



**Effects Of Energy Restriction And Macronutrient  
Composition On Weight Loss, Energy Expenditure, And  
Glucose, Insulin And Lipid Levels In Humans**

A thesis submitted to the University of Adelaide by

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For the degree of

**Doctor of Philosophy**

November 2002

# TABLE OF CONTENTS

	<b>Page</b>
<b>Summary</b> .....	<b>viii</b>
<b>Acknowledgement</b> .....	<b>xii</b>
<b>Declaration of Originality</b> .....	<b>xiv</b>
<b>Publications and Prizes Arising from this Thesis</b> .....	<b>xv</b>
<b>List of Figures</b> .....	<b>xvi</b>
<b>List of Tables</b> .....	<b>xviii</b>
<b>List of Abbreviations</b> .....	<b>xxi</b>

## **CHAPTER 1 Human Obesity: The Regulation Of Body Weight And Dietary Strategies For Weight Loss**

<b>1.1</b>	<b>Introduction</b> .....	<b>2</b>
<b>1.2</b>	<b>Definition and prevalence of obesity</b> .....	<b>4</b>
	1.2.1 Definition of obesity.....	4
	1.2.2 Prevalence of obesity.....	6
	1.2.3 Economic costs of obesity.....	7
<b>1.3</b>	<b>Health consequences of obesity</b> .....	<b>7</b>
	1.3.1 Type 2 Diabetes Mellitus.....	8
	1.3.2 Fatty liver disease.....	11
	1.3.3 Hypertension.....	13
	1.3.4 Cardiovascular disease.....	13
<b>1.4</b>	<b>The influence of body fat distribution on the health consequences of obesity</b> .....	<b>15</b>
<b>1.5</b>	<b>Role of insulin resistance in the development of hyperglycaemia and the risk factors of cardiovascular disease</b> .....	<b>17</b>
	1.5.1 Insulin resistance and the development of hyperglycaemia.....	18
	1.5.2 Insulin resistance and the development of cardiovascular disease.....	19
<b>1.6</b>	<b>The regulation of human body weight and factors influencing the development of obesity</b> .....	<b>21</b>
	1.6.1 Evidence that body weight is physiologically regulated.....	22
	1.6.2 The physiological system regulating body weight.....	24
	1.6.3 Macronutrient balance.....	28

1.6.4	The regulation of appetite and food intake.....	36
1.6.5	The role of energy expenditure in the regulation of body weight.....	46
<b>1.7</b>	<b>Dietary strategies for the treatment of obesity and its associated diseases.....</b>	<b>59</b>
1.7.1	Low-fat, high-carbohydrate diets.....	61
1.7.2	Low-fat, high-protein diets.....	72
1.7.3	Other types of popular fad diets.....	88
<b>1.8</b>	<b>Overall objectives.....</b>	<b>89</b>
1.7.1	Hypotheses.....	90
1.7.2	Aims.....	90

## **CHAPTER 2 Common Methodologies**

<b>2.1</b>	<b>Introduction.....</b>	<b>93</b>
<b>2.2</b>	<b>Ethics approval.....</b>	<b>93</b>
<b>2.3</b>	<b>Techniques for measurements.....</b>	<b>93</b>
2.3.1	Energy expenditure.....	93
2.3.2	Respiratory quotient.....	93
2.3.3	Energy intake, macronutrient composition, and food quotient.....	102
2.3.4	Body weight, height and body mass index.....	104
2.3.5	Body composition.....	107
2.3.6	Blood sampling and 24-hour urine collection.....	110
2.3.7	Biochemical variables.....	110

## **CHAPTER 3 Reproducibility and Reliability of Indirect Calorimetry for Measuring Resting Energy Expenditure, Respiratory Quotient and the Thermic Effect of Feeding**

<b>3.1</b>	<b>Summary.....</b>	<b>114</b>
<b>3.2</b>	<b>Introduction.....</b>	<b>114</b>
<b>3.3</b>	<b>Research design and methods.....</b>	<b>116</b>
3.3.1	Subjects.....	116
3.3.2	Experimental design.....	117
3.3.3	Measurements.....	117
3.3.4	Statistical analysis.....	118
<b>3.4</b>	<b>Results.....</b>	<b>119</b>

3.4.1	Subject characteristics.....	119
3.4.2	Resting energy expenditure, respiratory quotient and the thermic effect of feeding.....	119
<b>3.5</b>	<b>Discussion.....</b>	<b>123</b>
<b>3.6</b>	<b>Conclusion.....</b>	<b>126</b>

