



**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

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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 [¹⁴C]-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 [¹⁴C]-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 [¹⁴C]-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/m²) 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 ± 1.6 kg of which 8.4 ± 1.0 kg was fat mass. Lean mass was reduced 3.8 ± 0.7 kg. Resting energy expenditure was reduced $5.6 \pm 1.3\%$ (500 ± 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}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 22nd November 2002.

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

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.
2. Parker B., Luscombe N.D., Noakes M., Clifton P.M. The effects of a high-protein, high monounsaturated fat weight loss diet on glycemic control and lipid levels in Type 2 diabetes. *Diabetes Care* 2002; March 25(3): 425-430.
3. Luscombe N.D., Wittert G., Noakes M., Farnsworth E, Clifton P.M. Effect of a high-protein, energy restricted diet on weight loss and energy expenditure after weight stabilisation in hyperinsulinemic subjects. (Accepted for publication in the *International Journal of Obesity*, October 2002).
4. Farnsworth E, Luscombe N.D., Noakes M., Wittert G., Argyriou E, Clifton P.M. Effect of a high-protein, energy restricted diet on body composition, glycaemic control and lipid levels in hyperinsulinemic subjects. (Accepted for publication in the *American Journal of Clinical Nutrition*, September 2002).
5. Luscombe N.D., Kirkwood I., Bellon M., Tsoelas C., Clifton P, Wittert G. Measurement of energy expenditure in overweight men and women using [¹⁴C]-bicarbonate-urea before and after weight loss. (Submitted to *American Journal of Physiology* 2002)

Prizes

Award for Best Oral Presentation

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

The Australasian Society for the Study of Obesity

10th Annual Scientific Meeting

Gold Coast, Queensland, Australia

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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

CHAPTER 1

Human Obesity: The Regulation Of Body Weight And Dietary Strategies For Weight Loss

1.1 INTRODUCTION

The contribution of genetic factors to body weight regulation is approximately 25 to 40% (Bouchard, 1988; Bouchard, 1997a; Bouchard, 1997b; Bouchard and Perusse, 1988a; Bouchard and Tremblay, 1997; Stunkard et al. 1986a; Stunkard et al. 1986b). The rapid increase in the prevalence of over-weight and obesity in the past 20 years indicates that environmental and behavioral factors interact with genetic susceptibility in promoting and/or exacerbating obesity (Kopelman, 2000; WHO, 1997).

The adult human generally maintains a relatively stable body weight from year to year despite variations in daily energy intake and energy expenditure (Hervey G.R., 1969; Passmore R, 1971; Weigle, 1994). Studies of over- and under-feeding demonstrate that energy expenditure is increased or decreased, respectively, to compensate for, or to oppose changes in energy balance (Bouchard C. et al. 1990; Bouchard et al. 1988; Diaz et al. 1992; Jebb et al. 1996; Larson et al. 1995; Leibel et al. 1995; Ravussin et al. 1985b; Roberts et al. 1996; Sims E.A.H and Horton E.S., 1968). When over- or underfeeding ceases, subjects generally return towards their initial body weight indicating that regulation of energy intake and energy expenditure occurs.

The rapid increase in the prevalence of obesity indicates that in a permissive environment a gain in weight occurs- at least in genetically susceptible individuals. An “obesogenic” environment is one where there is an abundance of energy-dense foods and labour saving technology. It appears that these environmental factors exert a constant pressure to increase energy intake and decrease expenditure, which override the body’s defense mechanisms.

Dietary treatments for obesity involve energy restriction to in order to induce a negative energy balance, to promote weight loss and thereby reduce the risk of morbidity and

mortality from associated diseases such as Type 2 diabetes and cardiovascular disease. Moderate energy restriction and a subsequent 5 to 10% reduction in body weight may significantly improve the health risks associated with obesity (Williams and Kelley, 2001). Very few people, however, are successful at maintaining the weight loss for a number of reasons including: i) a reduced total energy expenditure resulting from either a decrease in resting energy expenditure and energy expenditure due to physical activity (Astrup et al. 1999; Saltzman and Roberts, 1995); ii) low levels of physical activity (Pavlou et al. 1989; Westerterp, 1998; Wilmore et al. 1999); iii) passive over-consumption of energy-dense foods (e.g. high-fat foods) (Bray and Popkin, 1998; Dreon et al. 1988; Stubbs et al. 1995b); and iv) a low degree of fat oxidation during fasting, or an inability adjust macronutrient oxidation to match the macronutrient composition of the diet consumed (Astrup et al. 1994; Cooling and Blundell, 2000; Toubro et al. 1998; Zurlo et al. 1990). Two or more of these factors may act in synergy to promote weight regain.

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%). There is some concern that the high-carbohydrate (50 to 60% of total energy), low-fat (< 30% of energy) diets that have traditionally been used for weight loss, may raise plasma glucose and triacylglycerol concentrations, and reduce LDL-cholesterol particle size. These controversies have led to a renewed interest in the use of alternative dietary strategies such as the popular high-protein diets.

This chapter examines the consequences, development and treatment of obesity. Section 1.2 examines the definition and prevalence of obesity. Section 1.3 reviews the health consequences of obesity and section 1.4 addresses the influence of body fat distribution on these. The role of insulin resistance in the development of obesity co-morbidities is discussed in section 1.5. Section 1.6 examines the regulation of body weight and the

development of obesity. Evidence that energy expenditure is an important physiological mediator of body weight, is the focus of the discussion. Section 1.7 discusses the role of low-fat, high-carbohydrate and low-fat, high-protein dietary strategies in the treatment of obesity and its associate diseases. Finally, the objectives of this thesis are outlined in section 1.8.

1.2 DEFINITION AND PREVALENCE OF OBESITY

1.2.1 Definition of obesity

Obesity is a condition of abnormal or excessive amount of adipose tissue, to the extent that health may be adversely affected (WHO, 1997). Because it is not easy to directly measure the amount of fat tissue in humans, we rely on proxy indicators to assess body fatness. The simplest and most frequently used indicator of obesity is 'Body Mass Index' or BMI. BMI is calculated by dividing weight in kilograms by height in metres squared (kg/m^2). The World Health Organisation (WHO) has devised a graded classification of overweight and obesity based on BMI values (Table 1.1).

Table 1.1 Cut-off points proposed by a WHO expert committee for the classification of overweight and obese (redrawn from (WHO, 1997)).

BMI (kg/m^2)	Classification	Popular description	Risk of Co-morbidities
18.5 – 24.9	Normal range	Healthy, acceptable	Average
25.0 – 29.9	Pre-obese	Overweight	Increased
30.0 – 34.9	Obese class 1	Obese	Moderate
35.0 – 39.9	Obese class 2	Obese	Severe
≥ 40.0	Obese class 3	Morbid obesity	Very severe

The classifications provide valuable information about the increasing body size of individuals; they are are-independent and the same for both sexes. The BMI values allow comparisons of weight status within and between populations, and help identify individuals and groups at increased risk of morbidity and mortality. The classification of overweight and obesity is, however, based on total mortality rates, which may be

misleading. People, who lost weight due to illness and died, may artificially inflate the mortality rate associated with lower weights. Confounding factors such as smoking may also distort the association between body weight and mortality. This point was illustrated by the Nurses Health Study which prospectively studied 116, 000 women in the USA over 17 years. The overall analysis of the data adjusted for age showed a U-shaped relationship between mortality and BMI but when an adjustment was made for smoking status, a simple positive association was observed for those had never smoked (Manson et al. 1995). Despite these shortcomings in the calculation of BMI, there is a positive association between an increased BMI and all-cause mortality in men (Lindsted et al. 1991; Seidell et al. 1996), and between an increased BMI and coronary heart disease mortality in both men and women (Seidell et al. 1996). BMI does not correspond to the same degree of fatness across different ethnic populations. For example, He et al. (2001) demonstrated that Hong Kong Chinese men and women (190 women/ 140 men) had a higher body fat content for a given BMI. A BMI of 25 and 30 kg/m² predicted using the WHO Caucasian-based formula corresponded to an actual BMI of 23 and 25 kg/m². These results, as well as those observed by Swinburn and colleagues (1996), support the recommendations of using lower BMI cut-offs to define obesity in the Asia Region. In addition, body fat content increases and lean mass decreases with ageing, in both men and women (Davies et al. 2002; Forbes and Reina, 1970; Jackson et al. 2002).

Waist circumference and the waist-to-hip ratio (WHR) are two other proxy indicators of the risk of complications of obesity. The World Health Organisation suggest that waist circumference or WHR should be used, in addition to BMI, because they provide information about the distribution of excess fat in the various adipose tissue compartments (WHO, 1997). A number of studies that have demonstrated that body fat distribution is an important factor contributing to cardiovascular risk, glucose intolerance and hyperinsulinemia (Despres et al. 1989c; Despres et al. 1988; Ducimetiere et al. 1986;

Kelley et al. 2000; Larsson et al. 1984; Wajchenberg B.L., 2000). The influence of body fat distribution on the health consequence of obesity, are discussed in section 1.4.

1.2.2 Prevalence of obesity

Obesity is a common and serious medical condition within both developed and developing countries around the world. Increasingly, obesity affects all ethnic groups, age and socio-economic groups. Current figures indicate that there are more than 250 million obese people worldwide which represents 7% of the adult population (Kopelman, 2000; WHO, 1997). In Australia, the prevalence of obesity (BMI > 30 kg/m²) has more than doubled over the last 20 years and currently affects one in five people. Latest Australian figures for overweight (BMI > 25 kg/m²) and obesity (BMI > 30 kg/m²) come from the Diabetes, Obesity and Lifestyle Study (AusDiab) of 11,247 men and women over the age of 25 years, during the year 1999 to 2000 (Australian Institute of Health and Welfare, 2000). The study found that 52.5% of Australian women were overweight and 21.8% were obese. For the men, 67.7% were overweight and 19.4% were obese. The figures for men were similar to those reported in the 1995 National Nutrition survey (Australian Bureau of Statistics and Commonwealth Department of Health and Aged Care, 1998), but for women the prevalence of obesity (BMI > 30 kg/m²) had increased a further 3.4% from 18.4% in 1995. The prevalence of overweight and obesity in Australian children has also increased over the last 10 years and currently it stands at 19 to 26% for boys and 21 to 28% for girls (Booth et al. 1999; Booth et al. 2001; Goodman et al. 2002). Extrapolations suggest that if current trends continue, between 25 to 50% of the adult population in countries including Australia, England, Mauritius, Brazil, and the USA will be obese by 2025 (Kopelman, 2000; WHO, 1997).

1.2.3 Economic costs of obesity

Conservative estimates of the direct economic costs (diagnosis, treatment and management) of obesity in developed countries are between 2 to 8% of the total health care costs (Brown, 2000). In Australia, Caterson and colleagues (2002; NHMRC, 1997) calculated that the direct cost of obesity in Australia is AU\$830 million. In the USA, it has been estimated to be US\$70 billion (Caterson, 2002). The indirect cost of obesity to the individual (e.g. psychological state, self-esteem, and employment) and to the society (e.g. loss of productivity, sick days, money spent of weight loss programs) has not been assessed. Undoubtedly, they would raise the total to even higher amounts.

1.3 HEALTH CONSEQUENCES OF OBESITY

Obesity causes and predisposes individuals to numerous health complications, ranging from non-fatal but debilitating disorders including breathlessness, sleep apnea, back pain, and osteo-arthritis, to life threatening diseases such as cardiovascular disease, Type 2 diabetes mellitus, gallbladder disease, fatty liver, and certain cancers (Colditz, 1999; Kopelman, 2000; WHO, 1997). Obesity is also a risk factor of psychological disorders including lowered self-esteem, eating disorders and clinical depression (WHO, 1997). The WHO Expert Committee has estimated that the relative risk of the particular diseases shown in Table 1.2 (for an obese person as compared to a lean person) are constant, even though the absolute prevalence of obesity-associated health problems may vary between different ethnic groups throughout the world.

Table 1.2 Estimated relative risk of health problems associated with obesity as proposed by the WHO Expert Committee [redrawn from (WHO, 1997)].

Greatly increased (relative risk > 3)	Moderately increased (relative risk 2-3)	Slightly increased (relative risk 1-2)
Type 2 diabetes mellitus	Cardiovascular disease	Cancer (breast, colon, endometrial and prostate)
Gallbladder disease	Hypertension	Reproductive hormone abnormalities
Dyslipidaemia	Osteoarthritis	Polycystic ovary syndrome
Insulin resistance	Hyperuricaemia and gout	Impaired fertility
Breathlessness		Low back pain
Sleep apnoea		Anaesthetic risk

Obese individuals who have at least two of the following conditions are defined as having 'Metabolic Syndrome' (or Syndrome X): high blood pressure (>140/80 mmHg), impaired glucose tolerance (refer to Table 1.3), hyperinsulinemia (>10 mU/L), high plasma triacylglycerol (>2 mmol/L), low HDL-cholesterol (<1.0 mmol/L), and abdominal obesity (Reaven, 2001). Metabolic syndrome is known to be highly predictive of a range of diseases including Type 2 diabetes, fatty liver, hypertension, and cardiovascular disease (Grundy, 2000; Hauner, 2002; Lopez-Candales, 2001; Reaven, 2001).

1.3.1 Type 2 Diabetes Mellitus

The prevalence of diabetes mellitus worldwide has paralleled the dramatic increase in body weight over the past two decades (Kopelman, 2000; Mokdad et al. 2001; Mokdad et al. 2000). The AusDiab study showed that almost one in 4 Australians over the age of 25 years now has impaired glucose tolerance or a form of diabetes mellitus (i.e. Type 1 or Type 2 diabetes mellitus) (Dunstan et al. 2002). Over the last 20 years, the number of Australians with diabetes has increased from 250,000 to one million persons. For all diagnosed cases of diabetes it has been estimated that there are an equal number of undiagnosed cases, placing the true incidence at 8% (Dunstan et al. 2002). Type 2 diabetes mellitus accounts for approximately 80 to 90% of all diagnosed cases of diabetes. Similar data is available in European and American populations. In Europe, diabetes affects

approximately one in 20 adults (~22 million) with a further 6 million expected to be affected by 2025 (Andersson et al. 1991; Gourdy et al. 2001; International diabetes federation, 2001; Riste et al. 2001). In America, it has been estimated that 14 to 15 million adults over the age of 20 years have Type 2 diabetes (Harris et al. 1998). The prevalence of Type 2 diabetes is highly-age dependent, increasing 2 to 3 fold every ten years after 40 years of age (Andersson et al. 1991; Harris et al. 1987; Laakso et al. 1991). In Australia, the estimated direct annual health care costs for Type 2 diabetes were \$217 million (Mathers and Penm, 1999). In America, the direct medical cost of diabetes is \$44 billion (American Diabetes Association, 1998).

Type 2 diabetes mellitus is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from a dual impairment of insulin resistance and pancreatic β cells function (Alberti and Zimmet, 1998). Insulin resistance of the liver, muscle, and adipose tissue prevents them from responding appropriately to insulin because either post-receptor events are impaired, or in relatively fewer individuals the number or affinity of insulin receptors on the plasma membrane are reduced (Kahn and Flier, 2000). In the pre-diabetic state, individuals are able to compensate for insulin resistance by secreting high levels of insulin. However, in individuals destined to develop diabetes there is a progressive loss of pancreatic β cells cell function (i.e. secondary failure of the pancreas), which leads to fasting and postprandial hyperglycaemia. Eventually the compensatory response declines so that there is a relative or absolute deficiency of insulin to regulate the metabolic actions of the liver, muscle, or adipose tissue and individuals have full-blown diabetes (Reaven, 1995). The exact cause of Type 2 diabetes remains unclear. However, the progression from insulin resistance to hyperinsulinemia to hyperglycaemia and then clinically diagnosed Type 2 diabetes (Table 1.3) is clearly associated with increased body fatness, particularly abdominal fatness (see section 1.4 for discussion of body fat distribution) (Carey, 1998; Chisholm et al. 1997; Despres, 1993).

The pathophysiology of insulin resistance in Type 2 diabetes, as well as in CVD, is discussed in greater detail in Section 1.5. Other factors implicated in the development of diabetes include: i) genetics (Tripathy et al. 2000), ii) age, iii) low level of physical activity (Kriska A.M. et al. 2001), iv) childhood obesity or progressive weight gain from 18 years (Dietz, 1998; WHO, 1997), and v) arguably a high fat diet (Marshall et al. 1991).

Table 1.3 Classification of normal and impaired glucose tolerance and diabetes, based on fasting and 2-hour plasma glucose values defined by the WHO, 1998.

	Fasting Plasma Glucose (mmol/L)	2-hour Plasma Glucose* (mmol/L)
Normal glucose tolerance	< 6.1	< 7.8
Impaired glucose tolerance	≥ 6.1 – 7.0	≥ 7.8 – 11.1
Type 2 diabetes mellitus	≥ 7.0	≥ 11.1

*2-hour plasma glucose levels measured after an oral glucose tolerance test.

Macrovascular complications are the most important cause of mortality and morbidity for Type 2 diabetes. The risk of CHD is 2 to 4-fold higher in individuals with diabetes than in those with normal glucose levels (Laakso, 2001). The risk of stroke and peripheral vascular disease is also increased, and consequently, CVD accounts for approximately 70% of all mortality in Type 2 diabetes. Moreover, Type 2 diabetes has been reported to abolish the protection that women have from CVD as compared to men. Because the classical risk factors of CVD (e.g. hypertension, low levels of HDL-cholesterol, elevated levels of triacylglycerol) already exist in the pre-diabetic state (Laakso, 1997) it has been postulated that hyperglycaemia, independent of insulin resistance, plays an important role in the increased risk for CVD in Type 2 diabetes (Laakso, 1999). A 7-year follow-up study of 1059 Finnish subjects with Type 2 diabetes aged 45 to 64 years at baseline, found that high fasting plasma glucose predicted CHD (Lehto et al. 1997), and high fasting plasma glucose and HbA_{1c} predicted fatal and non-fatal stroke (Lehto et al. 1996). The U.K. Prospective Diabetes Study (UKPDS), including 2693 subjects aged 25 to 65 years with

newly diagnosed type 2 diabetes, reported that HbA_{1c} was the third most important risk factor behind elevated levels of LDL-cholesterol and reduced levels of HDL-cholesterol, for CHD (Turner, 1998). Two-hour plasma glucose levels were not assessed in either of these studies, but there is evidence that postprandial hyperglycaemia is an independent risk factor for the development of macrovascular complications (American Diabetes Association, 1999; Barrett-Connor and Ferrara, 1998; Temelkova-Kurktschiev et al. 2000).

A number of potential mechanisms may explain the association between glycaemic control and CVD in Type 2 diabetes (Kuusisto and Laakso, 1999). The development of atherosclerosis may be enhanced by: i) glycation of the lipoproteins, ii) acceleration of lipoprotein oxidation, and iii) irreversible glycation of proteins in the arterial wall that can occlude the lumen (Bierman, 1992; Makita et al. 1999; Numano et al. 1997; Turner, 1998). Moreover, changes in the arterial wall induced by hyperglycaemia may promote the rupture of the atherosclerotic plaques and thrombosis (Libby, 1995).

1.3.2 Fatty liver disease

Normal liver contains small fat globules in the hepatocytes that can be seen using an electron microscope, ultrasound, or proton spectroscopy. Fatty liver is characterized by the accumulation of fat globules (formed mainly from triacylglycerol) in over 50% of the hepatocytes (Mach, 2000). In persons clinically diagnosed with fatty liver disease, the amount of fat exceeds 5% of the liver's total weight (Kawai et al., 1995; Mach, 2000). Non-alcoholic fatty liver disease is now recognized as the most common liver disease in the United States, with a prevalence of approximately 5% in the general population and up to 25% to 75% in patients with obesity and Type 2 diabetes mellitus (McCullough, 2000). Non-alcoholic fatty liver disease includes hepatic steatosis and non-alcoholic steatohepatitis (McCullough, 2000). Insulin resistance is associated with steatosis.

Increased oxidative stress, which produces lipid peroxidation and activates inflammatory cytokines, leads to non-alcoholic steatohepatitis (Mach, 2000; McCullough, 2000). Approximately 14 to 40% of patients with non-alcoholic steatohepatitis develop fibrosis, and an unknown percent of these patients progress to cirrhosis and liver-related death (Mach, 2000; Neuschwander-Tetri, 2001).

Liver fat content is positively associated with increasing body weight. A Japanese study of 816 white-collar workers over 35 years old showed that the prevalence of fatty liver disease diagnosed by ultrasonography was 17.9% in all subjects and was maximum (24.4%) in males 45-49 years of age. BMI were higher in the subjects with fatty liver than in the controls (Kawai et al. 1995). In 17 men and 3 women with Type 2 diabetes, Ryysy et al. (2000) also found that the individuals with high liver fat contents (measured by proton spectroscopy) were more obese than those with low liver fat contents. Moreover, non-alcoholic steatohepatitis occurs commonly in obese subjects and is several times more frequent in middle-aged women with diabetes (Mach, 2000). Cirrhosis is most common in the morbidly obese (McCullough, 2000).

Recent data suggests that there is a strong relationship between Type 2 diabetes and fatty liver, independent of overall body fatness. Clark et al. (2002) has shown that adults with non-alcohol fatty liver disease were two-times more likely to have diabetes than those without it, even after the adjustment for BMI, age, gender, and race. In addition, a Finnish group (Ryysy et al. 2000) demonstrated that hepatic fat content was significantly and positively correlated with the ability of intravenous insulin to suppress endogenous glucose production hepatic insulin sensitivity (measured using euglycemic insulin clamp), in 20 subjects with Type 2 diabetes. This finding suggests that variation in hepatic fat content may influence insulin requirements via an effect on the sensitivity of endogenous glucose production to insulin.

1.3.3 Hypertension

In Australia, the prevalence of hypertension ($\geq 140/90$ mmHg) is 30.9% for men and 28% for women, aged over 25 years (Australian Institute of Health and Welfare, 2000). Community surveys in the USA (NHANE II) show that the prevalence of hypertension in overweight American adults is 2.9 fold higher than normal weight individuals (Van Itallie, 1985). Obese individuals have a greater risk of developing hypertension than lean subjects (Han et al. 2002; Stamler et al. 1993a; Stamler et al. 1993b; Stamler et al. 1989; Tsao et al. 2002) and the Framingham Health Study demonstrated that approximately 75% of hypertension could be attributed to obesity (Kannel et al. 1967a; Kannel et al. 1967b).

Obesity is associated with an increase in both fat and lean mass, and in body surface area. This, and an associated increase in total blood volume are accompanied by increases in cardiac output and stroke volume. Ultimately, systemic vascular resistance is increased and it causes a sustained rise in systolic and diastolic blood pressure (Kopelman, 2000). There remains no clear reason for the association between increased weight and hypertension, but higher levels of insulin may enhance renal retention of sodium (Brenner et al. 1988; Prichard et al. 1992) and increase sympathetic nervous system activity (Reaven, 1991).

1.3.4 Cardiovascular disease

Cardiovascular disease (CVD) includes coronary heart disease (CHD), stroke and peripheral vascular disease. In Australia, the prevalence of CVD is 68% for men and 53% for women, who have a BMI ≥ 25 kg/m² and who are over the age of 25 years (Australian Institute of Health and Welfare, 2000). From 1993 to 1994, the estimated annual health care cost for CVD and its risk factors were \$3.9 billion (~12% of the total health care system), in Australia (Mathers and Penm, 1999).

The Framingham Heart Study has demonstrated that the incidence of CHD in men and women was proportionately related degree of overweight (Hubert et al. 1983). In this study, the 26-year incidence of CHD increased by a factor of 2.0 in obese men under the age of 50 years, and body weight was found to be the third most important predictor of CHD among males, after age and dyslipidaemia. In obese women, the 26-year incidence of CHD increased by a factor of 2.4. Similar findings were observed in other very large prospective studies (Rimm et al. 1995; Willett et al. 1995) including a 15-year follow-up study of 16,000 men and women in Eastern Finland (Jousilahti et al. 1996).

Few prospective studies have been conducted to investigate the relationship between obesity and stroke (MacMahon et al. 1990). The Honolulu Heart study that examined 1163 non-smoking men aged between 55 to 58 years found that increased BMI was associated with an increased risk of having a stroke (Abbott et al. 1994). There is no data, as yet, that conclusively shows a similar relationship in obese women.

Risk factors for CVD that are associated with obesity include hypertension, hypercholesterolaemia, hypertriglycerol, reduced HDL-lipoprotein. Data from the British Regional Heart Survey (Shaper et al. 1981; Walker, 1984) show how blood pressure and triglycerol concentrations increase, and HDL-cholesterol concentrations decrease, even at mild to moderate degrees of obesity. Although total-cholesterol levels may rise as BMI increases, CVD can occur in the absence of hypercholesterolaemia (Reaven and Chen, 1988). The combination of an increased triglycerol and low HDL-cholesterol concentrations as risk factors of CVD has been emphasized by results of two other epidemiological studies (Assmann and Schulte, 1992; Manninen et al. 1992). The size and density of LDL-cholesterol particles (Reaven et al. 1993) and prolonged and/or exaggerated lipidaemia following fat ingestion (Griffin, 1995; Wilson et al. 1985), may also contribute to the risk of CVD in obese individuals.

1.4 THE INFLUENCE OF BODY FAT DISTRIBUTION ON THE HEALTH CONSEQUENCES OF OBESITY

Obesity is the predominant factor leading to insulin resistance, but other factors, such as body fat distribution, physical activity, hormones, and a person's genetic make-up, also play important roles. This section will focus on the influence of body fat distribution on insulin resistance and the health consequences of obesity.

Obesity is a heterogeneous condition. Moreover, the regional distribution of adipose tissue is important to understanding the relationship between obesity and the disturbances in glucose-insulin homeostasis and lipid metabolism. A number of prospective studies examining middle-aged men have shown that fat in the upper part of the body (i.e. abdominal or android type obesity) is associated with greater mortality and risk of diabetes, dyslipidaemia, hypertension, and CVD than excess fat in the lower part of the body (i.e. gynoid obesity) (Ducimetiere et al. 1986; Larsson et al. 1989; Larsson et al. 1984; Ohlson et al. 1985). In these studies, the body fat distribution was assessed using anthropometric measurements including the waist-to-hip circumference ratio and skinfolds. Anthropometric measurements, although suitable for use in large-scale epidemiological studies, are not able to distinguish subcutaneous fat from deep abdominal (or visceral) fat.

The advent of imaging techniques, particularly computed tomography and magnetic resonance imaging, has enabled the investigation of the influence of visceral as compared to subcutaneous adipose tissue on insulin resistance and the Metabolic Syndrome. Computed tomography is considered the gold-standard technique for adipose tissue measurement as well as the measurement of body composition in other regions of the body (Wajchenberg, 2000). Several studies have shown that high levels of visceral fat are associated with increased glucose and insulin responses to oral glucose tolerance

challenges, in obese non-diabetic men and women (Despres et al. 1989d; Pouliot et al. 1992). The effect of increased visceral fat on glucose tolerance is independent of both total fat mass and subcutaneous abdominal adipose tissue (Despres et al. 1989d; Pouliot et al. 1992). In obese subjects with and without Type 2 diabetes as well as in subjects with impaired glucose tolerance, high levels of visceral fat have also been shown to correlate significantly with higher fasting triacylglycerol and reduced HDL-cholesterol concentrations (Despres, 1991; Despres et al. 1989a; Despres et al. 1989b; Laakso et al. 1990). While the available evidence indicates that there is an important association between visceral fat and hyperinsulinemia and dyslipidaemia, some debate exists regarding whether subcutaneous adipose tissue is an equally important predictor of insulin sensitivity as visceral adipose tissue. Abate et al. (1996) and Goodpaster et al. (1997) found that abdominal subcutaneous fat (determined by either magnetic resonance imaging or computed tomography) was as strongly correlated to insulin sensitivity (evaluated by euglycemic clamp) as visceral fat, in both non-diabetic subjects and in subjects with mild Type 2 diabetes. In addition, Goodpaster et al. (1997) showed that this association was retained after adjusting for visceral fat. Further studies are required to confirm the importance of increased subcutaneous adipose tissue on insulin sensitivity.

The metabolic activity and the vascular anatomy of visceral fat may be key factors predisposing susceptible individuals to the complications of obesity (Wajchenberg, 2000). Mobilization of free-fatty acids is more rapid from visceral than from subcutaneous fat cells because of the higher lipolytic activity in visceral adipocytes (Arner, 1998; Hoffstedt et al. 1997) in both non-obese and particularly in obese individuals. Arner et al. (1990) has demonstrated using abdominal and gluteal fat cells from non-obese men and women that the variation in lipolysis can be attributed to regional variation in the action of the major lipolytic hormones, catecholamines and insulin; the lipolytic effect of catecholamines is more pronounced and the antilipolytic effect of insulin is weaker in

visceral as compared to subcutaneous adipose tissue. Catecholamine-induced lipolysis is higher in the visceral tissue as a result of increased expression of β -adrenoreceptors (Arner et al. 1990). Furthermore, free-fatty acids released from visceral adipose tissue directly access the liver through the portal system whereas those released from the subcutaneous adipose tissue move through the systemic circulatory system (Wajchenberg, 2000). The release of excess free-fatty acids into the portal circulation may have a number of unwanted effects on the liver including: i) decreased hepatic insulin extraction which may lead to systemic hyperinsulinemia and increased hepatic glucose production (Bevilacqua et al. 1987; Ferrannini et al. 1983), ii) increased gluconeogenesis (Boden and Shulman, 2002), iii) increased synthesis and secretion of small VLDL particles (Brunzell and Hokanson, 1999), and iv) an increase in hepatic lipase activity which leads to removal of lipids from LDL and HDL making them smaller and more dense (Brunzell and Hokanson, 1999). Moreover, visceral adipocytes as compared to subcutaneous adipocytes differentially secrete factors with endocrine functions (Wajchenberg, 2000). For example, leptin is expressed at much lower concentrations in the visceral adipocytes of mildly obese subjects (Montague et al. 1997b). Conversely, higher concentrations of apoptosis protein-2, plasminogen activated inhibitor-1 and interleukin-6 are expressed in visceral adipocytes (Alessi et al. 1997; Mohamed-Ali et al. 1998; Montague et al. 1998). These factors may be another mechanism increasing the risk of diabetes and CVD in individuals with android type obesity as compared to those with gynoid type obesity.

1.5 ROLE OF INSULIN RESISTANCE IN THE DEVELOPMENT OF HYPERGLYCAEMIA AND THE RISK FACTORS OF CVD

Insulin has a number of actions in the human body but the primary roles are to: i) stimulate the uptake of glucose by muscle, fat and liver cells, ii) suppress the mobilization fat stores from the adipose tissue (i.e. lipolysis), iii) promote the storage of triacylglycerols from the plasma (i.e. lipogenesis), iv) suppress hepatic glucose output, v) promote vasodilation and

stimulate nitric oxide in the endothelial cells, vi) act as a growth factor for vascular tissue, and vii) mediates the migration of vascular smooth muscle cells, monocytes, macrophages and endothelial cells (Goldstein, 2002; Kahn and Flier, 2000; Reaven, 1995; Hsueh, 2002). Many obese and non-obese non-diabetic individuals are hyperinsulinemic (Haffner and Miettinen, 1997; Polonsky, 2000; Zavaroni et al. 1994). Accordingly, insulin resistance is fundamental to the etiology of the Metabolic syndrome, fatty liver, Type 2 diabetes and CVD.

1.5.1 Insulin resistance and the development of hyperglycaemia

The development of hyperglycaemia in insulin resistant individuals is the result of the complex interplay between muscle, pancreatic β cells, adipose tissue, and the liver. Insulin resistance affects glucose disposal in skeletal muscle and the adipose tissue, as well as suppression of hepatic glucose output (Goldstein, 2002; Kahn and Flier, 2000; Reaven, 1995). A simplified schema of the role of insulin resistance in the development of hyperglycaemia is depicted in Figure 1.1.

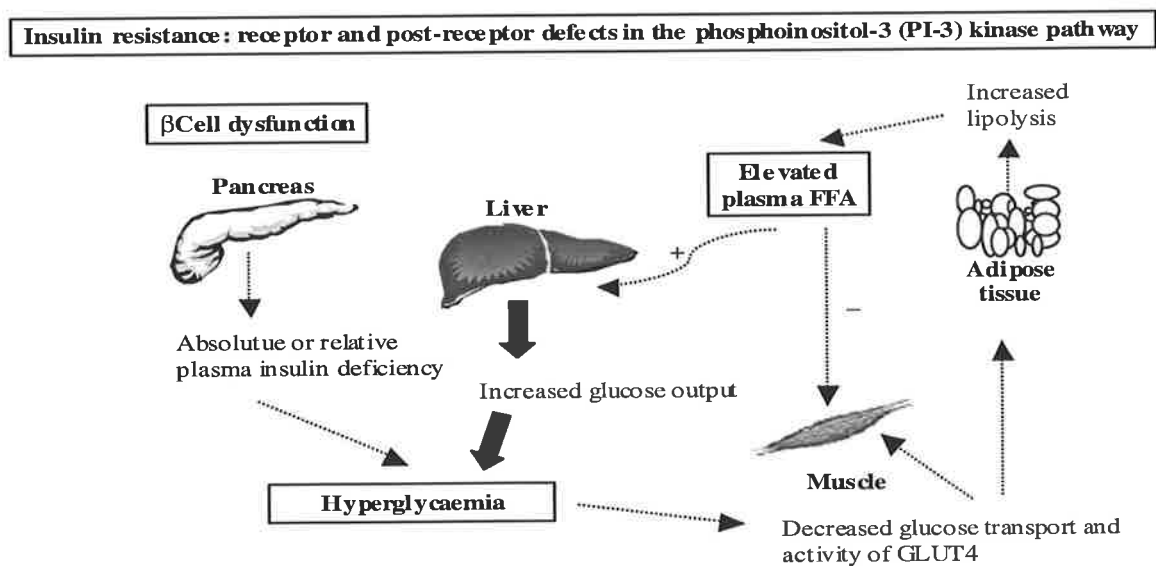


Figure 1.1 The role of insulin resistance in the development of hyperglycaemia

Many details of the mechanisms by which insulin resistance affects glucose disposal remain unknown. However, it is clear that activation by insulin of the phosphoinositol-3 (PI-3) kinase pathway is important for glucose transport in the muscle and adipose tissue. It has been postulated that defective signaling at one or more positions along this pathway is responsible for impaired glucose transport (Kahn and Flier, 2000). In adipose tissue, glucose disposal may be affected by a down-regulation of the major insulin-responsive glucose transporter, GLUT4 (Shepherd and Kahn, 1999). In skeletal muscle, GLUT4 expression is normal and it is speculated that impaired translocation, docking, or fusion of the GLUT4-containing vesicles within the plasma membrane may cause the reduction in glucose uptake (Shepherd and Kahn, 1999). Adding to the effect of insulin resistance on glucose disposal is the issue of increased intra-abdominal fat depots in obese individuals. It is hypothesized that intra-abdominal adipocytes are more lipolytic than peripheral adipocytes (Carey, 1998; Vidal, 2001). This leads to an increased rate of lipolysis, elevated plasma FFA levels and an increased flux to the liver and skeletal muscle (Carey, 1998; Vidal, 2001). Consequently, increased levels of FFA from the intra-abdominal adipose tissue may impair glucose uptake in the muscle, independent of insulin resistance (Bajaj et al. 2002; Boden and Shulman, 2002; Clerk et al. 2002). In addition, an increase in FFA flux to the liver may stimulate hepatic FFA oxidation and hepatic glucose production (gluconeogenesis) thereby enhancing hyperglycaemia (Boden and Shulman, 2002). As plasma FFA and glucose concentrations increase, β cell secretory function is further compromised (Boden and Shulman, 2002).

1.5.2 Insulin resistance and the development of cardiovascular disease

The precise mechanisms by which insulin resistance affects the development of dyslipidaemia and the vascular abnormalities that contribute to CVD morbidity and mortality are unclear. In obese and non-obese subjects with insulin resistance or in individuals with Type 2 diabetes, it has been shown that the insulin response to a glucose

load may not be sufficient to suppress lipolysis in the adipose tissue (Chen et al. 1987; Del Prato et al. 1990; Golay et al. 1990; Golay et al. 1986). The increased flux of FFA to the liver results in increased stimulation of hepatic very-low-density lipoprotein-triacylglycerol (VLDL-TG) secretion and elevated plasma triacylglycerol concentrations. The reduced HDL cholesterol concentrations observed in individuals with hyperinsulinemia is most likely due to higher concentrations of plasma triacylglycerol promoting the transfer of the cholesterol ester from HDL to apoprotein B containing lipoproteins (VLDL, IDL, VLDL) (Swenson,1991). Others have shown that hyperinsulinemia may directly influence the catabolism of apoprotein A1 thereby further lowering HDL concentrations (Golay et al. 1987).

There is also some evidence that arterial injury is aggravated by the elevated FFA concentrations that are associated with insulin resistance, in non-diabetic and diabetic individuals (Hsueh, 2002). It has been hypothesized that FFA levels may increase vascular damage by stimulating inflammatory factors such as peroxisome proliferator activated receptor- γ , tumor necrosis factor- α , C-reactive protein, and oxidised LDL-cholesterol (Grimble, 2002; Hsueh and Law, 1998; Hsueh and Law, 2001; Sack, 2002). So far this hypothesis remains unproven.

On the basis of the above evidence, it appears that insulin resistance and compensatory hyperinsulinemia are important in the pathogenesis of overweight and obesity. A moderate degree of weight loss can improve glucose, insulin and lipid levels. However, even after weight loss many individuals still have levels that put them at increased risk of developing Type 2 diabetes as well as morbidity or mortality from CVD. There is some data suggesting that an increased dietary protein content may have beneficial effects on these biochemical parameters, independent of weight loss (Section 1.7). The studies described in

Chapters 8 and 9 examine the effects of weight loss diets with either a high or standard protein content on glucose, insulin and lipid metabolism.

1.6 THE REGULATION OF HUMAN BODY WEIGHT AND FACTORS INFLUENCING THE DEVELOPMENT OF OBESITY

Most adults maintain a relatively stable body weight from year to year despite variations in daily energy intake and energy expenditure (Hervey, 1969; Passmore, 1971; Weigle, 1994). Such precise regulation indicates the existence of physiological mechanisms that act to maintain macronutrient balance (i.e. when fat, carbohydrate and protein intakes match the utilisation of each) and ultimately energy balance (i.e. when energy intake matches energy expenditure). In individuals with a genetic susceptibility to obesity these physiological mechanisms are altered in some way so that the body does not exert a strong defense against the numerous and constant “obesogenic” influences that are present in modern society (i.e. unrestricted access to highly palatable, energy-dense foods and energy-saving technology) (Flatt, 1995; Jequier and Tappy, 1999; Kopelman, 2000; WHO, 1997).

The following section examines the regulation of body weight and the development of obesity. Section 1.6.1 discusses that evidence supporting the existence of a physical system that regulates body weight and section 1.6.2 gives an overview of that physiological system. The fundamental principles regulating macronutrient balance are discussed in section 1.6.3. Appetite mechanisms involved in the regulation of energy intake and body weight will be examined in section 1.6.4. Section 1.6.5 will address the regulation of energy expenditure. Evidence arguing that energy expenditure is an important factor contributing to energy balance in non-obese individuals as well as to the dysregulation of energy balance that characterizes obesity, is presented.

1.6.1 Evidence that body weight is physiologically regulated

The concept that body weight is physiologically regulated within a predetermined range of weight, or “settling point”, has been the subject of discussion for several decades (Hervey, 1969; Passmore, 1971; Keesey, 1989; Harris, 1990; Weigle, 1994; Keesey and Hirvonen, 1997). The existence of a physiological system (described in section 1.6.2) acting to regulate human body weight is implied by several observations (Passmore, 1971; Harris, 1990; Hervey, 1969; Kinney, 1995; Weigle, 1994). Passmore (1971) calculated that eating an extra half slice of bread per day (~ 220 kJ) should result in a weight gain of 20 kg over 10 years. In reality, however, most men and women experience only a gradual increase in their weight of ~11 kg between the ages of 25 to 65 years (Harris, 1990; Hervey, 1969; Kinney, 1995; Weigle, 1994). Such weight gain represents a yearly energy surplus of less than 0.2%, or an average daily error of 350 mg of food (Weigle, 1994). It is unlikely that body weight could be maintained this precisely without the continual operation of multiple mechanisms that match the rate of macronutrient intake to the rate of utilisation and the rate of energy intake to the rate of energy expenditure. A second observation supporting physiological regulation of body weight is that variation in BMI among individuals has a strong hereditary component. The genetic contribution to the regulation of body weight has been well documented in studies of siblings, twins, and adoptees (Bouchard, 1988; Bouchard, 1997a; Bouchard et al. 1988b; Bouchard and Tremblay, 1997; Stunkard et al. 1986a; Stunkard et al. 1986b). Also providing strong evidence that body weight is regulated within a “settling-point” are the observations that humans forced to gain or lose weight by over- or under-feeding will return to their baseline weight once the stimulus to change is removed (Bouchard et al. 1990; Bouchard et al. 1988; Diaz et al. 1992; Jebb et al. 1996; Larson et al. 1995; Leibel et al. 1995; Ravussin et al. 1985b; Roberts et al. 1996; Sims and Horton, 1968).

The rapid rate at which the prevalence of obesity has risen in populations over recent years raises questions as to the physiology of the system that regulates body weight. Genotype-environment interactions most likely explain why certain individuals have become obese while others have not. Blundell's group from the University of Leeds, have recently identified two groups of individuals with similar habitual high-fat dietary intakes (assessed using a food frequency questionnaire) who have quite different BMIs (mean BMI of 21.8 vs 29.4 kg/m²) (Le Noury et al. 2002). To determine how the overweight and lean phenotypes differed in their responses to high-fat dietary patterns, all subjects were provided with a fixed high-fat lunch (> 55% fat) followed by an *ad libitum* high-fat test meal 4 hours later and high-fat snacks in the evening. The results of this study indicated that the overweight group probably gained weight because they showed a stronger preference for high-fat foods in choice situations, ate greater amounts of these energy-dense foods, and received greater rewards from their consumption (feelings of pleasantness, satisfaction and taste). It is speculated that the preference of dietary fat might be genetically determined (Reed et al. 1997). The preference of high-fat foods was probably advantageous for the survival of our ancestors during times of famine. In an "obesogenic" environment, however, the body's physiological defense mechanisms to limit fat intake appear to be over-ridden by behavioral responses. A second example highlighting the importance of gene-environment interactions in the development of obesity comes from twin studies of isocaloric overfeeding (Bouchard et al. 1990; Diaz et al. 1992; Sims et al. 1968). Bouchard et al. (Bouchard et al. 1990) overfed 12 pairs of healthy, male, monozygotic twins with no family history of obesity, an energy surplus of 4.2 MJ/day for 100 days. The mean body weight gain was 8.1 kg, but there was a 3-fold difference between the lowest and highest gains. Moreover, the heterogeneity in the response was not randomly distributed across genotypes. For instance, there was approximately 3 times more variance between pairs than within pairs for the changes in body weight. The results from Bouchard's work indicate that the genotype does not cause

obesity directly, but increases the expression of the obese phenotype when individuals are exposed to the right environmental conditions. It is likely that the physiological system defending the body from over-nutrition is more permissive than the system protecting the body from under-nutrition.

1.6.2 The physiological system regulating body weight

The numerous influences on body weight and the physiological system that mediates them are depicted in Figure 1.2 (Egger and Swinburn, 1996; Flatt, 1995; WHO, 1997). At the center of this model is the fundamental principle of human body weight regulation. It shows that in order to preserve a body weight (or more correctly body fat stores) within equilibrium levels (or “settling-point”), macronutrient balance (i.e. when fat, carbohydrate and protein intakes match the utilisation of each) and ultimately energy balance (i.e. when energy intake matches energy expenditure) must be maintained (Egger and Swinburn, 1996; Flatt, 1995; WHO, 1997). Macronutrient balance has been included in the model of body weight regulation for two reasons. In the 1980s, Jean-Pierre Flatt demonstrated that the body derives energy from the utilisation (oxidation) of its protein, carbohydrate, fat and alcohol stores, which come from the intake of each macronutrient (Flatt, 1995).

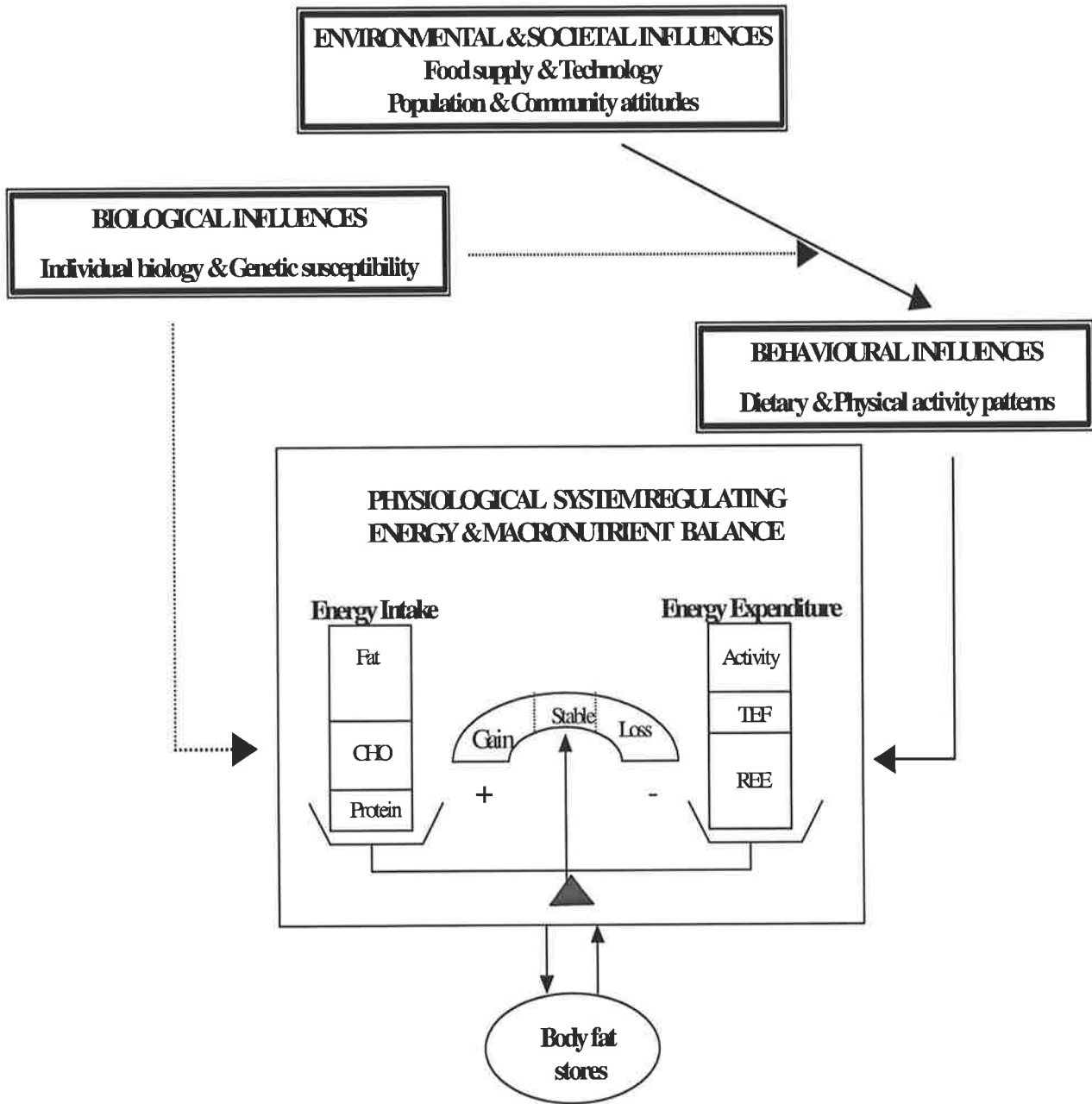


Figure 1.2 The physiological regulation of body weight. CHO, carbohydrate intake; physical, energy expenditure due to physical activity; REE, resting energy expenditure; TEF, the thermic effect of food. Adapted from (WHO, 1997).

Flatt's studies also found that macronutrient balance, particularly fat balance, equals energy balance (Flatt, 1995). Subsequently, Flatt proposed that a fat imbalance was the main cause of the imbalance in energy stores which results in a change in body weight (Flatt, 1995); a positive fat and energy balance promote and increase in body fat stores and weight whereas a negative fat and energy balance results in weight loss. Flatt's concept of

body weight regulation has been supported by numerous observations from epidemiological and intervention studies that dietary fat contributes significantly to overweight and obesity in humans (Bray and Popkin, 1998). As macronutrient balance is an important concept in the regulation of energy balance, it will be discussed in detail in section 1.6.3.

Aspects of macronutrient/energy intake or macronutrient utilisation/energy expenditure are affected (increased or decreased) by interactions between environmental, biological and behavioral influences (Egger and Swinburn, 1996; Flatt, 1995; WHO, 1997). Environments that influence the development of obesity in humans can be categorized based on size ('macro' or 'micro') or type ('physical' and 'socio-cultural') (Egger and Swinburn, 1996). A summary of some of the main environmental influences is presented in Table 1.4. Biological influences on body weight include age, gender, race, hormonal factors (such as puberty, pregnancy and menopause), and genetic factors (Egger and Swinburn, 1996). Genetic susceptibility in most cases is polygenetic, but in rare cases the cause of obesity may be monogenetic e.g. leptin-deficiency arising from a mutation in the *ob* gene that produces leptin (Montague et al. 1997a; Perusse and Bouchard, 2000). Behavioral influences, such as eating and physical activity habits, stem from an individual's beliefs (rational or irrational) about body weight (Egger and Swinburn, 1996). The effects of these influences are regulated by a number of identified physiological mechanisms (there are probably many unidentified mechanisms also). Figure 1.2 shows the main physiological mechanisms that adjust to changes in macronutrient and energy intake and/or expenditure so as to restore macronutrient balance and ultimately energy balance (Schutz, 1995; WHO, 1997); these include: i) appetite, ii) the thermic effect of food (TEF), iii) energy expenditure due to physical activity which includes both voluntary and involuntary activity (PAEE), iv) resting energy expenditure (REE), and v) substrate oxidation substrate oxidation (which is reflected by respiratory quotient i.e. RQ). It is

speculated that short-term adjustments to imbalances in macronutrient and energy intakes are made primarily by appetite mechanisms (Flatt, 1995; Schutz, 1995). The thermic effect of food may also play a role, albeit minor, in the short-term regulation of macronutrient and energy intake. A change in TEF depends on both the size of the meal eaten and the composition of the meal (Tappy et al. 1993; Westerterp et al. 1999; Westerterp et al. 1999). In the longer-term, as fat mass and lean mass change, so do REE, PAEE and RQ (Schutz, 1995). The key appetite mechanisms regulating daily energy intake will be examined in section 1.6.4 and changes in the components of daily energy expenditure are discussed in section 1.6.5. The hypothesis that physiological adjustments in energy expenditure are stronger in response to negative than positive energy balances may explain the presence of weight plateaus during energy-restriction as well as the phenomenon of weight-regain that many individuals experience. It may also account for the ease at which many people gain moderate amounts of weight during short-term periods of overfeeding. These issues will be the focus of section 1.6.5. Chapters 7 to 9 of this thesis will examine the effect of moderate weight loss on the changes in the mechanisms regulating energy expenditure.

Table 1.4 Some examples of environmental influences on body weight. Redrawn from (Egger and Swinburn, 1996).

Size of environment	Type of environment			
	Physical		Socio-cultural	
	Energy intake	Energy expenditure	Energy intake	Energy expenditure
Macro	Food supply Availability Economy Advertising Legislation Pricing	Technology Transport Recreation facilities School Physical Ed Weather Safety	Advertising Attitudes Fast food Festivities Customs Food variety	Social trend Recreation time Attitude towards physical activity
Micro	Food at home Meal sizes Low-fat food Budget Cooking skills	Number of cars Number of TVs Remote controls Electronics Exercise equipment	Gender influence Weekly habits Social circles Work and peer influences	Attitudes and support of peers, family, partners Encouragement of activity for children

1.6.3 Macronutrient balance

The need to match energy intake to energy expended has long been recognized as an essential condition for weight maintenance. Since the 1950s it has been hypothesized that acute positive and negative oscillations in energy balance are somehow corrected by physiological signals produced by dietary macronutrients, particularly carbohydrates and fat (Flatt, 1996). Mayer (Mayer, 1955) initially suggested that energy balance was regulated predominantly through short-term “glucostatic” (i.e. monitoring of blood glucose) responses that could be corrected by longer-term “lipostatic” (i.e. body fat stores) regulation, should energy balance be sufficiently disturbed. It was not until the 1980s that the body’s ability to adjust the composition of the fuel mix utilised (or oxidised), on average, to the composition of the fuel mix consumed was considered a critical concept in the maintenance of a stable body weight (Flatt, 1988).

The sum of macronutrients (protein, carbohydrate, fat and alcohol) from daily meals, constitute the daily energy intake. The body utilises the daily macronutrient mix to generate ATP for metabolism, maintenance of body temperature, and for movement. The process of ATP generation is termed substrate oxidation (or oxidative phosphorylation) (Flatt, 1995). The rate of fuel oxidation as well as the composition of the fuel mix oxidised, varies considerably both during the day and from day-to-day, depending on how much ATP is required (Schutz et al. 1989). Moreover, this happens in a way that minimizes changes in the body’s protein and carbohydrate content (Abbott et al. 1988; Flatt et al. 1985; Horton et al. 1995; Schrauwen et al. 1997; Smith et al. 2000b). The variability in substrate oxidation from day-to-day occurs because there is a hierarchy in the extent to which macronutrients can be stored and utilised (Flatt, 1995). The storage and oxidative capacities of the three main macronutrients will be discussed in section 1.6.3.1.

The relative rates of macronutrient oxidation can be determined from the measurement of CO₂ production and O₂ consumption. The respiratory quotient (RQ), which is the ratio of CO₂ produced to O₂ consumed, provides a good indication about the relative proportions of carbohydrate and fat being oxidised (Flatt, 1996). Protein also contributes to CO₂ production and O₂ consumption, but in relatively minor and constant amounts and therefore does not substantially affect RQ (always < 20%) (Flatt, 1996). During the oxidation of carbohydrates 1 mol of CO₂ is produced per 1 mol of O₂ consumed, whereas fat oxidation yields only 0.7 mol of CO₂. Therefore, an RQ of 1 indicates that carbohydrate is the primary fuel being oxidised, whereas an RQ of 0.7 reflects the oxidation of fat. By comparing the RQ to the diet's food quotient (FQ; defined by the ratio of CO₂ produced to O₂ consumed during the oxidation of a representative sample of the macronutrient composition of the diet), it is possible to assess the extent to which the relative proportions of carbohydrate and of fat in the fuel mix oxidised, differs from the carbohydrate-to-fat ratio of the diet. In order to maintain energy balance, the average RQ must match the FQ of the diet (i.e. $RQ/FQ = 1$). A ratio greater than 1 suggests that a positive energy balance exists and the body's glycogen and/or fat reserves will be increased. The opposite is true for a RQ/FQ less than 1.

1.6.3.1 Storage and oxidative capacities of protein, carbohydrate and fat

The storage and oxidative capacities of the three main macronutrients are depicted in Table 1.5 and each macronutrient will be discussed below.

Table 1.5 The storage and oxidative characteristics of dietary protein, carbohydrate and fat. Redrawn from (WHO, 1997).

	Protein	Carbohydrate	Fat
Storage capacity in the body	low	low	high
Ability to stimulate own oxidation Metabolic pathway to transfer to another compartment	excellent	excellent	poor (slow)
	yes	yes	no

Protein storage and oxidation

Dietary protein has structural (e.g. tissue protein such as collagen) and functional (e.g. regulatory proteins such as digestive enzymes) importance for the body. Daily protein intakes vary between 50 to 100 g in adults and are small compared with the body's total protein content of which about half (6 kg) is intracellular and engaged in active turnover (Flatt, 1995). After feeding, protein undergoes digestion in the stomach and small intestine to release amino acids, which are taken up by cells and used for synthesis of structural and functional proteins (Benyon, 1998). If circulating amino acids are present in excess of the body's requirements, they can be used directly to produce ATP or converted to glycogen or fat and stored for later use. When the dietary protein requirements are met, the body's protein content remains stable. Moreover, small gains or losses of the body's tissue protein stores influence amino acid oxidation and induce corrective responses so as to minimize losses during food deprivation, and prevent their build-up when high-protein diets are consumed (Benyon, 1998; Welle and Nair, 1990). In 7 healthy young men, Flatt and colleagues demonstrated that protein balance (i.e. the ratio of protein ingested to that oxidised) was re-established within 9 hours of consuming 3 breakfasts meals (consumed in random order separated by at least 1 week). The meals varied in their macronutrient composition (i.e. breakfast 1: 62% carbohydrate/27% protein/11% fat; breakfast 2: 35% carbohydrate/15% protein/50% medium-chain triacylglycerols; breakfast 3: 35% carbohydrate/15% protein/9% long-chain triacylglycerols/ 41% medium-chain triacylglycerols) (Flatt et al. 1985). Abbott and colleagues (1988) also demonstrated that

protein (and carbohydrate) stores were closely regulated by adjusting oxidation to intake over a 24-hour period, in 27 men and 27 women. Strict regulation of protein stores even occurs when an organism is recovering from disease or under nutrition, and during a period of growth (Flatt, 1995).

Carbohydrate storage and oxidation

The principal product of carbohydrate digestion is glucose. Glucose is converted to glycogen and is stored in most of the body's tissues, the largest depots being in the liver and skeletal muscles. When blood glucose concentration is low, glucose is released from the liver. Conversely, when blood glucose is high, glucose is stored as glycogen (Benyon, 1998). Glycogen stores are extremely important in the maintenance of a stable blood glucose concentration and in ensuring muscular responses. However, the body's total glycogen stores are generally not much greater than 1 day's carbohydrate intake (200-500g in adults) (Abbott et al. 1988; Acheson et al. 1984). Because of the importance of glycogen and the body's limited capacity to store it, regulatory mechanisms including endocrine signals have evolved that give priority to the adjustment of carbohydrate oxidation to carbohydrate intake (Flatt, 1995). Immediately after a meal rich in carbohydrate, carbohydrate oxidation increases as indicated by the prompt postprandial rise in RQ (Abbott et al. 1988; Bobbioni et al. 1997; Flatt et al. 1985). Bobbioni and colleagues (1997) demonstrated that RQ reached its highest value after an oral load of pure glucose as compared to either mixed load (glucose + cream) or a pure lipid load, in 5 non-obese men and 5 non-obese women. The RQ remained almost unchanged after the pure lipid load. This finding indicates that the rise in RQ reflects the amount and nature of the carbohydrates consumed. Large carbohydrate intakes over a period of several or more days lead to sustained high rates of carbohydrate oxidation and cause glycogen concentrations to return towards their usual range (Acheson et al. 1984). When the over-consumption of carbohydrate persists for more than 2 to 3 weeks then the body's glycogen

stores are raised considerably above their usual levels (Acheson et al. 1988). On the other hand, when ingestion of carbohydrate fails to replenish glycogen reserves (i.e. after strenuous exercise or an overnight fast) there is increased gluconeogenesis; free fatty acids or amino acids are converted to glucose in the liver. When considered over a period of a few days, however, carbohydrate oxidation and intake are matched. Jebb and co-workers (1996) performed an energy imbalance study in 6 lean men; 3 men were overfed (16.5 MJ/day) and another 3 men were underfed (3.5 MJ/day) a mixed diet for 12 days. Twenty-four hour protein, carbohydrate and fat balances were continuously measured for the 13 days while subjects were resident in a respiratory chamber. The study demonstrated that carbohydrate balance was close to zero by day 5 of both the over- and under-feeding protocols, indicating that carbohydrate intake exerts direct auto-regulatory feedback on carbohydrate oxidation. Protein balance was not as effectively regulated as carbohydrate balance, and fat oxidation was not at all sensitive to fat intake. These results indicate that the highly efficient regulation of carbohydrate stores in maintaining adequate glycogen reserves reduces the need to use fat as fuel. Consequently, carbohydrate is an important determinant of how much ingested dietary fat will be oxidised and retained as body fat.

Table 1.5 indicates that carbohydrate can be converted and stored in an alternative energy compartment. Glucose can be converted into fat (i.e. lipogenesis is reflected by an RQ > 1) to promote the build up of fat reserves when the diet is low in fat and high in carbohydrate (Benyon, 1998). It is important to note, however, that this rarely occurs. In fact, such *de novo* lipogenesis is only observed in humans during periods of forced massive overfeeding- a condition that does not occur in everyday life (Flatt, 1995). Furthermore, the conversion of glucose to fat is evolutionarily unfavourable because *de novo* lipogenesis is an energy-requiring process in which 25% of the energy from carbohydrate is converted into heat.

Fat storage and oxidation

Fat intake and fat oxidation are poorly regulated (Flatt et al. 1985; Griffiths et al. 1994; Schutz et al. 1989). Flatt and colleagues (1985) found that the insulin response, changes in glucose and fatty acid concentrations, and the postprandial RQ response were barely affected by the addition of 40g of fat to a meal containing 75% carbohydrate, in 7 young healthy men. Griffiths et al. (1994) also showed that the addition of 80g of fat to a meal did increase fat oxidation, but only marginally for the subsequent 6 hours, in 8 non-obese subjects. In this study it was also demonstrated that the addition of 80g fat markedly delayed gastric emptying and the absorption of glucose, and consequently reduced the rapid carbohydrate-induced postprandial rise in RQ. The adipose tissue store is the largest storage compartment of energy within the human body. In a lean 60 kg women, fat stores account for ~15 kg. This corresponds to ~500,000 kJ whereas the average glycogen stores contain ~1000 to 2000 kJ (Flatt, 1995a and 1995b; Schutz, 1995). Accordingly, the daily fat intake constitutes a small proportion (< 1%) of the energy content of the fat stores. Although protein and carbohydrate oxidation adjust to protein and carbohydrate intake over a period of 1 to 7 days (Schrauwen et al. 1997; Smith et al. 2000a; Smith et al. 2000b; Thomas et al. 1992), fat oxidation can take more than 7 days to match fat intake (Schrauwen et al. 2000a; Schrauwen and Westerterp, 2000b; Schutz et al. 1992b). This is because the rate of fat oxidation is determined primarily by the gap between total energy expenditure and the amount of energy ingested in the form of carbohydrate and protein, rather than by the amount of fat consumed on a given day (Flatt, 1995b). Accordingly, acute positive balances which are trivially small as compared to the energy stores within the body's fat compartment, are readily accommodated by an expansion in the body's fat stores (Flatt, 1993; Flatt, 1995b). However, substantial expansion of an individual's adipose tissue mass are not caused by small, independent daily positive balances in the body's fat stores (Flatt, 1995b). Instead, it is the cumulative (or chronic) effects of repeated positive balances (i.e. fat intake is chronically greater than fat oxidation) that can

in time lead to substantial increases in the size of the body's fat stores. In addition to the reduced rate at which the body oxidizes fat (as compared to carbohydrate and protein), expansion of body fat stores are facilitated by the deposition of chylomicrons (fatty acids in the circulation) because they are not metabolized like their free fatty acid counterparts (Flatt, 1995b). Substantial expansion of the fat mass enhances fat oxidation until a new equilibrium is reached between fat intake and oxidation (Flatt, 1995b). As a result protein, carbohydrate as well as fat balances are all close to zero at the new fat mass (reflected by RQ equal to FQ of the diet). When this point is reached, the individual is in a state of energy balance once more and body weight stabilizes, albeit at a higher level. During negative energy balance, the same physiological changes occur except in the reverse direction (Schutz et al. 1992a).

