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Dietary manipulation and weight management

Sze Yen Tan
University of Wollongong

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DIETARY MANIPULATION AND WEIGHT MANAGEMENT

A thesis submitted in fulfillment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

from

UNIVERSITY OF WOLLONGONG

by

SZE YEN TAN
MSc., Grad. Cert. Bus., B.D.(Hons), A.P.D.

SCHOOL OF HEALTH SCIENCES

2010
I, Sze Yen Tan, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualification at any other academic institution.

Sze Yen Tan

28 June 2010
For my Pa and Ma
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>%E</td>
<td>Percentage of energy intake</td>
</tr>
<tr>
<td>ΔEE</td>
<td>Changes in energy expenditure (kcal/d)</td>
</tr>
<tr>
<td>ΔW</td>
<td>Weight changes (kg)</td>
</tr>
<tr>
<td>BIA</td>
<td>Multiple frequency bioimpedance</td>
</tr>
<tr>
<td>CHO</td>
<td>Dietary carbohydrate</td>
</tr>
<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>CWC</td>
<td>The Californian Walnut Commission (USA)</td>
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<tr>
<td>AEE</td>
<td>Activity-related energy expenditure</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BMR</td>
<td>Basal metabolic rate</td>
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<tr>
<td>DEXA</td>
<td>Dual-energy x-ray absorptiometry</td>
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<tr>
<td>EI</td>
<td>Energy intake</td>
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<tr>
<td>EE</td>
<td>Energy expenditure</td>
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<tr>
<td>FFM</td>
<td>Fat free mass</td>
</tr>
<tr>
<td>FM</td>
<td>Fat mass</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>J</td>
<td>Joule</td>
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<tr>
<td>kcal</td>
<td>Kilocalories</td>
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<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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MRI  Magnetic resonance imaging
MRS  Mass spectroscopy
MUFA  Monounsaturated fatty acids
NCEFF  The National Centre of Excellence in Functional Foods
NEAT  Non-exercise activity thermogenesis
NHMRC  The National Health and Medical Research Council
P:S  Ratio of polyunsaturated to saturated fatty acids
PUFA  Polyunsaturated fatty acids
REE  Resting energy expenditure
RQ  Respiratory quotient
SAT  Subcutaneous adipose tissue
S.D.  Standard deviation
SFA  Saturated fatty acids
SPA  Spontaneous physical activity
SPSS  Statistical package for social sciences
TEE  Total energy expenditure
TEF  Thermic effect of food
TG  Triglyceride
VAT  Visceral adipose tissue
WHO  World Health Organization
WHR  Waist to hip ratio
WRC  Whole room calorimeter
PUBLICATIONS RELATED TO THIS THESIS

Journal articles:


3. Tan SY, Batterham M, Tapsell L: Comparison of methods used to predict energy requirements in a whole room calorimeter. *Obesity Research and Clinical Practice* 2010; doi: 10.1016/j.orcp.2010.02.005.

4. Tan SY, Batterham M, Tapsell L: Activity counts from accelerometers do not add value to energy expenditure predictions in sedentary overweight individuals during weight loss interventions. *Journal of Physical Activity and Health* [Accepted for publication April 2010].

Prepared manuscripts:

1. Tan SY, Batterham M, Tapsell L: High protein diets with differing protein sources produce similar weight loss profiles in overweight adults.
2. Tan SY, Batterham M, Tapsell L: A dynamic view of energy balance may help weight loss in practice.

Conference abstracts:


CONTRIBUTION TOWARDS STUDIES FROM WHICH DATA WERE EXTACTED FOR THIS THESIS

This thesis utilised data from a number of acute feeding and longer term dietary interventions. The relevant projects and funding sources are summarised in Appendix A. The candidate was involved in data collection by working with a team of clinical researchers in the Smart Foods Centre, University of Wollongong. The main responsibilities of the candidate included: a) the design and execution of experiment protocols involving the whole room calorimeter facility, b) the maintenance of the calorimeter laboratory, and c) the collection and analysis of energy expenditure and substrate oxidation data.

Below are the parent papers of these funded projects:


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ABSTRACT

Obesity results from a cumulative positive energy balance over a period of time with resultant increases in adipose tissue. Dietary intervention is crucial in inducing negative energy balance, which is the cornerstone to weight loss. Finding an optimal ratio of macronutrients in a weight loss diet is important as it can potentially enhance weight and fat mass loss. Dietary protein has been shown to be more thermogenic than other nutrients while polyunsaturated fatty acids (PUFA) are capable of elevating human fat oxidation rates. These properties are important in weight management and have received much attention in the scientific literature in recent years. This thesis argues that the dietary protein and PUFA fraction of the diet are especially significant and that manipulation of these fractions will result in metabolic advantages. The overall aim of this thesis was to investigate the position of dietary protein and PUFA in a weight loss diet, using measures of energy and substrate metabolism in studies involving the whole room calorimeter (WRC) facility. From a methodological perspective, the validity of triaxial accelerometers for estimating energy expenditure and in predicting energy requirement in the WRC was investigated. Following this, the usefulness of increased protein proportions in a weight loss diet was examined through two studies. The first study investigated the acute effects of high protein diets from three protein sources (meat, dairy and soy) on human energy and substrate metabolism, while the second study investigated longer term effects of high protein diets on the related clinical outcomes of weight and fat mass loss. The acute study demonstrated that meat protein was more retained in the human body which may result in less fat-free
mass loss during weight loss in the longer term. However, this was not observed where all study groups showed similar weight loss and body composition changes in a 12-week weight loss trial. Using a similar experimental approach, the usefulness of increased dietary PUFA in weight management was tested through acute and longer term dietary interventions. Fat oxidation was significantly increased following high PUFA meals and produced significant negative fat balance, but in the longer term this was not translated into greater fat mass loss. Fat mass loss was also not greater in the abdominal region although there is evidence linking higher PUFA intake with lower visceral adipose tissues. In both dietary protein and PUFA manipulation studies, the acute effects on energy expenditure and substrate oxidation did not appear to be sustained over a longer period of time. Therefore, in the final chapter of this thesis, reasons for this failure to extend acute effects to longer term clinical outcomes were explored. A dynamic energy balance framework was proposed to help manage weight more effectively in a clinical setting. Effective weight management should include more specific dietary strategies involving the manipulation of dietary components, instead of simple generic advice to eat less and exercise more.
CHAPTER 1. INTRODUCTION

The global prevalence of overweight and obesity is escalating at an alarming rate. According to the Australian Bureau of Statistics, 68% of males and 55% of females in Australia are overweight and obese [1]. This condition of over-nutrition is a known contributor to many other preventable metabolic conditions such as cardiovascular diseases, hypertension, and type 2 diabetes [2, 3]. Together, these metabolic conditions increase the mortality rates and impose a significant cost to the healthcare system.

Fundamentally, overweight and obesity are a result of disturbances in energy balance in the human body. When energy input is higher than energy output, excessive energy is stored in the human body as fat in the adipose tissues. Therefore, the main strategy to losing weight is to induce a state of energy deficit to promote the use of excessive energy stored in the body. In practice, dietary interventions are often used in the treatment of overweight and obesity [4]. Restricting energy intake and increasing physical activity have been proven to play important roles in achieving negative energy balance among overweight and obese individuals [5].

However, little weight loss success is achieved in a free-living environment over a long term [6]. In most cases, actual weight loss was much lower than the expected values based on the prescribed energy deficit, and over a longer term, there is a tendency for these individuals to regain weight [7]. This implies that reducing energy intake and increasing exercise alone are insufficient and more
research is needed to drive practice. In recent years, evidence indicates that dietary strategies for weight management need to be more specific and the manipulation of certain dietary macronutrients may further enhance a traditional hypoenergetic weight loss diet.

Chapter 2 summarises the reasons from the literature why the manipulation of both dietary protein and polyunsaturated fat (PUFA) becomes increasingly important in weight management. As a single nutrient, protein is the most thermogenic and this effect is shown to extend to high protein mixed diets. Evidence also suggests that the dietary source of protein within a high protein diet is an important determinant of its thermic effect. Dietary PUFA, when given as fish oil supplements, increases fat oxidation rates [8]. Although the evidence appears to be convincing, there are limitations in these studies that need to be addressed. Most of the acute studies in the literature applied extreme macronutrient levels in hypothesis testing, which do not reflect consumption in reality. The acute effects of dietary protein sources on energy expenditure have not been tested in a longer term to determine if they can be translated into greater weight loss. In the case of PUFA, it needs to be tested whether higher fat oxidation is associated with negative fat balance. There is a preferential loss of fat mass in the abdominal region following the consumption of high PUFA diets and the validity of this claim needs to be established. This thesis addresses the issues above and hypothesises that the manipulation of dietary protein and PUFA using commonly consumed foods is beneficial for weight management.
A whole room calorimeter (WRC) was selected to measure energy expenditure and substrate utilisation. A detailed description of the facility located at the University of Wollongong (Australia) is presented in Chapter 3. In the same chapter, two studies on this WRC method were reported. The first study compared three ways of predicting energy requirement in the WRC. The best method of prediction was used in the WRC studies reported in the later chapters of this thesis. The second study investigated the effects of leptin and insulin on energy expenditure, and determined if these variables need to be statistically adjusted for in the following studies involving the measurements of energy and substrate metabolism.

The role of dietary protein in weight management was examined in Chapter 4. This chapter consisted of one acute feeding study and a longer term dietary intervention. The acute feeding study was conducted in the WRC over an 8-hour period and it aimed to compare the thermic effect and substrate oxidation rates following consumption of three high protein meals from meat, dairy and soy sources. Outcomes from the acute study were then examined to see if they were sustained over a longer term period of time. The benefits of increasing PUFA in a diet, by incorporating PUFA-rich foods, were also tested (Chapter 5) using data obtained from an acute feeding study and two longer term dietary interventions. The acute study aimed to compare energy expenditure, substrate utilisation and substrate balance induced by a control and high PUFA diets. The acute effects of high PUFA diets were then examined over a longer period of time.
The studies in Chapter 4 and Chapter 5 included data obtained from the following acute feeding and longer term dietary interventions:

1. **Acute effects of dietary protein manipulation**: NCEFF study, funded by the National Centre of Excellence in Functional Foods (Australia).
2. **Long term effects dietary protein manipulation**: HIPHOP study, funded by the National Centre of Excellence in Functional Foods (Australia).
3. **Acute effects of dietary PUFA manipulation**: FAME study, funded by the Californian Walnut Commission (USA).
4. **Long term effects dietary PUFA manipulation**: a) HELP study, funded by the National Health and Medical Research Council (Australia), Project Grant #354111, and b) HERO study, funded by the Californian Walnut Commission (USA).

In Chapter 6, conclusions from the studies of dietary protein and PUFA and considerations from the literature were drawn and discussed using a dynamic energy balance framework. The reasons behind the failure to achieve expected weight loss in three longer term dietary interventions were explored using the framework proposed in this chapter. In practice, a dynamic view of energy balance is important as it provides a more holistic approach for weight management, by highlighting how the human body could potentially counteract the intervention strategies. This framework also highlights that specific strategies should be incorporated into a weight loss regime, and a simple ‘eat less, exercise more’ may be ineffective at an individual level.
In the final chapter (Chapter 7), conclusions are drawn based on the observations from the studies that were designed to test the hypothesis. Limitations of the studies reported in this thesis and future directions are discussed.
CHAPTER 2. DIETARY MANIPULATIONS: WHAT IS KNOWN, WHAT NEEDS TO BE DONE?

The purpose of this chapter is to discuss emerging evidence in the scientific literature for potential strategies that can be incorporated into the dietary management of overweight and obesity. It begins with a section outlining the methods used to diagnose overweight and obesity, its prevalence and burden on individuals and society. Weight problems are caused by a disturbance in human body energy balance, where energy input exceeds energy output over a prolonged period of time. Hence, an understanding of the energetics of the human body is crucial in seeking effective weight management strategies. Energy balance of the human body is counter-balanced by energy intake and energy expenditure and each is discussed in greater detail in this chapter. As this thesis involves the measurement of diet-induced energy expenditure and substrate oxidation, various methods of measurement were reviewed and the most appropriate method to support data collection was selected. Following this, current weight management strategies are summarised using an energy balance model. Dietary intervention has been shown to be the cornerstone to successful weight management as it can alter human energy balance through energy intake and energy expenditure. Emerging evidence reveals that the manipulation of dietary macronutrients especially dietary protein and fat sub-types may enhance the effects of a weight loss diet through higher energy expenditure and fat oxidation. However, studies investigating the effects of dietary protein and fat sub-types have their limitations and they are also
highlighted in this chapter. Based on the gaps in the literature, hypotheses and aims for this thesis are determined.

2.1 Overweight and obesity: a global health concern

The prevalence of overweight and obesity has increased by 16% in males and 18% in females since the National Health Survey conducted in year 1995 [1]. These conditions are assessed through anthropometric measurements. The body mass index (BMI), defined as the ratio of body weight (in kilogram) to the squared height (in meters), is the most commonly used method for determining overweight and obesity in a clinical setting. The World Health Organization (WHO) defines a body mass index (BMI) of over 25 kgm$^{-2}$ as overweight, while a BMI greater than 30 kgm$^{-2}$ indicates obesity [9]. Waist circumference is sometimes used in conjunction with BMI to define abdominal adiposity [10]. A waist circumference of >102m in males and >88cm in females has been associated with an increased risk of cardiometabolic diseases [11]. These cutoff points were derived from a regression model that associated the waist circumference with a BMI greater than 30 kgm$^{-2}$ [12]. With advancements in biomedical technology, more accurate methods have been developed to determine body composition. These methods include dual energy x-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), multiple frequency bioimpedance (BIA), spectroscopy (MRS), and computed tomography (CT) scans [13]. These methods are relatively expensive due to the cost of the equipment involved and may not be available to healthcare professionals in the diagnosis of overweight and obesity. The BMI and waist circumference
therefore remain the most commonly and relatively reliable methods to be used clinically.

Overweight and obesity have been associated with the pathogenesis of multiple diseases including cardiovascular and diabetes, gallbladder, pulmonary, and bone and joint diseases [14, 15]. The direct healthcare costs of obesity has been estimated to be approximately $830 million every year [16]. It has been shown that metabolic fitness of overweight and obese individuals can be improved by losing the excessive body weight [17-19]. This implies that by treating overweight and obesity the occurrence of other metabolic diseases may be delayed or prevented. For this reason, finding effective strategies for weight management is of highest priority.

However, finding effective weight loss strategies requires in-depth understanding of its causes. A recent review outlined all the factors that contribute towards overweight and obesity. In this review the authors proposed an epidemiologic model of obesity, where the susceptible individuals interact with various agents (for example drugs, food, toxins, ease of inactivity and viruses) which then lead to the development of obesity [20]. These factors can be funneled down as they eventually have an impact on the balance of energy in the human body. In other words, overweight and obesity occur as a result of disturbance to the energy balance over a prolonged period of time, where energy input exceeds energy output (also known as positive energy balance). The treatment of obesity therefore involves the reversal of positive energy balance. Before the various methods used to combat weight problems are
discussed, it is important to first understand how the human body obtains and expends energy.

2.2 Energetics of the human body

The human body requires energy to maintain basic physiological functions and to carry out daily activities. The regulation of energy input and energy output within the human body is controlled through a homeostatic process. When energy input exceeds energy output, the body serves as a reservoir to conserve the excessive energy so that life-sustaining physiological processes can continue when the absence of energy intake (fasting or starvation) over a short period of time occurs. The storage of excessive energy in the human body usually takes the form of fat stored in the human adipose tissues. However, not all excessive energy is stored. The human body is capable of increasing the metabolic rate and forced oxidation in the event of excessive consumption [21]. However, this response to over-consumption of energy is usually less efficient, and the storage of excessive energy as adipose tissues is hence inevitable. This process, over a period of time, causes overweight and obesity.

The balance of energy in the human body depends on the supply of energy (energy intake) and the use of energy (energy expenditure). This concept is portrayed graphically in Figure 2-1. Because human beings do not obtain energy from sources other than those in the chemical forms, foods are the only sources of energy. On the other hand, energy is expended through various ways which can be grouped into three categories. Energy intake and energy expenditure will be described in greater details as they indicate these
components can be targeted to enhance weight loss. The understanding of the various components of energy expenditure also provides rationales in the selection of appropriate measurement methods.

**Figure 2-1. Energy balance model**

2.2.1 Energy intake: Foods as energy sources

Foods are composed of macronutrients namely carbohydrate (CHO), protein and fat [22, 23] that provide the human body with a certain amounts of energy. A unit of energy is known as a calorie, which is equivalent to 4.184 absolute joule (J), and a food calorie refers to a kilocalorie (kcal). Dietary fat has the highest metabolisable energy-density of 8.9 kcal/g while each gram of protein and carbohydrate contain 4 kcal [24]. When ingested, foods are broken down by the alimentary system into macronutrients, then further into single unit nutrients such as glucose, amino acids and fatty acids. These single unit nutrients, also known as substrates, are then oxidised to generate energy or stored in the body when overfeeding occurs. Alcohol is another energy-
containing nutrient and, unlike the other three macronutrients, it undergoes first-
pass metabolism [25] where it is metabolised in the human stomach and liver
[26].

To release its energy, each substrate has to be oxidised. Substrate oxidation
describes a process in which single unit nutrients such as glucose, amino acids
and fatty acids are broken down into smaller molecules or atoms where energy
is generated through the release of adenosine triphosphate (ATP) [27]. As the
word “oxidation” suggests, this process occurs with the presence of oxygen.
When a biomolecule containing carbon, hydrogen, oxygen and nitrogen atoms
is oxidised, end-products such as carbon dioxide, water and nitrogen waste are
produced and excreted from the human body through lungs, skin, urine and
feces.

Each substrate is unique in the amount of oxygen required and carbon dioxide
produced during the oxidation process. To enable the differentiation of the
various substrates, the ratio of carbon dioxide to oxygen can be used and this
ratio is known as the respiratory quotient (RQ). Using the RQ values, it is
possible to predict what substrates are used as energy sources in the human
body. The RQ of carbohydrate as glucose (C₆H₁₂O₆) is 1.00, typical fat
(C₅₅H₁₀₄O₆) is 0.71, and alcohol as ethanol (C₂H₅OH) is 0.67 [28]. These values
differ slightly if carbohydrate other than hexose and different fatty acids are
considered [29]. Oxidation of protein requires empirical measurements and it
can be determined through the quantification of nitrogen excreted in the urine
[28, 29].
The energy converted from the breakdown of foods and oxidation of substrates is distributed for use in the human body. The next section describes human energy expenditure which is the second component of human energy balance.

2.2.2 Human energy expenditure

Based on how energy obtained from foods is utilised, human energy expenditure can be sub-divided into three major categories: the basal metabolic rate (BMR), thermogenesis, and activity-related energy expenditure (AEE) (Figure 2-2). Each component contributes towards different proportion of the human total daily energy expenditure.

2.2.2.1 Basal metabolic rate (BMR)

Basal metabolic rate (BMR) is the biggest component of the human energy expenditure. It applies particularly to humans and it is now rarely used other than in human studies [30]. BMR refers to the minimal amount of energy required to sustain life through the maintenance of the integrated systems of the body, and homeothermic temperature at rest [31]. BMR also includes the energetics of the chemical reactions plus those due to interactions of thyroid hormones and sympathetic nervous system [31]. BMR, or sometimes described as resting energy expenditure, usually represents approximately 70% of the total daily energy expenditure (TEE) [14]. The measurement of BMR has been described in details by Benedict in 1938 and the conditions under which BMR measurement should be done were extremely rigorous [30]. These strict criteria
were not easy to follow and hence sometimes not adhered to. In this case a set of standards or protocol, close to the conditions described by Benedict, was introduced and followed. The term BMR was then replaced by “resting energy expenditure (REE)” [22]. Technically BMR and REE refer to two different measurements however they were usually used interchangeably in the literature. Human BMR is influenced by both exogenous and endogenous factors but it is not affected by the composition of nutrient ingested.

Figure 2-2. Human energy expenditure and its three major components.
2.2.2.2 Thermogenesis

Thermogenesis represents the smallest component of energy expenditure and it can be sub-divided into two smaller components: facultative thermogenesis and the thermic effect of food (TEF). TEF is the component of energy expenditure that has a direct relationship with dietary intake. TEF describes the increase in energy expenditure above BMR following consumption of a meal. The increment in energy expenditure is due to the digestion, absorption and disposal of ingested nutrient processes [32]. On average, TEF contributes towards about 10% to 15% of the total daily energy expenditure.

The measurement of TEF is performed after an overnight fast to eliminate residual postprandial thermogenesis of previous meal. A review indicates that postprandial thermogenesis takes at least 5 hours [33] and is regarded to be completely terminated at approximately 10 hours after a meal [34]. There are different ways of expressing TEF in the literature: (i) as an absolute amount of energy in joule [35], (ii) as a percentage of the total energy intake [36], (iii) as a percentage increment from BMR [37], and (iv) as an increased volume of oxygen consumption [38].

Thermogenesis also includes a component known as the facultative thermogenesis which refers to the human body’s responses to environmental changes where the expended energy does not yield any physiological or mechanical work within the human biological system [31]. It was also perceived to be a mechanism that regulates energy imbalance in the human body. Thus, facultative thermogenesis is also known as “adaptive thermogenesis”. This
concept originated from overfeeding trials which observed lower-than-expected weight gain. Adaptive thermogenesis, which take place in the brown adipose tissues and skeletal muscle [39], was hypothesised to be the underlying mechanism that regulated and force-metabolised energy (homeostatically energy wastage) during overfeeding [40]. Since it is also related to dietary consumption, adaptive thermogenesis may be regarded as part of TEF. However, the evidence of facultative thermogenesis is still inconsistent [41, 42] and if present it is likely to be too small to be measured.

Thermogenesis has been suggested to be an essential determinant of obesity [43-46]. TEF, in conjunction with facultative thermogenesis, is now of major interest as it seems to be a definitive indicator of metabolic efficiency [44]. Thermogenesis is the energy expenditure component of interest in this thesis.

2.2.2.3 Activity-related energy expenditure (AEE)

AEE is the second biggest and the most varied component of energy expenditure between individuals [47]. AEE is therefore considered another component of energy expenditure that may influence the changes of body weight. Unlike BMR and TEF, the components of AEE are relatively hard to determine due to: a) the measurement errors which were usually carried over from 24-hour EE, BMR and TEF [48]; b) the interactions between AEE and TEF [49, 50]; and c) the difficulties in determining every single component of physical activity. In order to distinguish activities that are of a different nature, AEE is sub-divided into exercise and non-exercise related thermogenesis.
Exercise-induced thermogenesis refers to the energy expenditure associated with leisure physical activities. Leisure physical activities include intentional activities that are performed other than daily routine including occupation-related activities. There is ample data showing that the vast majority of people from developed nations have very little or no exercise-induced thermogenesis [51], and the decrease in exercise related energy expenditure has happened during the past two decades [52-54]. This could partly be due to the attractiveness of sedentary activities such as television watching, video games, and computer-use that compete with leisure time physical activities [51].

Non-exercise thermogenesis, also referred to as non-exercise activity thermogenesis (NEAT) or spontaneous physical activity (SPA), which includes all activities we undertake as vibrant, individual beings [51]. NEAT includes thermogenesis expended in activities related to occupation, sitting, standing, walking, talking, dancing, and shopping. Because NEAT covers a broad-spectrum of activities, its roles in human energy metabolism are difficult to define. AEE contributes to approximately 20% of TEE, including activities that vary considerably from day to day. In developed nations where exercise energy expenditure is limited, NEAT is an important determinant of TEE in humans [55]. The contribution of low NEAT levels in the pathogenesis of overweight and obesity was largely drawn from population and ecological data. This data tends to show a negative relationship between self-reported physical activity levels and BMI [56], regardless of gender and age.
2.2.3 Measurement of human energy expenditure

The measurement of energy expenditure can be performed in many ways, from the direct measurement of temperature differences from heat production to the indirect measurements through gas exchanges. This section provides brief descriptions of the various methods, highlighting the strengths and limitations of each method.

