

**Evaluation of appetite regulation in  
lean and obese individuals**

*A thesis submitted by*

**Ixchel Maya Brennan**

*For the degree of*

**Doctor of Philosophy**

**Discipline of Medicine**

**School of Medicine**

**University of Adelaide**

**June 2009**

# TABLE OF CONTENTS

|                                       |      |
|---------------------------------------|------|
| LIST OF ABBREVIATIONS .....           | i    |
| THESIS SUMMARY .....                  | ii   |
| STATEMENT OF ORIGINALITY .....        | ix   |
| DEDICATION .....                      | x    |
| ACKNOWLEDGEMENTS .....                | xi   |
| PUBLICATIONS ARISING FROM THESIS..... | xvi  |
| OTHER PUBLICATIONS .....              | xvii |

## CHAPTER 1

### REGULATION OF GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE

|   |    |
|---|----|
| 1.1 INTRODUCTION .....  | 1  |
| 1.2 ROLE OF THE CENTRAL NERVOUS SYSTEM IN THE REGULATION OF APPETITE AND ENERGY INTAKE..... | 2  |
| 1.3 ANATOMY AND FUNCTION OF THE GASTROINTESTINAL TRACT...5                                  |    |
| 1.3.1 Function of the stomach.....  | 5  |
| 1.3.2 Function of the pylorus .....   | 5  |
| 1.3.3 Function of the small intestine .....   | 6  |
| 1.4 ROLE OF NUTRIENTS IN THE REGULATION OF GASTROINTESTINAL MOTILITY.....                   | 7  |
| 1.4.1 Fasting gastrointestinal motility.....  | 7  |
| 1.4.2 Postprandial gastrointestinal motility.....   | 8  |
| 1.4.2.1 Gastric emptying.....   | 8  |
| 1.5 EFFECTS OF SMALL INTESTINAL NUTRIENTS ON GASTROINTESTINAL HORMONE SECRETION.....        | 11 |
| 1.5.1 Cholecystokinin .....   | 12 |
| 1.5.2 Peptide YY .....  | 12 |
| 1.5.3 Glucagon-like peptide-1.....  | 13 |

|         |   |    |
|---------|---|----|
| 1.5.4   | Ghrelin .....   | 13 |
| 1.5.5   | Insulin.....  | 14 |
| 1.6     | EFFECTS OF SMALL INTESTINAL NUTRIENTS ON APPETITE AND ENERGY INTAKE .....   | 14 |
| 1.7     | RELATIONSHIP BETWEEN APPETITE AND ENERGY INTAKE WITH GASTROINTESTINAL MOTOR FUNCTION .....  | 16 |
| 1.7.1   | Relationship between proximal stomach function with appetite and energy intake .....  | 16 |
| 1.7.2   | Relationship between distal stomach function with appetite and energy intake .....  | 17 |
| 1.7.3   | Relationship between appetite and energy intake with pyloric pressures.   | 19 |
| 1.8     | ROLE OF GASTROINTESTINAL HORMONES IN MEDIATING THE EFFECTS OF NUTRIENTS ON GASTROINTESTINAL MOTOR FUNCTION, APPETITE AND ENERGY INTAKE..... | 20 |
| 1.8.1   | Cholecystokinin .....   | 21 |
| 1.8.1.1 | <i>Effects of CCK on gastrointestinal motor function.....</i>   | 21 |
| 1.8.1.2 | <i>Effects of CCK on appetite and energy intake .....</i>   | 22 |
| 1.8.2   | Peptide YY.....   | 23 |
| 1.8.2.1 | <i>Effects of PYY on gastrointestinal motor function.....</i>   | 24 |
| 1.8.2.2 | <i>Effects of PYY on appetite and energy intake .....</i>   | 24 |
| 1.8.3   | Glucagon-like peptide-1.....  | 25 |
| 1.8.3.1 | <i>Effects GLP-1 on gastrointestinal motor function .....</i>   | 25 |
| 1.8.3.2 | <i>Effects of GLP-1 on appetite and energy intake .....</i>   | 26 |
| 1.8.4   | Ghrelin .....   | 27 |
| 1.8.4.1 | <i>Effects of ghrelin on gastrointestinal motor function .....</i>  | 27 |
| 1.8.4.2 | <i>Effects of ghrelin on appetite and energy intake.....</i>  | 28 |
| 1.8.5   | Insulin.....  | 28 |
| 1.8.5.1 | <i>Effects of insulin on gastrointestinal motility .....</i>  | 29 |
| 1.8.5.2 | <i>Effects of insulin on appetite and energy intake .....</i>   | 29 |
| 1.9     | INTERACTIONS BETWEEN GASTROINTESTINAL STIMULI WITH APPETITE AND ENERGY INTAKE .....   | 31 |
| 1.9.1   | Interactions between gastric distension with small intestinal nutrients ....  | 31 |

|         |  |    |
|---------|--|----|
| 1.9.2   | Interactions between gastric distension and CCK.....         | 32 |
| 1.9.3   | Interactions between small intestinal nutrients and CCK..... | 32 |
| 1.9.4   | Interactions between gastrointestinal hormones .....         | 33 |
| 1.9.4.1 | <i>Interaction between CCK and GLP-1</i> .....               | 34 |
| 1.10    | CONCLUSIONS.....   | 36 |