1.6.3.2 Implications for weight gain or weight loss

Considerable variability between individuals in the partitioning and oxidation of excess energy (whether it be primarily from carbohydrate or fat) suggests that other factors, in addition to diet composition, may influence the ratio of fat-to-carbohydrate oxidation. Cooling and Blundell (1998a; 1998b; 2000) demonstrated that young, lean males who usually consumed a high-fat diet were able to lower their RQ in response to a high-fat meal and increase their RQ in response to a high-carbohydrate meal. In contrast, the subjects of similar BMI, who usually consumed a high-carbohydrate diet, were not capable of switching from carbohydrate to fat oxidation after the high fat meal (i.e. their RQ remained elevated). Clearly, the habitual high-fat group, were capable of adapting to multiple dietary conditions whereas the habitual high-carbohydrate group were not. Consequently, the latter groups would be vulnerable to weight gain if the diet allowed occasional high-fat intakes, or if the diet changed suddenly. The investigators suggested that an increase in resting energy expenditure, which they observed in the former but not the latter group, may have in part, explained their adaptation to high-fat intakes. Factors

including physical activity, physical fitness (i.e. VO_2max), age, gender and genetics may also explain why some individuals can adapt to any diet composition. Concurrent physical activity has been shown to increase the rate of fat oxidation during a shift to a high-fat diet and thereby accelerate the re-establishment of fat balance, in 6 young, healthy men (Smith et al. 2000a). Smith et al. have also shown that fat balance was positively associated with baseline insulin concentrations and negatively associated with VO_2max during treadmill exercise, in 6 men shifted from a low-fat to an isco-caloric high-fat (37% vs 50% of energy) diet (Smith et al. 2000b).

A low ratio of fat-to-carbohydrate oxidation (i.e. a high RQ) has been found to predict weight gain in susceptible individuals such as the Pima Indians of Arizona (Weyer et al. 2000; Zurlo et al. 1990). In 111 non-diabetic subjects (87 men/65 women) who had measurements of 24-hour RQ and TEE measured 25 \pm 11 months apart, it was found that the subjects with higher 24-hour RQ (90th percentile), independent of TEE, were 2.5 times more likely to gain \geq 5 kg of weight than the individuals who had lower 24-hour RQ (10th percentile) (Zurlo et al. 1990). Subjects consumed a weight-maintenance, solid food diet (50% carbohydrate, 30% fat and 20% protein) for at least 2 days before their metabolic rate was measured. It is unlikely, therefore, that the higher RQ was the result of an increased dietary carbohydrate (subjects were in energy and macronutrient balance before all measurements). Of the variables measured in the study, family membership was the greatest determinant of 24-hour RQ. Astrup's group (Toubro et al. 1998) reported similar findings in a Danish population of 71 healthy siblings from 32 families. However, both studies found that body composition, fasting plasma insulin and free-fatty acid levels, and gender also impacted on RQ (Toubro et al. 1998; Zurlo et al. 1990).

Susceptibility to obesity not only depends on the initial rates of macronutrient oxidation, but also on how the rates change in response to changes in weight. Nelson et al. (1992)

demonstrated that fat oxidation was greater, carbohydrate oxidation less, and protein oxidation the same in 24 obese women, before weight loss, as compared to non-obese controls. Diet composition had no impact on these changes because all subjects were studied after 10 days of a weight maintaining diet that contained 55% carbohydrate, 22% protein and 23% fat. After weight loss, the post-obese subjects utilised carbohydrates, lipids, and proteins to the same extent as the non-obese controls. In addition, Weyer and colleagues (2000) observed a significant but weak ($r = -0.2$) relationship between the change in 24-hour RQ (adjusted for % body fat, age, gender and energy balance) and the change body weight after a mean follow-up period of 3.6 years, in 102 Pima Indians. These studies indicate that to oppose further weight change, adjustments in the oxidation of substrates do occur. Chapters 7, 8 and 9 of this thesis examine the effect of low-fat, energy-restricted diets on changes in fat and carbohydrate oxidation, in obese subjects with and without Type 2 diabetes.

Longitudinal studies also suggest an important relationship between the ratio of fat-to-carbohydrate oxidation and weight re-gain after energy restriction is ceased. Lean and James (1988) reported higher 24-h RQs in post-obese and obese women as compared with weight- and body composition-matched, non-obese controls. Froidevaux et al. (1993) also reported that women failing to maintain a body weight reduction had higher 24-hour RQs than those who successfully kept their weight down.

1.6.4 The regulation of appetite and food intake

Human appetite is a complex mixture of physiological and psycho-social phenomena. Physiological regulation of appetite arises from complex interactions between and within multiple central and peripheral sites (Blundell and Halford, 1994; Kalra S et al. 1999; Read N et al. 1994; Stubbs, 1998). From this wealth of feedback the brain must sort out relevant signals and make decisions about food intake. It appears that when hunger is high,

an individual's ability to inhibit inappropriate food choices is reduced; presumably because the appetite mechanisms such as satiety are easily over-ridden by ever-present cues to eat in our obesogenic society. Accordingly, some genetically susceptible individuals can become obese by eating too much.

The central and peripheral mechanisms regulating appetite indicate that the macronutrient composition of the diet is an important determinant of hunger and fullness. Section 1.6.3 has already discussed the body's ability to oxidize (or utilise) excess carbohydrate and protein at the expense of fat, and the significance of substrate oxidation in the etiology of obesity. Data also suggests that the amount of energy ingested over 24 hours depends on the macronutrient composition of the food eaten. It has been proposed that carbohydrate, protein and fat may differ in the effects that they exert on the regulation of 'satiation' and 'satiety' (Blundell and Halford, 1994; Rolls and Han, 1995). Satiation describes the series of sensations that terminate eating; it is primarily responsible for determining the size of individual meals. Conversely, satiety describes the absence of hunger and it signals to the individual not to commence eating. Hunger is defined as the sensation that drives a person to search for and eat food. Differences in the storage capacity and oxidation (Section 1.6.3), energy density, volume or weight, palatability, and the stimulation of hormones and neurotransmitters, between carbohydrate, protein and fat, may all affect satiety and satiety.

Sections 1.6.4.1 and 1.6.4.2 will provide a brief overview of the key central and peripheral mechanisms controlling food intake. Section 1.6.4.3 will examine the comparative effects of carbohydrate, protein and fat on appetite and food intake. Evidence will be presented to demonstrate that there is a satiety hierarchy for macronutrients. The different characteristics of the macronutrients and how they affect satiety will be briefly discussed.

1.6.4.1 Central mechanisms controlling appetite and food intake

The mammalian brain has a number of nuclei in the basal hypothalamus that are associated with neural mechanisms affecting appetite (Jequier and Tappy, 1999; Kalra et al. 1999; Read et al. 1994). Figure 1.3 illustrates some of the central sites in the regulation of appetite.

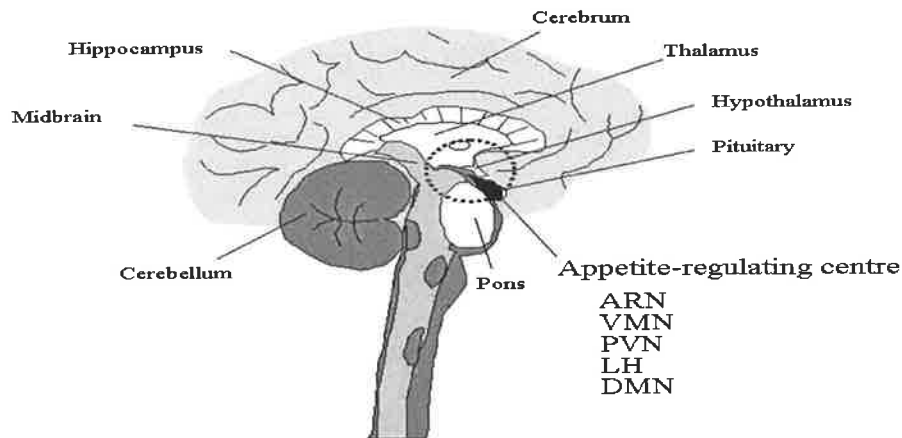


Figure 1.3 Key central sites involved in the regulation of appetite. Adapted from (Kalra et al. 1999). ARN, arcuate nucleus; VMN, ventromedial nucleus; PVN, paraventricular nucleus; LH, lateral hypothalamus; DMN, dorsomedial nucleus.

Using animal models and techniques such as hypothalamic lesions or surgical transactions of neural pathways, the ventromedial nucleus, dorsomedial nucleus, paraventricular nucleus and the lateral hypothalamus have been recognized as containing neural mechanisms affecting feeding behavior (Hoebel and Hernandez, 1993). They integrate and relay information between the peripheral autonomic/endocrine organs and the forebrain structures. Studies in rodents demonstrated that the lateral hypothalamus is involved in the onset of feeding and its stimulation results in overeating (Hoebel and Hernandez, 1993; Weigle, 1994). Conversely, the ventromedial nucleus mediates satiety in response to signals from the periphery and its stimulation causes under-eating. In addition, ablation of these regions of the brain produces the opposite effects in rodents (i.e. destruction of the

lateral hypothalamus results in under eating and in the ventromedial hypothalamus it causes hyperphagia). Nuclei in the midbrain and the thalamus are thought to interpret the information in relation to the sensory properties of foods (Jequier and Tappy, 1999). Numerous central and peripheral compounds involved in the central regulation of feeding have been identified. A comprehensive list depicting their stimulatory or inhibitory effects on food intake is presented in Table 1.6.

Table 1.6 Endogenous compounds implicated in the central regulation of mammalian feeding behavior. Adapted from (Hope, 2000; MacIntosh, 2001).

	Stimulatory effect on food intake	Inhibitory effect on food intake
Pancreatic hormones		Amylin Insulin Insulin-like growth factor-1 Pancreatic glucagon
Monoamines	α -adrenergic regulators (e.g. noradrenaline) Histamine	β -adrenergic regulators (e.g. adrenaline) Dopamine Serotonin
Gastrointestinal peptides	Ghrelin Motilin	Bombesin Cholecystokinin Gastrin releasing hormone Glucagon-like-peptide-1 Somatostatin
Gonadal hormones	Progesterone Testosterone	Estrogen
Adipose tissue signals		Leptin Tumor necrosis factor- α
Miscellaneous neurotransmitters	Nitric oxide γ -aminobutyric acid	N-methyl-D-aspartate
Miscellaneous peptides and hormones	β -endorphin Cytokines Galanin Growth hormone releasing hormone Melanin-concentrating hormone Neuropeptide Y Orexins Oxytocin Peptide YY Thyroid hormone Urocortin	α -melanocortin stimulating hormone Calcitonin Calcitonin gene related peptide Cocaine gene related peptide Corticotrophin releasing factor Melanocortin releasing factor Thyrotrophin-releasing hormone

1.6.4.2 *Peripheral mechanisms controlling appetite and food intake*

Major peripheral systems involved in the regulation of appetite include the gastrointestinal tract, autonomic nervous system, liver, and adipose tissue (Figure 1.4). The mouth is the first part of the gastrointestinal tract to be exposed to food and taste plays an important

role in food selection as well as determining the amount of food that is eaten (i.e. energy intake) (Rolls et al. 1988a). The upper gastrointestinal tract including the mouth and stomach appears to generate signals that induce satiation, whereas the rest of the gastrointestinal tract may regulate between-meal satiety (Read et al. 1994). Adipose tissue depots, the size of which are signaled by the circulating protein product, leptin, may also play a role in the control of long-term feeding patterns by interacting with metabolic pathways (Campfield and Smith, 1998).

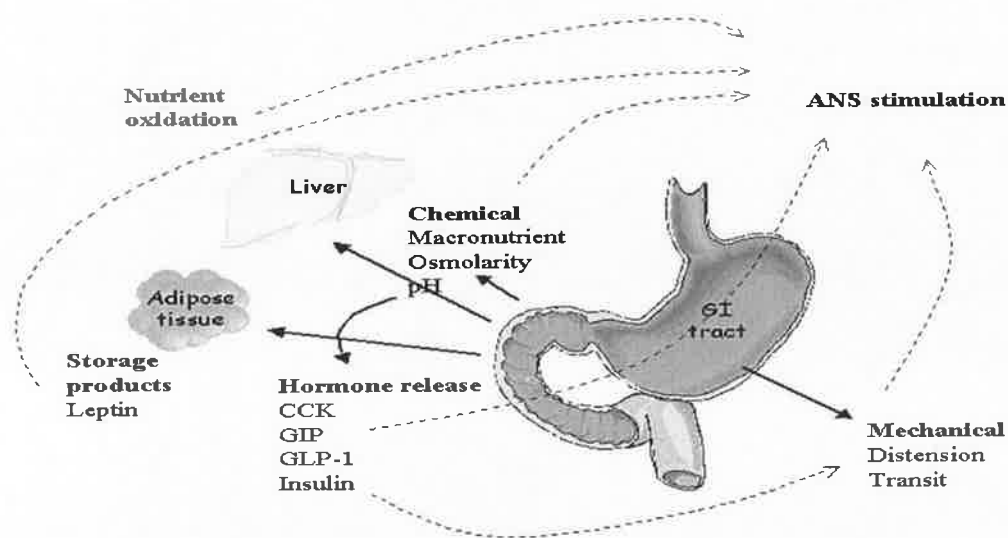


Figure 1.4 Overview of some major peripheral factors controlling appetite. Adapted from (Morley, 1987; Read et al. 1994). ANS, autonomic nervous system; GI, gastrointestinal; CCK, cholecystokinin; GIP, gastric inhibitory peptide; GLP-1, glucagons-like peptide-1.

Peripheral signals are relayed to central feeding centers via a variety of pathways, many of which are indicated in Figure 1.4. The intrinsic and extrinsic innervations of the upper gastrointestinal tract, together with the neural pathways and their target neurons in the central nervous system, comprise the gut-brain axis. The gut-brain axis describes the afferent signals produced by gut contact in response to ingested nutrients (Read et al. 1994). These signals travel from the upper gastrointestinal tract to the central nervous system to mediate food intake (Morley, 1987). A number of hormones released from the

pancreatic and gastrointestinal cells may act concurrently to mediate many of the consequences arising from the contact of nutrients within the small intestine. These are presented in Table 1.7 and the table shows that many of them depend on the vagal nerve to exert their satiating effects.

Table 1.7 Gastrointestinal and pancreatic hormones that modulate food intake after peripheral administration.

	Effect on food intake	Dependence on vagus nerve
Pancreatic hormones		
Glucagon	Decrease	Yes
Insulin	Increase	No
Gastrointestinal peptides		
Amylin	Decrease	No
Bombesin	Decrease	Partially
Cholecystokinin	Decrease	Yes
Gastrin-releasing peptide	Decrease	Unknown
Ghrelin	Increase	Unknown
Motilin	Increase	Unknown
Satielin	Decrease	Unknown
Somatostatin	Decrease or no effect	Yes
Thyrotropin-releasing hormone	Decrease	Yes

1.6.4.3 Dietary macronutrients and their comparative effects on appetite and food intake

There is some evidence that excess energy from fat has a greater obesogenic effect as compared to excess calories from other macronutrients. It remains unclear whether obesity results from excess energy per se, or over-consumption of dietary fat. The American Nationwide Food Consumption Survey shows energy intake has increased over the past 20 years (Nielsen et al. 2002). The data indicate that the increase in energy intake has arisen from a shift away from eating at home to eating away-from-home, as well as a shift from eating meals to eating energy-dense, low-nutrition snacks. Conversely, other American data that compared energy intake during the periods of 1976-80 and 1987-91, found that there was a 4% decrease in overall energy consumption and an 11% decrease in the

amount of energy consumed as fat (Heini and Weinsier, 1997). Despite this, a 4% increase in the mean BMI coincided with a 31% increase in the prevalence of overweight (Heini and Weinsier, 1997). Discrepancies in food intake data reflect poor methods for quantifying it. It is known that all individuals under-report their intake, the obese more than normal weight individuals (Heitmann and Lissner, 1995; Schoeller et al. 1990). It has also been suggested that obese under-report between meal snacks and fat-rich foods (Heitmann and Lissner, 1995). Regardless of whether energy intake has remained relatively constant or increased over the last few decades, it does appear that the macronutrient composition of the diet may be an important determinant of excess energy intake. The following section will examine the hypothesis that protein is more satiating than carbohydrate, which is more satiating than fat, and how satiation affects food intake.

Macronutrient satiating hierarchy

Laboratory studies utilizing pure nutrient infusions in rats support the concept that protein is more satiating than carbohydrate which is more satiating than fat (Geliebter, 1979; Walls and Koopmans, 1992). In humans, Rolls et al. (1988b) reported that iso-energetic pre-loads high in fat, carbohydrate (sweet or non-sweet), protein or a mixture of nutrients, had differing effects on food intake at a buffet lunch 2-hours later. In the 10 non-obese women investigated in this study, fullness (assessed using visual analogue scales) was greatest following ingestion of both the high-protein and the high-starch, compared with the high-fat, high-sucrose and mixed nutrient pre-loads. In addition, the weight and energy content of the food consumed at the self-selected buffet lunch were almost 40% lower after the high-protein than after the high-fat pre-loads. These findings indicate that high-protein followed by high-starch foods are better at suppressing over-consumption of energy than foods enriched with simple carbohydrates and fat. It is possible, however, that the different satiating effect of these pre-loads may have been influenced by preconceptions about food items offered. For example, the high-protein and high-starch

pre-loads were chicken and pasta, traditionally considered main meal type foods. The high fat and high sucrose pre-loads were chocolate and Turkish delight, which represent snack type foods and therefore possibly perceived as less satiating. A number of other acute feeding studies have confirmed that protein enriched meals are more satiating and reduce subsequent food intake to a greater extent than high-carbohydrate foods (although it appears to depend on the type of carbohydrate) (Barkeling et al. 1990; Booth et al. 1970; Porrini et al. 1995); none, however, have simultaneously compared protein, carbohydrate and fat preloads.

In a series of 3 studies, Blundell and colleagues (1993) compared the effects of fat and carbohydrate pre-loads on appetite responses and subsequent food intake. An important aim of these studies was to investigate the time effects of the different pre-loads on the suppression of food intake. Accordingly, the interval between the pre-load and subsequent test meal was varied. Breakfasts of orange juice, scones and fruit yoghurt [1.82 MJ control day], or the same supplemented to 3.36 MJ with fat (polyunsaturated margarine and dairy cream) or carbohydrate (glucose, sucrose and maltodextrin), were consumed by lean healthy men who then ate lunch ad libitum 4-hours later. Protein constant was constant for all meals. In the first study, only appetite sensations were assessed at the 4-hour time point. It was found that carbohydrate supplemented breakfast suppressed hunger, desire to eat, and prospective consumption, and decreased fullness. In contrast, the fat supplemented breakfast had no effect on these sensations of appetite. In the second study, food intake at lunch was suppressed by the carbohydrate supplemented breakfast but not the fat supplemented breakfast, that were eaten 90 minutes earlier. In the third study, food intake at lunch, presented 270 minutes after the breakfast meals, was unchanged. These studies support the hypothesis that carbohydrate is more satiating than fat, but suggest that the effect may differ over the day. Rolls et al. (1991) confirmed that differences in the

satiating properties between carbohydrate and fat, appear to depend on the time of measurement.

A limitation of the studies described above was that they examined the effect of pre-loads on subsequent food intake using buffet meals that were at fixed intervals after the pre-load. In free-living individuals, it remains unknown whether the satiating properties of the different macronutrients effect spontaneous eating, which have implications for daily energy intake. Marmonier et al. (2002) recently assessed the timing and quantity of food intake following the consumption of isoenergetic (~1 MJ) high-protein (64% of energy) and high-carbohydrate (66% of energy) afternoon snacks in the satiated state, in 8 young men. The protein snack (cooked chicken breast and low fat dressing) delayed the dinner request by 38 min, whereas the dinner request after the carbohydrate snack (rice pudding) was similar to the control. Energy ingested over the day was greater (by ~500 kJ) following the carbohydrate snack as compared to both the control and protein snacks. In a similar study by the same group, a protein snack delayed dinner by 60 min whereas a carbohydrate snack (rye bread and raisins) and fat snack (cream cheese and toasted bread) delayed dinner by 34 and 25 minutes respectively, as compared to the control day (Marmonier et al. 2000). In contrast to the first study, no difference in daily energy intake was observed between the protein, carbohydrate or fat treatment days. Neither of Marmonier's studies accounted for differences in the sensory properties, volume, or energy density of the snacks administered. A recent study by our group (Vozzo et al. 2002) found that there was no difference in time until the first spontaneous food request, following yoghurt pre-loads (~3 MJ) of either fat (40% of energy), carbohydrate (60% of energy), or protein (29% of energy). They also observed that food intake was suppressed to a greater extent following the protein pre-load, but there was no difference in food intake between the fat or carbohydrate preloads. All yoghurt preloads in this study were matched for taste, pleasantness, texture and colour. Therefore, discrepancies between

Vozzo et al. (2002) and Marmonier et al. (2000) may have been due to cognitive factors influencing appetite in the latter study.

Characteristics of foods affecting satiety

1) Energy density, volume and weight

In part, food intake may be regulated by the volume and or weight of food consumed (Read et al. 1994; Rolls et al. 1998). Since the energy density of the three macronutrients differ (fat provides ~33.5 kJ/g compared to ~16 kJ/g for carbohydrate and protein), eating by volume or weight has implications on the amount of energy ingested by individuals who eat high-fat diets as compared to those who eat high-carbohydrate or high-protein diets. People eating the same volume of high-fat foods as compared to those eating high-carbohydrate foods would consume more calories because the energy density of fat is higher (i.e. passive over-consumption). In healthy men, Stubbs et al. (1995b) demonstrated that the amount of calories ingested spontaneously increased as the amount of fat in the diet increased. In this study fat was covertly incorporated into the diet to maintain its palatability and volume while increasing its' energy-density.

2) Sensory properties

The taste, smell and texture of food play a role in the determining the amount and frequency of its consumption (Rolls et al. 1988a). An increase in palatability tends to promote over-consumption and thus causes a positive energy balance. Some people overeat high-fat foods simply because they are enjoyable and highly palatable, particularly those with a sweet/fat mixture (Golay and Bobbioni, 1997; Mela, 1997). However, individuals differ greatly in their preference of foods. Some studies suggest that obese individuals have an enhanced sensory preference for high-fat foods, but work done so far is not conclusive (Cooling and Blundell, 2001; Mattes, 1993). The texture of the food determines the amount of chewing required for particular foods. The degree of chewing required might affect satiety and help to determine how much food is consumed. Foods

rich in protein, fibre and complex carbohydrate tend to require more chewing than fat-rich foods and are therefore eaten more slowly and are more satiating. In contrast, fats that can be eaten quickly appear to promote over-consumption (Golay and Bobbioni, 1997).

Implications for the development of obesity and weight loss

A greater intake of dietary fat has been proposed as a cause of the over-consumption of energy that leads to obesity. The above discussion indicates that the greater energy density of fat coupled with the notion that it elicits a weaker satiety response than either protein or complex carbohydrates are two reasons explaining how fat can be easily over-consumed. Weight loss strategies have, therefore, advocated a reduction in dietary fat. Low-fat weight loss strategies are reviewed in Section 1.7.

1.6.5 The role of energy expenditure in the regulation of human body weight

Gluttony and/or sloth are frequently blamed as the cause of positive energy balances that, over time, result in obesity. It is, however, not clear why some individuals become obese, whereas others with the same diet and activity patterns, remain lean. Whether differences between individuals in their susceptibility to obesity are, in part, due to differences in energy expenditure, remains controversial. It is hypothesized that, for many people, weight gain in an 'obesogenic' environment is easy because compensatory adjustments in the physiological mechanisms regulating total energy expenditure, are weak. Conversely, it is hypothesized that, for many individuals, weight loss is difficult because compensatory adjustments in energy expenditure during a negative energy balance are stronger than during a positive energy balance. At present, the evidence for these two hypotheses, particularly the latter, is difficult to interpret because of methodological issues. Some research indicates that changes in energy expenditure, in response to perturbations in energy balance, do not exceed those predicted from the change in energy intake (and the resultant change in body composition) (Forbes et al. 1986; Garby et al. 1988; Prentice et

al. 1986; Prentice et al. 1991). Other data, particularly those investigating changes in energy expenditure in response to weight loss, indicate that the changes do exceed predictions and as such they appear to represent an adaptive response by the body to resist a further change in weight (Bessard et al. 1983; Froidevaux et al. 1993; Leibel et al. 1995; Ravussin et al. 1988; Saltzman and Roberts, 1995).

The following section reviews some of the findings from energy expenditure research during the last 15 years. Section 1.6.5.1 describes the components of energy expenditure that are responsible for matching total energy expenditure to total energy intake. Prospective studies suggest that lower rates of energy expenditure are a significant contributor to human obesity- these studies will be reviewed in Section 1.6.5.2. Intervention studies involving over- or under-feeding have been conducted to address the issue of whether changes in the components of total energy expenditure components are important physiological mechanisms regulating energy balance and body weight. Section 1.6.5.3 will present some evidence from over- and under-feeding studies showing that total energy expenditure may change in a direction so as to restore energy balance. Results from over- and under-feeding studies showing that changes in total energy expenditure may be mediated by changes in at least one or several of its components- resting energy expenditure, physical activity (voluntary and involuntary), and the thermic effect of feeding- will be reviewed in Sections 1.6.5.4 to 1.6.5.6. Section 1.6.5.7 will discuss the influence of the different macronutrients on energy expenditure.

1.6.5.1 Components of total energy expenditure

Total energy expenditure (TEE) refers to the daily amount of energy expended by the body while at rest and during all forms of activity. It comprises 3 components: i) Resting energy expenditure (REE), defined as the energy expenditure necessary to maintain basic physiological functions while lying at rest in a post-absorptive state. Depending on the

regularity and intensity of daily activities, REE accounts for ~60% of TEE and is strongly related to lean body mass (Keeseey and Hirvonen, 1997; Ravussin et al. 1986; Weigle, 1994); ii) Physical activity (PAEE), defined as energy expended during voluntary as well as in voluntary activities that occur during rest (e.g. fidgeting, maintenance of posture). Physical activity is the most variable component of TEE, explaining ~30 to 50% (Ravussin et al. 1986; Weigle, 1994); iii) Thermic effect of feeding (TEF), is the increase in energy expenditure above REE which results from the energy cost of digestion, absorption, transport, metabolism and storage of nutrients consumed. It contributes, on average, to ~10% of TEE (D'Alessio et al. 1988; Westerterp et al. 1999).

The measurement of TEE and its components was a significant aim of the studies presented in this thesis. Accordingly, the various methods used to measure TEE and PAEE are described in Chapter 5, and those used to measure REE and TEF are described in Chapter 3. The limitations of the methodologies used to measure each component are also discussed in considerable detail within these chapters. Methodological issues that contribute to the conflicting findings of the studies presented in the following sections will be briefly discussed.

1.6.5.2 Prospective studies implicating lower rates of energy expenditure in the etiology of obesity

Prospective studies, where normal weight individuals are followed over-time, have provided a valuable insight into how weight gain relates to initial measurements of energy expenditure and intake. Such studies are difficult to conduct (limited access to respiratory chambers or doubly-labeled water, expensive, each measurement is time consuming) and the timing of the measurements (whether they occur during a period of energy balance or imbalance) may mask the true relationship between measured parameters. Despite these

potential problems, the several prospective studies depicted in Table 1.8 have shown that reduced energy expenditure can precede weight gain.

Table 1.8 Does reduced energy expenditure precede weight gain in prospective studies? Redrawn from (Saltzman and Roberts, 1995).

Authors	Study population	Yes/No
(Ravussin et al. 1988)	Adult Pima Indians	Yes
(Roberts et al. 1988)	Infants of overweight mothers	Yes
(Griffiths and Payne, 1976; Griffiths et al. 1990)	Children of overweight parents	Yes
(Davies et al. 1991)	Infants of normal weight parents	No

In 1988, Ravussin et al. (1988) first reported a series of three studies in adult Pima Indians (1993; 1995; 1990). Ravussin et al. (1988) measured both TEE and REE (adjusted for body composition), in a respiratory chamber in a population of PIMA Indians. In the first study of 95 subjects, energy expenditure was negatively correlated with the rate of change in body weight over a two-year follow-up period. It was estimated that the risk of gaining more than 7.5 kg in body weight was increased fourfold in individuals with a low adjusted TEE (~800 kJ/day below predicted values) as compared with persons with a high TEE (~800 kJ/day above predicted values). In another 126 subjects, the adjusted REE at the initial visit was also found to predict the gain in body weight over a four-year follow-up period. In the third study, Ravussin and colleagues examined a group of 94 siblings from 36 families. It was found that values for adjusted TEE aggregated in families. However, the authors noted that the magnitude of the reduction in REE was not sufficient to account entirely for the surplus energy deposited in subjects who gained the most weight. This confirms that additional factors such as a low level of physical activity and/or a high energy intake, act synergistically with the reduced REE to lower TEE and thereby initiate weight gain.

Prospective studies investigating the causes of weight gain in infants and children have also indicated a role for energy expenditure in the regulation of body weight (Griffiths and Payne, 1976; Griffiths et al. 1990; Roberts et al. 1988). An advantage of Roberts et al. (1988) study was that it used doubly labeled water to measure the TEE and energy intake of 18 infants at 12 weeks of age who were born to lean or overweight mothers. Follow-up measurements of body composition and energy intake were performed until the infants were 1 year of age. All infants grew similarly until 12 weeks of age. However, 50% of the infants born to overweight mothers subsequently grew more rapidly, and by 1 year of age, they were defined as being overweight. The rapid weight gain in these infants was attributed to them having a 20.7% lower TEE at three months of age as compared to the infants who never became overweight. It was acknowledged, however, that there was an overlap of TEE values between the two groups of infants that was larger than could be accounted for by methodological error alone. Some of the normal weight infants had low rates of TEE and conversely some of the overweight infants had high TEE values. Furthermore, by 6 months of age, some of the infants destined to be overweight also displayed high energy intakes. The authors suggested two reasonable conclusions from the findings of this study: i) the low TEE is more likely a risk factor than the cause of weight gain, and ii) overeating may be the primary response when more energy is required, and that the decrease in TEE is a secondary response that only occurs when the demand for energy is not met (which is less likely in modern society) (Saltzman and Roberts, 1995). In two separate studies, Griffiths and colleagues (1976; 1990) also showed that 4 to 5 year old children with at least one obese parent had significantly reduced TEE, REE and energy intakes than children (who were of a similar weight and fat mass) of normal weight parents. When the same children were followed-up at 15 to 16 years of age, the children of the obese parent had subsequently gained more weight. This finding again suggests that a low TEE early in life may be a risk factor for subsequent weight gain. In contrast to these

findings, a prospective study by Davies et al. (1991) did not find a relationship between TEE (made using doubly labeled water) and body weight or composition at 9 and 24 months of age.

To our knowledge, the studies cited above are the only prospective studies examining the relationship between energy expenditure and weight gain that have been published. Accordingly, further prospective studies are warranted to determine the precise role of energy expenditure in the onset and development of obesity.

1.6.5.3 Changes in total energy expenditure in response to positive and negative energy balances

To understand the full adaptive response of energy expenditure to alterations in energy balance it is necessary that TEE, and not just REE, is measured. Only a relatively small number of studies have directly measured TEE using accurate methods such as doubly labeled water or whole-body indirect calorimetry, in response to over- and underfeeding. A number of these studies (most of which have included small heterogeneous subject populations) are summarised in Table 1.9.

In the overfeeding studies, Table 1.9 shows that the change in TEE in response to the positive energy balance is greater, by an average factor of 2.9, than the change in REE (i.e. the ratio of the changes in TEE to REE) (Saltzman and Roberts, 1995). This implies that increases in non-resting energy expenditure (i.e. TEF and PAEE) probably occur passively as a result of the increase in body mass (lean mass as well as fat mass increases as a result of positive energy balance) and macronutrient intakes. In contrast, the underfeeding studies exhibited much more variable responses in TEE. The decrease in TEE with underfeeding is usually, but not always (Bessard et al. 1983; Rumpler et al. 1991) (possibly due to measurement error or small heterogeneous population), larger than the

change in REE. The studies by Heyman et al. (1992) and Racette et al. (1995) demonstrated that the TEE response to a negative energy balance was considerably greater than the change in REE, by a factor of 4.4 and 2.8, respectively. These findings imply that in response to a negative energy balance, reductions in TEE may be due to reductions in energy expenditure due to physical activity, as well as to reductions in REE. Consequently, changes in TEE arising from changes in REE and PAEE, represent adaptive responses, which may limit further weight loss.

Table 1.9 Changes in total and resting energy expenditure during overfeeding and underfeeding*. Adapted and modified from Saltzman and Roberts (1995).

	Subjects			Excess EI (kJ/day)	Δ TEE (kJ/day)	Δ REE (kJ/day)
	n	Gender	Type			
Overfeeding studies						
(Roberts et al. 1990) ^{††}	7	M	N	4042	422	330
(Diaz et al. 1992) ^{††}	10	M	N+OW	6300	1797	899
(Goran et al. 1994) [†]	6	M	N	5041	907	481
Leibel et al. 1995 ^{§‡}	13	M+W	N	~3300	2629	615
Leibel et al. 1995 ^{§‡}	11	M+W	OW	~3300	3645	560
(Jebb et al. 1996) [†]	3	M	N	5500	750	420
Mean				~4580	1691	551
Mean ratio of Δ TEE: Δ REE = 2.9 [1.3 to 6.5]						
Underfeeding studies						
(Bessard et al. 1983) [†]	6	W	OW	~ -5600	-832	-995
(Heyman et al. 1992) [§]	7	M	N	-3285	-1237	-284
(Rumpler et al. 1991) [§]	8	M	OW	~ -6468	-273	-351
(Amatruda et al. 1993) ^{§‡}	33	W	OW	~ -8800	-966	-748
(Racette et al. 1995) [§]	13	F	OW	-1665	-1400	-497
Leibel et al. 1995 ^{§‡}	13	M+W	N	~ -3300	-1789	-924
Leibel et al. 1995 ^{§‡}	11	M+W	OW	~ -3300	-2303	-1212
(Jebb et al. 1996) [§]	3	M	N	-7000	-1200	-820
Weinsier et al. 2000 ^{§‡}	32	W	OW	~ -2750	-477	-289
Mean				~-4100	-1164	-617
Mean ratio of Δ TEE: Δ REE = 1.89 [0.7 to 4.4]						

*All values are study means. Subjects: M= males, F= females; Type: N = normal weight, OW = Overweight.

[†]Energy expenditure measurements made during active weight loss. [§]Energy expenditure measurements made after a period of weight stabilisation ranging from 1 week to 6 months. [‡]TEE measurements made using doubly labeled water instead of whole body indirect calorimetry.

An important consideration of the role of energy expenditure in the regulation of energy balance and body weight is whether the significant changes in TEE (and its components) observed during and/or immediately after the active period of weight loss, are maintained following a period of energy balance and subsequent weight maintenance. If so, such changes would predispose individuals to regain the lost weight and would be consistent with the hypothesis that energy expenditure is an effective component of the body's defense against weight loss and starvation.

Two different kinds of studies have been conducted to address this issue. The first kind of study has obtained measurements of energy expenditure during weight loss and after a period of weight stabilisation at the reduced weight (Bessard et al. 1983; de Boer et al. 1986; DeGroot et al. 1990; Froidevaux et al. 1993; Leibel et al. 1995; Rumpler et al. 1991; Weigle et al. 1988). A study of 8 women who maintained an 8.7 to 9.9 kg weight loss for more than 1 year showed that TEE adjusted for body weight and energy intake was not different from baseline when they were re-measured 1 month or 1 year after the initial weight loss period (DeGroot et al. 1990). In 8 obese men, Rumpler and colleagues (1991) demonstrated that TEE was 10% lower 28 days after the initiation of a moderate energy restrictive diet (6.2 MJ/day), but one week after the return to weight maintenance energy requirements TEE had returned to its baseline level. Conversely, other groups have reported persistent decreases in TEE following a period of weight maintenance (Bessard et al. 1983; de Boer et al. 1986; Froidevaux et al. 1993; Leibel et al. 1995; Weigle et al. 1988). Leibel et al. (1995), using doubly labeled water, measured TEE in 7 men and 11 women and observed that the stabilisation of body weight at a level 10% below the initial weight was associated with a 17% decrease in TEE normalized for lean mass. Using whole-body calorimetry, Froidevaux and colleagues (1993) also found that TEE remained 15% below the baseline value when TEE was measured at twelve weeks after the reduced body weight had been stabilised 19% (15.4 kg) below the initial weight.

The second kind of study that addresses the issue of whether changes in energy expenditure would predispose individuals to weight regain, are those that compare the energy expenditures of weight-stable, formerly obese subjects to never obese individuals who are matched for body size. These studies have, however, only examined REE and not TEE. These studies will be discussed further in Section 1.6.5.4.

1.6.5.4 Changes in resting energy expenditure in response to positive and negative energy balances

Several studies have reported changes in REE that are greater than predicted from the changes that occurred in body composition; consequently, these changes were considered as adaptive responses to protect the body against the full extent of overeating (Klein and Goran, 1993; Roberts et al. 1990) or undereating (Amatruda et al. 1993; Bessard et al. 1983; Leibel et al. 1995; Racette et al. 1995; Rumpler et al. 1991). The majority of these studies have included small heterogeneous subject populations, which may have caused a Type 2 error. Saltzman and Roberts (1995) performed a meta-analysis of 20 over- and underfeeding studies conducted during the period of 1985 to 1995. They found that the linear increase in REE with weight gain and the linear decrease with weight loss were greater than expected from the increase or decrease, respectively, in energy intake. This indicates that energy-conserving or energy-dissipating mechanisms may initially operate in proportion to alteration in energy stores, rather than in proportion to changes in energy balance. The mean increase in REE with weight gain was 116 kJ/kg of body weight and the mean decrease in REE with weight loss was 73 kJ/kg of body weight. In the same meta-analysis, Saltzman and Roberts also compared the relationship between changes in lean mass and REE during over- and underfeeding (with no exercise intervention) to cross-sectional measurements of the relationship between lean mass and REE in weight-stable individuals. The results indicated that in subjects being underfed, the decrease in REE was greater in relation to the loss of lean mass than predicted from the cross-sectional

relationship between REE and lean mass; the opposite is true for subjects being underfed. These findings indicate that the energy-conserving or energy dissipating mechanisms are greater than can be explained from obligatory changes in energy stores due to changes in lean mass. Accordingly, changes in REE can be regarded as a physiological mechanism defending the body against a change in fat stores.

As discussed in Section 1.6.5.3, studies that compare the energy expenditures of weight-stable, formerly obese subjects to never obese individuals who are matched for body size are designed to address the issue of whether changes in energy expenditure would predispose individuals to weight regain. All of the studies that have used this design have measured REE but not TEE. Astrup's group (1999) conducted an individual subject data meta-analysis of 124 formerly obese and 121 never-obese (control) subjects and found that REE (adjusted for differences in lean and fat masses and for differences between the studies) was 3% lower (mean difference of 178 kJ/day) in formerly obese subjects than in control subjects (i.e. 6073 vs 6252 kJ/day). A low relative REE was found in 3.3% of the control subjects and in 15.3% of the formerly obese subjects. They also conducted a traditional meta-analysis on 12 studies, including 3 that were not represented in the individual meta-analysis (total of 94 formerly obese and 99 control subjects). The traditional analysis found that relative REE was 5.1% lower in the formerly obese group than in the control group. These findings imply that the existence of a low REE (whether genetically acquired or diet-induced) may contribute to the high rate of weight regain in formerly obese individuals. In contrast, Wyatt et al. (1999) found that the majority of reduced-obese individuals (40 selected from the American National Weight Control Registry) did not have a REE that decreased beyond that accounted for by the reduced lean mass, when compared to a never-obese control group. Only subjects who had shown long-term success (≥ 1 year) in maintaining weight loss, however, were selected. By selecting only those subjects who successfully maintained their weight loss, the authors noted they

may have included only subjects with a normal REE before they had become obese. In some individuals the initial cause of obesity may have been a genetically determined low REE. In such individuals, it is likely that they would have regained weight before the follow-up measurement, and therefore they would have been excluded from the analysis.

1.6.5.5 Changes in physical activity in response to positive and negative energy balances

The role of physical activity on the regulation of energy balance and body weight is evident from the many studies that have reported lower body weights and body fat indices (e.g. % body fat, skinfolds and BMI) among individuals with high levels of physical activity (determined from self-reports, indirect measurements such as accelerometers, and estimation equations) (Rissanen et al. 1991; Saltzman and Roberts, 1995; Schutz, 1989; Wilmore, 1996). A recent compilation of 319 measurements of energy expenditure using doubly labeled water also showed that individuals who were massively obese engaged in lower levels of physical activity (Prentice et al. 1996). Furthermore, Rissanen et al. (1991) found that individuals who reported physical activity three or more times per week, lost weight between surveys (median 5.7 years), whereas those who reported undertaking little activity gained weight.

Many health professionals recommend implementing planned activity (exercise) into weight loss treatments for obesity. Physical activity may have a direct effect on TEE, and it may also elevate REE (Broeder et al. 1992a; Broeder et al. 1992b) and fat oxidation (Smith et al. 2000a). Ballor and Keesey (1995) recently performed a meta-analysis of 53 published studies. On average, the subjects examined participated in normal exercises (running, walking and cycling) for 125 to 155 min/week (as 3.5 to 4 sessions/ week), over a period of 12 weeks. The decrease in body fat resulting from this exercise was only 1 to 2%. This implies that physical activity has a modest influence on body fat content. Accordingly, if physical activity plays a causal role in the development of obesity, then the

amount of activity has to be substantially reduced. Other studies have, however, demonstrated that physical activity affects the rate of weight loss in a dose dependent manner that is dependent on the frequency and intensity of activity (Westerterp, 1998; Wilmore et al. 1999). While there is debate regarding the importance of physical activity for weight loss, Pavlou et al. (1989) showed that physical activity was an important factor in the maintenance of the reduced body weight of Boston policeman.

It is hypothesized that involuntary (or spontaneous) physical activity may be the best predictor of inter-individual differences in weight gain in response to overfeeding. Several studies have now examined the effect of overfeeding on physical activity (Levine et al. 1999; Ravussin et al. 1986). Levine et al. (1999) examined this issue in 16 non-obese, free-living adults (12 men/4 women) who underwent measurements of body composition and energy expenditure before and after 8 weeks of supervised overfeeding by 4180 kJ/day. Energy expenditure due to involuntary activity (i.e. non-exercise activity thermogenesis) was assessed by subtracting the sum of REE and TEF (measured by indirect calorimetry) from TEE (measured using doubly labeled water). In order to differentiate energy expended during involuntary activity from that expended during voluntary activities, subjects were instructed to stringently maintain voluntary activities at constant, low levels (confirmed using questionnaire). It was found that energy expended during involuntary activity significantly increased on average by 1.4 MJ/day (range -0.4 to 2.9 MJ/day), after 8 weeks of overfeeding. The increase accounted for two-thirds of the increase in TEE, and most importantly, the changes in energy expended during involuntary activity directly predicted resistance to fat gain (the predictive value was independent of the initial weight of the subject). No underfeeding study of this type has yet been conducted. Levine et al.'s (1999) findings warrant further research to determine the differences in energy expenditure due to involuntary activity, between individuals, and how it relates to the development and maintenance of obesity.

1.6.5.6 Changes in the thermic effect of feeding in response to positive and negative energy balances

Early reports that an increase in TEF was a mechanism that defended lean individuals against increases in body weight during overfeeding, have been criticized (Miller et al. 1967; Sims et al. 1973; Sims and Horton, 1968). The reason for this was because the investigators only inferred that an increased rate of heat production occurred as the weight gain was not proportional to the amount of excess energy ingested. More recent studies have found no association between TEF and changes in body weight during overfeeding (D'Alessio et al. 1988; Ravussin et al. 1985b; Tremblay et al. 1992) or underfeeding (Ravussin et al. 1985a). However, the same studies (all of which included only small numbers of subjects) have shown considerable variability between individuals in thermogenesis. Moreover, several studies have demonstrated that TEF is reduced in obese as compared to lean subjects (Nelson et al. 1992; Schutz et al. 1987; Segal et al. 1990; Segal et al. 1985). Based on their findings, the investigators once again suggest that a lower TEF (contributing to a lower TEE) may be a mechanism that prevents some individuals from successfully losing weight, or at least from maintaining the weight loss (i.e. maintains the obese state). Others, do not accept TEF as an important adaptive mechanism defending the body against over- or under-nutrition because it is limited to a maximum of 25% of energy consumed (the remaining 75% of excess energy is stored as fat) (Bessard et al. 1983; D'Alessio et al. 1988; Ravussin et al. 1985b). If a person ate 5000 kJ/day excess energy for a period of 1 week, then 'wasting' 25% (or 1250 kJ) of the excess energy would only prevent ~0.28 kg of fat from being gained. Given the large variation, within a person, in the measurement of TEF and the dominant effects of physical activity, the impact of TEF on either weight gain or weight loss is likely to be minimal. However, clearly more research is required to determine the importance of the relationship between TEF and the regulation of energy balance and hence body weight.

1.6.5.7 The influence of macronutrients on energy expenditure

Energy expenditure may be influenced by the macronutrient composition of the diet. It has been demonstrated that when single macronutrients are given, the TEF may reach 20 to 30% after protein, 5 to 10% after carbohydrate and 0 to 3% after fat ingestion (Astrup and Raben, 1995). These differences are related to the different costs of transporting, converting and storing of macronutrients (obligatory thermogenesis), and to an extra facultative thermogenesis after the intake of carbohydrate (Acheson et al. 1984). For glucose, the storage by glycogenesis has an energy cost of 7%, while glucose conversion to fat (lipogenesis) amounts to 25%; the latter process, however, occurs rarely. In studies where mixed-meals have been fed to both non-obese and obese subjects, it has been found that those rich in protein increase TEF more than those rich in carbohydrate and high-fat meals have the least effect on energy expenditure (Nair et al. 1983; Swaminathan et al. 1985; Westerterp et al. 1999). Whether the increases in TEF observed after protein enriched and carbohydrate enriched diets enhance TEE and/or REE remains unclear. This issue will be discussed in Section 1.7.2.2. The studies presented in Chapters 8 and 9 of this thesis compare two low-fat, isocaloric, energy-restricted diets either high in protein or high in carbohydrate, on TEE and its components.

1.7 DIETARY STRATEGIES FOR THE TREATMENT OF OBESITY AND ITS ASSOCIATED DISEASES

Weight loss is essential in the management of obesity and obesity related diseases such as Type 2 diabetes mellitus and cardiovascular disease. For successful long-term weight loss, it is widely recommended that individuals use a combination of caloric restriction and exercise (at least 150 minutes per week) (Serdula et al. 1999; Wing and Hill, 2001). Many popular weight loss plans emphasize diet more than physical activity, which may account for the high incidence of weight regain in many individuals. Moderate energy-restriction (~ 2000 to 4200 kJ less than average daily intake) and a subsequent reduction in weight by

as little as 5 to 10% of initial weight, may significantly improve fasting plasma glucose and serum insulin, fasting plasma lipids, and blood pressure, independent of an increased physical activity (Goodpaster et al. 1999; McLaughlin et al. 2001; Reaven, 2001; Williams and Kelley, 2001). The Da Qing Study of 577 patients with impaired glucose tolerance followed over 6 years, demonstrated that a modest diet-induced reduction in body weight can reduce the risk of developing Type 2 diabetes by 31% (Pan et al. 1997). Similarly, the Diabetes Prevention Program study found a 58% reduction in the risk of developing diabetes after modest weight loss (Knowler et al. 2000).

Approximately one in three individuals in Australia and America claim they are on some type of weight loss diet so that they look and feel better (Australian Bureau of Statistics and Commonwealth Department of Health and Family Services, 1997; Serdula et al. 1999). To lose weight, nutrition expert panels recommend moderately energy-restricted diets that are: i) low in total and saturated fat ($\leq 30\%$ of energy derived from total fat and $< 10\%$ from saturated fat), ii) have a limited alcohol and salt intake, iii) include plenty of vegetables and fruits, and iii) contain a wide variety of low-energy density cereals, meats (white and/or red), and dairy products (Assmann et al. 1999; Krauss et al. 2000; National Nutrition Committee CDA, 1999; NHMRC, 1991). Such diets promote a weight loss of 0.45 to 0.9 kg per week, and they try to teach individuals a new lifestyle of health eating.

A survey in America showed that more than one in five dieters used popular or 'fad' diets instead of the healthy eating plans recommended by nutrition experts (Jeffery et al. 1984). Presumably, the reason for using fad diets is because they claim to produce fast weight loss with seemingly minimum effort. In recent years, there has been resurgence of public interest in low-carbohydrate diets that emphasize a higher protein intake. The scientific evidence supporting the effectiveness and safety of such popular diets is limited. Several recent short-term clinical interventions have demonstrated that replacing some

carbohydrate with protein, in a low-fat diet, may be an effective dietary strategy for the treatment of obesity and its associated metabolic complications.

The following section will examine the advantages and potential health risks for a number of popular diets used for weight loss. Low-fat, high-carbohydrate diets will be reviewed in section 1.7.1. Section 1.7.2 will examine low-carbohydrate, high protein diets. Other types of popular fad diets will briefly be discussed in section 1.7.3.

1.7.1 Low-fat, high-carbohydrate diets

Plant-based, high-fibre, very-low fat foods have been the staple diet of our ancestors throughout the evolution of man. It was not until the Paleolithic period with the advent of stone tools and cooking methods, that the consumption of animal protein became a more prevalent in the human diet (Anderson et al. 2000). Accordingly, many nutritionists would argue that high-fibre plant foods, as well as exercise, shaped the human genome. The shift from the traditional dietary patterns of our ancestors (of which gathering food was a large part) plays a substantial role in the etiology of obesity and its associated diseases, in the majority of individuals.

Since the 1970s numerous epidemiological studies, laboratory studies, and clinical trials have indicated that higher fat intakes contribute to an increased body weight and risk of mortality from CVD (Astrup et al. 2000; Bray and Popkin, 1998; Hill et al. 2000). Bray and Popkin (1998) reported the prevalence of $\text{BMI} \geq 25 \text{ kg/m}^2$ for 20 countries from all regions around the world. In this study, regression analysis indicated a marked increase in overweight in countries with higher fat intakes. Data in China, Brazil, South Africa and Japan showed that when fat intake was low, the level of obesity was low; as the proportion of fat in the diet increased, however, the level of obesity increased (Monteiro et al. 1995; Popkin and Doak, 1998; Popkin et al. 1995a; Popkin et al. 1995b). The 1973 Ni-Hon-San

migration study reported that 2183 native Japanese, derived 15% of energy from the total fat intake whereas 8006 migrant Japanese living in either Honolulu or California, derived 33% and 38% of energy from fat (Kato et al. 1973). Two-fold greater fat intakes in the migrant groups were associated with a higher mean BMI and CVD. In addition to the impact of total fat intake on body weight and health outcomes, the 1970 Seven Countries Study conducted by Keys and colleagues showed that saturated fat was the strongest predictor of mortality from CVD (Keys, 1997). A review by Astrup and Raben (1995) also concluded that diets low in fat as well as rich in complex-carbohydrates potentially have a protective effect against body weight gain as compared to high-fat, carbohydrate diets. Furthermore, a long-term clinical trial of 28 men and women found that a vegetarian diet (consumed *ad libitum*) that restricted fat intake to 10% of daily energy and had 70-75% of energy from carbohydrate, in combination with moderate exercise, produced a 10 kg weight loss after 1 year (Ornish et al. 1990). In contrast, body weight remained unchanged in the 20 men and women in the control group who maintained their typical dietary habits and had a mean fat intake of 30% (Ornish et al. 1990).

Based on findings from studies like those reported above, expert nutrition committees recommend that complex-carbohydrates replace fat in the diet. Current guidelines advocate a diet that is: 1) low in total and saturated fat ($\leq 30\%$ of energy derived from total fat and $< 10\%$ from saturated fat), 2) high in complex carbohydrates that contribute 50 to 60% of total energy, 3) has an average protein content of 15% of total energy (or 50 to 100 g/day), and 4) contains < 300 mg of cholesterol per day (Assmann et al. 1999; Krauss et al. 2000; National Nutrition Committee CDA, 1999; NHMRC, 1991). For individuals with increased of Type 2 diabetes or CVD, it is advised that saturated fat intake be restricted to $< 7\%$ of total energy and cholesterol to < 200 mg per day (Krauss et al. 2000).

The philosophy and principles of several low-fat, high-carbohydrate diets will be reviewed in section 1.7.1.1. The effect of these diets on changes in body weight will be discussed in section 1.7.1.2, and sections 1.7.1.3 and 1.7.1.4 will review their effect on glycaemic control and lipid metabolism respectively. Finally, the effects of complex versus simple carbohydrates on obesity and CVD will be briefly discussed in section 1.7.1.5.

1.7.1.1 Philosophy and principles of several low-fat, high-carbohydrate diets

Popular low-fat, high-carbohydrate diets have been extensively reviewed by Anderson et al. (Anderson et al. 2000) and they include: i) the American Diabetes Association/American Dietetic Association (ADA) Exchange diet, ii) Dr Anderson's High-Fibre Fitness Plan, iii) Pritikin diet, and iii) Ornish diet. The macronutrient composition of these diets, are depicted in Table 1.10.

Table 1.10 Macronutrient composition of low-fat, high-carbohydrate weight loss diets of 6.7 MJ/day. Redrawn from Anderson et al. (Anderson et al. 2000)

	ADA	High Fiber	Pritikin	Ornish
Carbohydrate (%E)	60	63	73	74
Protein (%E)	20	16	18	18
Fat (%E)	20	21	9	7
SFA (%E)	6.1	4.2	2.9	2.0
MUFA (%E)	6.2	8.9	3.1	2.8
POLY (%E)	7.4	4.0	3.0	1.9
Alcohol (%E)	0	0	0	1
Cholesterol (mg/d)	112.1	57.6	57.1	29.5
Fiber (g/d)	22.3	28.5	40.7	49.1

1) ADA Exchange diet (ADA, 1995): This program is a “healthy eating” program that can be used for weight loss and long-term weight maintenance. It recommends an intake of 50 to 60% carbohydrate, 10 to 20% protein and fewer than 30% of calories from fat. Foods are categorized into exchanges of fruits, vegetables, meats, dairy, carbohydrates and fats. Individuals are encouraged to consume a specified number of items from each

exchange depending on their energy requirement. High fat animal products are prohibited and the use of oil is limited. Snacks should consist of fresh fruits, vegetable, nonfat yoghurt and whole-grain foods. Foods such as condiments, sugar-free lollies, jelly, and broth may be consumed freely as long as they contain < 84 kJ and < 5 g of carbohydrate per serving.

2) Dr Anderson's High-Fibre Fitness Plan (Anderson and Gustafson, 1994): The theory of this diet is that high-fat diets cause insulin resistance. The dietary principle is based on scientific evidence indicating that low-fat, high-carbohydrate, higher-fibre diets increase insulin sensitivity, lower insulin needs in diabetics and improve serum lipid. A wide variety of vegetables, fruits, whole-grain products and low-fat animal products are advocated. Anderson suggests many of the snacks and free-foods as listed in the ADA exchange diet, but energy and carbohydrate criteria are not specified.

3) Pritikin diet (Pritikin and McGrady, 1979): The philosophy of this diet is that "eating large amounts of fat damages the body's tissues by depriving them of oxygen, raises serum cholesterol and uric acid levels, and impedes carbohydrate metabolism causing insulin resistance". The Pritikin diet is low in fat, cholesterol, protein and highly refined carbohydrates, but high in complex carbohydrates and fibre. The diet recommends an intake of 5 to 10% of energy from fat, 10 to 15% from protein and approximately 80% as complex carbohydrates. The Pritikin diet allows unrestricted amounts of low-fat, low-energy soups, bouillon broth and most vegetables.

4) Ornish diet (Ornish, 1993): The Ornish diet is based on the fact that fat contributes to weight gain. The author postulated "the body converts dietary fat into body fat". It is advised that a vegetarian diet, high in complex carbohydrates and fibre is consumed, and <

10% of energy is derived from fat. Beans, legumes, fruit, vegetables and grains may be consumed in unlimited quantities.

1.7.1.2 Effect of low-fat, high-carbohydrate diets on weight loss

There is an increasing body of evidence that low-fat, high-carbohydrate diets promote weight loss; regardless of whether they are used as part of an energy-restricted dietary strategy or as an *ad libitum* dietary strategy. Bray and Popkin (1998) have extensively reviewed the findings of 28 clinical trials that ranged in duration from 2 weeks to 2 years. In these studies, low-fat diet groups were compared to control groups who maintained their usual diet, or consumed a medium- to high-fat diet (i.e. they examined studies where the fat-to-carbohydrate ratio varied and protein intake remained similar). Thirteen of the reviewed studies focused on non-obese individuals. In the non-obese populations, there was a mixture of successful weight loss and no weight loss. Of importance, however, was the finding that none of the 13 separate non-obese populations gained weight on the low-fat, high-carbohydrate diets (Bray and Popkin, 1998). The remaining 16 studies reviewed by Bray and Popkin (1998) examined overweight and obese populations. As expected, the reviewed data demonstrate that the average rate of weight loss on low-fat, high-carbohydrate, is generally greater for the overweight and obese than for the non-obese populations. Overall, Bray and Popkin's review of 28 clinical trials (that used a wide variety of low-fat, high-carbohydrate diets), found that a 10% reduction in energy from dietary fat was associated with a reduction in body weight of 16 g/day, or 2.9 kg if extrapolated over 6 months.

Meta-analysis is considered the strongest form of scientific evidence. A number of meta-analyses of *ad libitum* low-fat, high-carbohydrate diets in intervention trials consistently demonstrate a highly significant weight loss of 3 to 4 kg in normal-weight and overweight subjects (Astrup et al. 2002; Astrup et al. 2000a; Astrup et al. 2000b; Dattilo and Kris-

Etherton, 1992; Yu-Poth et al. 1999). For example, Astrup et al. (2000a) included 16 intervention studies (n= 1728 non-obese and overweight individuals; 1074 women and 654 men) conducted from 1966 to 1998 that examined weight loss occurring on *ad libitum* low-fat, high-carbohydrate diets. Studies were included in the meta-analysis if they fulfilled the following criteria: i) lasted between 2 to 12 months; ii) total energy intake was not voluntarily restricted; iii) included no other interventions that could affect weight loss [except physical activity which was recommended in 12 of the 16 studies]; iv) subjects with diabetes mellitus were prohibited; v) drugs that could affect weight loss were prohibited; vi) inclusion of a control group that were advised to maintain their regular diet or to consume a diet with a fat content that was typical of the Danish population (~35% of energy); vii) had made both pre- and post-treatment measurements of body weight. The results of this meta-analysis demonstrated that *ad libitum* low-fat interventions reduced fat intake from between 3.5 to 24.1% (mean reduction ~12%) of total energy intake, when compared to the control group. The reduction in energy from fat caused a mean weight loss of 2.9 kg (range -11.4 kg to +0.4 kg). In contrast, a mean weight gain of 0.14 kg (range 0 to +1.4 kg) was observed in the control groups. The low-fat intervention groups lost 2.4 kg more weight than the control groups (as assessed by fixed-effect meta-analysis). Regression analysis found that the reduction in percentage energy as fat was the strongest predictor of weight loss, but pre-treatment weight was also an independent predictor. The reduction in percentage energy as fat positively associated with weight loss in a dose-dependent manner. From the findings of this study, Astrup and colleagues (2000a) calculated that a 10% reduction in dietary fat might result in a 4 to 5 kg weight loss in an individual with a BMI of 30 kg/m².

In an earlier meta-analysis that used less stringent inclusion criteria, 37 studies conducted between 1991 and 1997 were analysed for the effect of low-fat diets on weight loss and subsequent CVD risk reduction (Yu-Poth et al. 1999). Studies were included if they met the

following criteria: i) the study was designed to lower cholesterol or reduce body weight for the primary purpose of preventing CVD; ii) used a randomized design; iii) a Step 1 diet (i.e. $\leq 30\%$ total fat, $\leq 10\%$ saturated fat, and ≤ 300 mg cholesterol per day), a Step 11 diet (i.e. $\leq 7\%$ saturated fat, and ≤ 200 mg cholesterol per day), or both diets were part of the intervention; iv) included free-living subjects who prepared their own food and who received dietary counseling by dietitians about low-fat diets; and v) the intervention lasted more than 3 weeks. The 37 studies analysed included 9276 subjects in intervention groups and 2310 subjects in control groups. The results of this meta-analysis showed that body weight was reduced, on average, by 2.79 kg in the low-fat, high-carbohydrate intervention groups that included no exercise, and by 5.7 kg when exercise was included as part of the dietary intervention (there was no difference in fat intake between the two groups, e.g. -11.6 vs -10.0% of energy). Regression analysis revealed that for every 1% decrease in energy as total dietary fat (overall mean reduction in fat of $\sim 10.8\%$ in both the low-fat interventions with and without exercise), there was a 0.28 kg decrease in body weight. The findings of Yu-Poth et al. (1999) are consistent with those reported by Astrup's group (2000a; 2000b). They indicate that a reduction in dietary fat without voluntary restriction of total energy intake causes weight loss in a dose-dependent manner and may produce modest, but clinically relevant, weight loss in overweight subjects.

It is important to note that the review by Bray and Popkin (1998) and the meta-analyses by Astrup (2000a) and Yu-Poth (1999) included several studies that reported weight gains after the low-fat, high-carbohydrate dietary interventions (de Lorgeril et al. 1994; Lyon et al. 1995; Weststrate et al. 1998). The study by Weststrate et al. (1998) was designed to compare the effect of a lower-fat and a full-fat diet on body weight and plasma lipids. After 6 months on a low-fat, high-carbohydrate diet, a weight gain of 0.4 kg was observed, in 58 women and 59 men with a mean BMI of 24.8 kg/m^2 . In the control group ($n = 51$ women and 52 men with a BMI of 25.0 kg/m^2) the weight gain was 1.1 kg. In this study,

failure to lose weight on the lower-fat diet was because energy intake was controlled (i.e. fixed energy intake of 10.1 MJ/day) and total fat intake was only reduced by 2% (from 35 to 33%). Also of importance were the findings of Lyon et al. (1995) that weight loss was positively correlated to dietary compliance. Using enrichment of dietary carbohydrate by ^{13}C -glucose and collection of an expired air sample to determination of $^{13}\text{CO}_2$, the investigators assessed the adherence of 8 moderately overweight women to *ad libitum* diets that varied in the fat-to-carbohydrate ratio. Lyon et al. found that when the fat content of the diet was reduced (from 44 to 31% of total energy) and the carbohydrate content increased (from 38 to 50% of energy), total energy was reduced, on average, by 1569 kJ/day and consequently fat mass fell by 1.7 kg. The patients who failed to lose weight on the low-fat, high-carbohydrate diet were those who did not adhere to the dietary principles. Such findings confirm the long held notion that people, particularly obese individuals, selectively underreport both total energy and fat intake (Black et al. 1993; Hebert et al. 1995; Heitmann et al. 2000; Pryer et al. 1997). They also indicate that the compliance of subjects to low-fat diets needs to be accurately measured when examining the relationship between dietary fat intake and body weight. Selective underreporting of fat intake could explain the paradoxical findings of long-term epidemiological data suggesting that fat intake has decreased despite an increasing prevalence of obesity (Heini and Weinsier, 1997).