2.2.3.1 Direct measurement of energy expenditure (direct calorimetry)

According to the first and second law of thermodynamics, energy transforms into heat when it is utilised by the human body. Heat is lost to the environment through non-evaporative heat loss (radiation, convection and conduction) and through evaporation of water. The direct measurement of energy expenditure therefore involves the quantification of whole body heat loss in a confined and insulated chamber [57, 58] or through a heat-exchanging body suit [59]. A detailed description of these apparatus can be found in a review by Murgatroyd and colleagues [60]. A direct calorimeter measures heat loss as temperature differences (using a wattmeter) which are induced by human metabolic processes. This method can be regarded as the most accurate and reliable way to measure human energy metabolism. Besides its accuracy, direct calorimetry also exhibits properties such as high precision and is fast-responding. However, direct calorimetry involves complex mechanical engineering in order to control the study environment. To ensure that the measured temperature differences are not induced by factors other than human body metabolism, heat transfer from food, drink, excreta, lighting and electrical appliances has to be accounted for. For this reason, direct calorimetry involves a very high equipment, operation
and labour cost. Furthermore, due to the tight controls necessary for this method, the study environment is highly mechanistic and artificial. Another limitation of this method is the inability to provide information on substrate oxidation, which is becoming more important in understanding the metabolism of overweight and obese individuals.

2.2.3.2 Indirect measurement of energy expenditure (indirect calorimetry)

Like direct calorimetry, indirect calorimetry also involves the measurement of heat. However, this method measures the rate of heat production in the human body instead of as temperature differences. The rate of heat production can be calculated through gaseous exchanges (between oxygen consumption and carbon dioxide production), together with the oxidation of energy-yielding substrates such as alcohol, carbohydrate, protein, and fat [61].

Based on these principles, methods such as whole room calorimetry and portable devices were developed. Portable devices measure gaseous exchanges through the differences between inlet and outlet gas compositions. The gas analysers are connected to either a hood that is placed over the head and shoulders to create a closed environment (Deltatrac™) or to a mouth piece (MedGem™ or BodyGem™) or a face mask (Metamax™) in combination with a nose clip to prevent air escaping through this route. The use of a hood, mouth piece or face mask can be uncomfortable and is therefore unsuitable for studies that require long-period measurements. Unlike the metabolic cart which measures both oxygen and carbon dioxide, handheld devices measure only oxygen consumption and calculate carbon dioxide production based on an
assumed RQ of 0.85. Therefore, handheld devices are not appropriate in studies requiring information on RQ or substrate oxidation (where exact oxygen consumption and carbon dioxide measurements are required). When metabolic cart and handheld devices were compared, only very small differences were observed [62] but the reproducibility of the handheld device was observed to be poor [63].

A whole room calorimeter requires study participants to be placed in an air-tight chamber which is constantly ventilated with fresh air. Mixed air is then extracted from the chamber to measure the participants’ respiratory gas by comparing oxygen and carbon dioxide concentration between the inlet and outlet gases. During this process pressure, temperature and humidity in the chamber are kept constant to eliminate their influences on the gas readings. Gas sampling does not involve a mouth piece or face mask hence is more comfortable for the participants. Whole room calorimetry also allows more control over the study environment such as temperature and amount of foods provided to the participants and is hence suitable for studies requiring longer periods of measurements of energy expenditure.

The results obtained from a whole room calorimeter have been shown to be comparable to those from direct calorimeter [60]. The only difference between these two methods is the slightly longer measurement time lag in the whole room calorimeter method. A change in energy expenditure can be detected faster as a difference in temperature, while it takes longer to be reflected as changes in gaseous exchanges. The measurement noise of a whole room calorimeter
calorimeter method was demonstrated to play only a small part in the total variability in the measurement of TEE which was analysed over one hour or less [64]. Measurement noise was mainly derived from the changes in gas concentrations and random noise of the analyser output signals.

Overall, variability within individuals and day-to-day 24 hour energy expenditure in a whole room indirect calorimetry is generally small (Table 2-1). It could therefore be regarded as a very reliable instrument to be used in the study of human energy metabolism. For comparison purposes, coefficients of variations (CV) obtained from a direct calorimeter (n=4) were reported to be 1.93% in 24-hour energy expenditure and 4.67% in BMR [64] whereas indirect calorimeter data from the University of Wollongong demonstrated a CV of 2.5% [65] and 5.37% (n=21) respectively. This implies that indirect calorimeter performs as well as a direct calorimeter in measuring energy expenditure.

Table 2-1. Reproducibility of human energy expenditure measurements (TEE) using a whole room calorimeter

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
<th>Subjects</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ravussin [48]</td>
<td>1986</td>
<td>12</td>
<td>10 males, 2 females</td>
<td>3.7%</td>
</tr>
<tr>
<td>Astrup [66]</td>
<td>1990</td>
<td>10</td>
<td>6 males, 4 females</td>
<td>5.0%</td>
</tr>
<tr>
<td>Rumpler [67]</td>
<td>1990</td>
<td>5</td>
<td>All males</td>
<td>2.9%</td>
</tr>
<tr>
<td>Toubro [68]</td>
<td>1995</td>
<td>22</td>
<td>7 males, 15 females</td>
<td>4.1%</td>
</tr>
<tr>
<td>Batterham [65]</td>
<td>2008</td>
<td>4</td>
<td>All females</td>
<td>2.5%</td>
</tr>
</tbody>
</table>
2.2.3.3 Doubly-labelled water

Doubly-labelled water is sometimes referred to as a “free-living indirect calorimetry” because it is a method used to measure energy expenditure in a free-living environment. The measurement of energy expenditure using this method usually requires 4 to 20 days, a period which is more likely to reflect habitual energy expenditure of an individual [69]. In this method, individuals are required to take an oral dose of water containing a known amount of stable isotopes deuterium $^2$H and $^{18}$O which will be excreted from the body through breath, urine and sweat [70]. At the end of the study period, the remaining isotopes in the body fluid are quantified using a mass spectrometry method. This analysis provides information on the total volume of carbon dioxide produced during the study period. Oxygen consumption is then calculated based on an assumed RQ. This method is thought to be the closest measure of free-living energy expenditure and its error is relatively low, estimated to be around 1 – 6% [71, 72]. There are nonetheless a few limitations to this method [69]: a) high costs of isotopes and expertise involved in the mass spectrometry analysis, b) an absence of information on acute period peak energy expenditure limits the study of TEF which occurs within a few hours postprandial, and c) the measurement of energy expenditure requires an assumption of RQ of the meal consumed. When RQ is assumed, it implies that the study of substrate oxidation is not possible. There is also a limitation on the timeframe of studies as it does not provide accurate energy expenditure of a period less than 4 days.
2.2.3.4 Motion sensors, heart rate monitors, activity questionnaires

Motion sensors are mechanical and electronic devices that pick up motion of a particular body part depending on where they are worn [73, 74]. Pedometers and accelerometers are among the several motion sensors available in the market. Among these devices, the triaxial accelerometer is the most precise device which measures acceleration in vertical, anterioposterior, and mediolateral planes as well as vector magnitude over a fixed time interval [69]. Together with age, body mass and gender [75, 76], it is a relatively good predictor of TEE [77]. All motion sensors predict TEE through the measurement of activities but they do not provide any information on gaseous exchanges.

There is a close relationship between heart rates and the amount of energy expended during exercise [78]. A record of heart rate is therefore thought to be useful in predicting energy expenditure. However, the relationship between energy expenditure and heart rate was found not to be linear and individual error of up to 52% has been reported [79] despite a mean group error as low as 10% [80, 81].

Many questionnaires have also been used to predict energy expenditure, only a few were validated against the doubly-labelled water method. Among them are the activity recall or log, Baecke questionnaire, Five-City questionnaire, Tecumseh questionnaire, and Yale Physical Activity Survey (YPAS) [69]. The questionnaires range from brief (one-page) to tedious efforts where participants are required to fill in the activity log every 15 minutes. From the activities reported or recorded in the questionnaires, energy expenditure is calculated by
multiplying the amount of time spent in each activity by the corresponding metabolic equivalent. In summary, all motion sensors, heart rate measurements and activity questionnaires may be more useful in a clinical setting but not in research because they are not objective measurements and do not provide information on gaseous exchanges which is important in the study of substrate oxidation.

2.2.3.5 Summary

Among all methods used to measure energy expenditure, the whole room calorimeter method appears to be the most appropriate for the study of human energy and substrate metabolism in the context of dietary manipulation. A whole room calorimeter is capable of providing more accurate and reliable energy expenditure measurements than the motion sensors, heart rate monitors, and activity questionnaires. At the same time, it provides information on gaseous exchanges which is not available by other methods such as doubly-labelled water and direct calorimeter methods. Finally, it is more comfortable to the subjects than a hood or mouthpiece when measuring postprandial energy expenditure which lasts for at least a few hours. Detailed descriptions of the whole room calorimeter located at the University of Wollongong, Australia will be presented in the next chapter.

With the understanding of how energy is obtained and utilised, the next section reviews various strategies currently used in the treatment of overweight and obesity. Each strategy is discussed using the energy balance model because
this thesis focuses on how dietary manipulations could increase the TEF in order to promote weight loss.

2.3 Dietary interventions: current and emerging strategies

As overweight and obesity originates from the long-term disturbance to the energy balance, the principal goal of weight management is to reverse the state of positive energy balance by manipulating energy intake and energy expenditure. A state of negative energy balance is induced when energy expenditure is greater than energy intake, and under this condition the human body utilises energy storage (in the adipose tissues) to meet the energy requirements. When sustained over a longer period of time, this will result in weight loss.

There are many strategies used to combat weight problems and these treatments include dietary interventions, increasing exercise, pharmacotherapy (the use of appetite suppressants such as gut hormones [82] and Reductil™ [83], and nutrient-absorption inhibitors such as Xenical™ [83]), and surgical procedures (jaw-wiring [84], jejunoileal bypass [85], and gastric-banding [86]). Both pharmacotherapy and surgical procedures are indicated only in extreme cases of obesity and pharmacotherapy is often linked to adverse side-effects [2]. While exercise remains important in weight management (by increasing energy expenditure through AEE), evidence suggests that exercise regime has to be implemented in conjunction with dietary interventions. Increasing exercise without dietary intake restriction was shown to be ineffective in weight management [87]. On the contrary, dietary interventions alone have been
observed to be as effective with or without exercise regime being incorporated into the treatment plan [88]. This implies that dietary intervention is the cornerstone to successful weight management. Dietary interventions manipulate the human energy balance mainly by restricting energy intake. To reduce energy intake, strategies such as the use of hypoenergetic diets [89], portion size control [90, 91], inclusion of foods that are low in energy-density [92], high in fibre [93] and nutrients that enhance satiation [94, 95] to reduce subsequent food intake have been proven to be effective.

While reducing overall energy intake remains crucial, the composition of a diet may very well influence the outcomes of weight management. This is because each macronutrient has different molecular structures and it is this difference that has an impact on how much energy is required in the oxidation process (TEF) and how each nutrient is selected by the human body as fuel. This is another aspect of the dietary interventions that has become increasingly important in the management of overweight and obesity in recent years.

2.4 Thermic effect and oxidation of single macronutrients

Of all nutrients, dietary protein is the most thermogenic [36]. When expressed as a percentage increment in BMR, the thermic effect of individual nutrients was reported to be the highest and most prolonged for protein (20 – 30%), followed by carbohydrate (5 – 10%) and fat (0 – 3%) [96]. The thermic effect of alcohol remains unclear. Attributing the highest thermic effect to protein is supported [97, 98] but the thermic responses to carbohydrate and fat were reported to be similar (6% vs. 7% respectively) by another study [99]. The higher thermic effect
of dietary protein has been suggested to be due to higher metabolic costs of amino acid metabolism such as deamination and urea formation [100].

Not only is the thermic effect different between macronutrients, it also appears to be a hierarchy on how macronutrients are selected as fuel by the human body. Like the TEF, the sequence and rate of substrate oxidation is determined by their chemical structures and their physiological functions in the human body. Substrates that are not retainable in the human body will be force-oxidised first, followed by substrates that could be stored at a limited amount and under certain circumstances, and lastly those substrates which can be stored easily will be oxidised last. The hierarchy of postprandial substrate oxidation was suggested to follow the sequence alcohol > protein > carbohydrate > fat [101]. However, another study observed that the oxidation of carbohydrate occurred faster than protein [99].

More interestingly, the hierarchy of oxidation exists not only between but also within each macronutrient. There is evidence suggesting that different fatty acid subtypes are oxidised in different orders and at different rates [102]. The first factor that influences the oxidation of individual fatty acids is the length of their carbon chains. This is supported by the animal models where the oxidation rates of saturated fatty acids in rats decreased with increasing length of carbon chain in the order of laureate (12:0) > myristate (14:0) > palmitate (16:0) > stearate (18:0) [103]. Another study using fat liver preparation also reported that the oxidation of butyrate (4:0) was higher than stearate (18:0) [104]. This trend
extends to human studies, where a higher oxidation of octanoate (8:0) than palmitate (16:0) and has been reported [105, 106].

The length of carbon chain is not the only factor that influences the rate of fatty acid oxidation, it is also determined by the saturation of the individual fatty acids. The oxidation of unsaturated fatty acids was higher in a rat study [107]. In mice, using fatty acids with the same carbon chain length, the highest oxidation of oleate (18:1n-9) was observed, followed by linoleate (18:2n-6) and stearate (18:0) [108]. These results were fully reproduced in a human study [109]. However, polyunsaturated fatty acids (PUFA) (linolenate 18:3n-3 and linoleate 18:2n-6) were observed to produce greater oxidation rate than monounsaturated fatty acids (MUFA) (oleate 18:1n-9), and saturated fatty acid (stearate 18:0) was observed to be least oxidised in a more recent study [110].

Another observation from this same study suggested that the stereoisomeric configuration of the double bonds may be another important determinant of oxidation rates of individual fatty acids.

Together, all evidence appears to suggest that the manipulation of dietary protein and fat subtypes may add benefits to a hypoenergetic diet in treating overweight and obesity [111]. Increasing dietary protein (the first substrate to be oxidised) will increase energy expenditure through higher TEF while increasing polyunsaturated fatty acids intake will enhance fat oxidation. Both these effects are favorable to obese individuals who suffer from positive energy balance and excessive fat accumulation.
However, it should be pointed out that the evidence presented thus far was obtained from studies that focused on single nutrients in their experiments. Doubtlessly, studies using a single or specific nutrient are essential in the understanding of underlying mechanisms how it works in the animal or human systems. However, they should not be regarded as the highest level of evidence for weight management in practice. This is because humans consume whole foods instead of single nutrients and it is impossible to prescribe a single nutrient intake since most foods consist of a mixture of nutrients in various proportions. Because foods consisting of various nutrients are consumed, synergistic effects between nutrients is inevitable and should therefore be considered [112]. Food is the basic unit in nutrition research and evidence obtained from single dietary intervention studies should be further assessed in the presence of other nutrients in the context of a diet or cuisine.

### 2.5 Experimental dietary studies using macronutrient manipulations

Studies involving the use of whole foods are far more complex than those of a single nutrient because most foods contained a combination of macronutrients in different proportions. It is hence impossible to manipulate one macronutrient at a time and therefore has to be done at the expense of other macronutrients.

A review paper identified and summarised observations from ten studies which manipulated the proportions of dietary carbohydrate and fat [113]. In these studies, the thermic effects of low fat diets (3% – 20% energy) and high fat (40% – 60% energy) meals were compared. The proportion of protein was held constant (10% – 20% energy) and dietary fat was substituted for carbohydrate.
The 24-hour energy expenditure induced by the two test diets was not significantly different. One study also confirmed that dietary carbohydrate did not affect the TEF [65]. This indicates that the manipulation of either dietary carbohydrate or fat does not enhance the thermic effects of the meals.

### 2.5.1 Dietary protein manipulation

In the case of dietary protein, the manipulation of protein was usually performed at the expense of dietary carbohydrate. To date, there appear to be no studies in the literature that compare high-protein and high-fat diets with a fixed carbohydrate proportion. When only two macronutrients, namely dietary protein and carbohydrate were manipulated, energy expenditure was significantly higher following the consumption of high-protein diets [114-117]. Similar observations were reported when all macronutrients were manipulated at the same time, where high-protein diets have been shown to be the most thermogenic [118-122]. In fact, a meta-analysis concluded that the thermic effect of food increases by approximately 7 kcal per 1000 kcal of energy consumption when the proportion of dietary protein in the diet increased by 10% [123]. The higher thermic effect of protein appears to extend to a context of mixed meal and higher energy expenditure induced by high protein diets suggest that these diets could potentially be used for weight management.

Not only is the amount of dietary protein important, there is emerging evidence to suggest that the dietary sources of protein can influence the TEF too. In an acute feeding study, the total daily energy expenditure induced by two high protein diets (from pork and soy sources) was shown to be 3% higher than
those induced by a high carbohydrate diet. Furthermore, TEF induced by the high pork protein diet was observed to be significantly higher (+248 KJ/day or 2%) than the high protein diet from soy sources [116]. However, to what extent the higher TEF induced by high animal protein diet has an impact on weight loss is yet to be investigated. Also, plant foods appear to be placed in an inferior position in the weight management context, despite the facts that these foods are usually nutrient-dense and good sources of fibre in the diet. A new enquiry in high protein diet research investigating which protein source may be the best in optimising TEF and assist in weight loss is therefore warranted.

While human trials have demonstrated that high protein diets were efficacious in promoting weight loss [124-132], it is important to note that the positive outcomes may not be solely explained by increased TEF or energy expenditure. There is also evidence that a high protein diet may enhance the oxidation of fat. The fat oxidation enhancing property of higher protein meals did not extend to the oxidation of carbohydrate [133]. When the proportion of fat in the high protein diet was increased, this fat oxidation enhancing property diminished. This implies that facultative thermogenesis of fat is less efficient than protein [134]. The enhancement of fat oxidation by high protein diets appeared to be another promising strategy in weight management which requires further investigation.

In addition to its TEF and fat oxidation enhancing properties, a high protein diet has been reported to also produce a high level of satiety, thereby reducing subsequent food intake [135-138]. Higher protein proportions in the diet have
also been reported to maintain or minimise the loss of metabolically active fat-free mass (FFM) during weight loss [139]. It was suggested that branched-chain amino acids such as leucine can stimulate muscle protein synthesis and inhibit protein degradation in skeletal muscle and liver [140, 141].

The use of a high protein diet was also supported by the protein leverage hypothesis [142] where living beings, when consuming an imbalanced diet, tend to first fulfill their protein requirement instead of other macronutrients. Although there are concerns over the adverse effects of long term consumption of high protein diets on health, several long term studies failed to observe harmful side-effects of higher protein intake (such as type 2 diabetes [143], some cancers [144], coronary heart diseases [145]). Cardiovascular risks [146], as measured using various clinical endpoints such as blood pressure [147, 148], lipids [149, 150], and homocysteine levels [151] remained unchanged after long term consumption of high protein diets. They also did not exhibit harmful effects on the kidneys [152, 153] or bone health [154-156].

The literature appears to support the use of high protein diets for weight management but there are a few limitations and gaps that need to be addressed. A majority of the core acute feeding trials using a WRC are dated (more than 10 years) and hence need to be revisited as methodology has changed and improved in the last decade. A number of the high-protein diet studies reported earlier were performed in an acute setting for hypothesis-testing and the test diets were designed to have extreme macronutrient proportions. For example, the proportion of protein was set as high as 87% in
the high protein diets and as low as 1% in the control diet of one study [157]. As a result, the proportions of other macronutrients were also unusually high or low. A typical Australian diet consists of 17% – 20% energy from dietary protein [158, 159] and the outcomes of these studies therefore have limited implications and applications in a free-living environment. If the high protein diets were to be used for weight loss purposes, the proportions of the macronutrients will have to be more realistic and whole foods should be used in the manipulation of the macronutrients. Whether or not the higher TEF and fat oxidation rate induced by high protein diets and its dietary sources can be translated into greater weight and fat mass loss over the longer term are the other two aspects that also warrant further investigation. These are the areas where this thesis focuses on.

### 2.5.2 Dietary fat manipulation

Polyunsaturated fatty acids have been shown to play an important role in regulating energy metabolism [160], substrate utilisation [161, 162], and adipocyte formation [163, 164]. Unlike dietary protein, the manipulation of dietary fat subtypes in a diet was less straightforward. On some occasions this was performed by substituting a macronutrient with one fat-subtype while in most cases it was done through dietary supplements. Diets higher in polyunsaturated to saturated ratio (P:S) have been reported to increase fat oxidation rate [161, 162]. A fish oil load of 6g/d was provided to subjects in one acute feeding trial. In this study preferential oxidation of fat over carbohydrate as the energy source was observed [165], where fat oxidation was shown to increase by 35%. It was proposed by the investigators that fish oil may affect the glucose transport mechanisms. The reduced carbohydrate oxidation rate
could also be explained by an increase in insulin sensitivity [166] and through the glycolytic pathways [165]. As a result, greater carbohydrate balance was achieved. Both higher fat oxidation and greater carbohydrate balance is important in weight management as they are negatively associated with adiposity [167].

When measured over a longer term, a diet higher in monounsaturated fat (when substituted for dietary carbohydrate) was observed to produce similar weight loss when compared to a high protein diet [120]. Fat mass was observed to decrease significantly following supplementation of PUFA rich fish oil [8]. Apart from higher fat oxidation rate [8], greater fat mass loss was found to be partly due to increased BMR and TEF [160, 168]. Further evidence suggests that not only fat mass loss was greater following higher consumption of PUFA but there is also preferential loss of fat mass around the abdominal region [166, 169]. On the contrary, saturated fat intake has been associated with higher body fat deposition [170, 171] and it is also associated with dyslipidemia and other health problems [172]. PUFA is hence the preferred fat subtype in the treatment of obesity and saturated fat has to be limited.

While evidence suggests that unsaturated fat may be beneficial in promoting weight and fat mass loss, there are a number of areas where high PUFA diet research needs to be refined. While the oxidation of dietary fat subtypes has been extensively studied in the animal model, evidence using human subjects remains weak and further investigations are necessary [173]. Furthermore, most studies supplemented the test diets with fish oil instead of identifying and
incorporating PUFA-rich foods into the test diets. In the case of supplementation, the proportion of dietary fat as a percentage of dietary energy intakes will change. It is hence important to increase PUFA intake and reduce intake of other fat subtypes such as saturated fat in order to keep the overall fat intake constant. The evidence on preferential loss of abdominal fat is drawn from observational study and is lacking support from other studies. These areas will be addressed in this thesis.

2.6 Summary

In summary, the review of literature reveals that:

1. Dietary intervention, on itself or in conjunction with other therapies, is the cornerstone to successful weight management.

2. A reduction in energy, fat and alcohol consumption are fundamental in weight management to reduce energy intake.

3. Differences between macronutrients and within each macronutrient group may have different effects on human energy and substrate metabolism.

4. The thermogenic and fat-oxidising effects of dietary protein and PUFA may add benefits to a low-fat hypoenergetic weight loss diet.

5. Past studies examining these nutrients used unrealistically high or low protein levels in the test diets and a majority of them were conducted in a well-controlled laboratory environments.

6. Weight loss studies conducted in free-living environments often have little success suggesting the possibilities of poor dietary compliance or adaptations of the human body to dietary interventions.
Based on the gaps in the literature, the following issues need to be addressed:

1. Whether the nutrient-focused studies can be translated into food-based research that uses more realistic macronutrient composition.