**CHAPTER 4    Reproducibility and Reliability of Dual-Energy X-ray Absorptiometry for Measuring Body Composition**

<b>4.1</b>	<b>Summary.....</b>	<b>128</b>
<b>4.2</b>	<b>Introduction.....</b>	<b>128</b>
<b>4.3</b>	<b>Research design and methods.....</b>	<b>130</b>
4.3.1	Subjects.....	130
4.3.2	Experimental design.....	131
4.3.3	Measurements.....	131
4.3.4	Statistical analysis.....	131
<b>4.4</b>	<b>Results.....</b>	<b>132</b>
4.4.1	Subject characteristics.....	132
4.4.2	Whole body bone mineral content, lean mass, fat mass, and percentage body fat as measured by DEXA.....	132
<b>4.5</b>	<b>Discussion.....</b>	<b>136</b>
<b>4.6</b>	<b>Conclusion.....</b>	<b>138</b>

**CHAPTER 5    The [<sup>14</sup>C]-Bicarbonate-Urea Method for Measuring Total Energy Expenditure**

<b>5.1</b>	<b>Summary.....</b>	<b>140</b>
<b>5.2</b>	<b>Introduction.....</b>	<b>140</b>
<b>5.3</b>	<b>Principle and background of the [<sup>14</sup>C]-bicarbonate-urea method.....</b>	<b>141</b>
5.3.1	Principle behind the [ <sup>14</sup> C]-bicarbonate-urea method.....	141
5.3.2	Accuracy of the [ <sup>14</sup> C]-bicarbonate-urea method.....	143
<b>5.4</b>	<b>The [<sup>14</sup>C]-bicarbonate-urea method in our laboratory.....</b>	<b>146</b>
5.4.1	Preparation of the [ <sup>14</sup> C]-urea and [ <sup>14</sup> C]-bicarbonate solutions.....	146
5.4.2	Administration of [ <sup>14</sup> C]-bicarbonate-urea.....	147
5.4.3	Twenty-four hour urine collection.....	149
5.4.4	Assay for measuring the specific activity of urinary-urea.....	149

5.4.5	Calculating the amount of CO <sub>2</sub> produced per day and total energy expenditure.....	155
5.5	<b>Conclusion.....</b>	<b>156</b>

**CHAPTER 6    Reproducibility, Reliability And Suitability of [<sup>14</sup>C]-Bicarbonate-Urea For Measuring Total Energy Expenditure in Non-obese and Obese Subjects**

6.1	<b>Summary.....</b>	<b>159</b>
6.2	<b>Introduction.....</b>	<b>160</b>
6.3	<b>Research design and methods.....</b>	<b>161</b>
6.3.1	Subjects.....	161
6.3.2	Experimental design.....	162
6.3.3	Measurements.....	163
6.3.4	Statistical analysis.....	164
6.4	<b>Results.....</b>	<b>165</b>
6.4.1	Subject characteristics and compliance.....	165
6.4.2	Total energy expenditure, resting energy expenditure, and physical activity energy expenditure.....	166
6.4.3	Predicted TEE and its' comparability to the [ <sup>14</sup> C]-bicarbonate-urea measurement of TEE.....	168
6.4.4	Suitability and comfort of the [ <sup>14</sup> C]-bicarbonate-urea method.....	169
6.5	<b>Discussion.....</b>	<b>174</b>
6.6	<b>Conclusion.....</b>	<b>179</b>

**CHAPTER 7    Use Of [<sup>14</sup>C]-Bicarbonate-Urea To Measure The Effect Of Weight Loss On Total Energy Expenditure After An Energy Restrictive 'Modifast' Diet In Overweight Subjects**

7.1	<b>Summary.....</b>	<b>181</b>
7.2	<b>Introduction.....</b>	<b>182</b>
7.3	<b>Research design and methods.....</b>	<b>182</b>
7.3.1	Subjects.....	182
7.3.2	Diet.....	184
7.3.3	Experimental design.....	185
7.3.4	Measurements.....	185

7.3.5	Statistical analysis.....	187
<b>7.4</b>	<b>Results.....</b>	<b>188</b>
7.4.1	Subject characteristics.....	187
7.4.2	Energy intake during the 3 days of energy expenditure measurements and urinary creatinine.....	187
7.4.3	Body weight and body composition.....	188
7.4.4	Total energy expenditure.....	189
7.4.5	Resting energy expenditure, the thermic effect of feeding and physical activity energy expenditure.....	189
7.4.6	Respiratory quotient.....	190
<b>7.5</b>	<b>Discussion.....</b>	<b>195</b>
<b>7.6</b>	<b>Conclusion.....</b>	<b>200</b>

**CHAPTER 8    Effect of a High-protein Weight Loss Diet on Resting Energy  
Expenditure and the Thermic Effect of Feeding, and Glycaemic  
Control and Lipid Levels in Men and Women with Type 2 Diabetes.**