## **CHAPTER 2**

### **THE EFFECTS OF DIETARY EXCESS, OR RESTRICTION, ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE**

|         |   |    |
|---------|---|----|
| 2.1     | INTRODUCTION .....  | 37 |
| 2.2     | ROLE OF A HIGH DIETARY FAT INTAKE IN THE DEVELOPMENT OF EXPERIMENTALLY INDUCED HYPERPHAGIA.....                             | 38 |
| 2.3     | EFFECTS OF EXPERIMENTALLY-INDUCED DIETARY EXCESS ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE .....             | 39 |
| 2.3.1   | Effects of experimentally-induced dietary excess on gastrointestinal motor function .....                                   | 39 |
| 2.3.2   | Effects of experimentally-induced dietary excess on the sensitivity to, and the release of, gastrointestinal hormones ..... | 41 |
| 2.3.3   | Effect of experimentally-induced dietary excess on appetite and energy intake .....   | 44 |
| 2.4     | EFFECTS OF EXPERIMENTAL DIETARY RESTRICTION ON GASTROINTESTINAL FUNCTION AND APPETITE.....                                  | 45 |
| 2.4.1   | Effects of short-term experimental dietary restriction .....  | 45 |
| 2.4.2   | Effects of long-term experimental dietary restriction.....  | 47 |
| 2.5     | PATHOPHYSIOLOGY OF OBESITY .....  | 49 |
| 2.5.1   | Significance of obesity.....  | 50 |
| 2.5.2   | Gastrointestinal function in obesity .....  | 51 |
| 2.5.2.1 | <i>Modulation of gastrointestinal motor function in obesity</i> .....   | 51 |
| 2.5.2.2 | <i>Modulation of gastrointestinal hormones in obesity</i> .....   | 52 |
| 2.6     | CONCLUSIONS.....  | 54 |

## CHAPTER 3

### THE EFFECTS OF ORAL MACRONUTRIENT INGESTION ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE

|         |   |           |
|---------|---|-----------|
| 3.1     | INTRODUCTION .....  | 56        |
| 3.2     | COMPARATIVE EFFECTS OF FAT, CARBOHYDRATE AND PROTEIN ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE IN LEAN SUBJECTS .....  | 57        |
| 3.2.1   | Effect of macronutrients on gastric emptying.....   | 57        |
| 3.2.2   | Effect of macronutrients on gastrointestinal hormones .....   | 58        |
| 3.2.3   | Effect of macronutrients on appetite and energy intake .....  | 60        |
| 3.3     | COMPARATIVE EFFECTS OF FAT, CARBOHYDRATE AND PROTEIN ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE IN OBESE SUBJECTS ..... | 62        |
| 3.3.1   | Effect of macronutrients on gastric motor function.....   | 63        |
| 3.3.2   | Effect of macronutrients on gastrointestinal hormones .....   | 63        |
| 3.3.3   | Effect of macronutrients on appetite and energy intake .....  | 65        |
| 3.4     | FACTORS THAT MAY INFLUENCE THE EFFECTS OF ORAL MACRONUTRIENTS ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE .....          | 67        |
| 3.4.1   | Day-to day variation in appetite and energy intake .....  | 67        |
| 3.4.1.1 | <i>Inter-individual variation in appetite and energy.....</i>   | <i>67</i> |
| 3.4.2   | Effects of the menstrual cycle on gastrointestinal function, appetite and energy intake.....  | 70        |
| 3.4.2.1 | <i>Hormonal changes during the menstrual cycle .....</i>  | <i>70</i> |
| 3.4.2.2 | <i>Effect of the menstrual cycle on gastric motor function .....</i>  | <i>72</i> |
| 3.4.2.3 | <i>Effects of the menstrual cycle on gastrointestinal hormones and glycaemia .....</i>  | <i>73</i> |
| 3.4.2.4 | <i>Effects of the menstrual cycle on appetite and energy intake .....</i>   | <i>74</i> |
| 3.5     | CONCLUSIONS.....  | 75        |

## CHAPTER 4

### COMMON METHODOLOGIES

|         |   |    |
|---------|---|----|
| 4.1     | INTRODUCTION .....  | 77 |
| 4.2     | SUBJECTS.....   | 77 |
| 4.2.1   | Healthy subjects .....                                      | 78 |
| 4.2.2   | Obese subjects.....   | 78 |
| 4.2.3   | Inclusion/Exclusion criteria .....                          | 78 |
| 4.3     | ETHICS COMMITTEE APPROVAL.....                              | 80 |
| 4.4     | STUDY ENVIRONMENT .....                                     | 80 |
| 4.5     | MEASUREMENT OF ANTROPYLORODUODENAL PRESSURES.....           | 81 |
| 4.5.1   | Catheter design.....  | 81 |
| 4.5.2   | Nasoduodenal intubation and manometry.....                  | 82 |
| 4.5.3   | Data acquisition and analyses .....                         | 83 |
| 4.6     | MEASUREMENT OF GASTRIC EMPTYING .....                       | 84 |
| 4.6.1   | Three dimensional (3-D) ultrasonography .....               | 84 |
| 4.6.1.1 | <i>Data acquisition and analyses .....</i>                  | 85 |
| 4.7     | MEASUREMENT OF HORMONES.....                                | 86 |
| 4.7.1   | Measurement of gastrointestinal hormones .....              | 86 |
| 4.7.1.1 | <i>Plasma cholecystokinin.....</i>                          | 86 |
| 4.7.1.2 | <i>Plasma ghrelin.....</i>                                  | 87 |
| 4.7.1.3 | <i>Plasma peptide YY.....</i>                               | 87 |
| 4.7.1.4 | <i>Plasma insulin.....</i>                                  | 88 |
| 4.7.2   | Serum oestradiol and progesterone .....                     | 88 |
| 4.8     | MEASUREMENT OF APPETITE PERCEPTIONS AND ENERGY INTAKE ..... | 89 |
| 4.8.1   | Visual analogue scale questionnaires.....                   | 89 |
| 4.8.2   | Energy intake .....   | 90 |
| 4.9     | PREPARATION AND ADMINISTRATION OF STUDY INTERVENTIONS ..... | 91 |
| 4.9.1   | Hormones for intravenous infusion.....                      | 91 |
| 4.9.1.1 | <i>Cholecystokinin.....</i>                                 | 91 |

|         |  |    |
|---------|--|----|
| 4.9.1.2 | <i>Glucagon-like peptide-1</i> .....   | 91 |
| 4.9.2   | Test meal and preload preparation..... | 92 |
| 4.9.2.1 | <i>Test meals</i> .....                | 92 |
| 4.9.2.2 | <i>Glucose preload</i> .....           | 93 |
| 4.9.3   | Intraduodenal nutrient infusion.....   | 93 |
| 4.9.4   | Very-low calorie diet.....             | 93 |
| 4.10    | DATA AND STATISTICAL ANALYSES.....     | 94 |

