lag} and AEE and compared by independent t-test to determine differences in characteristics between the upper and lower quartiles. Pearson (or Spearman where appropriate) correlations were used to determine relationships between GE and key variables in the whole group of participants (n = 44) and separately in active (n = 22) and sedentary (n = 22) groups. Associations between GE $t_{1/2}$ and t_{lag} and variables of interest were further explored using partial correlations after controlling for group. To identify factors associated with GE, variables of interest were included in multiple linear regression analysis with GE $t_{1/2}$ and t_{lag} as the dependent variables. The variance inflation factor (VIF) was checked for multicollinearity between predictors. Statistical analysis was performed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL) and Graph Pad Prism version 6.0 for Mac (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $P < .05$ unless otherwise stated.

4.3 RESULTS

4.3.1 Participant Characteristics

All participants completed all parts of the study (n = 22 per group), except for the TFEQ (where the mean of the active group refers to n =21, due to an incomplete questionnaire) and physical activity assessment (where the mean of the sedentary group refers to n = 19 due to 3 invalid accelerometry data sets). No participants were elite athletes. Participants in the active group reported taking part in various types of physical activity including aerobic exercise, resistance training, field sports and combinations of different modes of exercise. Mean anthropometric, body composition, resting metabolism and eating behaviour characteristics for active and sedentary groups are shown in **Table 4.2** below**.**

Table 4.2 Participant anthropometric, body composition, resting metabolism and eating behaviour characteristics.

	Active (n = 22)	Sedentary (n = 22)	*P*-value
Age (years)	29.4 ± 7.8	30.4 ± 8.5	0.56
Height (m)	1.80 ± 0.07	1.78 ± 0.08	0.55
Weight (kg)	79.2 ± 11.7	87.1 ± 15.8	0.07
BMI (kg/m^2)	24.5 ± 2.6	27.4 ± 4.2	**0.02**
BSA (m^2)	1.99 ± 0.18	2.08 ± 0.22	0.13
Body composition			
Body Fat (%)	14.3 ± 5.8	26.2 ± 8.7	**<0.001**
FFM (kg)	67.7 ± 8.9	63.3 ± 8.2	0.10
Resting HR (bpm)	52.7 ± 8.5	64.1 ± 9.3	**<0.001**
Fasting RQ	0.73 ± 0.03	0.76 ± 0.05	**<0.01**
RMR (kcal/day)	1933 ± 244	1970 ± 340	0.68
TFEQ[1]			
Restraint	10.1 ± 3.5	6.1 ± 3.7	**<0.01**
Disinhibition	5.4 ± 2.4	5.1 ± 3.4	0.75
Hunger	6.4 ± 2.1	4.9 ± 3.6	0.11

Data are means ± SD. [1]TFEQ refers to n =21 in active group.
BMI, body mass index; BSA, body surface area; FFM, fat free mass; HR, heart rate; RQ, respiratory quotient; RMR, resting metabolic rate; TFEQ, Three Factor Eating Questionnaire.

For a breakdown of the spread of individual anthropometric and body composition characteristics in each group see **Appendix E**. As expected, given the study design significant differences were found between active and sedentary groups for a number

of characteristics (**Table 4.2**). The active group had a significantly lower BMI and percentage body fat, as well as a trend towards a lower weight and higher absolute fat free mass compared to the sedentary group. In addition, resting HR and RQ were significantly lower in the active group. RMR on the other hand was similar between the 2 groups (**Table 4.2**). With regard to eating behaviours, dietary restraint was the only factor which differed significantly between the 2 groups - the active group had a higher level of dietary restraint than the sedentary group (**Table 4.2**).

Physical activity (as assessed by accelerometry) and EI (as assessed by diet recall) were also significantly different between active and sedentary groups for a number of variables. Mean energy intake and physical activity descriptive characteristics for the two groups are shown in **Table 4.3** below**.**

Table 4.3 Participant energy intake and physical activity characteristics

	Active (n = 22)	Sedentary (n = 22)	*P*-value
24h EI (kJ/d)	2541 ± 719	2131 ± 593	**0.03**
% Energy from:			
CHO	44 ± 9	46 ± 10	0.36
PRO	22 ± 6	18 ± 4	**0.02**
Fat	31 ± 8	33 ± 10	0.50
3d EI (kcal/d)	2470 ± 591	2157 ± 551	0.09
% Energy from:			
CHO	43 ± 8	47 ± 7	0.11
PRO	22 ± 5	19 ± 3	**0.04**
Fat	33 ± 6	32 ± 7	0.54
Physical Activity[1]			
Steps per day	9495 ± 2834	7046 ± 2973	**0.02**
AEE (kcal/day)	709 ± 239	525 ± 185	**<0.01**
TEE (kcal/day)	2890 ± 430	2665 ± 413	0.09
Time in activity			
Vigorous (min/day)	14 ± 9	6 ± 6	**<0.01**
Moderate (min/day)	58 ± 24	44 ± 17	**0.04**

Data are means ± SD. [1]Physical activity data refers to n =19 in sedentary group.
EI, energy intake; CHO, carbohydrate; PRO, protein; AEE, activity energy expenditure; TEE, total energy expenditure.
24hr EI refers to EI in the 24hrs prior to the GE test. 3d EI refers to average EI over three separate days.

For the day prior to the GE test (reported as 24h EI), the active group had a significantly higher EI and a higher percentage of total EI coming from protein compared to the sedentary group. Similar trends were seen when these variables were averaged over 3 days (3d EI). For both 24h EI and 3d EI, the percentage of total EI

coming from CHO and fat were not significantly different between groups (**Table 4.3**). When comparing physical activity characteristics over the week prior to the GE test, the active group were significantly more active, as indicated by a higher number of steps, AEE and time spent in moderate and vigorous activity per day. In addition there was a trend towards a higher TEE in the active compared to sedentary group (**Table 4.3**).

4.3.2 Gastric Emptying

4.3.2.1 Influence of VCO_2 prediction method on GE parameters

To rule out any differences in GE calculations due to possible errors in VCO_2 predictions GE outcomes were compared using 2 different methods - a VCO_2 value for each individual as measured during the RMR measurement and using a value of predicted VCO_2 derived from the formula of Shreeve et al. [332]. VCO_2 was significantly overestimated in the active group using the Shreeve formula (measured VCO_2: 557 (76) v predicted VCO_2, 597 (54), $p < 0.001$), but the difference between predicted and measured values did not reach significance in the sedentary group (measured: 597 mmol CO_2 h^{-1} v predicted 624 mmol CO_2 h^{-1}, $p = 0.12$). For both groups the percentage dose recovered was greater when using the predicted versus measured VCO_2 value (active: measured 38.4 v predicted 42.1%, $p < 0.01$; sedentary, measured 39.6 v predicted 42.0%, $p = .04$). However, this difference did not influence the values for any of the GE parameters. As GE outcome measures were identical regardless of the method used to calculate VCO_2, the VCO_2 value as predicted using the Shreeve et al. formula [332] was used to determine the results reported in this study. The latter is the method most frequently used in ^{13}C-OBT methodology including the original validation protocol [293] and is consistent with the other studies presented in this thesis.

4.3.2.2 Comparison of GE in active and sedentary groups

Mean GE data for active and sedentary groups are presented in **Table 4.4**. These data indicate that GE was significantly faster in the active compared to sedentary group for all parameters.

Table 4.4 Gastric Emptying Parameters in Active and Sedentary groups (n = 22 per group).

	Active	Sedentary	*P*-value
t_{lag} (min)	95 ± 13 (76-119)	110 ± 16 (85-158)	<0.001
$t_{1/2}$ (min)	157 ± 18 (125-195)	179 ± 21 (139-231)	<0.001
t_{lat} (min)	30 ± 7 (22-46)	35 ± 9 (20-60)	0.01
t_{asc} (min)	127 ± 15 (101-162)	143 ± 19 (110-179)	<0.01

Data are means ± SD. Range is indicated in brackets.
$t_{1/2}$, half time; t_{lag}, lag time; $t_{1/2s}$, t_{asc}, ascension time; t_{lat}, latency time.

A plot of individual values of GE $t_{1/2}$ in the active and sedentary groups is shown in **Figure 4.2**. GE $t_{1/2}$ was significantly faster in the active compared to sedentary group (157±18 v 179±21 min, $p < 0.001$).

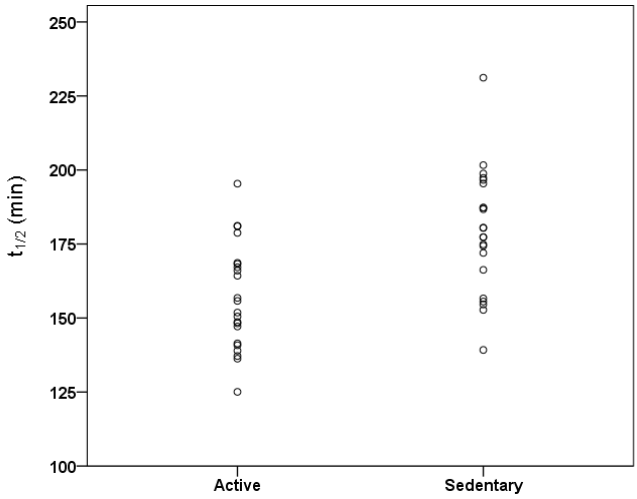


Figure 4.2 Gastric emptying half time ($t_{1/2}$) in individual participants in the active (n = 22) and sedentary (n = 22) groups.

Differences in GE between active and sedentary groups remained significant when both percent body fat and percent dietary energy intake from PRO were controlled for in covariate analyses (GE $t_{1/2}$, $p < 0.01$, GE t_{lag}, p 0<.01, for both). A subanalysis of the active and sedentary groups categorised as lean (BMI: 18 – 25

kg/m^2) sedentary and lean active, overweight/obese (BMI: 27 – 34 kg/m^2) sedentary and overweight/obese active was also undertaken. Although, a robust analysis was limited due to the small sample sizes (n = 6) in two of the sub groups, there was less than three minutes mean difference in GE half time between lean sedentary (n = 6) and overweight sedentary (n = 10) individuals. Similarly, there was less than three minutes difference in mean GE half time between overweight active (n = 6) and lean active (n = 16) individuals. However GE half time was 27 minutes shorter in the lean active versus lean sedentary group, and 24 minutes shorter in the overweight active versus lean active subgroup (**Table 4.5**), indicating GE was faster in both active subgroups.

Table 4.5 Body Composition and GE characteristics of 4 subgroups categorised as lean (BMI: 18 – 25kg/m^2) and overweight/obese (BMI: 27 – 34 kg/m^2) sedentary and active.

	Sedentary		Active	
	Lean (n = 6)	Overweight (n = 10)	Lean (n = 16)	Overweight (n = 6)
Age (y)	33.3 (14.6)	28.0 (4.6)	28.3 (3.5)	32.3 (11.5)
BMI (kg/m^2)	22.3 (1.0)	30.2 (5.0)	23.1 (1.1)	28.2 (1.5)
FFM (kg)	59.0 (8.5)	65.8 (7.7)	65.5 (7.9)	73.5 (10.4)
FM (kg)	11.9 (4.5)	29.9 (12.9)	9.1 (2.7)	18.0 (7.7)
FM (%)	16.9 (6.3)	31.4 (7.9)	12.3 (3.9)	19.4 (7.3)
GE $t_{1/2}$ (min)	183.3 (16.6)	180.7 (22.9)	156.0 (18.8)	158.9 (16.0)
GE t_{lag} (min)	110.1 (15.4)	113.7 (18.7)	93 (11.3)	99.8 (15.9)

BMI, body mass index; FFM, fat free mass; FM, fat mass; BF, body fat; GE $t_{1/2}$, Gastric Emptying half time; GE t_{lag}, Gastric emptying lag time.

4.3.2.3 GE $t_{1/2}$ in Groups Split by Median Body Composition Values

In addition to comparing GE between groups according to physical activity category (sedentary v active), a comparison of groups divided by the median of BMI (25kg/m^2), percent body fat (20%) and FFM (67kg) for GE $t_{1/2}$ is shown in **Figure 4.3**. GE $t_{1/2}$ was significantly faster in the group with a percent body fat (BF) below the median (< 20%) and significantly faster in the group with an absolute fat free mass (FFM) above the median (> 67kg). These data indicate GE was faster in individuals with a higher absolute FFM and also in individuals with a lower percent body fat. However, no statistically significant differences were observed when GE $t_{1/2}$ was compared in groups above and below the median BMI (**Figure 4.3**).

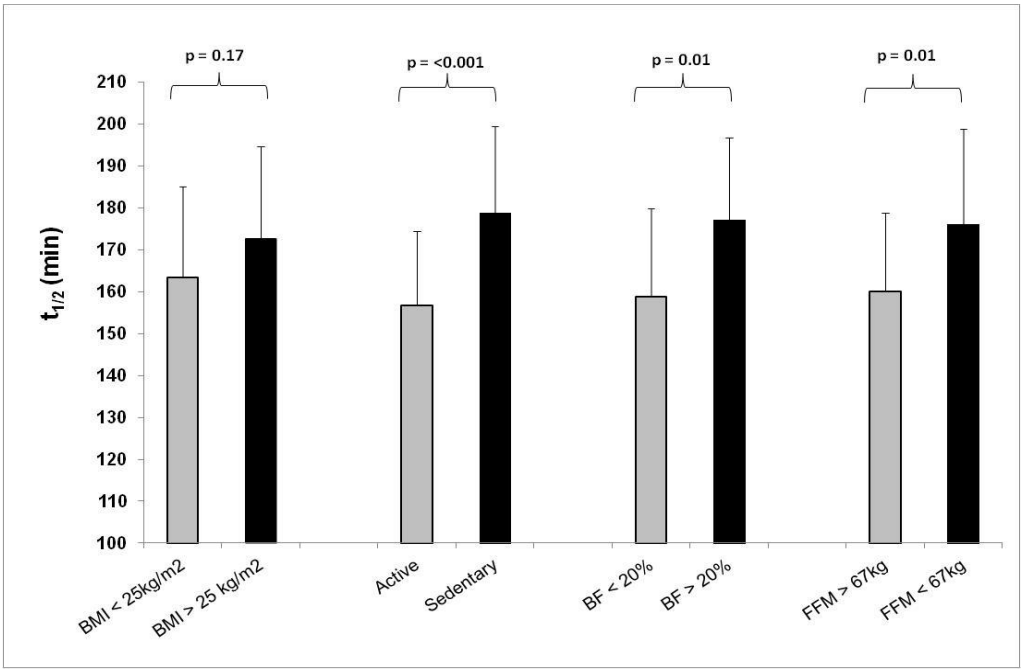


Figure 4.3 A comparison of GE $t_{1/2}$ in groups divided according to the median of BMI (25kg/m^2), activity level category (active or sedentary), the median of percentage body fat (BF) (20%) and the median of fat free mass (67kg). n = 22 per group for all categories. Error bars indicate SD.

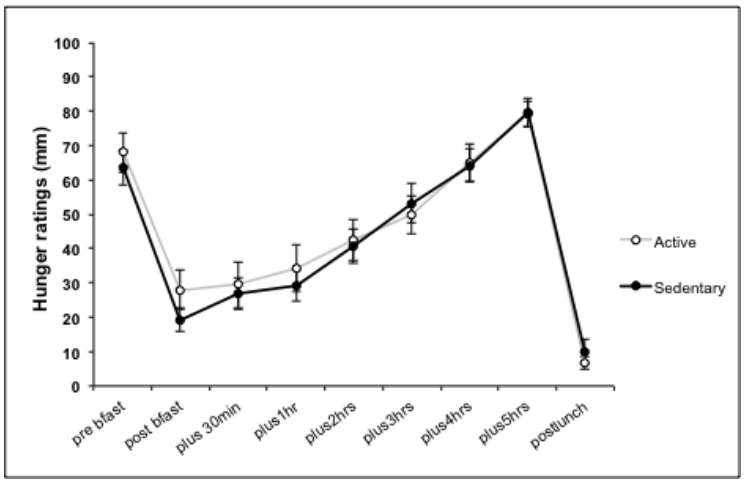
To test for any influence of cumulative percent ^{13}C dose recovered or RQ on outcomes [378], these parameters were compared between groups. There were no significant differences in cumulative percent dose recovered between groups when compared by physical activity level, or when divided by median BMI or median FFM ($p>0.05$). When divided by median percent BF, mean cumulative percent dose recovered was significantly higher in the group with a percent BF above the median compared to the group below the median (43 vs 41%, $p = 0.04$). There were no significant differences in RQ between groups when divided according to median FFM (FFM <67kg, RQ = 0.75 vs FFM >67kg, RQ = 0.74, $p = 0.74$). When divided by median percent BF and BMI, RQ was significantly higher in the group with a BF above the median (BF <20%, RQ = 0.73 vs BF >20%, RQ =0.76, p = <.01) and in the group with a BMI above the median (BMI <25kg/m^2, RQ = 0.73 vs BMI >25kg/m^2, RQ =0.76, $p < 0.01$). Adjusting for RQ did not influence the outcomes for any comparisons between groups. The difference in GE $t_{1/2}$ between groups divided by median BMI remained insignificant after adjusting for RQ ($F(2,41) = .39$, $p = 0.53$). Similarly the difference in GE $t_{1/2}$ between active and sedentary groups remained significant ($F(2,41) = 9.13$, $p < 0.01$) and the difference in GE $t_{1/2}$ between groups divided by median percent BF remained significant ($F(2,41) = 4.26$, $p = 0.04$)

after adjusting for RQ. Therefore any differences in RQ between groups did not significantly influence the differences in GE between groups shown in **Figure 4.3**.

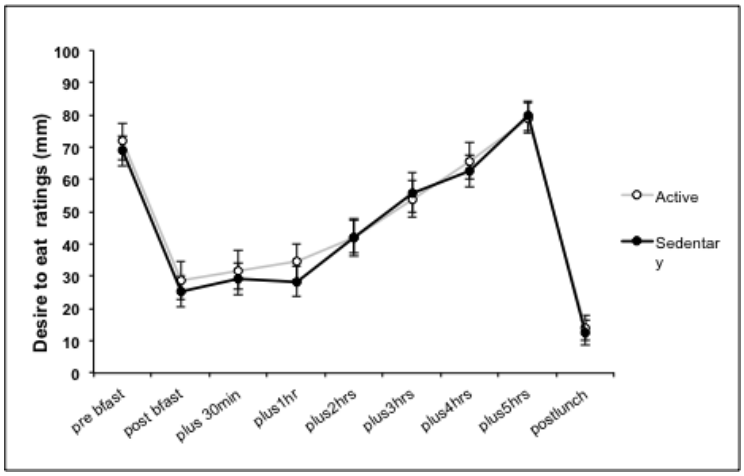
4.3.3 VAS Ratings

Subjective appetite ratings did not differ significantly between active and sedentary groups for fasting, mean 5h, 5h AUC and breakfast satiety quotient ($p > 0.05$, **Figure 4.4**). In addition, there were no significant differences between groups for ratings of alertness or for palatability ratings of the breakfast and lunch meals ($p > 0.05$, see Appendix F).

a)



b)



c)

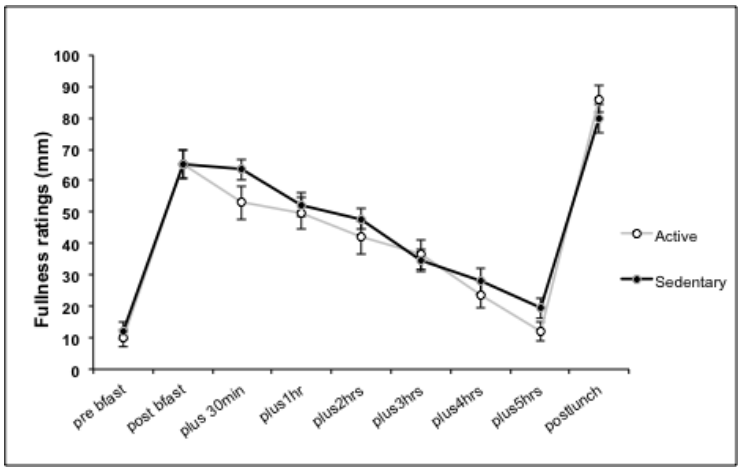


Figure 4.4 Mean (± SEM) subjective ratings for a) hunger, b) desire to eat and c) fullness in active and sedentary groups over the course of the GE test morning. n = 22 per group.

4.3.4 Ad libitum EI

The active group had a significantly higher EI at the *ad libitum* lunch meal (4946 ± 1254 kJ vs 3241 ± 885 kJ, p = 0.04) and took slightly longer to eat the meal (13.7 ± 2.9 vs 11.7 ± 4.0 minutes, p = 0.07). The amount of water consumed was not significantly different between groups (253 ± 113 vs 264 ± 109ml, p = 0.74, in active and sedentary groups respectively).

4.3.5 Food Preferences, 'Liking' and 'Wanting'

The active group had a greater preference for low fat savoury (LFSA) foods compared to the sedentary group at all time points (pre breakfast, p = 0.01; post breakfast, p = 0.03; pre lunch, (p = 0.01) (**Figure 4.5**). No significant differences were found between active and sedentary groups for other food categories. However, there was a trend towards a greater preference for high fat sweet (HFSW) foods prior to lunch in the sedentary group (p = 0.06).

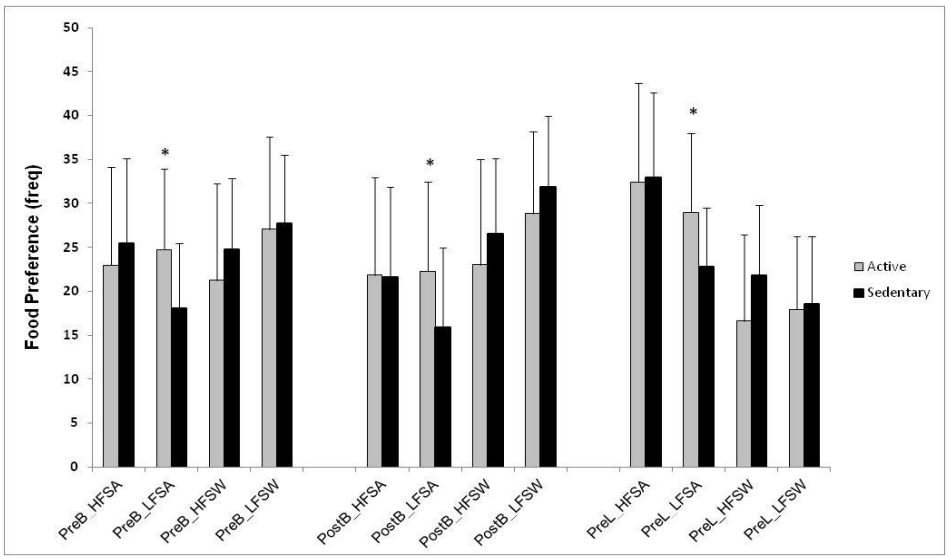


Figure 4.5 A comparison between active (n = 22) and sedentary (n = 22) groups of mean food preferences pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Food preferences were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$.

For ‘liking’ ratings, the sedentary group had a higher ‘liking’ for LFSW foods post breakfast compared to the active group ($p = 0.03$, **Figure 4.6**). In addition, there was a trend towards a significantly greater ‘liking’ for HFSW foods post breakfast ($p = 0.10$) and a lower ‘liking’ for LFSA food prior to lunch ($p = 0.10$) in the sedentary compared to active group.

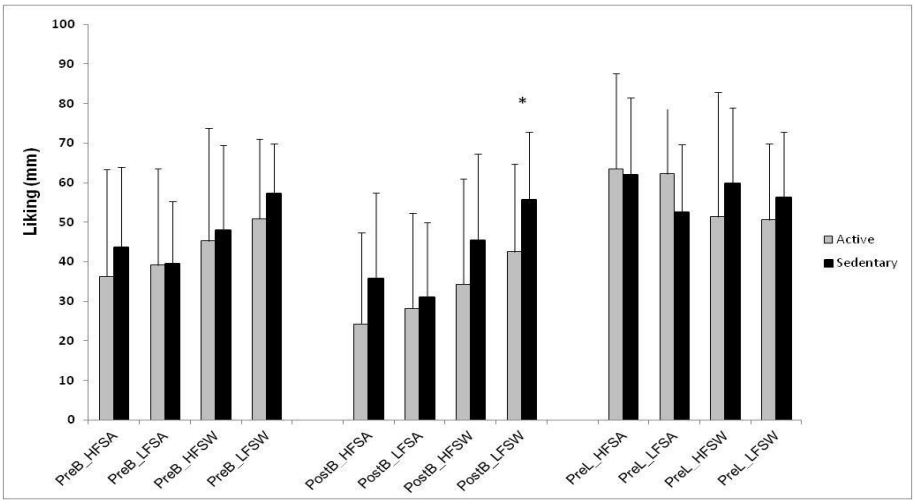


Figure 4.6 Mean ‘liking’ for different foods in active (n = 22) and sedentary (n = 22) groups assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$.

For ‘wanting’, the sedentary group had a greater ‘wanting’ for LFSW foods immediately post breakfast ($p = 0.02$) and a trend towards a greater ‘wanting’ for HFSA foods post breakfast compared to the active group ($p = 0.06$) (**Figure 4.7).**

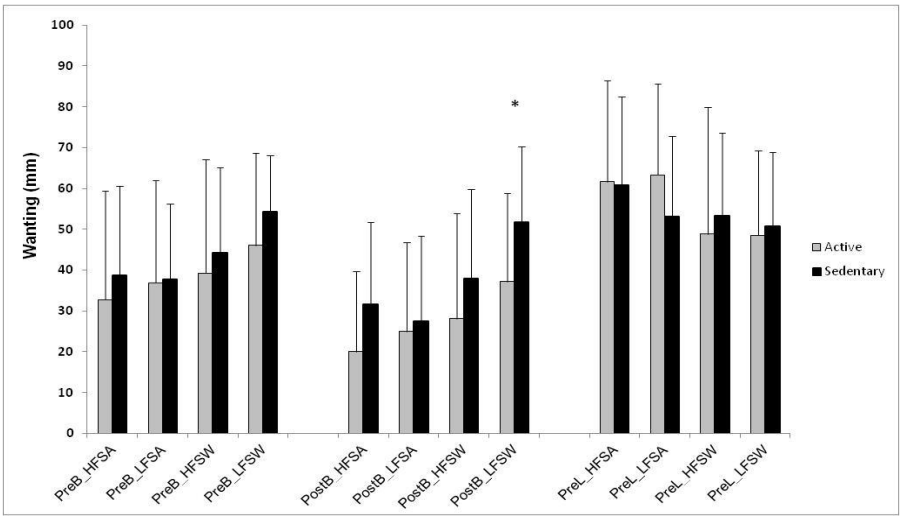


Figure 4.7 Mean ‘wanting’ for different foods in active (n = 22) and sedentary (n = 22) groups assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$.

4.3.6 Analysis of Groups by Quartiles

4.3.6.1 Characteristics of GE $t_{1/2}$ and GE t_{lag} upper and lower Quartiles

To further examine characteristics associated with differences in GE between individuals, the whole group (n = 44) were divided according to quartiles of GE $t_{1/2}$ and t_{lag}. When the upper and lower quartiles for GE $t_{1/2}$ (quartiles 1 and 4 shown in **Figure 4.8**) were compared, individuals in the lower quartile (reflecting a faster GE) had a significantly lower percent body fat, lower resting heart rate, lower RQ, higher dietary restraint and higher amount of physical activity, AEE and TEE compared to individuals in the upper quartile (reflecting the slowest GE) (see Appendix G, page 228). These differences remained significant when excluding the outlier in the upper quartile. Mean 5 hour and AUC fullness ratings were also significantly higher in the upper compared to lower quartile ($p < 0.01$ for both).