<b>8.1</b>	<b>Summary.....</b>	<b>202</b>
<b>8.2</b>	<b>Introduction.....</b>	<b>203</b>
<b>8.3</b>	<b>Research design and methods.....</b>	<b>206</b>
8.3.1	Subjects.....	206
8.3.2	Diets.....	207
8.3.3	Experimental design.....	208
8.3.4	Measurements.....	209
8.3.5	Statistical analysis.....	211
<b>8.4</b>	<b>Results.....</b>	<b>211</b>
8.4.1	Subject characteristics.....	211
8.4.2	Diet composition and subject compliance.....	211
8.4.3	Body weight and body composition.....	212
8.4.4	Glycaemic control and insulin sensitivity.....	213
8.4.5	Serum lipids.....	214
8.4.6	Resting energy expenditure, the thermic effect of feeding, and respiratory quotient.....	215
8.4.7	Blood pressure.....	216
<b>8.5</b>	<b>Discussion.....</b>	<b>227</b>
<b>8.6</b>	<b>Conclusion.....</b>	<b>234</b>

**CHAPTER 9**     **Effect of a High-protein Weight Loss Diet on Energy Expenditure, Glycaemic Control and Lipid Levels in Hyperinsulinemic Men and Women**

<b>9.1</b>	<b>Summary.....</b>	<b>236</b>
<b>9.2</b>	<b>Introduction.....</b>	<b>237</b>
<b>9.3</b>	<b>Research design and methods.....</b>	<b>240</b>
9.3.1	Subjects.....	240
9.3.2	Diets.....	241
9.3.3	Experimental design.....	241
9.3.4	Measurements.....	243
9.3.5	Statistical analysis.....	244
<b>9.4</b>	<b>Results.....</b>	<b>245</b>
9.4.1	Subject characteristics.....	245
9.4.2	Diet composition and subject compliance.....	245
9.4.3	Body weight and body composition.....	246
9.4.4	Glycaemic control, insulin sensitivity and free fatty acids.....	247
9.4.5	Serum lipids.....	248
9.4.6	Total energy expenditure, resting energy expenditure, and energy expenditure due to physical activity.....	249
9.4.7	Thermic effect of feeding and respiratory quotient.....	250
9.4.8	Bone turnover and blood pressure.....	251
<b>9.5</b>	<b>Discussion.....</b>	<b>264</b>
<b>9.6</b>	<b>Conclusion.....</b>	<b>273</b>

**CHAPTER 10**     **Conclusions**

<b>10.1</b>	<b>Introduction.....</b>	<b>275</b>
<b>10.2</b>	<b>Main findings .....</b>	<b>276</b>
<b>10.3</b>	<b>Conclusions and implications.....</b>	<b>279</b>
<b>10.4</b>	<b>Limitations .....</b>	<b>281</b>
<b>10.5</b>	<b>Future research.....</b>	<b>285</b>

<b><u>BIBLIOGRAPHY.....</u></b>	<b>287</b>
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**APPENDIX**

**A 1** Physical activity diary.....322

**A 2** Food diary.....326

**A 3** Daily food checklist.....330

**A 4** Questionnaire assessing the usefulness of the [<sup>14</sup>C]-bicarbonate-urea method for measuring total energy expenditure.....332

**A 5** An itemized composition of the low and high-protein test meals used in the thermic effect of feeding assessment in chapter 8.....334

**A 6** An itemized composition of the low and high-protein test meals used in the meal tolerance and thermic effect of feeding assessment in chapter 9.....335



## SUMMARY

Weight loss is essential in the management of obesity and obesity related diseases such as Type 2 diabetes mellitus and cardiovascular disease. Moderate energy restriction (~2000 to 4200 kJ less than average daily energy requirements) and a subsequent reduction in weight of as little as 5 to 10% may significantly improve blood pressure, fasting plasma glucose, and fasting serum insulin and lipids. There remains no consensus on the optimal macronutrient composition of weight loss diets apart from recommendations that the saturated fat content be kept low (< 10%). However, there is some concern that high-carbohydrate (50 to 60% of total energy), low-fat (< 30% energy) diets that are traditionally used for weight loss, may raise plasma glucose and serum triacylglycerol concentrations, and may reduce LDL-lipoprotein particle size.