As the above findings indicate, the reduction in total energy intake (relative to the energy content of the subjects usual diet) resulting from the decreased fat intake determines the amount of weight loss on *ad libitum* low-fat diets. However, acute dietary studies have shown that carbohydrate can potentially increase the energy deficit by independently affecting both the energy intake and energy expenditure sides of the energy balance equation. The mechanisms by which carbohydrates impact on energy intake and energy expenditure (in comparison to isocaloric amounts of fat) are still being debated (Sections

1.6.4.3 and 1.6.5.7). In brief, a decreased energy intake on a low-fat, carbohydrate-rich, high-fibre diet may be due to: i) a lower energy density (kJ/g) (Stubbs et al. 1995b); ii) an increased bulk or volume (due to water and fibre) (Duncan et al. 1983; Tremblay et al. 1991); iii) an increased satiety (Blundell et al. 1993; Stubbs et al. 1995a; Stubbs et al. 1995b); iii) an increased storage capacity (Flatt, 1993); iv) ability to stimulate own oxidation (Stubbs et al. 1995a); v) reduced energy availability due to high-fibre content (Miles, 1992). Furthermore, an increase in energy expenditure on high-carbohydrate as compared to high-fat diets could be the result of: i) increased TEF (Acheson et al. 1984; Flatt, 1993; Westerterp et al. 1999); ii) sympathetic nervous system activity (Astrup, 1996; Lean and James, 1988); and iii) physical activity (Stubbs et al. 1995b).

Recent evidence demonstrates that adherence to low-fat eating regimes that allow *ad libitum* consumption of carbohydrate-rich foods that are high in dietary fibre can be an effective dietary strategy for not only reducing body weight, but also for the long-term maintenance of weight loss and prevention of weight gain (Toubro and Astrup, 1997; Wing and Hill, 2001). Toubro and Astrup (1997) reported that the group of 17 men and women on the *ad libitum* low-fat, high-carbohydrate eating plan maintained 13.2 kg of the initial 13.5 kg weight loss (i.e. mean weight regain of 0.3 kg; range -3.0 to 3.6 kg) over a one year period. In contrast, the group of 16 men and women (who were similar with respect to weight loss and anthropometric characteristics) on the fixed energy intake low-fat, high-carbohydrate eating program maintained only 9.7 kg of the initial 13.8 kg weight loss (i.e. mean weight regain of 4.1 kg; range -0.4 to 8.0 kg). Interestingly, the reported percentage of energy from fat was lower in the *ad libitum* group than in the fixed-energy group. Moreover, 13 of the subjects from the *ad libitum* group and 15 from the fixed-energy group were assessed again, one year after the end of the supervised weight maintenance phase. Once more it was found that the maintained weight loss was greater in the *ad lib* group than in the fixed-energy group (i.e. *ad lib* group still a mean 8.0 kg lower

than their initial weight measured 2 years prior whereas the fixed-energy group were only 2.5 kg below initial weight). Furthermore, the American National Weight Control Registry set-up by Wing and Hill (2001) found that successful long-term weight loss maintainers (defined as those who had lost > 10% of their initial body weight and who had maintained it for more than 1 year) shared common behavioral strategies that included: i) eating a diet low in fat and high in carbohydrate (< 24% of energy as fat and ~ 56% as carbohydrate); ii) frequent self monitoring of weight and food intake; and iii) a high levels of physical activity (~1h/day). The registry of more than 3000 subjects calculated that about 20% of individuals successfully maintain their weight loss (average reported weight loss is 30 kg which is maintained for an average of 5.5 years).

1.7.1.3 Effect of low-fat, high-carbohydrate diets on glycaemic control

Available data from animal (Storlien et al. 1996; Storlien et al. 2000) and human (Marshall et al. 1997; Marshall et al. 1994; O'Dea et al. 1989) studies indicate that high-fat diets, particularly those with high saturated fat intakes, are associated with insulin resistance and adversely affect glycaemic control. In contrast, the consumption of high-carbohydrate diets may improve glycaemic control but this issue is still being debated somewhat. The work of Himsworth (1935; 1939), Brunzell (1971), Anderson (1977), Kiehm (1976), Simpson (1979) and their respective colleagues, provided early evidence of the beneficial effects associated with the high-carbohydrate diet. These studies examined both non-diabetic (Himsworth, 1935; Himsworth and Kerr, 1939) and people with Type 2 diabetes (Anderson, 1977; Brunzell et al. 1971; Kiehm et al. 1976; Simpson et al. 1979). Compared to moderate-carbohydrate diets (~45% of total energy as carbohydrate, 40% as fat), the high-carbohydrate diets (75-85% carbohydrate) used in these studies reduced fasting (Anderson, 1977; Brunzell et al. 1971; Himsworth, 1935; Himsworth and Kerr, 1939; Kiehm et al. 1976) and postprandial (Kiehm et al. 1976) plasma glucose concentrations, and improved insulin sensitivity (Anderson, 1977; Himsworth and Kerr,

1939; Kiehm et al. 1976). Since the subjects remained weight stable, weight loss did not impact on the results. In fact, no variables, other than the carbohydrate content of the diet, were found to be associated with the improvements in plasma glucose and insulin concentrations.

In contrast to the findings above, numerous short-term studies have reported that high-carbohydrate diets can worsen glucose homeostasis. Early work by Reaven, Brunzell, Ginsberg and their colleagues examined the effect of high-carbohydrate diets in non-diabetic (Ginsberg et al. 1976; Reaven and Olefsky, 1974) and diabetic (Brunzell et al. 1974) persons. When compared to moderate-carbohydrate (~40% energy), moderate-fat (~45%) diets, it was found that formula diets containing 55% carbohydrate-30% fat (Ginsberg et al. 1976; Reaven and Olefsky, 1974), or 85% carbohydrate-0% fat (Brunzell et al. 1974), elevated plasma glucose levels. In a more recent study of 42 patients with Type 2 diabetes, daylong plasma glucose and insulin values were elevated by 12% and 9%, respectively, after 1 week of a high-carbohydrate solid food diet (55% carbohydrate, 30% fat) as compared to a high-fat diet (40% carbohydrate, 45% fat) (Garg et al. 1994). Moreover, for 21 subjects who continued the original 6-week study for an additional 8 weeks, it was reported that the elevated levels persisted for 14 weeks.

Discrepant results regarding the effect of low-fat, high-carbohydrate diets as compared to higher fat diets clearly appear to be related to the fibre content of the diets. Many studies that have advocated the use of high-carbohydrate diets have concomitantly contained a large amount of dietary fibre (O'Dea et al. 1989; Riccardi and Parillo, 1993; Riccardi et al. 1984; Simpson et al. 1982; Simpson et al. 1979). O'Dea et al. (1989) and Riccardi et al. (1984) both compared two high-carbohydrate diets which differed in their fibre content, in subjects with Type 2 diabetes. O'Dea's two diets each contained approximately 60% carbohydrate and 10% fat and either 20 or 45 g of fibre per day, whereas Riccardi's

contained 53% carbohydrate and 30% fat and either 16 or 54 g of fibre per day. Riccardi found that only the diet containing 54 g of fibre significantly decreased postprandial plasma glucose and insulin concentrations. Similarly, O'Dea found that the most significant improvements in plasma glucose, insulin, total- and LDL-cholesterol occurred on the high-carbohydrate, low-fat, 45g fibre diet. Moreover, recent prospective (Meyer et al. 2000) and cross-sectional (Boeing et al. 2000; Marshall et al. 1997) studies have shown that dietary fibre intake is associated with reduced risk of Type 2 diabetes.

1.7.1.4 Effect of low-fat, high-carbohydrate diets on lipid metabolism

The major reason for recommending the replacement of total fat with carbohydrate was to curtail excess saturated-fat so that LDL-cholesterol, which is considered the most atherogenic lipoprotein particle, was reduced. There is substantial evidence that reductions in saturated fat, dietary cholesterol and weight loss offer the most effective dietary strategies for reducing LDL- and total-cholesterol, and cardiovascular risk (Anderson et al. 2000; Bray and Popkin, 1998). However, of concern to many health professionals is the finding that the reduction in LDL-cholesterol is often, but not always, accompanied by a reduction in HDL-cholesterol (Katan, 1998).

Several meta-analyses have been performed to determine how much impact such dietary strategies have on reducing cardiovascular risk (Dattilo and Kris-Etherton, 1992; Yu-Poth et al. 1999). Dattilo and Kris-Etherton (1992) analysed 70 studies that had reported inconsistent results for the effects of weight reduction by dieting on plasma triacylglycerol and total-, LDL-, VLDL- and HDL-cholesterol concentrations. The meta-analysis indicated that an average reduction in body weight of 16% (16.6 kg), was associated a significant decrease in triacylglycerol, and total-, LDL- and VLDL-cholesterol levels. Of importance, was the finding that for every 1 kg decrease in weight, a 0.009 mmol/L increase in HDL-cholesterol was reported when measurements were made after body

weight had been stabilised at the reduced level. In contrast, when HDL-cholesterol measurements were made during active weight loss, a 0.007 mmol/L decrease per kg of weight loss was observed. This may, in part, explain the discrepant observations for the effect of low-fat, high-carbohydrate weight loss diets on HDL-cholesterol. In the more recent meta-analysis conducted by Yu-Poth et al. (1999) (details discussed above in section 1.7.1.2), it was found that a reduction in saturated fat and cholesterol significantly improved the lipoprotein profile of individuals. It was also reported that the Step 1 diet (i.e. $\leq 30\%$ total fat, $\leq 10\%$ saturated fat, and ≤ 300 mg cholesterol per day) did not change HDL-cholesterol whereas the Step 2 diet (i.e. $\leq 7\%$ saturated fat, and ≤ 200 mg cholesterol per day) reduced it by 7%; the decrease in HDL-cholesterol, however, was found to be prevented when exercise was included in the meta-analysis as an independent factor. From the regression analysis, it was calculated that for every 1% decrease in energy consumed as dietary saturated fatty acid, total-cholesterol decreased by 0.056 mmol/L and LDL-cholesterol by 0.05 mmol/L. Moreover, independent of dietary modifications, every 1-kg decrease in body weight decreased triacylglycerol by 0.011 mmol/L and increased HDL-cholesterol by 0.011 mmol/L.

Whilst the above meta-analyses showed that low-fat, high-carbohydrate diets and weight loss have independent and additive effects on total- and LDL-cholesterol, only weight loss was found to reduce plasma triacylglycerol concentrations. In the absence of weight loss, low-fat, high-refined-carbohydrate diets increase triacylglycerol levels (Anderson, 2000; Anderson et al. 1980; Anderson and Ward, 1979; O'Dea et al. 1989; Riccardi et al. 1984). High-carbohydrate diets rich in fibre either have no effect or decrease fasting plasma triacylglycerol concentrations (Anderson, 2000; Anderson et al. 1980; Anderson and Ward, 1979; O'Dea et al. 1989; Riccardi et al. 1984). For example, the studies by O'Dea et al. (1989) and Riccardi et al. (1984) found that the lipid lowering effects of high-carbohydrate diets, particularly during weight maintenance, are also dependent on the

amount of fibre present in the diet (same studies showed improvements in glycaemic control; see section 1.7.1.3). This would partly explain why the only diets that have been associated with documented regression of atherosclerosis are very low-fat, high-carbohydrate diets such as the Ornish diet (Ornish et al. 1990).

1.7.1.5 Effect of complex versus simple carbohydrates

Many nutritionists speculate that an increase in carbohydrate consumed as refined carbohydrate (or simple sugars) over the last several decades, may play a role in the increasing prevalence of obesity and CVD (Bessesen, 2001; Bolton-Smith and Woodward, 1994b; Hudgins, 2000; Jimenez-Cruz et al. 2002; MacDiarmid et al. 1998; Saris et al. 2000). Bolton-Smith and Woodard (1994a; 1994b) analysed data from 11,626 men and women aged 25-64 who participated in the baseline Scottish Heart Health and MONICA studies. When the data was divided into quintiles according to the fat-to-sugar ratio, they found that there was a two- to three-fold higher prevalence of obesity in the highest versus the lowest quintile. With respect to CHD, neither extrinsic sugar, intrinsic sugar, nor the fat to sugar ratio were found to be significant independent predictors in this Scottish population, when the other major risk factors such as cigarette smoking, blood cholesterol concentration, and antioxidant vitamins intake were accounted for (Bolton-Smith and Woodward, 1994a). Long-term data that could be viewed as supporting the debate that an increase in simple carbohydrates contributes to the prevalence of obesity comes from the randomized controlled multi-centre CARMEN (Carbohydrate Ratio Management in European National diets) trial (Saris et al. 2000). The CARMEN trial investigated the effect of changes in dietary carbohydrate-to-fat ratio and simple versus complex carbohydrates, in 398 moderately obese men and women over a 6-month period. Subjects were randomly allocated either to a seasonal control group (no intervention), or to one of three experimental groups for 6 months: a control diet group (~50% carbohydrate, 35% fat); a low-fat high simple carbohydrate group; or a low-fat high complex carbohydrate

group. Compared to the two control groups who gained 0.6 kg of fat mass, the group that consumed the low-fat, high-complex carbohydrate experienced the greatest reduction in fat mass (-1.8 kg for fat mass). The low-fat, high-simple carbohydrate group lost 1.3 kg of fat. Although the difference in total weight and fat loss between the complex and simple carbohydrate groups was not significant, it is possible that the low-fat, simple-carbohydrate diet is less effective at inducing weight loss. Astrup et al. (2000) suggests that since there was no difference in energy-density between the two low-fat diets, it would be easier for individuals (particularly those with a genetically determined preference for sweet tasting food) to passively over-consume low-fat foods such as biscuits, cakes and breads that have been made more palatable by adding simple sugars. Consequently, although replacing fat with any carbohydrate source is beneficial, small differences in the impact of complex versus simple carbohydrates may play a role in maintaining obesity in certain individuals. Accordingly, until more information is known about the long-term effects of simple carbohydrates, advice should be to replace high fat foods with those rich in complex carbohydrates.

1.7.2 Low-carbohydrate, high protein diets

Low-carbohydrate, high protein diets have a long history (Denke, 2001). Greek Olympians ate high meat, low vegetable diets more than two thousand years ago to improve athletic performance. In the late eighteenth century Dr William Harvey recommended a diet prohibiting sweets and starchy foods and permitting *ad libitum* consumption of meats for patients who need suffered from water retention. As the understanding of essential vitamins developed, these diets fell out of favor. Resurgence of their popularity in modern society presumably has been influenced by the marketing of books that promote low-carbohydrate, high-protein diets which promise rapid weight loss within 7 days (Denke, 2001). The 'perceived' failure of low-fat, high-carbohydrate diets to reduced body weight and maintain the weight loss also encouraged many people to try fad diets (Roberts, 2001;

Stein, 2000). It appears that people thought they could eat as much low-fat food as possible and still lose weight because they did not understand that a reduction in total energy intake was important. Moreover, the controversy regarding the effect of low-fat, high-carbohydrate diets that are low in dietary fibre, on glycaemic control and lipid metabolism, have led to a renewed interest in low-carbohydrate, high-protein diets from within scientific community. The philosophy and principles of the various low-carbohydrate, high-protein diets will be reviewed in section 1.7.2.1. The role of these diets in weight loss will be discussed in section 1.7.2.2. Evidence that high-protein diets, emphasizing restriction of total and saturated fat, can have favourable effects on insulin sensitivity and lipid metabolism will be presented in section 1.7.2.3. Section 1.7.2.4 will discuss the long-term safety of high-protein weight loss.

1.7.2.1 Philosophy and principles of low-carbohydrate, high-protein diets

There are two types of low-carbohydrate, high-protein diets: i) very low-carbohydrate, high-protein concomitantly high in saturated fat (e.g. the 'Atkins' and 'Protein Power' diets; also termed ketogenic diets), and ii) low-carbohydrate, high-protein diets that emphasize fat restriction (e.g. the 'Zone' and 'Sugar Busters'). Table 1.11 summarizes the philosophy and principles of popular low-carbohydrate, high-protein diets.

Very low-carbohydrate, high protein diets concomitantly high in saturated fat

1) Atkins diet (Atkins, 1992): The 'Atkins diet' written and published by Dr Robert Atkins in 1972 was similar to the 'Banting diet' introduced by Dr William Harvey in approximately 1863. The theory of this diet is that "obesity exists for metabolic reasons and hyperinsulinemia and insulin resistance are the root causes". Atkins suggests that restricting carbohydrate can avoid the onset of this defect and that individuals can lose more weight eating a high-fat diet than with equivalent energy from a high-carbohydrate diet. He advocated < 20 grams of carbohydrate per day so that metabolic ketosis is

maintained. Emphasis is placed on high-fat foods and avoidance of vegetables, fruits, breads, cereals, starchy vegetable and most dairy products (Table 1.11). Atkins endorses snacking on meat, eggs, cheese or guacamole, in unlimited quantities. Two small green salads are allowed daily.

Table 1.11 Popular low-carbohydrate, high-protein diets [table modified from St. Jeor et al. (2001)].

	Atkins	Protein Power	Zone	Sugar Busters	Stillman
Philosophy	Too many carbohydrate causes obesity; ketosis leads to decreased hunger	Carbohydrates releases insulin in large quantities which contributes to obesity	Right food combinations leads to state at which body functions at peak performance; decreases hunger; increases weight loss and energy	Sugar is toxic and causes increased insulin levels which promotes fat storage	High-protein foods burn body fat; carbohydrates cause fat storage
Principle	Eat meat, fish, poultry, eggs, cheese, low-carb vegetables, butter, oil; no alcohol	Eat meat, fish, poultry, eggs, cheese, low-carb vegetables, butter, oil, salad dressing, moderate alcohol intake	Protein, fat and carbohydrates must be eaten in exact proportions (40/30/30); low glycaemic index foods; moderate alcohol intake	Eat protein and fat; low glycaemic index foods; olive oil, canola oil; moderate alcohol intake	Eat lean meats, skinless poultry, lean fish, eggs, cottage cheese, skim-milk cheeses; no alcohol
Composition	Protein 27%; carbohydrate 5%; fat 68% (saturated fat 26%)	Protein 26%; carbohydrate 16%; fat 54% (saturated fat 18%); alcohol 4%	Protein 34%; carbohydrate 36%; fat 29% (saturated fat 9%); alcohol 1%	Protein 27%; carbohydrate 52%; fat 21% (saturated fat 4%)	Protein 64%; carbohydrate 3%; fat 33% (saturated fat 13%)
Lose & maintain weight?	Yes but initial loss is mostly water. Promotes negative attitude to food groups. Difficult to maintain	Yes, via caloric restriction. Limited food choices; not practical for long term	Yes, via caloric restriction. Could achieve maintenance if followed carefully. Diet rigid	Yes, via caloric restriction. Limited food choices; not practical for long term	Yes, but mainly water lost; strict calorie counting; limited food choices; not practical for long term
Scientific evidence	No long-term validated studies	No long-term validated studies	No long-term validated studies	No long-term validated studies	No long-term validated studies
Compliance with AHA protein criteria	No; does not achieve recommended allowance for macronutrients or micronutrients	No; does not achieve recommended allowance for macronutrients or micronutrients	Yes, but may be low in copper	No; does not achieve recommended allowance for micronutrients	No; does not achieve recommended allowance for macronutrients or micronutrients

2) *Protein Power* (Eades and Eades, 1996): ‘Protein Power’ written by Drs Michael and Mary Eades closely resembles the Atkins diet in nutrient composition (Table 1.11). The

theory for this diet is that high levels of insulin in the blood cause metabolic disturbances in the body that lead to high blood pressure, high cholesterol and triacylglycerol levels, diabetes and obesity. The authors suggest that restricting carbohydrate intake to < 30 g/day will alleviate medical problems. The diet guidelines focus on the amount of protein rather than fat consumed, but they permit high-fat food choices. Snacks such as nuts and seeds, pork rinds, lean meat slices, boiled eggs, jerky, and cottage cheese are encouraged. Diet drinks, oils, avocado and unsalted butter are allowed to be eaten freely. Limited amounts of fruit and vegetables are advocated (Table 1.11).

Low-carbohydrate, high-protein diets that emphasize fat restriction

1) *The Zone* (Sears and Lawren, 1995): ‘The Zone’ written by Dr Barry Sears promotes a high protein, modestly restricted carbohydrate diet. Sears suggests “if you follow extremes of the fashionable low-fat, low-protein, high-carbohydrate diets, you may be putting yourself at risk of disease”. He states that it is “your body’s response to excess carbohydrate in the diet that makes you fat”; “eating fat does not make you fat”. The theory behind the Zone diet is that eating too many carbohydrates promotes the production of insulin, which destroys hormones known as eicosanoids. Seares postulates that production of specific eicosanoids resulting from the consumption of the appropriate balance of macronutrients (30% protein, 40% carbohydrate, 30% fat) help the body work at peak metabolic efficiency. The diet emphasizes the eating lean meats and can include three servings of poultry per day but avoids high-animal fat products, most grain products, starchy vegetables and some fruits (Table 1.11). Suggested snacks include cottage cheese with fruit and plain low-fat yoghurt or milk. Decaffeinated diet soft drinks can be consumed freely and the addition of fat to every meal is recommended.

2) *Sugar Busters* (Steward et al. 1995): The Sugar Busters diet states that “sugar is toxic!” The authors suggest that insulin insensitivity causes obesity and Type 2 diabetes. The diet

focuses on reducing high-glycaemic carbohydrates (insulin stimulating carbohydrates that are utilised quickly by the body) to lower insulin levels and decrease insulin-resistance. They state, “calories per se are not as important as the types of foods we eat, how we eat them, and what metabolic processes control their assimilation”. The diet emphasizes low-glycaemic carbohydrates (high-fibre vegetables, fruits and whole grains), lean meats and low-fat foods in moderation. Suggested snacks include most fruits and nuts. Only decaffeinated diet soft drinks are allowed in unlimited quantities. White bread, white rice, potatoes, corn, beetroot, carrots and many other root vegetables are not allowed. The authors advocate moderate alcohol consumption, primarily in the form of red wine, because they state that “compared to many other carbohydrates, a glass of wine is less fattening than a slice of white bread.” This statement is clearly misleading as gram-for-gram alcohol provides 29.3 kJ/g whereas carbohydrate provides 16.7 kJ/g. Consequently, it depends on how much of either food is eaten.

1.7.2.2 Mechanisms through which low-carbohydrate, high protein diets reduce body weight

Low-carbohydrate, high-protein diets typically elicit a rapid weight loss of approximately 2 to 3 kilograms within the first week of dieting (Denke, 2001; Yang and Van Itallie, 1976). This appears to be a major reason for the popularity of such diets. However, weight loss during the first week of severe carbohydrate restriction results because a significant amount of body water is lost (Denke, 2001; Yang and Van Itallie, 1976). Carbohydrate restriction forces the body to utilise its' glycogen stores to supply glucose. For each gram of glycogen mobilized, 2 to 3 grams of intracellular water are lost (Stryer, 1995). Anderson et al. (1977) suggested that diuresis resulting from decreased postprandial insulin levels may also facilitate initial weight loss; insulin is known to promote water retention. Water equilibrium is re-established in the second and subsequent weeks (Denke, 2001). Thereafter, further weight loss simply reflects a sustained energy deficit (i.e. weight

loss results from a negative energy balance). Severe restriction of carbohydrate, such as observed in the high-fat Atkins type diets, may induce metabolic ketosis (Denke, 2001; Yang and Van Itallie, 1976); but this does not always occur (Wing et al. 1995). Ketones bodies produced from the catabolism of dietary and endogenous fat may be used as a fuel source instead of glucose. Ketosis may enhance the energy deficit by suppressing appetite and causing nausea. It can also cause hyperuricaemia as ketones compete with uric acid (produced during the breakdown of purines from dietary protein) for renal tubular excretion (Denke, 2001; St.Jeor et al. 2001).

Only two studies using an Atkins type diet have been published. La Rosa and colleagues (1980) placed 24 obese men and women on the Atkins diet with an energy deficit of 2090 kJ/day, for 8 weeks. The mean weight loss was 7.7 kg at 8 weeks, which is no greater than that expected from caloric restriction alone. In a second study of six obese subjects, Yang and Van Itallie (1976) found that a 3.3 MJ/day low-carbohydrate, high-protein, high-fat diet resulted in 39% greater (i.e. 4.6 vs 2.8 kg) weight loss than a mixed diet (55% of energy as carbohydrate, 30% fat, 15% protein), over a 10-day period. However, using the energy-nitrogen balance method they also demonstrated that the composition of weight lost during the low-carbohydrate, high-fat diet was 61.2% water, 35.0% fat, 3.8% protein, whereas with the mixed diet the composition of weight loss was 37.1% water, 59.5% fat, and 3.4% protein.

Recent studies that have compared the effects of low-carbohydrate, high-protein diets to high-carbohydrate, standard-protein diets have also restricted dietary fat to $\leq 30\%$ of total daily energy. These studies have yielded mixed results; some studies have found a greater weight loss by 1 to 4 kg on a low-fat, low-carbohydrate, high-protein diet (Alford et al. 1990; Baba et al. 1999; Skov et al. 1999b) whereas others have observed no statistically advantage (Piatti et al. 1994; Vazquez et al. 1995; Whitehead et al. 1996). Baba et al.

(1999) showed that after 4 weeks, weight loss was 2.3 kg (38%) greater in 7 hyperinsulinemic men consuming a high-protein diet (45% of energy as protein, 30% fat) as compared to a group of 6 hyperinsulinemic men consuming an isocaloric (~7300 kJ/day) standard-protein diet (12% protein, 30% fat). The reduction in fat mass, however, was not different between the two diet groups. Of particular interest was the study by Skov et al. (1999b) in 65 healthy overweight men and women compared. They performed a randomized dietary intervention of 2 *ad libitum* low-fat diets (< 30% of energy from total fat, < 10% from saturated fat) varying in their protein and carbohydrate content (12 and 58 % of energy as protein and carbohydrate, respectively versus 25 and 45 % of energy as protein and carbohydrate); the higher protein diet was a similar composition as the Zone diet. Weight loss was greater with the high-protein than the standard-protein diet (8.9 versus 5.1 kg), over 6 months (Skov et al. 1999b). Moreover, 35% of subjects in the high-protein group lost more than 10 kg of weight as compared to only 9% of subjects in the standard-protein group. The larger weight loss in the high-protein group was mainly due to a greater reduction in fat mass (7.5 versus 5.0 kg after 3 months and 8.7 versus 5.0 kg after 6 months) (assessed using DEXA). After 6 months, Skov et al. (1999b) noted that the energy intake of the subjects consuming the low-fat, high-protein foods was 1.9 MJ/day less than those subjects consuming the low-fat, high-carbohydrate foods. The authors speculated that an increased satiating effect of high-protein foods, that has been observed in acute feeding studies (Latner and Schwartz, 1999; Marmonier et al. 2000; Rolls et al. 1988b), reduced the energy intake and subsequently caused the larger weight loss observed on the higher-protein diet. They also suggested that an increased thermogenesis caused by the higher protein foods (Karst et al. 1984; Westerterp et al. 1999; Westerterp et al. 1999), may have enhanced the negative energy balance on a low-carbohydrate, high-protein diet as compared to a high-carbohydrate, standard-protein diet. These findings suggest that satiety and the thermic effect of feeding are additional/or alternative

mechanisms (to metabolic ketosis) that may contribute to greater weight loss on low-carbohydrate, high-protein diets.

In addition, Dauncey and Bingham (1983) have shown that iso-energetic substitution of a high-carbohydrate (49% energy as carbohydrate, 3% as protein) by a high-protein (15% energy as carbohydrate, 37% as protein) diet resulted in a 12% increase in TEE (measured over 28 hours), in 2 men and 4 women who were healthy and weight stable. Mikkelsen et al. (2000) found that TEE was 3% greater when 17 to 18% of carbohydrate was substituted with either pork protein or soy protein for 4 days; the diet containing pork protein produced a 2% higher TEE than did the vegetable protein diet. Baba et al. (1999) also observed that the decrease in REE was 12% (252 kJ/day) less in the low-carbohydrate, high-protein group than in the standard-protein group, after 4 weeks of mild energy-restriction and subsequent weight loss. Furthermore, Whitehead et al. (1996) found that both REE and TEE were reduced significantly less after 7 days on a high-protein diet (36% of energy as protein, 32% as carbohydrate) as compared to two isocaloric standard-protein diets (both 15% protein and either 53 or 32 % carbohydrate), in 8 overweight subjects (i.e. the reduction in TEE was 285 vs 634 kJ/d and in REE the reduction was 207 vs 425 kJ/d, respectively). The reduced fall in REE may be related to a preservation of lean mass that has been observed with low-fat, high-protein diets (Hoffer et al. 1984; Piatti et al. 1994; Vazquez et al. 1995); however, that was not assessed in the aforementioned studies. Further research is required to determine whether the reduced decrease in energy expenditure is a consequence of a greater thermic effect of feeding, and/or a sparing of metabolically active lean mass, on the higher-protein diets.

It is important to emphasize that while high-protein intakes can elicit several mechanisms that induce a negative energy balance, ultimately it is the magnitude of energy deficit that is responsible for weight loss.

1.7.2.3 Evidence that high-protein diets emphasizing the restriction of total and saturated fat can favorably affect insulin sensitivity and lipid metabolism

As discussed above, high-protein diets with $\leq 30\%$ of energy from total fat and $\leq 10\%$ from saturated fat, may potentially reduce body weight more than low-fat, high-carbohydrate diets that contain only a standard amount of protein. There is some evidence that these types of high-protein diets (i.e. similar to the Zone diet) may also improve insulin sensitivity and plasma lipids.

Favourable effects on insulin sensitivity

Piatti et al. (1994) observed that a higher than recommended protein intake can ameliorate insulin resistance. This study compared two energy-restricted (3.3 MJ/day), low-fat (20% of energy) diets containing either 45 or 20% of energy as protein, in 25 obese hyperinsulinemic women. The change in plasma insulin concentrations was examined after 21 days on the prescribed diets. A significant improvement in insulin sensitivity after weight loss was observed on the high-protein diet (after a euglycaemic, hyperinsulinemic clamp, glucose oxidation significantly increased by 0.55 mg/kg FFM/min and the rate of disappearance of glucose significantly increased by 2.10% over the basal rate). No improvement was found with the lower protein diets (glucose oxidation was reduced by 0.55 mg/kg FFM/min and the rate of disappearance of glucose was reduced by 11.5% over the basal rate). Piatti et al. (1994) proposed that the improvement in insulin mediated glucose uptake in skeletal muscle resulted from the observed preservation of lean body mass after weight loss. Lean mass was on average reduced by 3 kg on the lower protein diet as compared to only 1.4 kg on the higher protein diet. Other studies have also observed that an increased dietary protein content can prevent the decrease in lean mass that is associated with weight loss (Hoffer et al. 1984; Piatti et al. 1994; Vazquez et al. 1995). A second study by Baba et al. (1999) also reported that mean fasting insulin

concentrations were reduced to within the normal range, following the high-protein (reduced from 38 to 20.5 $\mu\text{U/L}$) as compared to the standard-protein diet (41.5 to 27.4 $\mu\text{U/L}$).

Favourable effects on lipid metabolism

Two weight maintenance (Wolfe and Giovannetti, 1991; Wolfe and Piche, 1999) studies have shown that replacing some carbohydrate with protein improves the fasting lipid profile. Wolfe and colleagues (1991; 1999) demonstrated that two low-fat diets (~24% of energy as fat) with either a high-protein (22% energy) or lower protein content (~12% protein), reduced plasma triacylglycerol by ~27%, and total and LDL cholesterol by ~7%, and increased plasma HDL cholesterol by ~12%, in mildly hyperlipidemic and normolipidemic subjects. Skov et al. (1999b) also demonstrated that *ad libitum* consumption of a high-protein (25% of energy) diet from a clinic shop as compared to a normal protein (12%) diet, lead to significantly greater reductions in plasma triacylglycerol and free-fatty acid concentrations over a 6 month period; the high-protein diet reduced triacylglycerol by 0.37 mmol/l and free fatty acids by 30%).

1.7.2.4 Long-term safety of low-carbohydrate, high-protein diets

St Jeor et al. (2001) state that none of the high-protein diets meet the dietary guidelines of the American Heart Association (an average 50 to 100 g/day of protein, a minimum of 100 g/day of carbohydrate, and $\leq 30\%$ of daily energy derived from total fat and $< 10\%$ from saturated fat, and < 300 mg/day of cholesterol) (Table 1.11). Anderson et al. (2000) who devised menu plans for each of the popular high-protein diets based on a moderate energy-restriction (6.6 MJ/day), also showed that none met the recommendations of the American National Cholesterol Education Program. Both authors express concern that the protein content of popular high-protein diets, are almost double the recommended daily allowances. Association studies have linked high animal protein intakes to higher risks for

CHD (McGee et al. 1984), cancer (Zaridze et al. 1991), osteoporosis (Abelow et al. 1992), and renal disease (Brenner et al. 1982). However, evidence for all of these associations remains controversial (Henry, 1994; Hu et al. 1997; Munger et al. 1999).

Despite lack of evidence directly linking increased animal protein to the various diseases mentioned above, several short-term studies have shown that low-carbohydrate, high-protein diets may have untoward effects on metabolism. The unfavourable metabolic effects may arise from a number of factors including: i) ketosis, ii) high saturated fat intake, iii) high total fat intake, iv) high-protein intake, v) exclusion of fruits, vegetables, and grains (Denke, 2001).

Complications from ketosis

Potential complications of ketosis included dehydration, constipation, fatigue, nausea, sleep disturbances, kidney stones, hyperlipidaemia, impaired neutrophil function, optic neuropathy, osteoporosis, hypotension and protein deficiency (Denke, 2001). Wing et al. (1995) also observed that ketosis affected cognitive performance in obese women. They compared two liquid formula diets over 28 days. The diets were matched for energy (2.4 MJ/day) and protein (50-52 g/day). The diet classified as the “ketogenic” diet contained only 10g/day of carbohydrate whereas the diet classified as “non-ketogenic” contained 72g/day of carbohydrate. Performance on the trail making task, a neuropsychological test that requires higher order mental processing and flexibility was worse in the women consuming the ketogenic diet. The worsening in performance was observed primarily between baseline and week one of the ketogenic diet.

Complications from high saturated fat intakes

While weight loss decreases serum cholesterol, the type Atkins diets that promote a liberal intake of high fat meat and dairy products raise cholesterol levels. A study conducted by

LaRosa and colleagues (1980) examined the effect of lipid changes after following the Atkins diet for 8 weeks, in 24 obese men and women. Despite weight loss of 4.1 kg, total serum cholesterol increased 12.3 mg/100ml and LDL-cholesterol increased 23 mg/100ml. Uric acid and free fatty acid levels also increased significantly. Similar increases in total cholesterol (13%) were reported in a study of subjects following the Stillman diet (13% saturated fat) (Rickman 1974). Anderson et al. (2000) calculated that long-term use of the Atkins diet would increase serum cholesterol by ~25%, and an increase of this magnitude might increase the risk of CHD by > 50% if the diet is consumed over the long-term.

Complications from high total fat intakes

High-fat diets increase postprandial triacylglycerol levels and may increase the concentration of atherogenic chylomicron remnants (Cohen et al. 1988; Diwadkar et al. 1999). Consequently, long-term use of high protein, high-fat diets during weight-maintenance can potentially increase the risk of CHD. Furthermore, high-fat intakes are associated with insulin resistance. Atkins, Eades and their respective colleagues claim that high-protein, high-fat diets decrease insulin resistance (Atkins, 1992; Eades and Eades, 1996); no studies, however, have been conducted to test the claims. As discussed in section 1.7.2.3 Skov et al. (1999b) demonstrated that a high-protein diet that restricted total and saturated fat intakes may improve insulin sensitivity.

Complications from high-protein intakes

1) *Renal disease*: Kerstetter et al. (1998) has shown that increasing the protein content of the diet significantly increases glomerular filtration rate. However, if this compensatory response does not sufficiently clear by-products of protein metabolism, then a resultant increase in blood urea nitrogen and serum uric acid levels may occur (Denke, 2001). A reduction in urinary citrate may also result from high protein intakes, carbohydrate restriction and a limited intake of vegetables and fruits (Denke, 2001). There is concern

that such changes may potentially led to urine acidification, hyperuricosuria, and hypercalciuria which can all contribute to the development of nephrolithiasis (Denke, 2001). Evidence substantiating this concern is limited. A meta-analysis of 13 randomized, controlled dietary interventions (n = 1919 subjects in total) showed that dietary protein restriction retarded the progression of diabetic nephropathy to end stage renal disease (Kasiske et al. 1998); however, the rate of decline in estimated glomerular filtration rate was reduced by only 0.53 mL/min/yr with lower protein intakes suggesting that other factors are probably more important in the decline of renal function. In addition, Skov et al. (1999a) demonstrated that moderate changes in dietary protein intake caused adaptive alterations in renal size and function without indications of adverse effects. In this study, the effect of two low-fat (30% of energy) diets either high or lower in dietary protein (25 versus 12% of energy), were compared in 65 obese men and women. Protein intake changed from 91.1 g/d to a 6-month intervention average of 70.4 g/d in the standard-protein group, and from 91.4 g/d to 107.8 g/d in the high-protein group. In response to the diets, glomerular filtration rate decreased 7.1 ml/min in the standard-protein group and increased 5.2 ml/min in the high-protein group. Kidney volume decreased in the standard-protein group and increased by in the high-protein group, whereas albuminuria remained unchanged in all groups.

2) *Osteoporosis*: Metabolic acidosis promotes calcium mobilization from the bone (Barzel and Massey, 1998). Accordingly, there is concern that high-protein diets may increase bone resorption without affecting the rate of bone formation (Kerstetter et al. 1999). However, other studies have demonstrated that calcium loss only occurs when calcium intake is increased and the intake of phosphorous is fixed (Spencer et al. 1978). Moreover, two studies found that an increase in meat protein had no effect on bone turnover (Shapses et al. 1995; Spencer et al. 1978).

3) *Blood pressure*: Cross-sectional studies relating blood pressure to dietary protein intake have shown equivocal results (Cirillo et al. 2002; Havlik et al. 1990; Liu et al. 2000). In a

population of monozygotic twins, Havlik et al. (1990) identified a direct positive association between dietary protein intake and diastolic blood pressure that persisted after adjustment for known covariates of blood pressure. Adjusting for known covariates and holding total calories constant, a 9-g difference in daily protein intake was directly associated with a 1 mm Hg increase in diastolic blood pressure. For protein intake as a percentage of total calories, a 2.2% difference was directly associated with a 1 mm Hg increase in diastolic blood pressure. In contrast, both Liu et al. (2000) and Cirillo et al. (2002) revealed an negative association between blood pressure (both systolic and diastolic) and animal protein intake in their respective populations of Chinese and Italian men and women.

1.7.3 Other types of popular fad diets

Over the years an array of 'one food' diets have been promoted such as the rice diet, banana diet, and the grape fruit diet [cited in books by Stanton and Egger (Egger and Swinburn, 1996; Stanton, 1991)]. These types of diets limit the selection of foods in an effort to reduce temptation and often use foods that people believe are healthy or have special properties that burn fat. They are nutritionally unbalanced and encourage poor eating habits. Scientifically they are unsound and contrary to any research, and they are potentially dangerous which is probably why it is recommended that they be followed for no longer than 1 to 2 weeks. Other diets such as the 'Rotation diet', 'Fit for Life', 'Mayo Clinic diet', and 'Beverly Hills diet' base their theories on unproven information about physiology and metabolism [cited in books by Stanton and Egger (Egger and Swinburn, 1996; Stanton, 1991)]. For example, the 'Fit for Life' diet plan is low in milk, meat, breads and cereals, and very high in fruit and vegetables. Eating more fruit and vegetables is desirable, but the diet is based on the claim body fatness is caused by improper food combinations; they claim mixing protein and carbohydrates reduces the rate of gastric emptying and this contributes to accelerated fat gain. It also claims that certain

macronutrient combinations 'rot' in the stomach and release 'toxins'. These claims have no scientific evidence. Moreover, there are some diets based on the theory that blood type or personality influence the best eating pattern for individuals (e.g. 'Eat right for your type') (D'Adamo, 1997) and others that suggest that excess weight is caused by liver dysfunction and not energy imbalance (e.g. 'Liver cleansing' diet) (Cabot, 1996). Again, the claims are not supported by scientific evidence.

The above discussion highlights the controversy surrounding the optimal macronutrient composition of weight loss diets, particularly for persons who have or who are predisposed to Type 2 diabetes mellitus. The evidence strongly suggests that diets advocating high total and saturated fat contents should not be used as a weight loss strategy for obesity and its associated diseases. Whether the fat intake should be replaced by complex-carbohydrate or by protein, and in what quantities, remains unclear. Although some evidence suggests that low-fat, high-protein diets may be more affective at reducing weight and ameliorating insulin resistance than currently recommended low-fat, high-carbohydrate diets, there are no studies that comprehensively compared the benefits and potential risks of these two types of diet for subjects with Type 2 diabetes or hyperinsulinemia. The studies described in Chapters 8 and 9 of this thesis were conducted to compare the short-term efficacy of two moderately energy-restricted low-fat diets, with either a high-protein content or a standard protein intake, on weight loss, energy expenditure, and glucose, insulin and lipid levels. In Chapter 8, the subject population examined was overweight men and women with Type 2 diabetes. Non-diabetic, overweight men and women with hyperinsulinemia were investigated in Chapter 9.

1.8 OVERALL OBJECTIVES

There has been resurgence in the public popularity of high-protein, low-fat, 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 typically observed during and after weight loss. Consequently, low-fat, high-protein diets may be more beneficial than low-fat, high-carbohydrate diets for long-term weight management. Furthermore, several studies have found that high-protein diets can improve insulin sensitivity and plasma lipid levels.

The focus of this thesis was 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. To measure diet-induced changes in energy expenditure, a major objective of this thesis was to establish, in our laboratory, the novel [¹⁴C]-bicarbonate-urea method for evaluating total energy expenditure in free-living subjects. Indirect calorimetry for measuring resting energy expenditure, substrate oxidation and the thermic effect of feeding also had to be established. The hypotheses and aims of this thesis were:

1.8.1 Specific Hypotheses

1. After body weight is reduced and stabilised at a lower level, daily energy expenditure will be reduced, thereby predisposing individuals to weight regain.
2. Increasing the dietary protein content of energy-restricted diets will enhance weight loss and blunt the reduction in REE and/or TEE. An increase in the TEF and greater preservation of lean body mass are two mechanisms through which the increased dietary protein will act.
3. Increasing the dietary protein content of energy-restricted diets will preserve lean body mass, thereby improving insulin sensitivity and ameliorating insulin resistance.

1.8.2 Specific Aims

1. Establish the [^{14}C]-bicarbonate-urea method for measuring TEE in 'free-living' subjects and determine its' reproducibility.
2. Determine the reproducibility of indirect calorimetry for measuring REE, TEF and RQ.
3. Determine the reproducibility of DEXA for measuring changes in body composition.
4. Determine whether TEE and/or REE, TEF and PAEE decrease after body weight is reduced and stabilised at the lower level.
5. Determine the benefits of increasing the protein-to-carbohydrate ratio of moderately energy-restricted diets on weight loss and energy expenditure, and glucose, insulin and lipid levels, in adults with Type 2 diabetes or hyperinsulinemia.

CHAPTER 2

Common Methodologies

2.1 INTRODUCTION

The methodologies described in this Chapter are common to the studies presented in Chapters 3 to 9. Standard methods to evaluate energy expenditure, energy intake, body composition, and carbohydrate and lipid metabolism have been used where possible. When a standard technique has been used in our research group for the first time, the reproducibility of the method is discussed in a separate chapter. Where a new technique has been implemented (i.e. the [¹⁴C]-bicarbonate-urea method for measuring total energy expenditure), the principle and development of the method is described in detail in a separate chapter. The limitations of the methods used are discussed in the relevant chapters.

2.2 ETHICS APPROVAL

All protocols were approved by the Human Research Ethics Committee of the Royal Adelaide Hospital (Chapters 3 to 9) and where appropriate (Chapters 6 to 9) the Royal Adelaide Hospital Drug Sub-Committee and Radiation Protection Branch of the South Australian Health Commission. Protocols discussed in Chapters 8 and 9, were also approved by the Human Ethics Committee of the Commonwealth Scientific Industrial Research Organisation (CSIRO).

2.3 TECHNIQUES FOR MEASUREMENTS

2.3.1 Energy expenditure

Total energy expenditure has three main components: resting energy expenditure, the thermic effect of feeding, and physical activity (which includes both voluntary and involuntary activity). The proportion that each component contributes to total energy expenditure varies from day-to-day according to the regularity and intensity of physical activity. The ability to measure total energy expenditure and its' components is critical to understanding some of the processes that cause obesity.

2.3.1.1 Total energy expenditure (TEE)

A major aim of this thesis was to investigate the suitability of [¹⁴C]-bicarbonate-urea as a novel technique for measuring TEE. Given that the labeled-bicarbonate-urea method has been used in only a limited number of overseas studies, Chapter 5 has been devoted to describe the working principle and development of this method in our laboratory. In addition, the reproducibility and reliability of the method was determined and is described in Chapter 6. In brief, [¹⁴C]-bicarbonate-urea allows the assessment of CO₂ turnover and energy expenditure in humans under free-living conditions over 24 hours (or multiples of 1-day periods). It is essentially an isotopic dilution technique whereby a known concentration of [¹⁴C]-bicarbonate-urea is infused under the skin of the stomach over 48 hours, and is diluted by the endogenous CO₂ pools. A proportion of the infused [¹⁴C]-bicarbonate-urea is incorporated into urea, which is excreted in the urine; the remainder is exhaled as CO₂. The method involves the collection of a 24-hour urine sample from which the specific activity of urinary urea is measured and used to indirectly calculate TEE (Elia et al. 1995; Leijssen and Elia, 1996).

In Chapter 6, TEE was also predicted using an equation that is based on the factorial method for estimating daily energy requirements (NHMRC, 1991; FAO/WHO/UNU Expert Consultation, 1985):

$$\text{Predicted TEE (kJ/day)} = \text{REE (measured)} \times \text{physical activity index [i]}$$

Where REE is measured using indirect calorimetry (see section 2.3.1.2), and the physical activity index (which reflects voluntary activity) was determined from a three day activity diary (see section 2.3.1.4) in conjunction with a table of physical activity indexes for eight different activity levels (NHMRC, 1991; FAO/WHO/UNU Expert Consultation, 1985).

2.3.1.2 *Resting energy expenditure (REE)*

Resting energy expenditure is defined as the energy expenditure necessary to maintain basic physiological functions while at rest. Indirect calorimetry using a ventilated canopy and a Deltatrac I™ metabolic monitor (Datex Division Instrumentarium Corp., Helsinki, Finland) was used to measure REE for all studies reported within this thesis. The reproducibility and reliability of the Deltatrac I™ metabolic monitor for measuring REE, respiratory quotient, and the thermic effect of feeding was determined in our laboratory and is described in Chapter 3.

Measurements of the oxygen and carbon dioxide contents of expired air, together with either inspired or expired breathing volume, provide the basic data for determining respiratory gas exchange and oxygen uptake, and inferring the body's rate of energy expenditure (McKardle et al, 1996). In brief, the Deltatrac™ metabolic monitor uses an open circuit calorimetry technique whereby ambient air with a constant composition of 20.93% oxygen, 0.03% carbon dioxide, and 79.04% nitrogen is pushed through the canopy at a constant flow (~ 40 L/min) and the subject inhales this air. The monitor continuously measures inspiratory, expiratory and ambient values of oxygen and carbon dioxide concentrations, and their volumes. Because oxygen is used during energy-yielding reactions and carbon dioxide is produced, the expired air contains less oxygen and more carbon dioxide than the inspired air. The difference in the composition of the inspired and expired gas volumes reflects the body's constant release of energy through aerobic metabolic reactions. Utilising these values and multiplying by the flow of the respective gases [the flow of carbon dioxide is estimated using the Haldane transformation (Wilmore and Costill, 1973)], the Deltatrac automatically calculates the volumetric flows of oxygen and carbon dioxide in ml/min (i.e. the amount of oxygen removed from the inspired air each minute and the amount of carbon dioxide produced per minute).

Resting energy expenditure is then automatically calculated using the equation of the manufacturer:

$$\text{REE} = 5.50 \text{ VO}_2 + 1.76 \text{ VCO}_2 - 1.99 \text{ U}_N \text{ [i]}$$

Where REE is resting energy expenditure (kcal/24h); VO_2 is ventilatory oxygen consumption (ml/min); VCO_2 is ventilatory carbon dioxide production (ml/min); and U_N is urinary nitrogen (g/24 h) that was calculated from the concentration of urinary urea. The conversion of REE from kcal/day to kJ/day was done using a factor of 4.18.

The manufacturers equation for REE differs somewhat from the frequently cited Weir (1990) [ii] and Elia and Livessey (1992) [iii] equations for metabolic rate which are:

$$\text{REE} = 3.94 \text{ VO}_2 + 1.11 \text{ VCO}_2 - 2.17 \text{ U}_N \text{ [ii]}$$

$$\text{REE} = 22.78 \text{ VO}_2 + 7.45 \text{ VCO}_2 \quad \text{[iii]}$$

However, if values for VO_2 (ml/min) and VCO_2 (ml/min) are in the normal physiological range, results from equation [i] as compared to equations [ii] and [iii] differ by 1% or less (i.e. 8.87-17.9 kcal/day or 37-74.8 kJ/day).

The protocol for the measurement of REE was the same for all studies in this thesis. Following a minimum fast of eight hours, subjects lay quietly in the recumbent position on a bed in a thermo-neutral environment (approximately 24°C) with the ventilated canopy placed over their head and shoulders. Resting energy expenditure was determined every minute for 30 minutes (Figure 2.1). The first 10 minutes of REE data were discarded to ensure all subjects had reached equilibrium; the remaining 20 minutes of data were averaged and represented the value for fasting REE used in all analysis.

The Deltatrac™ was calibrated each morning before measurements commenced, with a gas mixture of carbon dioxide (5%) in oxygen (95%) (BOC Gases Australia LTD, Australia).

Airflow rates through the canopy were checked by means of ethanol burning tests as described by the manufacturer, and they were conducted once before the commencement of each study within this thesis. Performance of the Deltatrac™ monitor was also checked by monitoring the ratio of carbon dioxide produced to oxygen consumed, during ethanol burnings. The mean (SD) of the ratio for the last 15 minutes of the tests were always within the manufacturers recommended range of 0.64 to 0.69.



Figure 2.1 Measurement of metabolic rate and respiratory quotient using indirect calorimetry.

A ventilated canopy and a Deltatrac™ metabolic monitor are used to continuously measure the volumetric flows of oxygen and carbon dioxide from which resting energy expenditure and respiratory quotient are determined.

2.3.1.3 *The thermic effect of food (TEF)*

The thermic effect of food (or also termed diet-induced thermogenesis) describes the increase in metabolic rate above REE resulting from the energy costs of digestion, absorption, transport, metabolism and storage of food consumed. The exact protocol for

the measurement of TEF varied between the studies reported within this thesis depending on the subject population, or type of diet being investigated (the reason for this is discussed below). In general, subjects ate a fixed-intake test meal following the measurement of REE. Subjects were given 20 minutes to eat the meal and then returned to the ventilated canopy for further measurements of REE. Postprandial REE measurements were made for 120 minutes as described in Chapters 3 and 8, or for 180 minutes in the studies described in Chapters 7 and 9. A value for TEF was determined at 20-minute intervals from the measurement of postprandial REE (i.e. fasting REE was subtracted from the mean postprandial REE that was measured every 20 minutes). The 20-minute TEF values were then averaged to determine the mean TEF over the 120 or 180-minute period. The TEF was expressed as a percentage of energy consumed during the test meal.

Initially we thought it was reasonable to measure TEF for 2 hours because several studies had shown that the maximum increase in thermogenesis, as well as approximately 50 to 70% of the total thermogenic response, had occurred within 120 after eating (Segal et al. 1990; Weststrate, 1993). We believed that a 2-hour measurement instead of a 6-hour or 24-hour measurement, as is often used, would increase the participation and compliance of subjects who were already committed to a large number of study measurements during our strictly controlled weight loss studies. However, several reviewers of the manuscript for the study described in Chapter 8 expressed concern that 2-hours may be too short to see a difference in the TEF between two diets of different macronutrient composition. Subsequently, the measurement of TEF was increased to 3-hours. Segal et al. (1990) has shown that 3-hour measurement of TEF was similar to a 6-hour measurement in lean and obese individuals. An additional limitation of our calculation of TEF was that the contribution of protein metabolism during the postprandial measurement of REE was not assessed (i.e. changes in urinary nitrogen over the postprandial period were not measured). Assuming postprandial urinary nitrogen remained the same as fasting urinary nitrogen

may have introduced an additional error in TEF of approximately 1.5%, particularly during the assessment of the thermic effect of high protein meals (Chapters 8 and 9).

For all assessments of TEF, subjects were instructed to remain awake but quiet. They were allowed read and listen to the radio throughout the measurement.

2.3.1.4 Physical activity

Physical activity is defined as 'any bodily movement produced by the skeletal muscles that results in energy expenditure (Caspersen et al. 1985). In daily life, physical activity can be categorized into occupational, sports, conditioning, household, or other voluntary activities, and it also includes involuntary activity such as fidgeting. Energy expended during physical activity (PAEE) is therefore the most modifiable and variable component of total energy expenditure.

Traditional methods for measuring physical activity in adults range from self-reported activity histories, year-long activity frequencies, 7 day activity recalls and activity diaries to more objective assessments of movement such as accelerometers, pedometers and heart rate monitors (Geissler et al. 1986; Melanson and Freedson, 1996; Sallis et al. 1985; Starling et al. 1999). These methods however remain problematic because: i) they need to be validated against the gold-standard method for estimating physical activity (Melanson and Freedson, 1996; Starling et al. 1999), and ii) many of these methods are not capable of capturing both voluntary and involuntary forms of physical activity energy expenditure. With improvements in the precision of tracer techniques such as doubly labeled water for measuring TEE in a free-living environment, it has become possible to validate methods for measuring physical activity. The daily level of physical of an individual (PA index or PAL) can be computed by dividing TEE (measured by doubly labelled water) by REE (measured by indirect calorimetry) (FAO/WHO/UNU Expert Consultation, 1985). This

equation has now been accepted as a gold standard for determining the level of daily physical activity and it is capable of capturing both voluntary and involuntary activity. Improvement in the precision of tracer techniques for measuring TEE now also allows a semi-quantitative estimate of energy expenditure due to voluntary and involuntary physical activity to be computed.

Where measurements of TEE have been made using [¹⁴C]-bicarbonate-urea in this thesis, a semi-quantitative estimate of energy expenditure due to physical activity has been computed (Chapters 6, 7, and 9). For studies reported within Chapters 6 to 9, subjects were asked to maintain their normal levels of physical activity during the measurements of TEE. To determine that the subjects complied to this request the average daily level of physical activity was assessed using 3-day physical activity diaries and the level of physical activity was expressed as an index that was subjectively derived from a published table of physical activity indexes (Table 2.1). Although the daily level of physical activity in Chapters 6, 7 and 9 could have been computed as the ratio of TEE and REE, the aim of this thesis was to establish the suitability of the [¹⁴C]-bicarbonate-urea for measuring TEE as well as PAEE. Furthermore, the diary method of assessing the level of physical activity allowed us to remind the subjects what types of activities they did during the initial measurement of TEE; this could not have been possible if the level of physical activity was computed from the measurement of TEE.

Energy expenditure due to physical activity (PAEE)

Semi-quantitative estimates of PAEE (which includes energy expenditure from both voluntary and involuntary activities) were calculated using the following equation:

$$[^{14}\text{C}]\text{-bicarbonate-urea derived PA EE} = 0.9([^{14}\text{C}]\text{-bicarbonate-urea TEE}) - \text{REE [i]}$$

Where PAEE is expressed in kJ/day; total energy expenditure was measured using [¹⁴C]-bicarbonate-urea method; REE is determined using indirect calorimetry; and the constant

of 0.9 was based on the assumption that 10% of TEE is due to the thermic effect of feeding.

The use of the [^{14}C]-bicarbonate-urea method to semi-quantitatively estimate the average daily energy expenditure due physical activity has not been validated.

The average daily level of physical activity (PA index)

Three-day physical activity diaries (Appendix 1) were used to quantitatively evaluate the average daily level of physical activity [expressed as a multiple of REE]. Individuals were instructed how to complete diaries and written guidelines along with an example were included within the diaries. An investigator (myself) reviewed the diary with the subject on the third day and an average daily physical activity index (a multiple of REE) for the 3 days was estimated using Table 2.1 (NHMRC, 1991; FAO/WHO/UNU Expert Consultation, 1985).

Table 2.1 Physical activity index (expressed as a multiple of resting energy expenditure) for adult men and women at 8 different levels of activity.

Activity Level	Men	Women
Bed rest	1.2	1.2
Very sedentary	1.3	1.3
Sedentary	1.4	1.4
Light	1.5	1.5
Light – Moderate	1.7	1.6
Moderate	1.8	1.7
Heavy	2.1	1.8
Very Heavy	2.3	2.0

2.3.2 Respiratory quotient

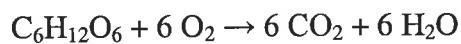
2.3.1.1 Fasting respiratory quotient (RQ)

Differences in the chemical compositions of carbohydrate, lipids and proteins, means that different amounts of O₂ are required to oxidise completely the molecule's carbon and hydrogen atoms to the end products of CO₂ and water. Accordingly, the quantity of CO₂ produced in relation to O₂ consumed varies depending on the substrate being metabolised (Flatt, 1996; McArdle et al, 1996). This ratio is termed the respiratory quotient (RQ) and is defined as:

$$\text{RQ} = \text{CO}_2/\text{O}_2$$

RQ for carbohydrate

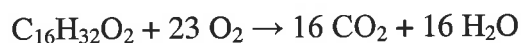
The complete oxidation of one carbohydrate molecule (e.g. glucose) is depicted in the equation:



During the oxidation of 1 molecule of carbohydrate, 1 mol of CO₂ is produced per mol of oxygen consumed. Accordingly, the RQ for carbohydrate equals 1.0 (i.e. RQ = 6 CO₂/ 6 O₂) (Flatt, 1996; McArdle et al, 1996).

RQ for fat

Lipids contain considerably fewer oxygen atoms than hydrogen atoms. Consequently, when 1 molecule of lipid is oxidised for energy, oxygen is required for the oxidation of carbon to CO₂ as well as for the oxidation of hydrogen atoms. When a typical lipid (e.g. palmitic acid) is oxidised to CO₂ and water, 16 CO₂ molecules are produced for every 23 O₂ molecules consumed. This exchange is summarised by the equation:



Accordingly, during the oxidation of 1 molecule of lipid, 0.7 mol of CO₂ are produced per mol of O₂ consumed. Hence, the RQ for lipid equals 0.7 (i.e. RQ = 16 CO₂/ 23 O₂) (Flatt, 1996; McArdle et al, 1996).

RQ for protein

Proteins are not simply oxidised to CO₂ and water during energy metabolism. Instead, protein is first deaminated in the liver, and the nitrogen and sulfur fragments are excreted in the urine and feces. The resultant “keto acid” fragments are then oxidised to CO₂ and water to provide energy. As for lipid, the keto acid fragments require more O₂ in relation to CO₂ produced. One molecule of protein (e.g. albumin) is oxidised as follows:



Accordingly, during the oxidation of 1 molecule of protein, 0.82 mol of CO₂ are produced per mol of O₂ consumed. Hence, the RQ for protein equals 0.82 (i.e. RQ = 63 CO₂/ 77 O₂) (Flatt, 1996; McArdle et al, 1996).

Nonprotein RQ

The RQ computed from the compositional analysis of expired air using indirect calorimetry and a ventilated hood generally reflects the catabolism of only carbohydrate and fat. This is because approximately 1 g of urinary nitrogen is excreted for every 6.25 g of protein metabolised, and 1 g of excreted nitrogen represents approximately 4.8 L of CO₂ production and 6.0L of O₂ consumption. Within this framework, the proportion of carbohydrate and fat being oxidised after the consumption of a mixed meal, can be assessed. For example, an RQ of 0.7 reflects pure lipid is being oxidised whereas an RQ of 1.0 reflects pure carbohydrate is being oxidised. Following a mixed meal however, an intermediate RQ is between 0.7 and 1.0. For example, an RQ of 0.82 following a mixed meal reflects that approximately 40% carbohydrate and 60% lipid are being metabolised (McArdle et al, 1996).

Within this thesis, RQ was calculated from the indirect calorimetry measurements of VO_2 (ml/min) and VCO_2 (ml/min). As for REE, fasting RQ was calculated as the average of the last 20 minutes of recorded data.

2.3.2.2 Postprandial respiratory quotient

Postprandial RQ measurements were collected for 120 minutes in the studies described in Chapters 8, or for 180 minutes in Chapters 7 and 9. From these measurements, a mean value for postprandial RQ was determined every 20 minutes. To calculate the increase in postprandial RQ at the specific time intervals, fasting RQ was subtracted from the mean postprandial RQ every 20-minutes. Thereafter, the 20-minute values were then averaged to determine the mean increase in RQ over the 120 or 180-minute period.

2.3.3 Energy intake, macronutrient composition, and the food quotient (FQ)

2.3.3.1 Energy intake and macronutrient composition

Numerous methods are available to assess the daily energy and nutrient intakes of individuals participating in studies that examine relationships between diet and disease (Bathalon et al. 2000; Stubbs et al. 1998; Thompson et al. 1997). In large population studies, the most common techniques used to determine dietary intake at an individual level include food diaries of varying duration (1-16 days) (Jorgensen et al. 1992; Luhrmann et al. 1999; Sempos et al. 1985), diet histories (Black et al. 2000), repeated 24 hour recalls (Beaton et al. 1983), food frequency questionnaires (Willett, 2001) and daily checklists (Murphy et al. 2001). All of these methods have been validated; either against more established self-reported methods such as weighed food records (Bingham et al. 1994), or more recently against established biomarkers of dietary intake such as doubly-labeled water (Trabulsi and Schoeller, 2001). A review of methodologies to assess habitual dietary intake by Trabuli and Schoeller's (2001) confirms the view that true energy intake, regardless of the instrument used to measure it, is consistently

underreported by most individuals, particularly those who are obese or who are post-obese. During intervention studies some obese persons may underreport their energy and specific macronutrient intakes to please the investigators. To a lesser extent, some individuals may eat less food than prescribed during dieting to enhance body weight loss thereby satisfying their own desire to be slimmer, as well as the investigators desire for them to loss weight.

For the purposes of the work conducted in Chapters 6 to 9, a 3-day weighed food diary (Appendix 2) was used to provide information on the habitual (baseline) diets of subjects before energy expenditure measurements were performed. For the weight loss studies described in Chapters 8 and 9, weighed dietary checklists were also completed throughout the study to assess compliance to the specified diets. The checklists were completed daily following the instructions provided (Appendix 3), and three days per fortnight (2 week and 1 weekend day) were analysed at the specified time points described within the relevant chapters. Urinary urea and creatinine were also measured to assess compliance to the study diets (see section 2.6.7.8).

Compliance of individuals to the energy restrictive diets used in Chapters 7 to 9 was assisted by the provision of key formula (Chapter 7) or foods (Chapters 8 and 9) as described in the respective chapters.

Energy (expressed as kJ) and macronutrient (expressed as g/day or as % of total energy) intakes were calculated using Diet 4 Nutritional software (Xyris Software, Highgate Hill, Queensland, Australia) based on Australian food composition tables and food manufacturers data (Cashell K, 1989).

2.3.3.2 *Food quotient*

Food quotient is the ratio of CO₂ produced to O₂ used for the biological oxidation of a representative aliquot of the diet and it indicates whether the fuel mix contains more or less fat. The FQ is used in conjunction with the RQ to indicate the state of energy balance. An average RQ > FQ reflects that less fat is oxidized than consumed and therefore implies a state of positive energy balance. Conversely, an average RQ < FQ implies a state of negative energy balance. The RQ/FQ ratio is positively correlated with energy balance, and weight maintenance corresponds to the situation where the average RQ is equal to the FQ (Flatt, 1996).