2. Whether the outcomes of food-based research will be similar to those obtained from nutrient-based studies.

3. Whether differences in nutrient subtype can be encompassed by different food items in a weight loss diet to achieve the desirable effects.

4. Whether the manipulation of dietary compositions will produce acute effects that are beneficial to energy and substrate metabolism.

5. Whether these acute effects can be extended into a longer term as metabolic and clinical outcomes.
2.7 Central hypothesis

The central hypothesis of this thesis is that, using specific commonly consumed foods, high protein and high PUFA diets are beneficial for weight management in a free-living environment.

This thesis focuses the following questions addressing this hypothesis:

1. What are the acute effects of high protein or high PUFA meals on energy expenditure and substrate oxidation?
2. Can the acute effects observed in the studies above be translated into weight loss and body composition changes in the long term?
3. Do the sources of dietary protein and subtypes of dietary fat matter in both study designs?

To answer these research questions, data from two acute feeding and four longer term dietary interventions were analysed. As this thesis focus primarily on the metabolic outcomes related to dietary manipulation, a whole room calorimeter was selected and used in the studies reported in this thesis. The calorimeter facility is described in greater detail in the following chapter.
CHAPTER 3. STUDIES OF METHODS USED

This chapter outlines the methodological principles that underpin the studies reported in this thesis. It starts with the description of the whole room calorimeter (WRC) facility used for data collection. The WRC measures oxygen consumption and carbon dioxide production, which are then used in the calculations of energy expenditure and substrate utilisation. These equations are presented following the description of the WRC facility. Human energy expenditure can be influenced by many factors and this implies that the study of the effects of dietary manipulation on energy expenditure will have to be controlled for these confounding factors. Many of them can be controlled for through careful experiment protocol development while others have to be accounted for during data analysis. Therefore, this chapter also consists of two data quality control studies. The first part examines methods that can be used to achieve energy balance during calorimeter studies through better prediction of energy requirement. The second part assesses the impact of insulin and leptin on energy expenditure. The knowledge in these two areas is important in making informed decision relating to WRC experiment protocol and data analysis. This in turn will improve the quality of data obtained from the WRC.
3.1 Whole room calorimeter facility in the University of Wollongong

For all acute and 24-hour energy and substrate metabolism studies reported in this thesis, a whole room calorimeter method was used. This facility is located in the School of Health Sciences, University of Wollongong. It consists of two separate but identical, air-tight, ventilated chambers measuring 2.1 m X 3 m. The total volume of each chamber is approximately 15000 L. Each chamber is furnished like a small hotel room with a chair, desk, fold-away bed, hand basin, toilet, television, video and DVD players, computer with internet access, and a phone. Each chamber has three windows with privacy curtains: one allows a view outside of the building, one to the surroundings of the laboratory, and one into the adjacent chamber. To ensure accurate measurements of gaseous exchanges, airlocks are used to pass items between the chamber and laboratory. Separate airlocks are used to pass foods and urine samples. Physical activity in the chambers is prescribed and monitored by accelerometers. Figure 3-1 illustrates the floor plan and some photos of the facility.
Figure 3-1. Whole room calorimeter facility located at the University of Wollongong, Australia.

Clockwise from the top: floor plan of the dual-chamber whole room calorimeter, interior of the chamber, and laboratory environment with laboratory supervisor’s working station.
3.1.1 Measurement of gas exchanges

The whole room calorimeter is an open-system respirometer which is constantly ventilated with fresh air from the atmosphere. Gas samples are dried using a Peltier dryer (Maastricht Instruments, Netherlands) and a PermaPure membrane dryer (PermaPure, NJ, USA). The ambient temperature in the chamber is maintained at 24°C through an air-conditioner. Oxygen concentration is measured using a paramagnetic oxygen analyser (Sable System Inc., PA-1B, Las Vegas, NV, USA) while carbon dioxide concentration is measured using an infrared analyser (Sable System Inc., CA-2A, Las Vegas, NV, USA). The calorimeter chambers maintain a stable sample flow at 190 ml/min with Sierra Mass Flow Meter controllers (Sierra Instruments Inc., Monterey, CA, USA). The measurements of gaseous exchanges are corrected to water vapour pressure and barometric pressure measurements. Data collection is performed through a programmable logic controller which is connected to a computer where data is stored and analysed. Oxygen consumption and carbon dioxide production are calculated from a differential between fresh air and outlet gas sample from the chamber. Gas analysers are calibrated manually prior to each study session against a span gas (with a fixed 18% oxygen and 0.8% carbon dioxide concentration) and nitrogen (0% oxygen and carbon dioxide) to ensure accurate readings of inlet and outlet gas concentration from the whole room calorimeter. The sampling of gas occurs every 2 minutes at a fixed sequence as shown in Figure 3-2. The rate of oxygen consumption and carbon dioxide production is calculated based on the inlet and outlet flows as measured by mass flow meters at 70 to 80 L/min (Honeywell Automation, AWM720P1, Golden Valley, MN, USA) and the differences in gas concentration.
concentrations according to Schoffelen [174] and Hill [175]. Measurement noise in the data is reduced by smoothing with cubic spline functions using JMP version 5.1 (SAS Institute Inc., NC, USA), where smoothed data has been shown to account for 97.3 ± 2.2% of the variance in outlet oxygen, and 99.96 ± 0.02% in carbon dioxide [65]. Analysis of the raw data provides the mean rate of gaseous exchanges for every 10 minute intervals. Between study sessions, the accuracy of the whole room calorimeter is monitored through methanol burns. The total amount of oxygen required and carbon dioxide produced are calculated from the total amount of methanol (g) burned. These values are then compared against the values measured by the whole room calorimeter to establish the recovery rates of oxygen and carbon dioxide.

**Figure 3-2.** Sequence of gas sampling

![Sequence of gas sampling](image)

*Note:* This figure is adapted from Shoffelen et. al. [174], with the time intervals re-aligned to the frequency of data collection in the WRC facility located at the University of Wollongong, Australia.
3.1.2 The calculations of energy and substrate metabolism

3.1.2.1 Energy expenditure

Energy expenditure is calculated based on the Weir equation [176], where:

\[
\text{Energy expenditure (kcal/day)} = 3.9 \times \text{O}_2 \text{ consumption (L/day)} + 1.1 \times \text{CO}_2 \text{ production (L/day)}
\]

The uniqueness of this equation is that it calculates energy expenditure from respiratory exchanges measurements, which are based on the changes in percentage oxygen content rather than the volumes of oxygen consumed. The advantage of using respiratory exchanges is that the changes in gas percentage can be measured with a higher degree of accuracy [177]. This equation also allows the exclusion of nitrogen excretion in the calculation of energy expenditure, where it yields an error of only 1% although protein metabolism contributes to approximately 12.3% of total energy expenditure. This is an important property of this equation because the measurement of urinary nitrogen excretion can be highly inaccurate, especially when the study period is short.

3.1.2.2 Substrate oxidation

The calculation of substrate oxidation is based on the volume of oxygen required to oxidise a gram of each substrate and the volume of carbon dioxide produced as an end product of oxidation. Protein oxidation can be estimated from the urinary nitrogen excretion, since most urinary nitrogen is in the form of
urea [178]. Total urinary nitrogen can be directly measured using the Kjeldahl method [179]. Alternatively, urine urea nitrogen is measured, and then being converted into total urinary nitrogen. Quality control data from the University of Wollongong WRC (n=19, unpublished) showed that urine urea nitrogen represented 83.59% ± 3.01% of the total urinary nitrogen (as measured by the Kjeldahl method), and this is used as a factor to convert urine urea nitrogen into urinary nitrogen. Based on the total urinary nitrogen, protein oxidation is calculated as:

\[
\text{Protein oxidation (g/day)} = \text{total urinary nitrogen (g/day)} \times 6.25
\]

Using this value, carbohydrate and fat oxidations can be calculated using the Frayn equations [29]:

\[
\text{CHO oxidation (g/day)} = 4.55 \times \text{CO}_2 \text{ production (L/day)} - 3.21 \times \text{O}_2 \text{ consumption (L/day)} - 2.87 \times \text{protein oxidation (g/day)}
\]

\[
\text{Fat oxidation (g/day)} = 1.67 \times \text{O}_2 \text{ consumption (L/day)} - 1.67 \times \text{CO}_2 \text{ production (L/day)} - 1.92 \times \text{protein oxidation (g/day)}
\]

3.1.3 Confounding variables in the study of energy expenditure and substrate utilisation

Energy metabolism is influenced by many factors. To examine the thermogenic effect of a test diet, it is therefore important to control for confounding variables. First of all, energy expenditure can be affected by temperature. It has been
observed that a decrease in ambient temperature resulted in increased BMR and TEF [180]. This highlights the importance of setting a thermoneutral environment in the study of energy metabolism. The definition of thermoneutral temperature appears to differ between age groups [181] but this can be overcome by maintaining a thermoneutral temperature in a crossover study design.

It is also important to recruit healthy participants absent of diseases that may affect energy metabolism and alter substrate oxidation pattern (for example endocrine disorders, diabetes mellitus, cancer, and acquired immune deficient syndrome AIDS). Pregnancy, lactation [182] and different phases during menstrual cycle [183] have also been found to affect energy metabolism in women and therefore need to be controlled for in calorimeter studies by scheduling all WRC visits of females participants at the same phase of menstrual cycle.

Studies have also reported an association between BMR and physical activity level [184-186] where BMR of active elderly was approximately 6% higher than those who are inactive. Therefore, physical activity level should be controlled for in the study of energy expenditure. This is less of an issue as there is limited space in the calorimeter and hence the performance of vigorous activities is unlikely. In addition, participants were asked to follow activities prescribed to them during their stays in the whole room calorimeter in order to ensure minimal energy metabolised as a result of intentional physical activities.
Energy and substrate metabolism is also affected by body compositions. Fat free mass (FFM) has been shown to be the best single determinant of BMR [187] which accounts for about 75% of the BMR variability within and across species.[188, 189] Basal oxygen consumption was also found to be better correlated with FFM [30]. Nevertheless, this does not imply that body fat is metabolically inert. Instead, there is a difference between the metabolic rate of fat and that of non-fat components in human body tissue. Adipose tissue can be regarded as an endocrine [190] and paracrine [191] organ which is capable of modulating its own metabolic activities [192]. Adipose tissue in humans mainly consists of white (for fat storage) and brown (metabolically active) adipose tissues [193]. Heaton studied brown adipose tissue distribution in humans and concluded that brown adipose tissues serve as a thermogenic jacket to human organs [194]. Therefore, both fat mass (FM) and FFM have an impact on human energy metabolism. There were also indications that organs are more metabolically active than muscle mass [195-198] and hence should be measured. However, measurement of organ mass requires the MRI and there was limited access to this equipment in WRC studies conducted in the University of Wollongong. Therefore, the measurements of energy and substrate metabolism as reported in the later chapters of this thesis were adjusted for FM and FFM, but not organ mass.

Other factors that affect energy expenditure include weight changes, gender differences and ageing. However, differences in energy expenditure due to these factors can be sufficiently explained through the differences in body composition. During weight gain or loss, the ratio of FM to FFM in the body
changes [41, 199-201] and this could explain the differences in energy expenditure. The same applies to gender differences because when energy expenditure was adjusted for fat and fat free mass, the differences between males and females diminished [202-204]. Finally, ageing has also been related to decreased organ and FFM [205]. In all these cases, adjusting for body composition will take into account these factors.

Finally, how foods are provided to the participants during calorimeter stay will also affect energy expenditure. Meal frequency and size have also been found to affect TEF where energy intake was positively correlated with TEF [111, 206, 207]. A study conducted by Tai and colleagues [208] observed that a meal eaten as a large portion in 10 minutes generated a higher TEF as compared to an isoenergetic amount of food was eaten as five separate smaller meals at 30 minute intervals over 3 hours. This implies that the higher TEF was partly due to larger meal sizes instead of thermogenic effects of a meal. To ensure that the measurements of energy metabolism were actually induced by test diets alone, it is therefore important to provide a diet with energy content that is as close to energy expenditure during calorimeter stays as possible, and being is distributed evenly throughout the study period instead of in one large meal.

The experiment protocols for all studies in the WRC will eliminate or minimise the effects of the confounding variables described earlier, and these protocols are outlined in the next section.
3.2 Whole room calorimeter protocols

Calorimeter studies reported in this thesis were carried over a period of eight or 24 hours and the protocols for both study design differ from each other.

For eight-hour studies, participants entered the WRC at 0800 hours after an overnight fast of approximately 10 hours to eliminate residual postprandial thermogenesis from the previous day. Participants’ energy requirements were estimated where 65% was provided as breakfast and lunch (at 60 and 300 minutes of study) during their eight-hour stays in the whole room calorimeter. Test meals were prepared by a dietitian throughout the study to ensure consistency in food preparation. In a crossover study design, males were asked to repeat the WRC measurements after three-day washout periods while females repeated their stays at the same stage of their menstrual cycles.

For 24 hour studies, participants also entered the WRC at 0800 hours after an overnight fast. Upon entrance, participants were allowed a 30-minute adaptation period in the WRC before being asked to rest in supine position for 30 minutes when resting energy expenditure was measured. Breakfast, lunch, and dinner were provided at approximately 0900, 1300, and 1800 hours respectively, and supper at 2100 hours. Participants were also asked to perform two sessions of 5-minute stepping exercise following a 40-beats-per-minute metronome during the WRC stays to replicate usual physical activity in a free-living environment.

These protocols were followed by participants, where specific instructions were given by trained supervisors in the laboratory.
3.3 Predicting energy requirements in the calorimeter

As the main focus of this thesis is to investigate the effects of dietary manipulation on energy expenditure and substrate oxidation, accurate prediction of energy requirements of participants is important to ensure adequate foods are provided during calorimeter stays. At the same time, it is important to prevent overfeeding or underfeeding. Both these conditions have been shown to be factors which significantly alter energy expenditure [21] and substrate oxidation [41] thus compromise the study outcomes. The overall aim of this section is to find a simple and inexpensive way of predicting energy requirements in the calorimeter. Three methods of prediction were compared and reported in this section based on free-living energy expenditure and by using an equation. The use of RT3 accelerometers was considered due to the absent of the doubly-labelled water method but their validity has to first be examined. This section is therefore arranged in two parts, where the first examines the validity of the RT3 while the second compares the three methods of energy requirement prediction.

3.3.1 Background

There are a number of protocols used by more than 20 whole room calorimeter facilities around the world to achieve energy balance in calorimeter research (Table 3-1). Each of them requires a range of resources and this poses challenges for reference standards for new facilities such as the Wollongong whole room calorimeter in Australia. A simple method requiring less resource to
better predict energy requirements in the whole room calorimeter was warranted.

Table 3-1. A summary of various methods used to predict energy requirement in a whole room calorimeter grouped into four categories

<table>
<thead>
<tr>
<th>1. Measurement of total daily or one component of energy expenditure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill [209]</td>
</tr>
<tr>
<td>Goldberg [210]</td>
</tr>
</tbody>
</table>
| de Jonge [211] | BMR X 1.4 (sedentary) and reduced to 85%  
| | BMR X 1.8 (with activity) and reduced to 85% |
| Alfonzo-Gonzalez [212] | BMR X 1.4 |
| Schrauwen [213] | SMR X 1.5 |
| Melanson [214] | 24-h energy expenditure prior to study |

<table>
<thead>
<tr>
<th>2. Using an equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bessard [215]</td>
</tr>
<tr>
<td>Abbott [216]</td>
</tr>
<tr>
<td>Mikkelsen [116]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Data from dietary intake and activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horton [217]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. Fixed dietary prescription</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaz [41]</td>
</tr>
</tbody>
</table>

BMR – basal metabolic rate; SMR – sleeping metabolic rate

One of the possible methods is to predict energy requirement based on free-living energy expenditure. Although the doubly-labelled water method is regarded the most appropriate measurement of free-living energy expenditure [218], it was not available and hence not used in this study. Accelerometers such as the RT3® triaxial accelerometers were therefore considered as an alternative to the doubly-labelled water method. The RT3® triaxial
accelerometers measure activities in three dimensions, which are then translated into energy expenditure. The predicted energy expenditure is based on the individual resting energy expenditure formulated from age, sex, height, and weight [76]. Before it is used, the validity of RT3 accelerometer in measuring energy expenditure has to be examined.

To date, studies reporting the validation of this device are limited. One study observed a significant correlation between RT3 activity counts and oxygen consumption (Douglas Bag) while walking and running on a treadmill in 19 boys and 15 men [219]. However, the measurements were performed over a short period of time and it was unclear if this correlation could be translated into correlations between RT3 counts and energy expenditure, although oxygen consumption is a determinant of energy expenditure. Here, accurate calculation of energy expenditure over the study period was not possible due to the absent of carbon dioxide measurements. A more recent study investigated the relative validity of RT3 by comparison to another accelerometer Actigraph [220] (Actigraph, LLC, USA). The RT3 was demonstrated to overestimate sedentary and underestimate other activities [221]. However, the appropriateness of using Actigraph accelerometers as a reference is questionable. It has also been suggested that RT3 accelerometers may not be suitable to measure energy expenditure of sedentary individuals because the values were significantly different from the energy expenditure measured by an AEI Moxus Metabolic Cart [220]. Due to the limitations, the RT3 accelerometers need to be validated against a more accurate method of energy expenditure such as whole room calorimetry, before they are used in the measurement of energy expenditure.
3.3.2 Validation of RT3 accelerometers

3.3.2.1 Experiment protocol

This study included data obtained from a 12-week dietary intervention (HIPHOP study). Data analyses were based on n=48 participants at baseline and n=31 at 12-weeks, who completed RT3 accelerometer (RT3, version 1.2, Stayhealthy Inc., Monrovia, CA, USA) measurements during their two 23½ hour stays in a WRC. The protocol for WRC study has been previously stated. All participants were overweight or obese. Smokers and individuals reporting medical conditions that may alter metabolic conditions were excluded. The study protocol was approved by the human research ethic committee and the trial from which the data were obtained was registered with the Australian Clinical Trials Registry (Registration number ACTRN12606000530527).

3.3.2.2 Measurements

*Anthropometry and energy expenditure*

Height, body weight (Tanita scales TBF622, Tanita Corp., Tokyo, Japan), and body composition (DEXA) (Hologic Discovery QDR Series, Hologic Inc., MA, USA) were measured during the baseline and 12-week visits of the trial. The DEXA method has been shown to have high precision, where the coefficients of variation were 1.2% for whole body fat mass, 1.6% for trunk fat mass, 0.5% for

---

1 This study included data from a longer term dietary intervention (HIPHOP study) which was funded by the National Centre of Excellence in Functional Foods of Australia.

This study has been written up, submitted and is accepted for publication (April 2010):

Tan SY, Batterham M, Tapsell L: Activity counts from accelerometers do not add value to energy expenditure predictions in sedentary overweight individuals during weight loss interventions. *Journal of Physical Activity and Health*
whole body lean mass, and 0.8% for appendicular lean mass [222]. Energy expenditure was assessed using a whole room calorimeter method as described in Section 3.2.

**RT3 accelerometers**

The RT3 accelerometer has dimensions of 68 X 48 X 18mm, weighs 65.2g, and is battery-powered. RT3 measures acceleration in three orthogonal dimensions (X – vertical, Y – anterioposterior, Z – mediolateral) periodically which is then converted to activity counts as well as energy expenditure based on the counts. This device has four operation modes which sample either activity counts or vector magnitude at 1s or 60s epoch intervals. Prior to the study, participant code, age, sex, weight and height were programmed into the device through a computer. During the study, it was initialised and worn to the right side of the anterior torso of participants at the level of waist. The mode of collecting activity counts and energy expenditure was set at 60s epoch intervals. This interval was chosen due to the sedentary lifestyle of the study population. Thus variation was expected to be low when compared to epoch interval of 1s data collection. This device also provided a value for non-activity energy expenditure which was assumed to be resting energy expenditure.

### 3.3.2.3 Data analysis

Total energy expenditure (TEE) between the two methods was compared, with WRC providing the reference method. Bias between the two methods was calculated and presented in absolute values as well as a percentage of the WRC energy expenditure value. To assess the agreement between the two
methods, a statistical analysis as described by Bland and Altman [223] was performed at baseline and 12-weeks of the trial. Correlations between energy expenditure, anthropometric measurements, and total counts (the sum of X, Y and Z plane activity counts) were examined and bias was tested using a paired-sample t-test. Linear multiple regression analysis was used to determine the predictors of total energy expenditure. All statistical analyses were performed using statistical analysis software SPSS (version 15.0, SPSS Inc., Chicago, IL, USA).

3.3.2.4 Results

Among the participants recruited in the original trial, 48 of the 61 and 31 of the 35 at baseline and 12-week completed both WRC and RT3 measurements. Twenty seven participants completed measurements at both time points. The average age of participants was 44.6 ± 8.6 years. Participants remained overweight after the 12-weeks intervention period even after significant weight loss (Table 3-2).

At baseline, significant correlations were found between WRC TEE (kcal/d) and body weight (R=0.797, P<0.001), and fat-free mass (R=0.876, P<0.001). The same was found for 12-weeks: body weight (R=0.763, P<0.001), and fat-free mass (R=0.890, P<0.001). Values for fat mass and activity counts were not significantly correlated with TEE values at both time points.

Significant correlations were found between the measured (WRC) and predicted (RT3) TEE (kcal/d) at both time points (Baseline: R=0.856, P<0.001; 12-week:
R=0.869, P<0.001). TEE values changed significantly (P<0.001) after the weight loss period (Table 3-2). TEE values between methods were not significantly different at baseline (N=48, P=0.677) but were significantly higher for the RT3 at 12-weeks after weight loss (N=31, P=0.007). Nevertheless, the bias between the two methods did not change significantly before and after weight loss (N=27, P=0.244). The extent of the bias was below 100 kcal/d (Figure 3-3). The trend for changes in bias was negligible.

**Table 3-2.** Anthropometric and metabolic measurements of participants at the baseline and 12-weeks of weight-loss trial.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (N=48)</th>
<th>12-week (N=31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>91.5 ± 12.9</td>
<td>86.9 ± 13.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BMI, kgm⁻²</td>
<td>32.3 ± 3.2</td>
<td>30.6 ± 3.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FM, kg (% weight)</td>
<td>32.0 ± 6.0 (36.2 ± 6.5)</td>
<td>28.7 ± 6.5 (33.9 ± 7.3)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FFM, kg (% weight)</td>
<td>57.4 ± 12.1 (63.8 ± 6.5)</td>
<td>56.9 ± 12.9 (66.1 ± 7.3)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>RT3 accelerometer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE, kcal/d</td>
<td>1903 ± 321</td>
<td>1871 ± 322</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TEE, kcal/d</td>
<td>2098 ± 352</td>
<td>2028 ± 341†</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total counts</td>
<td>92690 ± 28959</td>
<td>74716 ± 20962</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>%REE</td>
<td>90.7 ± 2.5</td>
<td>92.2 ± 2.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>Whole room calorimeter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEE, kcal/d</td>
<td>2110 ± 379</td>
<td>1962 ± 345†</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Bias between methods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kcal/d</td>
<td>-12 ± 197</td>
<td>67 ± 176</td>
<td>0.290</td>
</tr>
<tr>
<td>As % WRC TEE</td>
<td>-0.004 ± 8.972</td>
<td>3.823 ± 8.663</td>
<td>0.300</td>
</tr>
</tbody>
</table>

BMI – body mass index; FM – fat mass; FFM – fat free mass; REE – resting energy expenditure; TEE – total daily energy expenditure; %REE – percentage of REE to TEE; WRC – whole room calorimeter

* Significant changes after 3 months, P≤0.001
† Significant difference between WRC and RT3 methods, P=0.007
Figure 3-3. Bland-Altman plots showing bias between WRC and RT3 energy expenditure at baseline (A) and 12-weeks (B) of intervention.