## **CHAPTER 5**

### **INTRAVENOUS CCK-8, BUT NOT GLP-1, SUPPRESSES GHRELIN AND STIMULATES PYY RELEASE IN HEALTHY MEN**

|         |   |     |
|---------|---|-----|
| 5.1     | SUMMARY.....                                | 96  |
| 5.2     | INTRODUCTION.....                           | 97  |
| 5.3     | SUBJECTS AND METHODS.....                   | 98  |
| 5.3.1   | Subjects.....                               | 98  |
| 5.3.2   | Study design.....                           | 99  |
| 5.3.3   | Protocol.....                               | 99  |
| 5.3.4   | Measurements.....                           | 100 |
| 5.3.4.1 | <i>Plasma hormone concentrations</i> .....  | 100 |
| 5.3.5   | Statistical analysis.....                   | 100 |
| 5.4     | RESULTS.....                                | 101 |
| 5.4.1   | Plasma ghrelin concentrations.....          | 101 |
| 5.4.1.1 | <i>Effect of intravenous infusion</i> ..... | 101 |
| 5.4.1.2 | <i>Effect of meal</i> .....                 | 101 |
| 5.4.2   | Plasma PYY concentrations.....              | 102 |
| 5.4.2.1 | <i>Effect of intravenous infusion</i> ..... | 102 |
| 5.4.2.2 | <i>Effect of meal</i> .....                 | 103 |
| 5.5     | DISCUSSION.....                             | 106 |
| 5.6     | CONCLUSIONS.....                            | 109 |

## CHAPTER 6

### DOSE-DEPENDENT EFFECTS OF CHOLECYSTOKININ-8 ON ANTROPYLORODUODENAL MOTILITY, GASTROINTESTINAL HORMONES, APPETITE AND ENERGY INTAKE IN HEALTHY MEN

|         |  |     |
|---------|--|-----|
| 6.1     | SUMMARY .....  | 110 |
| 6.2     | INTRODUCTION .....   | 111 |
| 6.3     | SUBJECTS AND METHODS.....  | 113 |
| 6.3.1   | Subjects .....   | 113 |
| 6.3.2   | Study design.....  | 113 |
| 6.3.3   | Protocol .....   | 114 |
| 6.3.4   | Measurements .....   | 115 |
| 6.3.4.1 | <i>Antropyloroduodenal pressures</i> .....   | 115 |
| 6.3.4.2 | <i>Plasma hormone concentrations</i> .....   | 115 |
| 6.3.4.3 | <i>Appetite</i> .....  | 115 |
| 6.3.4.4 | <i>Energy intake</i> .....   | 115 |
| 6.3.5   | Statistical analysis .....   | 116 |
| 6.4     | RESULTS .....  | 117 |
| 6.4.1   | Antropyloroduodenal pressures .....  | 117 |
| 6.4.1.1 | <i>Antral pressures</i> .....  | 117 |
| 6.4.1.2 | <i>Pyloric pressures</i> .....   | 118 |
| 6.4.1.3 | <i>Duodenal pressures</i> .....  | 119 |
| 6.4.2   | Gastrointestinal hormone concentrations.....   | 119 |
| 6.4.2.1 | <i>Plasma CCK</i> .....  | 119 |
| 6.4.2.2 | <i>Plasma PYY</i> .....  | 120 |
| 6.4.2.3 | <i>Plasma ghrelin</i> .....  | 121 |
| 6.4.3   | Appetite.....  | 122 |
| 6.4.4   | Energy intake .....  | 122 |
| 6.4.5   | Relations between antropyloroduodenal motility, plasma hormones, appetite and energy intake..... | 123 |

|         |   |     |
|---------|---|-----|
| 6.4.5.1 | <i>Relationships between antropyloroduodenal motility, plasma hormones, appetite and energy intake with the dose of CCK-8 administered.....</i> | 123 |
| 6.4.5.2 | <i>Relationships between antropyloroduodenal motility with plasma hormones.....</i>   | 123 |
| 6.4.5.3 | <i>Relationships between energy intake with antropyloroduodenal motility and plasma hormones.....</i>   | 124 |
| 6.5     | DISCUSSION.....   | 130 |
| 6.6     | CONCLUSIONS.....  | 134 |

## **CHAPTER 7**

### **COMPARATIVE EFFECTS OF FAT, PROTEIN AND CARBOHYDRATE, AND DIFFERENT PROTEIN LOADS, ON APPETITE AND SUBSEQUENT ENERGY INTAKE IN LEAN AND OBESE SUBJECTS**

|         |  |     |
|---------|--|-----|
| 7.1     | SUMMARY.....   | 135 |
| 7.2     | INTRODUCTION.....                                      | 136 |
| 7.3     | SUBJECTS AND METHODS.....                              | 138 |
| 7.3.1   | Subjects.....  | 138 |
| 7.3.2   | Study design.....                                      | 139 |
| 7.3.2.1 | <i>Meal preload preparation.....</i>                   | 139 |
| 7.3.3   | Protocol.....  | 140 |
| 7.3.4   | Measurements.....                                      | 141 |
| 7.3.4.1 | <i>Plasma hormone concentrations.....</i>              | 141 |
| 7.3.4.2 | <i>Appetite.....</i>                                   | 142 |
| 7.3.4.3 | <i>Energy intake.....</i>                              | 142 |
| 7.3.5   | Statistical analysis.....                              | 142 |
| 7.4     | RESULTS.....   | 143 |
| 7.4.1   | Appetite.....  | 143 |
| 7.4.1.1 | <i>Lean subjects.....</i>                              | 143 |
| 7.4.1.2 | <i>Obese subjects.....</i>                             | 144 |
| 7.4.1.3 | <i>Comparison between lean and obese subjects.....</i> | 145 |