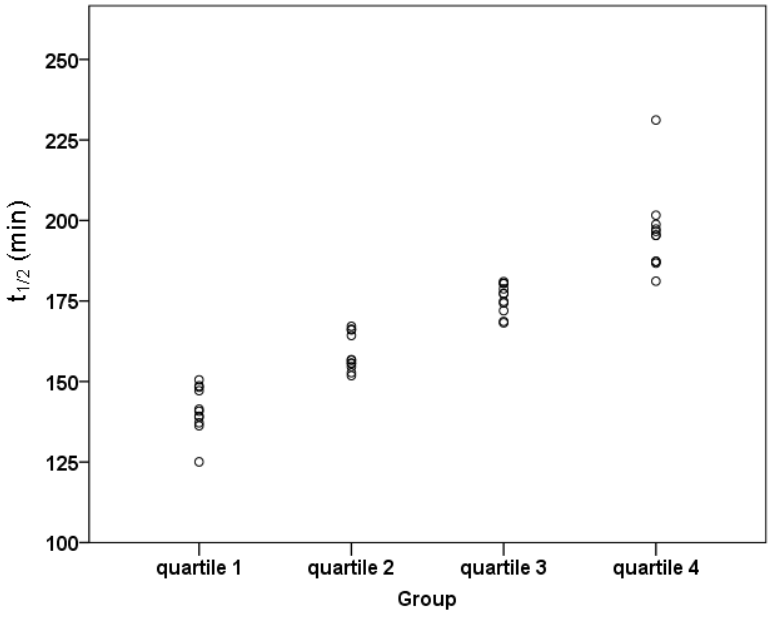


Figure 4.8 Plot of GE $t_{1/2}$ in individuals divided according to quartiles of GE $t_{1/2}$. n = 11 per quartile.

Similar differences were seen when comparing upper and lower quartiles of t_{lag} showing that individuals with a faster initial GE had a lower percent BF ($p < 0.01$), lower RQ ($p = 0.01$), lower RHR ($p < 0.01$), higher restraint ($p < 0.01$), higher physical activity (steps, average time in moderate and vigorous activity), AEE and TEE ($p < 0.05$ for all), compared to individuals in the upper quartile. Similar to $t_{1/2}$, mean 5 hour and AUC fullness ratings were significantly higher in the upper

compared to lower quartiles for t_{lag} ($p < 0.01$ for both), indicating individuals with the slowest initial GE had a greater fullness between breakfast and lunch.

4.3.6.2 GE characteristics of Activity Energy Expenditure (AEE) upper and lower quartiles

To further examine the effect of physical activity on GE, a similar analysis was undertaken to explore the differences in characteristics when analysing participants according to upper and lower quartiles of AEE. The upper quartile (reflecting those with the highest levels of AEE) had a higher absolute FFM and RMR and a faster initial and subsequent rate of GE compared to the lowest AEE quartile (see Appendix H, page 229). These data indicate that individuals with the highest AEE in the week prior to the GE test had a significantly faster GE than individuals with the lowest AEE.

4.3.7 Relationships between Variables and determinants of GE

4.3.7.1 Simple Correlation Analysis between Variables

To examine relationships between variables of interest, Pearson (or Spearman where appropriate) correlations were first undertaken among the group as a whole (n=44) and subsequently within each group separately (i.e. active (n=22) and sedentary (n=22)). To correct for multiple comparisons in the simple correlation analyses, only results with statistical significance at $p \leq 0.01$ are discussed. Despite the discrete nature of the 2 groups in terms of self reported habitual physical activity category (i.e. sedentary or active), it was evident that participants characteristics fell across a continuum when characteristics of the whole group were plotted (see Appendix D page 224**).**

4.3.7.1.1 Age

Age was positively correlated with t_{lat} ($r = .428$, $p < 0.01$) in the group as a whole.

4.3.7.1.2 Body composition

BMI, height and weight were not significantly associated with any GE variables in the group as a whole. t_{lag} was positively correlated with percent BF (r = .50, p = <.01) and absolute FM (r = .46, p < 0.01). t_{lat} was also positively correlated with percent BF (r = .47, p < 0.01) and absolute FM (r = .46, p < 0.01). $t_{1/2}$ was positively correlated with percent BF (r = .39, p < 0.01). Collectively, these data suggest that GE was faster in individuals in individuals with a lower absolute FM and in individuals with a lower percent BF. When analyses were undertaken in the separate groups no body composition variables were significantly correlated with GE (p > 0.01).

Body composition variables were not associated with 3 day average EI or *ad libitum* EI in the pooled or separate group data (p > 0.01).

4.3.7.1.3 RHR, RMR and RQ

In the group as a whole, RHR was positively correlated with t_{lag} (r=.37, p=.01), indicating initial GE was faster in individuals with a lower RHR. RQ was also positively correlated with t_{lag} (r = .41, p < 0.01), $t_{1/2}$ (r = .42, p < 0.01) and t_{asc} (r = .34, p = 0.03) and t_{lat} (r = .31, p = 0.04). In addition, a higher fasting RQ was associated with a higher absolute FM (r = .48, p < 0.01) and percent BF (r = .47, p = < 0.01). RQ had no association with the cumulative percent ^{13}C dose recovered (r = -.04, p = 0.80). There were no significant relationships between RMR and any of the GE or appetite variables in the group as a whole.

When relationships were assessed within groups, t_{lat} was negatively correlated with RMR (r = -.53, p = 0.01) in the active group, indicating active individuals with a higher RMR had a faster initial rate of GE. In the active and sedentary groups analysed separately, RQ and RHR were not associated with any GE variables.

4.3.7.1.4 Eating Behaviour, Appetite and Ad Libitum Test Meal EI

When all data were pooled, dietary restraint was negatively correlated with $t_{1/2}$ (r = -.38, p = 0.01). No significant relationships were found between disinhibition or hunger and GE variables. In addition, no significant associations between eating behaviour and GE were observed when the two groups were analysed separately.

Fasting hunger, fullness and desire to eat ratings were not associated with any GE variables or EI at lunch. AUC fullness was positively associated with t_{lag} (r = .38, p = 0.01) and $t_{1/2}$ (r = .41, p < 0.01), in the pooled data. When the groups were analysed separately, in the active group, AUC fullness was positively correlated with t_{lag} (r = .55, p < 0.01) and $t_{1/2}$ (r = .55, p < 0.01, **Figure 4.9**). Mean 5 hour fullness ratings were similarly positively correlated with t_{lag} (r = 52, p = 0.01) and $t_{1/2}$ (r = .53, p = 0.01). Taken together these findings suggest that a slower GE was associated with greater fullness between breakfast and lunch in active individuals. In contrast in the sedentary group, there were no significant correlations between subjective appetite ratings and GE. The correlation of fullness AUC ratings and GE $t_{1/2}$ in the sedentary group is shown in **Figure 4.10**.

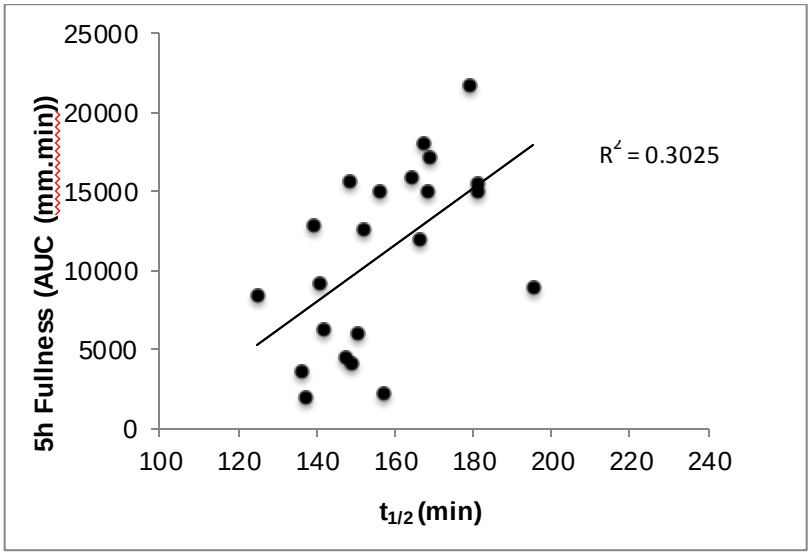


Figure 4.9 Scatter plot of the relation between gastric emptying half time ($t_{1/2}$) and 5h fullness AUC (Area Under Curve) ratings in **active males** (r = .55, p < 0.01) indicating a slower GE is associated with greater postprandial fullness. n = 22.

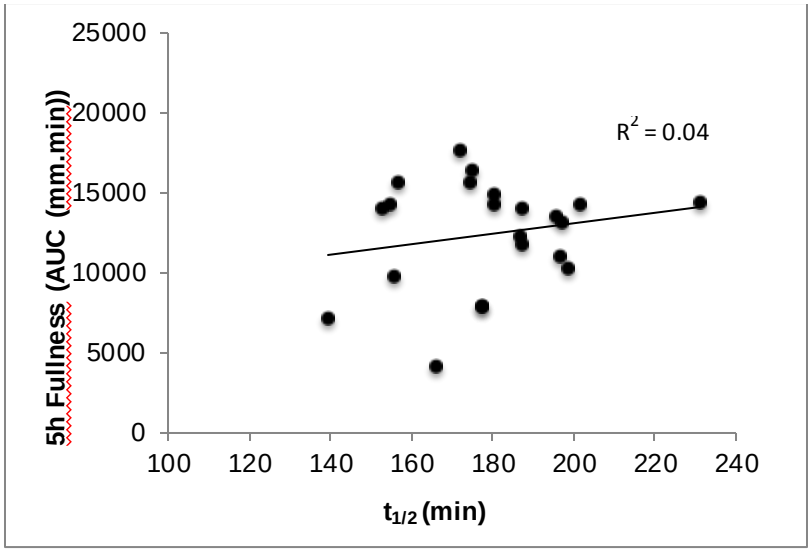


Figure 4.10 Scatter plot of the relation between gastric emptying half time ($t_{1/2}$) and 5h fullness AUC (Area Under Curve) ratings in **sedentary males** (r = .20, p = 0.38), indicating no association between GE and postprandial fullness. n = 22.

In the whole group, there were no significant associations between EI at the *ad libitum* meal and GE. However EI at lunch was associated with a number of appetite ratings (mean 5h hunger (r = .59, p < 0.001), mean 5h desire to eat (r = .61, p < 0.001), AUC hunger (r = .56, p < 0.001) and AUC desire to eat (r = .58, p < 0.001). These relationships indicate a greater hunger and desire to eat between breakfast and lunch was associated with a greater EI at the *ad libitum* lunch meal. When the separate groups were considered, higher lunch EI was associated with a lower intake of fat in the 24 hours prior to the GE test in the active group (r = -.58, p < 0.01). In the sedentary group no GE variables were associated with EI.

4.3.7.1.5 Dietary Intake, Food Preferences, 'Liking' and 'Wanting'

In the group as a whole, no dietary intake, food preference or 'liking' and 'wanting' variables as assessed individually at the three separate time-points were associated with GE.

4.3.7.1.6 AEE, TEE and Physical Activity

AEE was negatively correlated with $t_{1/2}$ (r = -.46, p < 0.01, **Figure 4.11**) in the whole group. Average time spent in vigorous activity per day was also negatively correlated with t_{lat} (r = -.50, p < 0.01), t_{lag} (r = -.53, p < 0.01) and $t_{1/2}$ (r = -.46, p = <

0.01). Similar negative correlations were observed between average time in moderate activity per day and GE variables (t_{lag}, r = -.42, p < 0.01; $t_{1/2}$, r = -.41, p < 0.01) and between the sum of moderate and sum of vigorous activity and GE variables (r = -.38 to r = -.43), p < 0.01 to p = 0.01). These correlations collectively indicate a higher amount of time spent in physical activity and a higher amount of energy expended in activity were associated with a faster GE. With regard to TEE, no significant associations were observed between GE and TEE.

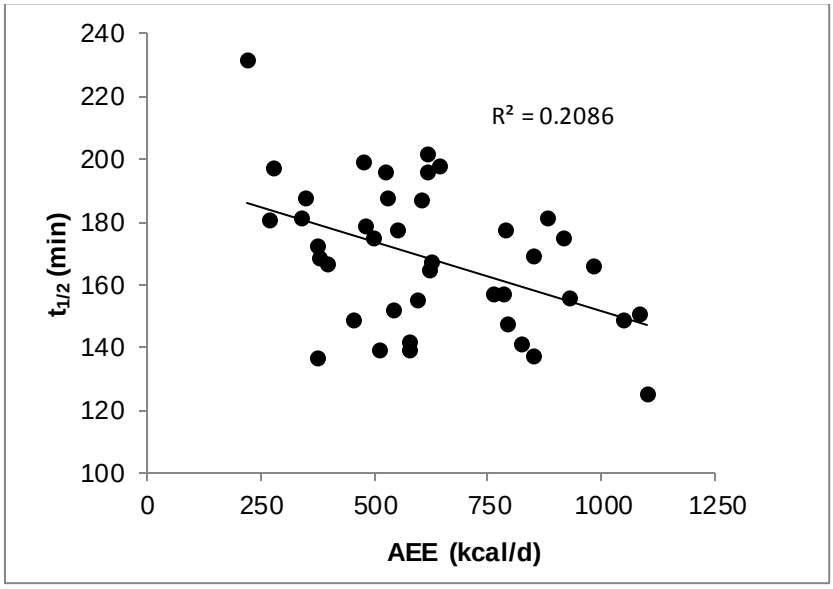


Figure 4.11 Scatter plot of the relation between the activity energy expenditure (AEE) and gastric emptying half time ($t_{1/2}$) (r = -.46, p < 0.01). n = 41.

In addition, no significant associations between EE and GE were observed when the two groups were analysed separately.

4.3.7.2 Partial correlations controlling for activity

Partial correlations of relevant variables with GE in the combined cohort (n = 44) were performed after controlling for group (i.e. active or sedentary) (**Table 4.6**).

Table 4.6 Partial correlations of age, body composition, resting metabolism variables and dietary restraint with GE t_{lag} and $t_{1/2}$ after controlling for activity group (sedentary or active)

	t_{lag}		$t_{1/2}$	
	r	p	r	p
Age	**.41***	**<.01**	.19	.21
BMI	.03	.86	-.05	.77
Body Fat (%)	.15	.34	.04	.80
FFM (kg)	-.21	.17	-.19	.23
Waist circumference	.07	.64	-.06	.70
RMR	-.22	.15	-.26	.09
RHR	.07	.67	.04	.77
RQ	.17	.29	.19	.22
AEE	**-.35***	**.03**	**-.31***	**.05**
TEE	-.30	.06	**-.31***	**.05**
Dietary Restraint	-.11	.48	-.18	.26
24h EI	-.18	.24	-.04	.78

AEE, activity energy expenditure, FFM, fat free mass, HR, heart rate, RQ, respiratory quotient, RMR, Resting metabolic rate, TFEQ, three factor eating questionnaire, $t_{1/2}$, half time; t_{lag}, lag time; t_{asc}, ascension time; t_{lat}, latency time.
n = 44, except for Dietary Restraint (n = 43) and AEE and TEE (n = 41).

The results of these analyses show that the significant associations between adiposity, RQ, dietary restraint and GE that were evident after a simple correlation analysis disappeared after correction for group. The positive correlation between age and t_{lag} however remained significant and the negative correlation between AEE and GE also remained significant after controlling for group (**Table 4.6**).

4.3.7.3 Multiple regression analysis

Based on relationships evident in the simple correlation analyses, FFM, percent BF, age, RQ, group (sedentary or active) and AEE were considered in separate multiple regression analyses with GE $t_{1/2}$ and t_{lag} as the dependent variables. Activity (i.e. active or sedentary) was a significant independent predictor of GE (Model adjusted R^2: .25, $p < 0.01$, $\beta = -.51$). When included in the same model as activity, FFM (kg) was not a significant predictor of GE $t_{1/2}$ ($\beta = -.16$, $p = 0.23$). When considering age, percent BF, activity and RQ as independent variables, activity was the only significant predictor of GE $t_{1/2}$ (**Table 4.7**).

Table 4.7 Regression model for the relation between GE $t_{1/2}$ and age, percent body fat, activity and respiratory quotient. n = 44.

Model	B	β	p	Model Adjusted R^2
GE $t_{1/2}$			<.01	0.24
Independent Variable				
Intercept	129			
Age	0.38	0.14	0.31	
BF (%)	-0.06	-0.03	0.88	
Activity[1]	-19.98	-0.46	0.01	
RQ	78.41	0.16	0.33	

GE $t_{1/2}$, gastric emptying half time; BF, body fat; RQ, respiratory quotient.
[1]Activity refers to group i.e. sedentary or active.

In addition to activity, AEE was a significant independent predictor of GE $t_{1/2}$ (β = -.40, $p < 0.01$). As there was no evidence of strong multicollinearity between AEE and activity (group) (VIF: 1.2) they were included in the same model. Together AEE and activity accounted for the greatest variance – 34% of GE $t_{1/2}$ (model adjusted R^2, .34, $p < 0.001$; activity: **β**, -.45, $p < 0.01$; AEE: **β**, -.28, $p = 0.05$).

For t_{lag}, activity and AEE together explained 31% of the variance (model adjusted R^2, .31, $p < 0.001$; activity: **β**, -.37, $p = 0.01$; AEE: **β**, -.33, $p = 0.03$). Similar to $t_{1/2}$, percent BF, FFM and RQ were not significant predictors of t_{lag} when included in the model. However, including age in the same model, increased the model adjusted R^2 to .38 (**Table 4.8**). Therefore, 38% of the variance in GE t_{lag} could be explained by age, activity and AEE.

Table 4.8 Regression model for the relation between GE t_{lag} and age, activity and AEE. n = 44.

Model	B	β	p	Model Adjusted R^2
GE t_{lag}			<.01	0.38
Independent Variable				
Intercept	116			
Age	0.60	0.29	0.03	
Activity[1]	-12.50	-0.37	0.01	
AEE (kcal/d)	-0.02	-0.26	0.07	

GE t_{lag}, gastric emptying lag time; AEE, activity energy expenditure
[1]Activity refers to group i.e. sedentary or active.

4.4 DISCUSSION

GE has long been implicated in the pathogenesis of obesity but with inconclusive findings. The influences of habitual physical activity level, body composition and EE on GE however are often overlooked and may explain some of this inconsistency. The present study aimed to compare GE in active and sedentary males, to further explore the influence of EE and body composition on GE and to characterise a number of factors which may account for potential differences in GE and eating behaviour between active and sedentary individuals. The major finding from the present study is that GE is significantly faster in active compared to sedentary males.

4.4.1 Physical Activity and GE

The present data add to previous work by demonstrating that faster GE is evident in habitual exercisers (participating in a range of activities) compared to sedentary individuals. Only two studies have previously investigated GE in active and sedentary individuals [40, 202]. The study by Carrio and colleagues (1989) [40] demonstrating faster GE in marathon runners compared to sedentary individuals is most frequently cited as evidence that physical activity level influences GE. GE was similarly previously reported to be faster in active compared to inactive elderly individuals [202]. However, limited descriptors of body composition were given in both studies, the sample sizes were small (n = 10 [40] and n = 7 [202] per group respectively) and age was not matched between the 2 groups in the latter study [202]. No other studies could be found which have investigated the effects of habitual exercise on GE. In contrast the present study included a larger sample size of active and sedentary males (n = 22 per group), age was similar between the 2 groups and body composition and associated characteristics that may differ between active and sedentary individuals were characterised. In addition, participants were involved in different modes of activity including resistance training, aerobic exercise and field sports. The findings therefore indicate that faster GE is a marker of an active lifestyle in males and is not limited to marathon runners [40].

Mechanisms previously proposed to contribute to differences in GE included enhanced parasympathetic tone [40] and gastric electroactivity [202]. Gastric electroactivity was not measured in the present study but has previously been shown

to be reduced in sedentary compared to active elderly subjects and accompanied by a slower GE [202] suggesting gastric myoelectrical activity may similarly have been reduced in the sedentary group in the present study. The parasympathetic nervous system can significantly influence gastric motility [244] and exercise training is known to improve vagal power [379]. Although many measures of autonomic nervous system function have been described the simplest to obtain is resting heart rate (HR) [380]. In the present study, the active group had a significantly lower resting HR which is consistent with higher levels of parasympathetic nervous system tone [380]. These findings support the hypothesis of Carrio et al. [40] that a training induced enhanced parasympathetic tone may be one mechanistic explanation for the faster GE observed in active individuals.

Differences in dietary intake, composition and eating behaviour may also contribute to differences in GE between active and sedentary individuals. Harris et al. [248] reported rapid orocecal transit time in active individuals with concomitant high EIs, and concluded that the high EI associated with chronic exercise may be associated with significant GI adaptations. In the present study, dietary intake was assessed by 24hr dietary recall on 3 occasions. On the day prior to the GE test, total EI was significantly higher in the active compared to sedentary group. Therefore, it is possible that faster GE in the active group was due in part to an adaptation to a higher habitual EI. However, the causal nature of this association is not possible to determine from these cross-sectional studies. With regard to macronutrient composition, the percentage energy coming from protein was significantly higher in the active compared to sedentary group but no differences were observed for other macronutrients. A 14 day high fat diet in humans and a 14 day high protein diet in rats have both been previously demonstrated to accelerate GE due to desensitised duodenal and CCK receptors [369, 381, 382] suggesting a similar mechanism could contribute to the present findings. However, others have shown no influence of a high protein diet on GE over 14 days in healthy adults [383]. However, the differences in % EI coming from protein were small in the present study (active, 22% v sedentary, 19%), and controlling for % EI from PRO did not alter the findings, suggesting that differences in macronutrient composition of the diet were unlikely to have played a major role in the differences in GE observed between the active and sedentary individuals.

In addition to differences in physical activity and habitual diet, eating behaviour differed between the active and sedentary groups. The active group had higher levels of dietary restraint compared to the sedentary group. No previous studies could be found which have investigated the effects of dietary restraint on GE. Some studies have shown a positive correlation between ghrelin and dietary restraint [384] whereas others have shown no association [71, 385]. This inconsistency is probably due to a lack of adjusting for disinhibition which has previously been shown to be related to ghrelin [71] and CCK levels [386]. These findings imply that restraint alone is unlikely to influence the release of appetite related hormones [387]. Given no differences in disinhibition between the active and sedentary groups in the present study, restraint may not therefore have influenced GE. However, the present findings are important to acknowledge as they highlight that in addition to physiological processes, a higher dietary restraint likely has a role in the control of habitual EI in active individuals.

Differences in GI hormones, blood glucose levels and insulin sensitivity may also contribute to differences in GE between active and sedentary individuals. Although these measures were not reported in the present study, previous findings of higher fasting ghrelin levels in active compared to sedentary adolescent girls [388] and higher acylated ghrelin levels after 12 weeks of exercise training [86] indicate fasting ghrelin levels could differ between active and sedentary individuals. Fasting ghrelin levels have been shown to be negatively correlated with GE half time in lean adults suggesting a direct influence of ghrelin on GE [159]. Blood glucose levels [389] and insulin sensitivity [390] can also influence GE and are known to change in response to exercise training [391]. Therefore, future characterisation of the blood profiles of active and sedentary individuals in addition to GE may yield further information on the mechanisms behind the differences in GE observed.

4.4.2 Body Composition and GE

Body composition was another factor that differed significantly between the active and sedentary groups in the present study. There was a trend towards a lower weight and a significantly lower BMI in the active compared to sedentary group. This was accompanied by a lower percentage body fat and trend towards a higher absolute FFM in the active compared to the sedentary group. Therefore, it is possible

that the differences in GE observed between the 2 groups are related to differences in body composition. In the 2 previous studies reporting faster GE in active compared to sedentary individuals, limited descriptors of body composition were given [40, 202]. Carrio et al. reported that body surface area was similar between the marathon runners and sedentary group in their study [32] but did not report FM and FFM. In the present study, body surface area was not significantly different between the 2 groups despite marked differences in percent body fat, suggesting differences in FM and FFM may also have existed in previous studies examining the effects of habitual physical activity on GE [40, 202].

Limited research has investigated the effects of body composition on GE. Numerous studies have compared groups by BMI but with inconsistent findings (See **Table 4.1** in Introduction). There is some evidence to suggest however that GE could be related to body composition (FM and FFM). In a cross sectional study of 45 males and females entering a weight reduction program, GE lag phase was negatively correlated with the percent body fat and half time was negatively correlated with fat free mass and waist/hip ratio. In addition the percentage solid emptying was correlated with height [18]. Mathus-Vliegen et al. [18] concluded that these findings indicated that GE was faster in taller and more muscular subjects, and in those with less intra-abdominal fat. This is consistent with findings from the present study showing that GE is faster in the active group - in which a lower percent body fat and trend towards a great FFM were evident.

To further examine the influence of body composition on GE in the present study, GE variables were compared in groups separated by the median values of BMI, FFM and percent BF. When the whole group were split by median BMI (25 kg/m^2), GE was not significantly different between the two groups. These findings are consistent with others showing no significant differences in GE between groups with a BMI below and above 25 [25, 26]. Recently Seimon et al. (2013) [26] reported no differences in GE or orocecal transit time between a group of 20 normal weight, 20 overweight and 20 obese males classified by BMI. No differences in habitual EI or macronutrient distribution were found between the three groups leading the authors to conclude that in the absence of differences in habitual EI GE is unaffected in obese individuals [26]. However, body composition was not reported in this study and differences in FM and FFM could represent an additional plausible

explanation for the lack of significant difference between groups. In the present study, when groups were split by median percent BF (20%) and median absolute FFM (67kg) the group below the median for percent BF and above the median for absolute FFM had a significantly faster GE, indicating that GE was faster in those with a lower percent BF and higher FFM. These findings are consistent with limited previous research examining the influence of body composition on GE [18]. The present findings highlight the importance of considering body composition in future studies, and support the hypothesis that differences in body composition represent one plausible explanation for the inconsistency in findings of previous studies investigating the role of GE in obesity.

4.4.3 Energy Expenditure and GE

One mechanism by which body composition may influence GE is through differences in EE. RMR is the largest component of daily EE, of which FFM is the major contributor to RMR [37]. Therefore it is conceivable that RMR could act as a mediating variable to reflect the influence of FFM on appetite control [37]. In the present study however RMR was nearly identical between the active and sedentary groups, suggesting differences in RMR are unlikely to be a contributing factor to the differences in GE observed between the two groups.

The current findings are compatible with a new formulation of appetite control recently proposed by Blundell et al. (2012) [37]. In this model, a tonic signal for the drive to eat arises mainly from RMR and FFM and tonic inhibition to this drive to eat is generated by leptin (reflecting the body's energy stores - mainly adipose tissue). In addition episodic signals arise from the GI tract and periodically suppress this drive to eat in response to food consumption [37, 39]. When the effects of episodic signals on satiety have worn off, the drive to eat from RMR then re-exerts its influence and food is again consumed [39]. In support of this formulation, FFM (but not BMI or FM) and RMR were recently demonstrated to be positively associated with meal size and EI in overweight and obese adults [36, 38]. Based on this model, RMR is linked to tonic appetite signals [37] whereas GE, which occurs periodically in response to food consumption, could be linked to episodic postprandial satiety signalling. The current findings suggest GE has a separate role to RMR in the relationship between EE and EI, and therefore support this recent model of appetite control [37].