(A)

(B)

Solid horizontal lines – means and 95% interval of the observations; Dotted horizontal lines – zero bias lines; Red dotted lines – trends of bias
Two models were used to determine the predictors of TEE at baseline (Table 3-3) and 12-weeks (Table 3-4). The Weight Model used standard characteristics (age, weight, and height), and Body Composition Model included age, fat mass, and fat-free mass. At baseline, the activity counts from the RT3 did not contribute to the explained variation in measured TEE in both models. Body composition appeared to be a better predictor of TEE rather than basic characteristics such as weight and height, where $R^2$ increased by 11.4% to 0.784 when fat-free mass was used. The Body Composition Model was also observed to better predict TEE at 12-week (Table 3-2). Contrary to the finding at baseline, activity counts were significant predictors of TEE at 12-week. However, activity counts increased the $R^2$ value by only 2.0%, resulting in a total $R^2$ of 0.824.

3.3.2.5 Discussion

This study aimed to investigate if RT3 accelerometers provide valid values for predicting the TEE of sedentary overweight and obese individuals. Before weight loss (baseline), values for the predicted TEE (RT3) were not significantly different from those of the measured TEE (WRC) and the magnitude and percentage of mean bias between methods was small (<100 kcal/d). Bland-Altman analyses further confirmed this observation and showed that the variances between the two methods were equal. This implied that RT3 accelerometers were a reasonably accurate device for predicting the TEE of sedentary overweight and obese individuals. They would be especially valuable in predicting TEE in the absence of more objective measures such as the WRC.
and doubly-labelled water methods. Also, this device is less expensive, making it appropriate to be used in a clinical practice.

However, activity measurements by the RT3 accelerometers did not add value to the assessment of TEE in our weight loss study. Activity counts were not significantly correlated to the WRC TEE and they were not a significant predictor of TEE regression models at baseline. Instead, only body weight and body composition were shown to be important in explaining variations in the measured TEE. This observation was different to those reported by Plasqui and colleagues [224], where activity counts explained an additional 19% of the variation in the measured TEE and 33% of activity energy expenditure. The different outcomes could be explained through the differences in study populations recruited in both studies. Overweight and obese individuals were recruited in the study, who were sedentary and TEE was likely to be contributed mainly by resting energy expenditure that depends on body fat-free mass [225], whereas healthy subjects were included in the study by Plasqui. Another study of older men (who spent most time on sedentary activities [226], also reported poor correlations between accelerometer output and measured energy expenditure in a free-living environment [227]. An additional 2% of variance in the WRC TEE explained by activity counts suggests that participants may have increased their spontaneous physical activity since the WRC protocol remained unchanged for both stays.

This study also aimed to investigate if RT3 accelerometers were sensitive to weight and body composition changes in the prediction of TEE. The
disproportionate loss of fat mass and fat-free mass during weight loss observed in our study was consistent with the scientific literature [228]. As a result, study participants substantially changed body composition after weight loss (Table 3-2). Body composition has been shown to be an important determinant of energy expenditure [229] and the fact that there was a significant difference between the measured and predicted TEE suggests that the RT3 was not sensitive to body composition changes. The measured TEE (WRC) was significantly higher. This was reflected in the higher percentage of fat-free mass after weight loss (Table 3-2), which may not have been picked up in the RT3 accelerometers predictions. This is because it utilises a built-in regression model to predict energy expenditure that only addresses body weight. Nonetheless, the bias between RT3 and WRC TEE was still considerably small (<100 kcal/d).

3.3.2.6 Conclusion

RT3 accelerometers were observed to be capable of providing reasonably accurate estimation of energy expenditure. Although RT3 and WRC TEE were significantly different at 12-weeks, the actual bias was small and acceptable. Physical activity (measured as activity counts) contributed minimally towards TEE at 12-weeks but not at the baseline. RT3 accelerometers are therefore a valid method to estimate energy expenditure of overweight and obese individuals with low physical activity levels (sedentary lifestyle). These devices were used to collect free-living environment energy expenditure, which was used in the next study to predict energy requirements in the WRC.
Table 3-3. Multiple linear regression analysis with TEE as the dependent variable at baseline.

<table>
<thead>
<tr>
<th>Independent</th>
<th>Coefficients</th>
<th>SE</th>
<th>P</th>
<th>Partial correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight Model</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>716.60</td>
<td>0.077</td>
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<tr>
<td>Age</td>
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<td>3.79</td>
<td>0.127</td>
<td>-0.388*</td>
</tr>
<tr>
<td>Weight, kg</td>
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<td>3.53</td>
<td>0.000</td>
<td>0.797*</td>
</tr>
<tr>
<td>Height, m</td>
<td>12.29</td>
<td>5.10</td>
<td>0.020</td>
<td>0.722*</td>
</tr>
<tr>
<td>Activity counts</td>
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<td>0.01</td>
<td>0.420</td>
<td>0.070</td>
</tr>
<tr>
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<td>R=0.844, adj. R^2=0.686</td>
</tr>
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<td>Weight, kg</td>
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<td>0.797*</td>
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<td>Height, m</td>
<td>13.39</td>
<td>4.91</td>
<td>0.009</td>
<td>0.722*</td>
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<td>Model</td>
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<td>R=0.826, adj. R^2=0.670</td>
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<table>
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</tr>
<tr>
<td>Fat mass, kg</td>
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<tr>
<td>Fat-free mass, kg</td>
</tr>
<tr>
<td>Activity counts</td>
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<tr>
<td>Model</td>
</tr>
<tr>
<td>Constant</td>
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<tr>
<td>Age</td>
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<td>Fat mass, kg</td>
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<tr>
<td>Age</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
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* Statistically significant, P<0.05
**Table 3-4.** Multiple linear regression analysis with TEE as the dependent variable at 12-weeks.

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<tr>
<th>Independent</th>
<th>Coefficients</th>
<th>SE</th>
<th>P</th>
<th>Partial correlation</th>
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<tr>
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<td>4.11</td>
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<td>0.763*</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>Height, m</td>
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<td>0.060</td>
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</tr>
<tr>
<td>Fat mass, kg</td>
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<td>4.24</td>
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<tr>
<td>Fat-free mass, kg</td>
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<td>0.890*</td>
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<tr>
<td>Activity counts</td>
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<tr>
<td>Fat-free mass, kg</td>
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<td>0.890*</td>
</tr>
<tr>
<td>Activity counts</td>
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<td>0.001</td>
<td>0.050</td>
<td>0.112</td>
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<td></td>
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<tr>
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<td>0.000</td>
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<td>-6.90</td>
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<td>Fat-free mass, kg</td>
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<td>0.000</td>
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<td><strong>Model</strong></td>
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<td></td>
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</tr>
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</table>

* Statistically significant, P<0.05
3.3.3 Comparison of methods to predict energy requirements

3.3.3.1 Experiment protocol

The aim of this study is to compare three methods of energy requirement prediction in the calorimeter. This study included baseline data collected from three longer term dietary interventions. The study protocols for these trials were registered with the Australian Clinical Trials Registry [HELP study: ACTRN12608000453381, SMART study: ACTRN12608000425392, HIPHOP study: ACTRN12606000530527]. All trials shared a similar study protocol where participants were asked to wear an accelerometer (RT3, version 1.2 Stayhealthy Inc., Monrovia, CA, USA) during their 24-h stays in a WRC and during a 3-day (two weekdays and one weekend day consecutively) period in a free-living environment prior to the WRC stays.

3.3.3.2 Measurements

Body weight and body compositions were measured using Tanita scales (TBF622, Tanita Corp., Tokyo, Japan) at baseline for all studies. Daily and mean 3-day energy expenditure was measured using RT3 accelerometers as described in the previous validation study.

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1 This study included baseline data from three longer term dietary interventions which were funded by the National Health and Medical Research Council of Australia (HELP study: Project Grant #354111 and SMART study #514631), and National Centre of Excellence in Functional Foods of Australia (HIPHOP study).

This study has been written up and accepted for publication (February 2010):

Tan SY, Batterham M, Tapsell L: Comparison of methods used to predict energy requirements in a whole room calorimeter Obesity Research and Clinical Practice 2010; doi: 10.1016/j.orcp.2010.02.005.
Methods to predict energy requirements were:

1. Using the Schofield equation [230] to predict basal metabolic rates and then multiplying by an activity factor of 1.2 to account for low physical activity in the whole room calorimeter, a confined space ($E_{SCHOFIELD}$).

2. Using a regression model to determine EE (WRC) where the mean value of 3-d data from RT3 accelerometers served as an independent variable ($E_{REGRESSION}$). Here baseline data from HELP and SMART studies ($n=97$) was used to develop the regression model to predict 24-h RT3 EE (in a WRC). Using this regression model, EE of participants in a WRC was predicted based on mean 3-day RT3 EE.

3. Multiplying mean 3-day RT3 EE with a factor of 85%, as described by de Jonge and colleagues [211]. ($E_{FACTOR}$)

3.3.3.3 Data Analysis

The WRC EE was used as the reference method and the individual differences between the three prediction methods and WRC EE ($\Delta$) were calculated and compared using data from HIPHOP study ($n=29$). Bland-Altman plots [223] were produced for comparison of each prediction method. Absolute individual difference ($|\Delta|$) between methods was grouped into one of the three categories: $<100$ kcal/d, 101 – 200 kcal/d, and $>200$ kcal/d. An absolute difference of under 100 kcal/d can be regarded as the ideal value to be achieved in the calorimeter, while differences between 100 to 200 kcal/d is acceptable based on the values reported by Grunwald and colleagues [231] when different methods were used.
3.3.3.4 Statistical analysis

All statistical analyses were performed using SPSS statistical software package for Windows (SPSS version 15.0.0: SPSS Chicago, IL, 2006). Relationships between 3-day and 24-h RT3 EE were analysed using Pearson Correlation Coefficient. Linear regression analysis was used to model EE\textsubscript{REGRESSION} using mean 3-day RT3 EE as the independent variable. Predicted energy requirements (EE\textsubscript{SCHOFIELD}, EE\textsubscript{REGRESSION} and EE\textsubscript{FACTOR}) were compared against the WRC method using a paired-sample t-test.

3.3.3.5 Results

Demographic characteristics of participants from the three studies are summarised in Table 3-5. Overall, mean values for 3-day RT3 EE was 2454.1 ± 491.9 kcal/d while those for the 24-h RT3 EE was 2022.7 ± 295.8 kcal/d. The 24-h and mean 3-d RT3 EE values were significantly correlated (R=0.763, P<0.001) and the 24-h RT3 EE values in the whole room calorimeter represented 84.0% ± 10.7% of those in a free-living environment for all participants. The regression model for energy requirements in the WRC was 

\[
EE\textsubscript{REGRESSION} = 0.405 \times [\text{mean 3-d RT3 EE}] + 1009.6 \text{ kcal/d} \quad (N=97, R^2=0.479, P<0.001).
\]

The mean differences between WRC EE were 7.5 ± 184.3 kcal/d for EE\textsubscript{SCHOFIELD}, 57.9 ± 286.8 kcal/d for EE\textsubscript{REGRESSION}, and -26.1 ± 377.5 kcal/d for EE\textsubscript{FACTOR}. Predicted energy requirements were not significantly different from the WRC EE (EE\textsubscript{SCHOFIELD} P=0.829, EE\textsubscript{REGRESSION} P=0.287, EE\textsubscript{FACTOR} P=0.712). Bland-Altman plots showing individual bias are presented as Figure 3-4. A
greater proportion of participants were able to achieve a smaller absolute individual difference ($|\Delta|$) using the Schofield method, followed by $EE_{FACTOR}$ and $EE_{REGRESSION}$ (Figure 3-5).

Table 3-5. Cross-sectional demographic characteristics of participants from three weight loss intervention trials at baseline.

<table>
<thead>
<tr>
<th>Study</th>
<th>Male, n</th>
<th>Female, n</th>
<th>Age, years</th>
<th>Weight, kg</th>
<th>BMI, $kg/m^2$</th>
<th>24-h EE, kcal/d</th>
<th>Mean 3-d EE, kcal/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>HELP</td>
<td>20</td>
<td>57</td>
<td>44.6 ± 10.5</td>
<td>88.2 ± 13.3</td>
<td>31.2 ± 3.7</td>
<td>2005 ± 298</td>
<td>2423 ± 501</td>
</tr>
<tr>
<td>SMART</td>
<td>2</td>
<td>19</td>
<td>44.4 ± 8.0</td>
<td>88.6 ± 10.4</td>
<td>31.3 ± 3.1</td>
<td>1958 ± 164</td>
<td>2455 ± 356</td>
</tr>
<tr>
<td>HIPHOP</td>
<td>16</td>
<td>27</td>
<td>44.9 ± 8.2</td>
<td>91.2 ± 12.1</td>
<td>32.1 ± 3.1</td>
<td>2100 ± 339</td>
<td>2510 ± 531</td>
</tr>
</tbody>
</table>

BMI – body mass index, EE – energy expenditure

Figure 3-4. Bland-Altman plot showing bias between predicted and actual (WRC) energy expenditure.
(A) EE\textsubscript{SCHOFIELD}, (B) EE\textsubscript{REGRESSION}, (C) EE\textsubscript{FACTOR}. Solid horizontal lines – means and 95\% interval of the observations; Dotted horizontal lines – zero bias lines; Red dotted lines – trends of bias.
Figure 3-5. Proportion of participants categorised into three groups of absolute individual difference ($|\Delta|$).

3.3.3.6 Discussion

This study aimed to compare a few simple and inexpensive methods to estimate energy requirements in the calorimeter. Results indicate that the Schofield equation gives better estimates than using free-living energy expenditure values measured by RT3 accelerometers.

The value of the energy expenditure measured by RT3 accelerometers while in the WRC was approximately 84% of the values obtained from measurements in the free-living environment. This figure is close to the 85% reported by de Jonge and colleagues [211]. The lower value is likely to be due to a lower physical activity level as a result of the confined space in the WRC (2.1 m X 3.0 m floor space). Using the energy requirement prediction regression model
(EE\textsubscript{REGRESSION}), the mean 3-d RT3 EE values accounted for 48% of the 24-h EE in the WRC.

Although energy requirements predicted by all three methods were not significantly different from the WRC EE, the mean bias and standard deviations from the Schofield method were observed to be the smallest (near zero) when compared to the other two methods. The bias between the methods was small and of limited clinical relevance. However, the small sample size limits firm conclusions. Previous research has shown that the Schofield equations overestimate basal metabolic rates of young Australians [232] but in this study applying the activity factor of 1.2 provided a good estimation of energy requirement in the WRC.

The comparison of the method is equally scaled Bland-Altman plots (Figure 3-4) confirms the Schofield method is a better method for predicting energy requirements in the WRC. The confidence interval is smaller and there appeared to be no trend in the bias. On the other hand, the values for the regression method (EE\textsubscript{REGRESSION}) tended to overestimate while the factor method (EE\textsubscript{FACTOR}) tended to underestimate energy requirements in the WRC when the daily energy expenditure exceeded 2000 kcal/day. The low agreement levels when energy expenditure was higher suggested that higher free-living energy expenditure may come from physical activities that are greatly limited in the WRC.
When the absolute individual differences (|Δi|) of the three methods were compared, the Schofield method again performed the best. Values for greater proportions of participants (76%) indicated energy balance of within 200 kcal/d (48.3% for <100 kcal/d; 27.6% for 100 – 200 kcal/d). Only 24.1% of values using the EE_{SCHOFIELD} method fell outside an energy balance of 200 kcal/d as compared to 44.9% for EE_{REGRESSION} and 41.4% for EE_{FACTOR} methods. This implies that the prediction of energy requirements in the WRC using free-living energy expenditure through both regression models and direct multiplication of estimated EE by a factor of 85% may not be valid approaches.

Other equations have also shown to produce accurate estimations of energy requirements for studies of human energy metabolism. One study predicted energy requirements based on calories per kilogram body mass [215] while another used body mass in the equation, which was then reduced by 20% to account for lower activity levels [216]. The advantages of using equations in the prediction of energy requirement include lower costs, time and resources required, making higher throughput possible.

3.3.3.7 Conclusion

The Schofield prediction equation proved to be an inexpensive, less laborious and feasible means for predicting energy requirement and it would be especially useful for studies requiring large sample sizes. This method of prediction was therefore used in the WRC studies reported in the following chapters.
3.4 Effects of insulin and leptin on energy metabolism

Many confounding variables to the measurement of energy expenditure and substrate oxidation can be eliminated though careful selection of study population and through standardised WRC protocol. However, some of these factors, such as body composition of study participants, cannot be eliminated or standardised, and therefore are accounted for using statistical methods. In recent years, there is evidence relating insulin and leptin hormones to energy expenditure. The study reported in this section investigates if these hormones affect human energy expenditure, and from there determines if levels of these hormones need to be accounted for statistically, like those in body composition, in WRC studies reported in this thesis.

3.4.1 Background

Adipose tissue is no longer considered metabolically inert as it has been demonstrated to influence metabolic processes through hormone secretions. The most important of all hormones (secreted by white adipose tissues) is leptin, a protein product of the ob gene which was discovered in the 1990s [233]. Leptin was first observed to be highly correlated with adiposity and it was found to play an important role in the regulation of appetite and food intake in both animals [234-236] and humans [237]. It was thus regarded as providing an important influence on the underlying mechanism for weight regulation. However, leptin infusion in an animal study produced a greater weight loss than would be expected due to reduced energy consumption alone [238].

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1 This study included baseline data from a longer term dietary intervention funded by the National Health and Medical Research Council of Australia (SMART study #514631).
observation suggested that leptin worked beyond the regulation of appetite and food intake, and possibly had an impact on the metabolic processes. However, a human trial failed to observe a relationship between leptin and energy expenditure [239]. Nevertheless, it was noted in the same study that the basal metabolic rate did not decrease despite weight loss. It was hence thought that the role of leptin in regulating energy metabolism may be more to prevent the fall in energy expenditure due to weight loss or energy restriction, rather than to induce energy expenditure above basal metabolic rate [240, 241]. An animal model study has shown that circulating leptin regulates energy metabolism through the sympathetic nervous system, where it increases noradrenalin concentrations, which in turn increases energy expenditure [242].

Insulin, like leptin, is affected by dietary intake. Insulin levels increase during re-feeding, and decrease during energy restriction or fasting [243]. However, it has been observed that insulin responses to energy consumption precede changes in circulating leptin levels. Insulin is therefore regarded as a regulator of leptin secretion by the white adipose tissues [243]. The mechanism appears to be that insulin increases ob gene expression leading to leptin production [244]. Based on this evidence, it appears that both leptin and insulin may influence human energy metabolism directly or indirectly. Therefore, the aim of this study was to investigate if leptin and insulin are confounding variables of energy expenditure and whether they should be adjusted statistically during data analyses of all WRC studies reported in this thesis.
3.4.2 Study protocol and measurements

This study utilised cross-sectional preliminary data obtained at the baseline of a 12-month longer term dietary intervention (SMART study) which included healthy overweight and obese adults. Data from participants who completed 24-hour stays in the WRC at baseline was included. Participants were weighed (Tanita scales TBF622, Tanita Corp., Tokyo, Japan), body composition was assessed using the dual-energy x-ray absorptiometry (DEXA) method (Hologic Discovery QDR Series, Hologic Inc., MA, USA) while fasting blood samples were obtained from participants for the analyses of blood insulin and leptin. Energy expenditure was calculated based on gaseous exchanges using the Weir equation. Using these measurements, the relationships between energy metabolism and body weight, fat mass, fat-free mass, insulin and leptin levels were investigated using a backward stepwise multiple linear regression analyses (SPSS version 15.0.0: SPSS Chicago, IL, 2006).

3.4.3 Results and discussion

From the analyses, either weight (P<0.001) or fat (P=0.006) and fat-free mass (P<0.001) were shown to be significant predictors of 24-h energy expenditure. Fasting leptin and/or insulin levels did not appear to be significant predictors of energy expenditure when combined with weight or body composition (Table 3-6).
Table 3-6. Predictors of energy expenditure using a multiple linear regression models

<table>
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<th>Adjusted R²</th>
<th>P</th>
<th>Factors</th>
<th>Adjusted R²</th>
<th>P</th>
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<td>0.046</td>
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<td></td>
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<tr>
<td>Weight</td>
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<td>0.000</td>
<td>Fat-free mass</td>
<td>0.249</td>
<td>0.000</td>
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<td></td>
<td></td>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.268</td>
<td>0.000</td>
<td>Fat-free mass</td>
<td>0.242</td>
<td>0.000</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.388</td>
<td></td>
<td>Fat mass</td>
<td>0.044</td>
<td>0.921</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leptin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.271</td>
<td>0.000</td>
<td>Fat-free mass</td>
<td>0.252</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fat mass</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

Consistent with the literature, the effects of leptin and insulin on human energy expenditure was not observed [239, 245]. From the multiple linear regression models that were conducted, only weight and body composition were shown to be significant predictors of energy expenditure. The possible explanation for this observation was that leptin was produced in the white adipose tissues and the effect of insulin on its production took place in white adipose tissues as well. Adjusting for fat mass statistically may sufficiently account for the leptin and insulin levels.

3.4.4 Conclusion

As leptin and insulin do not appear to be predictors of energy expenditure, data used in this thesis were only adjusted for body composition. This was the procedure followed in subsequent chapters of this thesis.
CHAPTER 4. ROLES OF DIETARY PROTEIN IN WEIGHT MANAGEMENT

This chapter consists of two studies, where the overall aim was to test the usefulness of high protein diets in promoting weight loss because high protein meals have been consistently reported to be more thermogenic than other isoenergetic lower protein meals. The justification for conducting these studies was limitations to the studies reported in the literature, where protein levels in the test meals have not been reflective of usual free-living consumption. In the two studies reported in this chapter, high protein diets were modelled at a level that was more realistic and whole foods were incorporated in the test diets. Thus a novel approach to the study design was taken. Further, the effects of high protein diets from different protein-rich food sources were compared. The investigation was conducted in two phases. First, the acute effects of high protein diets from different food sources on energy expenditure and substrate oxidation rates were examined. This phase was essential prior to the second, longer term study, as it informed on the underlying mechanisms that might explain the potential differences in clinical outcome measures (e.g. greater weight loss or higher fat-free mass retention). This two-step approach helped determine if the desired effects of the test diets such as higher energy and substrate metabolism shown in an acute setting could be extended into a free-living environment.

1 The studies reported in this chapter were funded by the National Centre of Excellence in Functional Foods of Australia. The conceptual framework and study descriptions have been previously published.

Journal article:
This chapter includes two empirical studies of different study designs to establish the mechanisms behind high protein diets from different food sources, and to examine their effects in the long term on clinical outcomes.
4.1 Acute effects of high protein meals from different sources on energy expenditure and substrate utilisation (NCEFF study)¹

4.1.1 Background

Dietary protein from different sources exhibited significantly different TEF [116], and increased level of dietary protein consumption has been reported to increase the rate of fat oxidation [133]. Therefore, the aim of this study was to examine the effects of protein sources (within a high protein diet context) on energy expenditure and substrate utilisation. Different from the past studies, three high protein diets from predominantly meat, dairy and plant sources were used. These test diets included whole foods and the protein proportion was set at a more realistic levels. A control low-protein arm was not included in this study as acute feeding trials involving humans have consistently reported higher thermic effect of high protein meals.

¹ The studies reported in this chapter were funded by the National Centre of Excellence in Functional Foods of Australia. The preliminary outcomes of this study were presented in a conference and the manuscript has been accepted for publication.