Within the dieting public, there has been a resurgence in the popularity of high-protein, low-carbohydrate weight loss diets. However, their efficacy in the treatment of obesity and Type 2 diabetes remains controversial. Several recent studies suggest that replacing some carbohydrate with protein, in low-fat diets, may blunt the diet-induced decrease in energy expenditure that is often observed after weight loss. Consequently, low-fat, high-protein diets may be more beneficial than low-fat, high-carbohydrate diets for long-term weight management. High-protein diets may also improve insulin sensitivity and thereby ameliorate insulin resistance. Accordingly, the focus of this thesis has been to investigate the effects of energy restriction and dietary macronutrient composition on weight loss and energy expenditure, as well as glucose, insulin and lipid levels, in obese adults with and without Type 2 diabetes.

A major aim of this work was to establish the [<sup>14</sup>C]-bicarbonate-urea method to measure total energy expenditure (TEE) in free-living subjects. Preliminary studies that evaluated the reproducibility and reliability of the method demonstrated that the intra-individual day-to-day variation in TEE was (mean ± SEM) 4.8 ± 1.0% for a non-obese group of men and 9.7 ± 1.3% in an obese group of men and women. The day-to-day reproducibility of the [<sup>14</sup>C]-bicarbonate-urea method was comparable to that of doubly labeled water (typically 8 to 14%). The reliability coefficient was high in both subject groups. Seventy-five percent of the non-obese and 73% of the obese individuals reported that the method allowed them to continue their normal lifestyle during the measurement period. These findings indicate that the [<sup>14</sup>C]-bicarbonate-urea method was well-tolerated by subjects under free-living conditions and is a reproducible and suitable method to measure TEE in normal and obese populations.

Preliminary studies were also conducted to determine the reproducibility and reliability of: 1) the Deltatrac™ Metabolic unit (indirect calorimetry) for measuring resting energy expenditure (REE), respiratory quotient (RQ) and the thermic effect of feeding (TEF), and 2) the Norland™ XR36 densitometer for measuring whole-body composition. In healthy men with a wide weight range (BMI 19.7 to 33.5 kg/m<sup>2</sup>) the within-subject day-to-day variation was  $1.7 \pm 0.41\%$  and  $3.1 \pm 0.8\%$  for fasting REE and RQ respectively, and  $7.8 \pm 1.5\%$  for the TEF measured over 2 hours. The reliability coefficient for REE was 0.97. For the RQ, a low reliability coefficient (0.35) may have reflected small differences in the composition of meals eaten the day prior to the study measurements, and it may also have reflect the high sensitivity of the Deltatrac for detecting small changes in RQ. These findings indicate that the Deltatrac metabolic unit was a reproducible and reliable instrument for measuring REE, RQ and TEF.

A separate study demonstrated that the measurements of total lean mass, total fat mass and body fat percentage using dual-energy X-ray absorptiometry had day-to-day variations of  $2.05 \pm 0.30\%$ ,  $2.34 \pm 0.73\%$ , and  $2.55 \pm 0.81\%$  respectively, in healthy men and women. The index of reliability was 0.99 for all body composition parameters. These findings indicate that the Norland™ XR36 densitometer is a reproducible and reliable method for measuring total body fat and lean mass in individuals that have a wide range of body weight. Subsequently, the above methods were used in three weight loss studies.

Resting energy expenditure is the major determinant of TEE in sedentary people. A small decrease in REE during energy restriction can lead to substantial decreases in daily energy-balance and consequently weight gain may occur. Relatively few studies have been conducted that assess, simultaneously, the impact of diet-induced weight loss on free-living TEE and its' components. The aim of the first weight loss study was to evaluate the effect of energy restriction on TEE and REE, the TEF, energy expenditure due to physical activity (PAEE) and RQ, after body weight is stabilized at a reduced level. Weight loss was induced using a combination of 'Modifast™' formula and one small meal per day (~3.3 MJ/day), in 6 men and 5 women who were overweight and obese. After 8 weeks of energy restriction and 2 weeks of weight maintenance, body weight was reduced  $12.2 \pm 1.6$  kg of which  $8.4 \pm 1.0$  kg was fat mass. Lean mass was reduced  $3.8 \pm 0.7$  kg. Resting energy expenditure was reduced  $5.6 \pm 1.3\%$  ( $500 \pm 128$  kJ/day) ( $p < 0.002$ ). Decreases in TEE ( $0.18 \pm 3.7\%$ ) and the TEF ( $1.4 \pm 0.9\%$ ), and the increase in PAEE ( $18.6 \pm 21.4\%$ )

were not significant. After the stabilization of the reduced body weight, the fasting and postprandial RQ remained unchanged. These findings suggest that after the stabilization of a moderately reduced body weight, REE but not TEE decreases. However, it is possible that decreases in TEE within the range of 0.1 to 10% were not detected because of the large degree of variability in the response, between-subjects.