In contrast to RMR which is relatively consistent between days [367] and could provide a tonic signal for the drive to eat, activity EE (AEE) tends to vary from day to day and would be expected to have a different role in appetite control [39]. Unlike RMR, AEE differed between the active and sedentary groups in the present study suggesting that differences in AEE could contribute to the differences in GE observed. No previous studies could be found reporting AEE and GE, however, it is reasonable to assume that the marathon runners in the study of Carrio et al. (1989) [32] had a higher AEE compared to the sedentary individuals. In the present study to further examine differences in GE in relation to AEE, the whole group were divided into quartiles according to AEE. The upper quartile representing individuals with the highest AEE had a significantly higher absolute FFM, a higher RMR and a faster GE compared to the lowest quartile. These data further support the contention that a higher AEE is associated with a faster GE and are compatible with the hypothesis that episodic signals arising from the GI tract may be more related to AEE [37].

4.4.4 Associations between Body Composition, EE, Eating Behaviour, EI and GE

The previous discussion has identified a number of factors that may contribute to the differences in GE between active and sedentary individuals observed in the present study. FFM, percent BF, dietary restraint, EI and AEE all differed between the active and sedentary groups. However, it is possible and likely in some cases that these variables may simply be correlates of one signal or various signals associated with differences in activity level. Indeed a similar observation was made with regard to the link between FFM, RMR and EI [38]. To further understand the role of these factors in relation to GE, a number of correlations were undertaken between each variable in the group as a whole, and within each group separately. This was followed by partial correlations controlling for activity and finally multiple regression analyses of the variables of interest.

The results of the simple correlation analyses revealed no significant association between BMI and GE but significant associations between several markers of body composition and GE. These findings are consistent with some previous studies showing no significant association between BMI and GE [15, 16, 18, 27, 156] but not others who reported increasing body surface area and BMI to be

associated with slower GE [153-155]. With regard to body composition, GE times were positively associated with percent body fat and absolute FM in the present study. Although few studies have reported correlations between body composition and GE, these findings are consistent with Mathus-Vliegen et al. [18] who reported similar associations in a cross sectional study of 45 males and females.

With regard to physical activity and AEE, significant associations were observed between a number of variables and GE in the group as a whole. The correlations collectively indicated that a higher amount of time spent in physical activity and a higher AEE were associated with a faster GE. Other associations between variables were also observed when conducting simple correlation analyses. Findings of a positive correlation between resting heart rate and GE are consistent with the hypothesis that faster GE may be due to a more predominant parasympathetic tone [40]. Moreover, a higher dietary restraint was associated with faster GE in simple correlation analyses.

After controlling for activity (active or sedentary) however a number of previously significant associations disappeared. The significant associations between adiposity, RQ, dietary restraint, HR and GE that were evident after a simple correlation analysis disappeared after correction for group (active or sedentary). The only associations that were significant were a positive correlation between age and t_{lag} and negative correlations between both AEE and TEE with GE. These findings therefore suggest that when the habitual activity level of the individual is considered increasing age is associated with a slower initial emptying and increasing AEE and TEE with a faster GE, but there is no association between adiposity and GE. Duval et al. (2008) [252] similarly showed in a large cross sectional analysis that the significant positive association between adiposity and eating frequency observed in their study was no longer significant after correction for AEE. The authors interpreted these findings to suggest that the observed relationship between adiposity and eating frequency may be an artefact of the higher AEE, and that this may explain why higher eating frequency was associated with a higher FFM despite a higher EI. A similar conclusion could be drawn with regard to body composition, AEE and GE in the present study. The significant association between increasing age and slower GE in the present study has been previously documented [392] but the negative association between AEE and GE is novel, and indicates that even when habitual

exercise category is controlled for higher average daily AEE (at least over the week prior to the GE test) is associated with faster GE.

The clear association of both AEE and habitual activity level (active or sedentary) with GE compared to other variables is further emphasised in the multiple regression analyses in the present study. Regression analyses were used to determine the extent to which age, habitual activity, AEE, REE, body composition variables, habitual EI and other variables which were found to be correlated with GE in the simple correlation analyses determined GE lag and half times. Multiple regression analysis revealed that habitual activity level was the strongest predictor of GE half time, explaining 25% of the variance. When considering age, FFM, percent BF, activity and RQ as independent variables, activity was the only significant predictor of GE $t_{1/2.}$ Collectively habitual activity and AEE accounted for 34% of the variance in GE $t_{1/2.}$ No other variables contributed to the variance in GE $t_{1/2}$. With regard to lag time, similar findings were demonstrated, however age also explained some of the variance in lag time. Activity and AEE together explained 31% of the variance in t_{lag}. 38% of the variance in t_{lag} could be accounted for by including age in the same model. These findings therefore suggest that of the variables measured in the present study habitual activity level and AEE account for the greatest differences in GE between individuals, and other factors may simply be correlates of activity and AEE levels. These findings ultimately highlight the influence of AEE and habitual activity on GE and underline the importance of considering habitual activity levels and AEE in future studies examining GE and parameters that may be affected by GE.

4.4.5 Appetite sensations

Despite the central role of the gut in appetite control, very few studies have reported subjective appetite ratings when investigating the effects of exercise on GE. In both previous studies comparing GE in active and sedentary individuals, energy intake and appetite were not reported [40, 202]. In the present study, no significant differences in any appetite variables were observed between the active and sedentary groups as assessed using visual analogue scales. A recent study involving a cross sectional analysis of males and females with a BMI <27.5kg/m^2, divided groups into

tertiles according to gender and AEE (measured by doubly labelled water) [393]. In the fasted state, a lower desire to eat and higher fullness was found in males in the highest AEE tertile compared to the lowest tertile. In addition, males in the middle tertile had higher satiety quotients reflecting a greater meal induced satiety compared to males in the other tertiles which the authors hypothesised could be due to differences in GE and gut peptides [393]. This is in contrast with the present study where no differences in appetite ratings were observed between males in the upper and lower quartiles of AEE. In the present study, however moderate exercisers were not included and it is therefore not possible to draw a direct comparison between these findings and the present study. The present findings are consistent with a previous cross sectional study of active and sedentary individuals showing no difference in fasting or postprandial hunger or fullness ratings between the groups [251]. In addition, the present findings are consistent with those of Long et al. (2002) [249] who demonstrated hunger and satiety responses did not differ between habitual exercisers and non-exercisers after both high and low energy preloads. However, a lower desire to eat before the preload in exercisers compared to non-exercisers was reported which contrasts with the present findings. One explanation may be that participants were asked to consume their usual breakfast prior to attending the laboratory in the latter study and this may have influenced the pre-meal ratings. Long et al. (2002) [249] concluded based on their findings that improved food intake regulation was not directly related to subjective feelings of hunger and satiety in habitual exercisers and that differences in appetite control may manifest themselves primarily as differences in intake. The present findings demonstrating no significant differences in subjective appetite ratings between active and sedentary individuals support this contention.

Other methodological factors may also account for the lack of significant difference in subjective appetite ratings between groups in the present study. One limitation of a cross-sectional analysis of appetite ratings relates to between subject reliability. As ratings are subjective, genuine differences in subject's interpretation of the scale could limit the inter-subject reliability [310]. Furthermore, the present study was powered to detect differences in GE and much larger sample sizes are required to detect differences in subjective appetite ratings using an unpaired design (see Chapter 3 page 58).

No significant differences in subjective appetite were observed between active and sedentary groups. However, in a simple correlation analysis within groups, GE was associated with postprandial fullness (5-hour area under the curve) in the active group, but no associations were demonstrated between GE and fullness in the sedentary group. These findings support the contention that exercise sensitises the physiological mechanisms involved in appetite control [11]

No significant association between RMR and appetite ratings was observed in the whole group including both active and sedentary individuals in the present study. This contrasts with reports of a positive association between RMR and hunger ratings in overweight and obese individuals [38]. One explanation could be the participant characteristics, in the present study - including both lean and obese and active and sedentary individuals. It is possible that these variables could exert a different influence on appetite and EI depending on the body composition or activity status of the individual. However, at present this remains speculation and the present findings should be interpreted with caution due to the sample size for within group correlations and the limitations associated with visual analogue scales in terms of between subject reliability. Furthermore, these variables may be correlates of another signal and the causal nature of these associations cannot be determined from this cross sectional analysis.

In summary, although many studies have examined the influence of acute exercise bouts and longitudinal exercise interventions on subjective appetite ratings [394, 395], few studies have compared subjective appetite ratings in individuals of different habitual activity levels. The present study demonstrated that despite differences in GE between active and sedentary individuals, no differences in subjective appetite ratings could be detected. However, fullness was associated with GE in active but not in sedentary individuals. These data support the contention that appetite may be better regulated in response to physiological signals in active individuals.

4.4.6 *Ad Libitum* Test Meal EI

Unlike appetite ratings, EI measured at an *ad libitum* lunch meal differed significantly between the active and sedentary groups in the present study. The active

group had a significantly higher EI compared to the sedentary group. Others have shown similar findings. Higher EI at an *ad libitum* meal was recently demonstrated in males in the highest tertile of AEE compared to lower AEE tertiles [393]. Further, although absolute FFM was not significantly greater in the active group in the present study, a trend towards a higher FFM in this group adds support to the hypothesis that active individuals with a larger muscle mass should have a tendency to consume larger meals [36]. However, no significant associations were observed between FFM and *ad libitum* EI in correlation analyses in the present study. In addition, the present findings are in contrast with two previous cross-sectional studies investigating *ad libitum* EI at a test meal in active and sedentary individuals [249, 251]. Van Walleghan et al. (2007) [251] reported no significant difference between active and sedentary individuals in EI at a buffet meal after high and low energy preloads. Long et al. (2002) [249] similarly reported no difference in EI after a low-energy preload but a significantly higher EI was demonstrated following a high energy preload in sedentary compared to active individuals. One explanation may be that in these studies the *ad libitum* meal was served at 30 and 60 minutes after the preloads respectively, which contrasts with the 5-hour time gap between breakfast and lunch in the present study. An additional intuitive explanation would be that the active individuals preferred the taste of the test meal (pasta) compared to the sedentary individuals in the present study. However, palatability ratings did not differ significantly between the two groups suggesting this did not account for the difference observed. The time interval between the preload and the *ad libitum* meal therefore represents the most likely factor to account for the inconsistency in findings with previous studies. When considering the kinetic and temporal pattern of GE in relation to appetite control [35] it is likely that appetite and EI are influenced by different mechanisms depending on the time interval following a preload. Future studies examining differences in *ad libitum EI* in active and sedentary individuals at different time intervals following a preload will assist in further understanding the effects of habitual exercise on *ad libitum* EI.

The causal nature and temporal pattern of the relationship between physical activity, GE and EI is interesting to consider. As previously highlighted evidence of rapid orocecal transit time in habitually active individuals with concomitant high EIs led the authors to conclude that the high EI associated with chronic exercise is associated with GI adaptation (i.e. accelerated orocecal transit time) [248]. However,

it could also be speculated that increased physical activity levels may have led to faster orocecal transit time and thus higher caloric intakes as a result of a shorter satiety period. The causal nature of this relationship is not possible to determine from this study or the present study due to their cross sectional designs. However, in the present study habitual EI (as assessed by diet recall) did not explain any of the variance in GE in a multiple regression analysis whereas AEE and activity did. Although longitudinal exercise intervention studies are necessary, the present findings strengthen the hypothesis that increased physical activity may lead to faster GE and thus higher EI as a result of a shorter satiety period.

4.4.7 'Liking' and 'Wanting'

In addition to homeostatic systems influencing food intake, food reward systems driving food choice may differ between active and sedentary individuals. In the present study, components of food reward ('liking', 'wanting', and food preference) were measured using a validated computer procedure [315] involving an array of images of foods in 4 different categories – high fat sweet and savoury and low fat sweet and savoury. Active individuals had a higher preference for low fat savoury foods compared to the sedentary group but no differences were observed between groups in the other categories. One explanation for this finding may be that active individuals are often motivated to consume healthier diets [396, 397] and it is likely that the low fat savoury food images (containing images such as chicken breast, boiled potatoes and tomatoes) were perceived as the 'healthy' option.

Interestingly 'liking' and 'wanting' for low fat savoury foods did not differ between active and sedentary individuals despite the increased preference for this category of food in the active group. One explanation for this finding may be that other factors such as the higher dietary restraint observed in this group influenced food preference. While people often want what they like and like what they want [311, 312], some individuals such as restrained eaters may habitually select less liked food items to prevent weight gain [313, 314]. The increased preference for low fat savoury foods in the active group despite no difference in 'liking' and 'wanting' compared to sedentary individuals supports this contention.

With regard to 'liking' and 'wanting', the only significant difference observed between active and sedentary individuals was in the low fat sweet category - the

sedentary group had a higher 'liking' for low fat sweet foods post breakfast and higher 'wanting' for low fat sweet foods prior to lunch. One potential explanation may be that differences in 'liking' and 'wanting' for sweet foods with habitual exercise may have resulted from differences in glucostatic metabolism or status [320]. Collectively, the present findings highlight that in addition to metabolic processes such as GE, hedonic processes of food reward and preferences also differed between active and sedentary individuals.

4.4.8 Methodological Considerations

There are various methodological aspects to the present study and studies investigating the effects of body composition and habitual exercise on GE and associated measures which are important to acknowledge. The ^{13}C-OBT has many advantages for the measurement of GE. It is non-invasive, non-radioactive, has been validated against scintigraphy, has a day to day variability comparable to scintigraphy [293][see Chapter 3] and is sensitive enough to detect pharmacological influences on GE [359]. The test has been shown to be unaffected by liver dysfunction in cirrhosis [398]. Further, conditions which might affect absorption or post absorptive metabolism of the tracer such as pancreatic exocrine insufficiency, liver and lung disease have been demonstrated to have no detectable effect on cumulative $^{13}CO_2$ excretion in a study of over 1000 individuals [357]. One limitation of the ^{13}C-OBT however is that unlike the 'gold standard' scintigraphy the ^{13}C-OBT does not permit direct imaging of gastric function. The rationale for the test is that ^{13}C-octanoic acid (as a medium chain fatty acid) passes through the stomach unabsorbed, is then rapidly absorbed upon emptying into the duodenum, oxidised in the liver and subsequently excreted in the breath as $^{13}CO_2$. The rate limiting step between the ingestion of the ^{13}C labelled meal and its appearance in the breath is GE [293].

The influence of various factors including VCO_2 predictions, RQ and post gastric metabolism on ^{13}C recovery have been investigated. As estimates of CO_2 production are used in calculations of the percent dose recovered it is possible that errors in CO_2 predictions may influence the results of ^{13}C-OBT GE studies. In the present study, to rule out any possible errors due to VCO_2 predictions, GE parameters were calculated using both the traditional VCO_2 prediction formula [332] and VCO_2 directly measured during the resting metabolic rate measurement. Despite

slight differences in the measured compared to the predicted VCO_2 values, this had no influence on the GE results reported, confirming earlier reports that GE time based parameters are independent of endogenous CO_2 production [399]. Therefore, it can be reasonably concluded that the VCO_2 prediction method did not influence the GE results in the present study. The respiratory quotient (RQ) is another factor which has been suggested may influence ^{13}C recovery. A significant positive correlation between ^{13}C recovery and RQ has previously been observed in a mixed population of lean, obese and diabetic individuals despite no difference between the groups [378]. In the present study, the active group had a significantly lower fasting RQ compared to the sedentary group. This finding is consistent with others who have reported greater fat oxidation at rest in endurance-trained compared to untrained individuals [400]. Using covariance analysis, after correction for RQ none of the GE findings were altered. Further, when included in the multiple regression analysis RQ did not explain any of the variance in GE in the present study. Therefore any differences in RQ are unlikely to have influenced the results. Similarly the thermic effect of feeding (TEF) is unlikely to have affected the results. No difference in TEF between trained and untrained individuals has previously been reported [384]. In addition, there were no differences in percent dose recovered between the active and sedentary groups in the present study further suggesting that the post-absorptive processing was similar between the 2 groups.

With regard to the validity of this technique in obese individuals, a previous study comparing the metabolism of medium and long chain fats in lean and obese individuals demonstrated that while there was a defect in the oxidation of long chain fats in obese individuals, no defect was observed for medium chain fatty acids [29, 401]. Moreover, reports of both faster and slower GE in obese compared to non-obese individuals using both the ^{13}C-OBT [29, 362] and scintigraphy [16, 27, 28, 200] indicate the method used to measure GE is unlikely to bias the GE results. In addition, the ^{13}C-OBT has been validated against scintigraphy in a cohort containing a large range of BMIs (19 – 42 kg/m^2) [402]. Based on the collective evidence, it can therefore be reasonably concluded that the method used to measure GE did not influence the findings in the present study and that the differences observed represent true differences in GE.

The sensitivity of the measurements of EE and EI could also have influenced the accuracy of the present findings. It is possible for example that more sensitive measures of physical activity or EI may have explained a greater variance in GE. Activity EE (AEE) was estimated in the present study using the Actigraph GT3X accelerometer and activity count cut-points for classifying physical activity intensity as recently proposed [373]. One limitation associated with accelerometers placed on the hip however is in detecting upper body exercise. In particular this may have underestimated AEE and time in activity in some individuals in the active group who recorded participating in resistance exercise. Nevertheless the cut point method is considered an effective way to monitor free-living physical activity [403] and the actigraph accelerometer has been demonstrated to reasonably correlate with EE measured by doubly labelled water [404].

Habitual EI was measured in the present study by 24h diet recall on 3 occasions, including the day prior to the GE test. The validity of 24h recall on a group level has been deemed satisfactory [405] and is therefore likely to have been sufficient to detect group differences in the present study. However, 24h recall has been found to be insufficiently valid on the individual level [405]. In a recent study, which reported a significant relationship between FFM and EI, EI was measured over the course of a probe day where all meals were provided to participants [36]. The authors highlighted that self-reports of food intake, by whatever method, are not sufficiently accurate and that food intake must be measured quantitatively and objectively under controlled conditions [36]. Limitations inherent in self report of food intake may therefore explain why no associations between body composition or GE and habitual EI were seen in the present study. Future studies involving quantitative and objective measurements of EI are needed to further investigate a possible association between habitual EI and GE.

Standardisation of diet and physical activity are also worth considering. In the present study, food intake was not standardised on the day prior to the GE test. In 2 recent studies, the reproducibility of *ad libitum* EI at experimental test meals have been demonstrated to be influenced very little by prior diet standardisation [305, 324], however, diet standardisation exerted a significant effect on *ad libitum* EI [324]. By instructing participants to consume their normal diet in the present study, this therefore ensured that participants were in their habitual state and that *ad libitum*

EI was not adversely influenced by diet standardisation. Further, it allowed for the effects of habitual EI on GE to be investigated.

With regard to physical activity, participants were instructed to avoid strenuous exercise for 24 hours prior to the GE test to avoid any effects of acute exercise on the parameters of interest, as is common in many protocols. This is an interesting consideration, with regard to appetite research. In an early study examining the relationship between EE and EI in twelve army cadets, Edholm et al. (1955) observed that there was no correlation between EE and EI of individuals on the same day but there was a significant correlation between mean daily EE and EI 2 days later [406]. Although, there is limited subsequent evidence to support such an effect, in a similar study Edholm et al. (1970) reported no within-day relationship between EE and EI, but a balance between EI and EE was evident over a one week period [407]. As summarised by King et al. 1997 [394] these studies point to the existence of a "gross" regulatory mechanism controlling EI but this control is not finely 'tuned' (i.e. no within day control). In the present study, it is possible that GE may have been influenced by exercise two days beforehand and faster GE in the active group may be one mechanism contributing to this 'gross' regulatory control of EI. However, it is not possible to compare the present findings to others as in the two previous studies which reported the effects of habitual exercise on GE [40, 202], no details on physical activity standardisation prior to the GE test were given. Future studies conducting GE tests at different time intervals following the last exercise session are therefore warranted to investigate the intriguing possibility that GE might contribute to a 'gross' regulatory mechanism controlling EI in habitually active individuals.

4.4.9 Future Directions

It is interesting that the influences of body composition, EE and habitual exercise on GE have received such little investigation to date, given the current obesity epidemic and associated urgency to better understand processes of appetite control and energy balance. One explanation may be that it has been assumed that GE is faster in active compared to sedentary individuals. The relationship between EE and EI however is not as straightforward as it might intuitively seem. As stated by Mayer in 1956, "it may be surprising to note that a fundamental mechanism – that of the regulation of EI fails to respond in a certain interval to variations of EE".

A number of questions have been raised based on the current findings, which are worthy of future study and may contribute to a better understanding of the complex relationship between EE and EI. Although, the present study demonstrates a clear effect of habitual physical activity level and AEE on GE, we did not find any clear mechanism that may account for the faster GE observed in habitually active individuals. A lower resting heart rate in the active group is consistent with the hypothesis that a more predominant parasympathetic tone may have a role [40] but this did not independently account for variance in GE. Other factors that were not measured in the present study must therefore account for some of the variance in GE observed. One such factor could be differences in GI hormones, blood glucose levels or insulin sensitivity as previously highlighted. In addition, exercise is known to improve leptin sensitivity via reducing fat mass [408, 409] which some evidence in animals suggests may interact with CCK and vagal afferent fibres to influence gastric motility [410]. Future analysis of these measures in addition to the variables in the present study could assist to better understand the mechanisms contributing to differences in GE with habitual exercise.

Whether faster GE with regular exercise has a mechanistic role in the improved appetite control (ability to compensate for prior energy intake), that has been observed with physical activity [214, 249, 251, 411] remains to be established. A faster emptying rate particularly in the initial postprandial period could have a beneficial role in appetite control when considered together with the critical role of GE in postprandial ghrelin suppression [139, 140], and evidence of increases in anorexigenic gut peptides in response to faster nutrient delivery to the small intestine [61, 130, 134, 181]. Studies testing GE and gut peptides in response to the preload paradigm (i.e. after both high and low energy preloads) in habitually active individuals are needed to test this hypothesis.

The present study did not include moderate exercisers or females, therefore findings cannot be extrapolated to these groups. Males only were studied to exclude any confounding effects of the phase of the menstrual cycle [277]. Although some studies report no sex-based differences in appetite responses to exercise [412], others suggest a difference may exist [393]. In addition, some evidence suggests moderate exercisers have different appetite and EI responses to others [249, 393] raising the possibility that the effects of habitual exercise on GE, appetite and EI may vary

depending on the level of habitual exercise. Future studies including females and moderate exercisers may therefore yield further information on the relationship between GE, EE and EI.

Finally, as previously highlighted while the present study shows a clear association between habitual physical activity level and AEE with GE, due to the cross sectional design it does not show that exercise is a causal factor in faster GE. Further studies are needed to determine the temporal patterns of changes in GE with exercise and the associated implications for appetite control.

Chapter 5: The Effect of a 4-Week Exercise Intervention on Gastric Emptying, Appetite and Energy Intake in Overweight and Obese Males

5.1 BACKGROUND

Exercise is an important factor in the regulation of appetite, energy balance and hence, body composition and is often recommended as a weight loss strategy. Adherence to an exercise program is one issue, however even when exercise is supervised there is a huge variability in weight loss response to exercise [12]. This may be due in part to compensatory responses in other components of energy balance such as resting metabolic rate, non-exercise spontaneous physical activity and energy intake (EI) (for a review of the complete range of responses to an exercise-induced energy deficit see King et al. (2008) [8]). Although it is intuitive that exercise drives an increase in appetite and EI, the relationship between exercise and appetite is more complex. Evidence indicates that exercise improves the sensitivity of the appetite control system [86, 99, 249, 251, 411] and that exercise influences at least two processes of appetite control: both the drive to eat and the satiating efficiency of a meal [99]. Following a twelve week exercise intervention, King et al. (2009) [99] observed an increase in the overall (orexigenic) drive to eat and a concomitant increase in the satiating efficiency of a fixed meal. As the strength of these processes may determine whether individuals lose weight with exercise [99], understanding the mechanisms behind changes in appetite with exercise is vital.

Signals arising from the GI tract appear to play a fundamental role in the physiologic regulation of appetite. Some appetite related gut peptides have been demonstrated to respond to short to medium term exercise interventions [89, 211, 212] (see **Table 2.3** page 25). Following exercise induced weight loss over 12 weeks, Martins et al., (2010) observed increases in fasting ghrelin, but also increased postprandial ghrelin suppression along with increased GLP-1 at 90-180 minutes after a fixed meal [86]. Other evidence suggests exercise may also influence GLP-1 and ghrelin independent of weight loss. Following just 5 days of exercise (i.e. before weight loss) GLP-1 levels were increased 30 minutes after a fixed meal in normal

and overweight adolescent boys [211]. Other short term studies indicate exercise appears to influence the fasting acylated ghrelin [212] and postprandial ghrelin response [89] independent of energy balance. As these peptides are known to both influence GE and be influenced by GE [35] it could be hypothesised that exercise influences GE independent of weight loss or changes in energy balance. However, this and the associated implications for appetite control and EI remain to be established.

Surprisingly, limited research has examined the influence of exercise on GE in relation to appetite and EI. Evidence of faster GE in marathon runners compared to sedentary individuals [40] is most frequently cited as evidence that physical activity influences GE. This was supported by the findings of the cross sectional study presented in Chapter 4. However, while cross sectional studies can provide important information, they do not allow for a causal relationship between changes in GE and EI with exercise to be determined. Differences in GE between active and sedentary individuals could also be attributed to differences in body composition, habitual diet or eating behaviours. Furthermore, gut adaptations observed in marathon runners [40] and habitual exercisers may only have occurred after a long period of training at high intensity and volume. One paradox relating to observations of a positive relationship between physical activity and EI in the long term free-living situation [364-366], is that in the shorter term evidence suggests exercise-induced energy expenditure (EE) and EI are only weakly coupled (i.e. EI is not matched to increased EE) [11, 41]. It is possible therefore that a period of transition or uncoupling of EE and EI occurs before a steady state (coupling of EE and EI) is achieved [42]. Consequently it could be expected that changes in GE and other mechanisms of appetite control may also differ over time (i.e. between transition and steady state) as exercise programs progress. Whether GE has a role in compensatory changes in EI as overweight and obese sedentary individuals' progress to a physically active lifestyle is unknown.

In light of this, the present study was undertaken to investigate the effects of a 4-week exercise intervention on GE, appetite and EI in overweight and obese sedentary males. By testing GE after 4 weeks of exercise, changes before significant weight loss was likely could be characterised.

5.1.1 Aims

The aims of this study were to determine in overweight and obese sedentary males:

(i) the effect of a 4 week exercise intervention on GE

(ii) associated changes (if any) in appetite, EI, eating behaviour, food preferences and 'liking' and 'wanting'

This knowledge will help to better understand processes of appetite control as sedentary overweight and obese individuals progress from a sedentary to a physically active lifestyle and may help to understand how exercise can be used more effectively in weight management.

5.2 METHODOLOGY

5.2.1 Participants

Participants were recruited through recruitment emails and flyers in the university and local area. Forty-one males completed an initial screening questionnaire to assess their eligibility to participate. Inclusion criteria were as follows: male, aged 18-60 years, BMI 25-40 kg/m^2, weight stable (± 4 kg over last 6 months), no history of GI surgery or disorder, non-diabetic, no medical conditions and not taking any medication known to influence any of the study outcome measures, willing to consume study test meals, not a heavy smoker (< 10 per day) and sedentary (participating in 1 structured exercise session or less per week and not engaged in strenuous work). One exercise session was defined as at least 40 minutes of moderate to high intensity activity [249]. Twenty three individuals were excluded after completing the initial questionnaire for various reasons including history of GI surgery (n = 1), egg allergy (n = 1), BMI < 25 (n = 6), BMI > 40 (n = 1), not weight stable (n = 4), unable to commit to testing schedule (n = 2), undertaking planned exercise > 1 times per week (n = 4), recent musculoskeletal injury (n = 1), aged > 60 (n = 1) and taking medication which may influence outcomes (n = 2). Eighteen males fit the inclusion criteria and were included in the study. Ethical approval for the study was granted by Queensland University of Technology Research Ethics Committee and all participants provided written informed consent prior to taking part.