Journal article:

Conference abstract:
Tan SY, Batterham M, Tapsell L: A plant-based high-protein diet appears to be as good as an animal-based high protein diet in promoting human energy expenditure: preliminary results, in Recent Advances and Controversies in Measuring Energy Metabolism (RACMEM), Denver, Colorado, USA 2008.
4.1.2 Methods

4.1.2.1 Experiment protocol

This was an acute crossover feeding study of three high-protein meals consumed in a WRC. The order of diets was randomised and the 8-hour WRC protocol has been previously stated. The experiment protocol was approved by the Human Research and Ethics Committee of the University of Wollongong (HE06/070).

4.1.2.2 Participants

Participants were volunteers recruited from both the University and local community. Inclusion criteria for this study were aged >18 years, normal or overweight, not a smoker, not pregnant or lactating, without food allergies, and generally well. Volunteers who had an acute illness, or conditions likely to alter metabolic rate such as thyroid abnormality were excluded from the study.

4.1.2.3 Test meals

The three test meals contained protein predominantly from meat, dairy and soy sources. The meat was lean beef and ham, the dairy comprised low-fat milk, cheese and yoghurt. Soy was chosen as a plant alternative option because the commercial availability of soy protein powder enabled dietary modeling to match for macronutrient properties in the test meals. Energy requirements were calculated using the Schofield equation where 65% was provided as breakfast and lunch meals. The macronutrient and micronutrient breakdown of the meals were obtained using the FoodWorks nutrient analysis software (FoodWorks
Professional 2007, Xyris Software, Brisbane). The carbohydrate, protein, and fat content in the test meals were kept consistent at 40%, 30% and 30% of total energy provided (Table 4-1).

**Table 4-1.** High protein meals provided to participants during the calorimeter stays (NCEFF study).

<table>
<thead>
<tr>
<th></th>
<th>Dairy</th>
<th>Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>Toast with ham &amp; tomato, fruit juice</td>
<td>Toast with butter &amp; jam, chocolate milk shake, soy powder (^1)</td>
</tr>
<tr>
<td></td>
<td>Toast with margarine &amp; jam,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>chocolate milk shake</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>Steak with potato &amp; vegetable, fruit juice</td>
<td>Salad with dressing &amp; grated cheese, yoghurt, chocolate milk shake, soy powder (^1)</td>
</tr>
<tr>
<td></td>
<td>Salad with dressing &amp; grated cheese,</td>
<td>Salad with dressing &amp; soy cheese,</td>
</tr>
<tr>
<td></td>
<td>yoghurt, chocolate milk shake</td>
<td>chocolate soy milk shake,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>soy yoghurt, chocolate soy milk shake,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>soy powder (^1)</td>
</tr>
</tbody>
</table>

\(^1\) Instant Natural Protein Powder (Nature’s Way, Australia)

An extra piece of fruit may be provided based on individual energy requirement.

### 4.1.2.4 Anthropometric measurements

Height, weight (Tanita TBF622, Tanita Corp., Tokyo, Japan), percentage body fat (Bodystat 1500, Bodystat Ltd., Douglas, IM, UK), and waist-hip circumference were measured during a pre-study assessment. Bodystat 1500 is a tetrapolar bioelectrical impedance device which has been validated against the dual-energy x-ray absorptiometry method [246]. Weight and body fat measurements were repeated prior to each of the three calorimeter stays.
4.1.2.5 Satiety level measurements

Satiety levels of participants during calorimeter stays were assessed on an hourly basis using an ‘appetite and desire for food’ questionnaire with visual analog scales (VAS), which has been previously validated [247]. The questionnaire consisted of eight questions, where the first four were used to assess satiety level (Appendix E). Participants were asked to make a vertical mark on each horizontal line, measuring 10 cm, for all questions. On the both ends of the lines were two opposite scenarios (for example full vs. hungry). The vertical marks on the lines were measured (to 1 mm) and indicated as scores for each question. As question 1 and question 4 of this questionnaire assessed hunger instead of fullness, the scores were inverted to represent satiety levels.

4.1.2.6 Measurement of energy metabolism

The measurements of energy expenditure were conducted using the WRC based on the calculations of gaseous exchanges, which have been previously described.

4.1.2.7 Data analysis

Nitrogen excretion, which was used to calculate protein oxidation, was estimated from measured urinary urea, and carbohydrate and fat oxidation rates were calculated using the equations outlined in Section 3.1.2.2. The hourly VAS scores from the appetite questionnaire were transformed into area under curve (AUC) for individual and combined scores for comparison. Differences in energy expenditure, rates of substrate oxidation and VAS AUC were compared using a
linear mixed model analysis (SPSS 15.0, SPSS Inc., Chicago IL USA). Fat mass (kg) and fat-free mass (kg) were used as covariates for energy expenditure and substrate oxidation rates.

4.1.3 Results

Twelve healthy participants (9 males, 3 females) were recruited and all completed three stays in the whole room calorimeter. Based on the World Health Organisation’s (WHO) [248] classification, one male participant was obese while the others were normal weight. The participants were aged 25.4 ± 5.2 years, with mean height = 175.3 ± 11.7cm, waist = 76.5 ± 10.9cm (n=11), hip = 98.9 ± 8.4cm (n=11), and waist-hip-ratio = 0.77 ± 0.05 (n=11). Anthropometric measurements, dietary intake, and energy and substrate metabolic rates are presented in Table 4-2. The energy expenditure (P=0.987), fat (P=0.997) and carbohydrate (P=0.951) oxidation rates were not significantly different between the three test meals. Satiety levels, both individual and combined scores, were also not different between test meals (Table 4-2). Protein oxidation was significantly different, with the difference found to lie between the Meat and Soy meals (P=0.012). In terms of capability to increase satiety levels, all test meals exhibited equal effects. The satiety levels peaked at an hour post meals (both breakfast and lunch), and lasted for 180 minutes when the satiety levels dropped to almost similar levels to the baseline of study (overnight fast) (Figure 4-2).
Table 4-2. Anthropometry, energy and substrate metabolism, and satiety levels of participants measured during the three test meals in the whole room calorimeter (NCEFF study).

<table>
<thead>
<tr>
<th></th>
<th>Meat</th>
<th>Dairy</th>
<th>Soy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71.1 ± 17.2</td>
<td>71.3 ± 16.9</td>
<td>70.7 ± 17.0</td>
<td>0.996</td>
</tr>
<tr>
<td>BMI, kgm(^2)</td>
<td>22.8 ± 3.5</td>
<td>22.9 ± 3.4</td>
<td>22.7 ± 3.4</td>
<td>0.990</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>17.8 ± 5.3</td>
<td>18.3 ± 6.7</td>
<td>17.7 ± 6.2</td>
<td>0.966</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kcal/8h</td>
<td>826.1 ± 135.4</td>
<td>814.0 ± 152.4</td>
<td>817.1 ± 139.0</td>
<td>0.987</td>
</tr>
<tr>
<td>Protein, g/8h</td>
<td>34.9 ± 11.1</td>
<td>36.2 ± 12.6</td>
<td>39.5 ± 10.7</td>
<td>0.038*</td>
</tr>
<tr>
<td>Carbohydrate, g/8h</td>
<td>105.9 ± 60.8</td>
<td>99.4 ± 34.1</td>
<td>97.7 ± 35.7</td>
<td>0.951</td>
</tr>
<tr>
<td>Fat, g/8h</td>
<td>26.6 ± 23.0</td>
<td>28.2 ± 17.6</td>
<td>28.3 ± 16.5</td>
<td>0.997</td>
</tr>
<tr>
<td><strong>Satiety levels, AUC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Question 1, inverted</td>
<td>2613.5 ± 369.6</td>
<td>2683.8 ± 694.0</td>
<td>2407.0 ± 433.5</td>
<td>0.283</td>
</tr>
<tr>
<td>Question 2</td>
<td>2318.8 ± 421.0</td>
<td>2392.3 ± 636.4</td>
<td>2196.5 ± 585.1</td>
<td>0.431</td>
</tr>
<tr>
<td>Question 3</td>
<td>2046.8 ± 644.9</td>
<td>2319.8 ± 577.0</td>
<td>2071.5 ± 700.1</td>
<td>0.288</td>
</tr>
<tr>
<td>Question 4, inverted</td>
<td>2306.3 ± 484.7</td>
<td>2414.5 ± 625.5</td>
<td>2153.5 ± 515.1</td>
<td>0.122</td>
</tr>
<tr>
<td>Combined score</td>
<td>9285 ± 1737</td>
<td>9727 ± 2236</td>
<td>8829 ± 2053</td>
<td>0.296</td>
</tr>
</tbody>
</table>

\(^*\) Significant difference between diets, linear mixed model, P<0.05
Figure 4-1. Changes in satiety level as measured by each of the four questions in the appetite and desire for food questionnaire (NCEFF study)

4.1.4 Discussion

Lower protein oxidation in the meat meal suggested a higher protein-sparing effect, which could be linked to the higher biological value of the meat protein [249]. This effect is important in weight loss, where there appears to be greater metabolic benefit in losing fat mass and also in retaining fat-free mass [32].
The energy expenditure following consumption of high protein meals from predominantly meat sources appeared to be slightly higher (but not significantly different) than those from dairy and soy sources. This is consistent with the observation by Mikkelson and colleagues [116]. However, the magnitude of differences was lower than those reported by the group (meat was 1.5% higher than dairy and 1.1% higher than soy). These differences translate to approximately 36 and 27 kcal/day higher in energy expenditure respectively. Based on an energy density assumption of 7700 kcal per kilogram body weight [250], these results could mean an extra weight loss in the meat meal of 32.7g compared to the dairy, and 24.5g compared to soy. These figures may not be of clinical importance. Moreover, the fat oxidation rate induced by the meat meal was lower than the other 2 diets although they were not significant. The magnitude of the difference is also not clinically statistically significant. Thus, even with a larger sample size, the effect on energy and fat metabolism would likely to have little impact on weight loss.

In term of dietary modelling, the dairy and soy meals were similar as they contained similar food types (yoghurt, cheese, milk) but the cuisine varied with the meat meal. Despite the similarity, the foods from soy sources still had lower protein contents [251] such that additional soy protein had to be used to achieve the protein target of these meals.
4.1.5 Conclusion

Although high protein diets were reported to be more thermogenic and may enhance fat oxidation, the differential effects of protein sources on these parameters and satiety level were not observed. High protein diet from plant sources is as thermogenic as those from animal or dairy sources. However, the higher protein retention in the high animal protein group suggests potential greater fat-free mass retention during weight loss, which in turn may maintain metabolic rate over a longer period of time.
4.2 The effectiveness of high protein diets from predominantly animal and plant sources on metabolic parameters, weight loss and body composition (HIPHOP study)¹

4.2.1 Background

The previous study suggests a value in high protein diets for promoting weight loss and fat-free mass retention which warrants further exploration. Animal protein appears to be better than plant protein based on its protein retention properties. However, safety issues of long-term consumption for weight loss purposes for example carcinogenicity [252] and environmental sustainability [253] may also need to be considered.

With all this in mind, the study reported in here further examined the effects of high protein diets from animal and plant sources over a longer period of time. It aimed to determine if the properties of high protein diets observed in the previous study could be extended into a free-living environment over a 12-week period of time to promote weight loss. This was assessed through their differing effects (low vs. high protein diets, and animal vs. plant sources) on weight loss, body composition, energy expenditure or metabolic risk indicators.

¹ The studies reported in this chapter were funded by the National Centre of Excellence in Functional Foods of Australia. The preliminary outcomes of this study were presented in a conference and the manuscript is currently under preparation.

Prepared manuscript:
Tan SY, Batterham M, Tapsell L: High protein diets with differing protein sources produce similar weight loss profiles in overweight adults.

Conference abstract:
Tan SY, Batterham M, Tapsell L, Quick C, Hietkamp K: Changes in energy expenditure of overweight and obese adults after following hypoenergetic high protein diets from either animal or plant sources: preliminary results, in Recent Advances and Controversies in Measuring Energy Metabolism (RACMEM), Denver, Colorado, USA 2008.
Only two high protein arms were included in this longer term dietary intervention, where dairy foods were allowed in both high protein arms. This was due to the fact that there were not significant effects of this protein source on any metabolic parameters that were measured in this first study. In addition, the weight loss diets were designed to be as realistic as possible based on the Australian Guide to Healthy Eating [254], where dairy foods were part of the healthy eating plan.

4.2.2 Methods

4.2.2.1 Experiment protocol

The study was a 3-month randomised, single-blinded, controlled trial with three study arms:

1. Control: hypoenergetic (2MJ/day deficit) standard protein cuisine based on usual eating pattern (20% protein, 50% carbohydrate, 30% fat)
2. Animal protein: hypoenergetic (2MJ/day deficit) high protein cuisine from predominantly animal sources (30% protein, 40% carbohydrate, 30% fat)
3. Plant protein: hypoenergetic (2MJ/day deficit) high protein cuisine from predominantly plant sources (30% protein, 40% carbohydrate, 30% fat)

Participants were recruited from the local community through local media advertisement. Eligible participants attended an assessment session where a diet history was taken and an accelerometer (RT3, version 1.2, Stayhealthy inc., Monrovia, CA, USA) supplied. At the baseline and when intervention was
completed, body weight (Tanita scale model TBF-622, Tanita Corp., Tokyo, Japan), blood biochemistry and calorimetry assessments were performed. All groups received support on a monthly basis to help maintain adherence to the prescribed diets. At baseline and post-intervention body composition was measured using dual-energy x-ray absorptiometry (DEXA) scans (Hologic Discovery QDR Series, Hologic Inc., MA, USA).

4.2.2.2 Participants

Inclusion criteria for this study were: aged 18-60 years, BMI>25 kgm$^{-2}$ and <37 kgm$^{-2}$. Volunteers were excluded if they were smokers, had major illness, food allergies, illiteracy and/or inadequate conversational English, and expressed inability to stay in the calorimeter. Participants were randomly assigned to one of the sex-stratified study arms by a researcher independent of the subject interface.

4.2.2.3 Ethics

The study was approved by the human research ethics committee of the University of Wollongong (HE06/332) and the protocol was registered with the Australian New Zealand Clinical Trial Registry (ACTR number 12606000530527) and American Clinical Trial Protocol Registration (identifier NCT00421616).
4.2.2.4 Dietary prescription and physical activity recommendations

The amount of food recommended was based on numbers of serves from core food groups to meet the target dietary nutrient composition profile of the prescribed diets (Table 4-3). All participants received individualised dietary advice and had same amount of contact with the dietitians over the 12-week study period. Participants were advised to include 5 X 30minute physical activity per week and level of free-living activities were monitored using the accelerometer (RT3, version 1.2, Stayhealthy Inc., Monrovia, CA, USA) and activity diary.

4.2.2.5 Measurements

Dietary intake

Usual dietary intake was assessed using a validated diet history interview [255] by qualified dietitians and three day food records. Dietary composition was analysed using the FoodWorks software system (Xyris Software, Professional Version, 2002, Brisbane, Australia).

Body weight and body composition

Body weight was measured using a Tanita Scale (model Tanita TBF622, Tanita Corp., Tokyo, Japan) [246] in an upright position with minimal clothing and without shoes at baseline, four, eight and 12 weeks. Body composition was assessed using the DEXA method (Hologic Discovery QDR Series, Hologic Inc., MA, USA) at baseline and the end of study.
Table 4-3. Comparison of dietary prescriptions to the Control, Animal protein, and Plant protein groups, based on an example of 1450 kcal/day dietary intake (HIPHOP study)

<table>
<thead>
<tr>
<th>Food/food groups</th>
<th>Control</th>
<th>Animal</th>
<th>Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables</td>
<td>5 serves/day</td>
<td>5 serves/day</td>
<td>5 serves/day</td>
</tr>
<tr>
<td>Fruits</td>
<td>2 serves/day</td>
<td>2 serves/day</td>
<td>2 serves/day</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>8 serves/day</td>
<td>4 serves/day</td>
<td>3½ serves/day</td>
</tr>
<tr>
<td>Bread, cereals,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pasta, rice, potato,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>corn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk, yoghurt,</td>
<td>1 serve/day¹</td>
<td>2 serves/day</td>
<td>2 serves/day</td>
</tr>
<tr>
<td>cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein sources</td>
<td>60g red meat/day</td>
<td>180g red meat or</td>
<td>120g meat</td>
</tr>
<tr>
<td></td>
<td>1 egg/week</td>
<td>chicken/day</td>
<td>substitute²/day</td>
</tr>
<tr>
<td></td>
<td>150g fish/week</td>
<td>145g oily fish/week</td>
<td>125g legumes/week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105g white fish/week</td>
<td>3 eggs/week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 eggs/week</td>
<td>300g tofu/week</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45g soy powder/day</td>
</tr>
<tr>
<td>Fats</td>
<td>5 serves/day</td>
<td>5 serves/day</td>
<td>6 serves/day</td>
</tr>
<tr>
<td>Oils, margarine,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nuts, avocado</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient analysis</td>
<td>Energy: 1445 kcal/day</td>
<td>Energy: 1437 kcal/day</td>
<td>Energy: 1452 kcal/day</td>
</tr>
<tr>
<td></td>
<td>Prot: 55g/day (16%)</td>
<td>Prot: 103g/day (30%)</td>
<td>Prot: 106g/day (30%)</td>
</tr>
<tr>
<td></td>
<td>CHO: 189g/day (55%)</td>
<td>CHO: 137g/day (40%)</td>
<td>CHO: 138g/day (40%)</td>
</tr>
<tr>
<td></td>
<td>Fat: 46g/day (29%)</td>
<td>Fat: 47g/day (30%)</td>
<td>Fat: 47g/day (30%)</td>
</tr>
</tbody>
</table>

¹ Calcium supplements provided to meet daily requirement
² Sanitarium vegetarian casserole mince
Energy expenditure and substrate oxidation

The WRC was used to assess 24-hour energy expenditure (EE) and substrate metabolism through gaseous exchanges at baseline and 12-weeks of intervention. Baseline diet was standardised while the 12-week diet was modified to match dietary target of study intervention. The operating conditions of the facility and its experiment protocol have been previously described. Urinary nitrogen excretion was measured using the Kjeldahl method. The oxidation rates of macronutrients were calculated based on gaseous exchanges and urinary nitrogen. Changes in EE after weight loss were calculated as $\Delta EE/\Delta W$ where $\Delta EE$ is the reduction in EE and $\Delta W$ is the weight changes from baseline to 12-weeks. $\Delta EE/\Delta W$ of this study was then compared with data reported from other weight loss studies.

Biochemistry analyses

Fasting blood samples were collected by trained staff at baseline and 12-weeks and sent to quality assured pathology laboratory (Southern IML Pathology) for analysis of blood lipids.

4.2.2.6 Data analysis

Data was analysed using SPSS (version 15.0.0; SPSS Chicago, IL, 2006) and SAS V9.1 (SAS Institute, Cary NC). Changes in weight loss were analysed using a linear mixed model for repeated measures (PROC MIXED)$^1$. This method was chosen as it uses all available data and therefore partial results

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$^1$ This statistical analysis was performed with the help from Dr Marijka Batterham who was a biostatistician.
from subjects who dropped out of the study or missed a study weigh-in were included. Secondary analyses were conducted using the general linear model for repeated measures, data at only two time points was available for secondary outcomes. Model assumptions were checked prior to analysis. Data on body fat was adjusted for sex, and energy expenditure and substrate oxidation data was adjusted for body composition using a linear regression model $Y = a_0 + a_1 X$ Fat-free mass (kg) + $a_2 X$ Fat mass (kg). A linear regression model was also used to investigate relationship between $\Delta$EE and $\Delta W$.

### 4.2.3 Results

A total of 102 volunteers expressed interest and were screened. Twenty-seven were not eligible, while 14 lost contact after obtaining information. Sixty one eligible participants were enrolled, and randomised into the Control (n=20), Animal protein (n=21), and Plant protein (n=20) groups. Ten participants withdrew their consent prior to the commencement of the study due to either lack of time or feeling claustrophobic in the calorimeter reasons. Of the 51 participants who started, 35 completed the study (Figure 4-3). Reasons given for loss to follow up were not related to dietary prescription and included lack of time, family issues and work commitment. At baseline, there were no significant differences between groups in demographic, anthropometric, biochemical parameters and dietary intakes. Mean age, BMI and percentage body fat was $45.6 \pm 8.5$ years, $32.22 \pm 3.28$ kgm$^{-2}$, and $35.45 \pm 6.67\%$ respectively.
Compliance to diet and physical activity intervention strategies were as prescribed. Through diet history, all groups reported reduced total energy, carbohydrate, and fat intakes after 3 months (time effect $P<0.001$) (Table 4-4). Participants assigned to the two high protein arms successfully maintained a significantly higher protein intake levels than the low protein group (interaction effect, $P=0.020$). Free-living physical activity measured by accelerometer did not change ($P=0.510$).
Table 4-4. Mean dietary intake, obtained using a diet history method, at baseline and 3 months of the study according to group randomisation (HIPHOP study).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3-month</th>
<th>Time P</th>
<th>Diet P</th>
<th>Interaction P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy intake (Kcal/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>2690±803</td>
<td>1698±366</td>
<td>0.000*</td>
<td>0.632</td>
<td>0.675</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>2403±827</td>
<td>1695±430</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>2584±678</td>
<td>1776±519</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein intake (g/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>120.5±29.5</td>
<td>78.2±20.4</td>
<td>0.003*</td>
<td>0.230</td>
<td>0.020*</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>110.8±35.6</td>
<td>112.7±30.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>121.5±25.0</td>
<td>107.9±24.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fat intake (g/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>94.2±35.4</td>
<td>51.1±17.3</td>
<td>0.000*</td>
<td>0.749</td>
<td>0.843</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>84.0±50.4</td>
<td>51.8±17.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>95.6±41.6</td>
<td>54.8±26.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate intake (g/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>320.1±110.4</td>
<td>211.2±54.2</td>
<td>0.000*</td>
<td>0.066</td>
<td>0.733</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>251.1±68.1</td>
<td>169.5±56.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>265.0±67.8</td>
<td>178.7±47.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P:S ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>0.516±0.190</td>
<td>0.442±0.199</td>
<td>0.138</td>
<td>0.229</td>
<td>0.120</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>0.466±0.156</td>
<td>0.284±0.155</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>0.418±0.161</td>
<td>0.462±0.262</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All groups lost weight (time effect, P<0.001) but there was no intervention effect for weight and body composition changes (Table 4-5). The sex by group (P<0.001) and time by sex (P=0.016) interactions were significant indicating that males lost more weight than females in all groups. An initial sample size calculation based on the average of the significant between group differences in the review paper by Halton and Hu [32] suggested a clinically and statistically relevant difference between groups was 3 kg. Based on a power of 80% it was determined that 11 subjects in each group would need to complete the study to detect this difference.
<table>
<thead>
<tr>
<th>Anthropometric &amp; biochemical parameters</th>
<th>Baseline (mean±sd)</th>
<th>3-month (changes from baseline)</th>
<th>Time P</th>
<th>Diet P</th>
<th>Interaction P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n=10</td>
<td></td>
<td></td>
<td>0.000*</td>
<td>0.866</td>
<td>0.6012</td>
</tr>
<tr>
<td>Animal, n=12</td>
<td></td>
<td></td>
<td>0.000*</td>
<td>0.986</td>
<td>0.826</td>
</tr>
<tr>
<td>Plant, n=13</td>
<td></td>
<td></td>
<td>0.000*</td>
<td>0.309</td>
<td>0.731</td>
</tr>
<tr>
<td>Weight, kg 91.4±14.7</td>
<td>-5.4±4.0</td>
<td>-6.1±4.6</td>
<td>-5.2±2.4</td>
<td>0.000*</td>
<td>0.866 0.6012</td>
</tr>
<tr>
<td>Fat-free mass, kg 59.4±13.6</td>
<td>-2.2±1.5</td>
<td>-2.1±1.9</td>
<td>-1.8±2.1</td>
<td>0.000*</td>
<td>0.986 0.826</td>
</tr>
<tr>
<td>Fat mass, kg 32.0±6.5</td>
<td>-3.0±3.0</td>
<td>-3.8±3.1</td>
<td>-3.6±2.1</td>
<td>0.000*</td>
<td>0.309 0.731</td>
</tr>
<tr>
<td>Waist, cm 98.0±9.8</td>
<td>-5.2±5.4</td>
<td>-5.3±4.4</td>
<td>-4.7±2.9</td>
<td>0.000*</td>
<td>0.309 0.731</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L) 5.06±0.82</td>
<td>-0.08±0.53</td>
<td>-0.13±0.47</td>
<td>-0.32±0.29</td>
<td>0.010*</td>
<td>0.442 0.200</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L) 1.54±0.75</td>
<td>-0.13±0.64</td>
<td>0.24±0.70</td>
<td>-0.05±0.46</td>
<td>0.793</td>
<td>0.944 0.163</td>
</tr>
<tr>
<td>LDL (mmol/L) 2.93±0.91</td>
<td>0.03±0.41</td>
<td>-0.17±0.44</td>
<td>-0.21±0.24</td>
<td>0.021*</td>
<td>0.559 0.332</td>
</tr>
<tr>
<td>HDL (mmol/L) 1.43±0.33</td>
<td>-0.05±0.17</td>
<td>-0.07±0.14</td>
<td>-0.10±0.15</td>
<td>0.038*</td>
<td>0.653 0.903</td>
</tr>
</tbody>
</table>

Despite stratified block randomisation an uneven dropout rate meant that the control group had 10 subjects. The small weight differences between groups led to the acceptance of the null hypothesis that the difference between the groups was not significant and it was concluded that the differences between the groups was not clinically relevant. Reductions in total cholesterol and LDL cholesterol were found in all groups (time effect, P=0.010). Triacylglycerol levels did not change throughout the study and HDL cholesterol decreased significantly in all groups (time effect, P=0.038).
After 3 months, energy expenditure measured by whole room calorimeter decreased significantly in all groups (time effect, $P=0.002$) and there was an effect of diet ($P=0.009$) where greater reduction was observed in the Control group. However, there was no interaction between groups (Table 4-6). Carbohydrate oxidation decreased in all groups (time effect, $P<0.001$) but with no diet or interaction effects. There was a diet effect on protein oxidation ($P=0.007$) but no time or interaction effects. There were no significant effects on fat oxidation. Although not statistically significant, post-intervention daily fat oxidation in the Animal protein and the Plant protein groups were about 10 g and 50 g higher than the low protein group, making the differences arguably of clinical importance. It should also be noted that the extra fat oxidation came from body storage as the oxidised fat exceeded reported fat intake (Table 4-4).