Since the 1960s, high-protein diets with emphasis on some degree of carbohydrate restriction have been popular with the dieting public. The efficacy of high-protein diets for facilitating weight loss and ameliorating insulin resistance, in subjects with type 2 diabetes and in those with hyperinsulinemia, remains unclear. The overall aim of both the second and third studies was to compare a high-protein diet (30% of energy) [HP diet] with an isocaloric diet that had 15% of energy as protein [SP diet], during 8 (study 2) to 12 (study 3) weeks of moderate energy restriction (6.7 MJ/day and 6.4 MJ/day, respectively) and 4 weeks of energy-balance. Dietary protein was supplied as red meat, poultry and dairy foods. The diets were compared in 54 obese subjects (19 men/ 35 women) with Type 2 diabetes (study 2) and in 57 obese subjects (14 men/ 43 women) with hyperinsulinemia (study 3). Body weight and fasting glucose, insulin, and lipids were assessed at weeks 0, 4, 8 and 12 (in study 3 only). At both week 0 and week 12 (for study 2) or week 16 (for study 3), body composition and postprandial glucose and insulin concentrations were measured after an oral glucose tolerance test (in study 2) or after a meal tolerance test that was representative of the study diet (in study 3). In addition, 26 subjects (11 men/ 15 women) in study 2 and 36 subjects (10 men/ 13 women) in study 3 had measurements for REE, TEF, and RQ made. The 36 subjects in study 3 also had TEE measured. Both study 2 and study 3 showed that energy restriction is the major determinant of weight loss, at least over the short-term (12 to 16 weeks). However, women with type 2 diabetes, that were on the HP diet lost more total body fat (5.3 vs 2.8 kg,  $p = 0.009$ ) and abdominal fat (1.3 vs 0.7 kg,  $p = 0.006$ ) than the women on the SP diet. For the women with hyperinsulinemia, there was no difference in total or abdominal fat loss between diets; however, total lean mass was preserved more on the HP than on the SP diet in the women with hyperinsulinemia. In both study populations, the TEF was greatest after the HP than SP meal; however, it was not associated with weight loss. After weight loss, an increased ratio of protein-to-carbohydrate did not significantly blunt the decrease in REE or alter the TEF. In the subjects with hyperinsulinemia, there was no change in TEE after weight loss (TEE was not measured in the diabetic population). In both study 2 and 3, insulin sensitivity (as depicted by a significant reduction in the HOMA index) increased in all subjects, however, the increase was not dependent on diet composition ( $p < 0.001$ ). In the subjects with

hyperinsulinemia, the glycaemic response to the HP meal was less than to the SP meal at weeks 0 and 16 ( $p = 0.027$ ), and the decrease in glycaemic response after weight loss was greater in the high-protein group ( $p = 0.049$ ). Despite improvements in insulin sensitivity in the diabetic subjects, there was no overall change in RQ after weight loss. In the subjects with hyperinsulinemia, fasting RQ also remained unchanged, and the small increase in postprandial RQ was not physiologically significant and was not related to the improvements in insulin sensitivity. There were some benefits of substituting protein for carbohydrate on the plasma lipid profile; the HP diet reduced total and LDL-cholesterol more than the SP diet in subjects with type 2 diabetes, and the triacylglycerol concentrations were reduced more on the HP diet in the subjects with hyperinsulinemia. No adverse effects of the increased protein content were observed on markers of bone turnover, blood pressure, or urinary protein, in either study population. The findings from studies 2 and 3 indicate that caloric restriction, rather than the macronutrient composition of the diet is the most important determinant of weight loss. Replacing some carbohydrate with protein however, may lower the incidence hyperglycaemia and improve lipid levels in individuals with Type 2 diabetes or in those with hyperinsulinemia.

In summary, the studies reported in this thesis demonstrate that: 1) after the stabilization of a moderately reduced body weight, REE but not TEE decreases, although there is substantial variability between individuals for the effect of diet-induced weight loss on TEE when measurements are made in the free-living environment, 2) the reduction in REE and a reduced capacity to enhance fat oxidation after weight loss may be the main mechanisms that may predispose individuals to weight regain on resumption of a normal diet, and 3) the macronutrient composition of diets have no benefits over and above energy restriction on weight loss and energy expenditure. Improvements in insulin sensitivity following a meal containing a high-protein content, combined with improvements in fasting glucose and insulin homeostasis that were a consequence of weight loss, suggest that HP diets may be a suitable diet choice for people with Type 2 diabetes as well as for obese adults who are at risk of developing diabetes. A diet with an increased protein-to-carbohydrate ratio may also reduce the risk of cardiovascular disease, in individuals with dyslipidaemia. These observations will contribute to advances in basic energy metabolism and clinically to dietary interventions in the treatment of obesity and Type 2 diabetes mellitus.