5.2.2 Design

Participants attended the laboratory on 2 separate test days in the week prior to the 4-week exercise intervention (baseline) and on 2 separate test days in the week following the exercise intervention (post-intervention) (see **Figure 5.1**). At one testing session, body composition, resting metabolic rate (RMR), VO_2max and eating behaviour (e.g. dietary restraint) were measured. At the second test session, GE, habitual EI, subjective appetite sensations, food preferences and 'liking' and 'wanting' were assessed. In addition, participants were given an accelerometer to wear for 7 days in the week prior to the exercise program and in the final week of the exercise program.

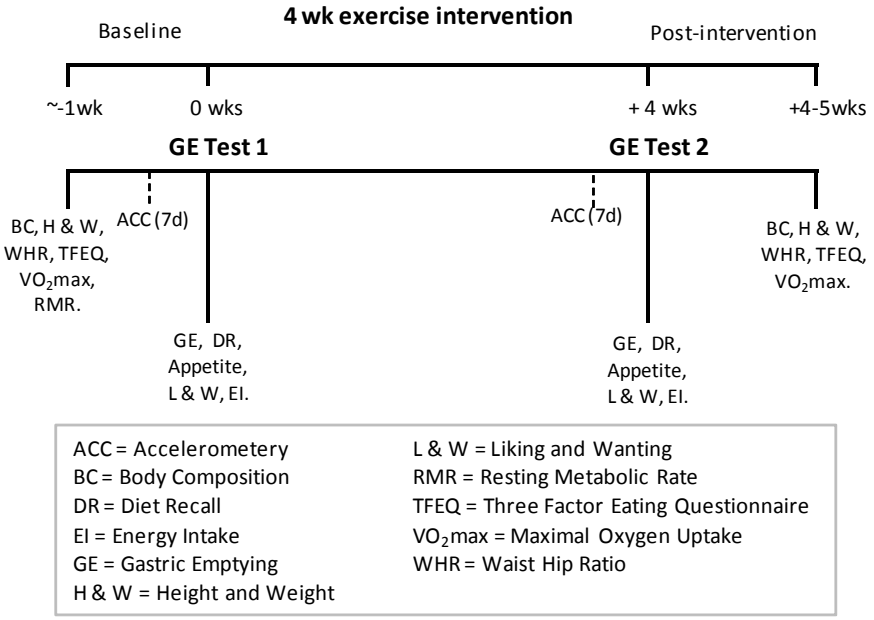


Figure 5.1 Schematic overview of Study 3 protocol. Baseline testing was conducted in the week prior to the exercise intervention. Post intervention testing was conducted ≥ 48h after the last exercise session.

5.2.2.1 Exercise Intervention

The exercise intervention consisted of 5 exercise sessions per week for 4 weeks (20 sessions in total). All exercise sessions were supervised at Queensland University of Technology and involved indoor cycling on a cycle ergometer (Monark 884E Ergomedic Sprint Bike, Monark Exercise AB, Vansbro, Sweden).

To provide variety, sessions alternated between continuous cycling at 50% VO_2max and high intensity interval exercise (HIIE) at 100% VO_2max. Workloads equivalent to 50% and 100% VO_2max were prescribed based on each individual's baseline VO_2max test. The continuous exercise sessions involved cycling at a constant workload equivalent to 50% VO_2max for the duration of the session. HIIE sessions consisted of 30 seconds cycling at 100% VO_2max followed by 30 seconds recovery (unloaded cycling or static recovery) each minute for the duration of the session. Most common HIIE protocols involve cycle exercise in excess of 90% VO_2max and have been demonstrated to significantly improve VO_2max [413].

Exercise duration progressed each week by 5 minutes from 30 minutes in week one to 45 minutes in week 4 as recommended by the American College of Sports Medicine [414]. Each session started with a 5 minute warm up of unloaded

cycling and finished with a cool down. Participants wore a heart rate monitor (Polar Electro Oy, Kempele, Finland) during each exercise session and were instructed to cycle at a cadence of 70rpm. Gymboss Timers(Gymboss, USA) were attached to each bike and used for the HIIE sessions to ensure correct timing for intervals. Distance cycled, heart rate (HR) and rating of perceived exertion (RPE) using the Borg Scale [415] were recorded every 5 minutes throughout each session.

5.2.3 Probe Measurements

The following measurements were taken on one test morning in the week prior to the exercise intervention and in the week following the exercise intervention (at least 48h after the last exercise session to avoid any acute effects of exercise). Participants attended the laboratory after a 12-hour overnight fast, and having avoided alcohol and strenuous exercise for 24 hours. Measurements were taken in the following order:

5.2.3.1 Anthropometry and Body Composition

Height was measured without shoes to the nearest 0.5cm and weight to the nearest 0.01kg. Waist and hip circumferences were taken and body composition was measured using air displacement plethysmography (Bodpod, Concord, CA). Procedures were identical to those described in Chapter 3.

5.2.3.2 Resting Metabolic Rate

RMR was measured by indirect calorimetry using a ventilated hood system (TrueOne 2400 Metabolic Cart, ParvoMedics, Utah, USA) using an identical procedure to that described in detail in Chapter 4. RMR was measured at pre-test only as it is well established that RMR is unlikely to change after 4 weeks of exercise [416]. The same pre-test RMR value was used in TEE calculations at both pre- and post-test.

5.2.3.3 Three Factor Eating Questionnaire

Restraint, Disinhibition and Hunger were assessed using the Three Factor Eating Questionnaire (TFEQ) [330]. For a detailed description, refer to Chapter 3.

5.2.3.4 Blood Pressure

Systolic and Diastolic blood pressure were assessed using an Omron IA1B blood pressure monitor (Omron Healthcare Singapore PTE Ltd, Singapore) in a seated position. Measurements were taken in duplicate following 10 minutes of sitting to ensure the participant was rested and relaxed.

5.2.3.5 Maximal oxygen consumption (VO_2 max)

VO_2 max was assessed using a TrueMax 2400 Metabolic Cart (ParvoMedics Inc, USA) and a two-way breathing valve and nose clip (Hans Rudolph, USA). All tests were conducted on the same cycle ergometer (Monark Bike 839E, Monark Exercise AB, Sweden) and consisted of 2 phases (similar to Wood et al. 2010 [417]). Phase 1 consisted of a graded exercise test performed to volitional exhaustion and phase 2 consisted of a verification test. Participants were instructed to maintain cycling cadence at 70rpm throughout. To ensure a sufficient duration for the test, the 1kg Monark weight basket was removed and replaced with a holder weighing 0.4kg. Participants began the graded test with a 2 minute warm up at 28W. Subsequently, workload was increased each minute by either 21 or 28W (determined prior to the test based on the participant's predicted VO_2max). An identical workload increase was used within each test and within each participant from pre to post test.

The continuous incremental exercise test (phase 1) was deemed to be a valid maximal test on the basis of achievement of at least three of the following criteria during the final 30 seconds of the last completed stage [417]:

- Increase in VO2 < 50% of that expected for the change in mechanical work
- Heart Rate (HR) within +/- 11bpm of age-predicted maximum, calculated as 220 –age
- Respiratory exchange ratio (RER) >/= 1.15

- RPE >/= 18

Following the first incremental graded test (phase 1), the participant was given a short (5 min) rest during which they were given a small glass of water. Participants then resumed cycling at the workload of the third last stage of the preceding maximal continuous incremental test for phase 2 (the verification test) [417]. As for phase 1, the workload was increased each minute until volitional exhaustion. This two-phase test was used as it has been suggested that a verification or ‘‘booster’’ test may provide a time- efficient means of verifying whether a VO_2peak is indicative of a true maximal VO_2 [417, 418].

5.2.3.6 Physical Activity and Energy Expenditure

Physical activity was monitored using a tri-axial GT3X accelerometer (Actigraph, Fort Walton Beach, FL, USA). Participants were provided with the activity monitor to wear for 7 days between the baseline assessment and pre GE test and again for 7 days between the first exercise session of the fourth week and the post GE test. A detailed description of the analysis procedures used is described in Chapter 4 (see page 86).

5.2.3.7 Diet Recall

A multiple-pass 24-hour diet recall was conducted on the morning of the GE test to assess participants’ habitual diet over the 24 hours prior to the GE test. The 24-hour recall aims to provide a complete record of all food and drink eaten on the previous day between midnight and midnight, and consists of three passes including 1) a quick list of food and beverages consumed, 2) a detailed description of type, amount, cooking method and time of consumption; and 3) review of intake to report any items that may previously have been forgotten, state whether intake was typical and list any dietary supplements used. Foodworks Professional Edition dietary analysis software (Foodworks; Xyris Software, Highgate Hill, Queensland, Australia) was used to quantify total energy intake and macronutrient composition of the diet over the 24 hours prior to the GE test.

5.2.4 GE Test Day Measurements

The GE test day took place on the week prior to the exercise intervention (pre) and in the week following the 4 week exercise intervention (post) and followed an identical protocol to Chapter 3 (for an overview see **Figure 3.2** page 48**)**. The test commenced between 6am and 9am and was identical for each participant between pre and post test. The test took place at least 48h following the last exercise session to avoid any effects of acute exercise. In addition, participants were instructed to avoid consumption of naturally ^{13}C-enriched foods (corn or corn products, pineapple, kiwi fruit, cane sugar and exotic fruits) for at least two days prior to the GE test, to refrain from alcohol for 24 hours, to eat a typical evening meal for them the night before and to then fast for 12 hours overnight until coming to the Human Appetite Research Centre the following morning. One glass of water was allowed upon waking. Participants were instructed to repeat these procedures prior to the post test.

5.2.4.1 Gastric Emptying

GE parameters were calculated using the ^{13}C-OBT [293], using an identical procedure to that described in detail in Chapter 3. In brief, the egg yolk of a standardized pancake breakfast meal [1676 kJ (400 kcal); 15g (15%) PRO, 17g (37%) Fat, 48g (48%) CHO)] was labelled with 100mg ^{13}C-octanoic acid (Cambridge Isotope Laboratories, Andover, USA). Participants consumed the meal together with 250ml of water within 10 minutes. Breath samples were collected in 10ml glass Exetainer tubes (Labco, Buckinghamshire, UK) prior to the breakfast, immediately after, and subsequently at 15-minute intervals for 5 hours after breakfast. Participants remained in sedentary activities throughout.

5.2.4.1.1 ^{13}C breath test analysis

Procedures for 13C breath and data analysis were identical to Chapter 3. In brief, ^{13}C enrichment of breath samples was measured by isotope ratio mass spectrometry (Hydra 20-20) and compared to a reference gas (5% CO_2, 75% N_2, 20% O_2 calibrated with a standard of $^{13}CO2$). Data were analysed according to Ghoos et al [293] and fitted to the original GE mathematical model by non-linear regression analysis. To calculate the cumulative percent of ^{13}C dose recovered and the

percentage $^{13}CO_2$ recovery per hour, enrichment values were multiplied by the estimated total CO_2 production (VCO_2) for each individual. Resting VCO_2 was predicted from body surface area [332]. Body surface area was calculated from height and weight using the formula of Haycock et al. (1978) [334]. The conventional uncorrected time based parameters (t_{lag} and $t_{1/2}$) proposed by Ghoos et al.[293] and the parameters latency time (t_{lat}) and ascension time (t_{asc}) proposed by Schommartz et al. (1998)[303] were calculated.

5.2.4.2 Subjective Appetite Sensations

Subjective appetite sensations were measured throughout the test day using an electronic appetite rating system. Participants were asked to rate feelings of hunger, fullness and desire to eat on 100 mm visual analogue scales, anchored at each end with the statements "not at all" and "extremely". Five hour postprandial area under the curve (AUC) was calculated using the trapezoidal rule. In addition, the satiety quotient (SQ) was calculated. The SQ relates the suppression of hunger, desire to eat or change in fullness to the amount of energy consumed. For a detailed description, see Chapter 3 page 52.

5.2.4.3 Food Preferences, 'Liking' and 'Wanting'

Food preferences and 'liking' and 'wanting' were measured on 3 occasions (pre-breakfast, post-breakfast and pre-lunch) during the test day using a computer-based procedure - the Leeds Food Preference Questionnaire (LFPQ) [339]. An identical procedure to that described in detail in chapter 3, page 52 was followed.

5.2.4.4 Ad libitum Energy Intake

At the end of the GE test, participants were provided with an ad libitum pasta lunch meal identical to that described in chapter 3 (47% CHO, 35% FAT, and 18% PRO, and an energy content of 7.6kJ/g) and water and told to consume as much as they wished until comfortably full. The amount (g) of food consumed from the *ad libitum* meal was determined by weighing the meal before and after consumption and energy intake (kJ) was calculated.

5.2.5 Statistical Analysis

Data are presented as mean values and standard deviations (SD). Changes in variables (GE, anthropometry and body composition, fitness, physical activity levels, eating behaviour related traits, 'liking and wanting', appetite and energy intake) from pre to post exercise intervention were assessed using paired sample t-tests. Pearson (or Spearman where appropriate) correlations were used to determine relationships between changes in key variables. Area under the curve for appetite ratings was calculated using the trapezoidal rule. Statistical analysis was carried out using PASW Statistics 18.0 (SPSS Inc., Chicago, IL) and statistical significance accepted at $p < .05$. Based on the study presented in Chapter 3 (see page 58), a minimum of 15 participants was sufficient to detect a mean change in GE $t_{1/2} \geq 14.40$min, $t_{lag} \geq 8.1$ min, $t_{asc} \geq 13.9$ min and $t_{lat} \geq 3.8$ min with a power of 80% and $\alpha = 0.05$.

5.3 RESULTS

Three participants did not complete the 4 week exercise intervention - two due to time commitments and personal circumstances and one participant was excluded due to insufficient attendance at exercise sessions. No significant differences in baseline characteristics between these individuals and those who completed the study were found. Results are presented for 15 males (BMI: 29.7 ± 3.3, Age: 31.1 ± 8.4 yrs) who completed all parts of the study.

5.3.1 Exercise Compliance

Participants completed 96 (3.9) % of the prescribed number of exercise sessions, with all participants completing a minimum of 90% (18 of 20) of the exercise sessions. Average HR across the 4-week intervention during interval sessions was 160 (13) bpm and during continuous sessions was 149 (12) bpm.

5.3.2 Anthropometry, Body Composition, Fitness and Blood Pressure

Weight, BMI, body fat, waist circumference and blood pressure were all significantly reduced after the 4-week exercise intervention (see **Table 5.1**). In addition, VO_2max was significantly higher at post-test indicating an improvement in fitness (**Table 5.1**). This change represented a mean 12.8% increase in VO_2max. Four participants did not meet the criteria for VO_2max at pre and post-test, however the verification test indicated that they could not complete any additional stages. Mean RER (Pre: 1.15 ± 0.04, Post: 1.13 ± 0.06) and HR (**Table 5.1**) during the final 30 seconds of the last completed stage did not differ significantly between pre and post test.

Table 5.1 Participant anthropometric, body composition, fitness and blood pressure characteristics pre and post 4 week exercise intervention (n = 15).

	Pre	Post	*P*-value
Weight (kg)	95.6 ± 13.0	94.7 ± 13.0	**<0.01**
BMI (kg/m^2)	29.7 ± 3.3	29.3 ± 3.2	**<0.001**
Body composition			
Body Fat (%)	30.0 ± 6.8	29.0 ± 6.7	**0.01**
FFM (kg)	66.4 ± 7.1	66.7 ± 6.8	0.5
Waist (cm)	97.1 ± 9.6	94.9 ± 8.7	**0.03**
Fitness			
VO_2max (ml/kg/min)	34.3 ± 5.9	38.7 ± 5.9	**<0.00001**
HR max (bpm)	183 ± 13	182 ± 8	0.51
Workload max (Watts)	270 ± 51	308 ± 48	**<0.00001**
Blood Pressure			
Systolic (mmHg)	122 ± 8	116 ± 9	**0.01**
Diastolic (mmHg)	79 ± 7	74 ± 9	**<0.01**

Data are means ± SD.
BMI, body mass index; FFM, fat free mass; VO_2max, maximum oxygen uptake; HR, heart rate;

There was a significant mean weight loss of -0.9 (1.0) kg after the 4 week exercise intervention. The inter-individual variability in weight change and body composition change is shown in **Figure 5.2** and **Figure 5.3** respectively**.** Weight change ranged from -2.8kg to +0.8kg over the 4 weeks.

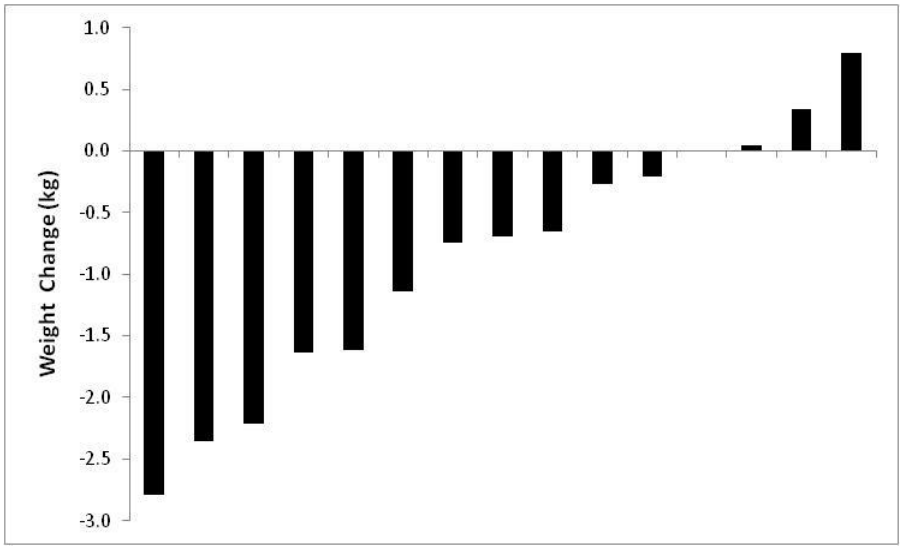


Figure 5.2 Variability in individual changes in body weight (kg) after the 4 week exercise intervention (n = 15).

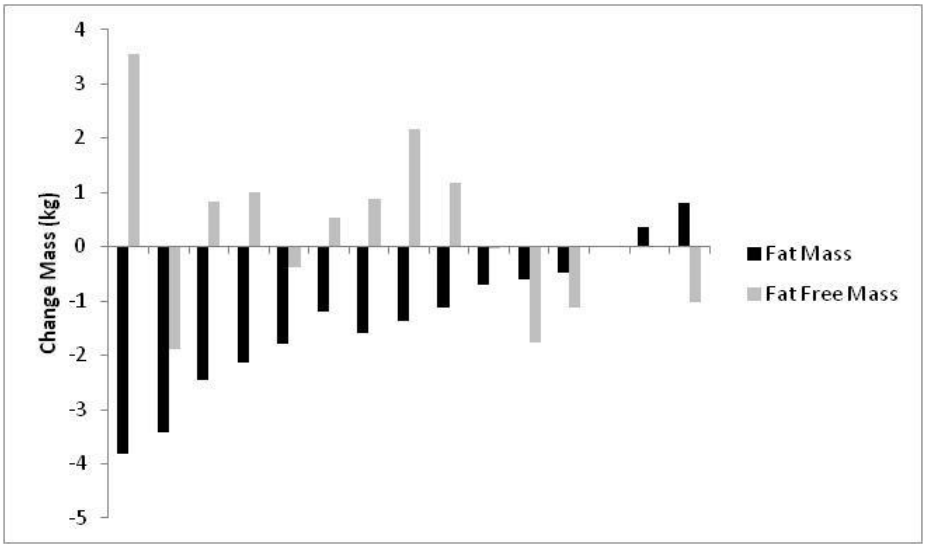


Figure 5.3 Variability in individual changes in fat and fat free mass (kg) after the 4 week exercise intervention (n = 15).

5.3.3 Participant Habitual Energy Intake and Physical Activity Characteristics

Habitual EI over the 24 hours prior to the GE test did not differ significantly between pre and post-test (**Table 5.2**). In addition, AEE and average time spent in physical activity in the week prior to the GE test did not differ between pre and post-test (**Table 5.2**). Due to two invalid accelerometery data sets, physical activity data is reported for n = 13.

Table 5.2 Participant energy intake and physical activity characteristics Pre and Post the 4 week exercise intervention (n = 15)

	Pre	Post	*P*-value
24h EI (kJ/d)	8597 ± 1970	8837 ± 1876	0.74
% Energy from:			
CHO	46 ± 11	43 ± 10	0.52
PRO	18 ± 4	20 ± 4	0.11
Fat	34 ± 11	33 ± 5	0.62
Physical Activity[1]			
Steps per day	6714 ± 2082	7914 ± 1670	0.22
AEE (kcal/day)	568 ± 196	702 ± 270	0.16
TEE (kcal/day)	2852 ± 373	3023 ± 420	0.28
Time in activity			
Vigorous (min/day)	5 ± 4	9 ± 8	0.27
Moderate (min/day)	42 ± 18	53 ± 26	0.23

Data are means ± SD. [1]Physical activity data refers to n =13
EI, energy intake; CHO, carbohydrate; PRO, protein; AEE, activity energy expenditure; TEE, total energy expenditure.
24hr EI refers to EI in the 24hrs prior to the GE test.

5.3.4 Gastric Emptying

Mean GE data at pre and post exercise intervention are presented in **Table 5.3**. These data indicate that GE did not significantly differ between pre and post exercise intervention.

Table 5.3 GE Time Based Parameters Pre and Post 4 week exercise intervention (n = 15)

	Pre (min)	Post (min)	*P*-value
t_{lag}	111 ± 17	110 ± 18	0.71
$t_{1/2}$	175 ± 22	179 ± 25	0.25
t_{lat}	37 ± 9	35 ± 8	0.09
t_{asc}	137 ± 17	144 ± 21	0.10

Data are means ± SD.
$t_{1/2}$, half time; t_{lag}, lag time; $t_{1/2s}$, t_{asc}, ascension time; t_{lat}, latency time.

A plot of individual changes in GE $t_{1/2}$ from pre to post test is shown in **Figure 5.4**. Six individuals had a faster $t_{1/2}$ at post test, ranging from 0.2 to 32.0 min faster. Eight individuals had a slower $t_{1/2}$ at post test, ranging from 5.5 to 25.0 min slower.

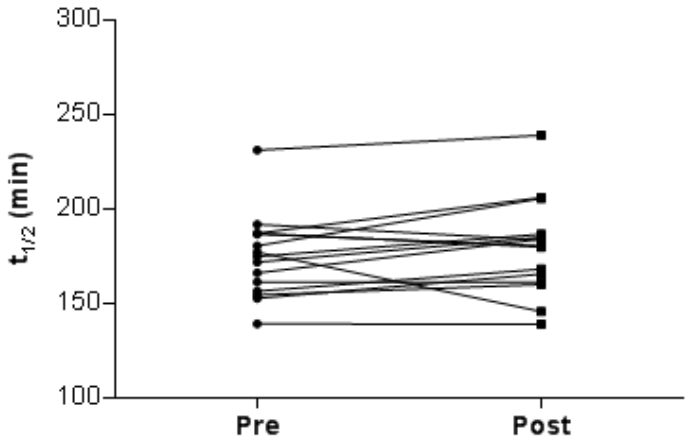
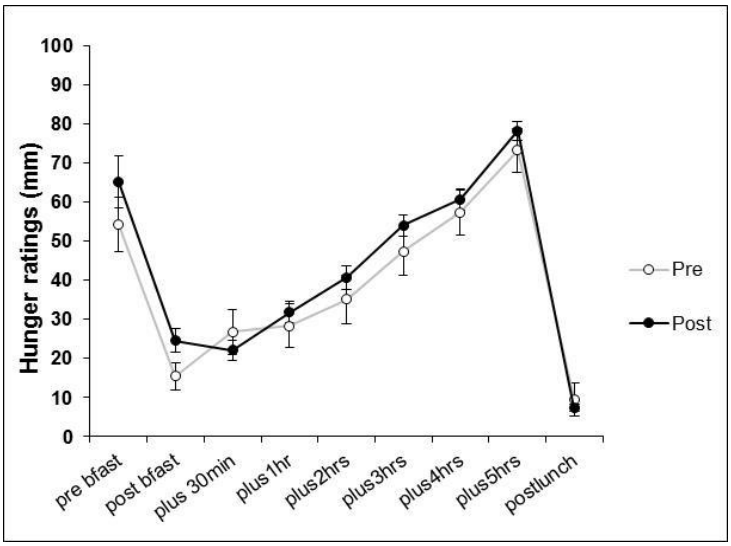


Figure 5.4 GE half time ($t_{1/2}$) pre and post the 4-week exercise intervention (n = 15). Each participant is represented by a solid line.

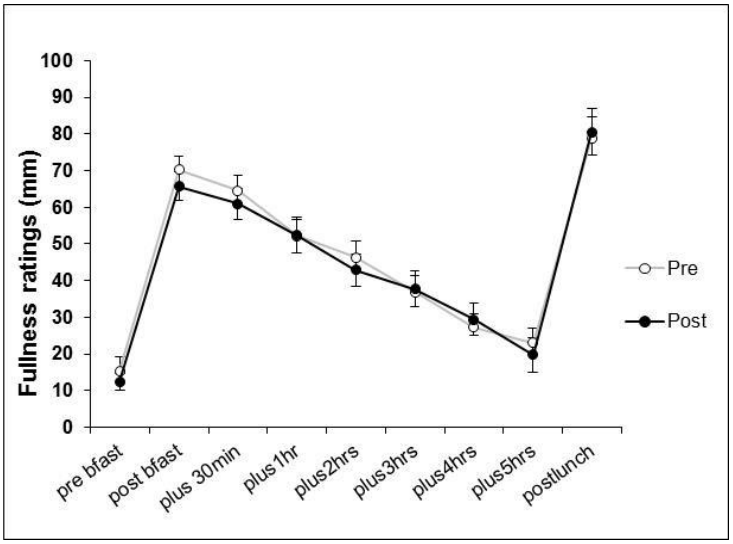
5.3.5 VAS Ratings

Subjective appetite ratings did not differ significantly between pre and post exercise intervention for fasting, mean 5h, 5h AUC and breakfast satiety quotient ($p > 0.05$, **Figure 5.5**). There was a trend towards an increased hunger SQ at 30mins post intervention ($p = 0.07$) indicating a trend towards a greater satiating efficiency of the breakfast meal at this time point. No other variables approached significance. Despite a mean increase between pre and post intervention of 10mm in fasting hunger ratings, this was not significant ($p = 0.14$). In addition, there were no significant differences between pre and post intervention for palatability ratings of the breakfast and lunch meals ($p > 0.05$ for all).

a)



b)



c)

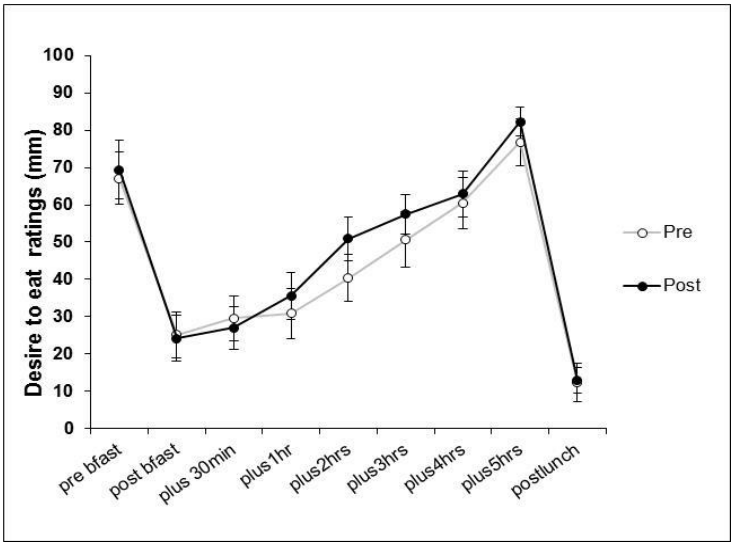


Figure 5.5 Mean (± SEM) subjective ratings for a) hunger, b) fullness and c) desire to eat over the course of the GE test morning pre and post the 4-week exercise intervention. n = 15.