For the studies described in Chapters 6, 7 and 9, the FQ was calculated from the average macronutrient composition of the diet consumed by the subjects over the 3-days of their energy expenditure measurements (i.e. macronutrient composition derived from their 3-day food diaries). FQ was calculated using the following equation (Toubro et al. 1998):

$$\text{FQ} = \frac{0.27 \times \% \text{CHO} + 0.159 \times \% \text{FAT} + 0.193 \times \% \text{PROT} + 0.137 \times \% \text{ALCOHOL}}{0.27 \times \% \text{CHO} + 0.226 \times \% \text{FAT} + 0.243 \times \% \text{PROT} + 0.206 \times \% \text{ALCOHOL}}$$

2.3.4 **Body weight, height and body mass index**

Body weight reflects the overall size of the body as well as the density of the combined body tissues including the fluid, bone, muscle and organs. Body weight (in kilograms) was measured in light clothing using Seca™ (Wedderburn Scales, Summer Hill, NSW, Australia) (Chapters 3, 6 and 7) or Mercury™AMZ14 (A&D Mercury, Australia) (Chapters 8 and 9) electronic scales. Height was measured at the initial visit to the nearest 0.5 cm using a stadiometer. Body mass index was determined from the measurements of weight (in kilograms) and height (in metres) using the equation, BMI = wt(kg)/ht(m)².

2.3.5 Body composition

Dual-energy X-ray absorptiometry (DEXA) and bioelectrical impedance analysis (BIA) are frequently used to measure total body fat and lean masses, and body fat distribution. In this thesis, bioelectrical impedance was used to determine body composition in one study that did not involve a dietary intervention (i.e. Chapter 6). For studies that involved a dietary intervention (Chapters 7, 8 and 9), changes in body composition were evaluated using DEXA because a preliminary study (described in Chapter 4) showed that the mean intra-individual coefficient of variations for the measurements of total lean and fat masses were smaller than was previously found for BIA (CVs shown in section 2.3.5.2).

2.3.5.1 Dual-energy X-ray absorptiometry (DEXA)

Dual-energy X-ray absorptiometry measures the masses of bone, fat, and lean tissue for the whole body as well as specific regions of the body. For the dietary intervention studies conducted in Chapters 7, 8 and 9 total fat and lean mass, and fat and lean masses on the arm, leg, trunk and abdomen were measured by whole-body dual-energy X-ray absorptiometry using a Norland™ densitometer XR36 (Norland Medical Systems, Fort Atkinson, Wisconsin, USA) with version Revision 2.4 software. Percentage body fat at the various regions were calculated from the measurements of fat mass and lean body mass. DEXA involved subjects (wearing only underwear and a light gown) lying in the supine position on the scanning bed (approximately 190 x 67 cm) while a very low dose of X-ray scans their body (effective dose of radiation was 0.18 μ Sv per scan). Individuals were scanned at 130 mm/s using a scan resolution of 6.5 x 13 mm. The entire procedure took approximately 20 minutes per individual. Figure 2.2 depicts a typical image for a whole-body DEXA scan. The reproducibility and reliability of the Norland™ XR36 densitometer used for the measurements of body composition reported within this thesis will be discussed in Chapter 4.

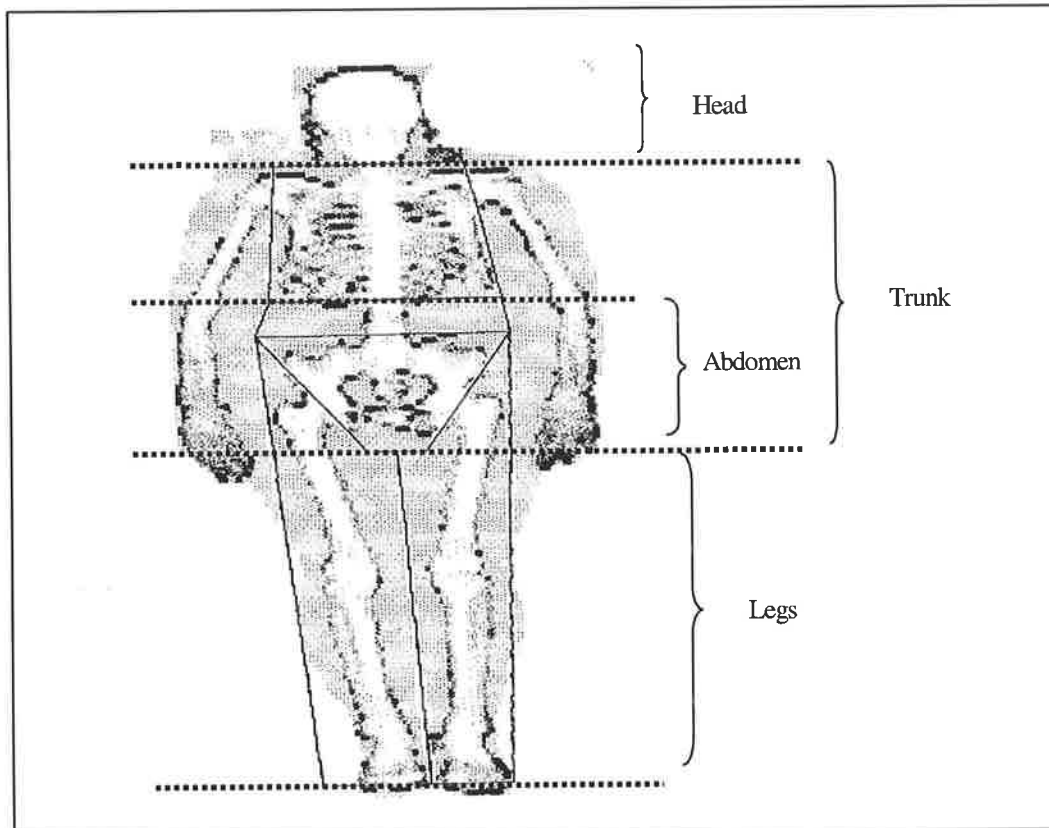


Figure 2.2 Image from a whole-body DEXA scan .

This is a typical image of a whole-body scan for the determination of the fat and lean masses of the head, trunk, abdomen, legs and arms. The Norland XR36 dual-energy X-ray densitometer defines the head region as the area from the top of the head to the bottom of the cervical vertebra; the trunk region from the top of the clavicle bone to the bottom of the pubic bone (i.e. the midriff + pelvis + chest areas); the abdominal region from the bottom of the rib cage to the bottom of the pubic bone (i.e. the midriff + pelvis). The leg region is defined as the area from the spiral line of the femur bone to the tip of the distal phalanx bone of the foot. The arm region is defined as the area from the top of the humerus bone to the tip of the distal phalanx bone of the hand. The left and right leg and arm are analysed separately, and then both masses are summed to calculate the total mass of the legs and arms, respectively. Total fat and lean mass are calculated as the sum of all the respective components.

2.3.5.2 Bioelectrical impedance analysis (BIA)

Bioelectrical impedance analysis indirectly calculates body fatness through the measurement of the electrical conductivity within the water compartment of the body. For studies described in Chapters 3 and 6, BIA was done on the right side of the body while

subjects were lying in the supine position using the Bodystat® 1500 Body Composition Monitoring Unit (Bodystat® Limited, Douglas Isle of Man, U.K.) (Figure 2.3). Impedance was measured using a current of 500 μA at a single frequency of 50 kHz (BODYSTAT (Isle of Man) LIMITED, 1992). Measurements of impedance and the height, weight, and gender of the individual are incorporated into the regression equations of the manufacturer (unpublished) to compute total body water and total lean mass (i.e. which includes the skeleton, muscles, viscera and total water content). Total fat mass is calculated as the difference between body weight that is measured using scales and total lean mass. The Bodystat® 1500 BIA unit has an accuracy of approximately 6 ohms. In our research group, the Bodystat® 1500 BIA unit has a mean intra-individual day-to-day variation (i.e. CV) of $1.3 \pm 0.33 \%$ (or expressed as an absolute value 0.9 kg) for total lean mass and for total fat mass the CV is $10.3 \pm 4.0 \%$ (or 1.1 kg).

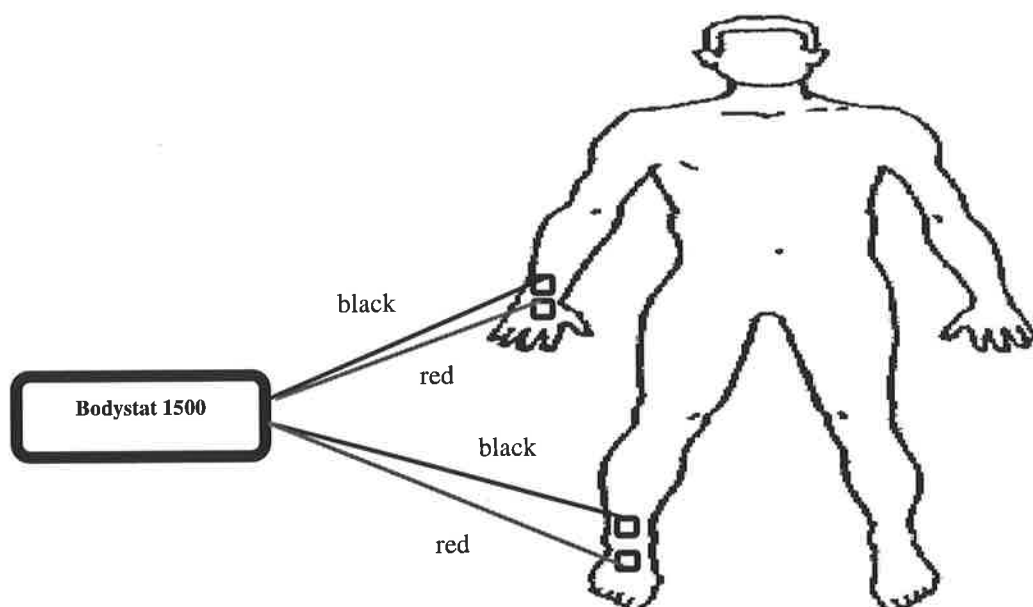


Figure 2.3 Bioelectrical Impedance Analysis.

This illustration depicts the positioning of the four electrodes on the dorsal side of the hand, wrist, foot and ankle, as well as the position of the legs and arms so that they are not touching any part of the body.

2.3.6 Blood sampling and 24 hour urine collection

In Chapters 8 and 9, blood was collected by venipuncture into tubes containing either sodium fluoride/EDTA (for glucose), or clot activator to induce serum separating (for all other biochemical variables). When difficult veins were encountered, or when blood was sampled at more than three time points over several hours, a catheter was inserted into either the forearm vein (20 Gauge, 1.1 x 30 mm i.v. catheter) or cephalic vein of the hand (21 Gauge, 0.8 x 19 mm i.v. catheter). Blood samples were drawn at time points specified in the relevant Chapters (Chapters 8 and 9), and tubes were placed immediately on ice. Plasma and serum was isolated within 3 hours of collection by low-speed centrifugation at 600 g for 10 min at 4°C (Beckman, Palo Alto, CA) and stored at -20°C until analysis.

In Chapters 6 to 9, twenty-four hour urine collections were made. The urine was collected in airtight 4L bottles with no added preservatives. Subjects noted the time that the first morning urine sample was passed, and thereafter, all urine passed over a 24-hour period, was collected. Twenty-four hour urine volumes were measured and aliquots were then stored in airtight vials with no preservatives at -20°C. At the end of each study, all samples from the same individual were analysed for each metabolic variable within the same run.

2.3.7 Biochemical variables

2.3.7.1 Plasma glucose

Plasma glucose concentration (mmol/l) was measured on an automated Cobas-Bio centrifugal analyser (Hoffman-La Roche, Basel, Switzerland) using a glucose hexokinase enzymatic kit (Boehringer-Mannheim, NJ, USA) and QCS 1 (Bio Rad Laboratories, Diagnostics Group, Irvine, California, USA) (Thomas, 1992). The intra-assay coefficient of variation (CV) was 0.8% at 5.1 mmol/l.

2.3.7.2 *Serum insulin*

Serum insulin concentration ($\mu\text{U/ml}$) was measured using a commercial radio-immunoassay kit (Pharmacia AB, Uppsala, Sweden) (McReynolds and Shah, 1973). The intra-assay CV was 4.6 to 9.4% at 20.1 to 78.7 $\mu\text{U/ml}$.

2.3.7.3 *Serum free fatty acids*

Serum free (non-esterified) fatty acid concentration (mmol/l) was measured on the automated centrifugal analyser using a free-fatty acid, half-micro enzymatic kit (Roche, NSW, Australia) and QCS 1 (Shimizu et al. 1980). The linearity limit is up to a concentration of 1.5 mmol/L .

2.3.7.4 *Serum triacylglycerols*

Serum triacylglycerol concentration (mmol/l) was measured on the automated centrifugal analyser using a triacylglycerol enzymatic kit (Boehringer-Mannheim, NJ, USA) and QCS 1 (Fossati, 1982). The intra-assay CV was 0.9% at 1.08 mmol/l .

2.3.7.5 *Serum total cholesterol*

Serum total cholesterol concentration (mmol/l) was measured on an automated centrifugal analyser using a total cholesterol enzymatic kit (Boehringer-Mannheim, NJ, USA) and QCS 1 (Allain, 1974). The linearity limit for this kit is 15 mmol/L .

2.3.7.6 *Serum high-density lipoprotein*

Serum high-density lipoprotein (HDL) cholesterol concentration (mmol/l) was measured after precipitation of the low-density and very-low-density lipoproteins with polyethylene glycol 6000 solution (Warnick et al. 1985) using the method cited in section 2.3.7.5.

2.3.7.7 *Serum low-density lipoprotein*

Serum low-density lipoprotein (LDL) cholesterol concentration (mmol/l) was calculated using the modified Freidewald equation (1972):

$$\text{LDL cholesterol (mmol/l)} = \text{Total cholesterol (mmol/l)} - \text{Triacylglycerols (mmol/l)/2.18} - \text{HDL cholesterol (mmol/l)}$$

2.6.7.8 *Twenty-four hour urinary urea and creatinine*

Twenty-four hour urine was analysed for urea and creatinine concentrations by the Quality Pathology Service Laboratory, I.M.V.S., Adelaide, Australia. Urinary urea is a by-product of protein metabolism. Twenty-four hour urinary urea reflects the utilisation of dietary protein by the body; excess protein not required by the body is degraded and excreted as urea. Urea was measured using the urease enzymatic assay that had an intra-assay CV of 2.03% at 6.7 mmol/l (Orsonneau et al. 1980). Urinary creatinine reflects the completeness of 24-hour urine saves. It was measured using the Jaffe reaction technique that had an intra-assay CV of 2.8% at 155 mmol/l (Narayanan and Appleton, 1980). The urinary urea/creatinine ratio reflects the magnitude of urea excretion over a 24-hour period.

CHAPTER 3

Reproducibility And Reliability Of Indirect Calorimetry For Measuring Resting Energy Expenditure, Respiratory Quotient And The Thermic Effect Of Feeding

3.1 SUMMARY

This study evaluated the reproducibility and reliability of the Deltatrac™ metabolic unit for measuring fasting resting energy expenditure (REE), the respiratory quotient (RQ), and the thermic effect of feeding (TEF), in our laboratory. On two occasions ~7 days apart, measurements of fasting REE and RQ, and the thermogenic response over 2 hours to a 4484 kJ test meal (55% of energy as carbohydrate, 26% fat) were made in 13 healthy men with a wide weight range (BMI 19.7 to 33.5 kg/m²). Paired t-tests showed that there were no significant differences in any of the variables between the measurements made on the first day as compared to those made 7 days later. The mean (\pm SEM) fasting REE and RQ were 8335 ± 317 kJ/day and 0.80 ± 0.01 , and the mean TEF was $4.47 \pm 0.29\%$. The intra-individual day-to-day variation was $1.7 \pm 0.41\%$ (range 0.1 to 4.5%) for REE, $3.1 \pm 0.8\%$ (range 0 to 8.7%) for RQ, and $7.8 \pm 1.5\%$ (range 0.1 to 14.7%) for TEF. The intraclass correlation coefficient [index of reliability] was 0.97 for fasting REE, 0.35 for fasting RQ, and 0.74 for TEF. Although the mean within-subject day-to-day variation in fasting RQ was 3.1%, the low (0.35) reliability coefficient suggests that there is considerable variability between successive measurements within some individuals. This may reflect differences in the composition of meals eaten the day prior to the study measurements. It may also reflect the high sensitivity of the Deltatrac™ for detecting small changes in RQ. We conclude that the Deltatrac metabolic monitor is a reproducible and reliable instrument for measuring fasting REE and RQ, and TEF. However, the within-individual variability for TEF was large and the reliability index for fasting RQ was low; both issues must be considered when designing protocols to measure changes in TEF and RQ during an intervention.

3.2 INTRODUCTION

Three mechanisms that may be implicated in the development and maintenance of obesity include (i) a low total and/or resting energy expenditure (Ravussin et al. 1988; Ravussin

and Swinburn, 1993), (2) a high 24-hour respiratory quotient (Ravussin and Swinburn, 1993; Toubro et al. 1998; Zurlo et al. 1990), and (3) a blunted increase in energy expenditure in response to the ingestion of a meal (Bessard et al. 1983; Schutz et al. 1984; Segal et al. 1992a; Segal et al. 1992b; Segal et al. 1990). Accordingly, it is of great importance in research settings to have practical and accurate methods that are reproducible and reliable for the measurement of energy expenditure and substrate utilization (reflected by the respiratory quotient).

Indirect calorimetry is widely accepted as a method to measure resting energy expenditure, respiratory quotient, and the thermic effect of feeding. Several techniques of indirect calorimetry are available. Measurements of 24-hour energy expenditure by whole-body indirect calorimetry in respiration chambers are reproducible and reliable (Astrup et al. 1990; Murgatroyd et al. 1987; Ravussin et al. 1986; Toubro et al. 1995). Respiratory chambers require specialised equipment, are expensive to build and maintain, and the methodology is time consuming. Consequently, the number of subjects that can be studied is limited. Instead, energy expenditure is often measured by indirect calorimetry using a portable metabolic monitor and ventilated canopy during a limited period of about 30 to 60 minutes. Other groups have shown that a number of commercially available metabolic monitors and ventilated canopy systems give reproducible and reliable measurements of energy expenditure when used under standardized conditions (Bukkens et al. 1991; Fontvieille et al. 1992; Segal et al. 1992b; Segal et al. 1990; Svendsen et al. 1993; Weststrate, 1993). Furthermore, two studies that directly compared the accuracy of ventilated canopies to a respiratory chamber for measuring resting energy expenditure, showed that the two methods are comparable (Kistorp et al. 1999; Soares et al. 1989).

The aim of this study was to determine, in our laboratory, the reproducibility and reliability of the Deltatrac™ metabolic monitor for measuring resting energy expenditure

(REE), respiratory quotient (RQ), and thermic effect of feeding (TEF). “Reproducibility” (which can be used interchangeably with the term “precision”) refers to the variability of a particular measurer using a particular method for the measurement of a given variable within the same subject. The term “reliability” has the same features as reproducibility but it also takes in account the variability between subjects. Knowledge of the intra-individual day-to-day variation in these variables is important for the design and evaluation of the subsequent dietary intervention studies within this thesis.

3.3 RESEARCH DESIGN & METHODS

3.3.1 Subjects

Male volunteers over the age of 18 years with a body mass index $> 19 \text{ kg/m}^2$ were recruited by advertisement within the Royal Adelaide Hospital and University of Adelaide. Subjects with cardiovascular, renal, liver or respiratory disease, hypertension, diabetes mellitus, or hyperthyroidism were excluded from participating in the study. Sixteen men were initially recruited; none were taking any medications or supplements. Three subjects dropped out of the study due to work commitments, after the first measurement period.

The reproducibility of REE, RQ and TEF using the Deltatrac™ ventilated canopy was validated only in male volunteers, because data from a meta-analysis of work performed by Weststrate et al. (1993) had shown that the day-to-day variability for these measurements was not affected by gender. Furthermore, studies reporting the intra-individual variability of these measurements generally report a ‘pooled’ coefficient of variation (Goran and Poehlman, 1992; Kistorp et al. 1999; Tataranni et al. 1995; Toubro et al. 1995; Welle and Nair, 1990). Weststrate (1993) has also shown that the phase of the menstrual cycle (pre-ovular vs post-ovular) had no impact on REE, RQ, or TEF measurements made in 23 women.

3.3.2 Experimental design

Measurements of body weight and composition, resting energy expenditure (REE), respiratory quotient (RQ), and the thermic effect of feeding (TEF) were made in each subject on two occasions, separated by at least 7 days. Subjects were instructed to maintain their typical diet and physical activity patterns on the day prior to each study visit, and on the morning of the measurements they were asked to arrive at the Department of Medicine by 9 am having fasted from 10 pm the night before.

3.3.3 Measurements

3.3.3.1 Body weight and body composition

Body weight was measured as described in Chapter 2.3.4. Total fat and lean masses were determined by bioelectrical impedance as described in Chapter 2.3.5.2.

3.3.3.2 Resting energy expenditure (REE), respiratory quotient (RQ), and the thermic effect of feeding (TEF)

Fasting REE and RQ were measured by indirect calorimetry using the ventilated canopy and Deltatrac I™ metabolic monitor as described in Chapters 2.3.1.2 and 2.3.2.1, respectively. Immediately following the measurements of fasting REE and RQ, each subject consumed, within 20 minutes, a 4484 kJ test meal which contained 20% of the total energy as protein, 55% as carbohydrate and 26% as fat. Thereafter, subjects returned to the hood for a further 120 minutes of REE measurements. The TEF over the 120-minute period was calculated as described in Chapter 2.3.1.3, and was expressed as the percentage increase per energy intake of the test meal.

The energy content of the test meal was designed to represent approximately one third of the average daily energy requirements of the group. At the screening visit, individuals

energy requirements were estimated by multiplying their resting metabolic rate [derived from the Schofield equation (1985)] by their reported level of physical activity (derived from Table 2.1 Chapter 2.3.1.4) (NHMRC, 1991; Warwick, 1989). The mean daily energy requirements for the group were found to be 12031 ± 560 kJ/day [range 10300 to 15872 kJ/day]. The test meal consisted of 30 g of corn flakes, 10 g of white sugar, 126 g reduced fat plain milk, 250 g of reduced fat chocolate milk, 3 slices of white bread, 5 g of table margarine, 70 g of lean leg ham, 40 g of cheddar cheese, 3 water crackers, and 1 banana.

3.3.4 Statistical analysis

All data are represented as means \pm SEM, unless specified otherwise as (\pm SD). Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). Reproducibility between the first and second energy expenditure measurements was determined from (i) a paired t-test with a 95% confidence interval, and (ii) the calculation of the intra-individual day-to-day variation of the variable, which is defined as the standard deviation of the repeated measurements taken independently on the same subject and is expressed as a percentage of the mean [may also be termed the intra-individual coefficient of variation (CV)] (Denegar and Ball, 1993; Peterson and Gore, 1996). A mean difference that falls within the 95% confidence intervals, and an intra-individual day-to-day variation of $< 5\%$ for fasting REE and RQ, and TEF would suggest that the methods were reproducible and could confidently be used to measure energy expenditure. The reliability of the measurement was calculated from a one-way ANOVA where the mean squares were combined in a ratio to give an intraclass correlation coefficient (ICC) (Denegar and Ball, 1993). The ICC ranges from 0 (zero reliability) to 1 (perfect reliability indicating successive measurements within individuals are in close agreement). Significance was set at $p < 0.05$.

3.4 RESULTS

3.4.1 Subject characteristics

The physical characteristics of the 13 men who completed the study are presented in Table 3.4.1. The mean age of the subjects was 34.1 ± 4.3 years and their mean BMI was 25 ± 1.2 kg/m². Body weight remained stable from the first to the second measurement period (81.4 ± 3.7 vs 81.3 ± 3.7 kg), as did total fat mass (13.3 ± 2.3 vs 12.8 ± 2.0 kg) and lean mass (67.7 ± 2.1 vs 68.5 ± 2.5 kg).

3.4.2 Resting energy expenditure (REE), respiratory quotient (RQ) and the thermic effect of feeding (TEF)

The values for repeated measurements and the intra-individual day-to-day variability for all energy expenditure variables are presented in Table 3.4.2. There was no difference between the first and second measurements of REE (8283 vs 8386 kJ/day, mean difference \pm SD was -102 ± 257 kJ/day, 95% CI -257 to $+53$, $p = 0.18$). The mean intra-individual CV for REE was $1.71 \pm 0.41\%$ and the ICC was 0.97.

Repeated measurements of RQ were similar (0.81 vs 0.80, mean difference \pm SD -0.014 ± 0.05 , 95% CI -0.014 to $+0.042$, $p = 0.30$). The mean intra-individual CV for RQ was $3.10 \pm 0.78\%$ and the ICC was 0.35.

There was no difference in TEF reported at the first as compared to second measurement period (4.61 vs 4.33%, mean difference \pm SD was $0.28 \pm 1.06\%$, 95% CI -0.36 to $+0.919$, $p = 0.357$). The mean CV for TEF was $14.7 \pm 7.1\%$, and the ICC was 0.82. However, when individual 4 (who had a CV of 98.5%) is excluded from the analysis, the mean variation of the group was reduced to $7.76 \pm 1.5\%$ [range 0.08 to 14.7%] (5 men had a variation of more than 10%). The ICC for the remaining 12 individuals was 0.74. The justification for removing individual 4 from the analysis is because the change in his TEF

was approximately 3 SD above the mean change for the group, thereby falling outside the 95% confidence intervals.

Table 3.4.1 Physical characteristics of the 13 men who completed the study

Subject	Age (yrs)	Height (m)	Weight (kg)	BMI (kg/m²)	Body fat %	Fat mass (kg)	Lean mass (kg)
1	22	1.85	67.5	19.8	8.1	5.5	62.0
2	22	1.89	99.8	28.2	15.5	15.5	84.3
3	28	1.80	74.0	22.8	9.6	7.1	66.9
4	46	1.64	90.1	33.5	21.1	19.0	71.1
5	21	1.82	75.1	22.5	4.2	3.2	72.2
6	30	1.86	80.9	23.5	14.9	12.2	68.7
7	29	1.76	61.7	19.8	10.3	6.3	55.0
8	51	1.73	89.0	29.7	25.1	22.3	66.7
9	54	1.74	76.3	25.3	23.8	18.2	58.1
10	23	1.80	86.2	26.6	18.1	15.6	70.6
11	31	1.72	62.3	21.1	4.9	3.1	59.2
12	19	1.81	95.7	29.3	13.9	13.3	82.4
13	68	1.82	99.6	30.1	26.3	26.2	73.4
Mean±SEM	34.1±4.3	1.79±1.89	81.4±3.7	25.5±1.2	15.1±2.1	12.9±2.1	68.5±2.4

Age and height were recorded upon admission to the study. Weight, BMI, body fat %, and total fat and lean masses were measured on two occasions that were separated by at least 7 days. There were no significant differences between the first and second measurements of weight, BMI or body composition variables ($p > 0.2$), and therefore the average values are presented.

Table 3.4.2 Repeated measurements and the intra-individual day-to-day variations for resting energy expenditure, respiratory quotient, and the thermic effect of feeding.

Subject	REE (kJ/day)			RQ			TEF (% EI)		
	Measure 1	Measure 2	CV	Measure 1	Measure 2	CV	Measure 1	Measure 2	CV
1	7896	8020	1.1	0.77	0.77	0	4.20	4.19	0.08
2	10087	9980	0.76	0.86	0.76	8.7	4.05	3.73	5.8
3	8476	8483	0.06	0.74	0.74	0	5.36	5.66	3.9
4	7705	8052	3.1	0.81	0.77	3.6	3.75	0.67	98.5
5	8221	8762	4.5	0.83	0.81	1.7	5.00	4.11	13.8
6	9085	8568	4.1	0.71	0.77	5.7	5.42	6.57	13.7
7	6432	6541	1.2	0.83	0.84	0.85	3.63	4.03	7.4
8	8125	8199	0.64	0.87	0.82	4.5	3.74	3.86	2.3
9	6865	6835	0.31	0.82	0.81	0.87	4.05	3.94	2.0
10	7795	7868	0.65	0.82	0.82	0	3.90	4.80	14.7
11	7251	7546	2.8	0.78	0.85	6.1	5.11	4.28	12.5
12	9913	10228	2.2	0.83	0.79	3.5	6.39	5.89	5.7
13	9835	9930	0.68	0.86	0.80	5.1	5.37	4.57	11.5
mean ± SEM	8284±323	8386±316	1.7±0.4	0.81±0.01	0.80±0.01	3.1±0.8	4.61±0.24	4.33± 0.39	14.7±7.1

REE, resting energy expenditure; RQ, respiratory quotient; TEF, the thermic response to a 4484 kJ test meal, expressed as the % increase per energy consumed over 2 hours. Measure 1 and measure 2 are the measurements of REE, RQ and TEF that were made at least 7 days apart. Paired t-tests indicated there were no significant differences between repeated measurements. Intra-individual day-to-day variation was expressed as the coefficient of variation [CV = (SD/mean)*100].

3.5 DISCUSSION

Day-to-day variation in REE did not exceed 5% for any individual and the reliability index was high (0.97) indicating that REE can be measured reproducibly and reliably within 30 minutes under standardized conditions using the Deltatrac™ metabolic monitor and ventilated canopy. Fontvieille and colleagues (1992) reported that the mean day-to-day variation in REE was 1.5% when they used a Deltatrac™ metabolic monitor to measure the energy expenditure of six children (4 male and 2 female). In addition, other studies have shown that a range of commercially available ventilated canopy systems give precise (mean intra-individual variations ranging from 2 to 6% and intraclass correlation coefficients of ~0.9) measurements of REE in both men (Segal et al. 1992b; Segal et al. 1990) and women (Svendsen et al. 1993), or in a combination of both genders (Goran and Poehlman, 1992; Isbell et al. 1991; Segal, 1987; Weststrate, 1993) with widely varying age and body weight.

The values for REE obtained in the current study were consistent with those published in studies where the mean age, body weight, and BMI were similar to our subject population and where REE was measured using either a respiratory chamber (Dionne et al. 1999; Poppitt et al. 1998) or a ventilated hood (Frankenfield et al. 1999). Furthermore, in a compilation study of 574 doubly labeled water measurements, Black et al. (1996) produced reference ranges for REE of (mean \pm SD) 7.5 ± 1.2 , 8.2 ± 1.8 , 7.0 ± 0.8 , and 6.9 ± 0.9 MJ/day for males with a mean BMI of around 25 kg/m^2 , who were aged 18-29, 30-39, 40-64, and 65-74 years, respectively. The majority of our subjects fell within these ranges, but subjects 2, 12 and 13 had a greater REE than expected (~10 MJ/day). At least for subject 1 and 12, the reason for the higher REE may be attributed to a large proportion of lean mass (~84% of their body weight). We observed a very close correlation between REE and lean mass ($r = 0.906$, $p < 0.0001$).

The mean intra-individual day-to-day variation in fasting RQ was 3.10%, but the intraclass correlation coefficient was only 0.35 suggesting that there is considerable variability between successive measurements within some of the individuals in this study. Two of the 13 men had a variation of more than 5%; for subject 11 it was 6.1% (represents a difference in RQ of 0.07) and for subject 2 it was 8.7% (represents a difference of 0.1). The low reliability coefficient for fasting RQ may reflect differences in the composition of meals eaten the day prior to the study measurements. Subjects were instructed to maintain their typical diet before each measurement but the composition of what they ate was not assessed in this study. If the subjects switched from a low-fat to high-fat diet in the day(s) preceding the second measurement, then it likely that the RQ would be lowered; the reverse would be true if they switched from a high-fat to low-fat diet. A low reliability coefficient may also reflect the high sensitivity of the Deltatrac™ for detecting small but statistically non-significant changes in RQ because of the small population size. Our findings are similar to those of Weststrate (1993) who reported mean fasting RQ values of 0.83 (range 0.75 to 0.87) for men, using a ventilated canopy. The mean intra-individual variation in fasting RQ that Weststrate (1993) observed was 4.5% for 37 non-obese men and 3.7% for a group of 22 non-obese and 32 obese women. A study of 15 female and 7 male subjects aged 27 to 64 years with a BMI of 30 to 39 kg/m², demonstrated that 24-hour RQ measured within a respiratory chamber, could have an intra-individual day-to-day variation of as low as 0.8% (Toubro et al., 1995).

In this study, the variability for TEF was considerably larger than for REE and RQ. The mean intra-individual variation in TEF was 7.76% and it is comparable to that reported by Segal et al. (1992b) (5.7%) for three groups of men (13 lean, 10 average and 12 obese) that were matched with respect to age, height, lean mass and level of cardiovascular fitness. Segal and colleagues also observed an intraclass correlation coefficient of 0.819 to 0.932 depending on the method used to calculate the TEF. The higher reliability index was

observed when TEF was calculated as the postprandial minus a 3-hour fasting REE measured on a control day when no test meal was consumed, whereas the lower reliability index was observed when fasting REE was derived from a 30-minute baseline measurement. Using a ventilated canopy, Bukkens and colleagues (1991) also showed a mean day-to-day variation of 16.4%, in 6 post-obese and 6 never overweight women. In contrast, others have reported variations of 32% (range 1 to 61%) when TEF was measured using a ventilated hood (Weststrate, 1993) and 48% (range 1 to 68%) when measurements were made in a respiratory chamber (Tataranni et al. 1995).

Poor reproducibility in the measurement of TEF may relate to i) variation in the conditions under which the measurements were assessed. For example, Weststrate and colleagues (1993) allowed their subjects to watch different films during repeated measurements which may have had an effect on behaviour; and ii) biological day-to-day variation in the postprandial processing of nutrients due to differences in food intake (Westerterp et al., 1999b) or physical activity (Segal et al., 1992a; Segal et al., 1992c) in the days prior to measurements. Weststrate (1993) however, has demonstrated that the intra-individual variability was not affected by antecedent diet. Therefore, changes in behaviour on the measurement day, and physical activity in the days prior to the measurement, presumably have the greatest impact on TEF.

Comparison of our values for TEF with data from other studies is difficult, because different methods are used to compute TEF. The methods used include: (1) 24-hour TEF (Tataranni et al. 1995); (2) 15-hour TEF (Schutz et al. 1984); and (3) TEF which is computed from the measurement of postprandial REE (made over shorter periods of time e.g. 3-hours) minus fasting REE (Segal et al. 1992b). These methods are very variable, and the first two are subject to the errors associated with the measurement of physical activity, which itself is problematic. In this study, we measured TEF for only 2 hours; Weststrate

(1993) and Segal et al. (1990) have shown that ~50 to 70% of the total TEF is observed within the first 2 hours of measurement. In this study, the thermogenic response to a 4484 kJ test meal ranged from 2.1 to 6.1% which is consistent with the range of values observed in men and women who had their TEF measured over 6 to 24 hours (Bessard et al. 1983; D'Alessio et al. 1988; Leibel et al. 1995).

The comprehensive body of work performed by Weststrate (1993) showed that the magnitude of intra-individual variation in REE, RQ and TEF was similar for men (6.0%, 4.5% and 27.6% respectively) and women (6.0%, 3.6% and 28.6% respectively). It is, therefore, likely that the intra-individual coefficients of variation for fasting REE and RQ, and TEF that were observed in this study of men would be similar for a population of women, providing the conditions for measurements remained the same. In fact, examination of data in a subsequent study (Chapter 6) shows that women with a BMI range of 28 to 40 kg/m² had similar mean within-subject day-to-day variations of $2.9 \pm 1.25\%$ for REE and $4.0 \pm 1.5\%$ for RQ.

3.6 CONCLUSION

This study demonstrated that the reproducibility and reliability of indirect calorimetry using the Deltatrac™ metabolic monitor is acceptable for measurements of fasting REE and RQ, and the TEF. The variation in fasting RQ is larger for some individuals than it is for others. The larger day-to-day variation in TEF for a group of subjects must be considered when designing protocols to measure changes in TEF during an intervention.

CHAPTER 4

Reproducibility And Reliability Of

Dual-Energy X-Ray Absorptiometry For Measuring Body Composition

4.1 SUMMARY

This chapter discusses the findings of a study that evaluated the reproducibility and reliability of dual-energy X-ray absorptiometry (DEXA) for measuring whole body composition, in our laboratory. On two occasions ~7 days apart, measurements of total lean mass (in kg), total fat mass (in kg) and body fat percentage were made using DEXA (Norland™ XR36 densitometer) in healthy Caucasian men (n = 3) and women (n = 5) with a wide weight range (BMI 20.9 to 33.5 kg/m²). There was no significant difference between repeated measurements for total lean mass, total fat mass, or body fat percentage. The mean (\pm SEM) intra-individual day-to-day variation was 2.05 \pm 0.30% for total lean mass, 2.34 \pm 0.73% for total fat mass, and 2.55 \pm 0.81% for body fat percentage. The intraclass correlation coefficient [index of reliability] was high for the measurements of body fat percentage, total fat mass and total lean mass (0.99 for all parameters). We conclude that DEXA is a reproducible and reliable method for measuring total body fat and lean mass in individuals that have a wide range of body weight.

4.2 INTRODUCTION

Accurate data on the change body composition are essential to the prescription and evaluation of the efficacy of clinical weight loss interventions, and therefore it is imperative that reproducible and reliable methods are used to measure body composition. A variety of body composition methods are available each with specific advantages and limitations.

For many years, underwater weighing (or hydrostatic weighing) has been used for the analysis of body composition and is commonly used to assess the accuracy of newer techniques (Sohlstrom et al. 1993; Fowler et al. 1991; Snead et al., 1993; Tothill et al. 1994). However, this method, involving the immersion of a subject underwater in a tank, is highly technical, time consuming and requires subject co-operation. Total body water

using isotope dilution (Lukaski and Johnson, 1985) is a second method for measuring whole body composition but it involves the administration of a known tracer (18-oxygen, deuterium, or tritium), requires a mass spectrometer, and is technically demanding. Computed tomography (Borkan et al. 1982) and magnetic resonance imaging (Engstrom et al. 1991) have the capacity to measure the mass of individual tissues, organs, or body segments. However the instruments are expensive, of limited availability, require specialist technicians to operate, and whole body analysis may take up to an hour for computed tomography and up to 3 hours for magnetic resonance imaging (Jebb and Elia, 1993). In addition, computed tomography involves a considerable radiation dose to the subject, and the measurement of whole body fat mass using magnetic resonance imaging has low precision (5-7% CV) (Jebb and Elia, 1993). Dual-energy X-ray absorptiometry is a relatively new method that is now frequently used in major hospitals and many large body composition laboratories around the world. The advantage of DEXA as compared to underwater weighing, total body water, and magnetic resonance imaging is that it is capable of simultaneously measuring whole body as well as regional tissue mass, bone mineral density and bone mineral content, with high precision. Body composition measurements made using DEXA take 20 minutes or less, results are available immediately, and the method is not as technically demanding as the aforementioned methods (Jebb and Elia, 1993). Furthermore, the dose of radiation provided by one DEXA scan is 0.18 μ SV, which is less than the amount of radiation provided by one chest x-ray (7 μ SV).

The principle underlying DEXA is the measurement of differential tissue attenuation of two energy levels of X-rays as they pass through the body (Mazess, 1989; Nord and Payne, 1995). DEXA is able to discriminate the X-ray absorbing characteristics of the body and minimizes errors from soft-tissue inhomogeneities and irregular body contours to provide measurements of soft-tissue mass and bone mineral content (also referred to as

bone mineral mass). Soft tissue mass is further divided into fat mass and lean mass using an equation resulting from the calibration of tissue-fat and water-fat mixtures in phantoms (Nord and Payne, 1995). The reproducibility of measurements for total lean mass, fat mass, and body fat percentage using DEXA is high; intra-individual day-to-day variations of approximately 1 to 4% have been reported (Hansen et al. 1999; Ley et al. 1992; Pritchard et al. 1993; Snead et al. 1993; Tothill et al. 1994). Several studies that compared the accuracy of DEXA to underwater weighing or total body water have observed strong correlations (>0.9) and mean relative errors of less than 3% (with limits of agreement being less than $\pm 9\%$) for the measurements of lean mass, body fat percentage and fat mass (Pritchard et al. 1993; Wellens et al. 1994).

The aim of the study described in this chapter was to evaluate the reproducibility and reliability of dual-energy X-ray absorptiometry (DEXA) using a Norland™ XR36 densitometer for measuring body fat percentage, total fat mass and total lean mass, in 5 men and 3 women with a wide weight range. As defined previously (in Chapter 3) “reproducibility” refers to the variability of a particular measurer using a particular method for the measurement of a given variable within the same subject. The term “reliability” has the same features as reproducibility but it also takes in account the variability between subjects.

4.3 RESEARCH DESIGN & METHODS

4.3.1 Subjects

Ten Caucasian members of staff (4 men and 6 women) over the age of 18 years with a body mass index $> 19 \text{ kg/m}^2$, were recruited from within the Endocrine Unit at the Royal Adelaide Hospital and the Department of Medicine at the University of Adelaide. Subjects with cardiovascular, renal, liver or respiratory disease, hypertension, diabetes mellitus, or hyperthyroidism were excluded from participating. None of the subjects were taking any

medications or supplements. Two subjects (1 man and 1 woman) did not have their second DEXA scan because of work commitments.

4.3.2 Experimental design

Measurements of body weight, percentage body fat, total body fat mass and total body lean mass were made in each subject on two occasions, at least 7 days apart. On each occasion, measurements were performed at the same time of the day. Subjects were asked to refrain from eating and drinking tea or coffee for at least 4 hours prior to the measurement. No alcohol was permitted for 24 hours prior to the DEXA measurement and subjects were asked to refrain from vigorous exercise for 12 hours prior to the measurement being made. The same member of staff at the Endocrine Unit, Royal Adelaide Hospital performed the repeated measurements for DEXA on each subject.

4.3.3 Measurements

4.3.3.1 Body weight

Body weight was measured at the Endocrine Unit using a digital scale accurate to within \pm 0.5 kg.

4.3.3.2 Body composition

Whole-body bone mineral content, lean and fat masses, and percentage body fat were determined by DEXA as described in Chapter 2.3.5.1.

4.3.4 Statistical analysis

All data are presented as means \pm SEM, unless specified otherwise as (\pm SD). Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). Reproducibility between the first and second body composition measurements (i.e. the intraindividual day-to-day variation or coefficient of variation [CV]) was determined

as described in Chapter 3.3.4. A mean difference that falls within the 95% confidence intervals, and an intra-individual day-to-day variation of < 5% for all variables would suggest that the methods were reproducible and could confidently be used measure body composition. The reliability of the DEXA measurements (i.e. the intraclass correlation coefficient [ICC] was determined as described in Chapter 3.4.4. Significance was set at $p < 0.05$.

4.4 RESULTS

4.4.1 Subject characteristics

The physical characteristics of the subjects who completed the study are presented in Table 4.4.1. Body weight remained stable from the first to the second measurement period (75.6 ± 5.0 vs 75.6 ± 5.1 kg).

4.4.2 Whole body bone mineral content, lean mass, fat mass, and percentage body fat as measured by DEXA

The whole body bone mineral content was similar on the first as compared to second measurement period (2.88 vs 2.87 kg, mean difference \pm SD was 0.004 ± 0.062 kg, 95% CI -0.048 to 0.056 kg, $p = 0.86$). The within-subject day-to-day variation was $1.2 \pm 0.32\%$ [range 0.05 to 3.1%] and the intraclass correlation coefficient was 0.98.

The values for repeated measurements of total lean mass, total fat mass and percentage body fat as measured by DEXA are presented in Table 4.4.2. There was no difference in the repeated measurements for total lean body mass (44.0 vs 43.9 kg, mean difference \pm SD was 0.113 ± 1.48 kg, 95% CI -1.1 to 1.35 kg, $p = 0.84$). The mean intra-individual CV for lean mass was $2.05 \pm 0.30\%$ and the ICC was 0.99. Total body fat mass was similar at the first as compared to the second measurement period (28.7 vs 28.9 kg, mean difference \pm SD was -0.149 ± 1.39 kg, 95% CI -1.31 to 1.01 kg, $p = 0.77$). The CV for fat mass was

$2.34 \pm 0.73\%$ and the ICC was 0.99. There was no difference in body fat percentage on the two separate occasions (38.3 vs 38.3%, mean difference \pm SD was $0 \pm 1.69\%$, 95% CI – 1.41 to +1.41%, $p = 1.0$). The mean intra-individual day-to-day variation was $2.55 \pm 0.81\%$. The ICC for body fat percentage was 0.99.

For all subjects, total body weight computed as the sum of the whole body bone mineral content, lean mass and fat mass equalled 75.6 ± 5.5 kg (mean \pm SD; range 58.4 to 98.9 kg) and was not significantly different from body weight measured using the scales (77.6 ± 4.6 kg).

Table 4.4.1 Physical characteristics of the 5 women and 3 men who completed the study.

Subject (female/male)	Age (yrs)	Weight (kg)	Height (m)	BMI (kg/m²)
1 (F)	35	64.0	1.75	20.9
2 (F)	24	58.0	1.61	22.4
3 (F)	38	69.0	1.74	22.8
4 (F)	36	58.0	1.57	23.5
5 (F)	34	92.0	1.68	32.6
6 (M)	31	82.5	1.79	25.8
7 (M)	41	85.0	1.72	28.7
8 (M)	59	98.0	1.71	33.5
Mean ± SEM	37 ± 3.2	75.6 ± 4.9	1.70 ± 0.02	26.3 ± 1.5

The values presented for body weight are the average of repeated measurements made on the two separate occasions.

There was no difference between the two measurements ($p > 0.5$).

Table 4.4.2 Repeated measurements and the intra-individual day-to-day variations for whole body composition as measured by DEXA for the 5 women and 3 men who completed the study.

Subject (Female/Male)	Lean mass (kg)			Fat mass (kg)			Body fat (%)		
	Measure 1	Measure 2	CV	Measure 1	Measure 2	CV	Measure 1	Measure 2	CV
1 (F)	42.5	43.9	2.3	18.4	18.2	0.7	29	28	2.5
2 (F)	34.0	34.8	1.7	22.0	21.6	1.4	38	37	1.9
3 (F)	33.0	31.5	3.4	31.12	32.3	2.7	46	48	3.0
4 (F)	26.3	27.1	2.1	29.3	29.1	0.6	50	49	1.4
5 (F)	42.8	42.1	1.1	45.6	44.7	1.5	50	50	0
6 (M)	57.3	58.8	1.8	21.5	20.0	5.1	26	24	5.7
7 (M)	54.2	53.6	0.9	28.0	28.3	0.8	33	33	0
8 (M)	62.1	59.5	3.0	33.7	36.7	5.9	34	37	6.0
Mean ± SEM	44.0 ± 4.5	43.9 ± 4.4	2.1 ± 0.3	28.7 ± 3.1	28.9 ± 3.2	2.3 ± 0.7	38.3 ± 3.1	38.3 ± 3.4	2.6 ± 0.8

Measure 1 and measure 2 are measurements of whole body lean mass, fat mass, and body fat % that were made ~ 7 days apart.

Paired t-tests indicated there were no significant differences between repeated measurements.

Intra-individual day-to-day variation was expressed as the coefficient of variation [CV = (SD/mean)*100].

4.5 DISCUSSION

A reproducible and reliable measurement of whole body bone mineral content is essential to the precision and accuracy of measuring body composition using DEXA, because a reduction in the whole body bone content can lead to a decrease in lean body density and therefore an underestimation of total lean body mass and an overestimation of total body fatness. In this study, the measurement of bone content was highly reproducible and reliable with a mean intra-individual day-to-day variation of 1.1% (~ 0.03 kg) and reliability index of 0.95. This result confirms others who have reported that the repeated measurement of bone mineral content within-subjects has a variation of ~1.0 % (Mazess et al. 1990; Pritchard et al. 1993).

In this group of 3 men and 5 women, the measurements of total lean mass, total fat mass, and body fat percentage were reproducible with mean day-to-day variations of 2.1% (~0.9kg), 2.3% (~0.7kg) and 2.6% (~1%), respectively. These findings are similar to Ley et al. (1992) who found day-to-day variations of $1.8 \pm 0.54\%$ for total lean mass and $2.9 \pm 1.2\%$ for total fat mass in 5 subjects who had measurements made on four separate occasions over an 8-week period. Snead and colleagues (1993) also reported that repeated measurements of body fat percentage over a 3-week period had a mean within-subject day-to-day variation of $1.5 \pm 1.0\%$ in 12 women aged 60 to 74 years.

Although the mean day-to-day variations in body composition measurements are similar to previously reported findings, the variation observed in total fat mass and body fat percentage was greater than 5% for subjects 7 and 8 who had BMIs of 33.5 and 25.8 kg/m², respectively. The reason for the increased variation is not clear. It could be due to an error in the proportion of total lean mass that is bone and therefore the variability in fat mass may have been increased (Laskey et al. 1992; Wellens et al. 1994). Other potential sources of error in DEXA measurements may include the attenuation coefficients for soft

tissue and bone due to DEXA's sensitivity to the tissue thickness of the body; especially for larger adults (Blake et al. 1992; Johnson and Dawson-Hughes, 1991). Anteroposterior thicknesses between 10 to 20 cm may be associated with a variation in percentage body fat expressed relative to soft tissue of 3 to 4%, and for tissue thicknesses greater than 20 cm the error may exceed 5% (Wellens et al. 1994). Changes in the hydration of soft tissue may also introduce error in the estimation of fat mass (Pietrobelli et al. 1998; Roubenoff et al. 1993); however hydration including fluid and electrolyte balance remains remarkably stable in healthy subjects and it is unlikely that it had an effect in this study. Body size and the height of a subject may also compromise the accuracy of body composition analysis using DEXA. For the Norland™ XR36 densitometer the scanning area is approximately 190 cm by 67 cm. For this reason it should not be used for persons who are grossly obese (i.e. BMI greater than 43 kg/m²) or taller than 190 cm.

The reproducibility of whole body DEXA is very good and the values of total fat and lean mass compare favourably to underwater weighing and total body water, on a group basis (Pritchard et al. 1993; Wellens et al. 1994). However, there is still some concern regarding the absolute accuracy of the different DEXA densitometers. The three manufacturers of DEXA machines are Norland, Hologic and Lunar. All three manufacturers use different algorithms and material for calibration, and accordingly they may yield different results. Tothill and colleagues have shown that body fat measurements determined using Norland densitometers is greater than that calculated using Lunar and Hologic densitometers by approximately $6.3 \pm 1.8\%$ (mean \pm SD) fat (Tothill et al. 1994). There is very little data comparing absolute measurements to chemical analysis of cadavers and therefore further research is required to determine which machine provides the most accurate body composition analysis. Accordingly, differences of fat proportion recorded by the three DEXA instruments should preclude interchangeability of machines for individuals or in clinical trials.

4.6 CONCLUSION

Measurements of total lean mass, total fat mass and body fat percentage as determined by DEXA using the Norland™ XR36 densiometer are reproducible and reliable, in men and women with a wide range of body weights. The mean within-subject day-to-day variation in the measurement of total lean and total fat masses was less than 2.3%. Accordingly, DEXA will be suitable to detect an approximate 5% reduction in lean mass and a 15% reduction in fat mass after a dietary intervention that should induce a weight loss of approximately 8% over 16 weeks.

CHAPTER 5

The [¹⁴C]-Sodium Bicarbonate-Urea Method For Measuring Total Energy Expenditure

5.1 SUMMARY

A focus of my PhD research was to investigate the effects of energy restriction and dietary macronutrient composition on weight loss and energy expenditure. Accordingly, a major aim of my PhD studies was to measure total energy expenditure using the [^{14}C]-sodium bicarbonate-urea method as described by Elia et al. (1995). This chapter describes the principle of the [^{14}C]-bicarbonate-urea method for measuring total energy expenditure. The assay for measuring the specific activity of urinary urea (Elia et al. 1995) from which total energy expenditure is indirectly determined, was established in our laboratory. Several steps within the protocol were modified. The modifications and reasons for them are discussed. Studies that were performed to determine the reproducibility and reliability of the method are described in Chapter 6.

5.2 INTRODUCTION

Studies in the American Pima Indians (Ravussin et al. 1988; Ravussin and Swinburn, 1993), and monozygotic and dizygotic twins (Bouchard and Tremblay, 1997; Dionne et al. 1999; Stunkard et al. 1986) provide strong evidence that a low total energy expenditure is, at least in part, under genetic control and may be implicated in the development and maintenance of obesity. In addition, several studies (Bessard et al. 1983; Leibel et al. 1995; Ravussin et al. 1985) have demonstrated that total energy expenditure decreased after significant diet-induced weight loss.

The most widely used methods currently available for measuring total energy expenditure are doubly labeled water and whole-body indirect calorimetry. Doubly labeled water uses the stable isotopes deuterium (^2H) and oxygen-18 (^{18}O) to accurately measure CO_2 production and energy expenditure over extended periods (typically 2 to 3 weeks), while individuals continue to live in their usual environment (Prentice et al. 1985; Prentice et al. 1984; Schoeller, 1999; Schoeller and Hnilicka, 1996). Doubly labeled water cannot

accurately assess CO₂ production over very short periods (i.e. 1-day periods or multiples thereof) so that changes in energy expenditure can be evaluated during acute dietary interventions. Furthermore, access to a mass spectrophotometer is required, and this coupled with the high cost of the stable isotopes, particularly oxygen-18, continues to limit the widespread use of doubly labeled water. Whole-body calorimetry is capable of measuring total energy expenditure over any time period (Astrup et al. 1990; Murgatroyd et al. 1987; Ravussin et al. 1986; Toubro et al. 1995), but requires specialised equipment that is expensive to build and maintain. Subjects are confined within a respiratory chamber and consequently the number that can be studied is limited.

A relatively new alternative to doubly labeled water and whole-body indirect calorimetry is the [¹⁴C]-sodium bicarbonate-urea method. The [¹⁴C]-bicarbonate-urea method is unique because it allows the assessment of CO₂ turnover and total energy expenditure under free-living conditions over a period of 24 hours (or multiples thereof) (Elia et al. 1995). Consequently, the method can potentially be used to measure changes in human energy expenditure during a variety of acute or short-term chronic dietary interventions, as well as to assess changes in the energy expenditure of individuals during periods of illness. The aims of this chapter are to: 1) describe the principle of the [¹⁴C]-bicarbonate-urea method and summarize the literature supporting its use, and 2) describe the development of method as it was used for the studies in this thesis.

5.3 PRINCIPLE AND BACKGROUND OF THE [¹⁴C]-BICARBONATE-UREA METHOD

5.3.1 Principle behind the [¹⁴C]-bicarbonate-urea method

The [¹⁴C]-bicarbonate-urea method allows the assessment of CO₂ turnover and energy expenditure in humans under free-living conditions of 24 hours (or multiples thereof) (Elia et al. 1992; Elia et al. 1995; Leijssen and Elia, 1996). It is an isotopic dilution technique

whereby a known concentration of labeled CO_2 (given as ^{14}C -bicarbonate) is continuously infused under the skin of the abdomen over 48 hours (or longer if energy expenditure is determined over multiple 1-day periods). A small bolus dose of ^{14}C -urea may also precede the continuous infusion of ^{14}C -bicarbonate to help establish a value for urea specific activity that is similar to the near steady-state value (Elia et al. 1995). The ^{14}C -bicarbonate gets incorporated into the body's CO_2 pathways and is diluted by the endogenous CO_2 pools. To ensure that the infused ^{14}C -bicarbonate reaches a near-steady state within the body's CO_2 pool, it is recommended that the infusion should commence at least 15 to 24 hours before the measurement of energy expenditure is to be made. Once the infused ^{14}C -bicarbonate reaches equilibrium with the body's endogenous CO_2 pools, approximately 95% of it is excreted as breath CO_2 and 1.5% as urinary urea (additional label is lost as acid-labile CO_2 in the urine and feces [$\sim 0.5\%$], and a small amount is considered to get fixed into fat, carbohydrate and protein and possibly enter CO_2 pools that turn over very slowly) (Elia et al. 1995; Leijssen and Elia, 1996).

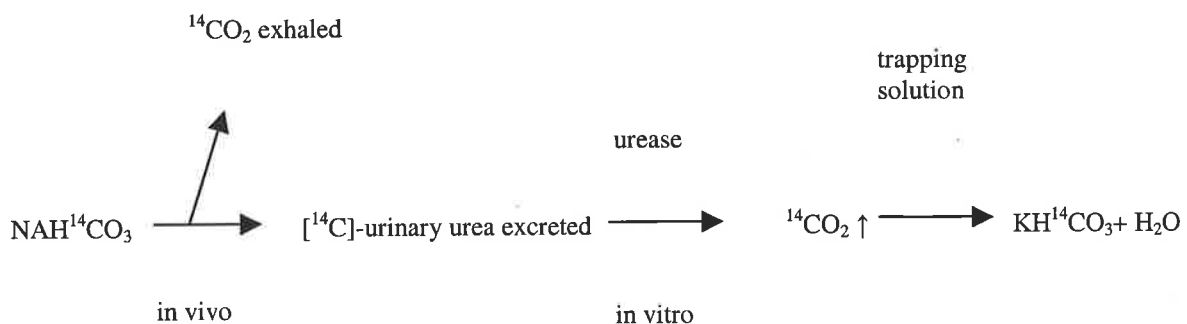


Figure 5.3.1 The fate of the infused ^{14}C -bicarbonate during the determination of the amount of CO_2 produced over a 1-day period. A 24-hour urine sample is collected from which a known amount of urinary urea (~ 12 mmol) is degraded to CO_2 using urease. A known quantity (~ 2.5 mmol) of the degraded CO_2 is trapped in a trapping agent that contains an indicator.

The specific activity of the trapped CO₂ (which reflects the specific activity of urinary urea) is measured using a liquid scintillation counter and the total amount of CO₂ produced over 24 hours is computed using equation [i] below (Elia et al. 1995):

$$\text{CO}_2 \text{ production (mol/day)} = \frac{0.95 \times 0.85 \times \text{infused bicarbonate (dpm/day)}}{\text{specific activity of urea (dpm/mol of CO}_2\text{)}} \quad [i]$$

The calculation of CO₂ production is based on two assumptions: that (i) the recovery of [¹⁴C] exhaled as gaseous CO₂ is 95% of the total amount of [¹⁴C] that was infused (Elia et al. 1992), and (ii) the specific activity of urinary urea is equivalent to 85% that of CO₂ in arterialised blood or breath (Fuller and Elia, 1989). Thereafter, total energy expenditure is calculated based on the assumption that CO₂ has an energy equivalent of 535 kJ/mol (this approximates the value obtained in subjects close to nutrient balance while consuming a diet with a food quotient of ~0.85) (Elia, 1991).

The accuracy of the [¹⁴C]-bicarbonate-urea method and evidence for the assumptions on which the calculation of net CO₂ production and total energy expenditure is based is discussed below in section 5.3.2.

5.3.2 Accuracy of the [¹⁴C]-bicarbonate-urea method

Three validation studies by Elia and colleagues have shown that the values of CO₂ production and energy expenditure measured using the bicarbonate-urea method are within 2 to 6 % of those obtained by whole-body indirect calorimetry over a 1-day or 4-day period (Elia et al. 1992; Elia et al. 1995; Gibney et al. 1997). In the first study (Elia et al. 1992), six healthy males received a constant infusion of [¹⁴C]-bicarbonate [500 μCi] administered over 36 hours while confined to a whole-body calorimeter. Elia et al. (1992) showed that the 95.6 ± 1.1% (SD) of the infused label was recovered in breath CO₂ between 12 and 36 hours (evidence for assumption 1), and that the values obtained for

total CO₂ production per day and total energy expenditure were approximately $\pm 6.0\%$ of the values measured within the respiratory chamber. In the second study (Elia et al. 1995) five healthy male subjects received a subcutaneous infusion of [¹⁴C]-bicarbonate (12.3 μ Ci/day) while in a whole-body calorimeter for 5 days. Similarly, the continuous daily collections of calorimeter air confirmed that $95.6 \pm 1.3\%$ of the infused label (substantially less label was infused in this study as compared to the first) was recovered as breath CO₂, and that the specific activity of urinary urea was approximately 85% of gaseous CO₂ (evidence for assumption 1 and 2). The estimated CO₂ production and total energy expenditure, calculated from the specific activity of urea in 24-hour urine samples was found to be $100 \pm 5.0\%$ of the calorimeter estimate for 1-day periods (20.8 \pm 2.1 mol CO₂/day and 11.1 \pm 1.1 MJ/day) and $100 \pm 2.0\%$ for 4-day periods (20.8 \pm 1.44 mol CO₂/day and 11.1 \pm 0.8 MJ/day). In the third validation study (Gibney et al. 1997), the method was applied to 8 free-living patients with small-cell lung cancer, for a period of 1 or 2 days. In five of these patients, Elias' group again showed that the 24-hour recovery of label from breath CO₂ was $95.6 \pm 0.5\%$ (evidence for assumption 1). The CO₂ production per day measured using [¹⁴C]-bicarbonate-urea was $102.1 \pm 3.4\%$ of that measured by whole-body indirect calorimetry, and total energy expenditure was $101.5 \pm 3.8\%$.

Fuller and Elia (1989) initially demonstrated that the specific activity of urinary urea is equivalent to 85% that of CO₂ in arterialised blood or breath (evidence for assumption 2). They compared the specific activity of urea, and that of CO₂ in breath, in urine and in arterialised blood, during a 36-h continuous infusion of 500 μ Ci of sodium bicarbonate into six normal male volunteers. After a period of equilibration, the mean specific radioactivity of urea was found to be only 16% below that of end expiratory CO₂ and a similar amount below that of CO₂ both in arterialised blood and in urine. This difference may be explained by isotopic dilution of ¹⁴CO₂ by metabolic CO₂ produced in the splanchnic tissues.

When subjects are close to nutrient balance (i.e. when the oxidation of fat, carbohydrate and protein match the intake of each, and which is indicated by a food quotient of approximately 0.85 for the diet) it may be assumed that the energy equivalent of 1 mol of CO₂ is 535 kJ (Elia et al. 1995; Gibney et al. 1997; Paton et al. 1996). In a group of 63 individuals living in a UK village, Elia (1991) demonstrated that the mean group value for the energy equivalent of CO₂ predicted the individual values to within 5%. Consequently, for individuals who have a food quotient of ≤ 0.8 or ≥ 0.9 , the error in measurement of energy expenditure would exceed 600 kJ/day or 6% (based on a person that produces 20 mol of CO₂ per day). However, of all the subjects who participated in the studies described in Chapters 6, 7 and 9, there were only 2 individuals who had a food quotient equal to 0.80; the remaining individuals had a value between 0.82 and 0.88. Accordingly, the error in the measurement of energy expenditure associated with the energy equivalent of 535 kJ/day would be less than $\pm 3\%$. Since the same energy equivalent was used at all measurement periods within the studies described Chapters 6, 7, and 9, and because the mean food quotient of the diets were similar at these time points (i.e. Chapter 6, 0.81 vs 0.82 for the non-obese and 0.84 vs 0.84 for the obese subjects; Chapter 7, 0.84 vs 0.85; and Chapter 9, 0.85 vs 0.85), the error would have been systematic and would not have affected our results.

The aforementioned validation studies for the [¹⁴C]-bicarbonate-urea method suggest that the assumptions on which the calculation of CO₂ production per day and total energy expenditure are dependent on, are robust, at least in healthy human populations. Errors associated with: i) the preparation and administration of the [¹⁴C]-bicarbonate solutions, ii) the assay to measure the specific activity of excreted urinary urea, and iii) the choice of energy equivalent for CO₂, should be minimized. As long as this is done the [¹⁴C]-

bicarbonate-urea method should provide a reproducible and reliable alternative to measure total energy expenditure.