## **ACKNOWLEDGEMENTS**

### **The following individuals and organisations made the studies in this thesis possible**

All the studies reported in this thesis were conducted in the Department of Medicine, Royal Adelaide Hospital and the Clinical Research Unit, CSIRO Health Science and Nutrition (1998 to 2002).

The studies reported in Chapters 8 and 9 were collaborative studies between Assoc Prof Gary Wittert, Assoc Prof Peter Clifton, and Dr Manny Noakes. The studies were funded by grants from the National Health and Medical Research Council and the Dairy Research and Development Corporation.

I was the recipient of a Dawes Scholarship, Royal Adelaide Hospital, (July 1998 to July 2001). The presentation of some of this work at scientific meetings was made possible by receipt of Travel Grants (2) awarded by the Australasian Society for the Study of Obesity.

### **The following honors students provided technical support on the studies indicated**

Ms Barbara Parker (Department of Physiology, University of Adelaide) collected the body composition, glycaemic control, and plasma lipids data reported in Chapter 8 and submitted this work as part of her Honor's thesis (University of Adelaide, 2000).

Ms Emma Farnsworth (Department of Physiology, University of Adelaide) collected the body composition, glycaemic control, and plasma lipids data reported in Chapter 9 and submitted this work as part of her Honor's thesis (University of Adelaide, 2001).

All data collected by Barbara and Emma was re-analysed and presented in a way that followed the aims and structure of this thesis.

### **I sincerely wish to thank**

My supervisors Assoc Prof Gary Wittert and Assoc Prof Peter Clifton. Thank-you for all your support throughout my professional development thus far. Your advice, sharing of knowledge, and enthusiasm for my work has encouraged me to pursue higher goals in scientific research. Your complementary natures have ensured that my PhD experience was rewarding. Working with both of you has been thoroughly enjoyable. Peter, thank-you for being the initial human 'guinea pig' in the development of the bicarbonate method and

in the practising of my venipuncture skills - better you than the volunteers! Once again, thanks to both of you.

Special thanks is also extended to Dr. Manny Noakes for the support and knowledge that she has provided throughout my honors and PhD, and for assistance she has provided in preparing and submitting manuscripts. Your chats regarding life after PhD and life in general have been much appreciated. Thanks for all the laughs in the clinic kitchen over the past four years.

Thank-you to the staff of the: i) Department of Medicine, RAH, ii) Human Nutrition Clinical Research Unit, CSIRO, and iii) Department of Nuclear Medicine, RAH. Collaborative studies between our Departments have introduced me to many wonderful and supportive people. In particular, many thanks to Dr Ian Kirkwood, Dr Chris Tsopelas and Max Bellon. Your expertise and guidance in establishing the [ $^{14}\text{C}$ ]-bicarbonate-urea technique, and your enthusiasm for presenting this work at nuclear medicine conferences, was greatly appreciated. Chris and Max, your humour and chats (even those on football-Go the Bombers!) while I was analysing way to many urine samples, kept me sane. To Anne McGuffin, Rosemary McArthur, Cherie Doran, Kay Pender, and Paul Foster, thank-you for all the hard work in running the weight loss trials that I have been involved with.

To fellow students/staff, Rosalie Vozzo, Barbara Parker, Jane Mudge, Deirdre O'Donovan, Christine Feinle, Selena Doran, Helen Turnbull, Zoe Holthouse, and Matthew Haren (Department of Medicine) and Ali Morris and Chris Bursill (CSIRO); conversations over coffee have made the past four years incredibly enjoyable. It has also been a delight to form many strong friendships away from the office. Thanks for the support and the memories!

A very special thank-you to my parents John and Georgine, Nan and Pa, Adam and Elizabeth, and Jamie. You have always encouraged me to aim high and have provided the greatest support to help me get there. Jamie, only a special man would keep me company in the lab, and in good humour, during the early hours of the morning. Thank-you for your love and patience over the past 5 years. Thank-you also to Rod, Ros, Paul, Jayne, Dan and Jo for your encouragement and delicious Monday night dinners!

Finally, thank-you to all the volunteers who participated in my studies.

## DECLARATION OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by any other person; except, in part, in the studies described in Chapters 8 and 9. The studies described in these two chapters were collaborative studies funded by a National Health and Medical Research Council grant and a large number of persons were involved in the data collection. Due reference has been made to the persons who significantly contributed to the collection of this data in the acknowledgement section. All data, however, was re-analysed and presented in a way that followed the aims and structure of this thesis.

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

Submitted for examination on the 22<sup>nd</sup> November 2002.

Amendments made in response to examiners comments and thesis re-submitted for graduation.