5.3.6 Eating Behaviour

Dietary restraint (p = 0.18), disinhibition (p = 0.19) and hunger (p = 0.83) did not differ significantly between pre and post exercise intervention (**Figure 5.6**).

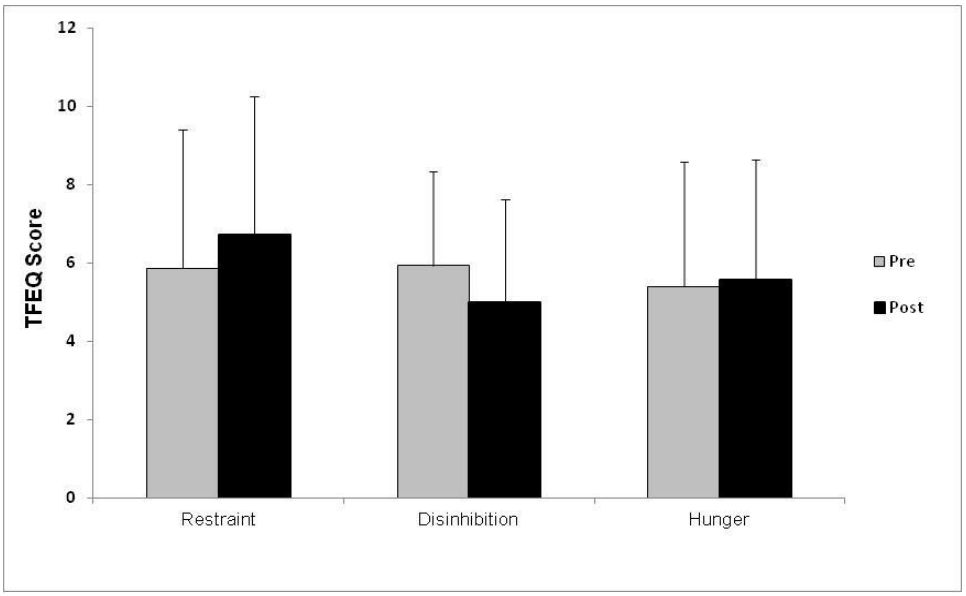


Figure 5.6 Dietary Restraint, Disinhibition and Hunger as assessed by the Three Factor Eating Questionnaire (TFEQ) Pre and Post the 4-week exercise intervention. (n = 15).

5.3.7 *Ad Libitum* Test Meal EI

EI at the *ad libitum* lunch test meal was significantly higher following the exercise intervention (Pre: 2978 ± 722 kJ, Post: 3695 ± 667 kJ, p = < 0.001). The amount of water consumed (Pre: 284 ± 109, Post: 242 ± 120 ml, p = 0.30) and the time taken to eat the meal (Pre: 11.1 ± 2.6, Post: 13.1 ± 3.9 min, p = 0.13) did not differ significantly.

5.3.8 Food Preferences, 'Liking' and 'Wanting'

No significant differences were found between pre and post exercise intervention for food preferences, except there was a small decrease in preference for LFSA foods post breakfast following the exercise intervention (p = 0.03) (**Figure 5.7**).

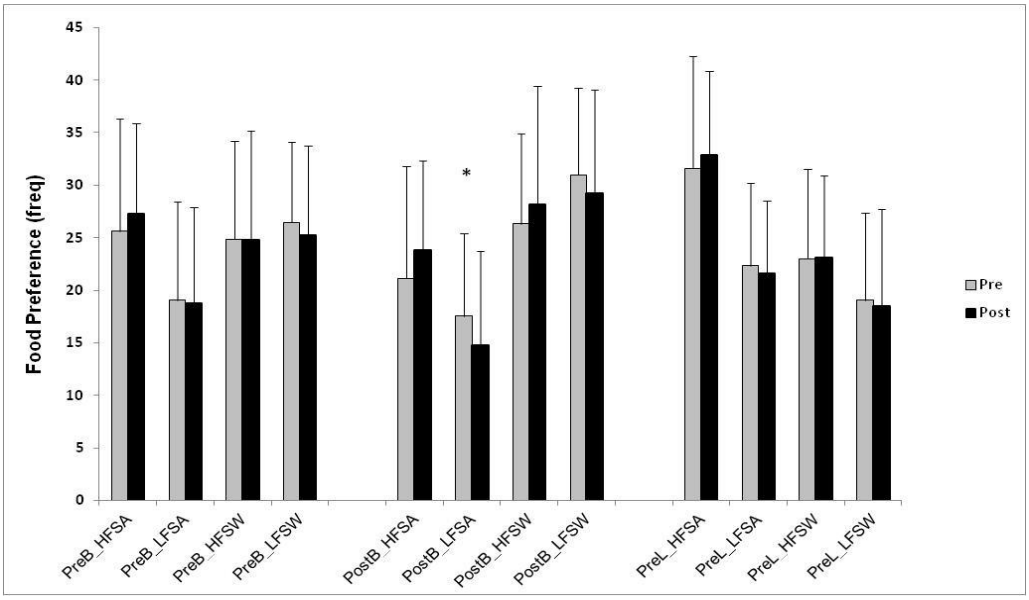


Figure 5.7 A comparison between pre and post exercise intervention of mean food preferences pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Food preferences were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. n = 15.

For 'liking' ratings, 'liking' for LFSW foods prior to breakfast was lower after the exercise intervention ($p = 0.02$, **Figure 5.8**) but no other changes were observed at any time points.

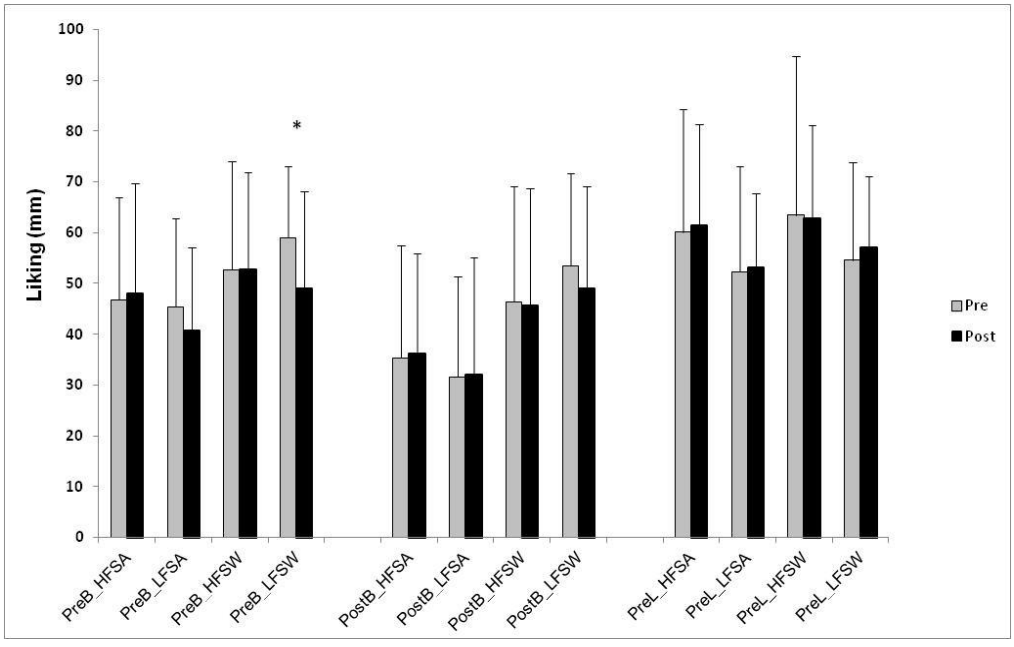


Figure 5.8 A comparison between pre and post exercise intervention of mean 'liking' for different foods assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. n = 15.

'Wanting' did not change between pre and post exercise intervention for any food categories (**Figure 5.9**).

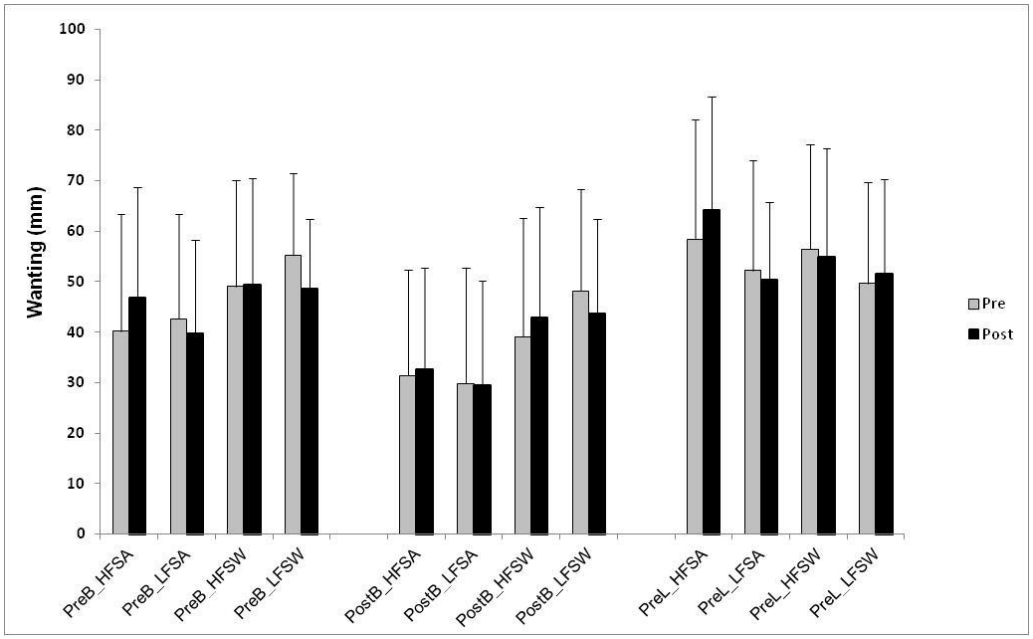


Figure 5.9 A comparison between pre and post exercise intervention of mean 'wanting' for different foods assessed pre breakfast (PreB), post breakfast (PostB) and pre lunch (PreL). Foods were divided according to frequency of choice for high fat savoury (HFSA), low fat savoury (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW) foods. Error bars indicate SD. * indicates $p < 0.05$. n = 15.

5.3.9 Relationships among anthropometric, AEE, GE and *ad libitum* EI changes

AEE was negatively correlated with GE at both pre test (t_{lag} r =-.56, p = 0.04; $t_{1/2}$ r = -.57, p = 0.04) and post test (t_{lag} r = -.63, p = 0.02; t_{lat} r = .66, p = 0.01; $t_{1/2}$, r = -.63, p = 0.02; t_{asc} r = -.58, p = 0.04). At pre test, there was no association between GE and *ad libitum* EI. However, at post test t_{lag} and t_{lat} correlated positively with *ad libitum* EI (t_{lag}, r = .59, p = 0.02, t_{lat}, r = .587, p = 0.02) indicating a slower initial GE was associated with increased EI at the lunch meal. In addition AEE, correlated negatively with *ad libitum* EI at post test (r = -.64, p = 0.01) but no association between AEE and *ad libitum* EI was evident at pre test.

Changes in GE and *ad libitum* EI were not correlated with any anthropometric or body composition variables at baseline. Change in t_{asc} was negatively correlated with change in AEE (r = -.67, p = 0.01, **Figure 5.10**) and similar negative correlations were found between change in t_{asc} and changes in steps per day (r = -.65, p = 0.02), mean time in vigorous activity per day (r = -.64, p = 0.02) and TEE (r = -

.68, p = 0.01). In addition, the change in absolute FFM was negatively correlated with *ad libitum* EI at the lunch test meal (r = -.604, p = 0.02, **Figure 5.11**).

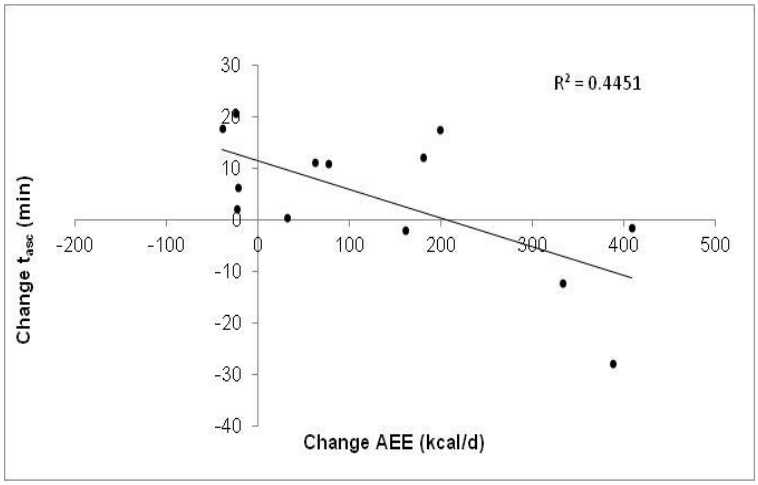


Figure 5.10 Correlation between change in AEE with change in t_{asc} after the 4 week exercise intervention.

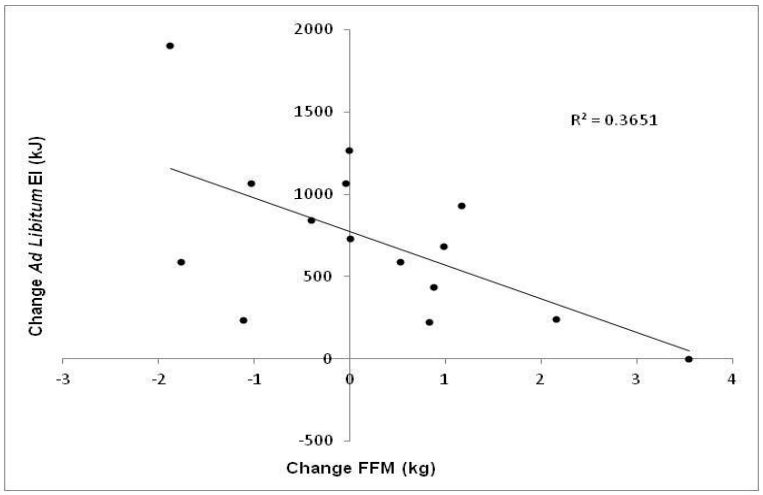


Figure 5.11 Correlation between change in absolute FFM (kg) and change in *ad libitum* EI at the lunch test meal after the 4-week exercise intervention.

5.4 DISCUSSION

Cross-sectional research indicates that GE is faster in habitually active compared to sedentary males [40, 202][Chapter 4]. However, the temporal and causal nature of this relationship cannot be determined from a cross-sectional analysis. The present study aimed to investigate the effects of a 4-week exercise intervention on GE in previously sedentary overweight and obese males. The findings demonstrate that a number of health markers improved but GE and subjective appetite ratings were unchanged following the 4-week intervention.

5.4.1 Gastric Emptying

Although many previous studies have examined the acute effects of exercise on GE [368], limited research has examined the longitudinal effects of exercise training on GE. No change in GE was observed following a 7-week intervention in adolescent girls [155]. However, the intervention consisted of three moderate intensity exercise sessions per week, which may not have been a sufficiently large volume of exercise. In the present study, compliance was high ($\geq$ 90% completion of all sessions), the intensity and frequency of sessions were high and each session was supervised in the laboratory. It can therefore be reasonably concluded based on the present findings that in the short to medium term (4 weeks), in the absence of acute exercise effects exercise training does not influence GE in overweight and obese males.

The present findings contrast with cross sectional evidence of faster GE in active compared to sedentary individuals [40, 202][Chapter 4], and may be explained by various reasons. In the study presented in Chapter 4, habitual exercisers were defined as individuals engaged in 4 or more exercise sessions per week for a minimum of 6 months, and in the study demonstrating faster GE in marathon runners, the runners were training for a mean 4.9 years [40]. Therefore, gut adaptations (i.e. faster GE) in response to regular exercise may only occur after a much longer period of time than the 4-week intervention in the present study. The findings from the present study indicate that faster GE is not an automatic compensatory response to four weeks of exercise training. Therefore, at least in the short-term, a significant improvement in fitness appears achievable without altering GE. Previous studies have demonstrated that in the shorter term exercise-induced EE

and EI are only weakly coupled (i.e. EI is not matched to increased EE) [11, 41], but when exercise is continued over several days, EI begins to partially track EE [419, 420]. These findings generate an optimistic view of the role of exercise in weight loss and weight control, as they indicate that EI is not automatically driven up to fully compensate for EE [41]. It is possible that a period of transition or uncoupling of EE and EI occurs before a steady state (coupling of EE and EI) is achieved [42]. The present findings indicate that GE is not a rapidly-acting mechanism to increase EI in response to exercise training.

It is also possible that GE may only respond to more substantial changes in body composition than observed in the present study. One hypothesis for evidence of no changes in EI with longer term exercise in overweight individuals [421-423] but increases in EI in lean individuals [424, 425] is that lean individuals may demonstrate a compensatory increase in EI to defend their relatively lower body fat reserves [368]. In support of this contention, post starvation hyperphagia is determined to a large extent by the degree of body fat and FFM depletion [426]. However, other evidence suggests habitual physical activity may influence GE and gut peptides independent of body composition or weight. In the study presented in Chapter 4, when activity was controlled for body composition was not associated with GE and body composition was not an independent predictor of GE. Further, changes in ghrelin and GLP-1, both of which are known to influence and be influenced by GE [35], have been demonstrated following just 5 days of exercise training (hence in the absence of significant weight loss) [211, 212]. In addition, Martins et al. (2010) [86] found no association between changes in gut peptide levels and the magnitude of weight loss following a 12 week exercise intervention. The authors highlighted that these data challenge the hypothesis that changes in gut peptides with exercise are part of a homeostatic compensatory mechanism to restore energy balance but cautioned that the sample size was small in their study for detecting such associations. As no studies could be found which have examined the longitudinal effects of more substantial exercise induced weight loss or changes in body composition on GE, further studies are warranted to investigate the hypothesis that GE may be one homeostatic compensatory mechanism contributing to changes in EI with longer term exercise.

It is also possible that faster GE in habitual exercisers is a correlate of another factor such as dietary habits, body composition or eating behaviour (e.g. dietary restraint), neither of which were significantly altered after the exercise intervention in the present study. However, in chapter 4, activity and AEE were independent predictors of GE whereas other factors including body composition were not.

A further explanation may be that differences in the time interval between the last exercise session and the GE test contributed to the contrasting findings. In the present study, the test was conducted ≥48h after the last exercise session whereas in the previous study (chapter 4) participants were asked to refrain from exercise for 24 hours before the GE test. In an early study examining the relationship between EE and EI, Edholm et al. (1955) observed that there was no correlation between EE and EI of individuals on the same day but there was a significant correlation between EE and EI 2 days later [406]. It is possible therefore that in the study presented in chapter 4, GE may have been influenced by exercise 2 days beforehand, whereas in the present study, exercise was avoided for a minimum of 2 days before the GE test. The contention that the time interval between the GE test and last exercise session may influence findings is supported by evidence of differences in insulin sensitivity depending on the time measured following 2 weeks of a HIIE intervention in 10 sedentary overweight and obese men [427]. Insulin sensitivity was higher when measured 24 hours post intervention but this was no longer significant when measured 72 hours post intervention [427]. Future studies conducting GE tests at different time intervals following the last exercise session are warranted to investigate this possibility. It can be concluded based on the present findings that GE (at least in the absence of strenuous exercise for 2 days beforehand) is not significantly influenced by 4 weeks of exercise training.

5.4.2 Appetite Ratings and *Ad Libitum* Test Meal EI

In addition to GE, subjective appetite sensations were unchanged after the 4-week intervention in the present study. This is consistent with some previous studies showing no change in subjective appetite ratings following both 7 days and 14 days of exercise in lean men and women [419, 420]. Stubbs et al. (2002) [420] concluded that following 7 days of an exercise induced EE of 1.6MJ/d and 3.2MJ/d, lean men appeared able to tolerate a considerable negative energy balance induced by exercise

without invoking compensatory responses in appetite. When a similar protocol was extended to 14 days of both moderate and high exercise levels, Whybrow et al. (2008) [419] similarly observed no changes in subjective appetite ratings. Others have also shown no change in subjective appetite ratings following a 6 week intervention despite improvements in short term EI regulation [411].

In contrast to these studies, changes in subjective appetite ratings have been documented after longer-term exercise interventions. Following a 12 week exercise intervention in 58 overweight and obese individuals fasting hunger levels increased while at the same time exercise improved satiation and satiety independent of weight loss [99]. Others have since shown similar findings [86]. Trends observed in the present study would support these findings but the changes observed were small and not statistically significant. Mean fasting hunger ratings were approximately 10mm higher following the 4-week intervention. Similarly, there was a trend towards a higher hunger SQ 30minutes post breakfast after the exercise intervention. This would indicate a trend towards a greater satiating efficiency of the breakfast meal. However, this was observed at this time point only. King et al. [99] on the other hand observed a higher satiating efficiency throughout the 4 hour postprandial period following 12 weeks of exercise [99]. Collectively, the present findings suggest that subjective appetite ratings might only respond to a longer period of exercise training than the 4-week intervention in present study. In addition, as highlighted by the reproducibility study presented in Chapter 3 larger sample sizes are necessary to detect changes in subjective appetite ratings of the magnitude observed in the present study.

Despite no significant changes in appetite ratings, there was a small but significant increase in *ad libitum* EI at the lunch test meal (mean 717kJ higher) following the exercise intervention. Others have shown partial compensation in EI of approximately 30% for exercise induced EE following 14 days of high exercise levels in lean men [419]. Whybrow et al. 2008 [419] concluded that these findings appeared to capture the first stages of an increase in EI to match large increases in exercise induced EE. Although, in the latter study subjects were resident in a human nutrition unit for the duration of the experiment and daily food intake was measured, the findings of a small but significant increase in EI at the *ad libitum* lunch in the present study could also be indicative of a partial compensation in EI. As GE was

unchanged, other factors may contribute to the change in *ad libitum EI* at the lunch test meal. A decrease in leptin has previously been shown to be associated with an increase in *ad libitum* EI in non-obese individuals [428] and a decrease in leptin has been demonstrated to occur in response to a 12 week intervention [214]. While leptin and appetite related gut peptides were not reported in the present study, reduced leptin levels represent one potential mechanistic explanation for the increase in *ad libitum* test meal EI following the exercise program in the present study. In addition, changes in cognitive factors such as attitudes and beliefs (e.g. exercise makes you hungry), a desire for self-reward after exercise and misjudgements about the amount of energy expended relative to EI [41, 42] could have a role. As GE was unchanged, it is important to address other factors, which may impede weight loss when individuals commence a physical activity program for weight management.

5.4.3 Food Preferences, 'Liking', 'Wanting' and Eating Behaviour

Changes in EI with exercise could also be explained by changes in macronutrient preferences, food choice or processes of food reward in response to exercise. In the present study, no changes in habitual macronutrient preference were detected by diet recall which is comparable with evidence of no consistent changes in macronutrient preferences with exercise [429]. With regard to food preferences, data from the computer based procedure [339] revealed the only significant change was a slightly lower preference for low fat savoury foods post breakfast following the intervention. The significance of this change is unclear as it occurred at this time point only and the change was small. With regard to 'liking' and 'wanting', 'liking' for low fat sweet foods pre-breakfast was lower following the exercise intervention but no changes were found when assessed post breakfast or prior to lunch.

Others have assessed changes in food reward in response to an acute exercise bout before and after a 12 week exercise intervention [321]. Acute increases in food preference after an exercise bout were associated with less weight loss [321]. However, it is not possible to compare these findings to the present study as responses were not measured in response to an acute exercise bout in the present study. In comparison to the cross sectional study presented in Chapter 4, where habitually active individuals had a higher preference for low fat savoury foods, a

lower 'liking' for low fat sweet foods and a higher dietary restraint, similar findings were not observed in the present study as sedentary individuals progressed to a physically active lifestyle. One explanation is that the differences observed in habitual exercisers could have occurred after a long period of time. In addition, the lack of significant change in food preference may be related to a lack of significant change in dietary restraint or disinhibition. Evidence has shown the effect of exercise on food preference can depend on eating behaviour traits [331, 368, 430] and both dietary restraint and disinhibition were unchanged following the 4-week intervention in the present study. However, although not statistically significant mean values for restraint were higher and disinhibition were lower. Others have shown increased dietary restraint and reduced disinhibition in response to longer duration (10-14wk) exercise interventions in overweight and obese individuals [431, 432]. The present findings while not statistically significant are in the same direction as those that have been reported previously [431, 432] and could suggest a larger sample size or longer duration of intervention than the 4 weeks in the present study may be required to detect significant changes in eating behaviour traits.

5.4.4 Body Composition, Blood Pressure and Fitness

The 4-week intervention had a positive impact on a number of health markers including body composition, blood pressure and fitness. Albeit small changes, body weight (-0.9 (1.1) kg), waist circumference (-2.3 (3.5) cm) and percent body fat (-0.9 (1.1) %) were significantly lower following the intervention, while fat free mass was maintained. In addition, both systolic (-6.2 (8.4) mmHg) and diastolic (-5.8 (2.2) mmHg) blood pressure were reduced and there was a mean 12.8% increase in VO_2max (+4.4 (2.1) ml/kg/min).

Emerging research has pointed to the efficacy of HIIE for improving body composition and health markers compared to other types of exercise [413]. Significant improvements in insulin sensitivity and a 7% increase in VO_2max have previously been demonstrated after just two weeks of three HIIE sessions per week in 10 overweight and obese sedentary males [427]. In the present study, VO_2max increased by approximately 13% following 4 weeks of exercise. The present findings are consistent with findings of significant increases in VO_2max of between 4 and 46% in HIIE programs lasting from 2 to 15 weeks [413]. Given the considerable

health benefits associated with small increases in cardio-respiratory fitness [433] (e.g. an increase in physical fitness of 1 MET (relative VO_2 3.5 ml/kg/min) has been demonstrated to be associated with a mortality benefit of about 20% [434]) the changes in cardio-respiratory fitness observed with 4 weeks of exercise in the current study are likely associated with many health benefits.

Previously, it has been demonstrated that VO_2max, blood pressure and waist circumference significantly improved both in individuals who achieved significant weight loss and in those who achieved a less than expected weight loss following a 12 week intervention [262]. King et al. (2009) [262] concluded that the data demonstrated that meaningful health benefits can be achieved independent of any change in body weight. The present findings add further support to these observations by demonstrating that following 4 weeks of exercise and in the absence of large weight losses, significant improvements in health markers are clearly evident.