5.4 THE [¹⁴C]-BICARBONATE-UREA METHOD IN OUR LABORATORY

The following section describes all aspects of the [¹⁴C]-bicarbonate-urea method as it was used for measuring total energy expenditure in the studies described within Chapters 7 and 9 of this thesis. Specifically, the preparation of the [¹⁴C]-urea and [¹⁴C]-bicarbonate solutions, and the protocols for the 48-hour infusion and 24-hour urine collection are outlined. Although the assay to determine the specific activity of urinary urea was based on the principle described by Elia and colleagues (1995), the apparatus used within our laboratory was different and therefore several steps of Elia et al.'s protocol were modified. As a consequence, the length of the assay per urine sample was shortened from ~3 hours for Elia et al. (1995) to ≤50 minutes, which means that 3 times the number of samples can be analysed in one day.

5.4.1 Preparation of the [¹⁴C]-urea and [¹⁴C]-bicarbonate solutions

Stock solutions of [¹⁴C]-urea and [¹⁴C]-bicarbonate were prepared before each study. Labeled-urea (250 μCi; Amersham Pharmacia Biotech; Buckinghamshire, UK) was reconstituted in 1 L of water for irrigation using aseptic technique to make a stock solution of 0.25 μCi/ml. Labeled-bicarbonate (1 mCi; Amersham Pharmacia Biotech; Buckinghamshire, UK) was reconstituted in 400 ml of water for irrigation to which 40 ml of 8.4% sodium bicarbonate was added to buffer the [¹⁴C]-bicarbonate solution (pH ~8) so that CO₂ did not form during preparation and storage. The reconstituted [¹⁴C]-urea and [¹⁴C]-bicarbonate solutions were passed through a 0.2 micron filter and aliquoted as 1 ml and as 8 ml aliquots, respectively. Aliquots were stored at -20°C until shortly before use.

Given that some loss of activity usually occurs during the preparation of radiolabeled solutions, the actual activity of the reconstituted [^{14}C]-urea and [^{14}C]-bicarbonate solutions was measured to determine whether it remained the same as the theoretical activity (i.e. the activity of the un-reconstituted product). Accordingly, the activity per 1 ml of solution was assessed before use. On several occasions, the activity of the reconstituted [^{14}C]-bicarbonate and [^{14}C]-urea solutions was less than the amount present in the un-reconstituted product. This highlights the importance of measuring the actual activity of the stock solutions that were going to be infused into subjects. After several months of storage at -20°C , there was no further loss of activity from the reconstituted aliquots of either solution. Each new batch of [^{14}C]-bicarbonate and [^{14}C]-urea solution was also tested for sterility and pyrogens (Australian Radioisotopes; Lucas Heights, NSW) to ensure that they were safe for use in humans.

5.4.2 Administration of [^{14}C]-bicarbonate-urea

An area of skin on the abdomen was anaesthetized with 2% lignocaine hydrochloride (Delta West Pty Ltd; Bentley, Western Australia) and a small flexible infusion cannula (MMT-316 Sof-set Infusion Set [60 cm tube]; MiniMed Technologies; West Chatswood, NSW, Australia) was inserted into the subcutaneous layer of fat while subjects lay in the supine position. A bolus priming dose of $0.205\ \mu\text{Ci}$ of ^{14}C -urea (concentration of $0.205\ \mu\text{Ci}/\text{ml}$) was administered and thereafter the infusion cannula was connected to a 20 ml syringe (Terumo Syringe with Leur Lock tip; Terumo Corporation, Springfield SA, Australia) that contained 15 ml of [^{14}C]-bicarbonate solution (the concentration of which is described below). The labeled bicarbonate solution was administered over 48 hours (i.e. from $\sim 9.30\ \text{am}$ on day 1 [immediately after a measurement of REE was made] to $\sim 9.30\ \text{am}$ on day 3) at constant infusion rate of approximately $0.3125\ \text{ml}/\text{hr}$ using a mini-pump syringe driver (SIMS Graseby MS16A Syringe driver; SIMS Australasia PTY.Ltd; Bundall QLD, Australia) that was worn in a pouch around the waist or over the shoulder.

In order to evaluate the precise volume and total dose of [^{14}C]-bicarbonate solution that was delivered over 48-hours, the 20 ml syringe containing the 15 ml of solution was weighed before and after the infusion was commenced, to the nearest 0.1 g.

In the study described in Chapter 6, the concentration of the [^{14}C]-bicarbonate solution administered to the non-obese subjects was 2.23 $\mu\text{Ci/ml}$ and for the obese subjects it was 1.86 $\mu\text{Ci/ml}$. For both of the studies described in Chapters 7 and 9, the concentration of [^{14}C]-bicarbonate was 1.74 $\mu\text{Ci/ml}$. Therefore, the dose of radioactivity administered was 15.4 and 13.0 $\mu\text{Ci/day}$ for the non-obese and obese subjects in Chapter 6, and 12.4 $\mu\text{Ci/day}$ for those subjects who completed the studies in Chapters 7 and 9. Within the respective studies, the total dose of activity administered to each individual was the statistically the same and accordingly the comparison of total energy expenditure before and after weight loss within-subjects was justified. Disparity in the dose of the [^{14}C]-bicarbonate solution given to the non-obese as compared to the obese individuals would not have affected the results discussed in Chapter 6 because the difference in energy expenditure between the two subject populations was not compared. For the studies within this thesis, the dose of radioactivity administered was based on the studies performed by Elia and colleagues; 5 healthy male subjects aged 34 ± 10 years with a BMI between 20 to 25 kg/m^2 were given 12.3 μCi of [^{14}C]-bicarbonate per day (Elia et al. 1995); 10 males with HIV who were aged 26 to 45 years and had a BMI of 15 to 30 kg/m^2 were given approximately 17 μCi of [^{14}C]-bicarbonate per day (Paton et al. 1996); and, 5 men and 3 women with lung cancer who were aged 68 ± 10 years and had a BMI of $25.2 \pm 4.4 \text{ kg/m}^2$ were given approximately 11 μCi of [^{14}C]-bicarbonate per day (Gibney et al. 1997).

The effective whole-body dose of radiation that subjects received due to the infusion of [^{14}C]-bicarbonate-urea (and DEXA for Chapter 9) was 213 and 220 μSv respectively, for the studies reported in Chapters 6 and 9. Subjects who completed the studies in both

Chapters 6 and 7, received 323 μSv of radiation. The maximum dose of radiation represents 16% of the annual natural background radiation (2000 μSv) and is equivalent to 44 hours of aeroplane travel or 6 chest x-rays. The doses of [^{14}C]-bicarbonate-urea administered were considered to be safe for research purposes according to NHMRC radiation guidelines.

5.4.3 Twenty-four hour urine collection

The morning after the labeled bicarbonate infusion was commenced (i.e. day 2), subjects noted the time that they passed their first urine sample. Thereafter, all urine passed over the next 24 hours was collected in a 4L airtight container that contained no preservative. Urine collections were deposited to the laboratory on the day morning they were completed. The volume of the 24-hour collection was measured and aliquots were stored in smaller airtight vials with no preservative, at -20°C . At the end of each study, all samples from the same individual were analysed for the concentration of urea and creatinine (see Chapter 2.6.7.8), and for the specific activity of urea (see section 5.4.4).

5.4.4 Assay for measuring the specific-activity of urinary urea

The apparatus used to measure the specific activity of urinary urea is illustrated in Figure 5.4.2. The volume of urine containing ~ 12 mmol of urea was added to a 500 ml round bottom flask containing 100 ml of sodium citrate buffer (1M; pH 5.5) and 5 ml of citric acid (1M; pH 1.4). Variable amounts of distilled water were added to achieve a constant volume of 300 ml. An airtight rubber stopper was firmly fixed into the opening of the flask and the assay mixture was flushed with nitrogen (UHP Grade Compressed Gas, Air Liquide Adelaide, Australia) for five minutes (at 4L/min for the first 3 min and then at 0.5L/min for the remaining 2 min) through a gas inlet tube to remove traces of air from the assay mixture and flask; the air from the assay mixture leaves via the open gas outlet tube. Thereafter, the gas outlet tube was immersed in a 50 ml volumetric flask that contains 25

ml of ethanol (HGDAAs/F3; ACE Chemical company, Camden Park, South Australia) and a known volume of CO₂ trapping solution (KOH/ethanol with 0.01% phenolphthalein and 0.001% thymolphthalein indicators; Department of Nuclear Medicine, Royal Adelaide Hospital, Australia) which is capable of trapping exactly 2.5 mmol of CO₂. The concentration of the CO₂ trapping solution (generally ~0.25 M/L) was precisely determined by volumetric titration with 1 M HCL (AnalaR reagents; Kilsyth, Victoria, Australia). The addition of 4 ml of urease (1000 units/ml; urease powder from Jackbeans; Sigma Aldrich, Castle Hill, NSW) initiated the conversion of urinary urea to CO₂, which occurred under nitrogen (0.5 L/min). The reaction was complete when the trapping solution changed from purple to colourless indicating that 2.5 mmol of CO₂ was trapped. The trapping solution containing the trapped CO₂ was then made up to 50 ml with ethanol, and a 5 ml sample of the final volume was aliquoted into a 20 ml scintillation vial (5 ml aliquots were done in triplicate under nitrogen). To each scintillation vial, 10 ml of scintillation fluid (Starscint; Packard BioScience Company, Meriden, USA) was added and gently mixed. Thereafter, the specific activity within 2.5 mmol of CO₂ was counted (in duplicate) using a liquid scintillation counter (Beckman Instruments; Gladesville, NSW) and the value (which was corrected for the background radioactivity of the scintillation counter; see section 5.4.4.2) was incorporated into equation [i] section 5.3.1 to compute the net amount of CO₂ produced per day.

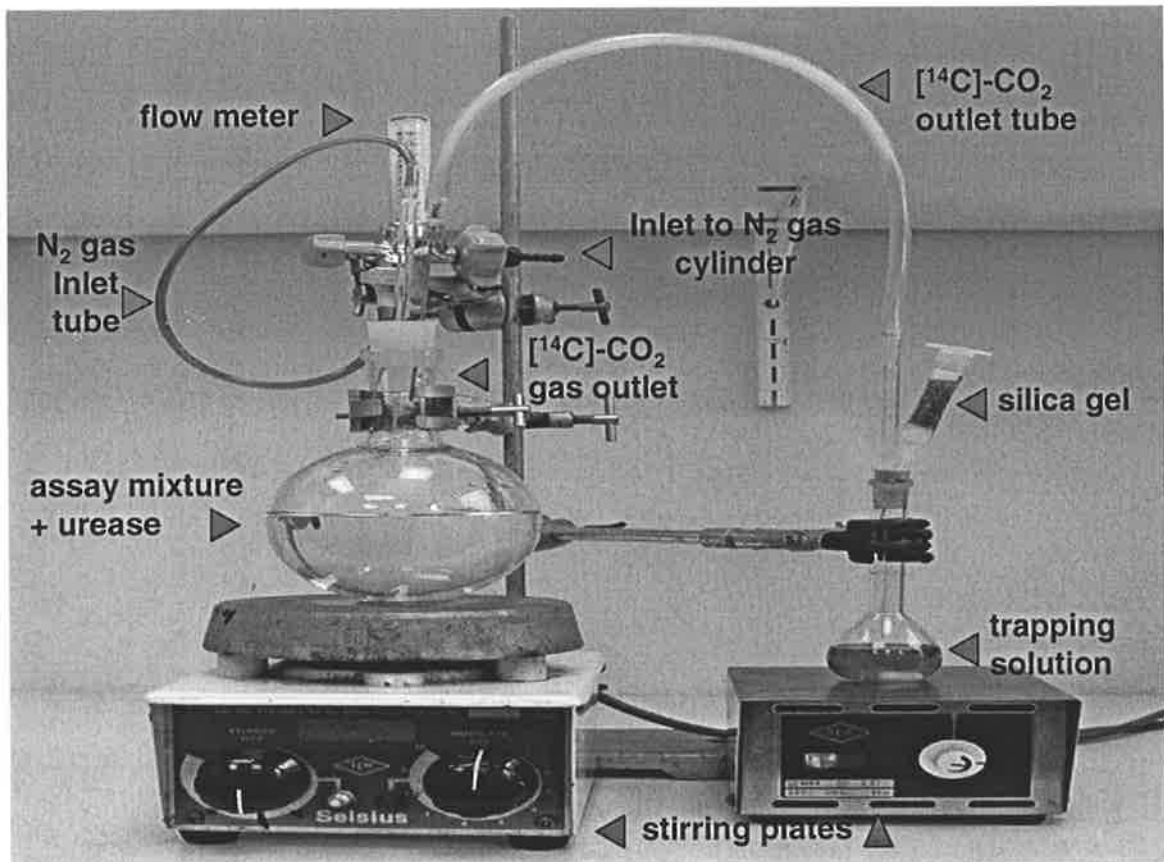


Figure 5.4.2 Apparatus used for the assay measuring the specific activity of urinary urea.

5.4.4.1 *Modifications made to the assay for measuring the specific activity of urinary urea and optimizing the assay conditions*

Several modifications were made to the assay for measuring the specific activity of urinary urea. First, the laboratory equipment was different to that described by Elia and colleagues (1995) and for that reason we had to alter the protocol for removing atmospheric CO_2 that was present in the assay mixture, before urinary urea could be converted to $^{14}\text{CO}_2$ by urease. Elia and colleagues (1995) used a vacuum pump for 60 to 90 minutes to remove atmospheric CO_2 from the assay mixture. In contrast, we flushed the apparatus (Figure 5.4.2) and assay mixture with nitrogen gas for 5 minutes (3 minutes at 4 L/min, followed by another 2 minutes at 0.5 L/min). To demonstrate that the apparatus removed all traces of CO_2 from the assay mixture, experiment 1 was performed.

Experiment 1: An assay mixture (containing 12 mmol of urinary urea [345 mmol/24h], 100 ml of citrate buffer, 5 ml of citric acid and 65 ml of distilled water) was flushed with nitrogen for 3 minutes at 4 L/min and then for a further 2 minutes at 0.5 L/min. Thereafter, the [¹⁴C]-CO₂ outlet tube was immersed into the 50 ml volumetric flask containing 2.756 mmol of trapping agent that had been titrated with 1 M HCL (Analar Grade; Kilsylh, Victoria, Australia) and shown to have a concentration of 2.483 M/L. The volumetric flask was made up to 50 ml with ethanol and therefore the concentration of the trapping agent within the volumetric flask was 0.0551 mmol/ml. Nitrogen gas (at 0.5 L/min) was bubbled through the assay mixture for a further 60 minutes. If excess CO₂ remained within the assay mixture after the initial 5-minute flushing period, then the concentration of the trapping agent would decrease because it would be pushed into the trapping agent during the subsequent 60 minutes. At the conclusion of the 60 minutes, three 2 ml aliquots of the trapping agent were titrated with HCL and it was found that the mean (\pm SEM) concentration was 0.0558 ± 0.002 mmol/ml. We concluded that the concentration of the trapping agent had not changed and therefore our method for removing atmospheric CO₂ from the assay mixture was successful.

A second modification to the assay was that the digestion of urinary urea to CO₂, using urease, was done under nitrogen. After the addition of urease to the assay mixture, the apparatus (Figure 5.4.2) was sealed and the pressure generated by feeding nitrogen into the apparatus forced the CO₂ liberated from the urinary urea through the [¹⁴C]-CO₂ outlet tube that was immersed in the 2.5 mmol of trapping solution. In contrast, Elia and colleagues (1995) relied on diffusion of the liberated CO₂ from the assay mixture into an open scintillation vial containing trapping solution that was suspended within the round bottom flask. Our technique for trapping the liberated CO₂ took between 12 to 40 minutes whereas Elia et al. (1995) reported that the assay took ~70 to 90 minutes (the time taken to trap 2.5 mmol of CO₂ depended on the properties of the urine that was being assayed e.g. pH).

Preliminary experiments revealed: (1) that the pH of the assay mixture affected the rate at which urease converted urinary urea to CO₂, and (2) that the rate of conversion of urinary urea to CO₂ was dependent on the amount of urease used. Therefore, experiments were performed to determine the optimal pH of the assay mixture, and the optimal dose of urease that would be used in all subsequent analyses.

Experiment 2: We hypothesized that during urease digestion, the pH of the assay mixture increases (due to the formation of ammonia and hydroxide ions) and when it becomes too basic the liberation of CO₂ from urea is inhibited. Therefore, the aim of this experiment was to determine whether lowering the pH of the assay mixture blunted the increase in pH that occurred during urease digestion, and reduced the time required to trap a known amount of CO₂. Three assay mixtures (final volume 300 ml) were prepared identically and all had a pH of ~5.97. Thereafter, one assay mixture had its' pH adjusted to 5.52 (with 1 M citric acid) and the other was adjusted to 6.55 (with 1 M NaOH). Urease (2 ml, 1000 U/ml) was added to the assay mixture and 0.5 mmol of CO₂ was trapped. Figure 5.4.3 shows that the time required to trap 0.5 mmol of CO₂ increased as the initial pH of the assay mixture increased to 6.55; the reason for this is presumably because the pH of the assay mixture rose to 8.97 during the urease reaction. Therefore, we concluded that the assay mixture must have an initial pH ≤5.5 in order to minimize the rise in pH during the conversion of urinary urea to CO₂.

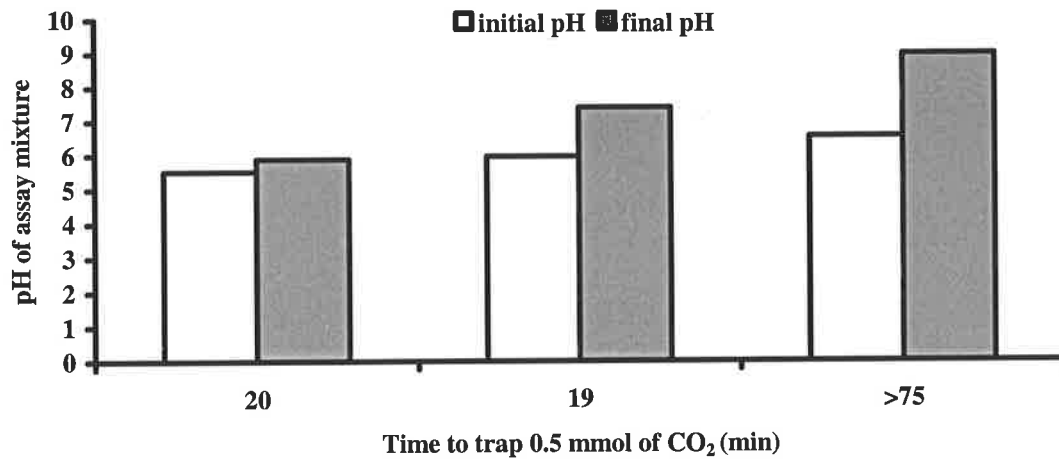


Figure 5.4.3 The effect of the initial pH of the assay mixture on the rate of urease activity

Experiment 3: To determine whether the rate of conversion of urinary urea to CO₂ was dependent on the amount of urease used. We hypothesized that doubling the dose of urease would halve the time required to trap an identical amount of CO₂. Two identical assay mixtures were prepared, and 2 ml of urease (1000 U/ml) was added to the first assay mixture and 4 ml was to the second mixture. The time required to trap 0.5 mmol of CO₂ was 20 minutes and 40 seconds when 2 ml of urease was used, whereas when 4 ml was used the time was halved to 10 minutes and 10 seconds. In a separate experiment we added 8 ml of urease to the assay mixture but the experiment had to be terminated, because excessive bubbling occurred and some assay mixture bubbled into the [¹⁴C]-CO₂ outlet tube. Therefore, we concluded that 4 ml (1000U/ml) was the optimal dose of urease required for the assay.

5.4.4.2 *Optimizing the conditions of scintillation counting*

Two experiments were performed to determine: (1) the background radioactivity of the scintillation counter, and (2) the coefficient of variation of the scintillation counter.

Experiment 1: To determine the background activity of the scintillation counter, 2.5 mmol of trapping agent (0.297 M/L) was aliquoted (under nitrogen) into a 50 ml volumetric flask that contained no activity. The trapping agent was neutralized by blowing through a straw into the flask until the solution was colourless, and an appropriate volume of ethanol was added to make the volume up to 50 ml. Thereafter, 5 ml aliquots of the neutralized trapping agent were aliquoted into three scintillation vials, and to each vial 10 ml of scintillation fluid was added. Each of the three scintillation vials was counted in duplicate to determine the amount background activity (i.e. [^{14}C]) present. The experiment was performed twice. The results showed that the mean \pm SEM background activity of the scintillation counter should be approximately 146.1 ± 1.97 disintegrations.min⁻¹.(dpm).vial⁻¹. To ensure this was the case, a new background was prepared each day test samples were analysed.

Experiment 2: To determine the coefficient of variation of the scintillation counter for measuring the specific activity of urea, eight 5 ml samples of trapped CO₂ were counted in duplicate. The mean coefficient of variation for the duplicate counts was $2.3 \pm 0.45\%$, range 0.21 to 4.4% (i.e. a difference of 0.7 to 17.8 dpm between the duplicates). From this experiment it was concluded that samples would be recounted when duplicate counts were different by more than 5% (i.e. ~20 dpm); this occurred rarely.

5.4.5 Calculating the amount of CO₂ produced per day and total energy expenditure

For the studies reported in Chapters 6, 7 and 9, CO₂ production and total energy expenditure was calculated as described above in section 5.3. As discussed previously in section 5.3.2, an energy equivalent of 535 kJ per mol of CO₂ was appropriate for our studies.

5.5 CONCLUSION

The [^{14}C]-sodium bicarbonate-urea method to measure total energy expenditure was successfully established following the principle and protocol described by Elia and colleagues. However, the apparatus used in our laboratory for the assay to determine the specific activity of urinary urea was different from that described by Elia's group and therefore several steps within the protocol were modified. Prior to the conversion of a known concentration of urinary urea to $^{14}\text{CO}_2$, all atmospheric CO_2 was flushed from the assay mixture using nitrogen gas. This modification reduced the assay time reported by Elia's group by approximately 55 to 85 minutes. The digestion of [^{14}C]-urinary urea to $^{14}\text{CO}_2$ was also done under nitrogen whereas Elia and colleagues relied on diffusion of the liberated $^{14}\text{CO}_2$ from the assay mixture into the trapping solution. A further 50 minutes was reduced from the assay time for measuring the specific activity of urinary urea. Hence, the modifications discussed in this chapter reduced the total length of the assay per urine sample from ~3 hours to ≤ 50 minutes.

In order to measure TEE precisely it is important to minimise the error associated with i) the preparation and administration of the [^{14}C]-bicarbonate solutions, ii) the assay to measure the specific activity of excreted urinary urea, and iii) the choice of energy equivalent for CO_2 . Subsequently, the study described in Chapter 6 was designed to determine the within-assay variation of the [^{14}C]-bicarbonate-urea method and the intra-individual day-to-day variation for the measurement of TEE, in both lean and obese subjects.

A limitation in developing the [^{14}C]-bicarbonate-urea method for measuring total energy expenditure in our laboratory was that we were not able to confirm the accuracy of the method against whole-body indirect calorimetry or doubly labeled water. New whole-body respiratory chambers at the University of Wollongong, Australia are almost complete. It is

intended that the measurement of 24-hour energy expenditure obtained using [^{14}C]-bicarbonate-urea will be validated against a measurement made in a whole-body indirect calorimetry in lean and obese subjects. We also intend to determine whether the [^{14}C]-bicarbonate-urea method can detect a marked increase in energy expenditure in response to different levels of physical activity. If the method can detect changes in total energy expenditure resulting from increased physical activity then it will be a valuable field technique.

CHAPTER 6

Reproducibility, Reliability And Suitability Of [¹⁴C]-Bicarbonate-Urea For Measuring Total Energy Expenditure In Non-Obese And Obese Subjects

6.1 SUMMARY

This study evaluated, in our laboratory, the reproducibility, reliability, and the suitability of the [^{14}C]-bicarbonate-urea method to measure TEE in free-living non-obese and obese individuals. On two occasions ~14 days apart, measurements of TEE and fasting resting energy expenditure (REE) [indirect calorimetry] were made in 8 non-obese men (mean \pm SEM age 50 ± 3.1 yrs; BMI 24.4 ± 1.3 kg/m²; fat mass 18.0 ± 4.2 kg; lean mass 61.3 ± 2.0 kg) and 15 obese men and women (6 men/ 9 women, age 48.3 ± 2.0 yrs, BMI 33.8 ± 1.8 kg/m², fat mass 42.0 ± 3.3 kg, lean mass 52.0 ± 3.9 kg). Energy expenditure due to physical activity (PAEE) was also computed [PAEE = $0.9(^{14}\text{C-TEE})$ -REE] and compared to activity diaries. The [^{14}C]-bicarbonate-urea method was evaluated using a questionnaire that rated suitability and comfort on a scale from 1 to 10. There was no significant difference in TEE on the two study days, in either the non-obese group or the obese group. The mean \pm SEM for TEE was 14844 ± 938 kJ/day (12187 to 20258 kJ/day) for the non-obese group and 11330 ± 837 kJ/day (range 8165 to 200048 kJ/day) for the obese group. The within-assay variation for the [^{14}C]-bicarbonate method was $3.9 \pm 0.31\%$ (range 1.7 to 6.0%) for the non-obese group, and $3.6 \pm 0.3\%$ (range 1.24 to 5.7%) for the obese group. The intra-individual day-to-day variation for the measurement of TEE was $4.8 \pm 1.0\%$ (range 0.95 - 9.24%) for the non-obese group, and $9.7 \pm 1.3\%$ (range 1.74 - 18.0%) for the obese group. The intraclass correlation coefficient was 0.9 for the non-obese group and 0.86 for the obese group, indicating that the method was reliable. Seventy-five percent of the non-obese and 73% of the obese subjects found that the method allows them to continue their normal lifestyle. We conclude that the [^{14}C]-bicarbonate-urea method is a reproducible and suitable method to measure TEE in both non-obese and obese populations. The day-to-day variation within some obese individuals is large. The reason for this is not clear, but it must be considered when designing protocols to measure changes in TEE.

6.2 INTRODUCTION

Total energy expenditure (TEE) has traditionally been measured using either whole-body indirect calorimetry (Astrup et al. 1990; Jequier et al. 1987; Ravussin et al. 1991; Rosenbaum et al. 1996; Rumpler et al. 1990; Seale et al. 1990; Toubro et al. 1995) or doubly labeled water (Prentice et al. 1985; Prentice et al. 1984; Roberts and Dallal, 1993; Schoeller and Hnilicka, 1996; Westerterp, 1998). Whole-body indirect calorimetry requires specialised equipment that is expensive to maintain, and individuals are confined within the artificial environment. It is, therefore, extremely difficult to study large groups of subjects using whole-body calorimetry. Doubly labeled water measures TEE in free-living individuals, but the measurement is made over 2-3 weeks. Furthermore, access to the stable isotopes deuterium (^2H) and oxygen-18 (^{18}O) and an isotope ratio mass spectrophotometer are required, and as a consequence the method is expensive.

As discussed in Chapter 5, [^{14}C]-bicarbonate-urea is a relatively new technique for measuring TEE that has been demonstrated to be as accurate as whole-body calorimetry and doubly labeled water (Elia et al. 1995; Gibney et al. 1997; Paton et al. 1996). The advantages of [^{14}C]-bicarbonate-urea are that it measures CO_2 turnover and TEE in humans under free-living conditions over 24-hours (or multiples thereof) and it does not require specialised equipment. Used in conjunction with a "Deltatrac" metabolic monitor to determine resting energy expenditure and the thermic effect of feeding, an estimate of energy expenditure due to physical activity can also be derived. Therefore, the [^{14}C]-bicarbonate-urea method may provide a potentially powerful investigative field tool for the assessment of energy requirements and for determining the components of TEE.

The aim of this study was to determine: (i) the reproducibility and reliability of repeated measurements of TEE using [^{14}C]-bicarbonate-urea, in free-living non-obese and obese subjects, (ii) the comparability of the [^{14}C]-bicarbonate method with TEE predicted using

an equation that is based on the factorial method for estimating daily energy requirements, and (iii) the effect of the [¹⁴C]-bicarbonate-urea method on daily activities and the suitability of the method to study participants. As previously discussed, “reproducibility” refers to the variability of a particular measurer using a particular method for the measurement of a given variable within the same subject. The term “reliability” has the same features as reproducibility but it also takes in account the variability between subjects.

6.3 RESEARCH DESIGN AND METHODS

6.3.1 Subjects

Two populations of subjects: i) 8 non-obese men, and ii) 15 obese men and women (6 men and 9 women). The reproducibility of the [¹⁴C]-bicarbonate-urea method was initially validated in non-obese male volunteers because the RAH human ethics committee was concerned by its’ use in women.

All subjects were recruited by public advertisement, and each attended a detailed information session and gave written informed consent to participate. Males were included if they were over 35 years of age, and women were included if they were postmenopausal or sterile. Subjects were classified as ‘non-obese’ if they had a BMI of 19-25 kg/m², or as ‘obese’ if their BMI was > 28 kg/m². Although the classification of ‘obesity’ recommended by the World Health Organization (WHO, 1997) is BMI ≥ 30 kg/m², a minimum cut-off of > 28 kg/m² for the ‘obese’ group was selected because we wanted to test the usefulness of the [¹⁴C]-bicarbonate-urea method in a population that was similar to the populations that we typically recruited for weight loss studies (i.e. a BMI > 27 kg/m²). Data from the American ‘National Health and Nutrition Examination Survey’ showed that a BMI of 27.8 kg/m² for men and 27.3 kg/m² for women were the cut-off points associated with a substantially increased risk of morbidity and mortality (Kuczmarski et al. 1994).

Exclusion criteria included malignancy; type 1 or 2 diabetes mellitus; renal or liver disease; hyperthyroidism; unstable cardiovascular, respiratory, gastrointestinal or disease; and significant weight loss in the month prior to commencing the study. None of the 8 non-obese subjects were taking any medication or supplements. Obese subjects on anti-hypertensive (2 subjects) or lipid lowering (1 subject) medication were asked to maintain all medication and supplements at pre-study doses.

6.3.2 Experimental design

Measurements of body weight, total energy expenditure (TEE), resting energy expenditure (REE), respiratory quotient (RQ), and energy expenditure due to physical activity (PAEE) were made in each subject on two occasions, separated by at least 14 days. A period of 14 days between the repeated measurements was chosen to ensure that the radioactive [^{14}C]-bicarbonate-urea was washed out of the subjects' system. On each occasion, subjects kept a 3-day food diary that commenced the day prior to the measurements being made and continued over the 48-hour [^{14}C]-bicarbonate-urea infusion. The food diaries were collected and later analysed to determine the subjects' energy and macronutrient intakes as described in Chapter 2.3.3.1. In addition, the food quotient (FQ) (as described in Chapter 2.3.3.2) of the subjects' diet over the 3 days was calculated. Three-day physical activity diaries were also collected to determine the subjects' daily activity levels [expressed as a multiple of REE] (see Chapter 2.3.1.4 for details). For the second series of measurements, subjects were given a photocopy of their first diaries to help them maintain similar diet and activity patterns. On two separate occasions, subjects also completed a questionnaire evaluating the intrusiveness of the [^{14}C]-bicarbonate-urea method. During the first measurement period, total body fat and total body lean mass were evaluated using bioelectrical impedance as described in Chapter 2.3.5.2. The same investigator made all repeated measurements.

6.3.3 Measurements

6.3.3.1 Body weight

Body weight was measured as described in Chapter 2.3.4.

6.3.3.2 Resting energy expenditure (REE) and respiratory quotient (RQ)

Fasting REE and RQ were measured by indirect calorimetry using the ventilated canopy and Deltatrac metabolic monitor as described in Chapters 2.3.1.2 and 2.3.2.1, respectively.

6.3.3.3 Total energy expenditure (TEE)

Total energy expenditure was measured using the [¹⁴C]-bicarbonate-urea method as described in Chapter 5. For the non-obese group 13.8 ± 0.4 ml of [¹⁴C]-bicarbonate-urea solution (2.23 ± 0.008 μCi/ml) was administered over 48 hours, and for the obese group 14.3 ± 0.1 ml (1.81 ± 0.005 μCi/ml) was administered over 48 hours.

TEE measured using [¹⁴C]-bicarbonate-urea was also compared to a prediction of TEE based on the factorial method for estimating daily energy requirements:

Predicted TEE (kJ/day) = REE (measured) x physical activity index

(see equation [i] Chapter 2.3.1.1. for details)

6.3.3.4 Energy expenditure due to physical activity (PAEE)

On the day that TEE was measured, energy expenditure due to physical activity (both voluntary and involuntary) was calculated using the semi-quantitative estimation equation:

[¹⁴C]-PAEE (kJ/day) = 0.9x[¹⁴C]-bicarbonate TEE - REE

(see equation [i] Chapter 2.3.1.4. for details)

6.3.3.5 *Questionnaire assessing suitability and comfort of the [¹⁴C]-bicarbonate-urea method*

The intrusiveness of the [¹⁴C]-bicarbonate-urea method and its' effect on usual daily activity was evaluated using a questionnaire (Appendix 4) that rated, on a scale from 1 to 10, the suitability and comfort of wearing the syringe infusion pump, and the intrusiveness of the 48 hour infusion. Subjects were also asked whether the method interfered with their normal lifestyle.

6.3.4 Statistical analysis

All data are represented as means \pm SEM, unless specified otherwise as (\pm SD). Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). The intra-individual variation for the [¹⁴C]-bicarbonate-urea assay (within-assay variation) was determined from triplicate samples of each subjects' urine that was assayed for the specific activity of urea (see Chapter 3.3.4 for details). Reproducibility between the first and second energy expenditure measurements (i.e. the intraindividual day-to-day variation or coefficient of variation [CV]) was determined as described in Chapter 3.3.4. A mean difference that falls within the 95% confidence intervals and an intra-individual day-to-day variation of < 10% would suggest that the methods were reproducible and could confidently be used to measure energy expenditure. The reliability of the TEE measurement (i.e. the intraclass correlation coefficient [ICC] was determined as described in Chapter 3.4.4. Agreement between the [¹⁴C]-bicarbonate-urea method and the prediction equation for measuring TEE was assessed using the 95% limits of agreement (i.e. mean difference between [¹⁴C]-bicarbonate and prediction equation \pm 2 SD of the difference) (Bland and Altman, 1986; Bland and Altman, 1995). A narrow limit of agreement (i.e. \pm 500-1000 kJ/day) would suggest that the two methods could be used interchangeably to measure TEE. Significance was set at $p < 0.05$.

6.4 RESULTS

6.4.1 Subject characteristics and compliance

Descriptive characteristics of the obese and non-obese groups are presented in Table 6.4.1. The mean age of the non-obese subjects was 50.0 ± 3.1 years and their mean BMI was $24.4 \pm 1.3 \text{ kg/m}^2$, whereas the mean age of the obese subjects was 48.3 ± 2.2 years and their mean BMI was $33.8 \pm 1.8 \text{ kg/m}^2$. Body weight remained stable from the first to the second measurement period for both of the non-obese (79.2 ± 3.08 vs 79.3 ± 3.09 kg, $p = 0.56$) and the obese (94.1 ± 6.2 vs 94.0 ± 6.1 kg, $p = 0.36$) groups. In the obese group, the 6 men had a mean weight of 106.8 ± 12.9 kg, BMI of $35.2 \pm 3.7 \text{ kg/m}^2$, fat mass of 36.7 ± 9.1 kg and lean mass of 70.1 ± 4.1 kg, and the 9 women had a mean weight of 85.6 ± 4.4 kg, BMI of $33.1 \pm 1.7 \text{ kg/m}^2$, fat mass of 39.3 ± 3.3 kg and lean mass of 46.6 ± 1.5 kg.

Energy intakes and the macronutrient composition of the subjects' usual diet during the first and second measurement periods are presented in Table 6.4.2 and there was no difference between the values reported on the two measurement occasions. The mean energy intake was 10747 ± 531 kJ/day [range 7969 to 12580 kJ/day] for the non-obese group and 7822 ± 677 kJ/day [range 5062 to 11485 kJ/day] for the obese group. Despite being asked not to consume alcohol during the study, several subjects in both the non-obese and obese group reported that they had consumed some at the first measurement and therefore they were instructed to consume the same amount during the second measurement (average %energy as alcohol was $3.8 \pm 3.1\%$ in the non-obese group and $4.0 \pm 2.2\%$ in the obese group). The mean FQ for the two groups (0.84 ± 0.009 for the non-obese group vs 0.84 ± 0.007 for the obese group) reflected the macronutrient mix of a 'typical' western diet. The mean RQ/FQ of 0.97 ± 0.02 [range 0.93 to 1.06] for the non-obese group suggests that most subjects were close to energy balance. The mean RQ/FQ

for the obese group was 0.94 ± 0.01 [range 0.85 to 1.03] and indicates that 9 individuals may have been in slight negative energy balance.

Since the mean food quotient of the diet for both groups of subjects was ~ 0.84 and because the RQ/FQ reflected that they were near energy balance, the assumption associated with the [^{14}C]-bicarbonate-urea that the energy equivalent of CO_2 was 535 kJ/mol (Elia et al. 1995) was justified (see Chapter 5.3.2 for discussion of justification).

The first and second measurements of twenty-four hour urinary creatinine were similar for both the non-obese group (12.4 ± 1.1 and 14.2 ± 1.6 mmol/L, $p = 0.2$) and the obese group (13.3 ± 1.1 and 13.2 ± 0.97 mmol/L, $p = 0.9$). This indicates that the subjects' 24-hour urine collections were presumably complete, as they had reported.

6.4.2 Total energy expenditure (TEE), Resting energy expenditure (REE), and Physical activity energy expenditure (PAEE)

The mean within-assay variation for the [^{14}C]-bicarbonate-urea method was $3.89 \pm 0.31\%$ [range 1.69 to 5.89%] for the non-obese group, and $3.63 \pm 0.28\%$ [range 1.24 to 5.69%] for the obese group.

The average values of the two repeated measurements for all energy expenditure variables are presented in Table 6.4.3. There was no difference between the first and second measurements of [^{14}C]-TEE for the non-obese group (14730 vs 14960 kJ/day, mean difference \pm SD was -229 ± 1234 kJ/day, 95% CI -1260 to $+803$, $p = 0.62$) and the obese group (10967 vs 11694 kJ/day, mean difference \pm SD -727 ± 1672 kJ/day, 95% CI -1653 to $+198$, $p = 0.11$). The mean intra-individual CV for TEE measured using the [^{14}C]-bicarbonate-urea method was $4.83 \pm 0.96\%$ [range 0.95 to 9.24%] for the non-obese group

and $9.68 \pm 1.31\%$ [range 1.74 to 18.03%] for the obese group. The ICC was 0.90 for the non-obese individuals and 0.86 for the obese individuals.

Repeated measurements of REE were similar for both the non-obese group (8061 vs 8097 kJ/day, mean difference \pm SD -36 ± 390 kJ/day, 95% CI -362 to $+290$, $p = 0.80$), and the obese group (8113 vs 8092 kJ/day, mean difference \pm SD $+21 \pm 490$ kJ/day, 95% CI -243 to $+299$, $p = 0.83$). The mean intra-individual CV for REE was $2.97 \pm 0.51\%$ [range 0.70 to 5.40%] for the non-obese group and $2.80 \pm 0.80\%$ [range 0.01 to 6.30%] for the obese group, and the ICC was 0.87 and 0.97 for the respective groups.

Semi-quantitative estimates of PAEE (i.e. $PAEE = 0.9 \times [^{14}C\text{-bicarbonate TEE} - REE]$) were not different at the first as compared to the second measurement period for the non-obese group (5197 vs 5366 kJ/day, mean difference \pm SD -170 ± 1251 kJ/day, 95% CI -1216 to $+876$, $p = 0.71$). For the obese group, 3 subjects (2 females and 1 male) were excluded from the analysis because a negative estimate of PAEE (or very small value i.e. 30 kJ/day) was obtained on one of the repeated measurements. Presumably the negative values arose because of the independent errors associated with the measurements of TEE and REE, and the assumption that the TEF accounts for 10% of TEE, which may be overestimated in some individuals. For the remaining 12 subjects in the obese group, repeated estimates of PAEE were similar (2286 vs 2924 kJ/day, mean difference \pm SD -638 ± 1321 kJ/day, 95% CI -1478 to $+201$, $p = 0.122$). The mean intra-individual CV for PAEE was $14.0 \pm 3.90\%$ [range 4.40 to 37.8%] for the non-obese group and $41.3 \pm 6.90\%$ [range 6.40 to 88.4%] for the obese group, and the ICC was 0.91 and 0.54, respectively.

Linear regression analysis on the combined the data of the non-obese and obese groups showed that there was trend for PAEE to increase with increasing BMI ($\beta = 0.16$, $p =$

0.085) after it had been corrected for the level of physical activity ($\beta = 0.99$, $p < 0.001$) which was the strongest predictor of PAEE [multiple $R = 0.94$, $F_{(2,18)} = 68.7$, $p < 0.001$].

The reproducibility and reliability of the physical activity diary for the measurement of the daily level of physical activity (PA index) was not analysed because subjects were given a copy of their first diary and asked to maintain a similar activity pattern during the second measurement. The first and second measurements of the PA index were similar for both the non-obese group (1.72 vs 1.71, mean difference \pm SD -0.006 ± 0.005 , 95% CI -0.038 to $+0.05$, $p = 0.75$) and the obese group (1.55 vs 1.54, mean difference \pm SD -0.007 ± 0.07 , 95% CI -0.03 to $+0.05$, $p = 0.72$).

6.4.3 Predicted TEE and its' comparability to the [^{14}C]-bicarbonate-urea measurement of TEE

Predicted TEE (i.e. predicted TEE = REE (measured) \times physical activity index) was not different at the first as compared to second measurement period in both the non-obese group (13857 vs 13879 kJ/day, mean difference \pm SD -22 ± 692 kJ/day, 95% CI -601 to $+557$, $p = 0.93$) and the obese group (12678 vs 12594 kJ/day, mean difference \pm SD $+84 \pm 716$ kJ/day, 95% CI -312 to $+481$, $p = 0.66$). Therefore, the averaged values (\pm SEM) for predicted TEE were 13868 ± 700 kJ/day for the non-obese group, and 12636 ± 991 kJ/day for the obese group. The averaged values of predicted TEE for all individuals are shown in Table 6.4.3.

In the non-obese group, there was no correlation between TEE measured using [^{14}C]-bicarbonate-urea and predicted TEE ($r = 0.07$). The two methods were strongly correlated in the obese group ($r = 0.92$, $p < 0.0001$). For the non-obese group, the mean difference \pm SD between the two methods was 977 ± 3199 kJ/day ($p = 0.42$). The limits of agreement for the difference as depicted in figure 6.4.3A were wide (-5504 to $+7458$) indicating that

the two methods were not comparable. For the obese group, [¹⁴C]-TEE was significantly different to predicted-TEE (mean difference \pm SD -1305 ± 1562 kJ/day, 95% limits of agreement -4430 to $+1819$ kJ/day, $p < 0.01$) (Figure 6.4.3B).

6.4.4 Suitability and comfort of the [¹⁴C]-bicarbonate-urea method

The responses to all questions were similar for the repeated measurements, in both the non-obese and the obese group, respectively. None of the subjects reported any adverse reaction during the infusion of the [¹⁴C]-bicarbonate-urea solution. Discomfort associated with the 48-hour infusion of the [¹⁴C]-bicarbonate-urea solution was rated from 1 (painless) to 10 (painful). The mean discomfort score was 2.9 ± 0.67 [range 1 to 7] for the non-obese group, and 2.5 ± 0.41 [range 1 to 6.5] for the obese group. The comfort of wearing the syringe infusion pump over 48-hours in the subjects' free-living environment was rated from 1 (uncomfortable) to 10 (comfortable). For the non-obese group, the mean comfort score was 5.5 ± 0.68 [range 3 to 9], and for the obese group it was 4.6 ± 0.44 [range 2 to 8.5]. The suitability of wearing the syringe infusion pump in the subjects' free-living environment was rated from 1 (not practical) to 10 (practical). For the non-obese group, the mean suitability score was 6.7 ± 0.88 [range 3 to 9], and for the obese group it was 5.2 ± 0.53 [range 2 to 8.5]. When asked if the method interfered with their normal lifestyle, 75% of the non-obese subjects and 73% of the obese subjects replied 'No'. The activities that subjects cited as difficult to perform while wearing the infusion pump included showering and bending over, and one individual was unable to swim.

Table 6.4.1 Physical characteristics of the non-obese and obese subjects

	Non-Obese subjects	Obese subjects
	(8 men)	(6 men/ 9 women)
Age (yrs)	50.0 ± 3.1 [39 - 62]	48.3 ± 2.2 [35 - 61]
Weight (kg)	79.3 ± 3.1 [68.1 - 93.5]	94.1 ± 6.2 [66.5 - 169.5]
Height (m)	1.79 ± 2.0 [1.73 - 1.90]	1.66 ± 0.02 [1.54 - 1.80]
BMI (kg/m ²)	24.4 ± 1.3 [22.2 - 25.9]	33.8 ± 1.8 [28.1 - 52.3]
% Total body fat	22.6 ± 1.2 [17.3 - 27.6]	40.0 ± 2.4 [23.3 - 53.5]
Fat mass (kg)	18.1 ± 1.5 [12.4 - 22.9]	38.2 ± 4.0 [21.1 - 79.5]
Lean mass (kg)	61.3 ± 2.0 [53.1 - 72.6]	56.0 ± 3.6 [43.0 - 90.0]

Data are means ± SEM. The minimum and maximum values are shown in brackets [].

Body fat percentage, fat mass and total lean mass were measured using bioelectrical impedance.

Table 6.4.2 Energy intake and macronutrient composition of subjects' 3-day food records

	Measurement 1	Measurement 2	p value
Non-obese group (8 men)			
Energy intake (kJ/day)	10598 ± 703	10897 ± 441	0.57
Carbohydrate (% E)	42.3 ± 44.3	44.3 ± 3.4	0.50
Protein (% E)	18.5 ± 0.8	18.0 ± 0.6	0.52
Total Fat (% E)	36.5 ± 2.1	34.3 ± 2.1	0.47
RQ	0.81 ± 0.01	0.82 ± 0.02	0.91
FQ	0.84 ± 0.01	0.84 ± 0.001	0.61
RQ/FQ	0.97 ± 0.02	0.97 ± 0.03	0.93
Obese group (6 men/ 9women)			
Energy intake (kJ/day)	7576 ± 566	8068 ± 537	0.33
Carbohydrate (% E)	41.6 ± 2.2	42.5 ± 1.7	0.58
Protein (% E)	18.0 ± 0.8	19.1 ± 1.0	0.22
Total Fat (% E)	33.6 ± 1.5	32.8 ± 1.6	0.67
RQ	0.78 ± 0.01	0.80 ± 0.02	0.20
FQ	0.84 ± 0.007	0.84 ± 0.005	0.36
RQ/FQ	0.93 ± 0.01	0.95 ± 0.02	0.25

Data are means ± SEM. % E, percentage of total energy intake; RQ, respiratory quotient; FQ, food quotient of the habitual diet. Measurements 1 and 2 were compared using students paired t-test. Significance was set at $p < 0.05$.

None of the variables measured on the two separate occasions were different.

Table 6.4.3 Averaged values of the repeated measurements for total energy expenditure, resting energy expenditure and energy expenditure due to physical activity

	BMI (kg/m ²)	[¹⁴ C]-TEE (kJ/day)	Estimated TEE (kJ/day)	REE (kJ/day)	[¹⁴ C]-PAEE (kJ/day)	PA index
Non-obese group (8 men)						
1	24.0	20258	14609	7678	10554	1.90
2	25.3	14072	12053	8203	4462	1.47
3	25.9	12187	12556	8389	2580	1.50
4	24.9	14571	12403	7295	5819	1.70
5	25.7	16485	15468	9099	5737	1.70
6	23.7	15583	11684	7050	6975	1.65
7	23.5	13369	17222	8611	3421	2.0
8	22.2	12234	14952	8307	2704	1.8
Mean ± SEM	24.4 ± 1.3	14845 ± 938	13868 ± 700	8079 ± 243	5281 ± 937	1.72 ± 0.07
Obese group (9 women/ 6 men)						
1	30.7	9464	9788	6315	2203	1.55
2*	31.8	9166	10571	7047	1202	1.50
3	28.0	8165	8412	5608	3934	1.50
4*	33.6	8302	10404	6936	536	1.50
5	36.6	11378	13511	8445	1796	1.60
6	28.3	9108	9044	6029	2168	1.50
7	40.3	10602	11458	7639	1904	1.50
8	28.0	9445	10889	7259	1241	1.50
9	40.9	16171	14119	9114	5440	1.55
10*	28.6	9894	12431	8287	617	1.50
11	34.8	11657	14865	9298	1193	1.60
12	28.2	10568	11467	7908	1603	1.50
13	37.4	14195	14215	9477	3299	1.50
14	29.6	11792	13882	8166	2447	1.70
15	52.2	20048	24487	14010	4033	1.75
Mean ± SEM	34 ± 1.7	11330 ± 837	12636 ± 991	8102 ± 519	2605 ± 376 [†]	1.55 ± 0.02
Mean ± SEM men	35 ± 3.7	13026 ± 1527	15224 ± 1921	9524 ± 934	2515 ± 524	1.59 ± 0.05
Mean ± SEM women	33 ± 1.7	10200 ± 819	10911 ± 632	7155 ± 375	2669 ± 559	1.52 ± 0.01

[¹⁴C]-TEE, total energy expenditure measured using [¹⁴C]-bicarbonate-urea; Estimated TEE, computed as measured REE multiplied by physical activity factor; REE, resting energy expenditure; [¹⁴C]-PAEE, energy expenditure due to physical activity calculated from the measurements of [¹⁴C]-TEE and REE, and the assumption that the thermic effect of feeding is ~ 10%; PA index, physical activity index derived from 3-day activity diary. In the obese group, subjects 1 to 9 are female and subjects 10 to 15 are male.

*Subjects excluded from analysis for PAEE (2 females and 1 male)

[†]n = 12 subjects (7 females and 5 males)

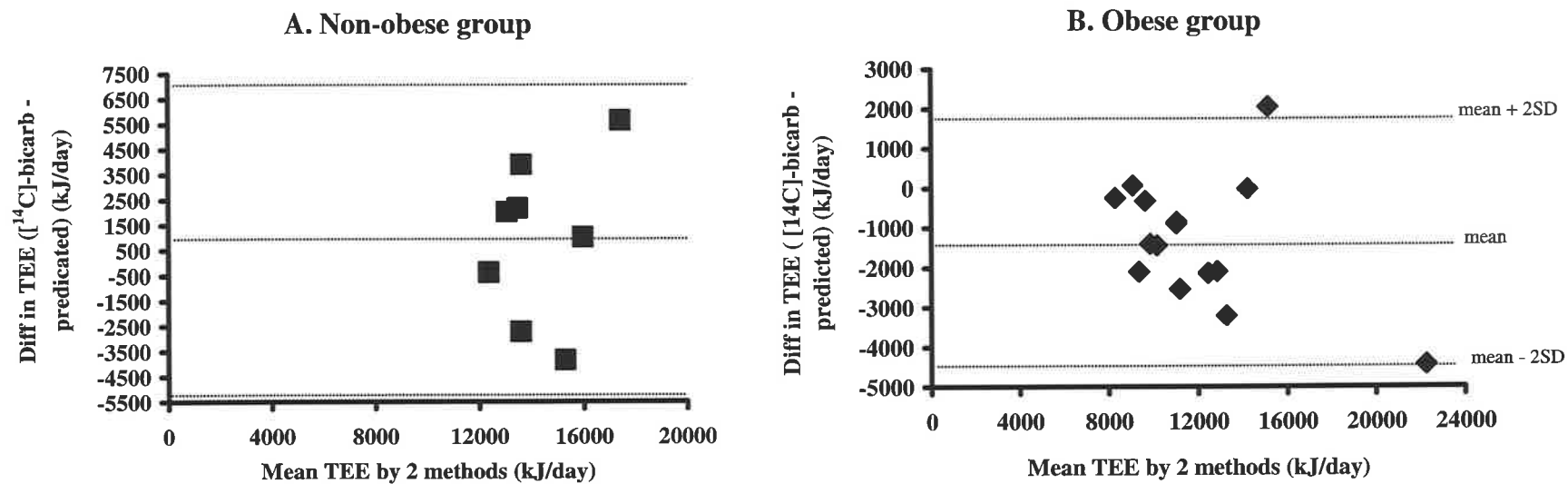


Figure 6.4.1 Bland and Altman plots showing the comparability of the [¹⁴C]-bicarbonate-urea method and a prediction equation for measuring TEE for the non-obese (A) and obese (B) group. Mean difference and the 95% limits of agreement (mean difference \pm 2 SD) are shown (-----).

6.5 DISCUSSION

The mean day-to-day variation in TEE for the non-obese group was 4.8%, of which ~3.89% was due to analytical error in the measurement of the specific activity of urea. Elia and colleagues (Elia et al. 1995; Gibney et al. 1997; Paton et al. 1996) have shown that the mean day-to-day variation in TEE for non-obese subjects was between 2.4 and 6% using the [¹⁴C]-bicarbonate-urea method. Day-to-day variation in TEE measured in non-obese individuals who are confined to a respiratory chamber generally never exceeds 5% (de Boer et al. 1987; Murgatroyd et al. 1987; Shetty et al. 1996; White et al. 1996). However, doubly labeled water studies show that that the mean intra-individual variation in measurements of free-living TEE is on average 8% but may be as high as 11% (Black and Cole, 2000; Schoeller and Hnilicka, 1996) in non-obese groups.

This is the first study to examine the use of [¹⁴C]-bicarbonate-urea in obese populations. The mean day-to-day variation in the measurement of TEE for the obese group was 9.68%, of which ~3.63% was due to analytical error within the assay for the specific activity of urea. Doubly labeled water studies reviewed by Schoeller and Hnilicka (1996) similarly show that the intra-individual day-to-day variation in TEE is greater in overweight and/or obese subjects as compared to non-obese subjects; three studies (n = 8, 9 and 19 subjects) reported a mean intra-individual day-to-day variation of between 8.8 to 13.6% for obese subjects. The reason why the day-to-day variation in the measurement of TEE is greater for obese persons as compared to non-obese persons is not known. It may, however, reflect greater day-to-day variation in the amount of energy they expend during physical activity. Furthermore, it may be that the amount of voluntary physical activity that obese persons carry-out fluctuates more from day-to-day than for non-obese individuals.

Two studies in healthy males (Elia et al. 1992; Elia et al. 1995) and one study in men and women with lung cancer (Gibney et al. 1997), have shown that TEE measured under “free-living” conditions using the [^{14}C]-bicarbonate-urea method was within 2 to 5% of values measured using whole-body indirect calorimetry. Total energy expenditure as measured using [^{14}C]-bicarbonate-urea has not been directly compared to that measured by doubly labeled water. In this study, however, the values of TEE as measured using [^{14}C]-bicarbonate-urea appear to be consistent with those reported in studies using doubly labeled water (Black et al. 1996; Prentice et al. 1996). In a compilation study of 319 doubly labeled water measurements, Prentice et al. (1996) produced reference ranges for TEE of (mean \pm SD) 12.9 ± 2.6 , 14.3 ± 2.8 , 16.4 ± 3.9 and 17.5 ± 2.7 MJ/day for males with a BMI of ≤ 25 , 25-30, 30-35, ≥ 35 kg/m², respectively. For females with a BMI of 25-30, 30-35, ≥ 35 kg/m², the reference ranges were 10.2 ± 1.6 , 11.4 ± 1.7 and 13.5 ± 1.8 MJ/day, respectively. The majority of our subjects fell within these ranges (i.e. the mean TEE [\pm SD] of the non-obese men was 14.8 ± 2.7 MJ/day, and for the obese men and women it was 13.0 ± 5.9 and 10.2 ± 3.2 MJ/day, respectively). Two of our male subjects (1 lean and 1 obese) had a TEE of ~ 20 MJ/day. The lean individual (BMI of 24.0 kg/m²) reported that he was actively training for triathlons, and the obese individual (52 kg/m²) was employed as a labourer that reportedly involved moderate occupational activity. It is, therefore, likely that the TEE of the lean individual is high because of his ability to perform activity of a high intensity, whereas the TEE of the obese individual is high because of his larger BMI. In a meta-analysis of data from 226 subjects, Westerterp (1999) illustrated how the upper limit of TEE measured using doubly labeled water was associated with both body mass and physical capacity (i.e. the ability to perform activity of higher intensity), whereas the lower limit of TEE related primarily to body size. For example, in Westerterp’s meta-analysis (1999) the lower limit of TEE (i.e. when the level of physical activity was minimal) clearly increased with BMI from <5 MJ/day in somebody with the lowest BMI of 12 kg/m² to >15 MJ/day in the morbid obese; a three-

fold difference. At the upper limit of TEE (i.e. when the level of physical activity was high) Westerterp (1999) observed a steep increase in TEE in the lower range of BMI from 5 MJ/day in someone with the lowest BMI of 12 kg/m² to a value of about 20 MJ/day in a person with a BMI of 22 kg/m².

The difference between measured TEE and reported energy intake was unlikely to be due to changes in energy balance because no significant changes in body weight occurred in this study. The difference between the two variables may indicate a degree of under-reporting of energy intake by our subjects. In fact, we found that magnitude of the difference between TEE and energy intake was positively related to increasing BMI (i.e. the level of underreporting energy intake increased as BMI increased $r = 0.45$, $p = 0.03$). Doubly labeled water studies have shown that individuals frequently under-report their usual energy intake by 20-44% when self report methods such as 3-day diaries are used (Black and Cole, 2000; Black et al. 1993; Poppitt et al. 1998; Trabulsi and Schoeller, 2001). In the present study, the non-obese group appeared to under-report their energy intake by $25.4 \pm 5.2\%$ (range 5.9 to 49%). Much of the variation in this error was due to 2 of the non-obese men (one of whom was training for a triathlon) having an energy intake of ~43% less than their measured TEE. Confirming previous observations (Black et al. 1993; Trabulsi and Schoeller, 2001), the degree of under-reporting energy intake by obese persons ($28.5 \pm 6.1\%$, range 1.4 to 62%) was greater (by 3.1%) as compared to non-obese persons. In deed, 7 obese subjects under-reported energy intake by more than 25%. Interestingly, it was the individuals who were the most obese, followed by those who appeared to be the most motivated to lose weight after the study, who showed the greatest discrepancy between their energy intake and expenditure.

An advantage of [¹⁴C]-bicarbonate-urea as compared to whole-body indirect calorimetry is that it can provide an estimate of daily energy expenditure due to physical activity

(PAEE), when it is used in conjunction with indirect calorimetry to measure REE. In this study, although repeated estimates of PAEE were similar in the non-obese group and the obese group, a large mean day-to-day variability was observed within-subjects, particularly in the obese group. It is not surprising that the estimated PAEE for an individual has a large degree of variability from day-to-day, because the value depends on TEE, REE, and the assumption that 10% of TEE is accounted for by the thermic effect of feeding; all have errors of measurement. In addition, variations in intensity, pace, and duration of activities may have a direct impact on the daily estimates of PAEE.

Comparisons of estimated PAEE between studies is problematic for a number of reasons. Some investigators have used the equation cited in this study (Paton et al. 1996; Starling et al. 1999) whereas others define it as the difference between TEE and REE (Black et al. 1996; Prentice et al. 1996; Shetty et al. 1996). However, PAEE values observed in this study (range 2.7 to 10.5 MJ/day and 1.1 to 5.4 MJ/day for the non-obese and obese individuals, respectively) did fall within the ranges of values derived from two meta-analyses of doubly labeled water studies. For example, both Prentice et al. (1996) and Westerterp (1999) reported that free-living PAEE ranges from ~1 to 10 MJ/day in individuals of varying body size. These two meta-analyses (Prentice et al. 1996; Westerterp, 1999) observed that PAEE was greater as BMI increased, despite the level of physical activity (PA index) remaining relatively stable across BMI categories. In the present study, the absolute values of PAEE suggest that PAEE was greater in the non-obese group as compared to the obese group. However, when PAEE was corrected for the level of physical activity (which was the strongest predictor of PAEE) a trend for PAEE to increase with increasing BMI was also apparent. Further work is warranted to confirm the significance of the relationship between BMI and energy cost of physical activity.

Predicted TEE was not comparable to TEE measured using [^{14}C]-bicarbonate-urea, in this study. In the non-obese group, predicted TEE underestimated [^{14}C]-TEE by 977 ± 3199 kJ/day (or $4 \pm 21\%$), and for 4 of the 8 men the underestimation was by more than 16%. In contrast, predicted TEE overestimated [^{14}C]-TEE by 1308 ± 1562 kJ/day (or $11 \pm 11\%$) in the obese group, with 8 of the 15 subjects (4 men and 4 women) having an estimated TEE 15% greater than [^{14}C]-TEE. Although discrepant results are, in part, due to error associated with the [^{14}C]-bicarbonate-urea method, we do consider the [^{14}C]-bicarbonate-urea TEE values more accurate than the predicted TEE values. It is likely that the greatest proportion of discrepancy between measured and predicted TEE results from error in computing the physical activity indexes used in the prediction equation. Physical activity indexes derived from activity diaries are prone to errors because of the difficulties associated with obtaining accurate details regarding the duration and intensity of activity, or information regarding the energy costs of specific activities for individuals of varying body mass and aerobic fitness (Warwick, 1989). In addition, subjects may not report certain activities (e.g. household duties such as ironing or gardening), they may forget to include all activities that they did over the day, or they may alter their daily routine during the study (LaPorte et al. 1985; Warwick, 1989). Interestingly, predicted TEE values were greater than measured TEE in the non-obese group, which is consistent with the notion that obese individuals may perceive themselves to be more active (or less sedentary) than they are.

Consistent with previous reports (Elia et al. 1992; Elia et al. 1995; Gibney et al. 1997; Paton et al. 1996) the 48-hour infusion of labeled bicarbonate did not cause a local inflammatory reaction and was well tolerated by our subjects who maintained their normal daily activities while at living at home. The infusion pump that subjects' wore in a waist pouch was considered comfortable, and even though some subjects reported that bending

over and showering was more difficult than usual, it did not prevent them from continuing their normal daily activities over the 2 days. The male subject who trained for triathlons, however, reported that he did have to substitute swimming with running and cycling.

6.6 CONCLUSION

The [^{14}C]-bicarbonate-urea method is well tolerated by subjects under free-living conditions, and apparently it does not restrict normal daily activities, even vigorous activities other than swimming. The method provides reproducible measurements of TEE, and the values obtained were consistent with those reported in studies using doubly labeled water. Therefore, this study confirms that the [^{14}C]-bicarbonate-urea method is a suitable field technique for the assessment of energy requirements in both non-obese and obese populations. When used in conjunction with indirect calorimetry, the [^{14}C]-bicarbonate-urea method may also provide an estimate of daily physical activity energy expenditure, albeit crude, for groups of people engaged in different occupations. As long as the within-subject day-to-day variability in TEE is considered in the study design, the [^{14}C]-bicarbonate-urea method may be used to detect alterations in TEE, in a variety of study populations during intervention studies.