As there was no treatment effect on $\Delta$EE/$\Delta$W (between 3 groups, $p=0.578$; between Control and combined high protein groups, $p=0.439$), a total group analysis was performed. Every kilogram of weight loss was found to result in a reduction in total daily energy expenditure of 26.67 kcal/day (linear regression, $p=0.006$).
Table 4-6. Mean energy expenditure and substrate oxidation of participants at baseline and 3 months of the HIPHOP study according to group randomisation.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3-month</th>
<th>Time P</th>
<th>Diet P</th>
<th>Interaction P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy expenditure¹ (Kcal/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>2003±169</td>
<td>1880±142</td>
<td>0.002*</td>
<td>0.009*</td>
<td>0.754</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>2075±126</td>
<td>1934±121</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>2161±175</td>
<td>2076±205</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein oxidation¹ (g/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>89.2±9.7</td>
<td>71.2±15.9</td>
<td>0.329</td>
<td>0.007*</td>
<td>0.230</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>87.3±14.8</td>
<td>88.0±20.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>99.2±28.5</td>
<td>101.8±24.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fat oxidation¹ (g/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>56.7±35.3</td>
<td>54.2±54.6</td>
<td>0.266</td>
<td>0.146</td>
<td>0.116</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>82.5±28.3</td>
<td>64.0±30.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>77.7±35.2</td>
<td>103.9±47.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate oxidation¹ (g/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n=10</td>
<td>276.9±56.8</td>
<td>163.3±55.6</td>
<td>0.000*</td>
<td>0.584</td>
<td>0.191</td>
</tr>
<tr>
<td>Animal protein, n=12</td>
<td>232.3±55.7</td>
<td>166.6±33.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant protein, n=13</td>
<td>252.6±87.9</td>
<td>129.2±42.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Adjusted for lean mass and fat mass

4.2.4 Discussion

The aim of this study was to determine if high protein diets differing in protein source had differing effects on weight loss, body composition, energy expenditure or metabolic risk factors. Compliance to dietary prescriptions and maintained physical activity levels confirmed the efficacy of reduced energy consumption on weight loss. Despite successful delivery of a higher protein diet, weight loss in all groups was not significantly different with similar levels of energy restriction.

Two weight loss studies comparing control and high protein diets over three-week [228] and nine-week [256] periods have reported significantly greater
retention of fat-free mass in the high protein groups while losing a similar amount of fat mass. A greater retention of metabolically active fat-free mass may imply less reduction in energy expenditure during weight loss. This may explain the successful weight maintenance with high protein ad libitum diet post weight loss [257]. However, higher fat-free mass retention and weight loss within and between the two high protein groups were failed to be observed in this study. This lack of difference in loss of fat-free mass is consistent with the similar daily energy expenditure found between groups post-intervention.

During weight loss, metabolically active fat-free mass decreases alongside fat mass. Consequently, energy expenditure decreases and the energy deficit will become smaller and weight loss will eventually reach a plateau. This may partly explain why expected weight loss was not achieved in some studies [6]. Our data revealed that total daily energy expenditure of participants reduced by 26.7 kcal/kg weight loss/day and this value was close to the values reported by two other studies [258, 259]. The decrease in energy expenditure may also be due to a loss in organ mass, not just fat-free mass [260]. This reduction in energy expenditure is a form of physiological adaptation and it will have negative impact on weight loss as body weight reduces. As expected, total and LDL cholesterol reduced with weight loss. An improvement in blood lipids following high protein diets has also been previously suggested [261]. Although statistically significant, reduction in HDL over time was minimal and not clinically significant. This reduction is likely to be related to the physical activity level which was maintained in this trial, instead of due to dietary intervention.
Acute metabolic studies have consistently demonstrated that the thermic effect of food increases with the proportion of protein in a meal [123], an effect attributed to the higher metabolic costs of amino acid metabolism [100]. It remains unclear if this thermic effect, at the magnitude of up to 14.6% [122], can be extended over longer period of time and beyond laboratory environments. Higher energy expenditure related to increased protein intake reported in the literature was not observed [32]. Possible explanations are the differences in study design and methodology (acute vs. longer term). Studies reporting higher energy expenditure following consumption of higher protein meals were either acute feeding trials, or used a more extreme protein level in their test meals. It was therefore speculated that differences between our control (approximately 20%) and high protein groups (30%) may not have been large enough to produce the desired thermogenic effects over longer periods of time.

Another explanation may be the thermogenic effect of high protein meals observed under well-controlled laboratory environment was "washed out" in the free-living environment. This may have been due to physiological adaptations to higher protein intakes over time or variability in food intakes compared to those in the laboratory environment. In the first case, there is little that can be done and it simply means that using a high protein cuisine may only work acutely but not over longer periods of time. In the latter case, the challenge is to make higher protein foods more desirable and available.

Fat oxidation rates have been reported to be significantly higher following the consumption of higher protein meals by overweight individuals [133]. This
indicated a value of high protein meals in weight management, as obese adults have been reported to have impaired fat oxidation compared to their lean counterparts [262]. In this study fat-oxidation enhancing effect in high protein groups was not observed, but so have others [96, 263, 264].

From a practical perspective, a challenge was faced in recruitment and in meeting the 30% protein target in the Plant protein group and soy protein powder supplementation was warranted. In plant foods the protein to non-protein ratio is generally much lower than animal food [251] which makes it difficult to achieve high dietary protein levels. Thus research of this kind is difficult to implement. However the results are promising and provide useful direction for future research.

### 4.2.5 Conclusion

The higher thermogenic effect from high protein diets did not translate into greater weight loss in high protein diet groups compared to low protein diet group. This may due to the wash-out effects in a free-living environment, or too small differences in dietary protein between low and high protein diets such that the desirable metabolic effects could not be observed. The higher protein retention in the high animal protein meal as demonstrated in the acute feeding trial was not seen in the long term study where fat-free mass was not different between the two high protein arms.
4.3 Summary of acute feeding and longer term high protein interventions

The outcomes from longer term study seem to suggest that there were no additional benefits in increasing the proportion of protein in a weight loss diet. However, the potential of this diet should not be dismissed based on these outcomes 

per se.

Since acute feeding studies have consistently demonstrated thermogenic effects from high protein diets, the failure to extend this observation into a free-living environment may largely be due to a lack of control. Dietary compliance may be a key limitation in long term studies. It is highly probable that high protein diets are useful in weight management, but they will have to be closely monitored regularly in term of clinical outcomes and compliance to the dietary intervention. In a later chapter of this thesis (Chapter 6) the possible use of high protein diets in practice is explored alongside considerations for achieving and maintaining successful weight loss. Having said this, it is recommended that a high protein diet should include protein-rich foods from all sources especially plant protein which is able to provide additional important nutrients such as phytochemicals and fibre.
CHAPTER 5. ROLE OF DIETARY POLYUNSATURATED FATTY ACID IN WEIGHT MANAGEMENT

Dietary protein is not the only macronutrient that may have a critical effect on weight loss. This thesis focuses on the manipulation of dietary macronutrients and studies on the manipulation of dietary protein have been reported in the previous chapter. Studies investigating the effects of dietary fat subtype manipulation are reported in this chapter. Dietary PUFA was observed to enhance fat oxidation humans [161, 162] and observations from a recent study also suggest a difference in thermic effect between fat subtypes [160]. Higher fat oxidation implies lower fat retention in the body. However, these trials either supplemented the test diets with fish oil, or used high-fat test meals. This means that these observations have limited application in the context of weight loss and test diets should therefore be low in fat, and PUFA should be increased by incorporating PUFA-rich foods to replicate food consumption pattern in a free-living environment.

On a population level, PUFA intake has been shown to be associated with lower body fat mass, especially in the abdominal region [166, 169]. Abdominal adiposity is a main predictor of the onset of other metabolic diseases [11] and hence needs to be treated among obese individuals. Based on the evidence from animal and human studies, it is likely that increasing the proportion of PUFA within a low-fat diet (30%E) may be beneficial for weight and loss of fat mass. A similar approach to that reported in the previous chapter was used to investigate the effectiveness of high PUFA diet, where the metabolic
mechanisms of high PUFA meals were first established using an acute feeding study design. Following that, whether or not the metabolic benefits of high PUFA diet could be seen in a longer period of time in a free-living environment was investigated. To determine this, anthropometric differences following high PUFA diets and a control diet were compared.

The overall aims of this chapter were to test the effectiveness of increased PUFA intake in promoting weight and fat mass losses, and to test the hypothesis that increased PUFA intake leads to loss of visceral adipose tissue. To achieve these aims, two studies are reported in this chapter. First, the effects of dietary PUFA manipulation were investigated using an acute feeding design, followed by a study of longer term changes in weight and fat mass associated with increased PUFA intake.
5.1 Acute effects of meals with increased PUFA on energy expenditure and fat balance (FAME study)

5.1.1 Background

Because overweight and obesity are characterised by the accumulation of body fat as a result of positive energy balance, the treatment of weight problems should focus on the promotion of weight loss especially in the form of fat mass. The loss of fat mass happens under two conditions, when negative energy balance occurs in the body (hence the use of body fat as fuel), and when the amount of fat oxidised exceeds fat intake. There is evidence suggesting higher thermic effect of PUFA and its preferential oxidation by the body. It remains unclear if higher fat oxidation is sufficient to induce negative fat balance. The study reported here therefore aimed to investigate the effects of increased PUFA intake on 24-hour energy expenditure and fat balance.

5.1.2 Methods

5.1.2.1 Experimental protocol

This study included data obtained from an acute feeding trial which investigated the effects of meals containing walnuts on 8-hour substrate oxidation [265]. This analysis differs from the parent paper where 8-hour data from the original trial was extrapolated into 24-hour values to enable comparison with 24-hour dietary consumption data. The original study was conducted in the WRC (two 8-hour stays). The order of the two test meals was randomised and the WRC protocol used for 8-hour study has been previously outlined. Participants for the study
attended a pre-study assessment where they were shown the facility, and anthropometric and dietary information was obtained. Prior to each of two stays in the whole room calorimeter, participants were asked to consume a standard dinner meal provided to them (35% energy based on Schofield equation) (Table 5-1). They were asked to fast for 10 hours following the standard dinner meal to eliminate residual postprandial thermogenesis. The remaining energy (65%) was provided as breakfast and lunch. Test meals were prepared by qualified dietitians to ensure consistency in food preparation. The study protocol was approved by the Human Research Ethics Committee of the University of Wollongong (approval number HE07/010).

5.1.2.2 Participants

Participants were volunteers recruited from the local community as well as from the University. To allow for dropouts, 24 participants (12 males and 12 females) were required. Inclusion criteria were aged >18 years, overweight (BMI 25 – 37 kgm^{-2}), not taking insulin, not pregnant or lactating, non-smoker, no food allergies, and generally well. Volunteers who had an acute illness requiring treatment or chronic conditions likely to alter metabolic rate such as thyroid abnormality were excluded from the study.

5.1.2.3 Test meals

Participants’ menus were modelled based on the individual’s energy requirement. The two test meals were either control (low PUFA) or high in PUFA. Meals were prepared using foods purchased from the local market and
were prepared by qualified dietitians in a metabolic kitchen. The control meal contained olive oil, rather than walnuts in the high PUFA meal. The nutrient breakdown of the test diets were analysed with FoodWorks Professional 2007 (Xyris Software, Brisbane, Australia) (Table 5-1).

5.1.2.4 Anthropometric and biomedical measurements

Height, weight and percentage body fat (Tanita TBF622, Tanita Corp., Tokyo, Japan) were measured during a pre-study assessment and prior to each calorimeter stay. Physical activity level in the chambers was monitored using triaxial accelerometers (RT3, version 1.2, StayHealthy Inc., Monrovia, CA, USA) to confirm the assumed sedentary state. Fasting blood samples were obtained by trained professionals and sent for analyses of glucose and insulin levels (Southern IML Pathology, Wollongong, NSW, Australia).

5.1.2.5 Measurements of energy and substrate metabolism

Energy expenditure was assessed based on gaseous exchanges measured in the WRC. Protein metabolism was estimated through urinary urea nitrogen, and energy expenditure and carbohydrate and fat oxidation rates were calculated using equations which have been outlined in previous chapter.
Table 5-1. Energy and nutrient composition and food content of test meals (FAME study)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>High PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/d</td>
<td>1839 ± 277</td>
<td>1803 ± 276</td>
</tr>
<tr>
<td>Carbohydrate, g/d (%)</td>
<td>259.9 ± 38.1 (57.8%)</td>
<td>216.8 ± 29.9 (49.2%)</td>
</tr>
<tr>
<td>Protein, g/d (%)</td>
<td>62.1 ± 9.6 (13.8%)</td>
<td>99.6 ± 16.7 (22.6%)</td>
</tr>
<tr>
<td>Fat, g/d (%)</td>
<td>56.8 ± 9.1 (28.4%)</td>
<td>55.2 ± 9.2 (28.2%)</td>
</tr>
<tr>
<td>SFA, g/d (%)</td>
<td>16.1 ± 2.2 (7.9%)</td>
<td>15.5 ± 1.9 (7.8%)</td>
</tr>
<tr>
<td>MUFA, g/d (%)</td>
<td>29.2 ± 4.9 (14.3%)</td>
<td>18.4 ± 3.6 (9.2%)</td>
</tr>
<tr>
<td>PUFA, g/d (%)</td>
<td>7.5 ± 1.4 (3.6%)</td>
<td>17.9 ± 3.1 (8.9%)</td>
</tr>
</tbody>
</table>

Meals

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muesli/cereal with low-fat milk</td>
<td>Ham, tomato toasted sandwich</td>
</tr>
<tr>
<td></td>
<td>Toast with margarine &amp; honey</td>
<td>Walnut</td>
</tr>
<tr>
<td></td>
<td>Fruit</td>
<td>Fruit</td>
</tr>
<tr>
<td>Lunch</td>
<td>Ham, cheese, tomato sandwich</td>
<td>Steak with potato and vegetables</td>
</tr>
<tr>
<td></td>
<td>Salad with olive oil</td>
<td>Orange juice</td>
</tr>
<tr>
<td>Dinner (prior to study)</td>
<td>Vegetarian lasagna</td>
<td>Vegetarian lasagna</td>
</tr>
<tr>
<td></td>
<td>Raisin toast</td>
<td>Yoghurt</td>
</tr>
</tbody>
</table>

5.1.2.6 Data analysis

Energy and substrate metabolism measured over the 8-hour period were extrapolated into 24-hour values. Although the extrapolation of energy and substrate metabolism values (by multiplying a factor of three) may introduce errors, these errors were nonetheless systematic in both meal tests and would be less important statistically in the repeated measure analysis. Energy and substrate balances were calculated as energy/substrate intake minus energy/substrate utilisation. Both energy and substrate metabolism were adjusted for body composition (fat mass and fat-free mass) using a regression model $Y = a_0 + a_1 \times \text{fat-free mass (kg)} + a_2 \times \text{fat mass (kg)}$. Weight, body
composition, energy and substrate metabolism between the two test meals were tested for differences using paired sample t-test (parametric data) and Wilcoxon sign-ranked test (non-parametric data) (SPSS, version 15.0.0; SPSS Chicago, IL, 2006). As participants with type 2 diabetes were included in this study, the effect of diabetes on energy and substrate metabolism was tested using the general linear model for repeated measures.

5.1.3 Results

Thirty-eight volunteers responded to the advertisements, of which 21 met the inclusion criteria and 16 completed the study. Twelve participants had normal glucose tolerance while the remaining four had type 2 diabetes. Reasons for withdrawal included time commitment (n=2), exited the calorimeter facility early (n=2), and feeling uncomfortable in a confined space (n=1). From the 21 participants at baseline (7 males and 14 females), 12 were randomised to receive a control low PUFA diet while the remaining received high PUFA meals during the first calorimeter stay. Anthropometric measurements of participants were presented in Table 5-2 and they were not different between the first and second WRC stays.
# Table 5-2. Characteristics of meal test FAME study participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>52.8 ± 10.0</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>7 / 9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88.8 ± 13.5</td>
</tr>
<tr>
<td>BMI, kg/m^2</td>
<td>31.2 ± 2.9</td>
</tr>
<tr>
<td>% body fat</td>
<td>38.0 ± 7.7</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>10.4 ± 5.3</td>
</tr>
</tbody>
</table>

Energy expenditure after the consumption of two test meals was not different (Table 5-3). Diabetes did not have an impact on energy expenditure (P=0.822) and RQ (P=0.181). Carbohydrate oxidation rates were significantly lower and fat oxidation rates were significantly higher following the consumption of high PUFA meals as compared to the control meals. Protein oxidation was not different between the two test meals. When energy expenditure and substrate oxidation were compared to energy and substrate intakes, both diets produced negative energy balance and positive protein balance. The high PUFA diet produced a significantly greater negative fat balance (P=0.002) while the control diet produced a significantly greater negative carbohydrate balance (P=0.018) (Figure 5-1).
Table 5-3. Energy and substrate metabolism following consumption of two test meals (FAME study)

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Control</th>
<th>High PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No adjustment (n=16)</td>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt; (n=14)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Energy, kcal/d</td>
<td>2033 ± 399</td>
<td>2013 ± 314</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>32.9 ± 8.2</td>
<td>31.7 ± 6.3</td>
</tr>
<tr>
<td>CHO, g/d</td>
<td>357.0 ± 82.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>345.3 ± 51.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>55.7 ± 40.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>58.7 ± 41.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for fat mass and fat-free mass  
<sup>b</sup> Body composition of 2 participants were not obtained, one due to physical disability (with diabetes) and one without diabetes (missing data)  
<sup>c</sup> Significant difference between diets, paired sample t-test, P<0.001  
<sup>d</sup> Significant difference between diets, Wilcoxon sign-ranked test, P=0.001  
<sup>e</sup> Significant difference between diets, Wilcoxon sign-ranked test, P=0.002

Figure 5-1. Substrate balance following consumption of control and high PUFA diets (FAME study).

*P<0.05, solid bars – control diet, white bars – high PUFA diet
5.1.4 Discussion

This study confirms the observations by Jones and colleagues [161, 162], where higher fat oxidation rates were observed following the consumption of meals with higher P:S ratios. Under the circumstances of energy deficit (control: -194 ± 298 kcal/d; high PUFA: -230 ± 345 kcal/d), negative fat and carbohydrate balance was observed. However, protein balance was not affected by the energy deficit. With an increased proportion of PUFA, there was a shift in the selection of fuel as the energy source where fat was oxidised preferentially instead of carbohydrate. As a result, a greater negative fat balance was observed, implying that endogenous fat storage was utilised. This property of a high PUFA diet may be particularly important to overweight and obese individuals where their fat oxidation capability has been observed to be compromised [262].

Despite the higher overall protein proportion in the high PUFA diet, energy expenditure was not different from the control diet. This may have been because the proportion of protein contained in the breakfast and lunch meals was not different (control: 51.5 ± 18.0 g/8h; high PUFA: 51.5 ± 22.1 g/8h, the difference only came from dinner meals) during the 8-hour calorimeter measurements. Similar levels of protein intake throughout the WRC measurements also imply that the higher fat oxidation rate observed during high PUFA feeding was a result of the PUFA but not other nutrients. The higher thermogenic effects of PUFA as reported by another study [160] were failed to be observed.
This study also demonstrated that the manipulation of dietary PUFA intake can be easily achieved by careful dietary modelling (based on core food groups) that incorporates PUFA rich foods. In this study, PUFA intake was elevated by simply adding walnut into the diets without changing the dietary pattern or increasing the overall proportion of fat. In fact, dietary fat was able to be maintained at a level of 30% of daily energy consumption, a level recommended by most healthy eating guidelines. The successful dietary modelling also implies that optimising PUFA intake can be achieved by inclusion of whole foods instead of supplements. This is important as food should be the basic unit in nutrition [112].

5.1.5 Conclusion

By increasing the proportion of PUFA from 3.6% to 8.9% of total daily energy intake from PUFA-rich foods, without changing the overall proportion of fat in the diet, fat oxidation rates were significantly elevated. Negative energy balance and positive protein balance under the circumstance of energy deficit were achieved and these conditions are imperative in weight management where they may result in greater fat mass loss while maintaining metabolically active fat-free mass over the long period of time. The longer term effects of longer term high PUFA consumption are investigated in the next study.
5.2 Long term effects of high PUFA intake\(^1\) (HELP and HERO studies)

5.2.1 Background

Although high PUFA meals have been shown to induce daily negative fat balance, it is essential to examine if this effect could be extended over a longer period of time to produce greater fat mass loss. This is because results observed from acute feeding trials are rarely shown to be extended over time periods [266, 267]. The study reported here therefore intends to compare fat mass loss induced by high PUFA and control diets. Higher energy expenditure among those who consumed a high PUFA diet was not observed in the previous study. It was expected that any weight loss observed with high PUFA diets would mainly be the result of the dietary energy deficit rather than increased thermogenesis.