5.4.5 Relationships between variables

As others have shown [12, 435-437], individual variability in responses to the exercise intervention were evident in the present study. Weight change ranged from -2.8kg to + 0.8kg over the 4 week intervention. In addition, changes in body composition differed between individuals. However, the changes in body weight and composition were not associated with changes in GE, appetite or EI. It is likely that a larger sample size would be required to detect such associations. Following a 12 week exercise intervention in 15 overweight and obese individuals, Martins et al. (2010) similarly found no associations between the magnitude of weight change and changes in appetite sensations or appetite related gut peptides, and suggested more power would be required to detect such associations [86].

Similar to the cross sectional study presented in Chapter 4, AEE was negatively correlated with GE times at both pre and post test in the present study. In addition, when changes in physical activity variables and GE were correlated, change in GE ascension time was negatively correlated with change in AEE, steps per day and time in vigorous activity indicating an increase in activity was associated with faster GE. The change in absolute FFM was the only variable associated with change

in EI at the *ad libitum* lunch test meal. Change in FFM was negatively associated with change in EI which contrasts with evidence of an association of increased FFM with increased EI previously demonstrated in a large cohort of overweight and obese individuals [36]. However, the present findings should be interpreted with caution due to the small sample size for correlations in the present study.

5.4.6 Methodological Considerations

As highlighted in the previous discussion, one limitation of the present study is that it was not powered to detect small but potentially relevant changes in some variables such as certain subjective appetite variables, eating behaviour traits and associations between variables. The findings of the reproducibility study presented in Chapter 3 for example showed that to detect a 10mm change in fasting hunger ratings, 65 participants would be needed whereas 11 participants would be needed to detect a 10mm change in mean ratings. The present study was powered to detect changes in the majority of variables and primarily GE as the primary outcome measure. Although a control group was not included, the study was powered to detect changes that would be considered clinically significant and outside of the normal day to day variability in these measures, based on findings from the reproducibility study presented in Chapter 3. The sample size of 15 participants was sufficient to detect at least an 8% change in GE half and lag times. Therefore, sample size was not a factor explaining the lack of significant change in GE in the present study. However, it is important to acknowledge that in the reproducibility study, the two tests were undertaken seven days apart. This is a shorter duration than between the two test days (pre and post the four-week intervention) in the present study and therefore it is possible the reproducibility of GE over this time frame may be different. Future studies including a control group would be ideal.

A limitation of this study when comparing findings to the cross sectional study presented in Chapter 4 is that participants were instructed to avoid exercise for different intervals of time prior to the GE test as highlighted earlier in this discussion. In the cross sectional study, participants were instructed to avoid exercise for 24 hours beforehand which is a common protocol used and has previously been used in studies examining appetite control in habitual exercisers [249]. However, in intervention studies examining the effects of exercise training on gut peptides, post

testing was conducted at 36 hours[211, 212] or > 48hours [86, 214] after the last exercise session. As a result the post GE test took place ≥ 48 hours after the last exercise session in the present study. Although this is a limitation when comparing the findings with the study presented in chapter 4, the strengths of using this protocol means findings are more applicable to compare to previous intervention studies examining changes in gut peptides with exercise [86, 211, 212, 214].

An additional methodological consideration in the present study is the mode of exercise. The exercise intervention consisted solely of indoor cycling on a cycle ergometer to minimise any potential confounding effects of mode of exercise on GE [243]. As a result the present findings cannot be extrapolated to other forms of exercise e.g. resistance training.

Changes in physical activity throughout the week prior to the GE test (including outside of the exercise sessions) could also have influenced the GE responses to the exercise intervention. However, while not statistically significant mean increases in physical activity and AEE were evident between pre and post test suggesting participants did not significantly compensate in terms of reduced activity outside of the intervention. One limitation which is evident retrospectively however is that in the week prior to the GE test, participants completed only 3 exercise sessions. Due to scheduling constrictions, the 4 week exercise program commenced and finished midweek and exercise sessions took place on weekdays. As a result 2 out of the 7 days prior to the GE post test were a weekend and hence exercise sessions were not scheduled and for 2 other days participants were instructed to avoid exercise prior to the GE test. Given the associations between AEE and GE evident in Chapter 4 and in the present study it cannot be discounted that a greater increase in the number of exercise sessions in the 7 days before the GE test may yield different findings. In addition, EI was only assessed prior to and post the exercise intervention, therefore it cannot be discounted that changes in habitual EI during the intervention influenced the findings. This is a common issue in exercise intervention studies as the reduced sensitivity of most available methods of assessing EI represents an ongoing challenge. Underreporting of EI is common in some individuals, regardless of the method [438]. Future studies involving quantitative and objective measurements of EI such as measuring EI over the course of a probe day

where all meals are provided to participants [36] may yield further information on associations between EI, GE and other variables.

The exercise intervention used in the present study (20 sessions over 4 weeks including HIIE) represented a considerable change in lifestyle for sedentary individuals. Although compliance was high in the present study a question that arises is how applicable the intervention would be outside of the laboratory environment. The optimal protocol for HIIE is still a matter of debate. The most commonly used protocol has been the Wingate test (30 second all out sprints), however it is likely unsuitable for most overweight sedentary individuals [413]. Studies have used various interval protocols ranging from 8 seconds cycle sprint followed by 12 seconds low intensity cycling [439] to a 2 minute cycle sprint followed by 15 seconds of low intensity cycling for a period of 20 minutes [440]. In the present study, the HIIE protocol of alternating between 30 seconds cycling at 100% VO_2max and 30 seconds of low intensity cycling for 30 minutes in week 1 was achievable. However, the participants found the sessions particularly difficult at the beginning of the intervention and would likely struggle to maintain adherence to such an intervention outside of the supervision of the laboratory environment. Others have shown that health benefits can be achieved with less intense protocols to that used in the present study [427], and therefore these protocols may have more ecological validity. Nevertheless, there is no consensus on the optimal protocol for HIIE for overweight and obese sedentary individuals and more research is needed to identify the optimal length and intensity of intervals for achieving varying health outcomes [413]. As the primary aim of the present study was to assess the effects of exercise training on GE and associated variables, a large volume of exercise was necessary to ensure physical activity was the major lifestyle change. In addition, each exercise session was supervised to minimise any confounding effect of adherence on the outcomes. Given the large volume of exercise and that each exercise session was carefully monitored these methodological aspects minimised the influence of other confounding factors on GE and thus increase the validity of the present findings with regard to the primary aim.

5.4.7 Summary and Future Directions

The findings from the present study demonstrate that GE is unchanged following 4 weeks of supervised exercise training in overweight and obese males. In addition, habitual EI, subjective appetite ratings, eating behaviour, food preferences and processes of food reward were largely unchanged but there was a small but significant increase in *ad libitum* EI at the lunch test meal. As GE was unchanged, it is important to address other factors, which may impede weight loss when individuals commence a physical activity program for weight management. Despite no changes in GE or appetite ratings, a number of health markers were significantly improved following the intervention. Albeit small, body weight, waist circumference and percent body fat were significantly reduced, while fat free mass was maintained. In addition, both systolic and diastolic blood pressure were significantly reduced and there was a significant increase in cardiorespiratory fitness. These findings highlight the health benefits that can be achieved with short-term exercise interventions.

Overall, the present findings indicate that at least in the short-term, a significant improvement in fitness appears achievable without altering GE. Whether changes in GE are different if measured 24 hours after the last exercise session remains to be established. In addition, future studies examining changes in GE, appetite and EI with longer term exercise interventions and in response to exercise induced weight loss are needed to further understand the temporal patterns of changes in GE, appetite and EI with exercise.

Chapter 6: General Discussion, Future Directions and Conclusions

The main focus of this thesis is concerned with developing an understanding of the influence of habitual physical activity level and short-term exercise training on processes implicated in appetite control, including gastric emptying (GE). In addition, this thesis seeks to examine the associations amongst energy expenditure (EE), body composition, GE, appetite and energy intake (EI). In this chapter, the findings from the preceding chapters are first summarised, the implications and methodological aspects of the thesis studies are then discussed, areas for future research are proposed and finally conclusions from the thesis are provided.

6.1 SUMMARY OF LITERATURE REVIEW AND EXPERIMENTAL STUDY FINDINGS

Chapter 2 highlights emerging evidence from some surgical procedures (e.g. sleeve gastrectomy and Roux-en-Y gastric bypass) indicating that in addition to gastric restriction [170], a faster emptying rate and earlier delivery of nutrients to the distal small intestine may improve appetite control [59, 61-63, 176]. The literature also suggests that energy restriction appears to slow GE [24, 162, 200, 201] and results in a blunted release of gut peptides - a response implicated to increase hunger [82, 204, 205, 207, 208]. However, with regard to habitual exercise, research examining GE was limited to only two cross sectional studies. These studies involved marathon runners [40] and the elderly [202], and provided little information on the characteristics of subjects. The review concluded that a better understanding of the effects of behavioural weight loss interventions such as exercise on GI targets of appetite control may be useful to improve the success of lifestyle interventions in weight management.

Three experimental studies were undertaken in this thesis, the first a methodological study, the second a cross-sectional study and the third a longitudinal study. A summary of the more significant specific findings is provided in **Figure 6.1**.

Specific findings from the three studies

Study 1 – *Chapter 3*

- GE is reproducible in overweight and obese males (CV_{intra} $t_{1/2}$ = 7.9%)
- A minimum of 10 participants is sufficient to detect a 10% change in GE $t_{1/2}$ in a paired design study in overweight and obese males
- VAS, an *ad libitum* lunch test meal and the LFPQ are sufficiently reliable for investigating changes in appetite ratings, *ad libitum* EI, food preferences and processes of food reward in overweight and obese males

Study 2 – *Chapter 4*

- GE is faster in habitually active males participating in a range of activities compared to sedentary males.
- GE is associated with postprandial fullness in active males but not in sedentary males.
- Active males have a higher dietary restraint and higher preference for low fat savoury foods compared to sedentary males.
- A higher percent BF and FM is associated with slower GE. These associations disappear after controlling for physical activity status. These data suggest an association between body composition and GE might be mediated by physical activity level.
- AEE is inversely associated with GE half time. Together AEE and physical activity status (active or sedentary) explain 34% of the variance in GE half time in a cohort of active and sedentary males. No other variables measured were significant independent predictors of GE half time.

Study 3 – *Chapter 5*

- GE was unchanged after 4 weeks of supervised exercise in previously sedentary overweight and obese males when measured 48 - 96 hrs post exercise.
- A number of health markers including waist circumference, body fat, weight, blood pressure and cardio respiratory fitness improved in response to the exercise intervention.

Figure 6.1 Summary of specific findings from the three thesis studies

The first experimental study presented in **Chapter 3** was a methodological study. The study aimed to determine the day-to-day variability of GE in overweight and obese males. This was necessary before undertaking further studies, to provide information for the design of studies which aim to investigate changes in GE in this population. Previously the day-to-day variability of GE in overweight and obese individuals had not been reported. Although it was hypothesised that the reproducibility of GE might be different in overweight and obese compared to lean individuals, the data demonstrate a mean intra-individual coefficient of variation of ~ 8% for GE half time in healthy overweight and obese males which is comparable to findings in lean individuals using test meals similar in energy content [276, 280, 283-285, 288, 294]. The 95% limits of agreement for GE $t_{1/2}$ between repeat visits were between -35.9 and 42.1 min. Other measures including subjective appetite ratings, *ad libitum* lunch test meal EI, food preferences and processes of food reward were found to be reproducible with no mean differences between test days for any parameters. Sample size calculations based on the day to day variations observed revealed a minimum of 10 participants is sufficient to detect a 10% change in GE half time in a paired design study and a minimum of 13 is sufficient in an unpaired design study with a power of 80% and $\alpha = 0.05$. In addition, the findings demonstrate that for some outcome measures the variability varies depending on the parameters reported.

The second experimental study presented in **Chapter 4** used a cross sectional design to examine the effects of habitual exercise on GE, appetite and EI. The findings demonstrate that GE is significantly faster in active compared to sedentary males. Active individuals reported participating in a range of types of activities, indicating that a faster GE with habitual physical activity is not limited to a specific type of exercise. In addition, AEE was negatively correlated with GE half time. Moreover, habitual activity and AEE were the only variables found to account for variance of GE half time in multiple regression analysis. These data further highlight the association between higher levels of physical activity and faster GE. Appetite ratings were not different between groups, but both habitual and *ad libitum* test meal EI were significantly higher in the active compared to the sedentary group. Postprandial AUC fullness ratings and lunch EI were associated with GE in the

active group, but not in the sedentary group. In addition, the active individuals had higher levels of dietary restraint, and a higher preference for low fat savoury foods. The influence of body composition on GE and appetite was also examined. Higher percent BF and FM was associated with slower GE. However, after controlling for physical activity status this association was no longer evident, suggesting that the association between GE and body composition might be mediated by differences in physical activity level. In addition, a comparison of four subgroups of the entire cohort categorised as lean (BMI: 18 – 25 kg/m^2) sedentary and lean active, overweight/obese (BMI: 27 – 34 kg/m^2) sedentary and overweight/obese active was undertaken. Although, a robust analysis was limited due to the small sample sizes (n = 6) in two of the sub groups, there was less than 3 min mean difference in GE half time between lean sedentary (n = 6) and overweight sedentary (n = 10) individuals. Whereas mean GE half time in overweight active (n = 6) and lean active (n = 16) individuals were 27 and 24 minutes shorter respectively than in the two sedentary subgroups. Together, these findings suggest slower GE is a marker of a sedentary lifestyle and that in the absence of differences in physical activity level, GE may not be influenced by body composition or BMI.

The third experimental study presented in **Chapter 5** used a longitudinal design to examine the effects of progressing from a sedentary to a physically active lifestyle on GE, appetite and EI. It was hypothesised that short-term exercise training (a 4 week intervention) would influence GE, based on prior evidence of changes in appetite related gut peptides in response to exercise [86, 211, 212]. Contrary to this hypothesis however, GE and appetite were unchanged despite significant changes in many other health markers following the exercise intervention.

6.2 PERSPECTIVES AND IMPLICATIONS

The findings from the reproducibility study presented in **Chapter 3** have implications for both clinical and research settings. The 95% limits of agreement for GE $t_{1/2}$ illustrate the variability that can occur in some individuals from day to day, and are useful to take into account when assessing individual changes in GE in clinical settings. In addition, the sample size calculations based on the day to day variations observed demonstrate that relatively small sample sizes are sufficient to detect clinically relevant changes in GE. As GE studies are often carried out in small numbers e.g. measured pre and post surgical procedure, these findings illustrate the

potential efficiency in undertaking smaller studies before larger studies are undertaken. Collectively, the findings from this study provide valuable information for research planning and support the use of these methods to evaluate changes in GE and associated measures in overweight and obese males in research and clinical settings. The immediate outcome of these findings for this thesis is that the sample size calculations informed the design of the subsequent studies to ensure they were appropriately powered.

The findings from the cross sectional study presented in **Chapter 4** have implications for an increased understanding of processes contributing to appetite control and weight regulation. Over the last 30 years a number of studies have investigated the hypothesis that GE has a role in obesity (see **Table 4.1** page 79**)**, and have shown inconsistent findings. Obese and non-obese groups have been categorised based on BMI or ideal body weight [15-18, 23-27, 29, 153-156, 200, 360-363, 441] and physical activity or body composition (FM and FFM) tend not to be controlled for. The results from Chapter 4 indicate that GE is faster in habitually active individuals, and that in correlation analyses GE is associated with AEE and body composition but not with BMI. The findings suggest slower GE is a marker of a sedentary lifestyle. It is hypothesised based on these findings that differences in participants' habitual physical activity levels may explain some of the inconsistency in findings of studies examining the role of GE in obesity. The findings appear significant as no previous studies could be found which have investigated the associations amongst habitual physical activity level, body composition, EE and GE. These findings highlight the importance of controlling for physical activity in studies examining GE (and parameters that may be influenced by GE) in various conditions, including obesity.

In addition, the implications of these findings for EI and weight management are worth considering. An obvious question that arises is whether it is beneficial to accelerate or delay GE for weight management? Perhaps, due to the inconsistency of findings from studies examining GE in obesity, there appears to be no concrete answer. Some therapies have been designed to slow GE to enhance gastric distension [442, 443] whereas others have been designed to accelerate GE thus targeting intestinal factors [161, 192], both with the aim to increase satiety. While causal relationships cannot be drawn from the cross-sectional study in Chapter 4, the

findings from this thesis and literature review allow for comment on the potential implications of manipulating GE for weight management. GE was faster in active males and it is therefore hypothesised that a faster GE could have a beneficial role in assisting in appetite control and weight maintenance with exercise.

One hypothesis on the role of exercise in weight maintenance is that exercise sensitises the physiological mechanisms involved in appetite control [11]. Unlike energy restriction, where it can be difficult to maintain weight after weight loss [444], studies consistently indicate exercise is crucial for weight maintenance [9]. In Mayer's 1956 study [10] of 213 workers in West Bengal, he demonstrated that food intake increased with activity only within a certain activity zone. In the sedentary range of occupations a decrease in activity was not followed by a decrease in EI but an increase. Mayer's findings are portrayed in **Figure 6.2** modified by Blundell (2011) [445]. Blundell [445] highlighted that people living in the 'zone of dysregulation' are at a greater risk of overeating than people who are more active due to the lack of physiological regulation that occurs within the 'sedentary range'. This contention is supported by findings from **Chapter 4** which indicate that postprandial fullness and EI are associated with a physiological process (GE) in the active group but not in the sedentary group.

Image removed for copyright reasons (Blundell, J.E., *Physical activity and appetite control: can we close the energy gap?* Nutrition Bulletin, 2011. **36**(3): p. 356-366.)

Figure 6.2 The relationship between energy expended in different occupations and food intake in calories per day in a study of 213 workers in West Bengal as found by Mayer et al. (1956) [10]. This figure is taken from Blundell (2011) [445] and adapted to show an interpretation of Mayer's data in light of more recent evidence on the impact of actively changing physical activity on accurately measured food intake

It is possible that a slower GE could be a predisposing factor to weight gain through a delayed or reduced release of gut peptides signalling satiety from the intestine [29]. Upper-gut hormonal profiles have been demonstrated to generally reflect changes in GE and a more rapid GE has been directly correlated with increases in gut peptides associated with increased satiety [25, 61, 130, 134, 135]. Indeed some evidence suggests a threshold rate of GE exists which must be exceeded to stimulate GLP-1 release [130]. The release of these satiety signals in response to a faster GE may prevent overeating and mean that food intake in active individuals is better regulated in response to physiological signals rather than other factors overriding sensations of satiety. In sedentary individuals, in contrast, a slower GE could result in the inactivation of GI signals and mean that other factors such as sensory cues or social values are more likely to influence EI. Exercise training could therefore serve to normalise or regulate GE as has been demonstrated with fasting blood glucose levels [446].

While it is clear from Chapter 4 that not all sedentary individuals with a slower GE are overweight, it is possible that a slower GE associated with a sedentary lifestyle reduces the sensitivity of appetite control and could increase the susceptibility to weight gain. The active group (Chapter 4) appears to have a more precise regulation of energy balance. Despite a faster GE, higher *ad libitum* test meal EI and a higher habitual EI, the active group had a lower body weight and lower percent BF than the sedentary group. Due to the cross-sectional design of this study, a causal link between EI and GE cannot be concluded, and it is possible that a faster GE may be a consequence of a higher habitual EI. However, habitual EI did not account for any variance in GE in multiple regression analysis, whereas AEE did. In addition, there was an inverse association between AEE and GE. Given evidence suggesting that GE influences appetite and EI [115-118], these data support the hypothesis that faster GE with habitual exercise may be a physiological mechanism to maintain energy balance in active individuals.

Of the factors measured in Chapter 4, in addition to a faster GE, the active group also had a higher dietary restraint and higher preference for low fat savoury foods compared to the sedentary group. Further, factors such as greater leptin and insulin sensitivity which were not measured in this thesis, have been reported as having a role in improved appetite control with physical activity [39]. Therefore, it is

proposed that a faster GE, among a combination of factors, contributes to the control of EI in active individuals.

Given that an increasing number of strategies are targeting the GI tract for the treatment of obesity, it seems pertinent to establish a better understanding of GI mechanisms of appetite control. Overall, findings from this study suggest slower GE is a marker of a sedentary lifestyle indicating it is important for studies examining GE (and parameters that may be influenced by GE) to take into account physical activity levels of individuals as potential confounding factors. The findings raise a number of questions and hypotheses for future investigation that could have ramifications for weight management strategies. These are discussed in Section 6.4 of this chapter.

The findings from the study presented in **Chapter 5** indicate that faster GE is not an automatic compensatory response to four weeks of exercise training. Therefore, at least in the short-term, a significant improvement in fitness appears achievable without altering GE. Previous studies have demonstrated that in the shorter term exercise-induced EE and EI are only weakly coupled (i.e. EI is not matched to increased EE) [11, 41], but when exercise is continued over several days, EI begins to partially track EE [419, 420]. These findings generate an optimistic view of the role of exercise in weight loss and weight control, as they indicate that EI is not automatically driven up to fully compensate for EE [41]. It is possible that a period of transition or uncoupling of EE and EI occurs before a steady state (coupling of EE and EI) is achieved [42]. The present findings indicate that GE is not a rapidly-acting mechanism to increase EI in response to exercise training. Other factors, which were not measured in this thesis, may contribute to the change in *ad libitum EI* at the lunch test meal and the inter-individual variability in weight change observed. These dimensions may include changes in cognitive factors such as attitudes and beliefs (e.g. exercise makes you hungry), a desire for self-reward after exercise and misjudgements about the amount of energy expended relative to EI [41, 42]. These factors were not examined in the present study. As GE was unchanged, it is important to address other factors, which may impede weight loss when individuals commence a physical activity program for weight management.

The findings from the final study also demonstrate a significant improvement in a number of health markers in the absence of substantial weight loss (mean weight loss of 0.9kg). Cardiorespiratory fitness increased by 12.8% (mean 4.4 ml/kg/min increase) following the intervention. Given an increase in physical fitness of 1 MET (relative VO_2 3.5 ml/kg/min) has been demonstrated to be associated with a mortality benefit of about 20% [434], the present findings add further support to evidence that marked improvements in health can be achieved in the absence of large weight loss [262]. These findings provide further rationale for promoting the health benefits of exercise and not using weight loss as a single measure of success.

6.3 METHODOLOGICAL ISSUES

A number of methodological considerations have been highlighted throughout this thesis and are discussed in detail in each individual chapter. A summary of these methodological considerations is provided in **Figure 6.3**, which are subsequently discussed in this section.

Methodological Considerations

- The ^{13}C-OBT does not allow direct imaging of gastric function. Studies using scintigraphy to examine the influence of body composition and habitual exercise on GE would be useful to confirm the findings of the studies presented in this thesis.
- More sensitive measures of physical activity or EI may explain a greater variance in GE.
- In Chapters 4 and 5, GE and appetite are assessed >24hrs and ≥ 48hrs after the last exercise session respectively. Studies conducting GE tests at different time intervals are warranted to investigate the possibility that changes in GE (and parameters influenced by GE) might differ depending on the time interval from the last exercise session.
- Gut peptides are not measured in this thesis due to cost limitations. Further studies should test GE in conjunction with gut peptides where feasible.
- Appetite control is a complex integration of a number of factors. The simultaneous assessment of physiological, psychological and behavioural factors involved in appetite control is a strength of the studies in this thesis.

Figure 6.3 A summary of the methodological considerations from the studies

The ^{13}C-OBT was selected as the method of measurement of GE for all three studies. Although scintigraphy is considered the 'gold standard' method for measuring GE, the optimal method of measuring GE in overweight and obese individuals remains unclear. Scintigraphic measurements may be hampered in obese individuals, as defining the gastric areas of interest can be difficult [291]. Further, scintigraphy requires the facilities of a nuclear medicine department and exposes subjects to radiation, thereby limiting its use in healthy individuals for repeated tests. Other non-invasive non-radioactive methods such as the paracetamol absorption test and ultrasound have only been validated for liquid emptying. In contrast, the ^{13}C-OBT measures solid meal emptying, has been validated against scintigraphy, has a day-to-day variability comparable to scintigraphy [293], is sensitive enough to detect pharmacological influences on GE [359], has previously been used to detect differences in GE in obese individuals [29, 362] and active individuals [202] and was found in chapter 3 to be a reproducible method of measuring GE in overweight and obese males. However, the ^{13}C-OBT does not allow direct imaging of gastric function as discussed in Chapter 4. Future studies using scintigraphy to examine the influence of body composition and habitual exercise on GE would therefore be useful to confirm the findings of the studies presented in this thesis.

As discussed in detail in Chapter 4, the sensitivity of the measurements of EE and EI could also have influenced the accuracy of the present findings. It is possible for example that more sensitive measures of physical activity or EI may explain a greater variance in GE in multiple regression analysis. In addition, in chapter 5, EI was only assessed prior to and post the exercise intervention, therefore it cannot be discounted that changes in habitual EI during the intervention influenced the findings. This is a common issue in exercise intervention studies as the reduced sensitivity of most available methods of assessing EI represents an ongoing challenge. Underreporting of EI is common in some individuals, regardless of the method [438]. Future studies involving quantitative and objective measurements of EI such as measuring EI over the course of a probe day where all meals are provided to participants [36] may yield further information on associations between EI, GE and other variables.

The issue of the timing of measurements in relation to the last exercise session is one that was raised in Chapters 4 and 5. In the cross sectional study in Chapter 4,

participants were instructed to avoid exercise for 24 hours beforehand which is a common protocol and has previously been used in studies examining appetite control in habitual exercisers [249]. However, in intervention studies examining the effects of exercise training on gut peptides, post testing has been conducted at 36 hours [211, 212] or > 48hours [86, 214] after the last exercise session to avoid any acute effects of exercise on responses. As a result in the intervention study in Chapter 5, the study was designed so that the post GE test took place ≥ 48 hours after the last exercise session. Although this is a limitation when comparing the findings between Chapters 4 and 5, the strength of using this protocol is that the findings are more comparable to previous intervention studies examining changes in gut peptides with exercise [86, 211, 212, 214]. Nevertheless, this raises the question of what is the best timing of measurements in order to test individuals in their habitual state. During an exercise program for example, individuals may be exercising daily or every second day. Therefore measuring GE ≥ 48 hours after the last exercise session may mean that alterations in mechanisms occurring within this time-frame, that may contribute to a change in EI, are not detected. Similarly instructing active individuals to avoid exercise for > 24 hrs would likely mean they would not be in their habitual state. The contention that the time interval between the GE test and last exercise session may influence findings is supported by evidence of differences in insulin sensitivity depending on the time measured following 2 weeks of a HIIE intervention [427]. Studies conducting GE tests at different time intervals following the last exercise session are therefore warranted to investigate the possibility that changes in GE might differ depending on the time interval from the last exercise session.

As highlighted in the literature review, it is important to measure GE in conjunction with gut peptides due to their important integrative relationship in appetite control. While GE can influence the release and suppression of some gut peptides [25, 130, 134, 135, 139], gut peptides in turn influence GE [136] and therefore, the question of which comes first is important to consider. Gut peptides were included in the original study designs, but due to the costs and resources, they were not measured. The rationale was that it is important to show meaningful associations between physical activity, EE, body composition and GE, and if significant associations were established to subsequently investigate hypothesised mechanisms in future work. This is particularly relevant to the findings from Chapter 4, where it is hypothesised that a faster GE may be associated with an increase in

intestinal postprandial satiety signalling. An alternative hypothesis could be that GE is better regulated in active individuals in response to GI signals such as ghrelin. As discussed below, future studies should ideally measure GE in conjunction with gut peptides.