CHAPTER 7

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

7.1 SUMMARY

The objective of this study was to measure the effect of a weight loss of more than 10% on TEE using [¹⁴C]-bicarbonate-urea after an energy restrictive 'Modifast' diet in overweight subjects. The effects of a 10% weight loss on the components of TEE were also evaluated. Eleven subjects (6 men, 5 women, aged 50 ± 3 yrs, BMI 34.1 ± 2.1 kg/m², body fat 38.7 ± 3%) were studied before and after a diet-induced decrease in body weight of more than 10% over a period of 8 weeks, followed by 2 weeks of a weight maintenance diet. Weight loss was induced using a combination of 'Modifast™' formula and one small meal per day (~3.3 MJ/day). Body composition, resting energy expenditure (REE) and the thermic effect of a 2.7 MJ test meal (TEF) were measured in addition to TEE, at week 0 (after an initial 2-week run in period on a weight maintenance diet), and again at week 10. Energy intake was assessed using diaries over 3 days during the measurements, and energy expenditure due to physical activity (PAEE) was calculated from the equation: PAEE = (0.9 x TEE) - REE, which assumes that 10% of TEE is accounted for by the TEF. After 8 weeks of energy restriction and 2 weeks on a weight maintenance diet, mean body weight decreased by 12.2 ± 1.6 kg (12.5%). Mean total fat mass, abdominal fat mass and lean mass decreased by 8.4 ± 1.0 kg (20.4%), 2.3 ± 0.4 kg (22.5%) and 3.8 ± 0.7 kg (6.7%), respectively. At week 10 as compared to baseline, mean REE decreased by 5.6 ± 1.3% (500 ± 128 kJ/day) (p < 0.002). Decreases in the mean TEE (0.18 ± 3.7%) and mean TEF (1.4 ± 0.9%), and the increase in mean PAEE (176 ± 412 kJ/day or 18.6 ± 21.4%) were not significant. We conclude that after the stabilization of a moderately reduced body weight, REE but not TEE decreases. There was, however, substantial variability between individuals when measurements are made in the subjects' normal environment.

7.2 INTRODUCTION

Most obesity treatments involve energy restriction to induce a negative energy balance and promote weight loss. Diet-induced weight loss however, is often (Bennett, 1995; Leibel et al. 1995; Ravussin and Bogardus, 2000; Ravussin et al. 1985a; Schoeller, 1998; Weyer et al. 2000), but not always (Amatruda et al. 1993; DeGroot LC et al. 1990; Rumpler et al. 1991) accompanied by a decrease in total energy expenditure that usually results in weight regain over time. Total energy expenditure is comprised of three components: resting energy expenditure which accounts for 60 to 75% of total energy expenditure (Keeseey and Hirvonen, 1997; Ravussin et al. 1988; Weigle, 1994), the thermic effect of feeding accounts for 6 to 15% (Bessard et al. 1983; D'Alessio et al. 1988), and energy expenditure due to physical activity (includes voluntary and involuntary activity) accounts for the remaining proportion. As resting energy expenditure is the major determinant of total energy expenditure in sedentary people, a small decrease can lead to substantial decreases in daily energy balance (Leibel et al. 1995; Ravussin et al. 1988). There is also evidence that the reduction in total energy expenditure involves decreases in the thermic effect of feeding because of smaller meals (Ravussin et al. 1985a), and decreases in the energy cost of activity because of a smaller body size (Froidevaux et al. 1993; Leibel et al. 1995). Relatively few studies, however, have been conducted that assess the impact of diet-induced weight loss on free-living total energy expenditure and each of its components (Amatruda et al. 1993; Leibel et al. 1995).

Total energy expenditure is usually measured by whole-body indirect calorimetry or doubly labeled water. The scarce amount of information regarding free-living total energy expenditure may relate to the expense associated with doubly labeled water. As discussed in Chapters 5, several studies have shown that the [^{14}C]-bicarbonate-urea method for measuring total energy expenditure in a subjects' naturalistic environment is comparable to whole-body indirect calorimetry (Elia et al. 1995; Gibney et al. 1997; Paton et al.

1996), and in Chapter 6 we demonstrated that the method, as used in our laboratory, was reproducible in both lean and obese populations. The [^{14}C]-bicarbonate-urea method has not previously been used to measure changes in energy expenditure after moderate weight loss.

The aim of this study was to determine whether changes in total energy expenditure, after a weight loss of more than 10%, could be measured using [^{14}C]-bicarbonate-urea. The effect of weight loss on resting energy expenditure (REE), the thermic effect of feeding (TEF) and energy expenditure due to physical activity (PAEE) will also be evaluated.

7.3 RESEARCH DESIGN AND METHODS

7.3.1 Subjects

Fifteen obese non-diabetic subjects (6 men/ 9 women) who completed the reproducibility study described in Chapter 6 were recruited to participate in this weight loss trial. Subjects were informed of the importance that they be highly motivated to lose at least 8% of their initial body weight. Exclusion criteria have been previously described in Chapter 6. All subjects gave informed written consent to participate in the study.

Of the fifteen subjects recruited, 11 (6 men/ 5 women) completed the study. Three subjects withdrew due to family and work commitments during the course of the study. Data from another subject was excluded from the analysis because she declined, for personal reasons, to have the final [^{14}C]-bicarbonate-urea measurement of TEE made. Subjects on anti-hypertensive, lipid lowering, respiratory or gastrointestinal medication were asked to maintain all medications and supplements at pre-study doses.

7.3.2 Diet

In order to induce a minimum 10% weight loss over 8 weeks all subjects were placed on an energy restrictive diet that consisted of a fixed energy intake of 3300 kJ/day. Energy was predominately derived from a liquid dietary formula (Modifast, Novartis, Australia) that contained 50% of the total energy as protein, 40% as carbohydrate and 10% as fat; all essential micronutrients were also incorporated into the formula. Although the majority of subjects had 5 sachets of formula per day, several subjects chose to replace two sachets of 'Modifast' with one very low calorie meal (~ 1470 kJ) at night. The low calorie meal consisted of 120g of lean meat, chicken or fish plus green vegetables or salad.

At the end of the energy-restrictive phase, subjects resumed a solid food weight maintenance diet, for an additional two weeks. From week 8 to 10, a weight maintenance diet was designed to maintain energy balance and therefore body weight at the newly reduced level. The energy level of the weight maintenance diet was individually prescribed based on each subject's resting metabolic rate multiplied by a physical activity index. For each individual, resting metabolic rate was calculated from the Schofield formula (1985) (where the value of weight was that measured at week 8) and a suitable physical activity index was derived from the subjects 3-day physical activity diary (collected at week 8) and a table that specified indexes (multiples of REE) for eight different levels of activity (NHMRC, 1991).

Prior to commencing the study, subjects received detailed dietary guidelines and a meal plan from a dietitian experienced in the use of Modifast. Once every week for the first two weeks of the energy-restrictive phase, subjects visited the same dietitian to receive the formula and dietary counseling. Thereafter, they received counseling once a fortnight until the commencement of the weight maintenance phase where they resumed counseling once a week. Subjects were asked to refrain from drinking alcohol throughout the study.

7.3.3 Experimental design

The study was conducted on an outpatient basis over 10 weeks. In Chapter 6, the second visit measurements of TEE, REE, and PAEE for the obese group constituted the pre-weight loss (week 0) recordings for the current study. Immediately after completing the week 0 measurements, subjects commenced 8 weeks of energy restriction and 2 weeks of weight maintenance at energy balance. Measurements for total energy expenditure, resting energy expenditure, the thermic effect of feeding and energy expenditure due to physical activity were repeated at the end of week 10 (the thermic effect of feeding at week 0 was not reported in Chapter 6). Body weight was recorded every two weeks and body composition was assessed at weeks 0 and 10. A 3-day food diary was collected at weeks 0 and 10 and later analysed to determine the subjects' energy and macronutrient intakes as described in Chapter 2.3.3.1, and the food quotient (FQ) of their habitual diet as described in Chapter 2.3.3.2. A three-day activity diary to determine the average daily activity (voluntary) levels [a multiple of REE] (described in Chapter 2.3.1.4) was also recorded during the measurements of energy expenditure. Both diaries commenced the day prior to the energy expenditure measurements being made and continued for a further 2 days until the end of the TEE measurement. Subjects were asked to continue their usual physical activity routines throughout the 10 weeks.

7.3.4 Measurements

7.3.4.1 Body weight and composition

Body weight was measured as described in Chapter 2.3.4 and total body fat and total body lean mass were assessed using dual-energy x-ray absorptiometry as described in Chapter 2.3.5.1.

7.3.4.2 *Total energy expenditure (TEE)*

Total energy expenditure was measured using the [¹⁴C]-bicarbonate-urea method as described in Chapter 5. In this study 14.19 ± 0.08 ml of [¹⁴C]-bicarbonate-urea solution (1.74 ± 0.008 μ Ci/ml) was administered.

7.3.4.3 *Resting energy expenditure (REE), Respiratory quotient (RQ), and the thermic effect of feeding (TEF)*

Fasting REE and RQ were measured by indirect calorimetry using the ventilated canopy and Deltatrac metabolic monitor as described in Chapters 2.3.1.2 and 2.3.2.1, respectively. Immediately following the measurements of fasting REE and RQ, each subject consumed, within 20 minutes, a 2791 kJ test meal. The meal consisted of 4 slices of white bread, 10 g of Flora Lite™ margarine, 50 g of lean leg ham, 21 g of Kraft Free™ cheese, 30 g of lettuce, 350 g of orange juice, and a 31g fruit museli bar, and the macronutrient composition was 18% of the total energy as protein, 62% as carbohydrate and 20% as fat. Thereafter subjects returned to the hood for a further 180 minutes of RQ and REE measurements. Postprandial RQ and the mean TEF over the 180-minute period were calculated as described in Chapters 2.3.2.2 and 2.3.1.3. Postprandial RQ was expressed as the mean change in RQ above baseline and TEF was expressed as the % increase per energy intake of the test meal.

7.3.4.4 *Energy expenditure due to physical activity (PAEE)*

On the day that TEE was measured, energy expenditure due to physical activity (which includes energy expenditure due to both voluntary and involuntary activity) was calculated using the semi-quantitative estimation equation:

$$[^{14}\text{C}]\text{-bicarbonate derived PAEE} = 0.9([\text{C}]\text{-bicarbonate TEE}) - \text{REE}$$

(see equation [i] Chapter 2.3.1.4. for details)

7.3.5 Statistical analysis

All data are represented as means \pm SEM, unless specified otherwise as \pm SD. Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). The effect of weight loss was assessed using repeated-measures ANOVA with variables measured at weeks 0 and 10 as the within-subject factor. Although the study was not powered to look for gender specific changes in energy expenditure, since there were approximately equal numbers of both genders we considered it reasonable to include gender as a between-subject factor. Stepwise linear regression was used to determine the relationship between the change in energy expenditure and body composition. Significance was set at $p < 0.05$. The study had 80% power to detect a decrease in TEE of 10%, a decrease in REE of 3%, an 8% decrease in TEF and a decrease in RQ of 3%.

7.4 RESULTS

7.4.1 Subject characteristics

Eleven overweight and obese subjects (6 men/5 women) completed the study. The physical characteristics of subjects at baseline are shown in Table 7.4.1. At baseline, lean mass was approximately 40% greater for men than women (66.1 ± 5.6 vs 39.6 ± 2.1 , $p = 0.003$).

7.4.2 Energy intake during the 3 days of energy expenditure measurements and urinary creatinine

The mean energy intake over the 3 days of the energy expenditure measurements was 8022 ± 682 kJ/day (range 6043 to 12619 kJ/day) at week 0 as compared to 5700 ± 317 kJ/day (range 3871 to 6992 kJ/day) at week 10 ($p < 0.001$); the mean change was 2326 ± 556 kJ/day (range -5627 to 790 kJ/day). The percentage of energy derived from carbohydrate (43 ± 2 vs $42 \pm 2\%$, $p = 0.8$), fat (29 ± 1.2 vs $32 \pm 2\%$, $p = 0.1$), and protein (22 ± 1.6 vs $20 \pm 1.1\%$, $p = 0.2$) were similar at week 0 and week 10. There was no effect

of gender on the energy intake or % of energy derived from fat and protein at either week 0 or at week 10. At week 0, however, women consumed more carbohydrate than men (46.7 vs 38.4%, $p = 0.37$). At week 10 there was no effect of gender on % carbohydrate consumed. The food quotient (FQ) was not different at week 0 as compared to week 10 (0.84 vs 0.85, $p > 0.1$) and there was no effect of gender. The RQ-to-FQ ratio was not different (0.95 vs 0.91) and there was no effect of gender at either week 0 or 10.

Twenty-four hour urinary creatinine was similar at week 0 (13.0 ± 1.3 mmol/L [range 7.5 to 19.8 mmol/L]) and week 10 (11.9 ± 1.2 mmol/L [range 7.2 to 18.7 mmol/L]) ($p = 0.23$) indicating that the subjects' 24-hour urine collections were likely to be complete.

7.4.3 Body weight and composition

The change in body weight over the 8 weeks of energy restriction and 2 weeks of energy balance is shown in Figure 7.4.1. After 8 weeks of energy restriction, mean body weight decreased by $11.8 \pm 1.0\%$ (11.7 ± 1.8 kg, range -6 to -27 kg) which accounted for 8.5 to 17.3% of original body weight ($p < 0.001$). After a further 2 weeks at prescribed energy balance, body weight had decreased an additional 0.51 ± 0.3 kg ($p = 0.04$). There was an effect of gender on weight loss from week 0 to week 8. From week 8 to the end of week 10, women lost an additional 1.2 ± 0.2 kg ($p = 0.004$) of weight, whereas the men maintained their weight.

Total fat mass and total lean mass at week 0 and week 10 are shown for all subjects in Table 7.4.1 and mean changes in body composition after 10 weeks are presented in Table 7.4.2. Total fat mass was reduced $20.4 \pm 1.6\%$ (range -13.2 to -27.2%) from week 0 to week 10 ($p < 0.001$) and this represented 68% of the total weight loss. Abdominal fat mass decreased $22.5 \pm 2.3\%$ (-12.2 to -34.8%) ($p < 0.001$). After 10 weeks, total lean mass was reduced $6.7 \pm 0.9\%$ (-0.8 to -8.5%) ($p < 0.001$) which represented 31% of total weight

loss. There was no affect of gender on the reduction in fat mass, but abdominal fat mass was reduced approximately 59% more in the men than in the women ($p = 0.003$). There was a trend for lean mass to be reduced approximately 51% more in the men than women ($p = 0.056$).

7.4.4 Total energy expenditure (TEE)

Individual values and the mean of the group for TEE at weeks 0 and 10 are shown in Table 7.4.3. After 10 weeks, there was a $0.18 \pm 3.7\%$ (or 181 ± 454 kJ/day) decrease in TEE (range -24% to 22%) that was not statistically significantly. There was no association between the change in TEE and the change in body weight, lean mass, or fat mass. There was no affect of gender on TEE.

7.4.5 Resting energy expenditure (REE), the thermic effect of feeding (TEF), and physical activity energy expenditure (PAEE)

Individual values and the means of the group for REE, TEF, and PAEE at weeks 0 and 10 are shown in Table 7.4.3. Resting energy expenditure (expressed as an absolute or normalized for fat and lean mass) was significantly reduced by 500 ± 128 kJ/day (or $5.6 \pm 1.3\%$, range -12 to $+2.8\%$) after 10 weeks ($p = 0.002$). Stepwise linear regression showed that the decrease in REE was best predicted by the decrease in fat mass [decrease in REE (kJ/day) = $101.37 + 72.37 \times \text{decrease in fat mass (kg)}$, $r^2 = 0.36$, $p = 0.049$]. The decrease in lean mass also made a small contribution to the decrease in REE [decrease in REE (kJ/day) = $-113.7 + 104.6 \times \text{decrease in lean mass (kg)}$, $r^2 = 0.35$, $p = 0.054$]. There was an overall effect of gender on REE at both week 0 (9634 ± 1043 vs 6575 ± 423 kJ/day) and week 10 (8920 ± 954 vs 6316 ± 373 kJ/day) such that men had a higher REE than women.

The mean TEF did not decrease significantly from week 0 to week 10 (mean decrease $1.4 \pm 0.9\%$, range -7.1% to 4.4%) (Table 7.4.3). There was no affect of gender on the TEF.

Data for the semi-quantitative estimate of physical activity energy expenditure (PAEE) from subject 10 (Table 7.4.3) was excluded from the analysis. At week 0, a negative value for PAEE for this individual may have arisen because of the independent errors associated with the measurements of TEE and REE, and the assumption that the TEF accounts for 10% of TEE which may be lower in many individuals. For the remaining 10 subjects, the mean energy expenditure due to physical activity (expressed as an absolute value) was similar at week 10 compared to week 0 (mean change 176 ± 412 kJ/day, range -2773 to 2274 kJ/day) (Table 7.4.3). The change in PAEE was not related to the changes in body weight, fat mass, or lean mass. The average daily physical activity index derived from the 3-day physical activity diaries was not different at week 10 as compared to week 0 (1.56 ± 0.03 vs 1.56 ± 0.03). Gender had no effect on the semi-quantitative estimate of PAEE, or the physical activity index.

7.4.6 Respiratory quotient

Fasting RQ was not significantly different at week 10 as compared to week 0 (0.78 ± 0.01 vs 0.80 ± 0.02 respectively). The mean increase in postprandial RQ also remained similar from week 0 to week 10 (0.05 ± 0.02 vs 0.06 ± 0.01). There was no effect of gender on the fasting or mean increase in postprandial RQ.

Table 7.4.1 Physical characteristics of the 11 subjects at baseline and after maintenance of the reduced body weight (week 10)

Subject/ Gender	Age	BMI		Weight		Fat mass		Lean mass		
	(yrs)	(kg/m ²)		(kg)		(kg)		(kg)		
	Week 0	Week 0	Week 10	Week 0	Week 8	Week 10	Week 0	Week 10	Week 0	Week 10
1/F	54	30.9	28.1	82.1	77.0	75.5	39.8	34.0	43.2	41.5
2/F	44	32.0	28.0	83.0	72.5	72.5	40.7	33.4	42.3	39.2
3/F	61	33.8	27.9	79.5	69.5	68.0	40.6	33.3	39.0	34.7
4/F	60	28.1	24.0	66.0	58.0	57.0	34.2	27.4	31.7	29.6
5/F	60	40.1	36.7	100.1	93.0	91.5	58.0	50.3	42.0	41.2
6/M	51	28.4	25.9	91.0	83.5	83.0	29.1	24.9	61.9	58.1
7/M	41	37.2	32.1	105.0	90.5	90.5	37.8	29.0	67.2	61.5
8/M	49	29.6	26.2	83.5	73.0	74.0	29.2	20.7	54.3	53.3
9/M	34	52.1	49.4	168.6	141.7	142.7	75.9	58.5	92.7	84.2
10/M	48	28.7	25.1	92.0	81.5	80.5	30.4	23.4	61.6	57.2
11/M	45	34.9	28.9	101.0	84.0	83.5	42.4	30.9	58.6	52.6
Mean ± SEM	50 ± 3	34.0 ± 1.7	30.2 ± 2.2	95.7 ± 8.0	84.0 ± 6.4	83.5 ± 6.6	41.7 ± 4.2	33.2 ± 3.5	54.0 ± 5.2	50.3 ± 4.6

Week 0 measurements were made prior to dietary intervention and were assessed using a one-way ANOVA with gender as the fixed factor.

BMI, body mass index.

Table 7.4.2 Changes in fat mass, abdominal fat mass and lean mass from week 0 to week 10

	Fat mass (kg)	Abdominal fat mass (kg)	Lean mass (kg)
Men (n = 6)	-9.6 ± 1.8	-3.2 ± 0.34 [†]	-4.9 ± 1.0 [‡]
Women (n = 5)	-7.0 ± 0.31	-1.3 ± 0.24	-2.4 ± 0.59
Combined (n = 11)	-8.4 ± 1.0*	-2.3 ± 0.39*	-3.8 ± 0.71*

Data are means ± SEM. Change, the difference in kg between week 10 and week 0 values that were derived from DEXA analysis. Week 0 and week 10 data were compared using repeated-measures ANOVA with gender as the between subject factor.

*Significant change from week 0 to week 10, $p < 0.001$.

[†]Significantly greater reduction in abdominal fat for men than women, $p = 0.003$

[‡]Trend for a greater reduction in lean mass for men compared to women, $p = 0.056$

Table 7.4.3 Energy expenditure variables at weeks 0 and 10 for the 11 individuals who completed the study

Subject/Gender	TEE (kJ/day)		REE (kJ/day)		PAEE (kJ/day)		TEF (% EI)	
	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10
1/F	10245	9618	6320	6149	2901	2507	9.2	8.8
2/F	10335	10041	6827	6290	2475	2747	9.0	4.3
3/F	8804	8578	6883	7074	1041	646	5.1	2.9
4/F	7954	7888	5121	5028	2038	2071	8.1	6.7
5/F	9863	11675	7683	7040	1194	3468	6.0	4.2
6/M	11231	10562	7819	7062	2289	2444	9.5	10.5
7/M	15955	12081	9791	9077	4569	1796	7.6	8.0
8/M	13013	13397	8165	7912	3547	4145	4.6	9.0
9/M	21207	21137	14628	13505	4458	5518	9.1	2.0
10/M	8740	10628	8221	7894	-355	1671	9.7	6.3
11/M	12360	12114	9184	8071	1940	2832	10.3	10.1
Mean ± SEM	11792 ± 1165	11612 ± 1070	8244 ± 749	7737 ± 664	2641 ± 389*	2817 ± 422*	8.0 ± 0.6	6.6 ± 0.9
Change		-181 ± 454		-500 ± 128 [†]		176 ± 412*		-1.4 ± 0.9

Week 0 and 10 data were compared using repeated-measures ANOVA with gender as the between subject factor. TEE, total energy expenditure as measured using the [¹⁴C]-bicarbonate-urea method; PAEE, energy expenditure as a result of physical activity calculated from the semi-quantitative estimation equation; TEF, the thermic response to a 2791 kJ test meal, expressed as the % increase per energy intake over 3 hours; Change, the difference between week 10 and week 0 values.

*Mean and SEM of 10 individuals because subject 10 was excluded from the analysis.

[†]Significant decrease in absolute REE from week 0 to week 10, $p = 0.002$.

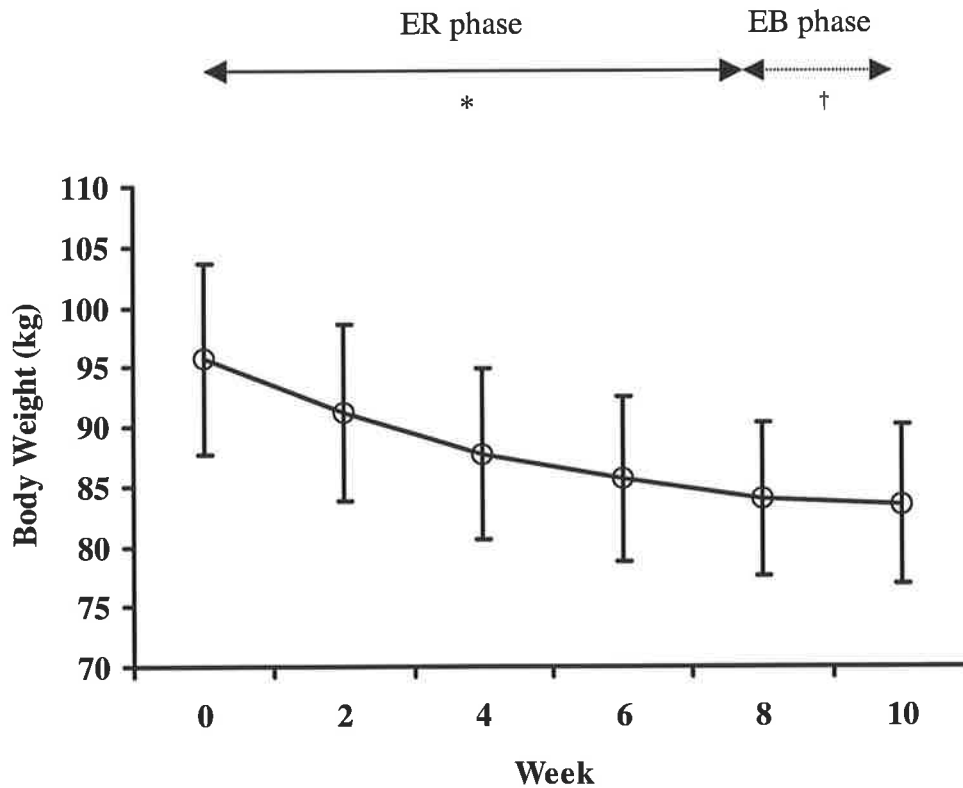


Figure 7.4.1 Change in body weight following 8 weeks of energy restriction (ER phase, weeks 0 to 8) and 2 weeks of weight maintenance at energy balance (EB phase, weeks 8 to 10) in the 11 subjects who completed the study.

Data are expressed as means \pm SEM. Weeks 0, 2, 4, 6, 8 and 10 were compared using repeated-measures ANOVA with gender as the between subject factor.

*Significant reduction in weight from week 0 to week 8, $p < 0.001$.

†Significant reduction in weight, $p = 0.04$.

7.5 DISCUSSION

This study represents the first use of the [¹⁴C]-bicarbonate-urea method to measure TEE before and after weight loss in free-living obese subjects. As discussed in Chapter 6, the method was well tolerated by subjects and by and large did not restrict normal daily activities. The major finding of this study was that the mean TEE in these “free-living” subjects was not reduced after body weight had been stabilized 12.5% below the initial weight. This confirms both doubly labeled water studies (Amatruda et al. 1993; Racette et al. 1995) and whole-body calorimetry (DeGroot et al. 1990; Rumpler et al. 1991). Amatruda et al. (1993) observed that the 8.5% decrease in TEE (966 kJ/day) with a weight loss of 22 kg (26.3%) was not statistically significant, in 18 obese women. Rumpler and colleagues (1991) demonstrated that TEE was 10% lower 28 days after the initiation of a moderate energy restrictive diet (6.2 MJ/day) in 8 obese men. One week after the return to weight maintenance energy requirements, however, TEE had returned to its baseline level (12.6 MJ/day at day 35 vs 12.3 MJ/day at baseline). Furthermore, a study of 8 women who maintained a 8.7 to 9.9 kg weight loss for more than 1 year showed that TEE adjusted for body weight and energy intake was not different from baseline when they were re-measured 1 month or 1 year after the initial weight loss period (DeGroot et al. 1990). In contrast to our finding for TEE, others have reported persistent decreases in TEE following a period of weight maintenance (Bessard et al. 1983; de Boer et al. 1986; Froidevaux et al. 1993; Leibel et al. 1995; Weigle et al. 1988). Leibel et al. (1995), using doubly labeled water to measure TEE in 7 men and 11 women, observed that the stabilization of body weight at a level 10% below the initial weight was associated with a 17% decrease in TEE normalized for lean mass. Using whole-body calorimetry, Froidevaux and colleagues (1993) measured TEE at baseline, 12 weeks after re-feeding at weight maintenance requirements, and 6 to 15 months after resuming an *ad libitum* diet, in 7 obese women. They found that TEE remained 15% (or 1.5 MJ/day) lower than the baseline value when TEE was measured at twelve weeks after the reduced body weight

had been stabilized 19% (15.4 kg) below the initial weight. Six to 15 months after these women resumed an *ad libitum* diet the decrease in TEE normalized for lean mass was not different from baseline (mean difference $3 \pm 5\%$).

Disparate findings regarding the impact of diet-induced weight loss on TEE may relate to errors associated with the measurement and hence the statistical power of the study. Studies that have examined the effect of energy restriction on TEE have generally included only small numbers of subjects (5 to 20 subjects) and none of the aforementioned studies reported the power of their observations; accordingly both type I and type II errors may have occurred. Based on the literature that had shown a reduction in TEE after weight loss (Bessard et al. 1983; Froidevaux et al. 1993; Leibel et al. 1995; Ravussin et al. 1985a) we expected that the fall in TEE would be approximately 0.8 to 1% per 1% decrease in body weight. Had this occurred, the present study would have had sufficient power (80%, alpha 0.05) to detect a mean decrease of 10% (1100 kJ/day) after a 10% reduction in body weight. However, the change in TEE in these 11 subjects was extremely variable and was not related to changes in body weight or composition. One of the 11 subjects had a decrease in TEE of 24%, and 2 experienced an increase in TEE of approximately 20%. For the remaining 8 individuals, the decrease (7 subjects) or increase (1 subject) in TEE ranged from 0.33% to 6.9%. Upon resumption of an *ad libitum* energy intake, a persistent decrease in TEE of 1 to 7% could potentially lead to a weight regain of approximately 2 to 9 kg over 1 year and therefore it is important that our future studies using the [¹⁴C]-bicarbonate method have sufficient subject numbers to detect small changes in TEE in heterogeneous populations.

Other reasons for conflicting findings may relate to the energy deficit of the diet. While we acknowledge that placing all subjects on a fixed intake of 3300 kJ/day may have caused considerable differences in the energy deficit between subjects, the diet was

designed to guarantee a weight loss of more than 10%. The aim of the study was to see whether the [¹⁴C]-bicarbonate method could measure changes in energy expenditure after weight loss of more than 10%. It is likely that a greater energy deficit would lead to greater weight loss and therefore if significantly TEE was reduced by weight loss, we would have expected that the decrease would have been greater in those individuals who lost more weight. However, as stated above the change in TEE was extremely variable and we did not observe an association between the reduction in TEE and body weight or composition. The timing of energy expenditure and body composition measurements may also in part explain disparate findings. Several studies have measured energy expenditure immediately after energy restriction (Bessard et al. 1983; Ravussin et al. 1985a) and as such the results may reflect a metabolic adaptation to an acute negative energy balance and/or the reduced cost of substrate oxidation due to the reduced energy intake. Studies that have assessed the changes in energy expenditure during energy restriction and after a period of weight loss have demonstrated that TEE returns to baseline after 6 to 15 months, even when weight loss has been maintained (DeGroot et al. 1990). Poehlman and Toth (1995) have demonstrated that the statistical procedure used to normalize energy expenditure for body size and composition may also lead to disparate results. They concluded that dividing energy expenditure by weight or body composition is not appropriate and data should be normalized using a regression-based approach.

Several studies have shown that the decrease in TEE (9 to 18%) after body weight was stabilized at a reduced level was due to a persistent and large reduction in REE (both 14%) (Bessard et al. 1983; Leibel et al. 1995). Leibel et al. (1995) originally reported that weight loss caused a reduced ratio of REE to lean mass; however, when their data was re-analysed for changes in lean and fat mass using a regression-based analysis, the changes in REE were not significant (Weinsier et al. 2000). In the present study, REE (expressed either as an absolute value or normalized for fat and lean masses) was reduced by $5.6 \pm$

1.3% after weight loss. Upon resumption of a normal free-living diet, a fall in REE of 500 kJ/day may predispose sedentary individuals to a weight regain of 5.6 kg over 1 year. The mean decrease in TEE, however, was not significant in our study population. It is possible that other factors that influence TEE, such as physical activity, may have offset some of the fall in REE. Several studies (Amatruda et al. 1993; Racette et al. 1995; Rumpler et al. 1991) have also demonstrated that REE may be reduced without a coincident fall in TEE after weight loss has been stabilized. Rumpler et al. (1991) found that the decrease in REE was small (3.2% or ~351 kJ) and TEE was not reduced, in 8 obese women who lost only 5 kg of body weight (of which 1 kg was lean mass). Furthermore, in 18 obese women, Amatruda et al. (1993) observed a 12% (748 kJ/day) decrease in REE but no significant fall in TEE (measured using doubly labeled water), after body weight was reduced and stabilized 26% below the initial weight.

The thermic effect of food, expressed as a percentage of ingested energy, is generally 6-15% (Bessard et al. 1983; D'Alessio et al. 1988). However, values as low as 2% have been observed in some individuals (Bessard et al. 1983; Leibel et al. 1995; Weststrate, 1993). In this study, the average thermogenic response to a standard 2791 kJ test meal before weight loss was 8% and it remained similar (6.6%) after weight loss. There was however substantial variability between individuals in the change in TEF; 8 subjects had a decrease in TEF of 0.2 to 7.1% whereas for 3 subjects the TEF increased by 0.4 to 4.4%. Since the mean error in the measurement of TEF is 7.8%, decreases smaller than 8% may not be detected. Consequently, no conclusions can be made regarding the effect of diet-induced weight loss on TEF, in this study.

In order to minimize the impact of variations in physical activity from obscuring the effect of weight loss on energy expenditure, subjects were asked to maintain a constant level of physical activity throughout the study. Our findings showed that the average daily level of

voluntary physical activity (as assessed from the 3-day activity diaries and a published table of activity indexes) remained similar before and after weight loss. We also found that energy expenditure due to physical activity (both voluntary and involuntary because it was calculated from the equation $0.9([\text{}^{14}\text{C}]\text{-bicarbonate TEE}) - \text{REE}$) remained similar before and after weight loss. There was, however, substantial variation in the change ($18.6 \pm 21.4\%$ increase) in PAEE between our individuals; three subjects had a decrease in PAEE ranging from 13.6 to 60% whereas six individuals had an increase of 6.7 to 46% and one subject had an increase of 190%. It is possible that the increase in PAEE for these 7 individuals may have offset the reductions in REE and therefore prevented a fall in TEE from being detected. However, since the mean error in the measurement of PAEE is 41%, changes of less than 55% could not be detected. Furthermore, it is also possible that changes larger than 55% were overestimated. Accordingly, it is difficult to make any definitive conclusions based on the available data as to whether the computed energy expenditure due to physical activity was reduced after diet-induced weight loss. Previous studies, many of which have been conducted within the confines of a respiratory chamber, have reported that the cost of physical activity for the same activity level is reduced after weight loss (Froidevaux et al. 1993; Leibel et al. 1995; Ravussin et al. 1985a; Schutz et al. 1982). However, in some people who lose weight in a less controlled environment, other issues such as does: i) the total amount of daily activity, or ii) the intensity at which the same activity is performed, increase as their self-esteem and body-image improves? If so, the variability in the estimate of PAEE in the free-living environment would be considerably greater than when PAEE is measured within a respiratory chamber.

After weight loss, we speculated that RQ may be reduced during the fasted state and increased in the postprandial state (in response to a high carbohydrate test meal). However, fat oxidation was not enhanced during fasting and carbohydrate oxidation was not increased after the test meal. The implications of having a reduced capacity to oxidize

fat during the fasting state, or increase carbohydrate oxidation after being fed a high carbohydrate meal, is that it is more difficult to maintain weight loss as compared to someone who has a larger capacity to switch between dietary substrates (Wyatt et al. 1999; Zurlo et al. 1990).

7.6 CONCLUSION

This study demonstrated that after the stabilization of a moderately reduced body weight, REE but not TEE is decreased. 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 between subjects. These preliminary findings suggest that [¹⁴C]-bicarbonate-urea is a suitable field technique to detect to changes in TEE in response to perturbations in energy balance, but studies with much larger and more homogeneous population will be required. The reduction in REE and a reduced capacity to enhance fat oxidation after weight loss may predispose individuals to weight regain on resumption of a normal diet.

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**

8.1 SUMMARY

The aim of this study was to determine the effect of a high-protein weight loss diet as compared with a standard-protein diet on body weight and composition, resting energy expenditure and the thermic effect of feeding, and glycaemic control and lipid levels in subjects with type 2 diabetes. We compared 2 groups of subjects randomly assigned to either a high-protein (HP) diet (28% energy as protein, 42% carbohydrate, 28% fat) or a standard-protein (SP) diet (16% protein, 55% carbohydrate, 26% fat) during 8 weeks energy restriction (6.7 MJ/d) and 4 weeks energy balance. Dietary protein was supplied as red meat, poultry and dairy foods. The 54 obese subjects (19 men/35 women) with type 2 diabetes had their body weight, and fasting glucose, insulin, and lipid concentrations measured at weeks 0, 4, 8 and 12. At weeks 0 and 12, body composition was assessed (DEXA) and postprandial glucose and insulin concentrations were measured after an oral glucose tolerance test. In addition, twenty-six subjects (11 men/15 women) had measurements for resting energy expenditure (REE) and the thermic effect of feeding (TEF) made. Weight loss (5.2 ± 1.8 kg) was achieved independently of diet composition. However, women on the HP diet lost significantly more total (5.3 vs 2.8 kg, $p = 0.009$) and abdominal (1.3 vs 0.7 kg, $p = 0.006$) fat when compared with women on the SP diet, whereas in men there was no difference in total fat loss between diets (3.9 vs 5.1 kg). Total lean mass decreased in all subjects independently of diet composition. For 26 subjects in the energy expenditure component of the study, the TEF was greatest after the HP than after the SP meal (6.4% vs 5.0% of energy intake, $p = 0.003$), but TEF was not associated with weight loss. After 12 weeks, both REE and TEF were reduced (effects of time, $p = 0.02$ and $p < 0.001$, respectively) but the decreases were not affected by diet composition. 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 ($p < 0.001$). The reduction in LDL-cholesterol was significantly greater on the HP (5.7%) than on the SP diet (2.7%) ($p = 0.001$). We conclude that in subjects with

type 2 diabetes, both HP and SP diets improve glucose and insulin homeostasis as a consequence of weight loss, at least over the short-term. However, the greater reduction in total and abdominal fat mass in women, and the greater reduction in LDL-cholesterol observed in both sexes, suggest that the HP weight loss diet is a suitable choice for reducing cardiovascular risk in type 2 diabetes.

8.2 INTRODUCTION

Type 2 diabetes is a major public health problem in the developed world (WHO, 1997) and is associated with obesity (Haffner, 1998) and cardiovascular disease (Stern, 1996). Insulin resistance with an inadequate insulin response to maintain normoglycaemia characterizes type 2 diabetes (Kahn, 1994). As approximately 90% of people with type 2 diabetes are obese, weight loss, particularly the loss of abdominal fat mass, is important in the management and prevention of the disease. A low-fat, high-carbohydrate diet has traditionally been advocated for type 2 diabetes (American Diabetes Association, 1994) but there is some evidence that this diet may increase plasma glucose and serum triacylglycerol concentrations, and reduce LDL particle size (Sharman et al. 2002; Garg et al. 1994; Riccardi and Parillo, 1993). Since the 1960s, high-protein diets with emphasis on some degree of carbohydrate restriction have been popular with the dieting public (St.Jeor et al. 2001). No studies, however, have yet determined the efficacy of high-protein diets for facilitating weight loss and ameliorating insulin resistance, in subjects with type 2 diabetes.

In comparison to high-carbohydrate, low-fat diets, high-protein, low-fat (< 30% total fat and < 10% saturated fat) diets have been demonstrated to enhance fat and weight loss in 65 healthy overweight and obese subjects during a 6 month structured *ad libitum* diet (Skov et al. 1999b). In addition, a 4-week randomized dietary intervention trial showed that 7 obese hyperinsulinemic normoglycaemic males lost 1.6% (2.3 kg) more weight on a

moderately hypocaloric (~7.4 MJ/day) high-protein diet (45% energy as protein, 25% carbohydrate) as compared to those (n = 6) on an isocaloric high-carbohydrate diet (12% protein, 58% carbohydrate) (Baba et al. 1999).

It has been proposed that weight loss on a high-protein diet may be facilitated, in part, by an increase in the thermic effect of feeding that may subsequently blunt the fall in resting and total energy expenditure which is frequently observed during weight loss (Baba et al. 1999). Acute feeding studies in lean and obese non-diabetic subjects have shown that protein can exert up to 3-times the thermic response as compared to isocaloric preloads of either carbohydrate or fat (Karst et al. 1984; Westerterp et al. 1999; Westerterp et al. 1999b). Two studies, one in 13 hyperinsulinemic men over 4-weeks (Baba et al. 1999) and a second in 8 overweight subjects (2 men/6women) over 7-days (Whitehead et al. 1996) showed that resting and total energy expenditure were reduced less after energy restrictive high-protein diets (45 and 36% of energy as protein, respectively) than after isocaloric standard-protein diets (15 and 12% protein, respectively). The effect of an energy restrictive high-protein diet on resting energy expenditure and the thermic effect of feeding in subjects with type 2 diabetes is not yet known. The reduced fall in resting energy expenditure may also be related to a preservation of lean mass that has been observed in obese non-diabetic women after energy restriction on low-fat diets containing an increased protein (>36% of energy) content (Hoffer et al. 1984; Piatti et al. 1994; Vazquez et al. 1995).

It has been established that both protein and amino acids added to a carbohydrate meal may stimulate insulin secretion in diabetics (Estrich et al. 1976; Nuttall et al. 1984) and non-diabetics (Spiller et al. 1987); the rationale that high-protein diets may ameliorate insulin resistance independent of weight loss, is based on this evidence. However, only one short-term (21 days) study that compared two hypocaloric (3.3 MJ/day) diets

containing either 45 or 20% of energy as protein, has observed an improvement in insulin resistance, in 25 obese non-diabetic women with hyperinsulinemia (Piatti et al. 1994). Furthermore, Piatti and colleagues (1994) found that the improvement in insulin sensitivity was due to an increase in insulin mediated glucose uptake in skeletal muscle that may have resulted from the preservation of lean body mass after weight loss. Two other studies reported that lean mass was preserved in healthy obese women following weight loss on an energy restrictive high-protein diet for 28 days (Vazquez et al. 1995) to 8 weeks (Hoffer et al. 1984). No study, however, has yet examined the effect of energy restrictive high-protein diets in sparing lean mass in subjects with type 2 diabetes, and neither Vazquez et al. (1995) or Hoffer et al. (1984) examined whether the preservation of lean mass was associated with an improvement in insulin resistance.

Two weight maintenance studies demonstrated that reductions serum LDL-cholesterol and serum triacylglycerol, and the increase HDL-cholesterol, were greater using diets containing 22% of energy as dietary protein as compared to diets containing 12% protein, in mildly hyperlipidemic (Wolfe and Giovannetti, 1991) and normolipidemic (Wolfe and Piche, 1999) subjects. Both of these studies however were of short duration (4-5 weeks) and contained only 10 subjects. Skov et al. (1999b) also demonstrated that *ad libitum* consumption of a high-protein (25% of energy) diet from a clinic shop lead to greater reductions in total-cholesterol, triacylglycerol, and free-fatty acid concentrations over a 6 month period when compared to a normal protein (12%) diet. No studies using energy restricted diets have yet confirmed the benefit of an increased protein content on the lipid profile of obese subjects with or without diabetes.

The aim of this study was to compare the effects of two fixed-intake diets, either high or lower in dietary protein (HP diet with 30% of energy as protein vs SP diet with 15% protein) on body weight and composition, resting energy expenditure and the thermic

effect of feeding, and glycaemic control and lipid levels in subjects with type 2 diabetes, in response to 8 weeks of moderate energy restriction and 4 weeks of energy balance. We hypothesized that: i) a high-protein diet will facilitate weight loss by blunting the decrease in resting energy expenditure as a consequent of an increase the thermic effect of feeding, as well as the preservation of lean mass, and ii) a greater depletion of fat mass and preservation of lean mass on the high-protein diet will improve insulin sensitivity, and reduce fasting and postprandial glucose and insulin concentrations, and improve fasting lipid levels, in type 2 diabetes.

8.3 RESEARCH DESIGN AND METHODS

8.3.1 Subjects

Sixty-six subjects with type 2 diabetes were recruited by public advertisement. Subjects were excluded if they had proteinuria (urinary protein of > 150 mg/day) or a history of liver, unstable cardiovascular, respiratory, gastrointestinal disease, or a malignancy. All subjects attended detailed information sessions and all gave written informed consent. Two subjects withdrew prior to commencement due to family commitments and ill health. A further 10 subjects (5 from each diet group) withdrew throughout the study citing ill-health that was unrelated to diabetes or dietary intervention, psychological and physical difficulties with dieting and/or venepuncture, and work and family commitments. Therefore, fifty-four subjects (19 men, 35 women) completed the study. Twenty-five subjects managed their diabetes by diet alone, 26 required oral hypoglycaemics (19 on metformin, 15 sulphonylureas alone or combination), and 4 required insulin. Four female subjects with fasting plasma glucose of 4-6mmol/l, were asked to cease medications prior to commencement of the diet to alleviate possible hypoglycaemic episodes with weight loss. Decreases in dosage occurred in 8 subjects at weeks 4 and 8 (5 from the high-protein and 3 from the standard-protein diet). Subjects on anti-hypertensive or lipid lowering medication were asked to maintain all medications and supplements at pre-study doses.

Subjects were asked to maintain their usual physical activity levels and to refrain from drinking alcohol throughout the study.

Thirty-two subjects also had measurements of resting energy expenditure, respiratory quotient, and the thermic effect of feeding. The subjects who had measurements of energy expenditure made were selected based on: i) their willingness to participate and ii) whether they had flexibility in their time and could stay in the clinic for approximately half-a-day. Twenty-six subjects (11 men, 15 women) completed this component of the study. Four subjects from this subpopulation withdrew during the course of the study, and a further 2 were not included in the energy expenditure analysis (1 subject was unable to comply, and data for another subject was incomplete due to a computer crash). Of these 26 subjects, 3 managed their diabetes by diet, 21 were taking oral hypoglycaemics, and 2 required insulin.

8.3.2 Diets

The high-protein (HP) diet consisted of 30% energy from protein (~110 g/day) and 40% from carbohydrate. The standard-protein (SP) diet consisted of 15% of energy from protein (~60 g/day) and 55% from carbohydrate. Both diets were matched for fatty acid profile (8% saturated, 12% monounsaturated, and 5% polyunsaturated fatty acids). The subjects were provided with prescriptive fixed menu plans and supplied with key foods which made-up 60 % of their energy intake to assist with dietary compliance. These included pre-weighed portions of beef and chicken suitable for 6 six meals per week, shortbread biscuits, Canola Lite™ margarine, and Sunola™ oil (MeadowLea Foods Ltd, Mascot, NSW, Australia)- plus, Kraft Free™ (3% of energy as fat) cheese (Kraft Foods Ltd, Melbourne, Vic, Australia), skim milk powder, and diet yoghurt for the HP diet, and sultanas and rice for the SP diet. The other differences between the diets lay in the amount of meat and chicken (200 vs 100g),

fruit (200 vs 300g), and wholemeal bread (3 vs 4 slices). Alcohol was not permitted and a list of free choice vegetables and salad (maximum 2.5 cups) was provided. During the weight maintenance phase, energy intake was increased by about 30% and protein intake increased by a further 20 g in the HP diet and 7 g in the SP diet (i.e. overall protein intakes during energy balance equated to approximately 131 and 69 g/day, respectively). Group training was provided in the use of scales and keeping food records. Each fortnight, subjects visited the same research dietitian who provided detailed dietary instruction and assessment. Weighed daily food records were completed on 3 consecutive days (2 week and 1 weekend day) at each 2-week period, and energy and macronutrient intakes were determined using Diet 1 Nutritional software (Xyris Software, Highgate Hill, Queensland, Australia) (Cashell K, 1989). This program is based on Australian food composition tables and food manufacturers data. The database has been extensively modified by the CSIRO clinical research dietitians to add new foods and recipes. There were no missing values for the nutrients of interest. Recipes were entered as proportions of the original ingredients.

8.3.3 Experimental design

The study was conducted on an outpatient basis over 12 weeks. Subjects were matched on the basis of fasting plasma insulin, BMI, age, sex and medication. The two matched groups were randomly assigned to either the HP or SP diet. Both the HP and SP groups underwent 8 weeks of energy restriction followed by 4 weeks at energy balance on the same macronutrient composition designed to maintain weight. During energy restriction, the energy intake of individuals was reduced by approximately 30% of their weight maintenance energy requirements (calculated as 1.3 multiplied by basal metabolic rate). Numerous weight loss studies conducted at the CSIRO have found that a 30% energy restriction is sufficient to successfully induce a weight loss of 0.5 to 1 kg per week.

On two consecutive days at weeks 0, 4, 8 and 12 subjects attended the CSIRO research unit to be weighed and to have venous blood samples and blood pressure taken after having fasted for 12 hours. At weeks 0, 8, and 12 all subjects underwent a 3-hour oral glucose tolerance test. Baseline venous blood samples were taken and a 75g, 300ml glucose drink was consumed. Blood samples were taken at 1, 2, and 3 hours to assess glucose and insulin concentrations. All subjects collected 24-hour urine samples at weeks 0, 8, and 12, one day prior to attending the clinic. Samples were assessed for the urea/creatinine ratio to determine dietary compliance.

At weeks 0 and 12 (after body weight had been stabilized), all subjects had their body composition measured by dual-energy x-ray absorptiometry. Also at weeks 0 and 12, the subjects participating in the energy expenditure assessment visited the Department of Medicine research unit at the Royal Adelaide hospital. At the Department of Medicine, subjects had their height, weight, resting energy expenditure, respiratory quotient, and thermic effect of feeding measured after having fasted for at least 6 hours. For each variable, the same investigator performed all measurements.

8.3.4 Measurements

8.3.4.1 Body weight, body composition, and blood pressure

Body weight was measured as described in Chapter 2.3.4, and total body fat mass and total body lean mass were assessed using dual-energy x-ray absorptiometry (DEXA) as described in Chapter 2.3.5.1. Two blood pressure measurements were recorded with an automated sphygmomanometer (Dinamap 1800, Critikon, FL, USA) after at least 5 minutes of seated rest.

8.3.4.2 *Resting energy expenditure (REE), respiratory quotient (RQ), and the thermic effect of feeding (TEF)*

Fasting REE and RQ were measured by indirect calorimetry using the ventilated canopy and Deltatrac metabolic monitor as described in Chapters 2.3.1.2 and 2.3.2.1, respectively. Immediately following fasting measurements of REE and RQ, a 2395 kJ HP (41% of energy as protein, 46% as carbohydrate) meal or a 2743 kJ SP (12% as protein, 69% as carbohydrate) (Appendix 5) was consumed within 20 minutes. The composition of the two test meals was different to the macronutrient composition of the overall study diets. The reason for this was because the meals were designed to maximize the exchange of carbohydrate for protein while at the same time matching caloric content of the two meals at approximately one third of the daily energy intake. After the test meals subjects returned to the hood for a further 120 minutes of RQ and REE measurements. Postprandial RQ and the mean TEF over the 120-minute period, were calculated as described in Chapters 2.3.2.2 and 2.3.1.3. Postprandial RQ was expressed as the mean change in RQ above baseline, and TEF was calculated as the mean increase in REE above baseline and expressed as the % increase per energy intake of the test meal.

8.3.4.3 *Biochemical sampling and analysis*

Blood sampling and the collection of 24-hour urine sample were done as described in Chapter 2.3.6. Biochemical assays were performed in a single assay at the completion of the study. Plasma glucose, and serum insulin, triacylglycerol, and total, LDL, and HDL cholesterol concentrations were determined as in Chapter 2.3.7. As a surrogate measure of insulin resistance, we used the HOMA insulin resistance index. HOMA is a simplest and least expensive of the alternate methods to the clamp technique (Bonora et al., 2000) and it has been shown to correlate highly with clamp techniques in several large non-diabetic populations (Yeni-Komshian et al. 2000). Urinary urea and creatinine concentrations were determined by methods described in Chapter 2.3.7.8. Glycated-haemoglobin samples were

frozen at -20°C and analysed by high performance liquid chromatography at the end of the study (Jeppsson et al. 1986).

8.3.5 Statistical analysis

All data are presented as means \pm SEM. Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). Baseline measurements were assessed for differences between the dietary groups and between men and women using two-way ANOVA. The effect of the diet intervention was assessed using repeated-measures ANOVA with variables measured at weeks 0, 4, 8, and 12. Diet and gender were the between subject factors. Inclusion of data from 8 subjects with medication changes significantly affected the results for the oral glucose tolerance test so these subjects were excluded from the analysis for this test. Incremental area under the glucose and insulin response curves during the 3-hour oral glucose tolerance test were calculated geometrically using the trapezoidal rule (Wolever et al. 1991). The homeostasis model assessment (HOMA) for insulin resistance was calculated as $[\text{Fasting insulin} \times \text{Fasting glucose} / 22.5]$ (Matthews et al. 1985). Significance was set at $p < 0.05$.

8.4 RESULTS

8.4.1 Subject characteristics

The mean values for the physical characteristics of the fifty-four subjects at baseline are shown in Table 8.4.1. There were no significant differences in any of the variables between diets. Baseline body weight, however, was significantly greater for males than females ($p < 0.01$).

8.4.2 Diet composition and subject compliance

Energy intake in the 8-week energy restriction phase and the 4-week energy balance phase was not statistically different between the two diets (Table 8.4.2). As prescribed, the

protein intake was higher and carbohydrate intake lower on the HP diet as compared to the SP diet, with no differences between the energy restrictive and energy balance phases (effect of diet, $p < 0.001$). Saturated fat intake was not different between the diets or the phases, but dietary fibre ($p < 0.001$) and dietary cholesterol ($p < 0.001$) were significantly different between the diets in both phases (Table 8.4.2).

Urine urea decreased from 450 mmol/day at week 0 to 420 mmol/day at week 8 on the HP diet and from 428 to 301 mmol/day on the SP diet (effect of diet, $p < 0.001$). At week 12 (end energy balance phase) as compared to week 8 (end of energy restriction phase), urine urea had risen to 461 mmol/day on the HP and to 344 mmol/day on the SP diet (effect of diet, $p < 0.001$). The urinary urea/creatinine ratio was significantly different between diets ($p < 0.001$) (Figure 8.4.1). Urinary albumin excretion did not change with weight loss on either diet. For the 11 subjects in the HP group with microalbuminuria, there was a small but non-significant decrease in albumin excretion from 25.3 to 22.1 mg/day, whereas for the 8 subjects in the SP group with microalbuminuria there was a non-significant increase from 3.6 to 4.5 mg/d.

8.4.3 Body weight, body composition, and blood pressure

After 8 weeks of energy restriction and 4 weeks of energy balance, the mean weight loss was 5.2 ± 1.8 kg ($p < 0.001$), but the decrease in weight was not affected by the diet composition (4.8 ± 3.1 vs 5.6 ± 2.5 kg on the HP and SP diets respectively) (Figure 8.4.2). There was no effect of gender on the decrease in weight, however there was a weak gender-by-diet interaction ($p = 0.04$) such that men lost more weight on the SP diet as compared to the HP diet (5.8 vs 4.7 kg) while the women lost more weight on the HP diet (6.0 vs 4.2 kg). Weight was maintained during energy balance, with no difference between diets or genders.

Total fat mass was reduced after 12 weeks ($p < 0.001$), but there was no effect of either diet composition or gender on the decrease (Table 8.4.3). A significant gender-by-diet interaction ($p = 0.032$) on the decrease in total fat mass was observed. Women lost 47.5% more total fat on the HP diet than on the SP diet (5.3 vs 2.8 kg, $p = 0.01$), while in the men there was no difference between the SP and HP diet (5.1 vs 3.9 kg) (Table 8.4.3). The reduction in abdominal fat mass ($p < 0.001$) was also independent of diet composition. The decrease in abdominal fat mass was affected by gender; the reduction being greater for men than women (effect of gender, $p = 0.001$). Furthermore, there was a significant gender-by-diet interaction such that women lost more abdominal fat on the HP diet as compared to the SP diet (1.3 vs 0.7 kg, $p = 0.006$) while men lost a similar amount on both diets (1.7 vs 1.4 kg) (Table 8.4.3). After 12 weeks of dietary intervention, total lean mass was significantly reduced by 1.0 kg ($p = 0.005$), but there was no effect of diet composition, or gender on the decrease (Table 8.4.3).

8.4.4 Glycaemic control and insulin sensitivity

Overall, fasting plasma glucose were reduced 12.2% from baseline to week 8 and remained 7.3% lower at week 12 as compared to baseline ($p < 0.001$). Neither diet composition nor gender affected fasting plasma glucose levels (Table 8.4.4). Fasting serum insulin was reduced by 20.1% at week 8 and by 14% at week 12, with no effect of either diet or gender ($p < 0.001$) (Table 8.4.4). The HOMA insulin resistance index decreased 28.7% from 6.1 ± 0.52 at week 0 to 4.35 ± 0.54 at week 8 ($p = 0.006$) and by 19.8% to 4.89 ± 0.54 at week 12 ($p = 0.039$). There was no effect of diet or gender on the HOMA index (Table 8.4.4).

The plasma glucose response after the ingestion of the oral glucose drink was significantly reduced at 8 week after energy restriction ($p < 0.001$), and remained reduced at week 12 after a further 4 weeks of energy balance ($p < 0.001$) (Figure 8.4.3A). From week 0 to

week 8, the plasma glucose concentration 2 hours after the ingestion of the oral glucose drink, was reduced by 17.4%, and at week 12 it remained 14.1% lower than at week 0 ($p < 0.001$). There was, however, no effect of diet composition on the decrease in post-load glucose concentrations and there was no effect of gender. The plasma glucose area under the curve (AUC) was reduced by 18.4% from $2454 \pm 85 \text{ mmol}^{-1} \cdot \text{L} \cdot 180\text{min}^{-1}$ at week 0 to $2003 \pm 90 \text{ mmol}^{-1} \cdot \text{L} \cdot 180\text{min}^{-1}$ at week 8, and by 14.3% to $2104 \pm 87 \text{ mmol}^{-1} \cdot \text{L} \cdot 180\text{min}^{-1}$ at week 12. Neither diet nor gender affected the glucose AUC (Table 8.4.4).

At both week 8 and week 12 as compared to week 0, serum insulin concentrations after the ingestion of the oral glucose drink were reduced ($p < 0.001$) (Figure 8.4.3B). At week 8 as compared to week 0, there was a 15.1% decrease in the insulin concentration at 2 hours after the glucose load, and by week 12 the reduction from baseline was 13.1%. Insulin AUC was reduced by 13.0% from $9602 \pm 923 \text{ mU}^{-1} \cdot \text{L} \cdot 180\text{min}^{-1}$ at week 0 to $8358 \pm 779 \text{ mU}^{-1} \cdot \text{L} \cdot 180\text{min}^{-1}$ at week 8 ($p = 0.009$), and by 10.5% to $8594 \pm 812 \text{ mU}^{-1} \cdot \text{L} \cdot 180\text{min}^{-1}$ at week 12 ($p = 0.016$). Neither diet composition nor gender affected post-load serum insulin concentrations or the insulin AUC (Table 8.4.4).

From week 0 to week 12, HbA1c decreased 0.6% (from 6.4% to 5.8%, $p < 0.001$) with no effect of diet or gender.

8.4.5 Serum Lipids

Total-cholesterol was reduced by 8.3% at week 8 as compared to week 0, and by 3.4% at week 12 ($p < 0.001$). The decreases in total-cholesterol were significantly greater on the HP diet than on the SP diet ($p = 0.032$ for time-by-diet interaction) (Table 8.4.5). There was no effect of gender on total-cholesterol. Overall, LDL-cholesterol concentrations were significantly reduced after 12 weeks of weight loss ($p = 0.004$). However, a significant time-by-diet interaction ($p = 0.035$) revealed the HP diet reduced LDL-C at all time points

whereas LDL-C at week 12 on the SP diet was greater than at baseline (Table 8.4.5). No effect of gender was observed for LDL-cholesterol. There was no effect of time, diet, or gender on HDL-cholesterol concentrations. Triacylglycerol concentrations had decreased by 20.9% at week 8 and by 13.3% at week 12 ($p < 0.001$) but there was no effect of diet or gender.

8.4.6 Resting energy expenditure, thermic effect of feeding, and respiratory quotient

For the 26 subjects participating in the energy expenditure component of this study, weight loss was representative of the larger study population (Table 8.4.6). However, in this subpopulation, we observed no beneficial effect of the HP diet as compared to the SP diet in reducing total or abdominal fat mass in women (Table 8.4.6). Furthermore, there was no significant decrease in total lean mass in these 26 subjects (Table 8.4.6).

After 12 weeks of dietary intervention, REE was reduced by 4.1% ($p = 0.023$), but the decrease was not related to diet composition or gender, regardless of whether REE was expressed as an absolute value or per kg of lean mass (Table 8.4.7). There was an overall effect of gender on REE such that absolute REE was greater in men than women at both week 0 (8814 vs 7295 kJ/day, $p = 0.003$) and week 12 (8613 vs 6876 kJ/day, $p < 0.001$).

The energy intake during the HP test meal was 2395 kJ whereas for the SP meal it was 2742 kJ, and therefore TEF was expressed as the % increase in energy expenditure per energy intake. After 12 weeks of dietary intervention, TEF was reduced ($p < 0.001$). There was a time-by-diet-gender effect (regardless of whether TEF was expressed as an absolute value or as TEF per energy consumed) with females on the HP diet experiencing an increase in TEF of 0.02 ± 0.3 % per energy intake, whereas the TEF of females on the SP diet was decreased by 1.3 ± 0.6 % per energy intake (Table 8.4.7). Furthermore, there was

an overall effect of diet on TEF such that the TEF produced after the HP meal as compared to after the SP meal was 1.2% greater at week 0 ($p = 0.008$) and 1.7% greater at week 12 ($p = 0.01$) (Table 8.4.7). At week 0, the TEF was 1.75% greater for men than it was for the women (effect of diet, $p = 0.004$) and by week 12 the TEF remained 0.5% greater for men as compared to women but the effect of diet was non-significant ($p = 0.5$) (Table 8.4.7). The TEF at weeks 0 or 12 was not related to the change in body weight.

At week 12, fasting RQ was not different from baseline. There was no effect of either diet or gender on fasting RQ (Table 8.4.7). Similarly, there was no change in postprandial RQ from week 0 to week 12. There was an overall effect of diet on postprandial RQ such that the increase in RQ after the SP meal was greater than after the HP meal at both week 0 (greater by 66%, $p < 0.001$) and week 12 (greater by 71%, $p < 0.001$) (Table 8.4.7). The change in postprandial RQ after 12 weeks was not significantly correlated to the decrease in fasting insulin (-2.14 ± 0.76 mU/L) ($r = 0.24$) and/or the change in area under the insulin curve after an oral glucose load ($-8.1 \pm 4.2\%$) ($r = -0.30$).

8.4.7 Blood pressure

From baseline to week 8, systolic blood pressure fell from 143 to 135 mmHg (5.6%, $p < 0.001$) and diastolic blood pressure fell from 82 to 76 mmHg (4.9%, $p < 0.001$). There was no differential effect of diet on blood pressure during energy restriction. During the weight stabilization period, systolic blood pressure rose by 3 mmHg and diastolic blood pressure by 1 mmHg. This was also not affected by diet composition.

Table 8.4.1 Physical characteristics of subjects at baseline*

	SP diet		HP diet	
	Male n = 10	Female n = 18	Male n = 9	Female n = 17
Age (yrs)	64.2 ± 3.8	60.9 ± 2.3	63.4 ± 1.7	58.7 ± 2.2
Weight (kg) [†]	101.2 ± 5.4	86.7 ± 4.1	107.6 ± 5.8	93.2 ± 3.7
BMI (kg/m ²)	33.4 ± 1.0	33.2 ± 1.4	35.4 ± 2.0	34.5 ± 1.4
Insulin (mU/L)	17.1 ± 3.0	16.1 ± 1.4	18.7 ± 2.6	15.8 ± 2.0
Glucose (mmol/L)	7.4 ± 0.4	7.4 ± 0.3	7.9 ± 0.9	7.5 ± 0.4
sBP (mmHg)	144 ± 6	137 ± 3	151 ± 5	143 ± 3
dBp (mmHg)	81 ± 3	78 ± 3	90 ± 3	83 ± 3

SP, standard-protein; HP, high-protein; BMI, body mass index; sBP, systolic blood pressure; dBp, diastolic blood pressure. Measurements were made at screening prior to dietary intervention and were assessed using two-way ANOVA with diet and gender as the fixed factors. *Data are expressed as means ± SEM

There were no significant differences between the SP and HP dietary groups.