Since there is evidence suggesting preferential loss of abdominal fat as a result of higher PUFA intakes [169], the changes in visceral adipose tissues (VAT) are also examined in this study. However, examining changes in VAT alone is insufficient to prove preferential oxidation of abdominal fat. Therefore, abdominal fat mass changes have to be examined alongside total fat mass loss using a mathematical model [268-270]. This model suggests that the loss of VAT is proportionate to the total fat mass loss which follows the relationship below:

\(^1\) This study utilised data obtained from two longer term dietary interventions. HELP study was funded by the National Health and Medical Research Council (Project Grant #354111) while HERO study was funded by the Californian Walnut Commission (USA).
\[
\frac{d\text{VAT}}{d\text{FM}} = k \frac{\text{VAT}}{\text{FM}}
\]

**Note:** \(d\text{VAT}\) and \(d\text{FM}\) refer to changes in visceral adipose tissue and fat mass; \(\text{VAT}\) and \(\text{FM}\) refer to initial visceral adipose tissue and fat mass. \(k\) is a dimensionless constant.

If there is preferential oxidation of \(\text{VAT}\) (higher \(d\text{VAT}\)) following consumption of high PUFA diets, the dimensionless constant \(k\) for high PUFA diets should be different from the \(k\) values from the control diets. This relationship is used in this study to test the hypothesis that there is preferential loss of \(\text{VAT}\) following high PUFA diets consumption.

The aims of this analysis were: a) to determine if increased PUFA intake would result in greater fat mass loss over a 12-week period, and b) to assess whether \(\text{VAT}\) was preferentially lost with increased PUFA intakes. To achieve these aims, data from two similar longer term dietary interventions [7, 271] were used.

### 5.2.2 Methods

#### 5.2.2.1 Experiment protocol

The two longer term dietary interventions (HELP and HERO studies) from which data was extracted had similar experimental protocols. The key intervention for both trials was increased dietary PUFA intake [7, 271]. HELP study was a 12-week randomised, controlled trial with four study arms while HERO study was a 52-week randomised controlled trial with two study arms (Table 5-4).
Table 5-4. Study arms of HELP and HERO studies

<table>
<thead>
<tr>
<th>HELP study</th>
<th>HELP study</th>
<th>HERO study</th>
<th>HERO study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat</td>
<td>High PUFA</td>
<td>Low fat &amp; Low energy</td>
<td>High PUFA &amp; Low energy</td>
</tr>
<tr>
<td>Isoenergetic low-fat diet with 20% protein, 50% carbohydrate, 30% fat (5% PUFA, 15% MUFA, 10% SFA)</td>
<td>Isoenergetic low-fat high PUFA diet with 20% protein, 50% carbohydrate and 30% fat (10% PUFA, 10% MUFA and 10% SFA)</td>
<td>A diet similar to LF but with a 500 kcal/d energy restriction</td>
<td>A diet similar to HPUFA but with a 500 kcal/d energy restriction</td>
</tr>
<tr>
<td>Isoenergetic low-fat high PUFA diet with 20% protein, 50% carbohydrate and 30% fat (10% PUFA, 10% MUFA and 10% SFA)</td>
<td>Isoenergetic high PUFA diet with 20% protein, 50% carbohydrate and 30% fat (10% PUFA, 10% MUFA and 10% SFA)</td>
<td>This study arm is the equivalent of the Low fat arm in the HELP study</td>
<td>This study arm was the equivalent of the High PUFA arm in the HELP study</td>
</tr>
</tbody>
</table>

For both trials, participants were recruited from the local community through media advertisement. Eligible participants attended a pre-study assessment session where anthropometric measurements, blood biochemistry, habitual dietary intake, and habitual physical activity levels were assessed. In both trials, participants in the high PUFA arms received structured dietary advice to incorporate PUFA-rich foods to achieve PUFA target of 10%. The intervention group in HERO study also received a supply of daily supplements of 30g walnuts. All participants received monthly supports from qualified dietitians to
help adhere to the prescribed diets for the first 12 weeks. After 12-week period, participants from the HERO study attended quarterly follow-up sessions.

### 5.2.2.2 Participants

In the HELP study [271], 20 participants were required in each of the four arms to provide a power of 80% (allowing three drop-outs per group), based on the published data on VAT changes of 20 cm$^2$ [166]. Inclusion criteria were aged >18 years and BMI > 25kgm$^2$. Participants were randomised into the four arms using random permuted blocks by a computerised random number generator. Exclusion criteria were: major illness, presence of food allergies or factors inhibiting the trials, illiteracy, and/or inadequate conversational English as dietary counselling was the key component of the interventions.

In the HERO study [7], 30 participants in each arm (allowing 10 drop-outs per group) were sufficient to provide a power of 96.7% based on the between group differences in percentage of body fat of 1.64 ± 2.26% from a previous study [159]. Inclusion and exclusion criteria were similar to the HELP study but participants diagnosed with type 2 diabetes mellitus (but not insulin-treated) were also included into this trial.
5.2.2.3 Ethics

The experiment protocols of both the HELP and HERO studies were approved by the University of Wollongong Human Research Ethics Committee (HELP: HE04/326; HERO: HE05/145). They were also registered with the Australian Clinical Trial Registry (HELP study: ACTRN12608000453381; HERO study: ACTRN12607000600448).

5.2.2.4 Dietary prescription and physical activity recommendations

Dietary recommendations for both HELP and HERO were based on core food groups to meet the target dietary nutrient composition profile of the different test diets. All participants received individualised dietary counselling and had the same amount of contact with qualified dietitians for the first 12 weeks of the studies. After that in HERO, participants were seen by dietitians at 6-month and 12-month. Participants in the HPUFA arms were asked to include PUFA-rich foods in the HELP study while participants in the HERO study received a daily supply of walnuts (30 g/d) to help achieve PUFA target. Participants were also advised to incorporate 5 X 30-minute physical activity per week.

5.2.2.5 Measurements

Body weight and body composition

Body weight and percentage body fat was measured using scales with bioelectrical impedance (Tanita TBF622, Tanita Corp., Tokyo, Japan) in an upright position with minimal clothing. Body fat measurements from these scales have been previously demonstrated to be comparable to those from
DEXA scans [246]. Single-slice abdominal CT scans were also taken at the fourth and fifth lumbar vertebra, as well as at the level of sacroiliac joints by a trained observer at a commercial x-ray facility in Wollongong (Southcoast X-Ray, NSW, Australia). Subcutaneous adipose tissues (SAT) and VAT areas were assessed at each level using SIENET Sky software (Siemens Corporation, NY, USA) that drives the CT scanner (Siemens AG, Munich, Germany). Using the VAT areas ($A_{VAT}$), total volume of VAT ($V_{VAT}$) at the abdominal region was calculated using equations described by Shen and colleagues [272]:

\[
\begin{align*}
\text{Men:} & \quad V_{VAT} = 0.0224 \times A_{VAT} + 0.162 \\
\text{Women:} & \quad V_{VAT} = 0.0205 \times A_{VAT} + 0.147
\end{align*}
\]

Dimensionless constant $k$ [269] was calculated for control and high PUFA diets using initial FM and VAT, as well as changes in FM (dFM) and VAT (dVAT).

Dietary intake

Habitual dietary intake was assessed using a validated diet history interview [255] conducted by dietitians and 3-day records. Dietary composition was analysed using the FoodWorks nutrient analysis software package (Xyris Software, Professional Version 2002, Brisbane, QLD, Australia). Intake of fatty acids were analysed using the AUSNUT fatty acid database (version 6, 2002) in the FoodWorks software package and Australian Fatty Acids Rev 6 2002 (RMIT, Melbourne, Australia).
5.2.2.6 Data analysis

For the purpose of this analysis, only data collected at baseline and 12-week was included. Data from the HELP and HERO studies were combined in order to test the relationship between fat mass and VAT changes. The study arms in both studies were collapsed into low fat (LF) and high PUFA (HPUFA) groups for comparison purposes. Data was analysed using SPSS (version 15.0.0, SPSS Chicago, IL, 2006). Baseline differences between diet groups were assessed using one-way ANOVA. Changes in the primary outcomes between study arms were compared using the general linear model for repeated measures ANOVA.

5.2.3 Results

5.2.3.1 Participants

From both the HELP and HERO studies, 273 participants were screened, where 200 of them were eligible and enrolled. They were randomly assigned to one of the study arms. Twenty-eight volunteers withdrew prior to the commencement of the study and 31 were lost further to follow-up visits. Of the original 200 participants, 141 completed the study (Figure 5-2). Baseline characteristics and habitual intake for these participants are presented in Table 5-5. All variables were not significantly different between the LF and HPUFA groups (one-way ANOVA, P>0.05).
**Figure 5-2.** Randomisation of participants into LF or HPUFA groups (HELP and HERO studies)

**Table 5-5.** Baseline characteristics of HELP and HERO studies participants

<table>
<thead>
<tr>
<th></th>
<th>LF</th>
<th>HPUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>47.8 ± 11.1</td>
<td>47.0 ± 10.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>90.8 ± 14.8</td>
<td>88.4 ± 13.9</td>
</tr>
<tr>
<td>BMI, kgm^2</td>
<td>32.1 ± 4.0</td>
<td>31.4 ± 3.9</td>
</tr>
<tr>
<td>Body fat, %‡</td>
<td>39.2 ± 6.6</td>
<td>39.4 ± 7.0</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>97.7 ± 12.4</td>
<td>96.6 ± 12.5</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>112.1 ± 8.5</td>
<td>111.6 ± 9.4</td>
</tr>
<tr>
<td>W:H ratio</td>
<td>0.87 ± 0.10</td>
<td>0.87 ± 0.09</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>2181 ± 694</td>
<td>2270 ± 744</td>
</tr>
<tr>
<td>Carbohydrate, %E</td>
<td>42.0 ± 7.0</td>
<td>43.8 ± 8.7</td>
</tr>
<tr>
<td>Protein, %E</td>
<td>20.3 ± 3.6</td>
<td>19.7 ± 3.7</td>
</tr>
<tr>
<td>Fat, %E</td>
<td>31.7 ± 6.1</td>
<td>31.7 ± 7.2</td>
</tr>
<tr>
<td>SFA, %E</td>
<td>10.9 ± 2.9</td>
<td>11.2 ± 2.7</td>
</tr>
<tr>
<td>MUFA, %E</td>
<td>12.3 ± 3.1</td>
<td>12.4 ± 4.3</td>
</tr>
<tr>
<td>PUFA, %E</td>
<td>5.5 ± 2.1</td>
<td>5.1 ± 2.0</td>
</tr>
</tbody>
</table>
5.2.3.2 Dietary change

After the 12-week intervention period, intakes of energy and macronutrients changed significantly within each study group (Table 5-6). Of all macronutrients, only the percentages of total fat (diet, \( P=0.018 \)) and PUFA (diet, \( P<0.001 \)) were significantly different between groups, where the LF group reduced fat intake and the HPUFA group increased PUFA consumption. The interaction effects were significant for carbohydrate (\( P=0.035 \)), SFA (\( P=0.003 \)) and PUFA (\( P<0.001 \)) proportions in the diets. The LF group increased the proportion of carbohydrate to substitute dietary fat while the HPUFA group increased their PUFA intake at the expense of SFA in the diet.

Table 5-6. Mean (± s.d.) dietary intakes at baseline and after 12-week of HELP and HERO studies

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12-week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>HPUFA</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>2181 ± 694</td>
<td>2270 ± 744</td>
</tr>
<tr>
<td>Carbohydrate, %E</td>
<td>42.0 ± 7.0</td>
<td>43.8 ± 8.7</td>
</tr>
<tr>
<td>Protein, %E</td>
<td>20.3 ± 3.6</td>
<td>19.7 ± 3.7</td>
</tr>
<tr>
<td>Fat, %E</td>
<td>31.7 ± 6.1</td>
<td>31.7 ± 7.2</td>
</tr>
<tr>
<td>SFA, %E</td>
<td>10.9 ± 2.9</td>
<td>11.2 ± 2.7</td>
</tr>
<tr>
<td>MUFA, %E</td>
<td>12.3 ± 3.1</td>
<td>12.4 ± 4.3</td>
</tr>
<tr>
<td>PUFA, %E</td>
<td>5.5 ± 2.1</td>
<td>5.1 ± 2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Time, P</th>
<th>Diet, P</th>
<th>Interaction, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal)</td>
<td>0.000*</td>
<td>0.243</td>
<td>0.639</td>
</tr>
<tr>
<td>Carbohydrate, %E</td>
<td>0.046*</td>
<td>0.804</td>
<td>0.035*</td>
</tr>
<tr>
<td>Protein, %E</td>
<td>0.000*</td>
<td>0.066</td>
<td>0.957</td>
</tr>
<tr>
<td>Fat, %E</td>
<td>0.000*</td>
<td>0.018*</td>
<td>0.066</td>
</tr>
<tr>
<td>SFA, %E</td>
<td>0.000*</td>
<td>0.470</td>
<td>0.003*</td>
</tr>
<tr>
<td>MUFA, %E</td>
<td>0.000*</td>
<td>0.909</td>
<td>0.242</td>
</tr>
<tr>
<td>PUFA, %E</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* Significant different, general linear model repeated measures ANOVA, \( P<0.05 \)
5.2.3.3 Anthropometric change

All groups lost weight significantly after the 12-week period. Subsequently, all other parameters were significantly reduced over time (time, \( P \leq 0.001 \)). However, these changes were not different between the LF and HPUFA groups and interaction effects were also not observed (Table 5-7).

Table 5-7. Comparison of weight, BMI, body fat percentage, waist circumference, area of VAT, and area of SAT before and after high PUFA intervention (HELP and HERO studies)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12-week</th>
<th>Time P</th>
<th>Diet P</th>
<th>Interaction P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF</td>
<td>HPUFA</td>
<td>LF</td>
<td>HPUFA</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>90.8 ± 14.8</td>
<td>88.4 ± 13.9</td>
<td>85.1 ± 13.7</td>
<td>84.0 ± 14.7</td>
<td>0.000*</td>
</tr>
<tr>
<td>BMI, kgm(^2)</td>
<td>32.1 ± 4.0</td>
<td>31.4 ± 3.9</td>
<td>30.2 ± 4.0</td>
<td>29.9 ± 4.4</td>
<td>0.000*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>39.2 ± 6.6</td>
<td>39.4 ± 7.0</td>
<td>35.8 ± 7.0</td>
<td>37.1 ± 8.3</td>
<td>0.000*</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>97.7 ± 12.4</td>
<td>96.6 ± 12.5</td>
<td>93.2 ± 11.5</td>
<td>93.4 ± 13.5</td>
<td>0.000*</td>
</tr>
<tr>
<td>VAT area, cm(^2)</td>
<td>156 ± 91</td>
<td>141 ± 81</td>
<td>130 ± 78</td>
<td>131 ± 90</td>
<td>0.000*</td>
</tr>
<tr>
<td>SAT area, cm(^2)</td>
<td>312 ± 102</td>
<td>338 ± 107</td>
<td>302 ± 108</td>
<td>316 ± 113</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* Significant different, general linear model repeated measures ANOVA, \( P \leq 0.05 \)

5.2.3.4 Relationship between fat mass and VAT changes

To test if the relationship between the changes of these two variables was allometric, 121 (58 LF and 63 HPUFA) complete data sets containing fat mass and VAT volume from the HELP and HERO studies were analysed. Baseline and post-intervention (12-week) measurements of weight, BMI, FM, FFM and VAT volume (calculated using equations outlined in Section 5.2.2.5) are presented in Table 5-8.
**Table 5-8.** Characteristics of participants (N=121) at baseline and 12-week of study (HELP and HERO studies)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12-week</th>
<th>Time</th>
<th>Diet</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF (n=58)</td>
<td>HPUFA (n=63)</td>
<td>LF (n=58)</td>
<td>HPUFA (n=63)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>48.4 ± 10.6</td>
<td>47.6 ± 10.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>88.0 ± 13.7</td>
<td>87.2 ± 14.0</td>
<td>84.7 ± 13.7</td>
<td>83.7 ± 14.6</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>BMI, kgm²</strong></td>
<td>31.3 ± 3.8</td>
<td>31.0 ± 4.0</td>
<td>30.1 ± 4.0</td>
<td>29.8 ± 4.4</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>FM, kg</strong></td>
<td>33.6 ± 8.2</td>
<td>33.9 ± 9.0</td>
<td>30.6 ± 8.2</td>
<td>31.3 ± 9.7</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>FFM, kg</strong></td>
<td>54.4 ± 9.9</td>
<td>53.3 ± 10.3</td>
<td>54.1 ± 10.2</td>
<td>52.4 ± 10.4</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>VAT volume, L</strong></td>
<td>3.5 ± 2.0</td>
<td>3.2 ± 1.8</td>
<td>2.9 ± 1.7</td>
<td>2.9 ± 2.0</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* Significant different, general linear model repeated measures ANOVA

Fat mass and volume of VAT decreased significantly in both groups after the 12-week period but there was no difference between LF and HPUFA groups. The dimensionless constant was \( k = 1.15 ± 3.59 \) for the LF group and \( k = 1.23 ± 2.86 \) for the HPUFA group, and they were not significantly different between groups (independent sample t-test, \( P=0.896 \)).

### 5.2.4 Discussion

From the diet history analyses, the HPUFA group showed an increased PUFA proportion in the diet while the LF groups did not change their PUFA consumption after 12-week. This indicates that the strategies employed to achieve target PUFA intake were effective and successful. One can increase the intake of PUFA either through careful dietary modelling to identify and incorporate PUFA-rich foods into the diet [271], or by supplementing with foods like walnuts [7].

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The fact that there was no significant difference in weight changes between the LF and HPUFA groups confirms the observations from the previous study (reported in Section 5.1), where diets higher in PUFA were not observed to be more thermogenic than the low PUFA diets. However, the fat-oxidising property of high PUFA diets observed in the same study did not appear to extend to a longer period of time, where body fat mass were not significantly lower in the HPUFA group. There was a significant interaction effect of treatment on FFM changes, however the differences were small between the LF and HPUFA groups (-0.3 kg/12 weeks vs. -0.9 kg/12 weeks) and were of limited clinical relevance.

Despite the reported link between higher PUFA consumption and lower abdominal adiposity [169], this was not observed in this study. First of all, the area of VAT that changed did not differ between the LF and HPUFA groups (Table 5-7), suggesting 12-week high PUFA consumption did not enhance the loss of adipose tissues in the abdominal region. Not only was there no preferential oxidation of abdominal fat from the high PUFA intake, the proportion of VAT loss to total fat mass loss also followed an allometric relationship (Section 5.2.3.4), where the dimensionless constant $k$ was not significantly different between the LF and the HPUFA groups. This implies that adipose tissues loss in the abdominal region depends on the total fat mass loss, rather than the intake of PUFA.
5.2.5 Conclusion

Energy restriction produced greater weight loss with or without increasing PUFA in the diet. High PUFA diets were not more thermogenic than low fat diets and the higher PUFA intake failed to produce greater fat mass loss despite greater fat oxidation rate observed in previous acute feeding trials. The dimensionless constant, $k$, was also not different implying that there was no preferential fat oxidation in the abdominal region by high PUFA diets. Finally, careful dietary modelling and supplement with PUFA-rich foods are effective strategies to increase PUFA intake.

5.3 Summary of acute feeding and longer term high PUFA interventions

Although the acute trial had demonstrated that higher fat oxidation rates and negative energy balance can be achieved acutely by increasing the proportion of PUFA within a diet, this longer term trial failed to observe them as significant clinical outcomes (fat mass and VAT loss). Preferential oxidation of adipose tissues at the abdominal region was also not observed. Failure to extend acute fat-oxidising effects to the longer term, however, does not mean that high PUFA diets are not beneficial in weight management. It was possible that the effects were washed out in the free-living environment, where there was more daily variation as compared to a laboratory environment. In the following chapter, possible reasons why expected changes in anthropometric measurements were not achieved are explored. It highlights the adaptations in the human body that may have produced lower-than-expected outcomes from the longer term dietary interventions reported in Chapter 4 and Chapter 5.
CHAPTER 6. USING A DYNAMIC ENERGY BALANCE FRAMEWORK TO PROMOTE AND MONITOR WEIGHT LOSS

In the previous chapters of this thesis, the acute effects of high protein and high PUFA meals on energy and substrate metabolism were observed but not extended to a longer period. These observations were consistent with other studies where weight loss decelerated over time and eventually reached a nadir [273, 274]. Actual weight loss was also observed to be smaller than the expected values [275]. Understanding the reasons behind these observations is therefore crucial otherwise there is no foundation to recommend dietary manipulation for weight management.

A number of reasons may explain why acute effects failed to be sustained over a longer term. On the one hand, there is less control in a free-living environment which may lead to lower compliance to the interventions while on the other hand there is evidence demonstrating adaptations of the human body [276-279] to the long-term changes introduced in order to promote weight loss. This implies that energy intake and energy expenditure, in fact, have an interactive relationship and a dynamic view of energy balance is hence essential in weight management. In this chapter, theoretical positions are considered and data from

1 The studies included in this chapter were funded by the National Health and Medical Research Council of Australia (HELP study, Project Grant #354111), the National Centre of Excellence in Functional Foods of Australia (HIPHOP study), and the California Walnut Commission of USA (HERO study).

Prepared manuscript:
Tan SY, Batterham M, Tapsell L: A dynamic view of energy balance may help weight loss in practice.

Conference Abstracts:
dietary interventions are reviewed to formulate ideas on how to manage weight more effectively from an energy metabolism and substrate utilisation perspective. This may help cut down the health costs related to obesity [280].

6.1 Energy balance and weight loss: new opportunities for clinical practice

The energy balance model in Figure 6-1 suggests that negative energy balance can be induced by decreasing energy intake and/or increasing energy expenditure. Energy expenditure is usually assumed to be equivalent to physical activity in practice, but in reality it also includes other components such as resting energy expenditure and the thermic effect of food, both of which could be manipulated in the intervention phase.

Figure 6-1. Simple energy balance model
Population-based lifestyle interventions for weight loss focus on strategies to reduce energy intake by eating better and less, and to increase energy expenditure by promoting physical activity. However, there may be opportunity to go into more detail in clinical practice when targeting weight loss in individuals. Weight loss is a dynamic process resulting from cumulative negative energy balance over time.[281] A more detailed approach in the clinical setting may be able to address the shifts in energy metabolism during the weight loss period.

A 14-week weight loss study reported that the observed weight loss was only 44% of the expected values based on the prescribed negative energy balance (energy deficit) [275]. This is a commonly reported phenomenon, where adaptive thermogenesis has been suggested to contribute to the observation of smaller-than-expected weight loss [278]. Applying this knowledge to clinical practice represents an opportunity to develop a more effective approach. This would take into consideration the physiological changes of the human body during weight loss and how these changes affect the outcomes of dietary and physical activity intervention. With this framework, practice could become more targeted over the time course of attempted weight loss.

Rather than a simple energy intake (diet) – energy expenditure (physical activity) counter-balance, the grouping of factors that promote weight loss onto one end of the scale, with inhibitory factors on the other (Figure 6-2) [282] is proposed. This means consideration of inhibitory factors (physiological adaptations that may inhibit or slow the weight loss process) and a detailed
analysis of promotional factors. What has been known for some time, that certain factors help to promote weight loss and others impede it, was built on.

**Figure 6-2.** Proposed dynamic energy balance framework for weight loss

<table>
<thead>
<tr>
<th>Promote</th>
<th>Inhibit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake deficit</td>
<td>Reduced energy expenditure during weight loss</td>
</tr>
<tr>
<td>Increased energy expenditure</td>
<td>Reduced energy expenditure during underfeeding</td>
</tr>
<tr>
<td>Body Composition</td>
<td></td>
</tr>
<tr>
<td>Fractional nutrient absorption</td>
<td></td>
</tr>
</tbody>
</table>

6.1.1 **Working with Factors that promote weight loss**

Mathematical models for addressing the dynamic aspects of energy exchange have recently emerged in the literature [283] this inspires hope that more effective practice can be developed. These models recognise a range of factors which promote weight loss, including: i) a deficit in energy intake, iii) increased energy expenditure, iii) body composition, and iv) fractional nutrient absorption. In view of developing approaches to practice, these were considered.
6.1.1.1 Achieving an energy intake deficit: another reason for increasing dietary protein?