The primary outcome measure of this thesis is GE. However, it is important to recognise that exercise may influence many aspects of the integrated regulatory process of appetite control including hormonal, neural, psychological and behavioural factors. Misjudgements concerning the amount of calories consumed in food compared to that expended with exercise, learned behaviours, the palatability of food and social values, among other factors no doubt have a role in the control of EI. Nevertheless, the series of studies presented in this thesis have included the assessment of body composition, metabolism, EE, GE, appetite sensations, eating behaviour traits, food preferences and processes of food reward. This approach allows for the potential emergence of associations between different areas of knowledge, which would normally belong to separate disciplines [447].

6.4 FUTURE DIRECTIONS

The results from the studies presented in this thesis provide significant information regarding the influence of habitual exercise and short-term exercise training on GE and associated measures in males. However, several issues remain which need to be addressed in future work. These include addressing whether GE has a critical role in obesity and the potential of manipulating GE for weight management. In addition, the underlying mechanisms and the temporal pattern of changes in GE with exercise remain to be established. Further, the wider implications outside of appetite control, including the relationship with postprandial glycaemia and alterations in GE in various medical conditions are relevant to consider. These proposed areas for further investigation are summarized in **Figure 6.4**, below and are subsequently discussed in more detail in the following sections.

Summary of Future Directions

- Larger studies examining GE in lean and obese individuals controlling for physical activity level (e.g. comparing lean sedentary and overweight sedentary individuals) are needed.
- Studies measuring gut peptides in conjunction with GE are needed to test the hypothesis that a slower GE in sedentary individuals could result in the inactivation of GI signals and reduced appetite control.
- Longitudinal studies examining GE and gut peptides are needed to test the hypothesis that longer-term exercise accelerates GE and increases postprandial satiety signalling thus facilitating weight maintenance.
- Longitudinal studies examining the effects of changing from an active to a sedentary lifestyle may yield further information on the mechanisms contributing to the lack of physiological regulation of appetite that occurs in the sedentary range
- To determine whether a causal relationship between GE and exercise exists, and the temporal patterns of changes in GE with exercise and the associated implications for appetite control, studies examining changes in GE, appetite and EI with longer term exercise interventions are needed.
- The potency of different satiety signals may vary depending on the population of interest or potentially the phase of weight loss. Few studies have investigated the proposition that targeting different signals could have separate roles depending on the phase of weight loss, therefore this represents an area for further study.
- Determining the intensity and modality of exercise, which minimises compensatory responses in EI will assist in exercise prescription for weight management. Future evaluation of the influence of AEE on GE and changes with different modes and volumes of exercise would be of interest.
- Future analysis of gut peptides, blood glucose levels and insulin sensitivity could assist to better understand the mechanisms contributing to differences in GE with habitual exercise. In addition, measuring postprandial blood glucose levels will assist in understanding the implications of alterations in GE for glycaemic control.
- Studying several aspects of the gastric and intestinal phase of appetite control at the same time would be ideal in future studies. MRI is a relatively new and promising technique that can measure several of these aspects and therefore could generate new insights into relevance of these parameters for appetite regulation [100].

Figure 6.4 Summary of recommendations for further research

6.4.1 Is there a role for GE in obesity?

Although the primary research theme of this thesis has concerned physical activity, the influence of body composition on GE, appetite and EI was also examined. The findings from this thesis suggest that associations between body composition and GE may be mediated by physical activity. In chapter 4, increasing percent body fat was associated with slower GE, but after controlling for physical activity level this association was no longer evident. Others have also shown associations between body composition and eating frequency to be mediated by physical activity [252]. A robust analysis of subgroups (categorised as lean sedentary, lean active, overweight sedentary and overweight active) in Chapter 4 was limited due to the small sample sizes (n = 6) in 2 of the sub groups. Nevertheless, the findings from this thesis collectively suggest that a slower GE is a marker of a sedentary lifestyle and that in the absence of differences in physical activity, GE appears unlikely to have a critical role in obesity. Larger studies examining GE in lean and obese individuals but controlling for physical activity level (e.g. comparing lean sedentary individuals and overweight sedentary individuals) are needed to test this hypothesis.

It is possible however that a slower GE associated with a sedentary lifestyle could be one predisposing factor to weight gain through a reduced intestinal satiety signalling and that therefore GE may indirectly have a role in obesity. As gut peptides were not measured in this thesis, future studies measuring gut peptides in conjunction with GE are needed to test the hypothesis that a slower GE in sedentary individuals could result in the inactivation of GI signals and a reduced sensitivity of appetite control. While it is not possible to establish a causal relationship from cross-sectional studies**,** longitudinal studies examining the effects of changing from an active to a sedentary lifestyle may yield further information on the mechanisms contributing to the lack of physiological regulation of appetite that occurs in the sedentary range [445].

6.4.2 GE, appetite and EI in weight maintenance

It is hypothesised here that a faster GE in combination with other factors could be beneficial for weight maintenance. Studies consistently show exercise is crucial for weight maintenance [9], unlike following energy restriction where it can be difficult

to maintain weight after weight loss [444]. Energy restriction appears to slow GE [24, 162, 200, 201] whereas the findings from this thesis indicate habitually active individuals have a faster GE. Martins et al. (2010) [86] demonstrated an increased GLP-1 response following 12 weeks of supervised exercise, whereas energy restriction has been shown to result in a blunted release of gut peptides - a response that is claimed to increase hunger [82, 204, 205, 207, 208] and to contribute to the relative lack of efficacy of sustaining weight loss after energy restriction. As GE and gut peptides appear to have an integrative relationship in appetite control, it is hypothesised based on this collective evidence that following energy restriction a slower GE could be a contributing factor to reduced intestinal satiety signalling, whereas exercise could facilitate weight maintenance by accelerating GE and producing an increased intestinal satiety signalling. However, no studies have been found which have measured both gut peptides and GE in response to energy restriction or habitual exercise. In addition, a causal relationship between exercise and GE has not been demonstrated in this thesis. Therefore, this linkage currently remains speculation and longitudinal studies examining both GE and gut peptides are needed to test the hypothesis that longer-term exercise accelerates GE and increases postprandial satiety signalling, whereas energy restriction has the opposite effects.

If faster GE has a facilitative role in weight maintenance, then this could have ramifications for weight management. The contention that faster GE may have a role in the prevention of weight gain is supported by findings from Roux-en-Y Gastric Bypass (RYGB) studies, which appear to accelerate the emptying rate and result in an enhanced release of anorexigenic gut peptides [59, 61, 62]. RYGB is associated with a greater and sustained weight loss compared to gastric banding [169], after which GE is unchanged [173-175] suggesting the difference in emptying rate could have a role in the enhanced sustained weight loss after RYGB. It could be proposed that a strategy which accelerates the emptying rate and increases the release of anorexigenic gut peptides could counteract the blunted gut peptide response to energy restriction [82, 204, 205, 207, 208] and assist in weight maintenance following energy restriction. Such strategies could include pharmacological strategies (e.g. [192]), functional foods or exercise.

There is no doubt that several factors influence weight maintenance and appetite control should not be limited to changes that occur in the GI tract. Many

factors (including psychosocial, behavioral, hormonal, and anatomical) influence weight loss after RYGB. Faster GE may have to be combined with other positive influences on eating behavior such as restraint and preference for low fat savoury foods to have a beneficial role in weight maintenance. As highlighted in the literature review - changes in gut peptides induced by strategies such as exercise are unlikely to be of the same magnitude as those following surgery, and other factors could more easily override signals from the GI tract. Combinations of diet and exercise are important to consider. For example, a high-fat diet-induced increase in GE is associated with diminished sensitivity to the appetite suppressing effects of gut peptides [250] and may therefore undermine any potential beneficial effects of exercise on GI mechanisms of appetite control. In contrast, reduced intake of fat and added sugars and increased intake of fibre is thought to transfer the intestinal absorption to the lower part of the small intestine and could increase intestinal satiety signalling [448, 449]. The findings from this thesis have demonstrated that active individuals have had a higher AEE, lower resting respiratory quotient, faster GE, higher dietary restraint and greater preference for low fat savoury foods and contribute to a growing body of work examining appetite control with a multifactorial approach [368]. Further characterisation of factors associated with energy balance in individuals who remain lean in the current 'obesigenic' environment will be useful for informing the design of weight management strategies.

6.4.3 GE, appetite and EI in weight loss

Based on the findings from this thesis and literature review, it is proposed that in the absence of gastric restriction, manipulating the emptying rate is unlikely to have a role in contributing to weight loss. While strategies which accelerate GE represent a reasonable target for weight maintenance by increasing the release of anorexigenic gut peptides, an overall faster GE time is likely to lead to decreased feelings of fullness arising from the stomach and shorten the onset to the next meal [192]. Hence, while appetite control between meals might be improved, net energy intake is unlikely to be reduced [192]. In Chapter 4 faster GE was associated with increased *ad libitum* EI in the active group in support of this hypothesis. Accelerating GE may therefore assist in weight maintenance by regulating intake, but not in weight loss.

Key unanswered questions concern which satiety signal, or mix of satiety signals, is most potent [450]. When a meal is eaten, a number of signals provide continuous information to the brain. Intestinal satiety signals are also influenced by insulin and leptin sensitivity. Therefore, targeting an increase in intestinal satiety may be more likely to be effective when improvements in leptin and insulin sensitivity have been achieved. In contrast it is possible that gastric distension may be a more potent target during initial weight loss. Few studies have investigated the proposition that targeting different signals could have separate roles depending on the phase of weight loss. This represents an area for further study.

In addition, the potency of different satiety signals may vary depending on the population of interest. Burton-Freeman (2008) [386] observed reduced CCK levels following a low glycaemic index meal in females which translated into reduced satiety sensations and suggested women seem to be more sensitive to intestinal phase satiety, than gastric distension. Similarly, Jones et al. (1996) [451] suggested that stimulation of intestinal receptors rather than gastric distension influences postprandial hunger in non-insulin dependent diabetes. Therefore, particular weight loss strategies may be more effective for certain populations.

Overall, the optimal strategy to reduce EI would appear to be one that maximises both the sensation of fullness from the stomach and the prolonged release of gut peptides from the intestine. Strategies targeting the 'ileal brake' mechanism, whereby under normal physiological situations undigested nutrients can reach the ileum earlier, and release satiety signals GLP-1 and PYY, which in turn act to delay GE, appear a promising target [33]. However, further work is needed as to how targeting the 'ileal brake' can be applied in practice [33]. On the basis of research to date, it appears that in the absence of gastric restriction, manipulating the overall emptying rate is unlikely to assist in reducing EI for weight loss – accelerating GE could increase intestinal satiety signalling but would shorten the satiety period and thus have no impact on overall intake [192], whereas delaying GE might reduce intestinal signalling and lead to a reduced physiological control over appetite.

6.4.4 Temporal relationship of changes in GE and food intake with exercise

The findings presented in this thesis do not show that exercise *per se* is a causal factor in faster GE. Although a clear association between habitual physical activity level and AEE with GE was demonstrated in the cross-sectional study (Chapter 4), it was not possible to establish a causal relationship due to the cross-sectional design. Further, in the longitudinal study (Chapter 5) GE was unchanged after 4 weeks of exercise. One hypothesis is that fat mass acts as an energy buffer and that EI rises markedly when lean mass is threatened by an energy deficit [264]. Therefore, it is possible that responses to exercise training in lean individuals or in response to exercise induced weight loss could be different. To determine the temporal patterns of changes in GE with exercise and the associated implications for appetite control, future studies examining changes in GE, appetite and EI during longer term exercise interventions and in response to exercise induced weight loss are needed.

6.4.5 Minimising compensatory responses to exercise training

An additional hypothesis is that GE may only adapt to a greater increase in AEE than that in the exercise intervention study in Chapter 5. Future evaluation of the influence of AEE on GE including changes with different modes and volumes of exercise would be of interest. Determining the intensity and modality of exercise which minimises compensatory responses in EI will assist in exercise prescription for weight management. If there is a threshold level of EE below which appetite appears not to be physiologically regulated, then it may be beneficial to only prescribe exercise EE below this level for weight loss, and focus on targeting other factors including dietary and behavioural factors during weight loss. Whereas, for weight maintenance, if there is a threshold amount of EE above which exercise appears to assist in physiologically regulating appetite, it would be beneficial to have an idea of the range and intensity of exercise that is required so that a sufficient exercise program is prescribed.

6.4.6 Underlying Mechanisms

No clear mechanism that could account for the faster GE observed in habitually active individuals or the association of GE with AEE was observed in Chapter 4. As discussed, a lower resting heart rate in the active group is consistent with the hypothesis that a more predominant parasympathetic tone may have a role [40] but this did not independently account for variance in GE. Other factors that were not measured in this study must therefore account for some of the variance in GE observed. One factor could be differences in GI hormones, blood glucose levels or insulin sensitivity as previously highlighted. In addition, exercise is known to improve leptin sensitivity via reducing fat mass [408, 409] which some evidence in animals suggests may interact with CCK and vagal afferent fibres to influence gastric motility [410]. Future analysis of these measures in addition to the variables in the present study could significantly expand current information on the mechanisms contributing to differences in GE with habitual exercise.

6.4.7 Other Roles of GE in Health

The focus of this thesis has been on GE in relation to appetite, food intake and other aspects of eating behaviour. However, GE has a number of other roles in health that are worth considering. GE has an important role in drug absorption [452] and is altered in various medical conditions (including Parkinson's disease [453], multiple sclerosis [454] and bulimia nervosa [455, 456]). For example, in bulimia nervosa, a slower GE [455, 456] delays the release of CCK [455], which may lead to less satiation and contribute to binge eating [457]. If exercise were found to have a causal relationship in altering GE, it would be one universally available therapeutic strategy, which could be used to assist individuals suffering from symptoms associated with delayed GE and to enhance appetite control and wellbeing.

In addition, GE is a major determinant of postprandial glycaemia [458] and a delay in GE is common in patients with diabetes and hyperinsulinemia [458]. The rate of GE appears to account for as much as 40-50% of variations in postprandial glycaemia [458]. Interactions between insulin and glucose explain the remaining 50% [459, 460]. In patients with type 2 diabetes mellitus, the inability to synchronise GE and insulin release contributes to postprandial hyperglycaemia [461]. In addition

to being influenced by GE, blood glucose levels in turn influence GE [462]. A delay in GE when blood glucose concentrations are high is observed in non-diabetic individuals and appears an appropriate response to hyperglycaemia, slowing further increases in blood glucose [186]. The close relation between GE and glucose absorption suggests that, if GE is accelerated by the use of prokinetics, or if the stomach is bypassed and nutrients are placed directly into the small intestine, the rate of glucose absorption may be increased which would be undesirable [463]. However, with bariatric surgery, increasing evidence indicates that alterations in circulating gut hormones mediate amelioration of type 2 diabetes following some surgical procedures [464] and that a shorter GE half time and small bowel transit time could contribute to better glucose homeostasis in patients with type 2 diabetes [64]. One explanation could be an increased GLP-1 response to surgery. GLP-1 stimulates the islet β cells in the pancreas to secrete insulin, thus contributing to the lowering of the blood glucose levels [465]. Incretin hormones (GLP-1 and glucose-dependent insulinotropic polypeptide [GIP]) are increasingly becoming a target of type 2 diabetes therapies [184]. Exenatide which has many of the same effects as GLP-1 has been demonstrated to slow GE, reduce hyperglycaemia and lower body weight [466, 467]. Therefore, alterations in GE with different strategies may have different implications for hormone release and glycaemic control. While postprandial glycaemia was not measured in this thesis, future studies measuring postprandial blood glucose levels in addition to other GI targets of appetite control are important to understand the implications of any alterations in GE for postprandial glycaemia and to ensure there are no undesirable effects. The primary goal should be to improve both appetite and glycaemic control.

6.4.8 Appetite control in females and other populations

Finally, it is important to acknowledge that healthy adult males were studied in all three studies in this thesis to exclude any confounding effects of gender and phase of the menstrual cycle on GE, appetite and EI [277]. The mechanisms discussed in this thesis may vary if a more varied subject population is investigated. Although some studies have reported no sex-based differences in appetite responses to exercise [412], others suggest a difference may exist [393]. Therefore findings in other populations could be different and are relevant to consider in future investigations.

6.5 CONCLUSIONS

GE and associated measures are reproducible in overweight and obese males, indicating the methods used are sufficiently reliable tools for assessing GE, appetite, *ad libitum* EI, food preferences and processes of food reward in this population. Habitually active males have a faster GE, higher dietary restraint and higher preference for low fat savoury foods compared to sedentary males. This combination of factors may contribute to a more precise regulation of energy balance in active males. In addition, AEE is inversely associated with GE half time. A 4-week exercise intervention did not significantly affect GE, appetite and eating behaviour, despite a significant increase in fitness in previously sedentary overweight and obese males.

The findings highlight the importance of controlling for physical activity level and AEE in studies examining the role of GE (and parameters that are influenced by GE) in obesity and provide insight into processes potentially contributing to the regulation of energy balance. A wide range of follow-up investigations may be relevant. Among them, further studies measuring gut peptides are needed to explore the hypothesis that faster GE may have a role in regulating food intake and energy balance in active individuals and to determine the temporal pattern of changes in GE with exercise and the implications for appetite and EI.

6.6 WIDER CONTEXTS

Appetite control and energy balance are influenced by a complex integration of social, cultural, psychological, genetic and physiological factors [13]. The focus of this thesis has been concerned with the influence of exercise on GE, eating behaviour, food preferences, processes of food reward, body composition and EE, which represent just a few aspects associated with the integrative process of appetite control. As the foregoing review of future directions indicates, there are many other factors that may override physiological signals of hunger and satiety. Nevertheless, a better knowledge of appetite physiology and responses to interventions will assist to understand how physiological processes can be manipulated to more readily resist other influences on food intake. The parameters assessed in this thesis contribute to a growing body of work examining factors influencing appetite and food intake with a multi-factorial approach [368]. While it is still controversial how sustained increases

in physical activity can be achieved at the population level [468], the provision of a clear rationale for the health benefits of exercise and a greater understanding of compensatory responses will be important to encourage and sustain long term participation in physical activity. By 2030, some estimates project that over 3 billion people may be overweight and obese [268]. Understanding the physiological and behavioural mechanisms influencing energy balance is essential for developing more effective weight management strategies.

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Appendices

APPENDIX A: OVERVIEW OF GASTRIC EMPTYING REPRODUCIBILITY STUDIES

Table A1 Overview of studies examining the reproducibility of gastric emptying measured by scintigraphy and breath test.

	Subject Characteristics			Test Meal Characteristics		Results		
Method (Reference)	**n**	**Population**	**Gender**	**Form**	**Size**	**Mean $t_{1/2}$[1]**	**t_{lag} CV_{intra}**	**$t_{1/2}$ CV_{intra}**
Scintigraphy								
Brophy et al. (1986) [278]	8	Healthy	M	S/L	208kcal	59min (S), 25min (L)	-	20% (S), 29% (L)
Chaudhuri et al. (1976) [469]	8	Healthy	M	S	257kcal	37min	-	14%
Choi et al. (1998) [279]	14	Healthy	M,F	S	240kcal	143min (median)	19%	14%
Collins et al. (1983) [280]	19	Healthy	M,F	S	270-410kcal	60min	-	15%
Cremonini et al. (2002) [281]	21	Healthy	M,F	S	296kcal	112min	-	14%
Degen & Phillips (1996) [282]	32	Healthy	M,F	S	219kcal	202min (F), 153min (M)	-	-
Jonderko et al. (1990) [283]	12	Healthy (n = 11), patient with duodenal bulb ulcer (n = 1)	M,F	S	394kcal	92min (median)	-	14% (ST), 17% (MT)
Kong et al. (1998) [284]	10	Healthy	M	S/L	400 kcal	137min (S), 31 min (L)	-	13% (S), 28% (L)
Latrigue et al. (1994) [276]	12	Healthy	M	S/L	418kcal	72min (S), 37min (L)	-	19% (S), 35% (L)
Latrigue et al. (1994) [276]	14	Diabetic	M,F	S/L	418kcal	133min (S), 50min (L)	-	29% (S), 67% (L)

Rasmussen et al. (1993) [285]	12	Healthy	M	S/L	334kcal	67min (S); 21min (L) (median)	25% (S), 54% (L)	12% (S), 33% (L)
Roland et al. (1990) [286]	6	Healthy	M	S	119kcal	55min	3-47% (range)	15–41% (range)
Scarpello et al. (1976) [287]	12	Healthy (n = 6), Diabetic (n = 6)	M,F	S	-	76min (healthy) 105min (diabetic)	-	16% (healthy), 39% (diabetic)
Tosetti et al. (1998) [288]	11 (S); 8 (L)	Healthy	M,F	S/L	638 kcal (S), 520 kcal (L)	92min (S), 83min (L)	39% (S), 34% (L)	11% (S), 7% (L)
^{13}C-Octanoic Acid Breath Test								
Barnett et al. (1999) [273]	28	Healthy preterm infants	M,F	L	20-24kcal	45min	-	24%
Chey et al. (2001) [345]	20	Healthy	M,F	S	350 kcal	209min	21%	25%
Choi et al. (1997)[290]	15	Healthy	M,F	S	240kcal	191min (median)	-	12%
Choi et al. (1998) [279]	30	Healthy	M,F	S	240kcal	191 min (median)	14%	15%
Deane et al. (2010) [275]	12	Critically ill	M,F	L	106kcal	127min	-	32%
Delbende et al. (2000) [291]	18	Healthy	M,F	S	324 kcal	90min	-	15%
Duan et al. (1995) [292]	7	Healthy	M,F	S	250kcal	148min	52%	20%
Ghoos et al. (1993) [293]	5	Healthy	M,F	S	250kcal	72min (n = 42)	45%	27%
Hauser et al. (2006) [301]	19	Healthy children	M,F	S	230kcal	149min	14%	13%
Kasicka-Jonderko et al. (2006) [294]	12	Healthy	M,F	S	378kcal	195min	8% (ST), 11% (MT)	7% (ST), 11% (MT)
Perri et al. (2010) [295]	30	Healthy	M,F	S	378kcal	88min	-	17%
Pfaffenbach et al. (1995) [296]	20	Healthy	M,F	S	280kcal	-	-	24%
Pozler et al. (2003) [300]	16	Healthy preterm infants	M,F	L	-	53min	-	11%

Ziegler et al. (1996) [297]	10	Healthy	M,F	S	250kcal	96min	47%	30%
^{13}C-Acetate Breath Test								
Barbosa et al. (2005) [272]	14	Healthy infants	M,F	L	-	69min	-	6%
Braden et al. (1995) [298]	5	Healthy	M,F	SS	200kcal	60min	25%	21%
Duan et al. (1995) [292]	5	Healthy	M,F	L	250kcal	83min	19%	6%
Gatti et al. (1998) [302]	60	Healthy children (n=30), with GERD (n=30).	M,F	L	105-160 kcal	74min (healthy), 104min (GERD).	6% (for both groups)	5% (for both groups)
Hauser et al. (2006) (medians reported) [301]	21	Healthy children	M,F	L	56kcal per 100g	81min	17%	8%
Other Breath Test								
Arts et al. (2005) [299]	20	Patients with functional dyspepsia.	M,F	S/L	250 kcal	95min (S), 67min (L)	-	73% (S), 67% (L)

[1] Mean $t_{1/2}$ from first gastric emptying test

M = Male, F = Female, S = Solid, L = Liquid, SS = Semisolid, ST = short term (tested 7 d apart), MT = medium term (tested 21d apart), GERD = Gastro Esophageal Reflux Disease.

APPENDIX B: REVIEW OF GASTRIC EMPTYING METHODS

Table A2 Summary of strengths and limitations of gastric emptying methods

Method of Measuring GE	Limitations	Strengths	References
Scintigraphy (the 'gold standard')	• Expensive • Requires a complex technique and facilities of a nuclear medicine department. • Exposes subjects to radiation	• Can assess liquid and solid GE simultaneously • Most frequently used and best validated method	Naslund et al. (2000) [470] Glerup et al. (2006) [471]
Intubation techniques	• Measures liquid GE only • Invasive • Nasogastric tube uncomfortable • Gastric tube interferes with GE	• Can be conducted even during intense exercise	Hunt & Stubbs (1975) [106] Medhus et al. (1999) [472] Naslund et al. (2000) [470]
Ultrasonography	• Only validated for measurement of liquid GE. • Need for a skilled operator. • Suboptimal examination in people not lean. • Generally impractical for prolonged observations.	• Provides information about gastric motility including emptying, gastroduodenal flow, contractility, and accommodation. • widely available equipment • modest running costs • noninvasive • good interobserver agreement • Closely correlated with scintigraphy results.	Bateman & Whittingham (1982) [473] Szarka et al. (2009) [474]
Paracetamol Absorption Test	• Drug interactions e.g. oral contraceptive alters paracetamol clearance. • Individual differences in metabolism not taken into consideration. • Indirect estimation of GE • Observations made using paracetamol (in liquids) cannot be used to draw inferences about the emptying of solids • the test can be used only with a liquid meal, and the grinding	• Requires no specialist equipment • Cheap • Detailed evaluation of GE dynamics • Real volume estimation • Ability to detect accelerated and delayed GE. • Test is well tolerated	Heading et al. (1973)[475] Medhus et al. (1999) [472]

	function of the antrum is therefore not tested • Requires repeat blood sampling		
Radiology	• Requires a hospital radiology unit • Emits irradiation • Cannot be used in pregnant and paediatric populations	• Readily available and easily performed but in a radiology unit. • Direct Measurement	Loreno et al. (2004) [476]
^{13}C Breath Test	• Indirect Estimation of GE • loss of accuracy in patients with severe diseases involving the intestinal mucosa, pancreas, liver, and respiratory system. • Isotope Ratio Mass Spectrometry facilities required.	• Non invasive • Non radioactive • Not operator dependent • Measures solid and liquid emptying, Correlated and validated with scintigraphy • Sensitive to pharmacological influences. • Used widely in healthy and patient children and adults	Ghoos et al. (1993) [293] Perri et al. (2005) [295]
^{2}H Breath Test	• Few (2) studies published using this method. • Tracer (^{2}H octanoic acid) very expensive.	• Results equivalent to scintigraphy	Bluck et al. (2002) [477]
Electrical Impedance Tomography	• Need to suppress gastric acid secretion prior to imaging • Measures Liquids only • Subjects required to lie on a bed • Need for sophisticated equipment	• Non invasive • Non-radioactive, electrodes easily attached to abdomen • Correlated with scintigraphy • Small and portable • Direct Measurement • Rapid Analysis	Nakae et al. (2000)[478]
Wireless pH and motility capsules	• No information on dynamics of GE such as in the early postprandial period, just total emptying times. • Not used in paediatrics. • Not widely used to date.	• GE time at 4 hours correlated with scintigraphy • ease of conduct of the study • reasonable discrimination between normal and delayed GE • Non-radioactive • Can determine small bowel, colon	Szarka et al. (2009) [474]

		and whole gut transit times, as well as gastric contractility	
Magnetic Resonance Imaging (MRI)	• Few studies in disease states or in response to different perturbations • Subject must be supine (eliminating the normal major force of gravity on GE). • cost and availability of equipment, imaging time, and technical expertise.	• non-invasive evaluation of both gastric volume and emptying of gastric contents independently. • No exposure to radiation • Validated and reproducible method.	Fruehauf et al. (2009) [479] Szarka et al. (2009) [474]

B.1 The ^{13}C-octanoic acid breath test

The ^{13}C-octanoic acid breath test (^{13}C-OBT) was selected as the most appropriate method for measuring GE in the studies presented in this thesis. ^{13}C breath tests have been used in research for over 30 years in both healthy and patient infants, children and adults and there are no known risks associated with the method. The ^{13}C-OBT was proposed as a safe, reliable, non-invasive and non-radioactive alternative to scintigraphy by Ghoos et al. (1993) [293]. The rationale of the test is that octanoic acid (a medium chain fatty acid) passes through the stomach without being metabolised, is then rapidly absorbed in the duodenum, metabolized in the liver and excreted in the expired air (**Figure A.1**), resulting in a rise in expired $^{13}CO_2$ over baseline. The underlying assumption is that emptying of the labelled test meal is the rate limiting step in breath $^{13}CO_2$ excretion, while all other metabolic processes involved in the absorption and metabolism of the tracer (^{13}C-octanoic acid) are not variable or negligible fast. Thus, $^{13}CO_2$ exhalation reflects gastric emptying of nutrients [293].