Signed: \_\_\_\_\_

Natalie Deanne Luscombe

Date: \_\_\_\_\_

11/07/2003

## PUBLICATIONS AND PRIZES ARISING FROM THIS THESIS

### Publications

1. Luscombe N.D., Wittert G., Noakes M., Parker B, Clifton P.M. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in Type 2 diabetes. *Diabetes Care* 2002; April 25(4):652-657.
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### Prizes

#### Award for Best Oral Presentation

Luscombe N.D., Kirkwood I., Bellon M., Tsopeles C., Wittert G. *Measurement of energy expenditure in overweight men and women using [<sup>14</sup>C]-bicarbonate-urea before and after weight loss.*

The Australasian Society for the Study of Obesity

10<sup>th</sup> Annual Scientific Meeting

Gold Coast, Queensland, Australia



## LIST OF FIGURES

	Page
<b>Figure 1.1</b>	The role of insulin resistance in the development of hyperglycaemia....18
<b>Figure 1.2</b>	The physiological regulation of body weight.....25
<b>Figure 1.3</b>	Key central sites involved in the regulation of appetite.....38
<b>Figure 1.4</b>	Overview of major peripheral factors controlling appetite.....40
<b>Figure 2.1</b>	Indirect calorimetry for the measurement of metabolic rate and respiratory quotient.....97
<b>Figure 2.2</b>	Image from a whole-body DEXA scan for the determination of body composition.....108
<b>Figure 2.3</b>	Bioelectrical impedance analysis for the measurement of body composition.....109
<b>Figure 5.3.1</b>	The fate of the infused [ <sup>14</sup> C]-bicarbonate during the determination of the amount of CO <sub>2</sub> produced over a 1-day period.....142
<b>Figure 5.4.2</b>	Apparatus used for the assay measuring the specific activity of urinary urea.....151
<b>Figure 5.4.3</b>	The effect of the initial pH of the assay mixture on the rate of urease activity.....154
<b>Figure 6.4.1</b>	Bland and Altman plots depicting the comparability of the [ <sup>14</sup> C]-bicarbonate-urea method and a prediction equation for measuring TEE in non-obese and obese groups of subjects.....173
<b>Figure 7.4.1</b>	Change in body weight following 8 weeks of energy restriction using “Modifast” and 2 weeks of weight maintenance at energy balance, in the 11 overweight men and women.....194
<b>Figure 8.4.1</b>	Urea excretion on the standard-protein and high-protein diets in subjects with Type 2 diabetes.....224
<b>Figure 8.4.2</b>	Change in body weight following 8 weeks of energy restriction and 4 weeks of energy balance on the standard-protein and high-protein study diets in subjects with Type 2 diabetes.....225
<b>Figure 8.4.3</b>	Glucose and insulin responses over 3-hours following an oral glucose tolerance test, in subjects with Type 2 diabetes.....226
<b>Figure 9.4.1</b>	Urea excretion on the standard-protein and high-protein diets in subjects with hyperinsulinemia.....260
<b>Figure 9.4.2</b>	Change in body weight following 12 weeks of energy restriction and 4 weeks of energy balance on the standard-protein and high-protein diets in subjects with hyperinsulinemia.....261

**Figure 9.4.3**    Glucose and insulin responses over 3-hours following meals that were representative of the standard-protein and high-protein diets, in subjects with hyperinsulinemia.....262

## LIST OF TABLES

Page

<b>Table 1.1</b>	Cut-off points proposed by a WHO expert committee for the classification of overweight and obese.....	4
<b>Table 1.2</b>	Estimated relative risk of health problems associated with obesity as proposed by the WHO Expert Committee.....	8
<b>Table 1.3</b>	Classification of normal and impaired glucose tolerance and diabetes, based on fasting and 2-hour plasma glucose values defined by the WHO, 1998.....	10
<b>Table 1.4</b>	Environmental influences on body weight.....	27
<b>Table 1.5</b>	The storage and oxidative characteristics of dietary protein, carbohydrate and fat.....	30
<b>Table 1.6</b>	Endogenous compounds implicated in the central regulation of mammalian feeding behaviour.....	39
<b>Table 1.7</b>	Gastrointestinal and pancreatic hormones that modulate food intake after peripheral administration.....	41
<b>Table 1.8</b>	Does reduced energy expenditure precede weight gain in prospective studies?.....	49
<b>Table 1.9</b>	Changes in total and resting energy expenditure during overfeeding and underfeeding studies.....	52
<b>Table 1.10</b>	Macronutrient composition of low-fat, high-carbohydrate weight loss diets.....	63
<b>Table 1.11</b>	Popular low-carbohydrate, high-protein diets.....	77
<b>Table 2.1</b>	Physical activity index for adult men and women at 8 different levels of activity.....	101
<b>Table 3.4.1</b>	Physical characteristic of the 13 healthy men who completed the indirect calorimetry reproducibility study.....	121
<b>Table 3.4.2</b>	Repeated measurements and the intra-individual day-to-day variations for resting energy expenditure, respiratory quotient, and the thermic effect of feeding in healthy men.....	122
<b>Table 4.4.1</b>	Physical characteristic of the 5 women and 3 men who completed the DEXA reproducibility study.....	134
<b>Table 4.4.2</b>	Repeated measurements and the intra-individual day-to-day variations for whole-body composition as measured by DEXA in 5 women and 3 men.....	135
<b>Table 6.4.1</b>	Physical characteristics of the non-obese and obese subjects who completed the [ <sup>14</sup> C]-bicarbonate reproducibility study.....	170