A dietary prescription for weight loss using a 2 MJ deficit per day can be found in both clinical and research settings, to promote approximately half a kilogram of weight loss per week. The basis for this practice is that a cumulative deficit of 32.2 MJ has been suggested to contribute to one kilogram of body weight loss, as proposed as far back as 1958 [250]. There are a few underlying assumptions associated with these values, based on conceptions of energy density. In this context ‘energy density’ refers to the energy value of body weight which in turn is dependent on the relative composition of the body weight. The energy density of fat mass is similar to energy density of fat which is approximately 39.5 MJ/kg [61], but there is a lack of consensus on the energy density of the lean mass (non-fat). The differences in energy density of lean mass are mainly derived from the various ways of determining the amount of water associated with protein mass (the protein hydration quotient) [284]. It is reasonable, however, to consider a protein hydration quotient of 1.6 gram water per gram of protein (as proposed by Hall [284]) to give an energy density for the lean mass component of 7.6 MJ/kg. Thus, assuming that the proportion of fat mass in every kilogram of weight loss is $f$, then the value for fat mass is $39.5f + 7.6(1 – f) = 32.2$. Using this equation, $f = 0.77$ or body fat is 77% of weight lost. Using the assumption that one kilogram of bodyweight accounts for 32.2 MJ, then 77% of body weight comes from fat mass while the remaining from lean mass. Now, the value for $f$ may not be constant as there are indications from studies that the proportion of fat mass loss per unit weight loss increases with the proportion of protein in the
diet [228, 285]. This suggests that the proportion of protein in the diet may be additionally advantageous in maintaining a weight loss process.

6.1.1.2 Increased energy expenditure: searching for food combinations that have higher energy costs

Increasing energy expenditure is one of the commonest strategies used in weight loss interventions. This is usually achieved through increased physical activity, through exercise programs or spontaneous physical activity [51]. Energy expenditure can also be increased through the thermic effect of food. It has been shown that diets higher in proportions of protein exhibit the benefit of higher thermic effect of food [32], although with diets used in practice, this effect was not observed [65, 286]. Recently studies have shown that energy expenditure can also be enhanced with other dietary components such as catechins, caffeine, capsaicin, L-tyrosine and calcium [287, 288], and that some hormones that regulate appetite may also enhance energy expenditure [289]. Thus dietary protein itself may not be acting in isolation, but rather other factors may also contribute to dietary thermogenic effects. More research on ‘whole of diet’ approach using specific food combinations and with multiple pathways in mind may help to capture this promotional factor more effectively.

6.1.1.3 Body composition: a need to target food combinations that support fat loss

Body composition is itself an important metabolic consideration in promoting (and extending) weight loss. As discussed earlier, it was assumed that a
kilogram of weight loss results from a 32.2 MJ dietary deficit [250]. Recently, this assumption has been shown to be true only for overweight and obese individuals and is highly inaccurate for their lean counterparts [284]. In his simulation model which has been verified with actual data, Hall pointed out that the energy deficit required to lose a kilogram of body weight depends on the initial fat mass of each individual [284]. The higher the initial body fat mass, the higher energy density per kilogram of the body weight. The simulation model also showed that as an obese individual loses weight, the energy density decreases and this means it becomes easier for that person to lose weight subsequently. Thus body composition itself becomes a factor that can promote weight loss. It suggests that targeting fat loss is an important detail in a metabolic weight loss strategy. For dietary prescriptions, it means that diets with compositions that favor fat oxidation will be more beneficial. Increasing the PUFA proportion in the diet has been demonstrated to be capable of enhancing fat oxidation rates acutely but the challenge remains in sustaining such effects over a longer term during the weight loss process.

6.1.1.4 Fractional nutrient absorption: including foods with an influence on energy bioavailability

Another factor to consider is the actual absorption of energy. Not all macronutrients in the diet are completely digested and absorbed, therefore the actual energy intake may be lower than what is consumed. This happens for two main reasons. First, there could be some components in the diet that inhibit digestion or absorption of certain nutrients. Secondly, incomplete digestion and absorption of certain nutrients can contribute to low levels of bioavailability. The
total energy absorbed from an average diet has been shown to be around 95% [290, 291] and the fraction of nutrients absorbed has been assumed to be 90.9% for protein, 94.8% for fat and 95.3% from carbohydrate [292]. Evidence suggests that dietary factors that influence the absorption of nutrients include: fibre, type of starch, antinutrients, type of dietary fat, and dietary calcium [293-296]. Thus, including these components in the diet may also contribute to creating the desired negative energy balance for weight loss.

The weight loss promotional factors of energy intake deficit, increased energy expenditure, body composition and fractional nutrient absorption, while inherent in traditional practice (reduce energy intake and exercise) could be considered for more effective practice.

6.1.2 Addressing factors that Inhibit weight loss

While researchers and healthcare professionals focus on promotional factors, those that inhibit weight loss should not be overlooked. They are the human physiological adaptations that react to changes in body weight and in energy intake, such as reduced energy expenditure.

6.1.2.1 Reduced energy expenditure during weight loss: a need to increase physical activity as time progresses

Data from weight loss studies have consistently demonstrated that energy expenditure decreases as an individual loses weight. Repeated measurements of various components of energy expenditure before and after weight loss have
revealed that resting energy expenditure [120, 297, 298], sleeping metabolic rate [299] and total daily energy expenditure [299-301] all drop after weight loss. A drop in resting energy expenditure is related to the loss of metabolically active tissues (organ and fat free mass) during weight loss [302] while decreased total daily energy expenditure could be due to lower energy required for daily activities with lower body weight. Our weight loss trial reported in Section 4.2 observed that the magnitude of reduction was about 26.7 kcal/kg weight loss/d. The reduced expenditure from resting and sleeping metabolic rates means that increasing physical activity becomes all the more important over time as weight loss progresses.

6.1.2.2 Reduced energy expenditure during underfeeding: smaller reductions in dietary calories might be better in the long run

While energy intake restriction is a commonly used strategy in weight loss, this strategy can exacerbate an energy-conservation state in the human body. Underfeeding has been shown to produce lower resting energy expenditure than when the person with the same amount of lean mass is fed sufficient energy [21]. This is a reactive mechanism addressing the insufficient energy input no matter if the person is normal weight or obese [303]. A study in 1971 reported an average daily fat loss of 170 g when an energy deficit of 2100 kcal/d was provided to 41 obese subjects. The body fat loss represented an energy deficit of 1190 kcal/d (170 g X 7 kcal/g) and the gap of 910 kcal/d (2100 kcal/d – 1530 kcal/d) was suggested to be due to reduction in energy expenditure due to underfeeding [304].
A more recent 6-month weight loss study also reported a reduction in total daily energy expenditure, after accounting for changes in body composition, by 135 kcal/d following 25% energy restriction from the diet, 117 kcal/d after 12.5% energy restriction plus 12.5% increase in exercise, and 125 kcal/d following a very-low-calorie diet of 890 kcal/d [305]. A further analysis of the data revealed that this reduction was partly contributed by a reduced basal metabolic rate, which averaged 91 kcal/d for the combined three study groups [306]. The magnitude of reduction in energy expenditure following energy restriction reported by this study is important and significant as it means that an overweight or obese individual may only be in less than 400 kcal/d actual deficit while an energy restriction of 500 kcal/d is followed. Another important message embedded in this study was that it appears that the magnitude of energy restriction from the diet does not determine the magnitude of such reduction. Also, maximising energy deficit through the inclusion of structured exercise may not be beneficial in reducing energy expenditure reduction from energy restriction.

To summarise the scientific evidence on human metabolic adaptations to both weight loss and energy restriction, it appears that extremes in under-feeding are likely to only be beneficial in the short term. For a longer term weight management maximising energy deficit through smaller reductions in energy intake plus the incorporation of thermogenic nutrients in the diet (to minimise effects of adaptation to energy restriction) together with increased levels of physical activity (to counteract adaptations to weight loss) may be preferable.
Counter-intuitively, plateau periods of no weight loss may also be of some value in the long term, as they represent adjustment phases.
6.2 Experience with weight loss interventions

Patterns of weight loss observed in longer term studies were found to be consistent with changes in energy expenditure as suggested above [7, 271]. Here, data from three longer term dietary interventions are reviewed (using the previously described framework) to examine the inter-relationships between approaches to weight loss and outcomes achieved. The experiment protocols of these studies (HELP, HIPHOP and HERO studies) have been described in the previous chapters.

6.2.1 Weight loss period (0 to 26 weeks)

In Figure 6-3(A), mean actual weight loss from the two 12-week trials (HIPHOP study and HELP study [271]) was plotted alongside with the predicted weight loss (dotted straight line), which was calculated based on a daily energy deficit of 500 kcal/d and the assumption that a kilogram of weight loss is equivalent to a 7700 kcal deficit. It appears that the actual weight loss closely matched the expected figures derived from a simple energy balance model. When the weight loss of the HERO study [7] is plotted (Figure 6-3(B)), there was also a sharp decrease in weight during the first 12-weeks. It is likely that both the physiological adaptations to weight loss interventions are minimal and dietary compliance maximal in a short term period of 12 weeks.

Weight loss in HERO study started to decelerate between 13 and 26 weeks. Weight loss for the first 13-weeks was 2 kg and that equals to 170 kcal/d energy deficits (7700 kcal/kg X 2 kg weight loss / 91 days). An additional 0.5 kg was
lost in the following 13 weeks, which means that the overall energy deficit over the first 26 weeks to be 106 kJ/d (7700 kcal/kg X 2.5 kg weight loss / 182 days). From 13 to 26 weeks, the energy deficit decreased by 64 kcal/d. As observed in a weight loss trial reported in this thesis (Section 4.2), the average decrease in energy expenditure per unit weight loss was approximately 27 kcal/kg weight loss/day. Reduction of energy expenditure after 2 kg weight loss (0 to 13 weeks) would be 54 kcal/d and this explains most of the decreased energy deficit (64 kcal/d) from 13 to 26 weeks. In other words, a decelerated rate of weight loss was likely to be mainly contributed by body adaptations to weight loss. The energy deficit gap not explained by reduction in energy expenditure following weight loss suggests that compliance to treatment plan needs to be checked and re-emphasised.

**Figure 6-3.** (A) Mean weight loss of overweight and obese participants in 12-week where dotted line represent expected weight loss from energy intake restriction, and (B) weight loss trend from a 52-week weight loss trial.
6.2.2 Weight regain period (26 weeks onwards)

In the HERO study [7], weight regain started after 26 weeks of intervention. This observation is consistent with the observations by other weight loss trials [273, 274]. During the first 26 weeks, mean weight loss was 2.5 kg. This was equivalent to a cumulative energy deficit of 19250 kcal (assuming 1 kg body weight equates to 7700 kcal), or 106 kJ/day energy deficit. Between the intervention periods of 26 to 39 weeks, mean weight regain was approximately 0.5 kg. Using the theoretical assumptions outlined thus far, to gain a body weight of 0.5 kg over a 3 month period, equivalent to a cumulative energy excess of 3850 kcal or 42 kcal/day (3850 kcal / 91 days) energy excess. However, to regain 0.5 kg after a 2.5 kg weight loss, the energy excess has to be ~150 kcal/day (106 kcal/day for weight loss plus 42 kcal/day for weight regain). This value is comparable to the value simulated by Kevin Hall [307], where his mathematical models suggested that a further reduction of 170 kcal/d from the initial diet may be required to prevent weight regain after 6 months. The amount of weight regained could partly be explained from the (weight loss related) reduction in energy expenditure. With the weight loss of 2.5 kg at 6 month, the energy expenditure might reduce by 67 kcal/day (27 kcal reductions in energy expenditure per kg weight loss as observed in the HIPHOP study in Section 4.2). By comparing the values (42 kcal/d for weight regain and 67 kcal/d reductions in energy expenditure), weight regain could be explained by the reduction in energy expenditure due to weight loss. However, this fails to explain the decelerating weight loss even if weight regain is prevented. The other two possible factors are the reduced energy expenditure during underfeeding or the poor compliance to dietary or exercise treatment plan. This
observation is supported by a study which has shown that dietary non-compliance was the major contributor to differences between expected and actual weight loss [275]. This provides support for reviewing and re-emphasising treatment plans to encourage compliance to the plan.

6.3 A new weight management approach

Integrating knowledge on energy metabolism may be useful for long term weight loss efforts. The proposed approach highlights a number of areas where this could be possible. In particular it shows that weight loss is not just about under-eating. Reviewing and modifying treatment protocols throughout the period of weight loss should be encouraged. Such a strategy may include the use of a higher protein cuisine at first, and a further reduced energy prescription after a few months (and/or significant increases in physical activity).

Finally, it should be acknowledged that the role of exercise (amount and intensity) should not be under-estimated. This is vital as there is evidence suggesting that exercise not only increases energy expenditure, but also may reduce the magnitude of reduced energy expenditure during weight loss [259, 308]. It also means that the initial plan for diet and exercise should be modified once a plateau effect is detected.

In summary, there appear to be 3 crucial periods to note in practice during weight loss treatment in a clinical setting. The first 12 weeks may well produce the most successful weight loss period. From 12 to 26 weeks, treatment compliance may start to deteriorate and physiological adaptations due to weight
loss may start to have an impact and weight loss may slow. At this stage reviewing dietary prescriptions to account for reductions in energy expenditure due to weight loss (27 kcal/kg weight loss/day) and motivating patients to adhere to the treatment plan may be crucial. From 26 weeks onwards a crucial period may emerge with weight regain. At this stage, accounting for the reduction in energy expenditure after weight loss may help to prevent weight regain. However, this alone is insufficient for further weight loss. This means that a further reduction of energy intake and/or a significant increase in exercise is warranted.

6.4 Conclusion

Failure to observe longer term effects of dietary manipulation (in Chapter 4 and 5) could partly be explained by adaptations of the human body to the changes, and not only because of non-compliant to interventions. The conceptual framework addressing weight loss presented in this chapter paints a more complete picture of energy balance for weight management. The manipulation of dietary components plays an important role in promoting weight loss. Not only does it enhance thermogenesis and substrate oxidation, but it also works on other pathways that add benefits in weight loss such as through appetite regulation. The push-pull relationship between intervention strategies and physiological adaptations could be manipulated to favour weight loss by using more specific dietary strategies.
CHAPTER 7. CONCLUSION

In treating overweight the simple generic advice to ‘eat less and exercise more’ appears to have limited success. This thesis hypothesised that using commonly consumed foods, high protein and high PUFA diets are beneficial for weight loss in a free-living environment. A mechanistic approach was considered, focusing on the effects of these manipulations on regulation of energy expenditure and substrate utilization (oxidation).

A whole room calorimeter (WRC) method was selected to measure energy expenditure and substrate oxidation rates and calorimeter data from a number of trials was accessed for analysis. In order to accurately measure the effects of dietary manipulation on energy expenditure and substrate oxidation, confounding variables were identified and controlled for through standard WRC experimental protocols. After comparing three methods of predicting energy requirements in the WRC the Schofield equation was found to provide the best predictions, and this method was used in all WRC studies reported in the thesis. In addition, values for leptin and insulin were found to have no association with energy expenditure and hence only fat and fat-free mass were adjusted for in the analysis of the data.

Acute feeding studies (8 hours in the WRC) manipulating dietary protein and polyunsaturated fat (PUFA) demonstrated favorable effects for overweight and obese individuals. Although high protein diets from meat, dairy and soy sources produced similar energy expenditure values, a higher protein retention was
observed in the high meat protein group. On the other hand, a high PUFA diet produced a significant negative fat balance (i.e. more fat was oxidized) but the energy expenditure was not different to the control diet. These observations suggested that high protein diets from meat sources could potentially help to minimise the loss of fat-free mass (e.g. muscle mass) under conditions of energy restriction, while high PUFA diets may induce greater fat mass loss over a longer period of time. These hypotheses were then tested by analyzing data from long term studies, but the expected outcomes were not observed. In the case of the proposed dietary protein influence, weight loss was not different between low and high protein groups, and changes in fat-free mass were not different between high protein diets from animal and plant sources. High PUFA diets also failed to produce higher fat mass loss, and there was no preferential loss of abdominal fat as suggested by the literature [166, 169]. In both cases, the acute effects of dietary protein and PUFA manipulation appeared to be washed-out over a longer period of time in a free-living environment.

While the long term effects were difficult to ascertain, results from the acute studies supported the hypothesis that the manipulation of dietary protein (and its food sources) and of dietary PUFA can lead to a greater energy expenditure and fat oxidation that would be favorable for weight management. There is a clear benefit of increasing dietary protein from predominantly meat sources (30% energy) as well as increasing daily PUFA intake (approximately 10%). A theoretical framework for addressing factors that promote and inhibit weight loss was seen from the analyses contained in the thesis and theoretical considerations in the literature [283, 284, 309]. This framework shows that
adaptations in metabolic processes and compliance with dietary manipulation are possible reasons contributing towards the wash-out effects seen in longer term interventions. While little can be done to overcome physiological adaptations, compliance to the treatment plan can be regularly supported and emphasised to promote and maintain weight loss.

Part of this thesis involved secondary analyses of data from trials designed for other purposes than those investigated here. Questions on whether the compliance-to-treatment or the adaptation of the human body plays a more important role in the failure to observe expected weight and body composition changes in the longer term studies remain unanswered because these trials were not originally conducted to quantify these parameters. Therefore, in future research on the effects of manipulating of dietary factors, a number of areas could be further investigated. This should focus on possible reasons behind the failure to sustain acute effects over time. The research should consider the dynamic environment in which human metabolism operates and therefore should focus on both compliance to the dietary prescription and adaptations to dietary changes over time. Each adaptive process needs to be quantified and appropriate strategies developed.

In this thesis the effects of dietary manipulation were examined separately for dietary protein and PUFA. Because these macronutrients acted on two different pathways, the synergistic effects of dietary protein and PUFA could also be investigated in future. This could also be done in consideration of the regulation of appetite. Although this aspect was not within the scope of this thesis, the
strategies to suppress hunger and to enhance satiety could be incorporated into
a proposed dynamic energy balance framework addressing the component of
energy restriction. Some of the thermogenic nutrients (for example dietary
protein) also possess satiating effects, and this could be considered
concurrently. In addition, there are many other food components that could be
examined, and their effects should also be tested through mechanistic studies
as well as dietary intervention trials. Adding the appetite regulation dimension to
the concept of dynamic energy balance will further enhance its utility and
effectiveness in dietetic practice to support better weight loss outcomes.

In conclusion, this thesis supports the hypothesis that the manipulation of
dietary protein and fat components, by incorporating commonly consumed
foods, is beneficial for weight loss. However, it appears that a better
understanding of long term dietary compliance and human metabolic
adaptations are required before overweight and obese individuals can benefit
from these kinds of dietary manipulation in a sustained manner.
REFERENCES


118. Johnston CS, Day CS, Swan PD: Postprandial thermogenesis is increased 100% on a high protein, low fat diet versus a high


263. Marmonier C, Chapelot D, Fantino M, Louise-Sylvestre J: Snacks consumed in a nonhungry state have poor satiating efficiency: influence


274. Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L: XENIlcal in the prevention of diabetes in obese subjects (XENDOS) study: a randomized


## Appendix A. Summary of acute feeding trials and longer term dietary interventions which data was used in support of this thesis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Funding Source</th>
<th>Design</th>
<th>Duration</th>
<th>Subject</th>
<th>Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCEFF</td>
<td>NCEFF</td>
<td>Acute crossover feeding trial</td>
<td>3 x 3-hour WR C stays</td>
<td>8 males, 3 females</td>
<td>HP (30% P, 30% F, 40% C) meals from meal, dairy and soy sources</td>
</tr>
<tr>
<td>FAME</td>
<td>CWVC</td>
<td>Acute crossover feeding trial</td>
<td>2 x 3-hour WR C stays</td>
<td>7 males, 0 females All overweight and 4 with type 2 diabetes</td>
<td>Control (30% P, 5% UFA) and HPUFA (30% F, 10% UFA) meals</td>
</tr>
</tbody>
</table>
| HIPHOP| NCEFF          | Randomised, controlled trial | 12 weeks | 36 overweight adults completed the study | Control 20% P, 30% F, 50% C, with a 2MJ/day energy restriction  
Animal HP (30% P, 30% F, 40% C) from animal protein sources, with a 2MJ/day energy restriction  
Plant HP (30% P, 30% F, 40% C) from plant sources, with a 2MJ/day energy restriction |
| HELP  | NHMRC (#354111) | Randomised, controlled trial | 12 weeks | 35 overweight adults completed the study | Low-fat 20% P, 50% C, 30% F (5% PPUFA, 15% MUFA, 10% SFA)  
HPUFA: 20% P, 50% C, 30% F (10% PPUFA, 10% MUFA, 10% SFA)  
Low-fat low-carbohydrate similar to low-fat diet but with a 2MJ/day energy restriction  
HPUFA low-carbohydrate similar to HPUFA diet but with a 2MJ/day energy restriction |
| HERO  | CWVC           | Randomised, controlled trial | 1 year | 35 overweight adults with type 2 diabetes but not insulin-treated | Control 20% P, 50% C, 30% F (10% SFA, 15% MUFA, 5% PPUFA)  
HPUFA: 20% P, 50% C, 30% F (10% SFA, 10% MUFA, 10% PPUFA) from 30g/d walnuts |
| SMART | NHMRC (#314031) | Randomised, controlled trial | 1 year | 128 overweight adults recruited | Control 25% P, 45% C, 30% F (10% SFA, 15% MUFA, 5% PPUFA), with a 2MJ/day energy restriction  
HPUFA: 25% P, 45% C, 30% F (10% SFA, 15% MUFA, 10% PPUFA), with a 2MJ/day energy restriction  
HPUFA + FO: 25% P, 45% C, 30% F (10% SFA, 10% MUFA, 10% PPUFA, plus 450mg EPA & 250mg DHA supplement per day), with a 2MJ/day energy restriction |

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*Study is ongoing, number of subjects recruited instead of completed is reported.
*NCEFF = the National Centre of Excellence in Functional Foods (Australia). CWVC = the California Walnut Commission (USA). NHMRC = the National Health and Medical Research Council (Australia).
Study group names are adjusted to match thesis purposes.
*P = protein, F = fat, C = carbohydrate, SFA = saturated fat, MUFA = monounsaturated fat, PUFA = polyunsaturated fat, HP = high protein diets, HPUFA = high PUFA diet, FO = fish oil supplement, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid
Appendix B. Parent paper of the HELP study


Please see print copy for this article, or click to access throught the publisher's web site

Appendix C. Parent paper of the HERO study


Please see print copy for this article, or click to access through the publisher's website

http://www.nature.com/ejcn/journal/v63/n8/full/ejcn200919a.html
Appendix D. Parent paper of the FAME study


Please see print copy for this article, or click to access through the publisher’s web site

http://www.jacn.org/content/28/5/611.full
Appendix E. Visual analog scales

Time: ___________________
Date: ________________

QUESTIONS ON APPETITE AND DESIRE FOR FOOD

INSTRUCTIONS: Make a vertical mark ( | ) on each line that best matches how you are feeling at the time of completing your meal.

I am not hungry at all
How hungry do you feel? I have never been more hungry

I am completely empty
How satisfied do you feel? I cannot eat another bite

Not at all full
How full do you feel? Totally full

Nothing at all
How much do you think you can eat? A lot

Yes, very much
Would you like to eat something sweet? No, not at all

Yes, very much
Would you like to eat something salty? No, not at all

Yes, very much
Would you like to eat something savoury? No, not at all

Yes, very much
Would you like to eat something fatty? No, not at all