Image removed for copyright reasons (Deane, A.M., Zaknic, A.V., Summers, M.J., Chapman, M.J., Lange, K., Ritz, M.A., Davidson, G., Horowitz, M., and Fraser, R.J., *Intrasubject variability of gastric emptying in the critically ill using a stable isotope breath test*. Clinical Nutrition, 2010. 29(5): p. 682-6)

Figure A1 Sequential metabolic steps after ingestion of a ^{13}C-labelled test meal. The rate limiting step of breath $^{13}CO_2$ excretion is represented by the gastric emptying of the meal. Adapted from Deane et al. (2010) [275] .

APPENDIX C: MEASUREMENT AND VALIDATION OF MASS SPECTROMETRY METHODS

Closely following the work of Slater (2004) [480], a number of steps were taken to validate the laboratory methods that were used in the studies and therefore ensure that measurement of isotopic abundance would not be a major source of error. The isotope ratio mass spectrometer (IRMS) in QUT used for the analysis of breath samples is composed of a Hydra 20-20 IRMS interfaced to an automated breath carbon analyser (ABCA, PDZ Europa, Crewe, UK). The abundance of ^{13}C and ^{18}O in breath CO_2 is measured by monitoring the ion beams at m/z 44, 45 and 46. The source tuning was optimised for CO_2 analysis by small adjustment of the HT, ion repeller and beam focus settings.

CO_2 calibration curve and Linearity of $^{13}CO_2$ abundance

As not all breath samples contain the same amount of CO_2, it is important to ensure that the percent CO_2 in the exetainer tube did not affect the ^{13}C results. The CO_2 calibration curve (R^2 = 1.0) was prepared over the expected range of CO_2 concentration in exhaled breath of 1-6 % CO_2 by injecting known amounts of the laboratory reference gas into evacuated Exetainers using a gas-tight syringe.

If the isotope ratio (abundance) is constant over the expected concentration range, a measurement is said to be linear [480]. For the calculated CO_2 concentrations in the calibration curve, measured ^{13}C abundance (ppm) was found to be constant (standard deviation = 1.8 ppm ^{13}C) indicating linearity of ^{13}C measurements.

C.1 Accuracy of measured $^{13}CO_2$ abundance

To ensure accuracy of measured $^{13}CO_2$ abundance, the laboratory CO_2 reference gas (5% CO_2 in N_2) was calibrated against secondary references that are traceable to the international standards (VPDB, Vienna Pee Dee Belemnite for ^{13}C and VSMOW, Vienna Standard Mean Ocean Water for ^{18}O) at Isoanalytical, Crewe, UK.

Exetainer tubes containing the reference gas were placed at intervals during each analytical run and the reported isotope ratio corrected for drift between references. Precision of $^{13}CO_2$ measurements was checked at intervals by analysing

batches of reference gases and calculating the standard deviation of at least 3 replicates. An SD < 0.5 ppm was regarded as acceptable [480, 481].

C.2 Reproducibility of procedure for filling reference gas samples

A procedure for filling reference gas samples was established and the reproducibility tested. Reference samples were prepared on a manifold which was flushed for 5 minutes with the reference gas before preparing the samples. All samples were prepared in 10 ml Exetainer tubes (Labco, High Wycombe, UK) which were filled through a straw attached to a needle and luer-lock stopcock on the manifold. Each exetainer was uncapped and filled with the reference gas for 15 seconds (timed using a stopwatch) by opening and closing the stopcock. Using this procedure for filling reference tubes, it was possible to achieve a coefficient of variation on the total beam size of 1.07%.

C.3 Precision of breath $^{13}CO_2$ analysis and inter-laboratory check

A gastric emptying (^{13}C-octanoic acid) breath test using the test meal and protocol described in Chapter 3 was performed on a single participant and multiple breath samples were obtained at each time point at baseline and over 6 hours postprandially throughout the test. The abundance of $^{13}CO_2$ was measured as previously described (see page 49). Three replicates were analysed on the HYDRA-20-20 IRMS at QUT, and one on the 20-20-IRMS (Europa Scientific) at Isoanalytical, Crewe, UK. The precision of replicate analyses of breath $^{13}CO_2$ enrichment at QUT is shown in **Figure A.2**, along with the enrichment of samples analysed at Isoanalytical. One set of samples were stored for 3 months before analysis (QUT 3 in **Figure A.2**) and analysed following the same procedure as the other analyses which were undertaken within one week of the test (QUT 1 and 2 in **Figure A.2**). The mean standard deviation of $^{13}CO_2$ abundance in breath CO_2 was 0.96 ppm excess ^{13}C over the range 7-211ppm excess ^{13}C (n = 3 analyses on the Hydra 20-20).

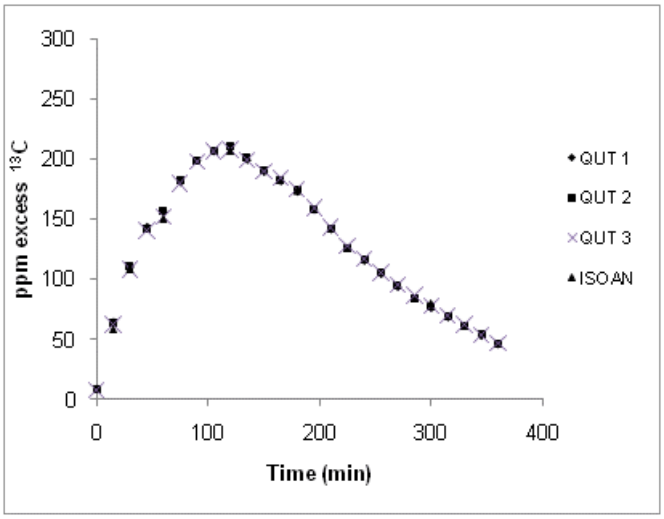


Figure A2 Precision of replicate analyses of breath $^{13}CO_2$ enrichment. Three replicates were analysed at QUT (shown as QUT 1, 2 and 3 in the legend) and one at Isoanalytical, Crewe, UK (shown as ISOAN in the legend).

C.4 Summary

The laboratory methods that were used to measure isotopic abundance were validated by ensuring that measurements were accurate (traceable to international standards or gravimetrically prepared standard curves), precise (SD of three replicate analyses < 1 ppm) and linear (a constant isotope ratio over the expected concentration range). Therefore, measurement of isotopic abundance should not be a major source of error in the studies.

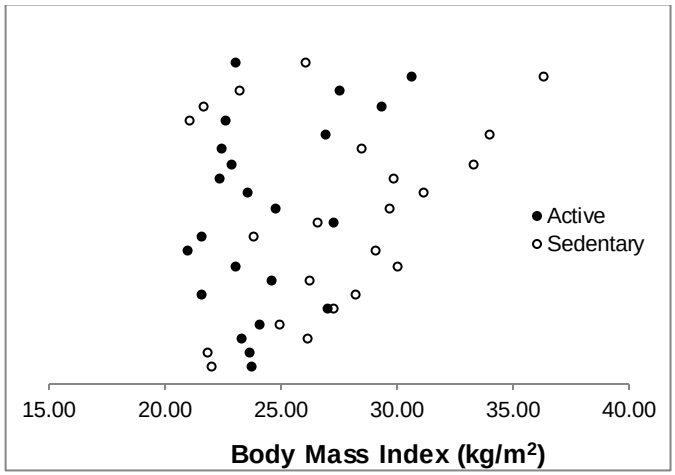
APPENDIX D: CHAPTER 3 CORRELATION COEFFICIENTS (R) OF VAS RATINGS VERSUS LUNCH ENERGY INTAKE (EI) AND GASTRIC EMPTYING (GE) PARAMETERS. (N = 15)

	Energy Intake (kJ)				GE t_{lag} (min)				GE $t_{1/2}$ (min)				GE t_{asc} (min)				GE t_{lat} (min)			
Variable	**Visit 1**	**Visit 2**	***Mean***	***Change***	**Visit 1**	**Visit 2**	***Mean***	***Change***	**Visit 1**	**Visit 2**	***Mean***	***Change***	**Visit 1**	**Visit 2**	***Mean***	***Change***	**Visit 1**	**Visit 2**	***Mean***	***Change***
Fasting Ratings																				
Hunger (mm)	0.2	0.3	0.31	-0.01	<-0.01	0.40	0.35	-0.14	<0.01	0.28	0.29	-0.17	0.02	0.13	0.27	-0.16	-0.04	0.39	0.16	-0.09
Fullness (mm)	-0.17	-0.05	-0.07	-0.24	0.14	-0.12	-0.16	0.2	0.03	0.03	0.03	0.04	-0.02	0.18	0.13	-0.03	0.13	-0.22	-0.22	0.27
Desire to Eat (mm)	0.35	0.16	0.30	0.06	0.09	0.33	0.32	-0.03	0.18	0.27	0.31	-0.02	0.16	0.12	0.21	<-0.01	-0.03	0.30	0.22	-0.07
Breakfast Satiety Quotient																				
Hunger (mm/kcal)	**-0.51***	-0.07	-0.27	-0.2	**0.53***	**0.54***	**0.59***	0.29	0.33	**0.53***	**0.61***	0.09	0.18	0.38	0.43	-0.18	0.34	0.44	0.39	0.47
Fullness (mm/kcal)	**0.54***	0.19	0.39	0.33	-0.30	-0.25	-0.37	0.07	-0.39	-0.42	**-0.69****	0.26	-0.31	-0.42	**-0.66****	0.32	-0.08	-0.09	-0.06	-0.18
Desire to Eat (mm/kcal)	-0.47	-0.23	-0.40	-0.06	**0.60***	0.43	**0.55***	0.22	0.43	**0.56***	**0.78*****	-0.1	0.26	0.51	**0.65****	-0.23	0.36	0.23	0.24	0.45
Mean 5h VAS Ratings																				
Hunger (mm)	**0.55***	0.46	**0.52***	0.36	-0.36	0.05	-0.06	-0.43	-0.15	-0.12	-0.12	-0.30	-0.04	-0.19	-0.12	-0.19	0.27	0.13	-0.01	-0.43
Fullness (mm)	**-0.52***	-0.29	-0.40	-0.47	0.32	0.09	0.11	0.34	0.45	0.35	**0.53***	-0.01	0.38	0.44	**0.60***	-0.16	0.02	-0.01	-0.17	**0.54***
Desire to Eat (mm)	**0.60***	0.41	**0.52***	0.25	0.41	0.07	-0.12	-0.18	-0.23	-0.14	-0.24	-0.04	-0.12	-0.2	-0.23	0.05	-0.27	0.18	<-0.01	-0.3
5h AUC VAS Ratings																				
Hunger (mm.min)	**0.53***	0.45	0.5	0.35	-0.34	0.09	-0.03	-0.36	-0.12	-0.1	-0.1	-0.25	-0.02	-0.18	-0.1	-0.16	-0.28	0.17	0.01	-0.35
Fullness (mm.min)	-0.48	-0.30	-0.39	-0.41	0.27	0.08	0.08	0.38	0.43	0.37	**0.52***	0.02	0.38	0.46	**0.60***	-0.13	0.03	-0.12	-0.08	**0.57***
Desire to Eat (mm.min)	**0.54***	0.38	0.50	0.22	-0.42	0.07	-0.09	-0.1	-0.15	-0.12	-0.2	-0.02	-0.02	-0.21	-0.2	0.02	-0.35	0.17	<-0.01	-0.18
Prelunch Ratings																				
Hunger (mm)	0.39	0.46	0.44	0.34	-0.37	-0.14	-0.18	-0.36	-0.28	-0.06	-0.08	-0.36	-0.19	<-0.01	-0.08	-0.34	-0.17	-0.15	-0.16	-0.13
Fullness (mm)	-0.31	-0.24	-0.20	**-0.61***	0.4	-0.08	0.05	0.20	**0.44**	<-0.01	0.19	0.18	0.35	0.05	0.21	0.15	0.11	-0.13	-0.05	0.11
Desire to Eat (mm)	0.28	0.27	0.28	0.25	**-0.53***	-0.12	-0.24	-0.29	**-0.53***	-0.06	-0.24	-0.34	-0.41	<-0.01	-0.17	-0.32	-0.15	-0.13	-0.14	-0.12
Lunch Satiety Quotient																				
Hunger (mm/kcal)	**-0.66****	**-0.55***	**-0.66****	-0.2	-0.2	0.24	0.32	-0.32	-0.17	**0.54***	0.43	-0.37	-0.27	**0.59***	0.35	-0.35	0.35	<-0.01	0.16	-0.13
Fullness (mm/kcal)	0.45	**0.60***	**0.58***	0.19	0.19	-0.25	-0.36	0.33	0.36	-0.28	-0.13	0.40	0.45	-0.23	0.03	0.39	-0.43	-0.17	-0.37	0.1
Desire to Eat (mm/kcal)	**-0.72****	**-0.70****	**-0.78*****	-0.41	-0.41	0.24	0.28	<-0.01	-0.27	0.42	0.25	-0.1	-0.38	0.44	-0.14	-0.14	0.45	0.06	0.22	0.12
Energy Intake																				
Lunch EI (kcal)					-0.32	-0.28	-0.27	-0.37	-0.02	**-0.55***	-0.32	-0.38	0.09	**-0.60***	-0.26	-0.34	-0.33	-0.02	-0.19	-0.19

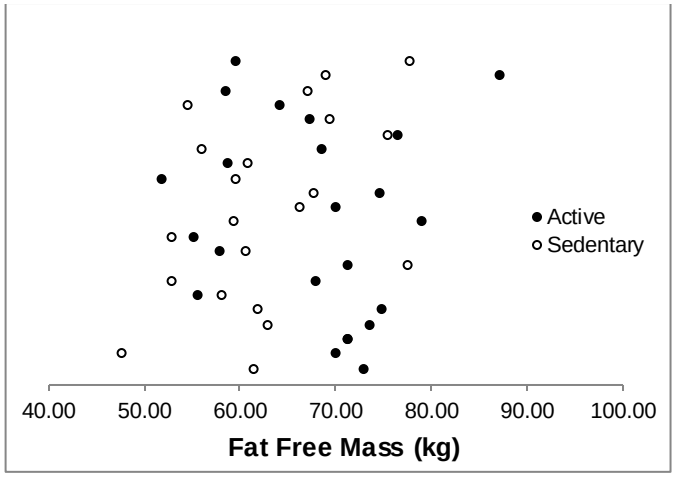
*Visit 1: variables correlated at Visit 1, Visit 2: variables correlated at visit 2, Mean: mean of both visits correlated, Change: Changes between visits correlated, EI, Energy Intake, * P<0.05, ** P<0.01, ***P<0.001.*

APPENDIX E: CHAPTER 4 SPREAD OF A) BMI, B) FAT FREE MASS, C) BODY FAT, D) RMR, E) AEE AND F) RESTRAINT IN ACTIVE (N = 22) AND SEDENTARY (N = 22) GROUPS.

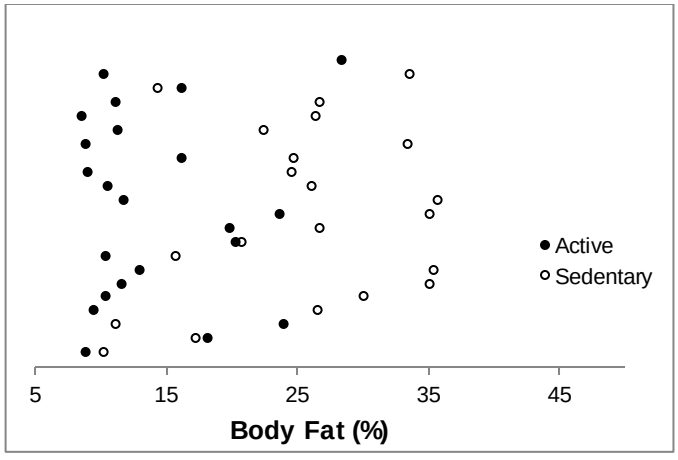
a)



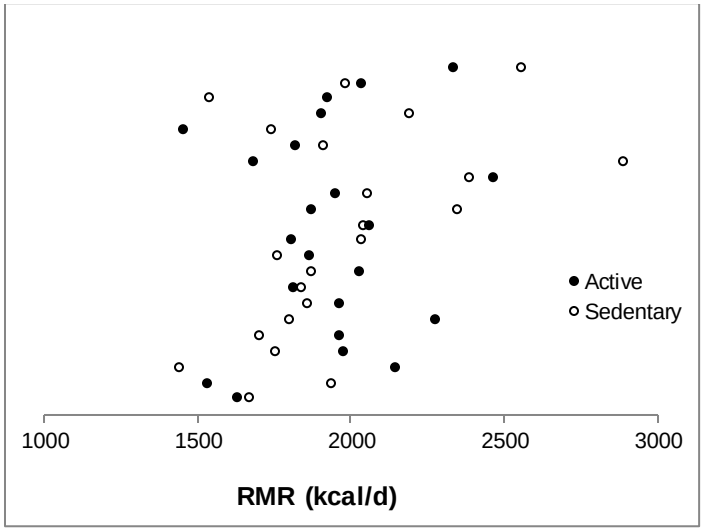
b)



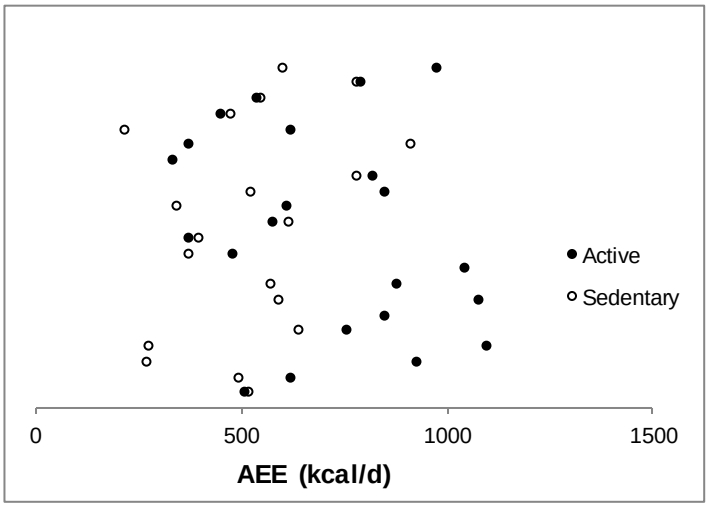
c)



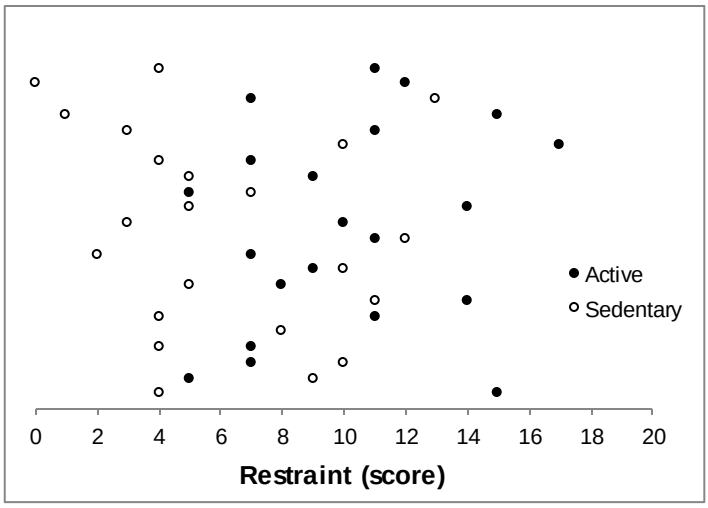
d)


Active
Sedentary
1000
1500
2000
2500
3000
RMR (kcal/d)

e)


Active
Sedentary
0
500
1000
1500
AEE (kcal/d)

f)


Active
Sedentary
0
2
4
6
8
10
12
14
16
18
20
Restraint (score)

APPENDIX F: CHAPTER 4 PALATABILITY RATINGS AND VAS ALERTNESS RATINGS

Table A3 Palatability ratings from 100mm visual analogue scales for breakfast and lunch meals in active and sedentary groups

	Active (n = 22)	Sedentary (n = 22)	*P*-value
Breakfast			
Sweet	56 ± 20	54 ± 20	0.11
Savoury	46 ± 25	53 ± 27	0.65
Tasty	59 ± 27	60 ± 23	0.79
Pleasant	68 ± 21	63 ± 21	0.16
Filling	64 ± 26	64 ± 17	0.97
Satisfying	66 ± 24	55 ± 24	0.81
Lunch			
Sweet	24 ± 25	31 ± 19	0.47
Savoury	79 ± 21	76 ± 18	0.70
Tasty	82 ± 17	69 ± 23	0.23
Pleasant	86 ± 14	70 ± 20	0.17
Filling	92 ± 12	82 ± 17	0.20
Satisfying	81 ± 27	80 ± 11	0.63

Data are Mean ± SD. Values are in mm.

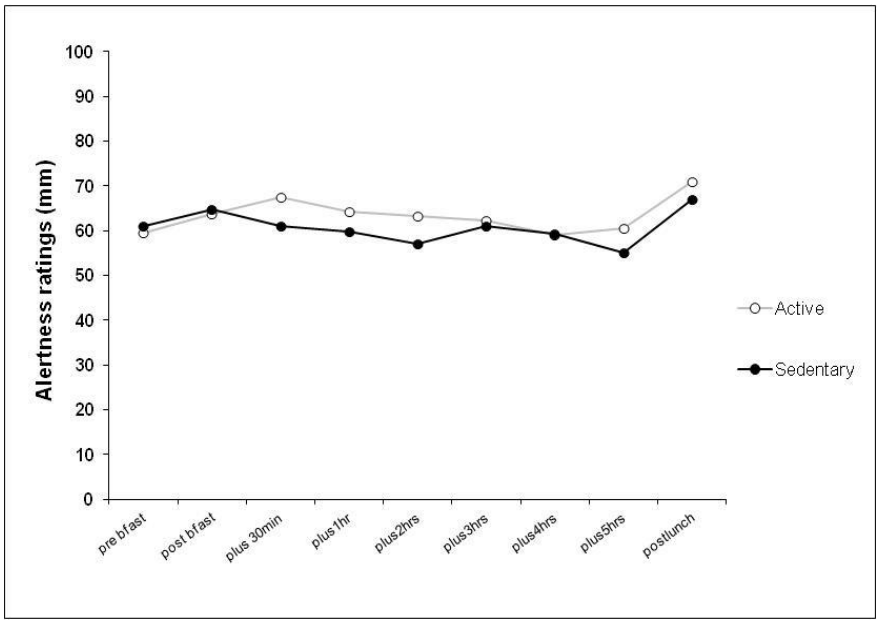


Figure A3 Subjective ratings for alertness in active and sedentary groups over the course of the GE test morning. n = 22 per group.

APPENDIX G: CHAPTER 4 COMPARISON OF QUARTILES FOR GE $T_{1/2}$

Table A4 Age, body composition, resting metabolism and physical activity characteristics of the lower and upper quartiles of gastric emptying half time.

	$t_{1/2}$		
	Lower quartile	**Upper quartile**	***P*-value**
Age (years)	28.6 ± 7.1	30.5 ± 24.6	.60
Height (m)	1.8 ± 0.1	1.5 ± 0.1	.66
Weight (kg)	78.5 ± 7.7	85.1 ± 16.2	.23
BMI (kg/m^2)	24.1 ± 1.8	26.4 ± 4.0	.10
Body composition			
Body Fat (%)	12.4 ± 4.6	22.8 ± 9.5	**.04**
FFM (kg)	68.8 ± 7.1	64.8 ± 9.3	.41
Resting HR (bpm)	53 ± 8	65 ± 10	**<.01**
Fasting RQ	.72 ± .04	.76 ± .04	**.01**
RMR (kcal/day)	2000 ± 227	1966 ± 301	.77
TFEQ			
Restraint	10.7 ± 3.2	5 ± 3.5	**<.01**
Disinhibition	4.8 ± 2.3	4.7 ± 3.4	.94
Hunger	6.4 ± 1.7	5.7 ± 4.0	.64
Physical Activity			
Steps per day	9833 ± 270	6624 ± 219	**.01**
AEE (kcal/day)	743 ± 261	472 ± 151	**<.01**
TEE (kcal/day)	2970 ± 408	2598 ± 292	**.02**
Time in activity			
Vigorous (min/day)	15.6 ± 10.1	6.8 ± 7.9	**.03**
Moderate (min/day)	60.7 ± 29.6	34.9 ± 15.1	**.02**

AEE, activity energy expenditure, FFM, fat free mass, HR, heart rate, RQ, respiratory quotient, RMR, Resting metabolic rate, TFEQ, three factor eating questionnaire, $t_{1/2}$, half time; t_{lag}, TEE, total energy expenditure. n = 11 per group for all.

APPENDIX H: CHAPTER 4 COMPARISON OF QUARTILES FOR AEE

Table A5 Age, body composition, resting metabolism and physical activity characteristics of the lower and upper quartiles of activity energy expenditure measured by accelerometery.

	AEE		
	Lower quartile	**Upper quartile**	***P*-value**
AEE (kcal/d)	343 ± 69	945 ± 101	**<.001**
Age (years)	32.9 ± 8.6	28.0 ± 8.5	.22
BMI (kg/m^2)	24.9 ± 3.5	26.4 ± 2.5	.29
Body composition			
Body Fat (%)	20.5 ± 8.2	17.4 ± 6.9	.36
FFM (kg)	60.3 ± 7.9	72.3 ± 7.9	**<.01**
Resting HR (bpm)	60 ± 13	55 ± 7	.27
Fasting RQ	.74 ± .03	.74 ± .05	.68
RMR (kcal/day)	1830 ± 238	2088 ± 212	**.02**
TFEQ			
Restraint	8.6 ± 5.2	9.1 ± 2.6	.79
Disinhibition	5.3 ± 3.2	6.0 ± 2.80	.61
Hunger	4.8 ± 2.7	6.2 ± 2.5	.25
t_{lag} (min)	114.9 ± 20.2	95.8 ± 15.4	**.03**
$t_{1/2}$ (min)	176.8 ± 26.2	154.8 ± 17.8	**.04**
t_{lat} (min)	40.35 ± 9.5	31.2 ± 8.3	**.03**
t_{asc} (min)	136.4 ± 18.4	123.7 ± 12.4	.09

AEE, activity energy expenditure, FFM, fat free mass, HR, heart rate, RQ, respiratory quotient, RMR, Resting metabolic rate, TFEQ, three factor eating questionnaire, $t_{1/2}$, half time; t_{lag}, lag time; t_{asc}, ascension time; t_{lat}, latency time. n = 10 per group.