|         |   |     |
|---------|---|-----|
| 7.4.2   | Energy intake .....   | 145 |
| 7.4.2.1 | <i>Lean subjects</i> .....  | 145 |
| 7.4.2.2 | <i>Obese subjects</i> .....   | 146 |
| 7.4.2.3 | <i>Comparison of lean and obese subjects</i> .....                                | 147 |
| 7.4.3   | Relationships between energy intake with appetite in lean and obese subjects..... | 148 |
| 7.5     | DISCUSSION.....   | 153 |
| 7.6     | CONCLUSIONS.....  | 157 |

## **CHAPTER 8**

### **REPRODUCIBILITY OF ENERGY INTAKE, GASTRIC EMPTYING, BLOOD GLUCOSE, PLASMA INSULIN AND CHOLECYSTOKININ RESPONSES IN HEALTHY YOUNG MALES**

|         |  |     |
|---------|--|-----|
| 8.1     | SUMMARY .....  | 158 |
| 8.2     | INTRODUCTION .....   | 159 |
| 8.3     | SUBJECTS AND METHODS.....  | 162 |
| 8.3.1   | Subjects .....   | 162 |
| 8.3.2   | Study design.....  | 162 |
| 8.3.3   | Protocol.....  | 163 |
| 8.3.4   | Measurements .....   | 163 |
| 8.3.4.1 | <i>Gastric emptying and intragastric meal distribution</i> .....         | 163 |
| 8.3.4.2 | <i>Blood glucose, plasma insulin and plasma CCK concentrations</i> ..... | 164 |
| 8.3.4.3 | <i>Appetite perceptions and energy intake</i> .....                      | 164 |
| 8.3.4.4 | <i>Energy intake</i> .....   | 164 |
| 8.3.5   | Statistical analysis .....   | 164 |
| 8.4     | RESULTS .....  | 166 |
| 8.4.1   | Gastric emptying.....  | 166 |
| 8.4.1.1 | <i>Total gastric emptying</i> .....                                      | 166 |
| 8.4.1.2 | <i>Intragastric meal distribution</i> .....                              | 166 |
| 8.4.2   | Blood glucose and plasma hormone concentrations.....                     | 167 |
| 8.4.2.1 | <i>Blood glucose</i> .....   | 167 |

|         |   |     |
|---------|---|-----|
| 8.4.2.2 | <i>Plasma insulin</i> .....   | 167 |
| 8.4.2.3 | <i>Plasma CCK</i> .....   | 168 |
| 8.4.3   | Appetite and energy intake .....  | 168 |
| 8.4.3.1 | <i>Appetite</i> .....   | 168 |
| 8.4.3.2 | <i>Energy intake</i> .....  | 169 |
| 8.4.4   | Relations between gastric emptying, blood glucose, hormones and energy intake .....                                 | 169 |
| 8.4.4.1 | <i>Relationships between gastric emptying, blood glucose, and plasma insulin and CCK with energy intake</i> .....   | 169 |
| 8.4.5   | Calculation of minimum effect sizes for the parameters assessed, based on observed intra-individual variations..... | 171 |
| 8.5     | DISCUSSION .....  | 178 |
| 8.6     | CONCLUSIONS.....  | 182 |

## **CHAPTER 9**

### **EFFECTS OF THE PHASES OF THE MENSTRUAL CYCLE ON GASTRIC EMPTYING, GLYCAEMIA, PLASMA GLP-1 AND INSULIN, AND ENERGY INTAKE IN HEALTHY LEAN WOMEN**

|         |  |     |
|---------|--|-----|
| 9.1     | SUMMARY .....  | 183 |
| 9.2     | INTRODUCTION .....   | 184 |
| 9.3     | SUBJECTS AND METHODS.....  | 187 |
| 9.3.1   | Subjects .....   | 187 |
| 9.3.2   | Study design.....  | 188 |
| 9.3.3   | Protocol.....  | 188 |
| 9.3.4   | Measurements .....   | 190 |
| 9.3.4.1 | <i>Gastric emptying and intragastric meal distribution</i> .....   | 190 |
| 9.3.4.2 | <i>Blood glucose, plasma GLP-1, insulin and CCK, and serum estradiol and progesterone concentrations</i> ..... | 190 |
| 9.3.4.3 | <i>Appetite</i> .....  | 190 |
| 9.3.4.4 | <i>Energy intake</i> .....   | 190 |
| 9.3.5   | Statistical analysis .....   | 190 |

|         |   |     |
|---------|---|-----|
| 9.4     | RESULTS .....   | 192 |
| 9.4.1   | Gastric emptying .....  | 192 |
| 9.4.1.1 | <i>Total gastric emptying</i> .....   | 192 |
| 9.4.1.2 | <i>Intragastric meal distribution</i> .....   | 193 |
| 9.4.2   | Blood glucose and plasma hormone concentrations .....   | 193 |
| 9.4.2.1 | <i>Blood glucose</i> .....  | 194 |
| 9.4.2.2 | <i>Plasma GLP-1</i> .....   | 194 |
| 9.4.2.3 | <i>Plasma insulin</i> .....   | 195 |
| 9.4.2.4 | <i>Plasma CCK</i> .....   | 196 |
| 9.4.3   | Appetite and energy intake .....  | 196 |
| 9.4.3.1 | <i>Appetite</i> .....   | 196 |
| 9.4.3.2 | <i>Energy intake</i> .....  | 197 |
| 9.4.4   | Relations between gastric emptying, hormones, blood glucose, appetite and energy intake ..... | 198 |
| 9.4.4.1 | <i>Relationships between hormones and blood glucose with gastric emptying</i> .....           | 198 |
| 9.4.4.2 | <i>Relationships between energy intake with gastric emptying and hunger</i> .....             | 198 |
| 9.4.5   | Intra-subject reproducibility between FOL-P1 and FOL-P2 .....                                 | 199 |
| 9.5     | DISCUSSION .....  | 208 |
| 9.6     | CONCLUSIONS.....  | 212 |

## **CHAPTER 10**

### **EFFECTS OF ACUTE ENERGY RESTRICTION ON ANTROPYLORODUODENAL MOTOR, APPETITE AND ENERGY INTAKE RESPONSES TO SMALL INTESTINAL FAT IN THE OBESE**

|        |                           |     |
|--------|---------------------------|-----|
| 10.1   | SUMMARY .....             | 214 |
| 10.2   | INTRODUCTION .....        | 215 |
| 10.3   | SUBJECTS AND METHODS..... | 218 |
| 10.3.1 | Subjects .....            | 218 |
| 10.3.2 | Study design.....         | 219 |

|          |  |     |
|----------|--|-----|
| 10.3.3   | Very-low calorie diet (VLCD)   | 219 |
| 10.3.4   | Protocol   | 220 |
| 10.3.5   | Measurements   | 220 |
| 10.3.5.1 | <i>Antropyloroduodenal pressures</i>   | 220 |
| 10.3.5.2 | <i>Plasma hormone concentrations</i>   | 221 |
| 10.3.5.3 | <i>Appetite</i>  | 221 |
| 10.3.5.4 | <i>Energy intake</i>   | 221 |
| 10.3.6   | Statistical analysis   | 221 |
| 10.4     | RESULTS  | 222 |
| 10.4.1   | Antropyloroduodenal pressures  | 223 |
| 10.4.1.1 | <i>Antral pressures</i>  | 223 |
| 10.4.1.2 | <i>Pyloric pressures</i>   | 223 |
| 10.4.1.3 | <i>Duodenal pressures</i>  | 225 |
| 10.4.1.4 | <i>Pressure wave sequences</i>   | 225 |
| 10.4.2   | Appetite   | 225 |
| 10.4.3   | Energy intake  | 226 |
| 10.4.4   | Relationships between energy intake with antropyloroduodenal motility and appetite | 226 |
| 10.5     | DISCUSSION   | 232 |
| 10.6     | CONCLUSIONS  | 235 |

## **CHAPTER 11**

|                    |     |
|--------------------|-----|
| <b>CONCLUSIONS</b> | 236 |
| APPENDIX I         | 241 |
| APPENDIX II        | 245 |
| APPENDIX III       | 246 |
| APPENDIX IV        | 247 |
| REFERENCES         | 262 |

# LIST OF ABBREVIATIONS

|       |                                     |
|-------|-------------------------------------|
| 3-D   | three-dimensional                   |
| AP    | adequate-protein                    |
| APD   | antropyloroduodenal                 |
| AUC   | area under the curve                |
| BMI   | body mass index                     |
| CCK   | cholecystokinin                     |
| CHO   | carbohydrate                        |
| CV    | coefficient of variation            |
| FSH   | follicle stimulating hormone        |
| GLP-1 | glucagon-like peptide-1             |
| HC    | high-carbohydrate                   |
| HF    | high-fat                            |
| HP    | high-protein                        |
| IPPWs | isolated pyloric pressure waves     |
| IV    | intravenous                         |
| LH    | luteinizing hormone                 |
| LOX   | loxiglumide                         |
| LP    | low-protein                         |
| MMC   | migrating motor complex             |
| PWs   | pressure waves                      |
| PWSs  | pressure wave sequences             |
| PYY   | peptide YY                          |
| SEM   | standard error of the mean          |
| TMPD  | transmucosal potential difference   |
| VAS   | visual analogue scale questionnaire |
| VLCD  | very-low calorie diet               |

## THESIS SUMMARY

The research presented within this thesis has focussed on the complex and interrelated postprandial gastrointestinal mechanisms involved in the regulation of appetite and energy intake. The three broad areas that have been investigated include: (i) the effect of gastrointestinal hormones on gastric motility, gastrointestinal hormone release/suppression, appetite and energy intake in healthy lean subjects, (ii) the effect of oral macronutrients on appetite and energy intake in both lean and obese subjects and (iii) the effects of acute energy restriction on gastrointestinal motility, gastrointestinal hormone release, appetite and energy intake in obese subjects.

Following meal ingestion, the presence of nutrients in the small intestine stimulate small intestinal receptors that trigger a number of gastrointestinal mechanisms within ~ 15 minutes; these include the modulation of gastric emptying and gastrointestinal motility and the release, or suppression, of gastrointestinal hormones i.e. cholecystokinin (CCK), peptide-YY (PYY), glucagon-like peptide-1 (GLP-1) and ghrelin. Hence, it is conceivable that interactions occur between one or more of these stimuli. The study in **Chapter 5** assessed possible interactions between intravenous CCK (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) that may modulate ghrelin and PYY release. At the doses evaluated, exogenous CCK-8 and GLP-1 had discrepant effects on the secretion of ghrelin and PYY; CCK-8

markedly suppressed ghrelin whereas GLP-1 had no effect, and the stimulation of PYY by CCK-8 was attenuated markedly by GLP-1.

Of the gastrointestinal hormones modulated following nutrient ingestion, CCK and its role in appetite regulation has been studied the most comprehensively. A recent study from our laboratory using exogenous CCK-8 suggested that the ability of CCK to suppress appetite and energy intake were mediated, at least in part, by its actions on the gastrointestinal tract. However, the plasma CCK concentrations resulting from this study were moderately supraphysiological and infusion of CCK-8 was associated with an increase, albeit modest, in nausea. The effects of increasing doses of CCK-8 on gastrointestinal motility, gut hormone release and the relationships between these effects with those on hunger and energy intake had not hitherto been assessed in humans. In **Chapter 6**, exogenous CCK-8 stimulated pressures in the pylorus, increased plasma PYY concentrations and suppressed desire-to-eat and energy intake in a dose-dependent manner, while all CCK-8 doses equally suppressed ghrelin. There were relationships between plasma CCK with basal pyloric pressure and isolated pyloric pressure waves, and energy intake with isolated pyloric pressure waves.

The prevalence of obesity is rapidly increasing, the cause of which is related, in part, to the readily available supply of high-fat, energy-dense foods. Recent data indicate that there are more than 250 million obese people worldwide, representing ~ 7 % of the adult population. There is evidence that gastrointestinal function in obesity is modified, which may be the result of the eating habits of obese

individuals, and in turn, may also contribute to the maintenance of obesity by causing insufficient suppression of energy intake. However, much of the literature relating to gastrointestinal function in the obese is inconclusive and controversial. A better understanding of any adaptations that occur in obesity is important, particularly in regards to treatment approaches for weight loss.

Protein is considered to be the most satiating macronutrient and studies have demonstrated that consumption of dietary protein reduces appetite and *ad libitum* energy intake when compared with either carbohydrate or fat. One option in the dietary management of obesity has been to replace some carbohydrate in the diet with protein, which has been demonstrated to facilitate loss of fat and blunt loss of lean mass. However, there are discrepancies in the ranking of macronutrients and not all studies demonstrate that protein is more satiating than carbohydrates or fat. Furthermore, studies that have demonstrated effects of high-protein preloads on appetite and energy intake have often used preloads consisting of ~ 60 % protein. Thus, it is plausible that the observed effects may have been due to excessive amounts of protein in the test meal; such meals would be less palatable, which may also lead to reduced energy intake. Since there may be differences in the regulation of gastrointestinal motor function, gastrointestinal hormone release, appetite and energy intake between lean and obese individuals, it is likely that ingestion of individual macronutrients may also have different effects on these parameters, which might have implications for the dietary treatment of obesity.

The study in **Chapter 7** evaluated the effects of high-protein, high-fat and high-carbohydrate test meals, and increasing amounts of protein in a test meal, on appetite and energy intake in lean and obese subjects. In addition, the study compared these responses between lean and obese subjects. In lean, but not obese, subjects, hunger was less, and fullness increased, following ingestion of the HF and HP meals. In addition, energy intake was reduced in lean subjects following the HF and HP meals when compared with the HC meal, while in obese subjects, the HP and AP meals reduced energy intake when compared with the HF and HC meals, and HC meal, respectively. When these responses were compared, the percentage change in energy intake between the HF and AP test meals was significantly different between lean and obese, suggesting that obese subjects may be less sensitive to the satiating effects of fat.

The studies presented in the subsequent two chapters (**Chapters 8 and 9**) investigated the contribution of factors that may influence the effects of oral macronutrients on gastrointestinal function, appetite and energy intake. While young, lean males are the subject group most capable of adjusting their energy intake in response to caloric manipulation, it has been observed that significant inter-individual variation occurs within this group. Therefore, it was important to evaluate whether there was a day-to-day variability in gastrointestinal function, including gastric emptying and gastrointestinal hormone secretion, and if so, how these variations influenced temporal changes in appetite and energy intake. The study in **Chapter 8** demonstrated that, in a laboratory setting, appetite perceptions and energy intake in response to a nutrient preload in healthy lean men were

highly reproducible, and that this consistency in energy intake was associated with reproducible patterns of gastric emptying and insulin and CCK secretion.

A major reason that females are used less frequently than males in research studies assessing gastrointestinal function, appetite and energy intake is the perceived confounding effect of the menstrual cycle on these parameters. There is evidence that fluctuations in hormone levels over the menstrual cycle affect energy intake, such that hunger and energy intake are less during the follicular phase and increased during the luteal phase. How this modulation of appetite and energy intake would be related to changes in gastrointestinal function, i.e. gastric emptying and gastrointestinal hormone release, remained unclear. The study described in **Chapter 9** demonstrated that gastric emptying was slower, and glycaemia, plasma GLP-1 and insulin responses, hunger and energy intake were less, during the follicular when compared with the luteal phase. Moreover, energy intake and the glucose, plasma GLP-1 and insulin responses were related to gastric emptying. In addition, these parameters were reproducible when assessed twice within the follicular phase of the menstrual cycle.

There is evidence that both previous patterns of macronutrient intake and fasting affect gastrointestinal function. In the context of obesity, both are of relevance. For example, in humans after a high-fat diet for 2 weeks, gastric emptying and mouth-to-caecum transit in response to a high-fat test meal were faster. In contrast, fasting has the opposite effect and a 4-day fast slowed gastric emptying of a glucose drink in both lean and obese subjects, suggesting that a reduction in

nutrient exposure may increase the sensitivity of gastrointestinal responses to nutrients in the obese. The study in **Chapter 10** demonstrated that following a four-day very-low calorie diet (VLCD) there was a significant increase in basal pyloric pressure and the number and amplitude of isolated pyloric pressure waves, and a decrease in the number of antral and duodenal pressure waves and pressure wave sequences, during a 120 minute intraduodenal lipid infusion. In addition, following the four-day VLCD, hunger and prospective consumption scores were lower, and energy intake was reduced, indicating that gastrointestinal function, appetite and energy intake in the obese can be modified over a short period of time.

The studies reported in this thesis provide new information relating to the regulation of appetite and energy intake by gastrointestinal motor function and hormone release and/or suppression, in healthy lean and obese subjects. These observations will contribute to advances in basic appetite physiology and have clinical implications for further development of dietary interventions for successful treatment of obesity.

# STATEMENT OF ORIGINALITY

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder/s of those works.

Ixchel Brennan

June 2009

# DEDICATION

This thesis is dedicated to  
my mother, Kathleen Winifred Brennan  
and to  
my grandmother, Winifred Royal Brennan.

My first teachers.

I am indebted to your selfless, extraordinary commitment to my education.  
I am forever grateful to you both for giving me the courage to think for myself,  
for helping me learn to persevere and work hard to succeed and  
achieve my goals, and for instilling within me the confidence  
that I am capable of doing anything I put my mind to.

# ACKNOWLEDGMENTS

The studies reported in this thesis were performed in the Discipline of Medicine University of Adelaide at the Royal Adelaide Hospital.

Whilst conducting this research I was financially supported by a Dawes Postgraduate Research Scholarship (2006 – 2009), jointly provided by the Royal Adelaide Hospital and the University of Adelaide, and also by the Discipline of Medicine.

It is a pleasure for me to thank the many people who have made this thesis possible, and supported me throughout its progress. Firstly, I would like to start by thanking my supervisors Associate Professor Christine Feinle-Bisset and Professor Michael Horowitz. Your ideas and tremendous support have had a major influence on me and this thesis. To Christine, I am grateful for your invaluable comments, constructive criticisms and continuous encouragement and enthusiasm. For me, you have not only been a wonderful teacher, but also a friend and advisor. To Michael, thank you for your continued guidance and kindness and for always having the time to share your knowledge with me. I have no doubt that you have both significantly contributed to my desire and ability as a scientist to investigate, read, write and learn! Thank you for always encouraging me to give everything I attempted my best effort, no matter how small the task may have seemed. I am also very grateful to you both for providing me with the opportunity to travel and attend conferences in different parts of the world – an amazing experience. I have always felt extremely privileged to work closely with two individuals who are successful, dedicated and passionate about their field of research. In your different ways, you have taught me so much about the nature of scientific research, and also a great deal about myself. This experience has been invaluable and I will carry lessons I have learnt with me forever.

Within our 'Team Feinle-Bisset' research team there are some wonderful individuals who I have had the privilege of working with. To Dr Kate Smith, Dr Tanya Little and Dr Amelia Pilichiewicz, at last I join you in the 'Dr Club'! Kate, the opportunity to work with such a close friend was an incredible experience. Thank you for your perspective and honesty, for the many laughs with and/or at me! and for always being there to listen and offer some advice, especially when I have needed it most. I am excited about continuing to share with you all that lies ahead in this next phase of our lives! To Tanya and Amelia, thank you both for your friendship, support and encouragement (even from afar!). Amongst all the studies, tubes and data, I look back very appreciatively at the fun and great times we have all shared, which has helped make this experience all the more enjoyable! I am so proud of each of you and all that you have, and continue to, accomplish, and I look forward to sharing many more happy memories together. More recently, thank you to Radhika Seimon, for your all help, thoughtfulness and encouragement.

To Dr Diana Gentilcore and Dr Natalie Luscombe-Marsh, thank you both for your friendship and support. Di, I have valued our chats and I am grateful for all your help along the way, the hugs just at the right time and for always reminding me that I can do it! Nat, thank you for always having the time for me to pop my head in! In particular, thank you for your help and input with Chapter 7. Thank you both for sharing with me your experiences and honest advice over the years. I have learnt a great deal professionally, and personally, and am truly very appreciative.

To Associate Professor Karen Jones, thank you for your support and for your help with the gastric emptying studies.

Thank you to Judith Wishart for your hard work analysing the blood samples in Chapters 6, 8 and 9. Many thanks also to Kylie Lange who provided help and advice with statistical analyses.

## *Acknowledgments*

Thank you to all the staff and students in the Discipline of Medicine who have been a great source of advice, help, humour and perspective across the years. I have enjoyed getting to know and working along side, many of you. In particular, thank you to Paul Cavuoto and Lora Vanis. Paul, you really are a great bloke! Many thanks for all the footy related banter and all the other chats/debates and funny times we have shared! Lora, thank you for your encouragement and the many laughs and fun times we have had. I have appreciated our friendships and you have both provided me with a great deal of assurance and support. I would also like to thank Dr Paul Kuo, Sean Martin, Dr Jing Ma, Dr Julie Stevens, Antonietta Russo, Lisa Philp and Emily Woodridge for your friendship, help and encouragement. And to those who have moved on: Dr Kamilia Tai, Anne Maddox, Niva Nair and Dr Reawika Chaikomin, but whose support and contributions around the office, or to my work, I remain very grateful for.

To the International collaborators whom I have had the pleasure of working with: Professor Trygve Hausken – thank you for being an excellent teacher, for adding much humour into our work and for your help with the gastric emptying studies, Associate Professor Baerbel Otto – thank you for the analysis of PYY and ghrelin in Chapter 5 and Professor Andre Smout – thank you for your assistance with motility analysis; basal pyloric pressure has never been simpler to analyse for which many individuals are very grateful! And finally, Professor James Meyer, I am very grateful for your ideas and intellectual contributions to my work and a number of our manuscripts.

A special thank you to all of the individuals who volunteered their time to participate in the studies presented in thesis. A particular thank you to those individuals who participated in numerous studies across the years – I am grateful for your time, trust and the many interesting conversations that helped make studies go that bit faster!

I have often considered myself extremely blessed to have in my life, best friends who inspire me, and who are such confident, intelligent and compassionate

## *Acknowledgments*

women. To Josie Flynn, Michaela del Campo, Kate Fuss, Brigid Kinloch and Suzanna Parisi – thank you for sharing your lives with me. I am so grateful that you have always been there to listen, for your open and honest advice, the many, many laughs we have shared together and for your constant encouragement of me, and my goals. I look forward to continuing to share all that lies ahead for each of us.

To Philip Portella and Dr Hoa Nguyen – over many years you have been an enormous source of support and friendship. Thank you for always watching out for me, for the hilarious emails that make me laugh so loudly the whole office stares, and for teaching me that there is also plenty of time in life for some fun! Thank you also to Monika Laskowski and Dr Libby Markwick, for your friendship and the great times we have shared. Thank you to Dan Adey and Kimberley Kingsborough, James Bosley, Cheryl and Elly Rabbah and Diana Salerno and Andrew Iarossi. -You are wonderful friends, and I am grateful for your advice and encouragement, and all the happy memories spent together – here is to many more!

To all the Brennan families – thank you to my Uncles, Aunties and precious cousins who continue to reinforce to me the importance and wonderful gift that is family life. Thank you beautiful cousins, whose beaming smiles and open arms have never, ever failed to brighten my day.

To the Tittoto family, thank you for warmly welcoming me into your homes and hearts. I am grateful for the love and friendship you have offered me and I look forward to continuing to share many more years together as part of the Tittoto family.

To my beloved grandparents, Bob and Winnie Brennan. Not a day goes by when you don't cross my mind, and in all the good, and the tough times, I know you are close to me. From an early age you impressed upon me the importance of an education and the wonderful opportunities it can provide. But you were also

powerful examples to me of many qualities you cannot be ‘taught’ – generosity, faithfulness, respect, thoughtfulness, forgiveness and patience to name a few, and for these lessons, I will be forever grateful.

To my mum, Kathy and my brother, Carlos. Mum, I believe I am the woman I am today so much because of your influence, constant support and love. You have not only been my guardian, but also my confidante, my carer and my friend. The depth of your selflessness, courage and determination knows no bounds – you have taught me not just through your words, but your actions, how to be thoughtful and considerate, how to persevere and overcome and how to have faith and trust. Carl, thank you for being my friend – the one who has always humoured me even when I have been tired and grouchy! You have often helped me put things into perspective and I am grateful for your openness, respect and all the wonderful times we share together!! Thank you both for all the car rides to and from work – quite often filled with listening to me and my work related developments, the many dinners (and desserts!) and the nights filled with laughter and jokes! Thank you both also for being so proud of me, for your words of encouragement and for the many little things, that at the time often went unnoticed, but that have made an enormous difference in how confidently I have been able to approach these past few years. I will be forever grateful and I love you both.

Finally, to my husband Silvano. You are my best friend and my love, and a constant reminder of what is most important to me in this life. Thank you for your quick wit and humour, which has helped dissipate many a stressful moment! I am so grateful for your continued and wholehearted encouragement of me, your unconditional support and the love you show me. You bring out the best in me, and you help me value each and every moment of life just by being a part of mine. I am very excited about facing all the ups and downs, challenges and adventures that life has in store with you by my side. I love you more than I can ever say.

## PUBLICATIONS ARISING FROM THESIS

**Brennan IM**, Otto B, Feltrin KL, Meyer JH, Horowitz M, Feinle-Bisset C. Intravenous CCK-8, but not GLP-1, suppresses ghrelin and stimulates PYY release in healthy men. *Peptides*: 28 (3): 607-611, 2007.

Nair NS, **Brennan IM\***, Little TJ, Gentilcore D, Hausken T, Jones KL, Wishart JM, Horowitz M, Feinle-Bisset C. Reproducibility of energy intake, gastric emptying, blood glucose, plasma insulin and cholecystokinin responses in healthy young males. *Br J Nutr*: 101 (7): 1094-1102, 2008. \*Denotes equal first authorship

**Brennan IM**, Little TJ, Feltrin KL, Smout AJ, Wishart JM, Horowitz M, Feinle-Bisset C. Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite and energy intake in healthy men. *Am J Physiol Endocrinol Metab*: 295 (6): E1487-E1494, 2008.

**Brennan IM**, Nair NS, Little TJ, Gentilcore D, Hausken T, Jones KL, Wishart J, Horowitz M, Feinle-Bisset C. Effects of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin and energy intake in healthy lean women. *Am J Physiol Gastro Intest Liver Physiol*: 297 (3): G602-G610, 2009.

## OTHER PUBLICATIONS

**Brennan IM**, Feltrin KL, Horowitz M, Smout AJPM, Meyer JH, Wishart J, Feinle-Bisset C. Evaluation of interactions between CCK and GLP-1 in their effects on appetite, energy intake and antropyloroduodenal motility in healthy men. *Am J Physiol Regul Integr Comp Physiol*: 288 (6): R1477-R1485, 2005.

Pilichiewicz AN, Little TJ, **Brennan IM**, Meyer JH, Wishart JM, Otto B, Horowitz M, Feinle-Bisset C. Effects of load, and duration, of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men. *Am J Physiol Regul Integr Comp Physiol*: 290 (3): R668-R677, 2006.

Pilichiewicz AN, Papadopoulos P, **Brennan IM**, Little TJ, Meyer JH, Wishart JM, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men. *Am J Physiol Regul Integr Comp Physiol*: 293 (6): R2170-R2178, 2007.

Pilichiewicz AN, Chaikomin R, **Brennan IM**, Wishart JM, Rayner C, Jones K, Smout AJPM, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. *Am J Physiol Endocrinol Metab*: 293 (3): E743-E753, 2007.

Cukier K, Pilichiewicz AN, Chaikomin R, **Brennan IM**, Wishart JM, Rayner C, Jones KL, Horowitz M, Feinle-Bisset C. Effect of small intestinal glucose load on plasma ghrelin in healthy men. *Am J Physiol Regul Integr Comp Physiol*: 295 (2): R459–R462, 2008.

Seimon RV, Feltrin KL, Meyer JH, **Brennan IM**, Wishart JM, Horowitz M, Feinle-Bisset C. Effects of varying combinations of intraduodenal lipid and carbohydrate on antropyloroduodenal motility, hormone release, and appetite in healthy males. *Am J Physiol Regul Integr Comp Physiol*: 296 (4): R912-R920, 2009.