[†]Significant effect of gender, $p < 0.01$

Table 8.4.2 Composition of study diets derived from subjects' daily weighed food records*

	SP diet		HP diet	
	n = 28		n = 26	
	ER	EB	ER	EB
Energy (MJ/day)	6483 ± 173 [†]	7498 ± 300 [†]	6744 ± 150 [†]	8074 ± 244 [‡]
Protein (% E)	16.4 ± 0.3 [†]	16.0 ± 0.3 [†]	28.1 ± 0.3 [‡]	27.7 ± 0.3 [‡]
Carbohydrate (% E)	54.8 ± 0.4 [†]	55.0 ± 0.4 [†]	42.1 ± 0.2 [‡]	42.6 ± 0.4 [‡]
Total fat (% E)	26.3 ± 0.4 [†]	26.7 ± 0.5 [†]	27.8 ± 0.2 [‡]	27.6 ± 0.3 [‡]
SFA (% E)	7.5 ± 0.1 [†]	7.6 ± 0.2 [†]	8.2 ± 0.1 [‡]	8.2 ± 0.2 [‡]
MUFA (% E)	11.2 ± 0.2 [†]	11.6 ± 0.3 [†]	12.1 ± 0.1 [‡]	12.2 ± 0.2 [‡]
PUFA (% E)	4.9 ± 0.1 [†]	4.8 ± 0.1 [†]	4.9 ± 0.1 [†]	4.7 ± 0.1 [†]
Fiber (g/day)	28.3 ± 0.8 [†]	33.5 ± 1.3 [†]	24.3 ± 0.7 [‡]	29.3 ± 1.2 [†]
Cholesterol (mg/day)	93.9 ± 5.6 [†]	98.6 ± 5.9 [†]	175.7 ± 6.1 [‡]	202.9 ± 8.5 [‡]

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Three days (2 week days and 1 weekend day) of dietary data were analysed at weeks 2, 4, 6 and 8 during the energy restrictive (ER) phase of the study, and at weeks 10 and 12 during energy balance (EB). No significant differences were found between the four diet records in the energy restrictive phase or between the two records for the energy balance phase, so data for recordings in each phase were averaged. *Data are expressed as means ± SEM.

Common superscripts denote that the composition of the allocated diets were not different ($p > 0.05$).

Table 8.4.3 Total fat mass, abdominal fat mass and total lean mass in the entire study population at weeks 0 and 12*

	SP diet		HP diet	
	Male n = 10	Female n = 18	Male n = 9	Female n = 17
Total fat mass (kg) ^{† ‡}				
Week 0	34.8 ± 5.2	39.9 ± 3.2	38.7 ± 5.0	42.8 ± 2.6
Week 12	30.4 ± 2.4	37.0 ± 3.1	35.5 ± 2.6	37.5 ± 2.5
Change	-5.1 ± 0.8	-2.8 ± 0.6	-3.9 ± 0.4	-5.3 ± 0.7
Abdominal fat mass (kg) ^{† §}				
Week 0	10.4 ± 0.7	9.7 ± 0.8	10.9 ± 1.3	10.9 ± 0.7
Week 12	8.7 ± 0.8	9.0 ± 0.8	9.5 ± 1.5	9.58 ± 0.7
Change	-1.7 ± 0.2	-0.7 ± 0.1	-1.4 ± 0.3	-1.3 ± 0.2
Total lean mass (kg) [¶]				
Week 0	61.5 ± 3.2	43.9 ± 1.4	63.7 ± 2.1	47.0 ± 1.3
Week 12	60.5 ± 2.9	42.2 ± 1.6	62.7 ± 2.3	46.6 ± 1.1
Change	-0.9 ± 0.8	-1.7 ± 0.6	-1.0 ± 0.7	-0.5 ± 0.5

Change, difference between weeks 12 and 0. Values were derived from DEXA analysis performed at week 0 and 12. Week 0 and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. *Data are means ± SEM.

[†]Significant effect of time from week 0 to week 12, $p < 0.001$

[‡]Significant time-by-diet-by-gender interaction for women, $p = 0.01$.

[§]Significant time-by-gender effect for men, $p = 0.001$.

^{||}Significant time-by-diet-by-gender interaction for women, $p = 0.006$.

[¶]Significant effect of time from week 0 to week 12, $p = 0.005$

Table 8.4.4 Fasting and 2 hour postprandial glucose and insulin concentrations*

	SP diet n = 28	HP diet n = 26
Fasting glucose (mmol/L)		
Week 0	7.76 ± 0.24	8.44 ± 0.41
Week 8	7.02 ± 0.21 [†]	7.30 ± 0.28 [†]
Week 12	7.33 ± 0.30 [†]	7.70 ± 0.31 [†]
Fasting insulin (mU/L)		
Week 0	16.5 ± 7.7	16.3 ± 7.2
Week 8	14.0 ± 10.8 [†]	12.1 ± 6.5 [†]
Week 12	14.8 ± 9.2 [†]	13.3 ± 6.8 [†]
HOMA index		
Week 0	5.87 ± 0.67	6.36 ± 0.82
Week 8	4.71 ± 0.94 [†]	3.97 ± 0.48 [†]
Week 12	5.11 ± 0.91 [†]	4.64 ± 0.54 [†]
Glucose AUC (mU ⁻¹ .L.180min ⁻¹)		
Week 0	2335 ± 104	2595 ± 135
Week 8	1894 ± 117 [†]	2134 ± 138 [†]
Week 12	1940 ± 108 [†]	2299 ± 132 [†]
Insulin AUC (mU ⁻¹ .L.180min ⁻¹)		
Week 0	10064 ± 1085	9051 ± 1578
Week 8	8711 ± 917 [†]	7938 ± 1333 [†]
Week 12	9000 ± 902 [†]	8160 ± 1438 [†]

Fasting glucose and insulin concentrations were measured on 2 consecutive days at weeks 0, 8 and 12, and the average of the two values at each week was recorded (determined not to be different using a student's paired t-test). HOMA index of insulin resistance was calculated as [Fasting glucose x Fasting insulin]/ 22.5. Glucose and insulin areas under the curve (AUC) were after the ingestion of 75 grams of glucose at weeks 0, 8 and 12. Weeks 0, 8, and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. *Data are means ± SEM.

[†]Significant effect of time from week 0, p < 0.05.

There was no effect of diet composition on any of the variables.

Table 8.4.5 Fasting serum lipids at weeks 0, 8, and 12 of dietary intervention*

	SP diet n = 28	HP diet n = 26
Total cholesterol (mmol/L)		
Week 0	5.16 ± 0.25	5.16 ± 0.17
Week 8	4.82 ± 0.22 [†]	4.64 ± 0.18 ^{†§}
Week 12	5.15 ± 0.25	4.81 ± 0.16 ^{†§}
LDL cholesterol (mmol/L)		
Week 0	3.23 ± 0.20	3.32 ± 0.16
Week 8	3.12 ± 0.20 [†]	3.02 ± 0.15 ^{†§}
Week 12	3.32 ± 0.22	3.13 ± 0.15 ^{†§}
HDL cholesterol (mmol/L)		
Week 0	0.95 ± 0.05	0.93 ± 0.03
Week 8	0.91 ± 0.04	0.92 ± 0.03
Week 12	0.96 ± 0.03	0.92 ± 0.04
Triacylglycerol (mmol/L)		
Week 0	2.17 ± 0.21	2.02 ± 0.15
Week 8	1.76 ± 0.11 [†]	1.56 ± 0.13 [†]
Week 12	1.94 ± 0.16 [†]	1.68 ± 0.14 [†]

Weeks 0, 8, and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Comparison between diets within the same week was made using 1-way ANVOVA. *Data are means ± SEM.

[†]Significant effect of time from week 0, $p < 0.004$.

[§]Significant effect of diet, $p < 0.05$.

Table 8.4.6 Weight, total fat and lean mass changes for the 26 subjects in the energy expenditure component of the study*

	SP diet	HP diet
	n = 11	n = 15
Body weight (kg)[†]		
Week 0	87.8 ± 4.9	91.5 ± 3.6
Week 12	83.2 ± 4.4	86.5 ± 3.7
Change	-4.6 ± 0.9	-4.9 ± 0.5
Total fat mass (kg)[†]		
Week 0	34.4 ± 2.8	40.0 ± 2.8
Week 12	30.2 ± 2.6	35.3 ± 3.0
Change	-4.2 ± 0.5	-4.7 ± 0.6
Total lean mass (kg)		
Week 0	53.4 ± 3.4	51.5 ± 2.8
Week 12	53.0 ± 1.3	51.2 ± 2.6
Change	-0.4 ± 0.8	-0.2 ± 0.4

Change, difference between weeks 12 and 0. Values were derived from DEXA analysis performed at week 0 and 12. Week 0 and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. *Data are means ± SEM.

[†]Significant effect of time from week 0 to week 12, $p < 0.05$

Table 8.4.7 Energy expenditure variables at weeks 0 and 12*

	SP diet			HP diet		
	Male	Female	Combined	Male	Female	Combined
	n = 6	n = 5	n = 11	n = 5	n = 10	n = 15
Fasting REE ^{†‡}						
Week 0	8256 ± 579	7315 ± 684	7828 ± 445	9476 ± 536	7296 ± 199	8023 ± 346
Week12	8286 ± 458	7042 ± 305	7721 ± 335	9010 ± 569	6803 ± 179	7538 ± 349
Change	-30 ± 229	-273 ± 524	-115 ± 257	-466 ± 50	-494 ± 102	-482 ± 69
TEF ^{†§ ̈}						
Week 0	6.3 ± 0.4	4.8 ± 0.5	5.6 ± 0.4	8.1 ± 1.0	6.1 ± 0.3	6.8 ± 0.5
Week12	5.0 ± 0.7	3.4 ± 0.4	4.3 ± 0.5	5.5 ± 0.8	6.1 ± 0.4	6.0 ± 0.4
Change	-1.3 ± 0.7	-1.3 ± 0.6	-1.3 ± 0.5	-2.6 ± 0.9	0.02 ± 0.3	-0.9 ± 0.5
Fasting RQ						
Week 0	0.76 ± 0.01	0.75 ± 0.01	0.76 ± 0.01	0.76 ± 0.02	0.78 ± 0.01	0.77 ± 0.01
Week12	0.77 ± 0.02	0.79 ± 0.02	0.78 ± 0.01	0.77 ± 0.02	0.78 ± 0.01	0.78 ± 0.01
Change	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.004 ± 0.02	0.01 ± 0.01
Posprandial RQ [¶]						
Week 0	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Week 12	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Change	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	-0.01 ± 0.01	0.002 ± 0.01	-0.001 ± 0.01

TEF, thermic response to a 2742 kJ SP, or 2395 kJ HP test meal over 3 hours, expressed as the % increase per energy intake; Change RQ, mean increase in RQ over 2 hours after the HP or SP test meal; Change, difference between weeks 12 and 0. Week 0 and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. *Data are means ± SEM. †Significant effect of time from week 0 to week 12, p < 0.05.

‡Significant effect of gender at both weeks 0 and 12, p < 0.04. §Significant effect of diet at both weeks 0 and 12, p < 0.01. ̈Significant effect of gender at week 0, p = 0.004. ¶Significant time-by-diet-by-gender interaction, p = 0.041. ¶Significant effect of diet at week 0 and week 12, p < 0.001

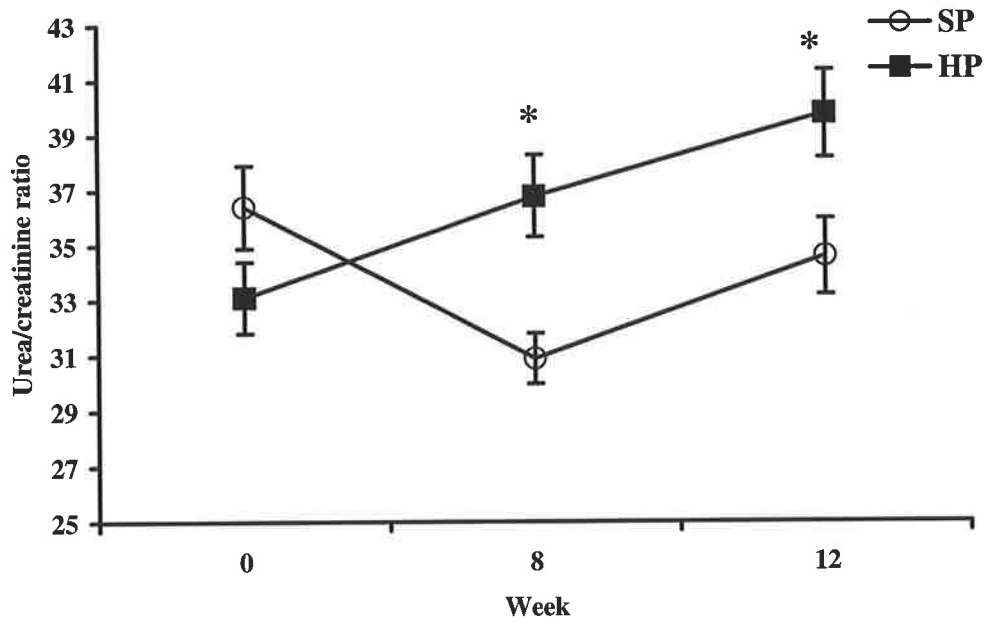


Figure 8.4.1 Urea excretion calculated from 24-hour urine saves and expressed as the ratio of urea/creatinine, in subjects with type 2 diabetes on the SP (n = 28) and HP (n = 26) study diets.

Urine samples were collected at weeks 0, 8, and 12 to determine compliance with the study diets. Week 0, 8 and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Data are expressed as means \pm SEM.

*Significant effect of diet at weeks 8 and 12, $p < 0.01$.

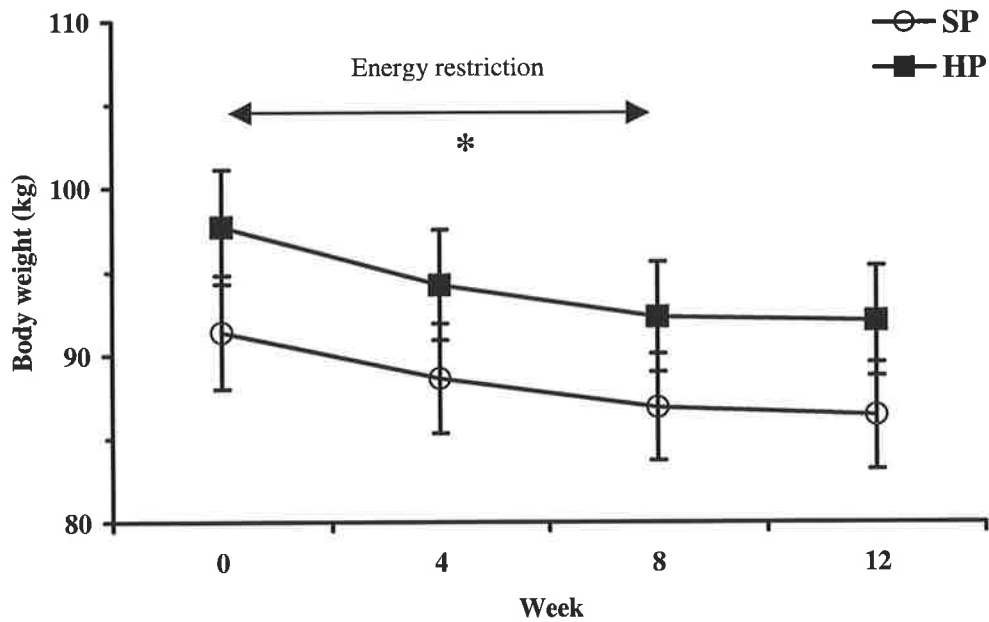


Figure 8.4.2 Change in body weight following 8 weeks of energy restriction (weeks 0 to 8) and 4 weeks of energy balance (from week 8 to 12) in subjects with type 2 diabetes on either the SP (n = 28) or HP (n = 26) study diet.

Week 0, 4, 8 and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Data are expressed as mean \pm SEM.

*Significant decrease in weight from week 0 to week 8, in all subjects, $p < 0.001$.

Weight was maintained from week 8 to week 12 during energy balance.

There was no effect of diet.

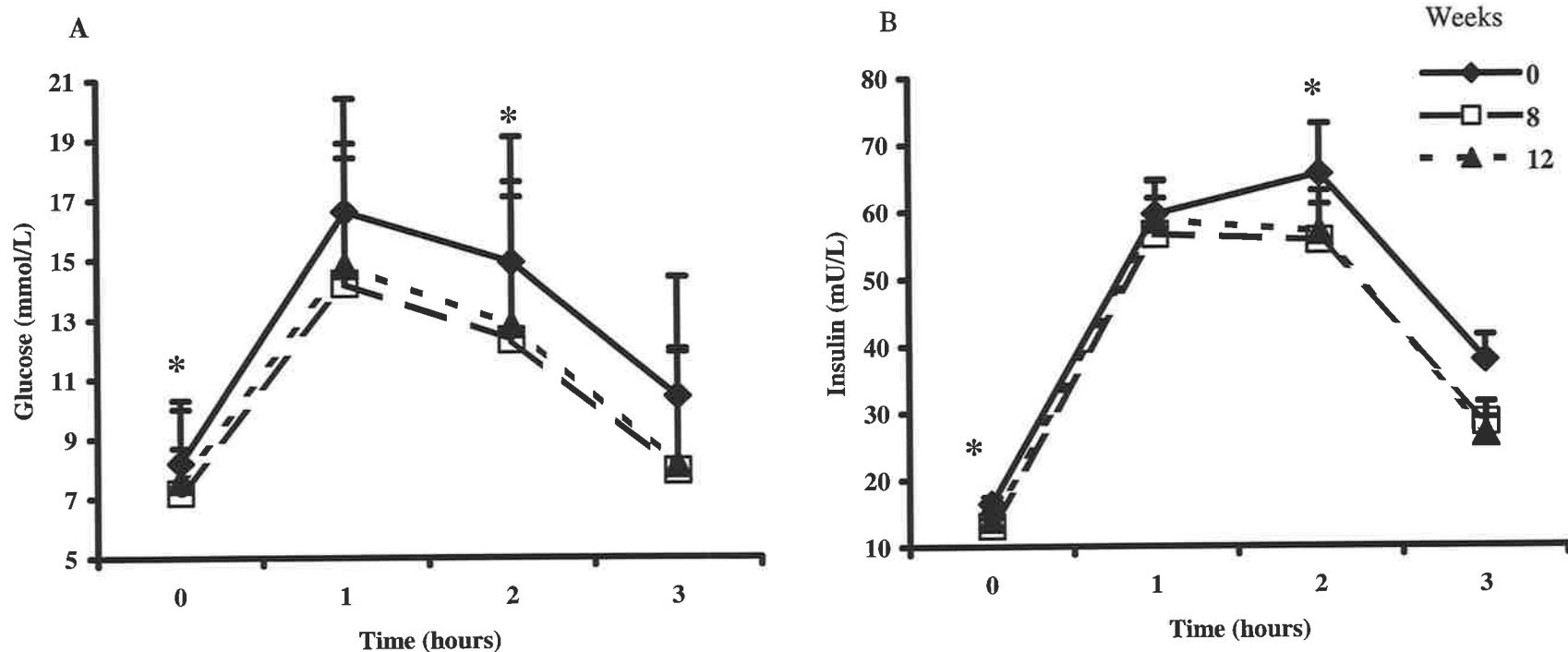


Figure 8.4.3 Glucose (A) and insulin (B) responses during the 3-hour oral glucose tolerance test.

Glucose and insulin concentrations were measured at baseline and after 1, 2, and 3 hours after the ingestion of 75 grams of glucose, at week 0, week 8 (at the end of the energy restriction phase), and week 12 (at the end of the energy balance phase). Data was excluded from 8 subjects who had medication changes throughout the study. Week 0, 8 and 12 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Data are expressed as the mean \pm SEM ($n = 46$). *Significantly different from week 0 to week 8 and from week 0 to week 12, $p < 0.001$. There was no effect of diet or diet-by-gender on the fasting or postprandial glucose or insulin concentrations.

8.5 DISCUSSION

The findings from this study show that total energy intake and not the protein-to-carbohydrate ratio of the diet is the most important determinant of weight loss, in subjects with type 2 diabetes, at least over 12 weeks. This observation is in accordance with several short term studies (21-28 days) in obese non-diabetic subjects that also showed equivalent weight loss on energy restricted (2.5-3.4 MJ/day) diets containing 45-49% of energy as protein as compared to diets with lower protein contents (20-37%) (Vazquez et al. 1995; Piatti et al. 1994). Furthermore, despite observing a significantly greater fall in sleeping metabolic rate and total energy expenditure after 7 days on a diet containing 36% of energy as protein as compared to two separate 7 day periods on isocaloric diets (4.2 MJ/day) with 15% protein and either 53% or 32% carbohydrate, Whitehead et al. (1996) found no difference in weight loss between the three dietary periods. In contrast, a recent study of 13 hyperinsulinemic men showed 28% more weight loss (8.3 vs 6.0 kg) over 4 weeks on a diet containing 45% of energy as protein as compared to an isocaloric diet with 12% protein (Baba et al. 1999). A significant difference in total body water loss (-1.0 vs 0.3) between the high and standard-protein diets, rather than fat loss (-7.1 vs -6.3 kg), may have explained some of the differential weight loss in the aforementioned study (Baba et al. 1999). In the present study, total body water was not measured, and the 1.2 kg (~25%) greater loss of fat mass and the 0.78 kg (~55%) smaller loss of lean mass in the HP as compared to the SP group, was not statistically significant. Small, statistically non-significant changes in body composition may in part, be the reason why weight loss was not different between our HP and SP groups.

Baba et al. (1999) also observed that the decrease in REE was 11.5% less (equates to a difference of 1053 kJ/day) on the HP diet as compared to their SP diet. A blunting in REE of this magnitude is sufficient to completely explain the differential weight loss between the two diets over the 4 weeks of energy restriction.

Data from the 26 subjects who had energy expenditure measured in this study showed no beneficial effect of an increased protein-to-carbohydrate ratio in preventing the decrease in REE. The observed 4.1% decrease in REE after a 5.3% loss of body weight in subjects with type 2 diabetes was comparable to a study in 8 obese non-diabetic men (Rumpler et al. 1991). In Rumpler et al's study (1991), an energy restriction of 6.3 MJ/day was maintained for 28 days and using solid food diets varying in the carbohydrate-to-fat content. The present study had sufficient power (80%, alpha 0.05) to detect a 7% difference in the mean REE of the HP and SP groups after the intervention. Disparity in outcomes between Baba et al. (1999) and the present study may have been a consequence of them having 33% more protein in their HP diet than in their SP diet, whereas the HP diet had 15% more protein than the SP diet in this study. Furthermore, they included only normoglycaemic men, while in the present study men and women with type 2 diabetes.

Acute feeding studies in non-diabetic subjects have shown that dietary protein exerts up to 3-times the thermic response as compared to isocaloric preloads of either carbohydrate or fat when the TEF is measured from 7.5 to 24 hours using indirect calorimetry (Tappy et al. 1993; Westerterp et al. 1999; Westerterp et al. 1999b). We hypothesized that the high-protein diet may facilitate weight loss by reducing the decrease in REE due to an increase in TEF. We measured TEF for only 2 hours; Weststrate (1993) and Segal et al. (1990) have shown that a 2-hour measurement of TEF accounts for approximately 50-70% of ingested energy. It is likely that our observations for the TEF were qualitatively correct and permit comparison between the groups. In accordance with previous findings (Westerterp et al. 1999; Westerterp et al. 1999b), we observed that the TEF was greater after the HP meal than after the SP meal. However, the increased TEF in the HP group only accounted for approximately 1.2 to 1.7% more energy over the 2 hours than the TEF in the SP group, which was not sufficient to blunt the decrease in REE or facilitate weight

loss in this study. Over 6 months, during energy balance, and assuming that our subjects eat three 2.7 MJ meals per day (or a total of 8.1 MJ/day), the extra 97 to 138 kJ/day being burnt on the high-protein as compared to the standard-protein diet, may equate to a difference of only 1.1 to 1.6 kg between the two diets. Accordingly, given the large within-subject day-to-day variation in the measurement of TEF and the dominant effect of both energy intake and physical activity on energy balance, the impact of TEF on either weight loss or weight maintenance is likely to be minimal, at least in the intermediate term.

Although there was no overall difference between the diets for the change in TEF after weight loss, in women there was a smaller decrease in TEF on the HP diet as compared to the SP diet (54 kJ/day difference). Since the study was not specifically powered to determine gender-by-diet interactions, and since the magnitude of TEF observed in our 26 subjects appears to have had a trivial impact on the energy balance equation, the significance of this finding remains unknown.

The observation of an effect of the HP diet on total and abdominal fat loss in women but not men requires confirmation. Although this result may also reflect the small number of male subjects in both dietary interventions, there was no suggestion that the HP diet was advantageous in the men. Skov and colleagues (1999b) observed a 3.3 kg greater loss of fat mass over 6 months on an *ad libitum* diet that contained 25% of total energy as dietary protein as compared to one that contained 12% protein, 65 healthy men and women. The differential fat loss in their study was a consequence of reduced energy intake on the HP as compared to SP diet, and it is possible that increased satiety following the consumption of the HP meals was responsible for the observed decrease in energy intake. In the present study, the HP and SP diets were fixed menu isocaloric diets, and as expected we did not observe a difference in energy intake for the females in our two dietary groups.

Consequently, it is unlikely that satiety played a role in the differential loss of fat mass in the females in the HP as compared to SP group. Accordingly the mechanism for the effect of a HP diet on total and abdominal fat loss in women with type 2 diabetes is unclear.

In the present study, subjects lost approximately 2.0% lean mass overall with no significant difference between the two diets. Greater energy restriction (3.2 vs 6.0 MJ/day), higher protein levels (45% of energy as protein vs 28%), and the inclusion of only glucose tolerant women may have impacted on the disparity in outcomes between the study of Piatti et al. (1994) who found that high-protein weight loss diets spared lean body mass and our study. Dual-energy X-ray absorptiometry was used for estimating body composition in the present study whereas Piatti et al. (1994) used anthropometry. Anthropometric methods such as skinfold thickness and waist-hip ratio have been shown to be less reproducible and less accurate than DEXA (Erselcan et al. 2000; Hansen et al. 1999). Thus, the effect of HP moderately energy-restricted diets in preserving lean body mass still remains to be clarified.

Consistent with previous research in subjects with type 2 diabetes (Kelley et al. 1993; Markovic et al. 1998), our study showed that fasting plasma glucose and insulin concentrations were reduced in both dietary groups after energy restriction and subsequent weight loss. Markovic et al. (1998) observed that after 4 days on an energy restrictive (4.6 MJ/day), the decrease in fasting plasma glucose levels was affected by the carbohydrate content of the diet in 10 subjects with normal glucose tolerance (0.4 mmol/L decrease) and in 10 subjects with type 2 diabetes (1.1 mmol/L decrease). Accordingly, the reduction in fasting glucose in both the HP and SP groups may, in part, reflect the reduced carbohydrate content of the energy-restricted diets. A reduction in hepatic glucose production in subjects with type 2 diabetes (Kelley et al. 1993; Markovic et al. 1998) may

further explain how fasting glucose levels decrease after energy restriction and weight loss. Hepatic glucose production was not measured in the present study.

Insulin resistance can be measured by using the glucose clamp technique (DeFronzo et al. 1979), which is regarded as the reference method for the accurate assessment of in vivo insulin sensitivity (American Diabetes Association, 1998a). However, this method is laborious, expensive and therefore is not suitable for large-scale studies or those that are measuring an extensive number of outcome variables. As a surrogate measure of insulin resistance, we used the HOMA insulin resistance index. HOMA is a simplest and least expensive of the alternate methods to the clamp technique (Bonora et al., 2000) and it has been shown to correlate highly with clamp techniques in several large non-diabetic populations (Yeni-Komshian et al. 2000). In the present study, the HP diet had no benefit over the SP diet in ameliorating insulin resistance. This finding is in contrast to the improvement in insulin resistance reported by Piatti and colleagues (1994) who used a euglycaemic hyperinsulinaemic clamp to evaluate changes in insulin sensitivity; they showed a 17% as compared to a 15% decrease in serum insulin concentrations after weight loss on a HP and SP diets, respectively. The HOMA index only provides a qualitative estimate of insulin resistance and therefore small changes in insulin sensitivity may not have been detected. This may explain why the women in the HP group, who had lost the greatest amount of body fat, were not found to have the greatest improvement in insulin sensitivity. Alternatively, the fat loss for these women may not have been from visceral fat stores, which have been shown to be more strongly associated with insulin action than subcutaneous fat stores (Despres, 1993).

In the present study, there was no difference in the reduction of post-load glucose and insulin levels between the HP and SP groups, and therefore the lower glucose responses following the OGTT in both dietary groups at week 8 and week 12 largely reflect an

improvement in insulin action after energy restriction and weight loss. Prospective data from the Rancho Bernardo (Barrett-Connor and Ferrara, 1998) and the DECODE (The DECODE study group, 1999) studies have reported that post-load glucose levels after an oral glucose challenge are related to cardiovascular disease and death. Lowering post-load glucose levels may reduce the risk for future cardiovascular morbidity and mortality. It has been established that both protein and amino acids added to a carbohydrate meal may stimulate insulin secretion in diabetics (Estrich et al. 1976; Nuttall et al. 1984) and non-diabetics (Spiller et al. 1987). We did not measure insulin secretion during the OGTT and therefore the whether an increase in insulin secretion persists after repeated exposure to HP meals is not known.

Insulin resistance is associated with lower carbohydrate oxidation and higher fat oxidation (Zurlo et al. 1990). In the 26 subjects in whom RQ was measured, the greater increase in postprandial RQ probably reflected the greater carbohydrate load of the SP as compared to the HP meal (Flatt, 1995). The magnitude of the increase in postprandial RQ after the SP meal was smaller than expected. This suggests that the ability to switch from predominantly lipid oxidation during fasting, to increased glucose uptake, oxidation and storage after feeding, was reduced because of the decreased effectiveness of insulin action in our subjects with type 2 diabetes. After weight loss, we speculated that an improvement in insulin sensitivity may enhance carbohydrate oxidation as a consequent of improvements in glucose uptake and the suppression of both hepatic glucose release and the release of free fatty acids from the adipose tissue (Zurlo et al. 1990). In the subpopulation in which energy expenditure was measured, even though there were significant decrease in fasting insulin and the area under the insulin response curve after an oral glucose load, it is reasonable to suggest that a defect in carbohydrate oxidation may have been present. There was no relationship between changes in postprandial RQ and insulin. The implication of having a reduced capacity to switch from fat to

carbohydrate oxidation is that it may be more difficult to maintain weight loss as compared to someone who has a larger capacity to switch between dietary substrates.

In the present study, a greater decrease in LDL-cholesterol concentrations at all time points was observed after 12 weeks, on the HP diet as compared to the SP diet. There was no impact of body composition changes on this result. As saturated fat intake was not different between our two diets, the saturated fat content was not the cause of the differential LDL-cholesterol reduction. The mechanism for the hypolipidaemic effect of a high-protein intake on LDL-cholesterol concentrations is unclear. The 5.7% decrease in LDL-cholesterol concentrations on the HP diet may lead to decrease in the risk of CVD in these subjects. This is relevant, since in people with type 2 diabetes, the risk of CVD is increased 2 to 4 times that of the normal population (Mathers and Penm, 1999). However, as this effect on LDL-cholesterol has not been observed before with energy restrictive high-protein diets, further confirmatory work is required.

In addition to the significant effect of the HP diet on lowering LDL-cholesterol concentrations, total cholesterol was also lowered more on the HP than SP diet, in this study. Our findings confirm the results from two weight maintenance studies; one in 4 men and 6 women with mildly high cholesterol levels (Wolfe and Giovannetti, 1991) and a second in 2 men and 8 women with normal lipid levels (Wolfe and Piche, 1999). On two separate occasions, Wolfe and colleagues (1991; 1999) contrasted a HP (~22% energy as protein) with a SP (~12% energy) diet and randomly assigned free-living subjects, in a crossover design, to both dietary interventions for 4-5 weeks. Both the HP and SP diets were low in saturated fat and cholesterol. In the men and women with hypercholesterolaemia, total cholesterol was lowered by 6.5% and LDL-cholesterol was lowered by 6.4%. The magnitude of the effect of the HP diet was similar in their normolipidaemic subjects (Wolfe and Piche, 1999). In contrast to our findings, Wolfe and

colleagues also found greater reductions in triacylglycerol concentrations (reduced by ~27%), as well as greater increases (by ~12%) in HDL-cholesterol concentrations, in their HP dietary group.

8.6 CONCLUSION

The replacement of some dietary carbohydrate with protein, under energy restrictive conditions, did not blunt the diet-induced fall in REE, or increase the TEF to a level that was large enough to facilitate weight loss in patients with type 2 diabetes. Thus, the reduction in calories appears to be the most important determinant of weight loss in this population, at least in the short-term. Improvements in glucose and insulin homeostasis on both HP and SP diets were a consequence of weight loss in this study. The greater reduction in total and abdominal fat mass in women, however, and the greater reduction in LDL-cholesterol observed in both sexes, suggest that the HP diet is a suitable choice for reducing cardiovascular risk in type 2 diabetes. Long-term studies, in large study populations are required to confirm or refute the gender specific effects that we observed in this study, and to determine how these may impact on the maintenance of body weight, glycaemic control and lipid levels in individuals with type 2 diabetes.

CHAPTER 9

Effect Of A High-Protein Weight Loss Diet On Energy Expenditure, Glycaemic Control And Lipid Levels, In Hyperinsulinemic Men And Women

9.1 SUMMARY

The aim of this study was to determine whether diets with a high-protein content have any benefit over and above energy restriction on weight loss, or the amelioration of insulin resistance. We compared 2 groups of obese hyperinsulinemic, non-diabetic subjects randomly assigned to either a high-protein (HP) diet (27% energy as protein, 44% carbohydrate, 27% fat) or a standard-protein (SP) diet (16% protein, 57% carbohydrate, 27% fat) during 12 weeks of energy restriction (6.4 MJ/d) and 4 weeks of energy balance (8.2 MJ/day). Dietary protein was supplied as red meat, poultry and dairy foods. The 57 obese subjects with hyperinsulinemia (14 men/ 43 women, aged 22-65 yrs, BMI 27.5-42.6 kg/m², fasting insulin 12.0-47.8 mU/L) (n = 28 on the HP diet, and n = 29 on the SP diet) had their body weight, and fasting glucose, insulin and lipid concentrations measured at weeks 0, 4, 8, 12 and 16. At weeks 0 and 16, body composition (DEXA) and postprandial glucose and insulin concentrations were measured after a meal tolerance test that was representative of the study diet. In addition, 36 subjects (10 males/13 females) had measurements for total energy expenditure (TEE), resting energy expenditure (REE), and the thermic effect of feeding (TEF) made. After 16 weeks, weight loss was similar in response to each diet; the overall decrease was 7.9 ± 0.5 kg of which 6.9 ± 0.4 kg was fat loss ($P < 0.001$). Total lean mass was preserved to a greater extent in the female subjects on the HP diet (-0.1 ± 0.3 kg) as compared to the SP diet (-1.5 ± 0.3 kg) ($p = 0.02$). For 36 subjects in the energy expenditure component of the study, REE fell similarly with each diet; the overall decrease was 719 ± 106 kJ/day. The TEF was 2% greater after the HP meal than after the SP meal at baseline ($p = 0.009$) and 0.8% greater at week 16 ($p = 0.35$), but TEF was not associated with weight loss. There was no change in TEE after 16 weeks. 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 ($p < 0.001$). 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 ($p = 0.049$). The reduction in serum triacylglycerol concentrations was significantly greater on the HP diet (23%) than on the SP diet (10%) ($p < 0.05$). After 16 weeks, markers of bone turnover, calcium excretion and systolic blood pressure were unchanged, whereas diastolic blood pressure decreased similarly in both diet groups. We conclude that caloric restriction, rather than the macronutrient composition of the diet is the most important determinant of weight loss in subjects with hyperinsulinemia. However, improvements in insulin sensitivity following meals containing a high-protein content, combined with the greater decrease in triacylglycerol concentrations and a greater preservation of total lean mass in females, suggests that an energy restrictive high-protein diet may reduce the risk of cardiovascular disease and delay the onset of type 2 diabetes in subjects with hyperinsulinemia.

9.2 INTRODUCTION

Insulin resistance affects approximately 25-35% of the population in developed countries (Kelly, 2000) and is associated with obesity (Belfiore and Iannello, 1998; Kopelman, 2000), type 2 diabetes (Boden, 1997), and cardiovascular disease (Despres et al. 1996). Compensatory hyperinsulinemia, decreased insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscle, and impaired suppression of hepatic glucose output are the metabolic abnormalities that characterize insulin resistance (Kahn and Flier, 2000). In overweight patients, moderate weight loss (~4.5 to 13 kg) (Reaven, 2001; Goodpaster et al. 1999; McLaughlin et al. 2001), particularly reductions in visceral adipose tissue (Golay et al. 1996; Pascale et al. 1992) improves insulin resistance. Whether varying the macronutrient content of the diet has any benefit over and above energy restriction on weight loss, or the amelioration of insulin resistance, remains unclear.

The replacement of some dietary carbohydrate for protein, combined with a low total (< 30%) and saturated (< 10%) fat content, has been shown to enhance weight loss (Baba et al. 1999; Skov et al. 1999b). It has been proposed that weight loss on a high-protein diet may be facilitated, in part, by an increase in thermogenesis that may subsequently blunt the fall in resting and total energy expenditure that is usually observed during weight loss (Baba et al. 1999). Two studies, one in 8 overweight subjects (Whitehead et al. 1996) and a second in 13 hyperinsulinemic men (Baba et al. 1999) showed that resting and total energy expenditure were reduced less after 7 days or 4 weeks of energy restriction on high-protein diets (36 and 45% protein) as compared to isocaloric standard-protein diets (12 and 15%). Besides these two small studies, no others have examined the effect of energy restriction and increased dietary protein on the changes in body weight, energy expenditure and thermogenesis, in obese subjects with or without insulin resistance. Neither study however, simultaneously examined the changes in total and resting energy expenditure, and the thermic effect of feeding. The reduced fall in resting energy expenditure may also be related to a preservation of lean mass that has been observed in obese non-diabetic women after energy restriction on low-fat diets containing an increased protein content (>36% of energy) (Piatti et al. 1994; Vazquez et al. 1995; Hoffer et al. 1984). No studies have as yet examined the relationship between changes energy expenditure and lean mass in response to high-protein diets.

There is minimal evidence for the amelioration of insulin resistance independent of weight loss using a high-protein diet (Piatti et al. 1994). Piatti et al. (1994) examined the effects of two hypocaloric (3.3 MJ/day) diets containing either 45 or 20% of energy as protein, on insulin sensitivity and lean mass in 25 obese non-diabetic women with hyperinsulinemia. After 21 days, they found that the improvement in insulin sensitivity was due to an increase in insulin mediated glucose uptake in skeletal muscle that may have resulted from the preservation of lean body mass after weight loss (Piatti et al. 1994). Two other studies

reported that lean mass was preserved in healthy obese women following weight loss on an energy restrictive high-protein diet for 28 days (Vazquez et al. 1995) to 8 weeks (Hoffer et al. 1984). However, neither examined whether there was an associated improvement in insulin resistance.

Two weight maintenance (Wolfe and Giovannetti, 1991; Wolfe and Piche, 1999) studies have also shown that replacing some carbohydrate with protein improves the fasting lipid profile. Wolfe and colleague demonstrated that high-protein (~22% of energy) diets as compared to diets containing ~12% protein, lowered plasma triacylglycerol by ~27%, and total and LDL cholesterol by ~7%, and increased plasma HDL cholesterol by ~12%, in mildly hyperlipidemic (Wolfe and Giovannetti, 1991) and normolipidemic (Wolfe and Piche, 1999) subjects. All of the aforementioned studies were of short duration (4 to 5 weeks) and contained only 10 to 13 subjects. Skov et al. (1999b) also demonstrated that *ad libitum* consumption of a high-protein (25% of energy) diet from a clinic shop lead to greater reductions in total-cholesterol, triacylglycerol, and free-fatty acid concentrations over a 6 month period when compared to a standard-protein (12%) diet. The benefit of high-protein diets on plasma lipids during either energy restriction or weight maintenance requires confirmation.

Concern has been expressed over the safety of high-protein diets (St.Jeor et al. 2001). High-protein diets may enhance calcium excretion and increase bone resorption (Kerstetter et al. 1999). However, it has been demonstrated that calcium loss only occurs when calcium intake is increased and the intake of phosphorous is fixed (Spencer et al. 1978). Moreover, two studies found that an increase in meat protein had no effect on bone turnover (Spencer et al. 1978; Shapses et al. 1995). There is also concern that diets high in animal protein may increase blood pressure (Havlik et al. 1990; Sacks et al. 1981). Cross-sectional data however, reveals an inverse association between blood pressure and animal

protein intake (Liu et al. 2000; Cirillo et al. 2002). There is no data reporting the effects of high-protein diets on the changes in bone and blood pressure after moderate weight loss.

The aim of this study was to compare the effect of two 30% fat, isocaloric diets, either high or lower in dietary protein (30 vs 15 % of energy as protein) on body composition, energy expenditure, glucose and insulin homeostasis, lipid levels, blood pressure and bone turnover, in obese subjects with hyperinsulinemia, during 12 weeks of energy restriction and 4 weeks of energy balance. We hypothesize that a high-protein diet will: (i) facilitate weight loss by blunting the decrease in resting and total energy expenditures as a consequent of an increased thermic effect of feeding and the preservation of lean muscle mass, (ii) enhance the increase insulin sensitivity after weight loss, because of a greater depletion of fat mass and preservation of lean mass, and (iii) improve dyslipidaemia.

9.3 RESEARCH DESIGN AND METHODS

9.3.1 Subjects

Sixty-six obese hyperinsulinaemic subjects were recruited by public advertisement. Subjects attended detailed information sessions and all gave written informed consent. Subjects were included if they were aged between 20 to 65 years, had a fasting plasma insulin greater than 12 mU/L, and a body mass index between 27 and 43 kg/m² (43 kg/m² was the upper cut-off point because the accuracy of DEXA for measuring body fatness is limited at higher BMIs). Exclusion criteria included type 1 or 2 diabetes, proteinuria, or a history of liver, unstable cardiovascular, respiratory, gastrointestinal disease or malignancy. Six subjects withdrew prior to commencement due to family and work commitments. During the course of the study, one subject withdrew due to pregnancy, while another subject lost contact with the clinic. Fifty-eight subjects (14 male, 44 female) who participated in the study. None of the subjects were taking medication known to affect glucose or insulin metabolism. Subjects on anti-hypertensive or lipid lowering

medication were asked to maintain all medications and supplements at pre-study doses. Subjects were asked to continue their usual physical activity levels and to refrain from drinking alcohol throughout the study.

Thirty-six subjects (10 male, 26 female) subjects also had measurements of total and resting energy expenditure, respiratory quotient, and the thermic effect of feeding made. The subjects who had measurements of energy expenditure made were selected based on: i) their willingness to participate, ii) whether they had flexibility in their time and could stay in the clinic for approximately half-a-day, and iii) women had to be postmenopausal or infertile. All 36 subjects completed this component of the study.

9.3.2 Diets

Dietary prescriptions for the high-protein (HP) diet (30% of energy as protein, 40% carbohydrate, 30% fat) and standard-protein (SP) diet (15% protein, 55% carbohydrate, 30% fat) were the same as detailed in Chapter 8.2.2. Dietary instruction and assessment was also the same as described in Chapter 8.2.2.

9.3.3 Experimental design

The study was conducted on an outpatient basis over 16 weeks. Subjects were matched on the basis of fasting plasma insulin, BMI, age, and gender. Subjects in the two matched groups were randomly assigned to either the HP or SP diet. Both the HP and SP groups underwent 12 weeks of energy restriction followed by 4 weeks at energy balance on the same macronutrient composition as during the energy restriction phase of the study. During energy restriction, the energy intake of individuals was reduced by approximately 30% of their weight maintenance energy requirements (calculated as 1.3 multiplied by basal metabolic rate). Numerous weight loss studies conducted at the CSIRO have found

that a 30% energy restriction is sufficient to successfully induce a weight loss of 0.5 to 1 kg per week.

On two consecutive days at weeks 0, 4, 8, 12 and 16 subjects attended the CSIRO research unit, having fasted overnight. Body weight and blood pressure was recorded, and venous blood samples taken for assessment of insulin, glucose and lipid concentrations. At weeks 0 and 16, all subjects underwent a 3-hour meal tolerance test, with the meal representative of the diet to which they were assigned. Baseline venous blood samples were taken prior to consuming a HP (2715 kJ, 32% protein, 54% carbohydrate, 14% fat) or SP (2747 kJ, 10% protein, 77% carbohydrate, 13% fat) test meal (Appendix 6). The composition of the two test meals were different to the macronutrient composition of the overall study diet because the aim was to match the caloric content while maximizing the protein-to-carbohydrate exchange between the two single meals. Thereafter blood was sampled at 30, 60, 120 and 180 minutes to assess plasma glucose, insulin, and free-fatty acid concentrations. All subjects had their body composition measured by dual-energy X-ray absorptiometry and collected 24-hour urine samples, prior to attending the clinic at weeks 0 and 16. The urea/creatinine ratio was measured from urine samples to determine dietary compliance, and markers of bone turnover and calcium excretion were also measured. The 36 subjects participating in the energy expenditure assessment had their total and resting energy expenditure, respiratory quotient, and thermic effect of feeding measured, and their energy expenditure due to physical activity estimated, at weeks 0 and 16 after having fasted for at least 6 hours. These 36 subjects, completed a 3-day food diary to determine the food quotient (FQ) (as described in Chapter 2.3.3.2) of their diet over the energy expenditure measurements at weeks 0 and 16. For each variable, the same investigator performed all measurements.

9.3.4 Measurements

9.3.4.1 *Body weight, body composition, and blood pressure*

Body weight was measured as described in Chapter 2.3.4, and total body fat mass and total body lean mass were assessed using dual-energy X-ray absorptiometry (DEXA) as described in Chapter 2.3.5.1. Two blood pressure measurements were recorded with an automated sphygmomanometer (Dinamap 1800, Critikon, FL, USA) after at least 5 minutes of seated rest.

9.3.4.2 *Total energy expenditure (TEE)*

Total energy expenditure was measured using the [^{14}C]-bicarbonate method as described in Chapter 5. In this study, 14.3 ± 0.1 ml of [^{14}C]-bicarbonate solution (1.74 ± 0.01 $\mu\text{Ci/ml}$) was administered over 48 hours.

9.3.4.3 *Resting energy expenditure (REE), Respiratory quotient (RQ), and the thermic effect of feeding (TEF)*

Fasting REE and RQ were measured by indirect calorimetry using the ventilated canopy and Deltatrac metabolic monitor as described in Chapters 2.3.1.2 and 2.3.2.1, respectively. Immediately following measurements of fasting REE and RQ, each subject consumed, within 20 minutes, a test meal that was representative of the diet to they were assigned (Appendix 9.3.3). The composition of the test meal was different to the macronutrient composition of the daily study diet because of the difficulty associated with keeping the energy intake of the two test meals similar when the protein-to-carbohydrate ratio was increased. Thereafter subjects returned to the hood for a further 180 minutes of RQ and REE measurements. Postprandial RQ and the mean TEF over the 180-minute period were calculated as described in Chapters 2.3.2.2 and 2.3.1.3. Postprandial RQ was expressed as the mean change in RQ above baseline, and TEF was calculated as the mean increase in REE above baseline and expressed as the % increase per energy intake of the test meal.

For the subjects who participated in the energy expenditure component of this study, postprandial RQ and TEF were measured at the same time as glucose, insulin and FFA responses were assessed.

9.3.4.4 Energy expenditure due to physical activity (PAEE)

On the day that TEE was measured, energy expenditure due to physical activity was calculated using the semi-qualitative estimation equation:

$$[^{14}\text{C}]\text{-PA EE} = 0.9([^{14}\text{C}]\text{-bicarbonate TEE}) - \text{REE}$$

(see equation [i] Chapter 2.3.1.4. for details)

A three-day physical activity diary was also collected to quantitatively evaluate whether or not average daily levels of physical activity remained stable over the 3 days of energy expenditure measurements (see Chapter 2.3.1.4 for details).

9.3.4.5 Biochemical sampling and analysis

Blood sampling and the collection of 24-hour urine samples were done as described in Chapter 2.3.6. Biochemical measurements were performed in a single assay at the completion of the study. Concentrations of plasma glucose, and serum insulin, free-fatty acids, triacylglycerol, and total, LDL and HDL cholesterol were determined using methods described in Chapter 2.3.7. Urinary urea and creatinine concentrations were determined by methods described in Chapter 2.3.7.8. Urinary pyridinium crosslinks (markers of bone turnover) were measured using high performance liquid chromatography.

9.3.5 Statistical analysis

One subject was excluded from all data analysis due to non-compliance with the dietary intervention, while one subject was excluded from the lipid analysis due to the

commencement of lipid lowering medications. In addition, one subject requested not to be included in the body composition analysis for personal reasons. All data are presented as means \pm SEM. Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). Baseline measurements were assessed using two-way ANOVA with diet and gender as the fixed factors. The effect of the diet intervention was assessed using repeated-measures ANOVA (with covariates of baseline weight, total fat mass and total lean mass in specific analyses) with variables measured at weeks 0, 4, 8, 12 and 16. Diet and gender were the between subject factors. Incremental area under the glucose and insulin response curves during the 3-hour meal tolerance tests, were calculated geometrically using the trapezoidal rule (Wolever et al. 1991). The homeostasis model assessment (HOMA) for insulin resistance was calculated as [fasting insulin x fasting glucose/22.5] (Matthews et al. 1985). Pearson correlation analysis was used to determine the relationship between two variables. Significance was set at $p < 0.05$.

9.4 RESULTS

9.4.1 Subject characteristics

Fifty-seven subjects complied too the study protocol. The physical characteristics of the subjects at baseline are shown in Table 9.4.1. There were no significant differences in any of the variables between diet groups. Baseline body weight and fasting glucose was significantly greater in males than in females ($p < 0.0001$). There was no effect of gender on any of the other variables at baseline.

9.4.2 Diet composition and subject compliance

Energy intake was not different between the two diets during the 12-week energy restrictive and 4-week energy balance phases (Table 9.4.2). The protein intake was higher and the carbohydrate intake lower in the HP diet as compared to SP diet, with no difference between energy restrictive and energy balance phases (effect of diet, $p <$

0.0001). Total and saturated fat content was not different between the diets or phases, but dietary fibre was lower and dietary cholesterol was higher on the HP compared to SP diet during both the energy restrictive and energy balance phase (Table 9.4.2).

The urinary/creatinine ratio rose from 30.3 ± 1.5 at week 0 to 36.5 ± 1.6 at week 16 on the HP diet, and remained constant from week 0 to week 16 on the SP diet (32.2 ± 1 vs 31.8 ± 1) ($p < 0.001$ for diet effect) (Figure 9.4.1).

9.4.3 Body weight and body composition

After 12 weeks of energy restriction and 4 weeks of energy balance the mean weight loss was 7.9 ± 0.5 kg ($p < 0.0001$), but the decrease in weight was not effected by the diet composition (7.8 ± 0.7 vs 7.9 ± 0.6 kg on the HP and SP diets respectively) (Figure 9.4.2). Overall men lost more weight than women (10.5 ± 1.9 vs 7.0 ± 0.5 kg, $p < 0.0001$). After controlling for baseline weight, however, there was no difference in the reduction in weight between the two genders. Weight was maintained during energy balance, with no difference between diets or genders.

Total fat mass was reduced 6.9 ± 0.4 kg after 16 weeks ($p < 0.0001$), but the decrease was not affected by diet composition (Table 9.4.3). There was, however, a significant time-by-gender interaction such that the men lost more fat as compared to the women (8.3 ± 1.6 vs 6.8 ± 1.2 kg, $p = 0.023$). The 3.1 ± 0.2 kg decrease in abdominal fat mass ($p < 0.0001$) was also independent of diet composition. The decrease, however, was affected by gender ($p = 0.005$); the reduction being greater for men than women (Table 9.4.3). After 16 weeks of dietary intervention, total lean mass had significantly decreased by 1.2 ± 0.3 kg ($p < 0.0001$). Although there was no effect of diet on the reduction in lean mass, there was a significant time-by-diet-by-gender interaction; women lost significantly more lean mass

on the SP diet than on the HP diet ($p = 0.002$) while men lost a similar amount on both diets (Table 9.4.3).

9.4.4 Glycaemic control, insulin sensitivity and free fatty acids

Overall, fasting plasma glucose had increased by 3% in the HP diet group and decreased 2% in the SP group (time-by-diet effect, $p = 0.017$) (Table 9.4.4). There was no effect of gender on fasting glucose. Fasting serum insulin was reduced by $33 \pm 3.3\%$ at week 12 and by $29 \pm 3.4\%$ at week 16 ($p < 0.001$) (Table 9.4.4), with no effect of either diet composition or gender. The HOMA insulin resistance index decreased $32 \pm 4\%$ from 4.3 at week 0 to 2.5 at week 12 ($p < 0.001$), and by $27 \pm 4\%$ to 2.8 at week 16 ($p < 0.001$). Neither diet nor gender affected the HOMA index.

Overall, the area under the plasma glucose curve (glucose AUC) was less following the HP meal as compared to the SP meal, at both weeks 0 and 16 ($p = 0.027$) (Table 9.4.5). After 16 weeks, the response of plasma glucose to the test meals was reduced ($p < 0.001$) (Figure 9.4.3A), and there was a time-by-diet interaction ($p = 0.049$) such that the plasma glucose response following the HP meal was reduced more than after the SP meal (Figure 9.4.3A). Post-hoc analysis revealed that at week 16, plasma glucose concentrations at all time points were lower following the HP meal compared to the SP meal ($p < 0.03$ for diet effect), whereas at week 0 only the 120 minute plasma glucose concentration was lower on the HP diet ($p = 0.02$). After weight loss, the glucose AUC was reduced at week 16 as compared to week 0 ($p < 0.001$) (Table 9.4.5), but the reduction was greater in the HP as compared to SP group ($-8.7 \pm 2.2\%$ vs $1.9 \pm 2.1\%$) ($p = 0.08$).

At week 16 as compared to week 0, the response of serum insulin to the test meals was reduced ($p < 0.001$) (Figure 9.4.4B). There was no effect of diet composition on the

reduction of postprandial serum insulin at week 16 (Figure 9.4.4B) or on the insulin AUC (Table 9.4.5).

After 16 weeks, fasting serum free-fatty acids decreased 26% ($p < 0.001$), with no effect of either diet or gender (Table 9.4.4). At week 0 during the meal tolerance test, serum free-fatty acid concentrations decreased from 0.43 ± 0.02 mmol/L at baseline to 0.006 ± 0.003 mmol/L at 120 minutes, but no further decrease occurred after 16 weeks. There was no effect of either diet or gender on the free-fatty acid response curves following the meal tolerance tests.

9.4.5 Serum lipids

Overall, fasting serum total cholesterol was reduced by 10.0% at week 12 as compared to week 0, and by 5.3% at week 16 ($p < 0.0001$), with no effect of diet (Table 9.4.6). There was an effect of gender on the decrease in fasting serum total cholesterol from week 0 to week 12 ($p < 0.005$) such that total cholesterol was reduced more in men than women (-1.0 ± 0.2 vs -0.42 ± 0.1 mmol/L). Fasting serum LDL cholesterol was reduced by 12% at week 12 and by 6% at week 16 ($p < 0.0001$), with no effect of diet (Table 9.4.6). At both weeks 12 and 16, the fall in LDL cholesterol was greater for men than women ($p < 0.02$). Fasting serum HDL cholesterol increased 2% by week 12 and 5% by week 16 ($p = 0.001$), with no effect of either diet or gender (Table 9.4.6). Fasting serum triacylglycerol concentrations decreased 15.8% by week 12 and 14.1% by week 16 ($p < 0.0001$). A time-by-diet effect was observed ($p < 0.05$) such that the decrease in serum triacylglycerol concentrations was 29% by week 12 and 23% by week 16 on the HP diet, while on the SP diet the decrease was 12% and 10% at weeks 12 and 16 respectively (Table 9.4.6).

9.4.6 Total energy expenditure (TEE), resting energy expenditure (REE), and energy expenditure due to physical activity (PAEE)

For the 36 subjects participating in the energy expenditure component of this study, weight loss was representative of the larger study population (Table 9.4.7). In this subpopulation, however, we observed no beneficial effect of the HP diet as compared to the SP diet in preserving lean mass in either women, or men.

The food quotient (FQ) was not different over the 3 days of energy expenditure measurements at week 0 as compared to week 16 (0.85 ± 0.003 vs 0.85 ± 0.003) and there was no effect of diet or gender. The RQ/FQ ratio was lower at week 0 as compared to week 16 (0.92 ± 0.09 vs 0.96 ± 0.009), but there was no effect of diet or gender on the RQ/FQ ratio at either week 0 or 10.

Overall, there was a non-significant decrease (4.1 %) in TEE from 11187 ± 356 kJ/day at week 0 to 10582 ± 409 kJ/day at week 16 (Table 9.4.8). Diet composition had no effect on TEE. At both weeks 0 and 16, the TEE was greater in men than women (13360 vs 9955 kJ/day, $p < 0.001$ for overall effect of gender), but there was no effect of gender on the decrease in TEE.

Resting energy expenditure was reduced by 8.8% after 16 weeks (from 7833 ± 251 at baseline to 7114 ± 206 kJ/day, $p < 0.001$). The decrease in REE was not related to diet composition (Table 9.4.8), regardless of whether REE was expressed as an absolute value, or normalized for body composition. At both weeks 0 and 16, men had a greater REE than women (9117 vs 6842 kJ/day, $p < 0.001$ for overall effect of gender), but there was no effect of gender on the decrease in REE resulting from weight loss.

At baseline as compared to week 16, energy expenditure due to physical activity was not significantly different (2331 ± 202 vs 2397 ± 265 kJ/day). Diet composition had no effect on energy expenditure due to physical activity (Table 9.4.8). In accordance with this result, we also observed that the average daily physical activity index derived from a 3-day physical activity diary was not different at week 16 as compared to week 0 (1.56 ± 0.02 vs 1.55 ± 0.02). There was no significant effect of gender on physical activity.

9.4.7 The thermic effect of feeding (TEF) and respiratory quotient (RQ)

At week 0, the TEF (expressed either as an absolute value, or as a % of energy intake at the test meal) after the HP meal was 2% greater than for the SP meal (effect of diet, $p = 0.009$) (Table 9.4.8). At week 16, the TEF remained 0.8% greater following the HP meal as compared to after the SP meal, but the effect of diet was no longer significant ($p = 0.35$) (Table 9.4.8). The reduction in TEF from week 0 to week 16 was not significant, in either the HP or SP group (Table 9.4.8). There was no effect of gender on TEF (Table 9.4.8). There was no correlation between TEF at weeks 0 and 16, and the change in body weight.

Overall, the mean fasting RQ increased from 0.78 ± 0.007 at baseline to 0.82 ± 0.008 at week 16 ($p < 0.001$). Diet composition had a significant effect on fasting RQ such that the increase in the SP diet group (from 0.77 ± 0.009 at baseline to 0.83 ± 0.010 at week 16) was 62% greater than in the increase in the HP group (from 0.80 ± 0.012 at baseline to 0.82 ± 0.013 at week 16) (time-by-diet effect, $p = 0.02$) (Table 9.4.8). There was no effect of gender on fasting RQ (Table 9.4.8).

At week 0, postprandial RQ after the SP meal was approximately 54.5% greater than after the HP meal (0.011 ± 0.001 vs 0.005 ± 0.001 , $p < 0.001$) (Table 9.4.8). At week 16, postprandial RQ remained 33.3% greater following the SP meal as compared to the HP meal but the effect of diet was no longer significant (0.009 vs 0.006 , $p = 0.06$). There was

a significant time-by-diet effect on postprandial RQ ($p = 0.003$); in the SP group postprandial RQ decreased from 0.011 ± 0.001 at baseline to 0.009 ± 0.001 at week 16, and increased from 0.005 ± 0.001 to 0.006 ± 0.001 in the HP group (Table 9.4.8). There was no effect of gender on postprandial RQ (Table 9.4.8). Postprandial RQ was not related to either the 5.3 mU/L decrease in fasting plasma insulin ($r = 0.33$) or the 11.3% decrease in insulin area under the curve ($r = 0.15$).

9.4.8 Bone turnover and blood pressure

Urinary calcium excretion was unchanged at week 16 compared to week 0 in the 57 subjects who completed this study (4.7 ± 0.4 vs 4.4 ± 0.3 mmol/24h). There was no effect of diet or gender on urinary calcium. Bone turnover markers (pyridinoline/creatinine 62.5 ± 2.2 vs 62.1 ± 2.6 nmol/mmol and deoxypyridinoline/creatinine 18.6 ± 0.7 vs 18.5 ± 0.9 nmol/mmol) remained similar from baseline to week 16, for both dietary groups.

Systolic blood pressure decreased from 130 ± 1.9 mmHg at week 0 to 126 ± 1.8 mmHg at week 12 ($p = 0.022$), but week 16 as compared to week 0 systolic blood pressure (126 ± 2.3 mmHg) was not different. Diastolic blood pressure was reduced at both week 12 and week 16 as compared to week 0 (72 ± 1.3 and 72 ± 1.4 mmHg, respectively, versus 74 ± 1.4 mmHg, $p < 0.04$). There was no effect of either diet or gender on systolic or diastolic blood pressure.

Table 9.4.1 Subject characteristics at baseline*

	SP diet		HP diet	
	Male n = 7	Female n = 22	Male n = 7	Female n = 21
Age (yrs)	48.6 ± 3.2	50.6 ± 2.1	51.9 ± 3.3	50.6 ± 2.7
Weight (kg) [†]	109.4 ± 5.2	88.3 ± 2.2	107.4 ± 5.8	89.5 ± 2.1
BMI (kg/m ²)	34.5 ± 1.5	33.6 ± 0.8	33.7 ± 1.1	34.5 ± 0.9
Insulin (mU/L)	21.6 ± 2.2	17.1 ± 1.6	17.6 ± 1.4	18.1 ± 1.6
Glucose (mmol/L) [†]	5.9 ± 0.4	5.2 ± 0.1	5.4 ± 0.1	5.3 ± 0.2
sBP (mmHg)	128 ± 6	129 ± 3	132 ± 6	132 ± 3
dBP (mmHg)	76 ± 3	72 ± 2	81 ± 5	72 ± 2

SP, standard-protein; HP, high-protein; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure. Measurements were made at screening prior to dietary intervention and were assessed using two-way ANOVA with diet and gender as the fixed factors. *Data are expressed as means ± SEM

There were no significant differences between the SP and HP dietary groups.

[†] Significant effect of gender, $p < 0.001$

Table 9.4.2 Composition of study diets derived from subjects' daily weighed food records*

	SP diet		HP diet	
	n = 29		n = 28	
	ER	EB	ER	EB
Energy (MJ/day)	6.5 ± 0.1 [†]	8.2 ± 0.2 [‡]	6.3 ± 0.1 [†]	8.0 ± 0.2 [‡]
Protein (% E)	15.7 ± 0.2 [†]	15.4.0 ± 0.3 [†]	27.4 ± 0.2 [‡]	27.3 ± 0.3 [‡]
Carbohydrate (% E)	57.3 ± 0.3 [†]	56.9 ± 0.3 [†]	44.4 ± 0.4 [‡]	44.6 ± 0.5 [‡]
Total fat (% E)	26.8 ± 0.3 [†]	27.5 ± 0.4 [†]	27.0 ± 0.3 [†]	26.9 ± 0.5 [†]
SFA (% E)	7.9 ± 0.1 [†]	8.4 ± 0.3 [†]	8.5 ± 0.1 [†]	8.3 ± 0.2 [†]
MUFA (% E)	13.3 ± 0.2 [†]	13.0 ± 0.3 [†]	13.3 ± 0.2 [†]	13.1 ± 0.3 [†]
PUFA (% E)	3.0 ± 0.1 [†]	3.5 ± 0.2 [†]	2.8 ± 0.1 [†]	2.9 ± 0.1 [†]
Fiber (g/day)	29.4 ± 0.6 [†]	35.8 ± 0.8 [†]	21.8 ± 0.7 [‡]	28.4 ± 1.1 [†]
Cholesterol (mg/day)	87.4 ± 2.5 [†]	117.2 ± 6.4 [†]	178.0 ± 6.1 [‡]	239.4 ± 13.5 [‡]

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Three days (2 week days and 1 weekend day) of dietary data were analysed at weeks 2, 4, 6 8 and 12 during the energy restrictive (ER) phase of the study, and at weeks 14 and 16 during energy balance (EB). No significant differences were found between the four diet records in the energy restrictive phase or between the two records for the energy balance phase, so data for recordings in each phase were averaged. *Data are expressed as means ± SEM.

Common superscripts denote that the composition of the allocated diets were not different ($p > 0.05$).

Table 9.4.3 Total fat mass, abdominal fat mass and total lean mass in the entire study population at weeks 0 and 16*

	SP diet		HP diet	
	Male n = 7	Female n = 21	Male n = 7	Female n = 21
Total fat mass (kg) ^{†§}				
Week 0	38.2 ± 3.3	42.7 ± 2.0	37.8 ± 3.3	43.2 ± 1.3
Week 16	30.6 ± 2.9	35.6 ± 1.9	28.8 ± 2.0	36.6 ± 1.4
Change	-7.6 ± 3.1	-7.1 ± 2.0	-9.0 ± 2.7	-6.6 ± 1.4
Abdominal fat mass (kg) ^{†§}				
Week 0	15.7 ± 1.6	15.6 ± 0.6	15.6 ± 1.4	15.4 ± 0.5
Week 16	12.2 ± 1.3	12.7 ± 0.7	11.1 ± 0.8	12.5 ± 0.5
Change	-3.5 ± 0.7	-2.6 ± 0.2	-4.5 ± 1.0	-2.8 ± 0.3
Total lean mass (kg) ^{†¶}				
Week 0	67.0 ± 2.1	42.4 ± 0.9	66.1 ± 3.1	42.5 ± 1.1
Week 16	65.1 ± 2.1	40.9 ± 0.9	63.6 ± 2.4	42.4 ± 1.2
Change	-1.9 ± 2.1	-1.5 ± 0.3	-2.5 ± 2.8	-0.1 ± 0.3

Change, the difference in week 16 values from week 0. Values were derived from DEXA analysis performed at week 0 and 16. Week 0 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. *Data are means ± SEM.

[†]Significant effect of time from week 0 to week 16, $p < 0.0001$

[§]Significant time-by-gender effect for men, $p < 0.03$.

[¶]Significant time-by-diet-by-gender interaction for women, $p = 0.002$.

Table 9.4.4 Fasting glucose, insulin, and free fatty acid concentrations for the SP (n = 29) and HP (n = 28) diet groups*

	Week 0	Week 4	Week 8	Week 12	Week 16
Fasting glucose (mmol/L)					
SP diet	5.3 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
HP diet	5.3 ± 0.1	5.3 ± 0.1	5.6 ± 0.09 [†]	5.4 ± 0.06	5.5 ± 0.08 [‡]
Fasting insulin (mU/L)					
SP diet	17.7 ± 2.4	12.5 ± 1.3 ^δ	10.7 ± 0.9 ^δ	10.4 ± 0.6 ^δ	11.5 ± 0.9 ^δ
HP diet	17.5 ± 1.2	12.6 ± 1.1 ^δ	13.5 ± 1.4 ^δ	10.5 ± 0.7 ^δ	11.9 ± 1.1 ^δ
Fasting FFA (mmol/L)					
SP diet	0.4 ± 0.03	0.4 ± 0.02	0.3 ± 0.03 ^δ	0.3 ± 0.02 ^δ	0.3 ± 0.02 ^δ
HP diet	0.4 ± 0.03	0.4 ± 0.02	0.3 ± 0.02 ^δ	0.3 ± 0.03 ^δ	0.3 ± 0.03 ^δ

Fasting glucose, insulin and free-fatty acid (FFA) concentrations were measured on 2 consecutive days at weeks 0, 4, 8, 12 and 16, and the average of the two values at each week was recorded (determined not to be different using a student's paired t-test). Weeks 0, 4, 8, 12 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Comparison between diets within the same week was made using 1-way ANOVA.

*Data are means ± SEM.

[†]Significant effect of diet at week 8, p = 0.008.

[‡]Significant overall time-by-diet interaction, p = 0.017

^δSignificantly different from baseline, p < 0.0001.

Table 9.4.5 Glucose and insulin response areas under the meal tolerance test curves for the SP (n = 29) and HP (n = 28) diet groups at weeks 0 and 16*

	SP diet n = 29	HP diet n = 28
Glucose AUC (mmol ⁻¹ .L.180min ⁻¹)		
Week 0	1300 ± 49	1202 ± 32 [‡]
Week 16	1265 ± 48 [†]	1099 ± 40 ^{†‡}
Insulin AUC (mU ⁻¹ .L.180min ⁻¹) [†]		
Week 0	9108 ± 1094	8719 ± 718
Week 16	7935 ± 762 [†]	5922 ± 533 [†]

AUC, area under the glucose and insulin response curves during the 3-hour meal tolerance test. Week 0 and week 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors.

*Data are means ± SEM.

[†]Significantly different from week 0 to week 16, p < 0.001.

[‡]Significant effect of the HP diet at both weeks 0 and 16, p < 0.05.

Table 9.4.6 Fasting serum lipid concentrations for the SP (n = 28) and HP (n = 28) diet groups*

	Week 0	Week 4	Week 8	Week 12	Week 16
Total cholesterol (mmol/L)					
SP diet	5.59 ± 0.16	5.10 ± 0.19 [†]	5.09 ± 0.18 [†]	5.12 ± 0.22 [†]	5.34 ± 0.19 [†]
HP diet	5.57 ± 0.21	4.82 ± 0.19 [†]	4.87 ± 0.22 [†]	4.89 ± 0.19 [†]	5.17 ± 0.19 [†]
LDL cholesterol (mmol/L)					
SP diet	3.76 ± 0.16	3.41 ± 0.18 [†]	3.33 ± 0.17 [†]	3.37 ± 0.20 [†]	3.53 ± 0.18 [†]
HP diet	3.75 ± 0.18	3.27 ± 0.17 [†]	3.22 ± 0.14 [†]	3.27 ± 0.16 [†]	3.49 ± 0.17 [†]
HDL cholesterol (mmol/L)					
SP diet	0.98 ± 0.05	0.95 ± 0.04	0.95 ± 0.04	0.99 ± 0.04 [†]	1.03 ± 0.04 [†]
HP diet	0.98 ± 0.04	0.94 ± 0.03	0.96 ± 0.04	1.02 ± 0.03 [†]	1.04 ± 0.04 [†]
Triacylglycerol (mmol/L)					
SP diet	1.87 ± 0.11	1.60 ± 0.10 [†]	1.62 ± 0.12 [†]	1.66 ± 0.13 [†]	1.70 ± 0.12 [†]
HP diet	1.84 ± 0.13	1.33 ± 0.08 ^{†‡}	1.39 ± 0.11 ^{†‡}	1.31 ± 0.10 ^{†‡}	1.42 ± 0.11 ^{†‡}

Fasting lipid concentrations were measured on 2 consecutive days at weeks 0, 4, 8, 12 and 16, and the average of the two values at each week was recorded (determined not to be different using a student's paired t-test). Weeks 0, 4, 8, 12 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Comparison between diets within the same week was made using 1-way ANOVA. *Data are means ± SEM.

[†]Significantly different from week 0, p < 0.01

[‡]Significant effect of diet, p < 0.05.

Table 9.4.7 Weight, total fat and lean mass changes for the 36 subjects in the energy expenditure component of the study*

	SP diet n = 19	HP diet n = 17
Body weight (kg)[†]		
Week 0	93.7 ± 3.7	94.3 ± 3.5
Week 16	85.8 ± 3.4	86.4 ± 2.9
Change	-8.0 ± 0.7	-7.9 ± 1.1
Total fat mass (kg)[†]		
Week 0	40.4 ± 1.9	42.3 ± 1.7
Week 16	33.9 ± 1.8	35.1 ± 2.0
Change	-6.5 ± 0.7	-7.1 ± 0.7
Total lean mass (kg)[†]		
Week 0	50.5 ± 3.0	49.1 ± 3.1
Week 16	48.8 ± 3.0	47.4 ± 2.7
Change	-1.7 ± 0.3	-1.2 ± 0.6

Change, difference in week 16 values from week 0. Values were derived from DEXA analysis performed at week 0 and 16. Week 0 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors.

*Data are means ± SEM.

[†]Significant effect of time from week 0 to week 16, $p < 0.05$

Table 9.4.8 Energy expenditure variables at weeks 0 and 16*

	SP diet			HP diet		
	Male n = 6	Female n = 13	Combined n = 19	Male n = 4	Female n = 13	Combined n = 17
TEE (kJ/day) [†]						
Week 0	13429 ± 550	10122 ± 550	11166 ± 544	13520 ± 1211	10500 ± 283	11210 ± 462
Week 16	13854 ± 500	9323 ± 475	10753 ± 609	12060 ± 1601	9878 ± 487	10392 ± 550
Change	425 ± 287	-799 ± 726	-413 ± 515	-277 ± 1239	-621 ± 425	-819 ± 375
Fasting REE ^{†‡}						
Week 0	9704 ± 498	6895 ± 180	7782 ± 362	9546 ± 873	7382 ± 262	7890 ± 354
Week 16	8656 ± 180	6238 ± 153	7002 ± 289	8498 ± 573	6854 ± 279	7240 ± 299
Change	-1047 ± 334	-657 ± 113	-780 ± 132	-1048 ± 565	-528 ± 146	-650 ± 171
TEF (%EI) [§]						
Week 0	6.8 ± 1.5	7.3 ± 0.4	7.1 ± 0.5	10.2 ± 1.3	8.8 ± 0.5	9.1 ± 0.5
Week 16	8.0 ± 0.7	7.7 ± 0.8	7.8 ± 0.6	11.3 ± 0.66	7.8 ± 0.4	8.6 ± 0.5
Change	1.3 ± 1.3	0.43 ± 0.74	0.69 ± 0.64	1.1 ± 0.90	-1.1 ± 0.61	-0.56 ± 0.55
PAEE (kJ/day)						
Week 0	2385 ± 317	2208 ± 510	2264 ± 357	2623 ± 230	2062 ± 198	2194 ± 168
Week 16	3803 ± 337	2138 ± 462	2664 ± 375	2346 ± 1315	2022 ± 324	2099 ± 371
Change	1418 ± 318	-70.3 ± 640	400 ± 471	-277 ± 1239	-39.6 ± 419	-95.2 ± 411
Fasting RQ ^{†¶}						
Week 0	0.79 ± 0.01	0.77 ± 0.01	0.77 ± 0.01	0.82 ± 0.02	0.79 ± 0.01	0.80 ± 0.01
Week 16	0.82 ± 0.02	0.83 ± 0.01	0.83 ± 0.01	0.80 ± 0.34	0.82 ± 0.01	0.82 ± 0.013
Change	0.034 ± 0.024	0.06 ± 0.008	0.052 ± 0.010	-0.02 ± 0.02	0.03 ± 0.01	0.020 ± 0.011
Postprandial RQ ^{†§¶}						
Week 0	0.0070 ± 0.001	0.013 ± 0.0012	0.011 ± 0.001	0.001 ± 0.003	0.006 ± 0.001	0.005 ± 0.001
Week 16	0.0068 ± 0.002	0.010 ± 0.002	0.009 ± 0.001	0.005 ± 0.002	0.006 ± 0.001	0.006 ± 0.001
Change	-0.0002 ± 0.001	-0.003 ± 0.001	-0.002 ± 0.001	0.004 ± 0.001	0.0005 ± 0.001	0.001 ± 0.001

TEE, total energy expenditure as measured using the [¹⁴C]-bicarbonate-urea method; Change, difference between weeks 16 and 0; PAEE, energy expenditure due to physical activity calculated from the estimation equation; TEF, thermic response to a 2747 kJ SP, or 2715 kJ HP test meal expressed as the % increase per energy intake over 3 hours; Postprandial RQ, mean increase in RQ over 3 hours after the HP or SP test meal. Week 0 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. *Data are means ± SEM. †Significant effect of gender at both weeks 0 and 16, p < 0.001. ‡Significant effect of time from week 0 to 16, p < 0.001.

§Significant effect of diet at week 0, p < 0.01. ¶Significant time-by-diet effect, p < 0.02.

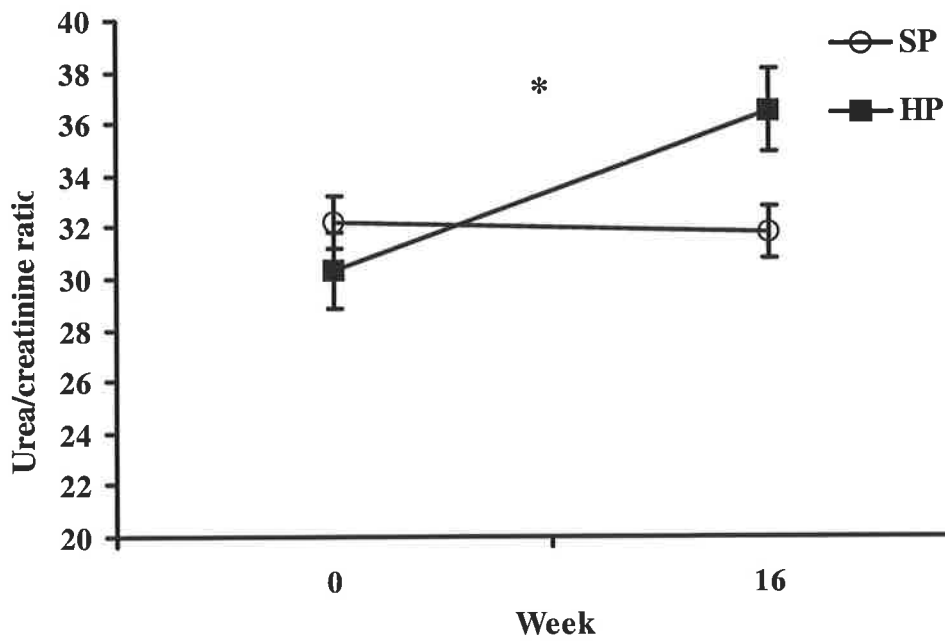


Figure 9.4.1 Urea excretion calculated from 24-hour urine saves and expressed as the ratio of urea/creatinine, in subjects with hyperinsulinemia on the SP (n = 29) and HP (n = 28) study diets.

Urine samples were collected at weeks 0 and 16 to determine compliance to the study diets. Week 0 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Data are expressed as means \pm SEM.

*Significant time-by-diet effect, $p < 0.0001$.

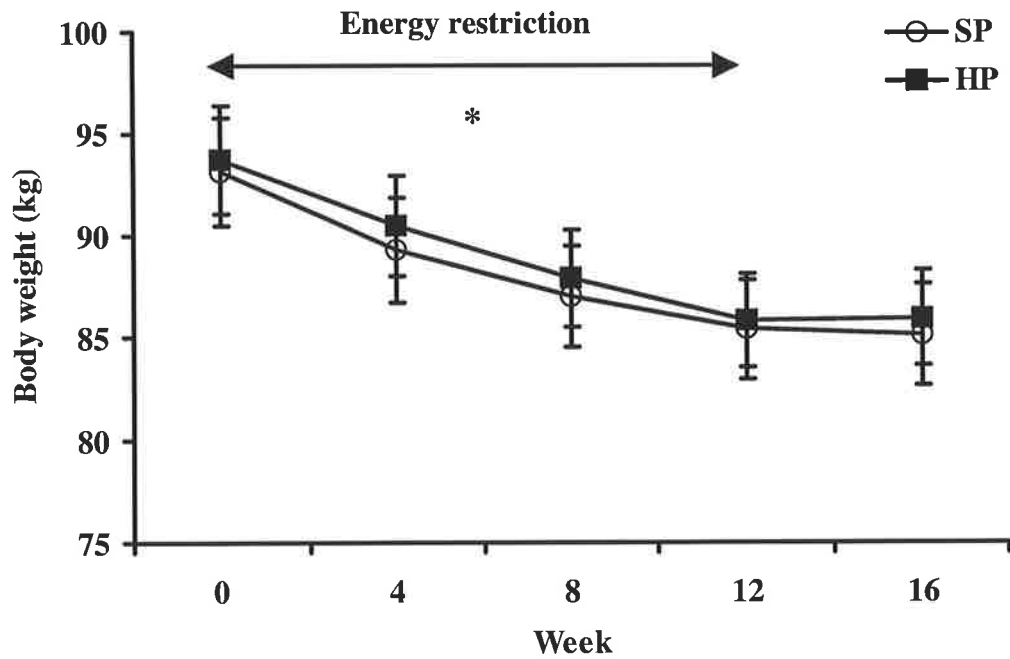


Figure 9.4.2 Change in body weight following 12 weeks of energy restriction (weeks 0 to 12) and 4 weeks of energy balance (from week 12 to 16) in subjects with hyperinsulinemia on either the SP (n = 29) or HP (n = 28) study diet.

Week 0, 4, 8, 12 and 16 data were compared using repeated-measures ANOVA with diet and gender as between subject factors. Comparison between diets within the same week was made using 1-way ANOVA. Data are expressed as mean \pm SEM.

*Significant decrease in weight from week 0 to week 12, in all subjects, $p < 0.001$.

Weight was maintained from week 8 to week 16 during energy balance.

There was no effect of diet.

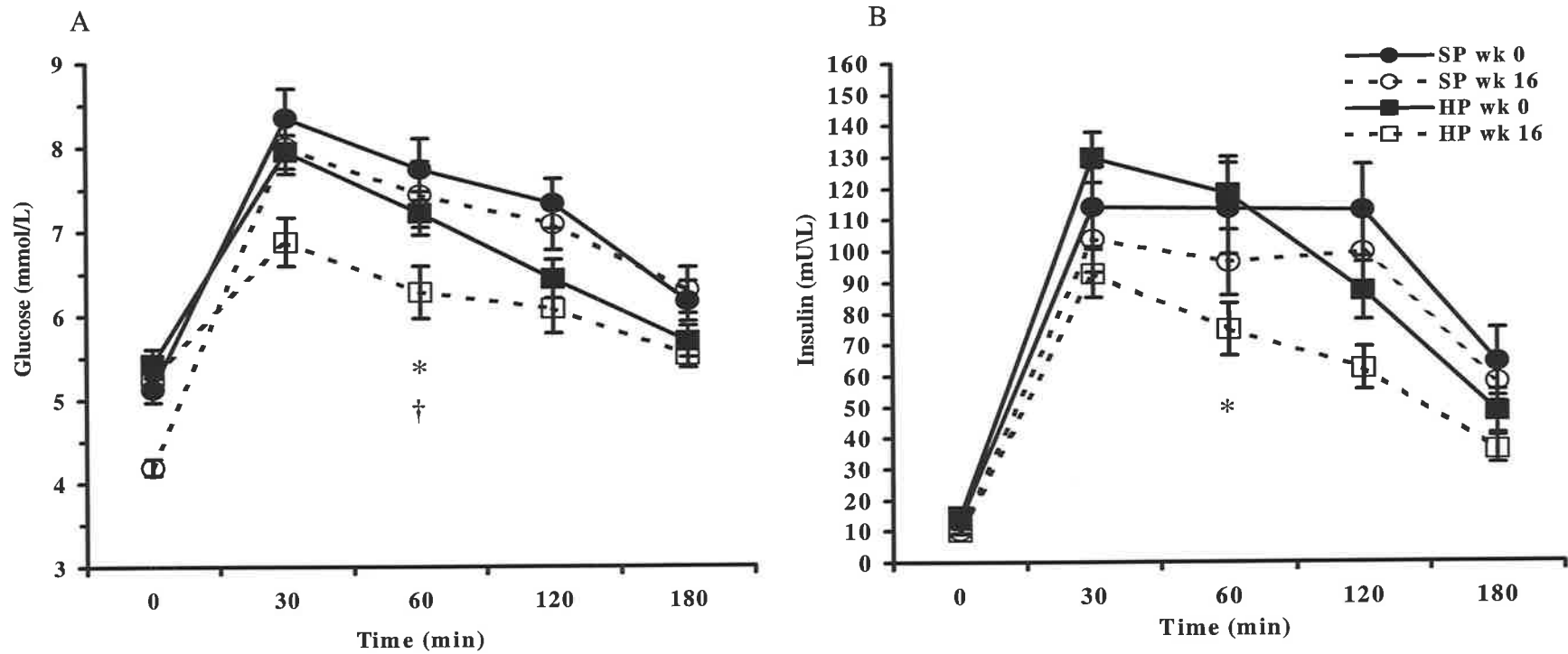


Figure 9.4.3 Glucose (A) and insulin (B) responses during the 3-hour meal tolerance test.

Glucose and insulin concentrations were measured at baseline and after 30, 60, 120 and 180 minutes after the ingestion of a SP or HP test meal, that was representative of the subjects' study diet, at weeks 0 and 16. Week 0 (wk0) and 16 (wk16) data were compared using repeated-measures

ANOVA with diet and gender as between subject factors. Comparison between diets at the same time point was made using 1-way ANOVA. Data are expressed as the mean \pm SEM. n = 29 in the SP group and 28 in the HP group.

*Significantly effect of time from week 0 to week 16, $p < 0.001$.

†Significant time-by-diet interaction such that glucose concentrations were reduced more greatly on the HP diet than on the SP diet by week 16, $p = 0.049$.

9.5 DISCUSSION

The findings from this study showed that, over 16 weeks, total energy intake and not the protein-to-carbohydrate ratio of the diet is the most important determinant of weight loss. This observation is in accordance with previous findings in obese subjects with type 2 diabetes that were reported in Chapter 8 (Parker et al. 2002). It also agrees with several other short-term studies (7 days to 10 weeks) in obese non-diabetic subjects that showed equivalent weight loss (mean change 3-8%) on energy restricted diets (3.3-4.2 MJ/day) with increased protein (36-49% of energy as protein) as compared to isocaloric diets with lower protein contents (10-37%) (Whitehead et al. 1996; Piatti et al. 1994). In contrast, a study of 13 hyperinsulinemic men showed 28% more weight loss (8.3 vs 6.0 kg) over 4 weeks on a diet containing 45% protein as compared to an isocaloric (~7.4 MJ/day) diet with 12% protein (Baba et al. 1999). A significant difference in total body water loss (-1.0 vs 0.3 L) between their high and lower protein diet, rather than fat loss (-7.1 vs -6.3 kg) may explain some of the differential weight loss in Baba et al's study (1999). In the present study, total body water was not measured. The 0.7 kg (~10%) greater loss of fat mass and the 0.8 kg (~52%) smaller loss of lean mass in our HP as compared to our SP group were not statistically significant. This may, in part, explain why the decrease in body weight was not different between our two dietary groups. In Baba et al.'s study (1999), there was also an 11.5% smaller decrease in REE (equates to a difference of 1053 kJ/day) on the HP as compared to their lower protein diet. A blunting in REE of this magnitude would be sufficient to explain the differential weight loss between Baba et al's (1999) two diets over the 4 weeks of energy restriction.

In the present study, data from the 36 subjects who had their energy expenditure measured showed that the fall in REE was not blunted by the increased protein content of the HP diet (even when adjusted for body weight or lean mass). This confirmed the findings reported in Chapter 8 for subjects with type 2 diabetes (Luscombe et al. 2002). Both of our studies had sufficient power (80%, alpha 0.05) to detect a 3% difference in the mean REE, a 2.7 kg difference in fat mass and a 1.8 kg difference in lean mass between the HP and SP groups. There was no difference in lean mass between the groups in our study. Ravussin and colleagues (1985a) demonstrated that a 9% decrease in REE after a 13% decrease in body weight, was not significant once it was normalised for changes in lean mass. Therefore, disparity in outcomes between the study of Baba et al. (1999) and our study may have been a consequence of the 33% greater protein content in their HP diet as compared to their lower protein diet, whereas in our two studies the HP diet had only 12 to 13 % more protein than the SP diet.

One small study (Whitehead et al. 1996) has shown that an increased protein intake during energy restriction may blunt the fall in TEE by more than 50%. We observed a small (4.2%) non-significant decrease in TEE after weight loss. Our study had enough power (80%, alpha 0.05) to detect an overall fall in TEE of 6.5%. Fifteen of the 36 subjects had a decrease in TEE of 6.5% or more, but for the remaining 21 subjects there was a large degree of variance for the change in TEE; 6 individuals experienced a decrease of less than 6.5% and 15 experienced an increase in TEE (0.28 to 36%). Our findings are not consistent with those of Whitehead et al. (1996). They measured TEE and sleeping metabolic rate in 2 men and 6 women who consumed a high-protein (36% energy as protein, 32% carbohydrate) diet and two standard-protein (15%

energy as protein, and either 53 or 32% carbohydrate) diets, each for 7 days. Since the fall in sleeping metabolic was significantly less in their HP group than in their SP group, it is not surprising that the fall in TEE was significantly blunted (i.e a 285 kJ reduction in TEE on the HP diets as compared to a 541 to 634 kJ decrease in TEE on the two SP diets)(Whitehead et al. 1996). Discrepant findings between Whitehead et al. (1996) and our study may be the result of: 1) a larger difference in the protein content between the HP and SP diets (21% difference in their study vs 12% difference in this study), or 2) the larger variability observed for the change in TEE in this study. We used the [¹⁴C]-bicarbonate-urea method to measure TEE whereas Whitehead et al. (1996) used whole-body indirect calorimetry. The [¹⁴C]-bicarbonate-urea method, which does not restrict subjects to an artificial living environment, has been shown to measure TEE within 2-5% of the value derived from whole-body indirect calorimetry (Elia, 1991; Gibney et al. 1997; Paton et al. 1996). Our observation that TEE was not significantly decreased after weight loss is consistent with our findings from Chapter 7 and also the findings reported by others (Amatruda et al. 1993; Rumpler et al. 1991). Both Amatruda et al. (1993) and Rumpler et al. (1991) measured TEE after their subjects' body weights had been stabilized at the reduced level for a period of time. Therefore, the timing of the energy expenditure measurements (ie. during dynamic weight loss versus after stabilisation at reduced weight) may have also impacted on the disparity in outcomes between the study of Whitehead et al. (1996), and our study. Additionally, it is possible that our study and the aforementioned studies, are committing type 2 errors because of their small sample sizes and variability within their study populations. To ensure that our 4.2% decrease in TEE after weight loss was not significant and a type 2 error had not been made, 81 subjects would have been required.

We hypothesized that the high-protein diet might facilitate weight loss by blunting the decrease in resting and total energy expenditures as a consequent of an increased thermic effect of feeding. In accordance with previous findings reported in Chapter 8 (Luscombe et al. 2002), as well as others (Westerterp et al. 1999; Westerterp et al. 1999b), we observed that the TEF was greater after the HP meal than after the SP meal. However, the increased TEF in the HP group only accounted for approximately 0.8 to 2 % more energy over the 3 hours than the TEF in the SP group, which was not sufficient to influence weight loss in this study. Over 6 months, assuming that our subjects eat three 2.7 MJ meals per day (or a total of 8.1 MJ/day) and are in energy balance, the extra 65 to 162 kJ/day being burnt in the HP as compared to the SP group, may equate to a difference of only 0.7 to 1.9 kg between the two diets. Accordingly, given the large within-subject day-to-day variation in the measurement of TEF as well as the dominant effects of energy intake and physical activity on energy balance, the impact of TEF on either weight loss or weight maintenance is likely to be minimal, at least in the intermediate term.

Since one objectives of the current study was to determine the effect of a HP energy restrictive diet on weight loss and the changes in energy expenditure, our subjects were asked to maintain physical activity constant throughout the study. Our findings in the subpopulation of 36 individuals showed that the average daily level of voluntary physical activity, assessed from the 3-day activity diaries and a published table of activity indexes, were similar during the 3 days of energy expenditure measurements at both week 0 and week 16. We also found that the average energy expended due to physical activity (includes both voluntary and involuntary activity)

was similar at week 0 and week 16. There was, however, substantial variation in the change in PAEE between our individuals and since the mean error in the measurement of PAEE is 41% (~950 kJ/day), it is possible that small changes in PAEE were not detected.

Our observation that all subjects lost a similar amount of fat mass on both diets (16.3 vs 17.5% on the SP and HP diets respectively) contrasts with our findings reported in Chapter 8. In the diabetic population, women in the HP group lost more body fat and abdominal fat than the women in the SP group. The reason for the differing results for fat and abdominal mass between the non-diabetic women in the present study and the diabetic women, is not clear. The findings for our hyperinsulinemic population also differ from a recent study that observed a 3.3 kg greater fat loss over 6 months on a diet that contained 25% of total energy from dietary protein as compared to one that contained 12% protein (Skov et al.1999b). Skov and colleagues (1999b) aided the compliance of the subjects over the long term by using *ad libitum* feeding. The differential fat loss in their study was a consequence of reduced energy intake on the high compared to standard-protein diet. They proposed that an increased satiety following the consumption of HP meals was responsible for the observed decrease in energy intake. Since our HP and SP diets were fixed menu isocaloric diets, the effect of satiety on energy-intake would have been negated, and would explain the disparity in weight and fat loss between Skov et al. (1999b), and our study.

Our finding that total lean mass was preserved in females on the HP diet is consistent with the findings of Piatti and colleagues (1994). That the effect was only observed in our female subjects may reflect the small number of males included our study, or it

may have been because the protein content of the HP diet for the men was not sufficient to preserve lean mass. Piatti et al. (1994) and others (Hoffer et al. 1984) have shown that proteolysis of lean tissue was suppressed in subjects consuming 1.5 g of protein per kg of ideal body weight. Proteolysis was not measured in the current study, but the reported protein intake in the HP group was 1.1 g/kg of ideal body weight for the males and 1.4 g/kg for the females. It is possible that there was sufficient dietary protein to suppress proteolysis and preserve lean mass in females, while for the men the lower protein intake was not adequate. We have previously shown that lean mass was not spared in either women or men with type 2 diabetes when an average 1.3 g/kg of protein was consumed during energy restriction (see Chapter 8) (Parker et al. 2002). In the diabetic women, however, there was a trend for the decrease in lean mass to be less on the HP diet as compared to the SP diet; the result may not have reached significance because there were fewer women in both dietary groups in the diabetic study than in the present study.

Fasting plasma glucose concentrations were not significantly reduced during energy restriction or after weight loss in the current study. Despite the small increase in plasma fasting glucose with the HP diet, all subjects remained normoglycaemic throughout the study. Linn et al. (1996) showed that a HP diet increased gluconeogenesis and hence hepatic glucose release, in subjects with and without Type 1 diabetes.

Consistent with the findings of Chapter 8 (Parker et al. 2002) as well as others (Kelley et al. 1993; Markovic et al. 1998; Atkinson and Kaiser, 1985), fasting insulin was reduced during energy restriction and subsequent weight loss. However, the HP diet

had no benefit in ameliorating insulin resistance (assessed using the HOMA insulin resistance index) over and above energy restriction and weight loss, in the present study or in Chapter 8. This finding is in contrast to the improvement in insulin resistance reported by Piatti and colleagues (1994) who used a euglycaemic hyperinsulinaemic clamp to evaluate changes in insulin sensitivity. The HOMA index only provides a qualitative estimate of insulin resistance and therefore small changes in insulin sensitivity may not have been detected.

When protein is added to a carbohydrate meal, a significant attenuation in the glucose response has been reported by some investigators (Spiller et al. 1987; Nuttall et al. 1984; Estrich et al. 1976), but not by others (Day et al. 1978; Westphal et al. 1990). In the present study, there was no difference in postload insulin levels between the HP and SP groups. Therefore, the lower glucose response following the HP meals probably reflects the smaller carbohydrate load of the HP as compared to SP meal. After weight loss, the glucose response area decreased 6.8% more in the HP group than in the SP group. Prospective data from the Rancho Bernardo (Barrett-Connor and Ferrara, 1998) and the DECODE (The DECODE study group, 1999) studies have reported that post-load glucose levels after an oral glucose challenge are related to cardiovascular disease and death. Lowering post-meal glucose, even in “glucose tolerant” subjects, may reduce the risk for future cardiovascular morbidity and mortality.

Insulin resistance is associated with lower carbohydrate oxidation and higher fat oxidation (Zurlo et al. 1990). For the 36 subjects who had their RQ assessed, the greater increase in postprandial RQ after the SP meal, as well as the greater increase

in fasting RQ after 16 weeks on the SP diet, probably reflects the of the greater carbohydrate content of the SP diet used in this study. This is consistent with our previous findings in subjects with type 2 diabetes (Chapter 8) (Luscombe et al. 2002). As in Chapter 8, the magnitude of the increase in postprandial RQ was smaller than expected. This supports the suggestion made in Chapter 8 that in subjects with insulin resistance the ability to switch from predominantly lipid oxidation, during fasting, to increased glucose uptake, oxidation and storage after feeding, was reduced. After weight loss, we speculated that an improvement in insulin sensitivity may enhance carbohydrate oxidation as a consequent of improvements in glucose uptake and the suppression of both hepatic glucose release and the release of free fatty acids from the adipose tissue (Zurlo et al. 1990). After 16 weeks, however, the increase in postprandial RQ was not related to a significant decrease in fasting insulin (-5.3 mU/L) or postprandial insulin area under the curve (-16.5%).

Our finding of a 14.1% greater reduction in fasting triacylglycerol concentrations in both men and women on the HP diet is similar to that observed by Wolfe and colleagues during weight maintenance studies in subjects with mildly high cholesterol levels (Wolfe and Giovannetti, 1991) and normal lipid levels (Wolfe and Piche, 1999). In the present study, the beneficial effect of the HP diet on triacylglycerol concentrations probably reflected the lower carbohydrate content of the diet. Low-fat, high-carbohydrate diets have been reported to elevate triacylglycerol levels (Kasim-Karakas et al. 2000; Schaefer et al. 1995), particularly in insulin resistant subjects (Jeppesen et al. 1997). The greater carbohydrate content of the SP diet may have enhanced de novo synthesis of triacylglycerol from carbohydrate (Hudgins et al. 2000) and decreased the clearance of triacylglycerol-rich very low density

lipoproteins due to a reduction in the efficiency of lipoprotein lipase (Kasim-Karakas et al. 2000). In Chapter 8, it was reported that a HP diet had no effect on serum triacylglycerol concentrations. We did however, observe reductions in fasting serum total- and LDL-cholesterol that were ~5% and 8.6% greater, respectively, on the HP as compared to the SP diet in subjects with type 2 diabetes (Parker et al. 2002). Disparate findings between the present study and the study described in Chapter 8 (Parker et al. 2002) may be due to the inclusion of subjects with lower baseline triacylglycerol concentrations and higher baseline cholesterol concentrations, in this study.

We observed no deleterious effect of the HP diet on bone turnover in this study. Our findings are consistent with Shapses et al. (1995) who showed no change in urinary hydroxyproline, pyridinoline and deoxypyridinoline when protein was increased from 0.44 to 2.71 g/kg, in 15 young subjects. In the Framingham osteoporosis study (Hannan et al. 2000) persons in the highest quartile of protein intake (1.24 to 2.78 g/kg/day or 17 to 27% of energy) lost the least bone mineral density, over 4 years.

One study reported an increase in systolic blood pressure following an increase in animal protein intake over 4 weeks, in 21 subjects (Sacks et al. 1981). We observed that the protein content had no effect on systolic or diastolic blood pressure. This finding was consistent with that reported in subjects with Type 2 diabetes in Chapter 8. No other studies have examined the effect of HP protein energy restrictive diets on blood pressure. Accordingly, our finding warrants further investigation.

9.6 CONCLUSION

Total energy intake and not the protein-to-carbohydrate ratio of the diet is the most important determinant of weight loss in insulin resistant subjects. The HP diet did not blunt the fall in energy expenditure, or increase the thermic effect of feeding that may be associated with weight loss. Improvements in fasting insulin homeostasis on both the HP and SP diets were a consequence of weight loss. After weight loss, however, improvements in postprandial insulin sensitivity were significantly greater following the HP meal. This finding, combined with the greater preservation of lean mass in females and the greater decrease in triacylglycerol concentrations, suggests that replacing carbohydrate for protein in the diet of obese insulin resistant subjects may delay the onset of type 2 diabetes and reduced the risk of cardiovascular disease. It remains to be determined whether increased protein diets are of benefit in the maintenance of body weight, glycaemic control and lipid levels in subjects with insulin resistance.

CHAPTER 10

Conclusions

10.1 INTRODUCTION

Within the dieting public there has been resurgence in the popularity of high-protein, low-fat, 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 typically observed during and after weight loss. Consequently, low-fat, high-protein diets may be more beneficial than low-fat, high-carbohydrate diets for long-term weight management. Furthermore, several studies have found that high-protein diets can improve insulin sensitivity and plasma lipid levels.

The focus of this thesis was 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. To measure diet-induced changes in energy expenditure, a major objective of this thesis was to establish, in our laboratory, the novel [^{14}C]-bicarbonate-urea method for evaluating total energy expenditure as well as energy expenditure due to physical activity, in free-living subjects. Indirect calorimetry for measuring resting energy expenditure, substrate oxidation and the thermic effect of feeding also had to be established.

The specific hypotheses addressed in this thesis were:

1. After body weight is reduced and stabilized at a lower level, daily energy expenditure will be reduced, thereby predisposing individuals to weight regain.
2. Increasing the dietary protein content of energy-restricted diets will enhance weight loss and blunt the reduction in REE and/or TEE. An increase in the TEF and greater preservation of lean body mass are two mechanism through which the increased dietary protein will act.

3. Increasing the dietary protein content of energy-restricted diets will preserve lean body mass, thereby improving insulin sensitivity and ameliorating insulin resistance.

In order to investigate these hypotheses the aims of this work were to:

1. Establish the [¹⁴C]-bicarbonate-urea method for measuring TEE and PAEE in “free-living” subjects and determine its’ reproducibility.
2. Determine the reproducibility of indirect calorimetry for measuring REE, TEF and RQ.
3. Determine the reproducibility of DEXA for measuring changes in body composition.
4. Determine whether TEE and/or REE, TEF and PAEE decrease after body weight is reduced and stabilized at the lower level.
5. Determine the benefits of increasing the protein-to-carbohydrate ratio of moderately energy-restricted diets on weight loss and energy expenditure, and glucose, insulin and lipid levels, in adults with Type 2 diabetes and hyperinsulinemia.

The first part of this chapter summarises the findings, conclusions and implications of this research. The second part of the chapter highlights the methodological limitations of energy metabolism and dietary intervention studies. Recommendations for further research are presented at the end of the chapter.

10.2 MAIN FINDINGS

The [¹⁴C]-bicarbonate-urea method is a novel and alternative method for measuring TEE to doubly labeled water and whole-body indirect calorimetry. We have demonstrated that the [¹⁴C]-bicarbonate-urea method for measuring TEE (Chapter 6) has an intra-individual day-to-day variation of $4.8 \pm 1.0\%$ (mean \pm SEM) for a non-obese group of men, and $9.7 \pm 1.3\%$ for an obese group of men and women. The day-to-day reproducibility of the [¹⁴C]-bicarbonate-urea method was comparable to that of doubly labeled water (typically 8 to 14%). In addition, the values of TEE obtained using [¹⁴C]-bicarbonate-urea, were

consistent with those reported in studies using doubly labeled water. 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; vigorous activities other than swimming were not restricted.

Three weight loss studies using [¹⁴C]-bicarbonate-urea (Chapters 7 to 9) were performed to evaluate the effects of diet-induced weight loss on total energy expenditure and its components, after body weight had been stabilised at a reduced level. Reductions in total fat mass ranged from 4.5 to 8.4 kg and reductions in lean mass ranged from 0.3 to 3.8 kg. All studies demonstrated that after a reduction in body weight of 5.4 to 12.5% below the initial level, REE was reduced by 4.1 to 8.8% (~ 296 to 719kJ/day). In Chapters 7 and 9, the changes in TEF (-1.4 and +0.13%, respectively), PAEE (-18.6 and +6.2%) and TEE (-0.18 and -4.1%) were not significant, largely because of large variations between individuals (the origin of which would be of considerable interest to investigate further). In men and women with Type 2 diabetes (Chapter 8), however, the 1.1% reduction in TEF that occurred after weight loss was statistically significant. TEE was not measured in Chapter 8 because the method was still being established in the lab. In Chapters 7 and 8, neither fasting nor postprandial RQ (reflects the ratio of fat to carbohydrate oxidation) were altered after diet-induced weight loss. In the non-diabetic men and women with hyperinsulinemia (Chapter 9), fasting RQ also remained unchanged. In this population, the statistically significant, albeit small, increase in postprandial RQ reflected the higher carbohydrate content of the SP meal.

The effect of low-fat (30% of energy), fixed energy intake diets, with either a high or standard protein content, on weight loss and energy expenditure, was the focus of the first part of the studies described in Chapters 8 and 9. After 8 to 12 weeks of energy restriction followed by 4 weeks of weight maintenance, the reduction in body weight was similar in

the HP and SP dietary groups for both the subjects with Type 2 diabetes (4.8 vs 5.9% respectively), and the non-diabetic subjects with hyperinsulinemia (7.9 vs 8.0% respectively). The women with Type 2 diabetes in the HP group lost more total body fat (12.4 vs 7.0 %) and abdominal fat (11.9 vs 7.2%) than the women in the SP group. This may have been a consequence of the women in the HP group having a marginally higher starting total and abdominal fat mass than the women in the SP group (e.g. 42.8 vs 39.9 kg for fat mass, or 10.9 vs 9.7 kg for abdominal fat mass). Alternatively, it may have been that several women in the SP group lost less weight than others. For the women with hyperinsulinemia, there was no difference in total or abdominal fat loss between diets. The reason for the differing results for fat mass between the non-diabetic and diabetic women, is not clear. For the non-diabetic women with hyperinsulinemia, total lean mass was preserved more on the HP than on the SP diet (0.2 vs 3.5 % reduction). The diabetic women showed a trend for lean mass to be preserved more on the HP diet. The absence of a statistically significant result may have been because of fewer women in both the HP and SP groups, in the diabetic study as compared to the non-diabetic hyperinsulinemic study. In the non-diabetic hyperinsulinemic population, diet composition had no effect on the decrease in TEE (7.1% on the HP vs 1.4% on the SP) [TEE was not measured in the diabetic population]. The reduction in REE was similar in the HP and SP groups for both the subjects with diabetes (6.0 vs 0.36 % decrease respectively) and for those with hyperinsulinemia (8.2 vs 10 %). In both populations, TEF was greater after the HP meals than after the SP meals (6.4 vs 5.0 and 8.9 vs 7.5 % of consumed energy, for subjects with and without diabetes, respectively). There was, however, no association between the increased TEF in the HP diet groups and weight loss in either subject population.

The second part of the weight loss studies described in Chapters 8 and 9 compared the effects of the HP and SP diets on glycaemic and insulinemic responses, and lipid levels. The improvement in fasting insulin sensitivity (as depicted by a significant reduction in the

HOMA index) was not dependent on diet composition, in either the subjects with diabetes (reduction of 27.3% in HP vs 26.4% in SP group), or in those with hyperinsulinemia (reduction of 21.0% in HP vs 9.0% in SP group). For the subjects with hyperinsulinemia, the glycaemic response to the HP meal was ~10% less than to the SP meal, both before and after weight loss. Furthermore, after weight loss, the decrease in glycaemic response was greater in the HP group than in the SP group (8.7 vs 1.9% reduction) for these subjects. In subjects with Type 2 diabetes, the reduction in total- and LDL-cholesterol was 6.1% and 8.5% greater, respectively, in the HP group than in the SP group. For the non-diabetic hyperinsulinemic subjects, triacylglycerol concentrations were reduced 13.8% more on the HP diet. The differential effect of the HP diet on the lipid metabolism of the non-diabetic hyperinsulinemic population, as compared to the diabetic population, may have been due to the inclusion of subjects with lower baseline triacylglycerol concentrations and higher baseline cholesterol concentrations in the former population. There were no deleterious effects of higher protein intakes on blood pressure or bone turnover in either of the subject populations examined in the studies described in Chapters 8 and 9.

10.3 CONCLUSIONS AND IMPLICATIONS

The [¹⁴C]-bicarbonate-urea method was a reproducible and therefore suitable field method for measuring the daily energy expenditures of overweight and obese populations while they continue their usual lifestyle. Since the [¹⁴C]-bicarbonate-urea method does not require expensive equipment that is of limited availability to most research centres, it can be used as alternative to doubly labeled water; particularly when energy expenditure needs to be assessed over 1 day periods (or multiples thereof) or in response to a short term intervention. Further research however, is warranted to validate its' accuracy for measuring "free-living" TEE against doubly labeled water. It must however, be stated that validation of [¹⁴C]-bicarbonate-urea against doubly labelled water would be technically difficult, time consuming and costly. These factors will probably prohibit such a study being conducted.

It is therefore likely that doubly labeled water, will remain the most important field tool for measuring integrated TEE over many days.

The three dietary intervention studies demonstrated that caloric-restriction, rather than the macronutrient composition of the diet, is the most important determinant of weight loss. Replacing some dietary carbohydrate for protein did not blunt the fall in REE during diet-induced weight loss. Accordingly, the reduction in REE that is consistently observed after weight loss may be one important mechanism contributing to the failure of many individuals to maintain the reduced body weight after energy restriction is ceased. In the obese populations that were investigated in these studies, however, TEE was not significantly reduced after body weight was stabilized 5 to 13% below its initial level. Nevertheless, it remains possible that the observed reductions in REE (4.1 to 8.8 %) did contribute to physiologically important decreases in TEE, although they may have been obscured because of some methodological issues (e.g. the small number of subjects examined in each study and the large variability between subjects in the response of TEE to weight loss). On a group basis, decreases in TEE ranged from 181 to 605 kJ/day. Reductions in TEE of this magnitude may lead to a weight regain of 2 to 6.8 kg over a year.

In the obese subjects studied, neither fasting nor postprandial RQ were altered after diet-induced weight loss. We speculated that an improvement in insulin sensitivity may enhance postprandial carbohydrate oxidation as a consequence of improvements in glucose uptake and the suppression of both hepatic glucose release and release of free-fatty acids from the adipose tissue. This was not the case and therefore the mechanisms regulating carbohydrate oxidation in obese populations require further research. The inability to increase carbohydrate oxidation after feeding may also contribute to the difficulties in maintaining weight loss.

Moderately higher protein intakes that are also low in fat (i.e. from lean meat, poultry and dairy foods) than usually consumed by Western society had the benefit of reducing postload glucose and fasting triacylglycerol concentrations in non-diabetic subjects with hyperinsulinemia; fasting total- and LDL-cholesterol concentrations were reduced in subjects with Type 2 diabetes. There was no deleterious effect of these high-protein diets on blood pressure, urinary protein excretion, or markers of bone turnover. These findings, combined with the improvements in fasting glucose and insulin homeostasis that were attributable to weight loss, suggest that low-fat diets, with an increased ratio of protein to carbohydrate, may be a useful dietary strategy for people with Type 2 diabetes as well as for obese adults who are at risk of developing diabetes or cardiovascular disease. This requires further investigation in clinical trials that implement an *ad libitum* high-protein, low-fat diet as compared to an *ad libitum* standard protein, low-fat diet, over at least 3 to 5 years in subjects with hyperinsulinemia and Type 2 diabetes.

10.4 LIMITATIONS

Despite the success of many individuals in losing a significant amount of excess weight and improving their glucose, insulin and lipid levels, the long-term maintenance of weight loss is an obstacle for most people. The weight-cycling phenomenon led to the speculation that metabolic efficiency develops during or after weight loss and defends the body from falling below a genetically pre-determined 'settling-point' of weight (or body fat stores). Evidence for this hypothesis, however, remains controversial because very few studies have measured TEE as well REE, TEF and PAEE, which is necessary to assess the full adaptive response of energy expenditure to diet-induced weight loss. In addition, the characteristics and number of subjects, the duration and degree of energy restriction, the macronutrient composition of the diet, and the timing of energy expenditure measurements are important issues that need to be considered when examining the study results. One or

more of these issues may account for the differing results between the studies that have examined the impact of weight loss on energy expenditure and also the impact of macronutrient composition on weight loss and the control of glucose, insulin, and lipid metabolism. The main limitations of the work presented in this thesis are discussed below.

1. Genetic heterogeneity

In each of the three weight loss studies, the heterogeneous nature of the subject populations presumably increased the variability of the response to energy restriction for many of the outcome measures. Although the our studies used populations that were larger than in other studies, they too were of a small to moderate size (11 to 57 subjects) and of mixed gender. Obesity was classified according to BMI. Despite the degree of obesity being matched when two groups of subjects were to be statistically compared, we did not take differences in the distribution of body fat (i.e. gynoid versus android, or visceral versus subcutaneous fat stores) into account. Furthermore, neither a family history of obesity, nor the individuals' history of obesity, was considered in the design of the studies. All of these factors may have masked physiologically important changes in some outcome measures.

2. Methodological error and power of the studies

Where possible the amount of methodological error for the techniques use to measure body composition, energy expenditure, and insulin, glucose and lipid metabolism was described. Due to a large degree of variability between subjects, it appears that the methods used to measure energy expenditure and insulin sensitivity were not sensitive enough to detect smaller than were anticipated changes in the overall small to moderate study populations. Moreover, unequal numbers of men and women were included in the weight loss studies (from our experience it is more difficult to get men to participate in strictly controlled weight loss trials). As a result none of the studies were specifically powered to compare

differences between men and women. Consequently, the significance of the diet-by-gender interactions in the protein and weight loss studies, are not clear and require confirmation.

3. *Degree of energy restriction*

The amount of body weight lost by individuals participating in short- to moderate-term weight loss studies is largely determined by the degree (i.e. severe versus moderate) of energy restriction. The aim of the studies described in Chapters 8 and 9 was to compare the effects of moderately energy restricted diets (~30% restriction) that were either high or lower in dietary protein on weight loss and energy expenditure, and glucose, insulin and lipid levels. Therefore, diets containing ~6.0 MJ/day were prescribed. With respect to energy expenditure, some studies suggest that a decrease in energy expenditure in response to diet-induced weight loss is a secondary response that occurs only when the minimum amount of energy required by the body cannot be supplied from the body's energy stores. For the work presented in this thesis, it is possible that no effect of weight loss was observed on TEE because the severity of energy restriction did not cause the subjects to reach the critical level of body weight that may signal that a reduction in energy expenditure is necessary to minimize further weight loss. Insufficient weight loss, or more specifically abdominal fat loss, may also explain why we did not observe an improvement in insulin sensitivity.

4. *Fixed energy intake versus ad libitum intake*

Skov's group demonstrated that *ad libitum* consumption of high protein foods reduced the overall amount of energy consumed over 6 months. Therefore, the increased satiety on the HP diet may have led to the greater reduction in weight and fat mass, in Skov's study. However, because a reduced energy intake, independently effects body weight and composition, energy expenditure, and glucose, insulin and lipid metabolism, the HP and SP diets compared in this research were matched for energy intake. This would have nullified

the satiety effect of the increased protein content from affecting weight and fat loss. As body weight and composition were not reduced more greatly on our HP as compared to our SP diet, it is likely that satiety and not an increase in energy expenditure, is the main mechanism through which the increased protein content acts.

4. *Size of the exchange of carbohydrate for protein between the HP and SP diets*

For the studies reported in this thesis, the exchange of carbohydrate for protein from the SP to the HP diet was ~12% of total energy intake. This exchange represented a moderate manipulation of the recommended macronutrient composition of diets for adult Australians and may have limited the effects of an increased protein content of weight loss, energy expenditure, and insulin sensitivity. Several fixed-intake studies used more extreme carbohydrate for protein exchanges (e.g. 25% and 33% of the total energy) and the greater amount of protein in the HP energy restricted diets had effects on fat and lean mass loss, energy expenditure, and insulin concentrations that were independent of the degree of energy restriction. On the other hand, recommending a protein level that is not excessively different from average has important implications with respect with respect to long-term compliance to the diet.

5. *Timing of the energy expenditure measurements*

Two studies that reported a similar amount of weight loss as compared to this work, observed significant reductions in TEE and it may be due to the fact that they measured energy expenditure during the energy restricted period. During energy restriction, a decrease in energy expenditure presumably reflects a metabolic adaptation to the acute negative energy balance and /or the reduced cost of substrate oxidation. Accordingly, the timing of measurements may also explain why we did not observe a decrease in energy expenditure, whereas others have.

10.5 FUTURE RESEARCH

Future research investigating the role of energy expenditure in the dysregulation of energy balance that characterizes obesity, needs to focus on improving field methods for measuring TEE. The advent of doubly labeled water has considerably aided advances in the understanding of “free-living” energy regulation over the past decade. Further advances can be anticipated if doubly labeled water and/or technically uncomplicated, time efficient and less expensive methods such as [¹⁴C]-bicarbonate-urea become more widespread. Accordingly, effort must be invested to refine and validate such techniques so that their intra-individual measurement error is as low as for whole-body indirect calorimetry (typically less than 5%). Advantages of the [¹⁴C]-bicarbonate-urea over doubly labeled water is that it is also capable of measuring free-living TEE over 1-day periods or multiples thereof, it can be combined with indirect calorimetry to simultaneously measure REE and TEE, and it uses a lower dose of radioactive tracer. Consequently, the [¹⁴C]-bicarbonate-urea has the potential to be used in prospective studies, as well as acute underfeeding, overfeeding, or exercise intervention studies.

Progress in the development of the optimal dietary treatment for obesity and its’ associated diseases may be facilitated by conducting research in much larger populations than are typically used (e.g. populations of several hundred). This will ensure that small differences would be detected. It is also important because different diets may suit different people in different ways. Consequently, some research questions may best be addressed using populations that are as genetically homogeneous as possible. Identification of a group of subjects sharing the same allelic variant(s) of a particular candidate obesity/diabetes gene would be required and then a dietary intervention within that population would be examined. For example, populations could be screened for candidate genes such as leptin, PPAR γ , and UCPs, or for polymorphisms within the candidate genes such as Gln223Arg in the leptin receptor and Trp64Arg in the β_3 adrenergic receptor. Such efforts may provide a

new basis for targeting obesity/diabetes prevention programs in susceptible individuals. Regardless of the size or nature of the population examined, obesity research might also be aided by acknowledging the statistical power of results so that they can be appropriately compared between different groups.

Further investigations into the efficacy of HP diets are required. Prospective studies that match subjects by insulin levels, age, gender, fat distribution, BMI, and family history of diabetes before subjects are placed on high and standard protein diets might delineate the effectiveness of altering dietary protein intake on delaying the progression to Type 2 diabetes and at reducing the risk of cardiovascular disease. Further studies are also warranted to determine if these findings are enhanced using *ad libitum* consumption of higher protein, low-fat foods. Whether the mechanism(s) relate to the higher protein content, or are a function of lower carbohydrate intake also requires clarification. Until these issues are clarified I would recommend that individuals are cautious in their choice of popular high protein diets such as the “Atkins”, “Protein Power” and “Stillman” diets. I would however, suggest that replacing a moderate proportion of the daily carbohydrate intake with low-fat, high protein food choices is a safe dietary option that may have some benefits for people wanting to reduce their weight and improve their glycaemic control and blood fat levels.

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3 DAY PHYSICAL ACTIVITY DIARY

NAME:

**Department of Medicine
Natalie Luscombe**

Royal Adelaide Hospital

(08) 8222 5039

NAME:

SEX:

AGE:

WEIGHT:

HEIGHT:

OCCUPATION:

GUIDELINES

1. This is a diary for you to record **all activity** that you do for **3** days.
2. From the time you get up in the morning until you go to bed at night we would like you to record **all activity that you do and the time spent doing it.**
3. If doing one form physical activity for a prolonged period (ie. over 5 mins), rate the intensity of the activity as
SEDENTARY/EASY/MODERATE/HARD/VERY HARD
Sedentary activity is sleeping, lying in bed, watching TV
Moderate intensity is similar to how you feel when you are walking at a normal pace
Hard is activity that feels harder than walking at a normal pace but not as hard as jogging/running
Very hard is activity that feels as hard as jogging/running
Easy is all other activity done during the waking hours
4. Fill in the diary each time you have a meal break ie. breakfast, lunch and tea, or immediately after exercising. Try to make your physical activity pattern as **typical** as possible.
5. Don't forget to record the **time you spend sleeping** overnight or if you have a sleep during the day, the time you spend a computer or in front of the TV, the time spent walking between home and the bus-stop.
6. List separate activities on a different line.
7. Please take the diary with you if you are away from home for the day.
8. Use **the example** given as a guide to record your activities.

EXAMPLE
Date: **Day of the week:**

Time of Day	Description of activity	Duration	Intensity
6.30am	Shower/ breakfast	30min	easy
7am	Brisk walk	30min	hard
7.30am	Walk to bus	15min	moderate
8.15am	Walk to bus	5min	moderate
8.30-12pm	Office duties	3.5hr	easy
12.15-1.15pm	Lunch. Walk about city	15min	easy
1.20-5.15pm	Walk to bus	5min	moderate
5.20pm	Walk to gym	5min	moderate
6pm	Gym: Bike (10min) treadmill (30min) aerobics (40min)	10min 30min 40min	easy moderate very hard
7-9pm	Dinner, shower,TV	2hr	easy
9-10pm	Reading	1hr	easy
10.30pm-7am	Sleep	8.5hr	sedentary

Date: Day of the week:

Time of Day	Description of activity	Duration	Intensity

3 DAY FOOD DIARY

NAME:

Department of Medicine

GUIDELINES

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

EXAMPLE

Date: **Day of the week:**

Time	Description of food and drink consumed	Quantity
7 am	Weetbix	3 biscuits
	Full-cream milk	½ cup
	Bread (white, toasted)	1 slice
	Margarine (polyunsaturated)	2 tsp
	Orange juice (unsweetened)	1 glass
10 am	Coffee (instant)	1 cup
	Sugar (white)	2 tsp
	Milk arrowroot biscuits	2 biscuits
12.30 pm	Bread (white)	2 slices
	Margarine (polyunsaturated)	2 tsp
	Ham (lean, shoulder)	1 slice
	Cheese (low fat cheddar)	1 slice
5.30 pm	Steak (beef, raw)	200 g
	Potato (with skin, baked)	200 g
	Beans (French, boiled)	60 g
	Bread (white)	1 slice
8 pm	Milk- full cream	250 ml
	Milo	2 tsp
	Milk coffee biscuits	2 biscuits
Recipe	TUNA MORNAY	SERVES 2
	Tuna (in brine)	250 g
	Flour (plain white)	1 Tbsp
	Cheese (maturated cheddar)	20 g
	Breadcrumbs (white)	1 Tbsp
	Margarine (polyunsat.)	3 tsp
	Milk (skimmer)	½ cup

Date: **Day of the week:**

Time	Description of food and drink consumed	Quantity

HOW TO FILL IN THE CHECKLIST

1. You will need to fill the checklist in each day.
2. Put a tick [✓] in the box if you have eaten the food listed in the correct amount along with the specific food type
Example [✓lamb] or [✓apple].
3. If you have eaten the food, but have had more or less than specified, write in the amount eaten.
Example [✓lamb 100g cooked]
or [✓apple 200g].
4. Put a cross (X) if you have not eaten that food at all.
5. List **ALL** foods eaten (allowed or not!) that are not specified on the checklist under OTHER.

Example [diet soft drink 1 can]

CHECKLIST DIET ① DATE:	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Weetbix 1							
Milk Light Start 250 mls							
Wholemeal Bread 3 slices							
Salad veg ½ cup							
Fruit 1 (100g)							
Fruit 2 (100g)							
Fruit 3 (100g)							
Meat etc 100g raw							
Rice 1 cup cooked							
Veg 1 (½ cup)							
Veg 2 (½ cup)							
Veg 3 (½ cup)							
Veg 4 (½ cup)							
Canola Lite margarine 2 tsp							
Sunola oil 3 tsp							
Shortbread biscuits 4							
<i>OTHER – list</i>							

A 4 Questionnaire assessing the usefulness of the [¹⁴C]-bicarbonate-urea method for measuring total energy expenditure

Name: **Study visit:**

Date:

Questionnaire relating to the practicality and comfort of the labelled bicarbonate-urea method for measuring 24-hour energy expenditure

***1. On a scale from 1 to 10 rate the discomfort experienced when the infusion needle was inserted into the stomach.**

Painless Very Painful
1 2 3 4 5 6 7 8 9 10

***2. On a scale from 1 to 10 rate the discomfort associated with the infusion of the priming [¹⁴C]-urea solution.**

Painless Very Painful
1 2 3 4 5 6 7 8 9 10

***3. On a scale from 1 to 10 rate the discomfort associated with the 48-hour infusion of [¹⁴C]-bicarbonate.**

Painless Very Painful
1 2 3 4 5 6 7 8 9 10

4. During the 48 hour infusion of [¹⁴C]-bicarbonate, were any days more uncomfortable than another? Yes / No

Describe if yes:

***5. Did you feel ill at any time during the infusion of either the [¹⁴C]-bicarbonate or [¹⁴C]-urea solutions? Yes / No**

Describe if yes :

6. On a scale from 1 to 10 rate the comfort associated with wearing the syringe infusion pump.

Uncomfortable Comfortable
1 2 3 4 5 6 7 8 9 10

***7. On a scale from 1 to 10 rate the practicality of wearing the syringe infusion pump in your free-living environment.**

Limits activity Does not limit activity

1 2 3 4 5 6 7 8 9 10

***8. Did wearing the syringe infusion pump interfere with your normal lifestyle?**

Yes / No

Describe if yes:

.....

9. On a scale from 1 to 10 rate the difficulty associated with collecting urine over 24 hours

Not difficult Very difficult

1 2 3 4 5 6 7 8 9 10

10. Please explain the difficulties you experienced with the urine collection.

.....

.....

***11. On a scale from 1 to 10 rate the difficulty associated with maintaining a food diary over the three days.**

Not difficult Very difficult

1 2 3 4 5 6 7 8 9 10

***12. On a scale from 1 to 10 rate the difficulty associated with maintaining an activity diary over the three days.**

Not difficult Very difficult

1 2 3 4 5 6 7 8 9 10

13. Please explain the difficulties you experienced in keeping the diaries.

Food diary:

.....

.....

Activity diary:

.....

.....

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

Food	Measure	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)
SP TEST MEAL					
Wholemeal bread	4 slices	1129	12	3	47
Cream cheese	30 g	422	3	10	1
Strawberry jam	20 g	217	0	0	13
Raw tomato	100 g	54	1	0	2
Dried apricots	20 g	163	1	0	9
Orange juice	500 g	757	3	0	44
Total		2743	20	14	116
% Energy			12	19	69
HP TEST MEAL					
Wholemeal bread	3 slices	848	9	3	35
Lean leg ham	50 g	230	9	2	0
Kraft Free cheese□□™	60 g	414	13	2	7
Feel Good flavoured milk™	500 g	903	24	2	24
Total		2395	56	8	66
% Energy			41	13	46

* Kraft Free cheese™ (Kraft Foods Ltd, Melbourne, Vic, Australia); Feel Good flavoured milk™□ (Farmers Union, National Foods Ltd, Mile End, S.A., Australia).

A 6 An itemized composition of the standard and high protein test meals used in the assessment of the meal tolerance and thermic effect of feeding tests in chapter 9.

Food	Measure	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)
SP TEST MEAL					
White bread	4 slices	1165	10	3	53
Cream cheese	20 g	282	2	7	1
Strawberry jam	20 g	218	0	0	18
Dried Apricots	40 g	328	2	0	18
Orange juice	500 g	755	3	0	44
Total		2747	16	10	129
% Energy			10	13	77
HP TEST MEAL					
White bread	2 slices	582	5	1	26
Lean leg ham	50 g	226	9	2	0
Kraft Free™ cheese	60 g	410	13	2	7
Pura Classic Lite™ flavoured milk	525 g	1496	23	5	58
Total		2715	51	10	92
% Energy			32	14	54

*Kraft Free™ cheese (Kraft Foods Ltd; Melbourne, Australia); Pura Classic Lite™ flavoured milk (National Foods Ltd; Salisbury, South Australia)