<b>Table 6.4.2</b>	Energy intake and macronutrient composition of the non-obese and obese subjects' habitual diet.....	171
<b>Table 6.4.3</b>	Repeated measurements of total energy expenditure, resting energy expenditure and energy expenditure due to physical activity in non-obese and obese subjects.....	172
<b>Table 7.4.1</b>	Characteristics of the 11 obese subjects who completed the "Modifast" diet, at baseline and after the maintenance of a reduced body weight .....	191
<b>Table 7.4.2</b>	Changes in fat mass, abdominal fat mass and lean mass for the 11 obese subjects who completed the "Modifast" diet .....	192
<b>Table 7.4.3</b>	Energy expenditure variables at weeks 0 and 10 for the 11 obese subjects who completed the "Modifast" diet .....	193
<b>Table 8.4.1</b>	Characteristics of 44 subjects with Type 2 diabetes at baseline.....	217
<b>Table 8.4.2</b>	Composition of study diets derived from daily weighed food records for the subjects with Type 2 diabetes.....	218
<b>Table 8.4.3</b>	Total fat mass, abdominal fat mass and total lean mass at weeks 0 and 12 for the 44 subjects with Type 2 diabetes .....	219
<b>Table 8.4.4</b>	Fasting and 2-hour postprandial glucose and insulin concentrations for 36 subjects with Type 2 diabetes.....	220
<b>Table 8.4.5</b>	Fasting serum lipids at weeks 0, 8, and 12 for the 44 subjects with Type 2 diabetes.....	221
<b>Table 8.4.6</b>	Weight, total fat and lean mass changes for the 26 subjects in the energy expenditure component of the study.....	222
<b>Table 8.4.7</b>	Energy expenditure variables at weeks 0 and 12 for the 26 subjects with Type 2 diabetes.....	223
<b>Table 9.4.1</b>	Characteristics of the 57 subjects with hyperinsulinemia at baseline...	252
<b>Table 9.4.2</b>	Composition of study diets derived from daily weighed food records for the subjects with hyperinsulinemia.....	253
<b>Table 9.4.3</b>	Total fat mass, abdominal fat mass and total lean mass in the 57 subjects with hyperinsulinemia at weeks 0 and 16.....	254
<b>Table 9.4.4</b>	Fasting glucose, insulin, and free fatty acid concentrations for the standard-protein and high-protein diet groups.....	255
<b>Table 9.4.5</b>	Glucose and insulin response areas under the meal tolerance test curves for the standard-protein and high-protein diet groups.....	256
<b>Table 9.4.6</b>	Fasting serum lipid concentrations for the standard-protein and high-protein diet groups.....	257

<b>Table 9.4.7</b>	Weight, total fat and lean mass changes for the 36 subjects with hyperinsulinemia who had energy expenditure measured.....	258
<b>Table 9.4.8</b>	Energy expenditure measurements at weeks 0 and 16 for 36 subjects with hyperinsulinemia.....	259

## LIST OF COMMONLY USED ABBREVIATIONS

WHO	World Health Organisation
CVD	Cardiovascular disease
CHD	Coronary heart disease
TEE	Total energy expenditure
REE	Resting energy expenditure
TEF	Thermic effect of feeding
PAEE	Energy expenditure due to physical activity
PA	Physical activity
RQ	Respiratory quotient
DEXA	Dual-energy X-ray absorptiometry
BIA	Bioelectrical impedance analysis
BMI	Body mass index
sBP	Systolic blood pressure
dBp	Diastolic blood pressure
HP	High-protein diet
SP	Standard protein diet
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
FQ	Food quotient
RQ/FQ	Respiratory quotient to food quotient ratio
OGTT	Oral glucose tolerance test
MTT	Meal tolerance test
AUC	Area under the curve
HOMA	Homeostasis model of insulin resistance
FFA	Free-fatty acids
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein