Exercise and nutrient interactions:

Effects on skeletal muscle and body fat mass

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Statement of Sources

This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No other person's work has been used without due acknowledgment in the main text of the thesis. This thesis has not been submitted towards the award of any other degree or diploma in any other tertiary institution. All research procedures reported in the thesis received the approval of the relevant Ethics Committees (where required).

Unless otherwise stated, all work comprising of this thesis has been undertaken by the candidate.

This candidature began at Royal Melbourne Institute of Technology (RMIT) University in July 2011. The candidate transferred to Australian Catholic University in January of 2014 following the move of the Principal Supervisor and laboratory group.

There have been several collaborations and ancillary staff involved in the studies of this thesis. These are listed in order of importance/contribution:

Study 2

- Two staff members were directly employed through the DHNC funding to complete the Clinical Trial Manager (participant recruitment and initial screening) and Dietitian (fortnightly participant meetings, dietary counselling) roles in *Study 2*.

- The dietary intervention for *Study 2* was designed by Prof Louise Burke (co-supervisor) and her team of dietitians based in the Sports Nutrition Discipline of the Australian Institute of Sport.

- Dr Vernon Coffey assisted throughout the first year (2012) in the primary and secondary analyses.

- Around ~120 Exercise Science students from RMIT University assisted with the daily resistance training sessions for all participants.

- Dietary record entry was completed by the study Dietitian with assistance from PhD student Kristyen Tomcik.

- Dietary analysis was initiated by Accredited Sports Dietitian Adam Zemski and completed by myself.

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- The blood samples (fasting glucose, insulin, cholesterol and triglycerides) were analysed commercially (Dorevich Pathology, Melbourne).

- Prof Stuart Phillips (McMaster University) was involved in the design of *Study 2*.

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Study 3

- Dr Donny Camera assisted with the design, initial analysis of the c-miRNA plates, and the interpretation of results of *Study 3*.

Signed:

Evelyn B Parr

Date: 27th October 2015

Supervisory Panel

It is acknowledged that the work within this thesis was supervised by the following ACU staff and external individuals:

Principal Supervisor: Professor John A Hawley (ACU, Fitzroy, VIC / RMIT University, Bundoora, VIC)

Associate Supervisor: Dr Vernon G Coffey (Bond University, Gold Coast, QLD / RMIT University, Bundoora, VIC)

Co-Supervisor: Professor Louise M Burke (ACU / Australian Institute of Sport, Canberra, ACT)

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1.1　　　Published

Parr, E.B., Coffey, V.G., Hawley, J.A. 'Sarcobesity': a metabolic conundrum, *Maturitas*, 2013, 74(2): 109-113.

1.2　　　Under review

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1.3　　　In preparation

Parr, E.B., Camera, D.M., Coffey, V.G., Phillips, S.M., Burke, L.M., Hawley, J.A. Modulation of circulating microRNAs between 'high' and 'low' responders following a 16-wk diet and exercise weight loss intervention

Parr, E.B., Coffey, V.G., Phillips, S.M., Burke, L.M., Hawley, J.A. Maintenance of body composition in the 12 months following a 16 wk diet and exercise intervention in overweight/obese middle-aged adults.

1.4　　　Others completed during candidature but not included in thesis

Parr, E.B., Camera, D.M., Areta, J.L., Burke, L.M., Phillips, S.M., Hawley, J.A., Coffey, V.G. Alcohol ingestion impairs maximal post-exercise rates of myofibrillar protein synthesis following a single bout of concurrent training, *PLoS One*, 2014, 9(2): e88384.

List of conference presentations

1. **Parr, E.B.,** Camera, D.M., Areta, J.L., Burke, L.M., Phillips, S.M., Hawley, J.A., Coffey, V.G. Alcohol consumption after a bout of concurrent training impairs rates of muscle protein synthesis, *Sports Dietitians Australia National Conference*, Melbourne, October 18th, 2013. [Oral presentation]

2. **Parr, E.B.,** Camera, D.M., Areta, J.L., Burke, L.M., Phillips, S.M., Hawley, J.A., Coffey, V.G. Alcohol consumption after a bout of concurrent training impairs rates of muscle protein synthesis, *Sports and Exercise Science New Zealand Conference*, Christchurch, November 29th, 2013. [Oral presentation; **Awarded best student presentation**]

3. **Parr, E.B.,** Coffey, V.G., Burke, L.M., Phillips, S.M., Hawley, J.A. Effect of increased consumption of dairy protein on weight loss during a diet and exercise intervention, *International Sports and Exercise Nutrition Conference*, Newcastle upon Tyne, December 17th, 2014. [Oral presentation]

4. **Parr, E.B**., Camera, D.M., Burke, L.M., Coffey, V.G., Hawley, J.A. Changes in circulating microRNAs between high and low responders to a 16-wk diet and exercise weight loss intervention, *European College of Sports Sciences (ECSS),* Malmo, Sweden, June 24-27, 2015. [Poster presentation]

Abstract

The worldwide prevalence of overweight and obesity continues to rise and will soon place unsustainable demands on the healthcare systems of most developed nations. Sarcopenia, the age-related loss of muscle mass, is commonly exacerbated in overweight/obese individuals causing loss of function and independence. Accordingly, a critical goal for overweight/obese adults is to lose fat mass while preserving lean mass to prevent the deleterious effects of inactivity and age-related metabolic diseases.

Although numerous studies have manipulated combinations of diet and/or exercise training to promote weight loss, the optimal diet to improve body composition remains controversial. Furthermore, the composition of tissue losses (i.e. fat versus lean mass) is not always examined and individual responses to weight loss interventions have, to date, received little scientific enquiry. Further, the success of a weight loss intervention should be determined not only acutely, but also in terms of its efficacy in maintaining body composition changes. This thesis comprised a series of independent but related studies that investigated the role of exercise and energy-restricted diets of varying macronutrient composition on the maintenance of skeletal muscle mass and body composition.

Study 1 was formative work that measured the reliability of the dual-energy x-ray absorptiometry (DXA) scanner in estimating total, lean and fat masses. The GE Lunar DXA scanner and operator had a coefficient of variation of less than 1.3%, representing technological and biological variability, allowing differences of lean and fat masses as small as ~500 g to be detected.

Study 2 determined the effect of energy-restricted high-protein diets with variable carbohydrate and fat content, in combination with exercise training, on changes in body composition. A clinical trial of 115 overweight/obese, middle aged (~35-59 years) men and women undertook one of three energy-restricted diets (two high protein (30% energy intake) and one moderate protein (~20% energy intake)) for 16 wk whilst completing daily exercise

(3 d/wk resistance and 4 d/wk aerobic). Main outcome measures were body composition using DXA and selected risk factors of obesity. There was a significant loss of fat mass (-7.3 ± 3.4 kg; P<0.05) coupled with the preservation of lean mass along with improvements in health-related parameters for all intervention groups. Furthermore, favourable changes in body composition were attained when protein intake met or exceeded RDIs with mild energy restriction and an appropriate exercise stimulus.

Study 3 determined the divergent individual responses to a diet and exercise intervention through epigenetic measures. From the *Study 2* participant cohort, a subgroup of high responders (>10% body mass loss, *n*=22) and low responders (<5% body mass loss, *n*=18) were identified and plasma samples analysed for the expression of 13 circulating microRNAs (c-miRNAs) previously shown to be altered by energy-restricted diet or exercise interventions. Four c-miRNAs were found to be differentially expressed, two pre and post intervention and two between high and low responders. These findings provide novel mechanistic information that may promote the beneficial effects of both exercise- and diet-induced energy restriction for body mass loss.

Study 4 investigated the influence of the 16-wk diet and exercise intervention on long-term weight loss maintenance through the pattern of body composition changes in the 12-month follow-up period. Participants from *Study 2* were invited back for DXA body composition measurements at 3, 6, 9 and 12 months post-intervention. No significant changes in body composition (i.e. fat or lean mass regain) were measured across the 12-month period. Further, a strong association was found between the body mass change in the first 3 months post-intervention and the 12-month follow up period.

The conclusions arising from this research are: 1) varying the macronutrient composition of energy-restricted diets does not alter the magnitude of body composition changes when undertaken with regular exercise, 2) an appropriate exercise stimulus maintains lean mass in the face of energy restriction, 3) select c-miRNAs may have potential as

biomarkers to predict the magnitude of weight loss to an exercise and diet intervention, and 4) education on dietary and exercise practices are associated with maintenance of weight loss after intervention controls are removed. This new knowledge will inform "best practice" to treat overweight and obese individuals embarking on weight loss programmes.

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Abbreviations

7DD	7 day food diary
ANOVA	analysis of variance
AUC	area under the curve
BM	body mass
BMI	body mass index
BP	blood pressure
c-miRs	circulating microRNAs
CHO	carbohydrate
CON	control diet (moderate protein, high carbohydrate)
CV	coefficient of variation
DXA	dual-energy x-ray absorptiometry
EI	energy intake
ELISA	enzyme linked immunosorbent assay
EXT	exercise training
FNDC5	fibronectin type III domain containing 5
FM	fat mass
FFM	fat free mass
FSR	fractional synthetic rate
HDL	high density lipoprotein
HDPMC	high dairy protein, moderate carbohydrate diet
HDPHC	high dairy protein, high carbohydrate diet
HOMA	homeostasis model assessment
miRNA	microRNA
mRNA	messengerRNA
MPS	muscle protein synthesis

LCD	low calorie diet
LDL	low density lipoprotein
LM	lean mass
OGTT	oral glucose tolerance test
RDI	recommended daily intake
REX	resistance exercise
RNA	ribonucleic acid
SD	standard deviation
VLCD	very low calorie diets
VO$_2$peak	rate of peak oxygen uptake
y	years of age

Chapter ONE

1   Introduction and Overview

The prevalence of overweight/obesity in Australia is approaching 60% and has more than doubled in the past 20 years (Cameron et al., 2003). Obesity is multi-faceted with many characteristics that predispose an individual to overweight/obesity including inactivity, positive energy balance and genetic predisposition. Sarcopenia, defined as the loss of skeletal muscle mass associated with aging (Baumgartner et al., 1998), is commonly exacerbated in overweight and obese individuals resulting in a debilitating loss of function and independence. Skeletal muscle loss is accelerated at around 60 years (y) of age (Sayer et al., 2008), which also corresponds to the highest prevalence of overweight or obesity in Australia (Australian Bureau of Statistics, 2013). Accordingly, a critical issue for the majority of Australian adults is how to lose fat mass while preserving lean mass in order to prevent the deleterious effects of inactivity- and age-related metabolic diseases.

Appropriate nutrition and adequate contractile loading are important determinants of skeletal muscle mass throughout the life cycle. The issue of muscle mass retention is complicated in individuals attempting to lose body mass (i.e. fat mass) during mild energy restriction. While energy-restricted diets have the greatest efficacy in terms of inducing body mass loss (Villareal et al., 2011), such body mass loss is indiscriminate: both fat and lean mass are lost. Adequate protein intake has been identified as an essential factor in maintaining skeletal muscle mass when dietary intakes are energy-restricted, but the precise quantity and timing of protein intake ensuring muscle retention is unclear.

While there are differences in the individual responses to body mass loss interventions, the underlying mechanism/s responsible for these differences are not well characterised. Previous intervention studies have typically recruited large numbers of participants to ensure adequate statistical power and often report measures of central tendency

(such as average values of the cohort under investigation). While some of the variability in body mass loss responses can be attributed to inconsistent adherence to diet and exercise training, environmental (epigenetic) factors and differences in an individual's genetic predisposition are likely play a role in some of the variable responses to diet/exercise interventions.

In addition to inter-individual differences to diet/exercise interventions, the efficacy of body mass loss strategies needs to be evaluated in terms of the chronic maintenance (i.e. several years) of a desirable physique (i.e. body mass and lean tissue mass) once the intervention is complete and support systems (i.e. diet counselling and personal training advice) are removed. Long-term maintenance of body mass loss is integral for reducing the incidence of obesity and its impact on individual quality of life and economic strain. Australian data from the early 2000s estimates the direct cost for overweight and obesity to be ~$21 million, with a further ~$35 billion in indirect costs (Colagiuri et al., 2010). Thus, from a national health perspective reducing obesity incidence in an ageing population is an important health and economic outcome for society.

The work undertaken for this thesis comprised a series of four independent but related studies examining the role of diet and exercise on changes in body composition:

Study 1: Formative work to determine the reliability of dual energy x-ray absorptiometry (DXA) for estimation of body composition (Chapter 3).

Study 2: The effect of dairy foods within high-protein, variable carbohydrate energy-restricted diets combined with exercise training on changes in body composition in middle-aged adults (Chapter 4).

Study 3: Individual responses to a diet and exercise body mass loss intervention and the association with circulating microRNAs (Chapter 5).

Study 4: Characterising changes of body composition up to 1 year after a 16 wk body mass loss intervention (Chapter 6).

Chapter TWO

2   Literature Review

The following review of literature comprises two sections. For the purpose of this thesis, sections 2.1 and 2.2 have already been published in an invited review detailing comorbidities of sarcopenia and obesity and the roles that diet and exercise play in minimising these conditions (Parr, Coffey, & Hawley, 2013). However, where appropriate these sections have been updated to include studies published after October 2012. Integral to this publication, and relevant to this thesis, is the notion that there is no "magic pill" to maintain skeletal muscle mass and that appropriate exercise and nutrition are the primary preventative therapies to combat muscle wasting. The review of literature also includes a discussion of the role of dairy products in dietary interventions, individual epigenetic responses to loss of body mass and the potential for body mass regain after cessation of an intervention.

2.1   Incidence and effects of sarcopenic obesity on body composition

Two independent but inter-related conditions that have a growing impact on healthy life expectancy and health care costs in developed nations are an age-related loss of muscle mass (i.e., sarcopenia) and obesity. Sarcopenia affects approximately one-third of adults over 60 years of age and more than 50% of those over 80 years (Baumgartner et al., 1998), with the progression of sarcopenia strongly associated with the age-related trend towards lower levels of habitual physical activity. With regards to obesity, the World Health Organisation (WHO) projected that by 2015 over 700 million adults will be obese (a body mass index [BMI = body mass /(height)^2] $\geq$30 kg/m^2) (World Health Organisation, 2012). In 2014, over 600 million were already classified as obese (World Health Organisation, 2015). Obesity is a major risk factor for chronic diseases including type 2 diabetes mellitus and cardiovascular disease. Sarcopenia is commonly exacerbated in overweight and obese individuals and progression

toward obesity promotes an increase in fat mass and a concomitant decrease in muscle mass, producing an unfavourable ratio of fat to muscle. The coexistence of diminished muscle mass and increased fat mass is referred to as sarcopenic obesity and is ultimately manifested by impaired mobility and/or development of life-style-related diseases (**Figure 2.1**). Accordingly, the critical health issue for a large proportion of adults in developed nations is how to lose fat mass while preserving muscle mass in the face of reduced levels of daily activity.
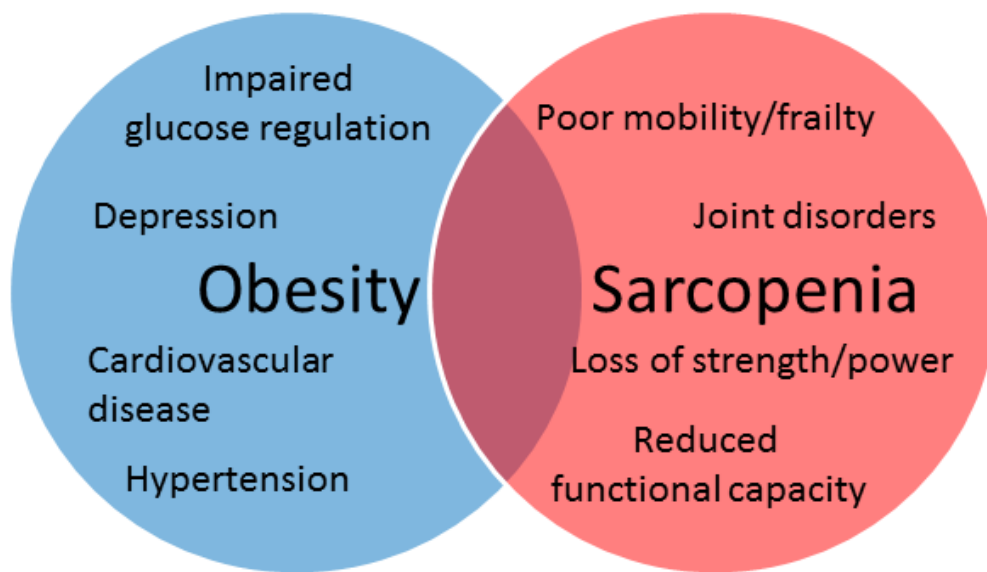
Figure 2.1. Lifestyle-related chronic diseases or disease states associated with sarcopenia and obesity, and their synergistic negative effect on health and functional capacity.

Skeletal muscle makes up ~50% of body mass and plays key roles in whole-body glucose disposal, insulin sensitivity, thermoregulation and locomotion (Zierath & Hawley, 2004). As such, skeletal muscle mass is critical to metabolic health. Peak skeletal muscle mass is attained within the first three decades of life and begins to decline thereafter. The incidence and severity of sarcopenia increase progressively from age 30 y over the remaining lifespan. This loss of skeletal muscle mass accelerates to a rate of approximately 1% per year after the age of 65 y (Frontera et al., 2000; Goodpaster et al., 2006) and is associated with a corresponding 2-3 fold loss in functional strength (Goodpaster et al., 2006). Therefore,

preventing the loss of muscle tissue in middle-aged adults (35-60 y) is of particular importance given the subsequent accelerated age-related decline in muscle mass.

When considering strategies to combat sarcopenia and obesity, it is important to consider that losses of muscle mass with aging are exacerbated by failure to maintain peak muscle mass attained in mid-adulthood (~30-50 y) (Sayer et al., 2008). One of the barriers to attaining and preserving healthy muscle mass is that the levels of habitual physical activity in developed nations are low and decline further with age (Haskell et al., 2007). Inactivity, combined with greater energy intake, increases the prevalence of obesity. Thus, it is clinically relevant to acknowledge that individuals are not only losing skeletal muscle mass but concomitantly gaining fat mass (Wannamethee, Shaper, Lennon, & Whincup, 2007). One of the mechanisms by which greater fat mass exacerbates sarcopenia is via the effect of lipid accumulation in preventing the incorporation of amino acids into the muscle. Briefly, several animal models have now demonstrated that high fat feeding impairs the activity of several proteins involved in protein synthesis (Sitnick, Bodine, & Rutledge, 2009; Deldicque et al., 2010; Masgrau et al., 2012), thus impairing the long-term maintenance of skeletal muscle mass.

The double-edged sword defining sarcopenic obesity is that sarcopenia acts synergistically with obesity in older adults to decrease health status and functional capacity beyond either condition in isolation (Baumgartner et al., 2004). In their study of 451 older (>60 y) Americans, Baumgartner et al., (2004) showed the differential effects of sarcopenic obese (6% of subject population) compared to non-sarcopenic obese (32%), sarcopenic non-obese (18%) and non-sarcopenic non-obese (44%). Regardless of sarcopenia, the obese groups had a lower physical activity score, were more likely to have hypertension at baseline and a higher incidence of metabolic syndrome. However, when sarcopenia was evident, regardless of obesity status, no lifestyle-related chronic diseases were exacerbated. The concomitant diagnosis of sarcopenic obesity indicated a greater likelihood of a decrease in

functional status and the highest prevalence of metabolic syndrome. Therefore, sarcopenic obesity presents a complex challenge for healthcare professionals trying to prescribe appropriate treatment to reduce the health risks associated with excess fat mass, while simultaneously preserving muscle mass to reduce the risk of further disability. From Baumgartner's (2004) work, the degree of obesity was of a greater detriment to other chronic lifestyle-related diseases and therefore reducing fat mass should be a general strategy to improving health status. However, to preserve independence in the home and maintain adequate functional status concomitantly with age, the loss of fat tissue should not be prioritised without employing a strategy to concurrently maintain muscle quality and quantity (mass).

2.2 Exercise and dietary strategies to combat sarcopenic obesity

2.2.1 Methodological considerations for body composition measures

Quantifying the success of weight loss interventions is largely dependent on the ability to accurately measure body composition and detect the (small) changes in the various tissues (lean and fat) that determine the overall decline in body mass. As Newman and colleagues (2005) showed, a retention of body mass in 70-79 y olds over a four-year measurement period was largely due to the loss of lean mass and concomitant gain of fat mass – a trend that would not be evident when only observing changes in total body mass. In terms of accuracy, a four compartment model, utilising DXA for bone mineral content, air-displacement plethysmography or hydrodensitometry for body density, total body water using deuterium dilution for lean mass and body mass to calculate fat mass, has greater precision than the two-compartment model provided by DXA technology (Fuller, Jebb, Laskey, Coward, & Elia, 1992). However, as the use of the four-compartment model is limited by cost and practicality, DXA has become the most commonly used "gold standard" technique for measuring body composition.

Although originally developed as a tool to estimate bone mineral density, DXA allows the estimation of whole body and regional measurements of lean and fat tissue mass when certain assumptions are met. As DXA is widely available, relatively inexpensive in terms of a single scan, and the radiation dose is minimal (Pietrobelli, Formica, Wang, & Heymsfield, 1996), it provides an alternative to other radiographic methods such as computed axial tomography (CT) and magnetic resonance imaging (MRI) (Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002). It assumes, however, that lean mass is representative of all lean tissue that is fat- and bone mineral-free (**Figure 2.2**).

DXA systems provide measures of the appendicular (limb) lean soft tissue (fat- and bone mineral-free) components that include muscle, skin, tendons and connective tissue (Kim et al., 2002). As the latter three components are unlikely to significantly change, DXA is considered to be a reliable tool for measuring lean mass (bone mineral-free) as long as hydration status is controlled (Nana et al., 2014). Although DXA is now commonly used to monitor changes in body composition as a result of weight loss interventions, no study to date has taken multiple-day scans at each measurement time point to ascertain the reliability of using DXA for research investigating weight loss.
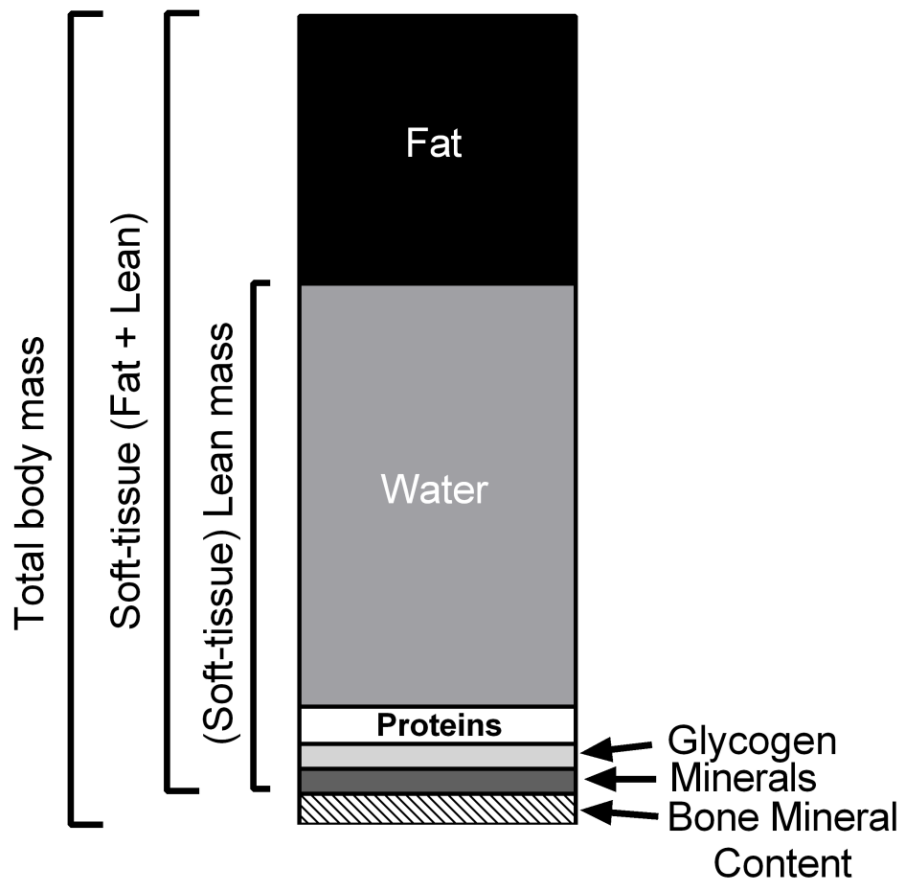
Figure 2.2. Example of body composition compartments for a 65 kg female using a DXA two-compartment model. Redrawn from Figure 7 of Pietrobelli et al., Am J Physiol Endocrin Metabol, 1996:271;6(1):E941-951.

2.2.2 Low energy diets

Health professionals frequently prescribe low energy diets for obese individuals as a 'first line of defence' in the battle to lose body mass. While energy restriction in isolation may be a rapid, short-term strategy for substantial weight loss, it results in a simultaneous reduction of both fat and lean muscle mass. Accordingly, the obese individual is ultimately predisposed to an unhealthy body composition. Up to 25% of weight loss achieved through short-term energy-restricted diets is lean muscle mass, largely investigated in post-menopausal women (Ballor, Katch, Becque, & Marks, 1988; Campbell et al., 2009; Nicklas et al., 2009) but has also been observed in a mixed sex cohort (Hoie, Bruusgaard, & Thom, 1993). Not surprisingly, it has been suggested that weight loss *per se* should not be the sole objective for obesity-related disease risk reduction (Ross & Janiszewski, 2008).

The negative consequences of indiscriminately focusing on weight are highlighted by the results from several investigations of mass regained after low energy diet-induced losses. It has been demonstrated that weight regain is comprised of up to 80% fat mass which compounds an already unfavourable body composition (K. M. Beavers et al., 2011; Newman et al., 2005). Newman and co-workers (2005) also demonstrated that in elderly men and women who maintained their body mass across a four-year period, this was only achieved because the loss of lean mass was replaced with fat mass (**Figure 2.3**). This has negative consequences for metabolic health but also creates a deceptive paradigm when body mass (weight), and not body composition, is the sole focus. Indeed, failure to recognise and educate health professionals about the importance of body composition penalises patients whose sole criteria for gauging success of obesity reduction is through measures of BMI or the bathroom scale.
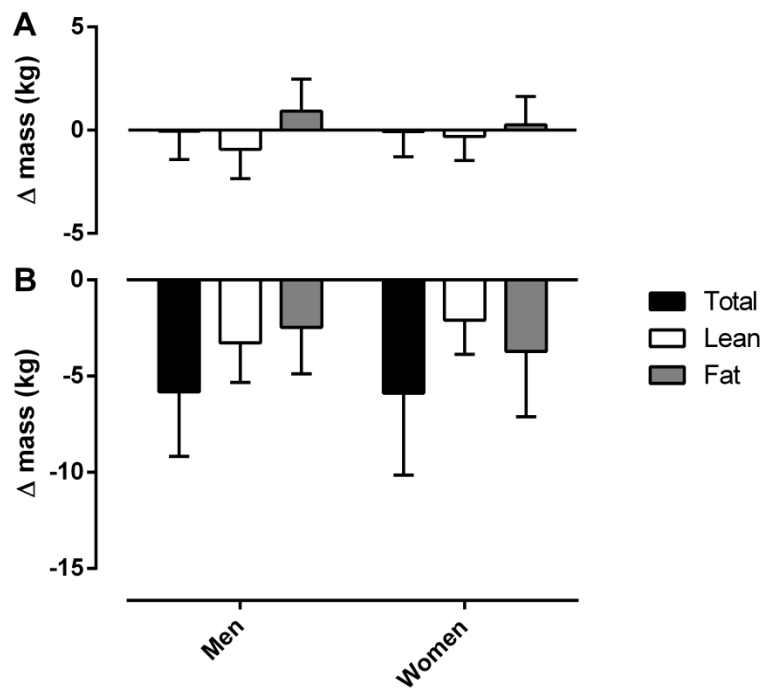
Figure 2.3. Absolute changes in body composition for 70-79 y olds followed up over a four-year period for: A) those who were body mass stable (*n*=495 men, *n*=490 women) and B) those who lost body mass (*n*=320 men, *n*=372 women). Redrawn from *Table 2* of Newman et al., Am J Clin Nutr., 2005;82:872-8.

The optimal energy deficit to reduce body mass is controversial with energy-restricted diets fitting broadly into two categories: Low calorie diets (LCD) in which daily energy intake is typically reduced by up to 1000 kcal/d or very low calorie diets (VLCD) in which the total energy intake is <1000 kcal/d. A recent meta-analysis from studies of low energy diets resulting in >10 kg loss of body mass show that VLCDs result in greater losses in the percentage of fat-free (lean) mass compared to LCDs (Chaston, Dixon, & O'Brien, 2007). Of the lean mass lost with VLCDs, not all is recovered when individuals return to energy balance. This topic will be discussed further later in this chapter. There is evidence to suggest that LCDs may be more beneficial than VLCDs for managing healthy body mass changes in overweight or obese populations. Despite the prevalence of severe energy restriction for weight loss, the most beneficial long-term outcomes for body composition have been achieved with LCDs inducing moderate energy deficits of 500-1000 kcal/d (Strychar, 2006).

2.2.3 Diet macronutrient composition

The optimal macronutrient composition of low energy diets is also highly contentious. As highlighted by Katz and Meller (2014), rigorous long-term studies comparing the efficacy of the "best diet" are unlikely. Thus, specific dietary interventions claiming advantage are often largely exaggerated. Instead, a theme of "healthful eating" is supported by a strong evidence base (Katz & Meller, 2014). Dietary intake consists of three major macronutrients, carbohydrate (CHO), fat and protein, each of which can be manipulated through changing either absolute or relative proportions of energy intake.

Fat intake in a Western diet makes a substantial contribution (~40% energy intake (EI)) to total energy consumption and reducing fat intake has been shown to result in weight loss (Astrup, Grunwald, Melanson, Saris, & Hill, 2000). A consequence of reduced fat intake in the diet is a compensatory increase in the contribution of CHO to EI. Diets with a greater CHO content (>40-45% of EI) have been shown to reduce the extent of body mass loss and associated loss of body fat (Abete, Astrup, Martínez, Thorsdottir, & Zulet, 2010; Volek et al., 2004). The mechanism for higher CHO diets attenuating body mass losses remains unclear, although Feinman and Fine (2007) have suggested that it is related to daily variations in blood glucose and insulin concentrations. High protein diets have also been shown to increase fat loss during energy restriction compared with high CHO, low protein equivalents (Layman, 2004). Rather than reducing fat intakes, increasing protein intake typically comes at the expense of CHO intake. The increased protein intake has been suggested to exhibit positive effects through increased satiety from protein and lower glycaemic load (Layman, 2003). Consequentially, when energy is restricted, a high protein diet elicits more favourable reductions of body and fat mass over a standard protein diet (Wycherley, Moran, Clifton, Noakes, & Brinkworth, 2012). However, when fat intakes are low (<30% EI), a systematic review has shown there is no benefit of high versus low protein ($\geq 25\%$ vs $\leq 20\%$ EI) diets for biomarkers of obesity (Schwingshackl & Hoffmann, 2013).

High protein intakes are generally recommended for the ageing (post-peak muscle mass, >30 y) population to attain a healthy body composition and therefore maintain muscle mass for functional outcomes. However, as many as 40% of adults do not meet the worldwide current recommended dietary allowance (RDA) of 0.8 g/kg/d of protein in their habitual diet (Campbell, Trappe, Wolfe, & Evans, 2001). The guidelines of the World Gastroenterology Organisation for obesity recommend a moderate energy deficit with sufficient high quality protein (1.0 g/kg/d) to maintain lean muscle mass (Mathus-Vliegen et al., 2012). In a meta-regression analysis of protein intake on body composition during energy restriction, the degree of lean mass retention increased as protein intake increased, and it was recommended that >1.05 g/kg/d is required to maintain lean mass (Krieger, Sitren, Daniels, & Langkamp-Henken, 2006). The age-related decline of skeletal muscle mass means that physical function in middle-aged (~40-60 y) and older (>60 y) adults may be more affected by reductions in lean mass (Janssen & Ross, 2005) and therefore higher protein intake in the diet is warranted (Wycherley et al., 2012).

The efficacy of protein consumed at current RDA levels has been questioned for minimising muscle mass losses during healthy ageing, irrespective of exercise (Campbell et al., 2001; Houston et al., 2008). The RDA is based on a healthy young population who are able to maintain muscle mass, compared to an older population (+60 y) where dietary protein intakes are more important for preventing muscle loss. High habitual intakes of protein (i.e. above RDA values) in elderly men and women has been shown to attenuate the loss of lean mass by up to 40% (Houston et al., 2008; Robinson, Cooper, & Aihie Sayer, 2012). In overweight/obese older men, a combination of a balanced distribution of protein intake across the day combined with resistance exercise was better at maintaining rates of muscle protein synthesis during a short term (~2 wk) energy-restricted intervention (Murphy et al., 2015). Although this study lacked measurement of functional outcomes, the results suggest that balanced protein ingestion in an ageing population is integral to maintaining lean mass, at

least when energy intakes are mildly restricted. Therefore, in order to achieve "healthy" ageing (i.e. conservation of lean mass and function) it is essential that the minimum RDA for protein is met and that protein intake is distributed appropriately throughout the day (Breen & Phillips, 2011). Furthermore, these results (Campbell et al., 2001; Houston et al., 2008; Murphy et al., 2015) form a strong case for increasing protein intake above the current RDA as an essential component and modifiable life-style factor to combat sarcopenic obesity.

2.2.4 Exercise training

Exercise promotes multiple benefits in almost every tissue and organ (Hawley, Hargreaves, Joyner, & Zierath, 2014) and, when undertaken regularly, is a cost-effective and potent stimuli for the prevention and treatment of many chronic diseases (Booth, Gordon, Carlson, & Hamilton, 2000). However, only a small proportion of the adult population currently meet the recommended guidelines of ≥30 min per day (Haskell et al., 2007) and the lack of adherence to regular exercise can promote a vicious cycle of inactivity and the progression of chronic diseases (**Figure 2.4**).
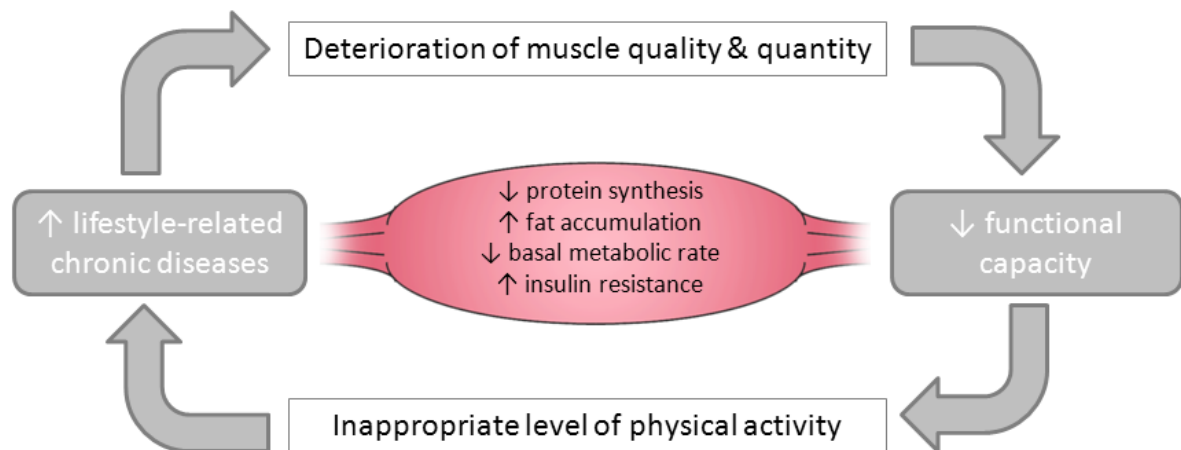
Figure 2.4. Inappropriate levels of physical activity combined with a low protein diet lead to a cascade of events in skeletal muscle that predispose the individual to a greater risk of many chronic metabolic diseases, resulting in a substantial loss of functional capacity.

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Aerobic- or endurance-based exercise (e.g., walking, swimming, cycling) is an accessible form of training, due to requiring little guidance for most overweight and/or obese individuals. The benefits of aerobic training on skeletal muscle include improved oxidative metabolism via an increase in both capillary density and mitochondrial content (Holloszy & Coyle, 1984), and at a whole body level enhance cardiovascular function, increase basal metabolic rate, increase rates of fat metabolism and improve aerobic capacity (Slentz et al., 2011). Regular exercise may also induce changes in eating behaviours that may reduce energy intake in an individual's habitual diet (Bales et al., 2012). Such changes in whole body metabolism and dietary habits are beneficial to maintain a healthy body composition.

Aerobic exercise can also elevate muscle protein synthesis and elicit modest increases in muscle mass (Donges et al., 2012; Harber et al., 2010; Wilkinson et al., 2008). However, the capacity of aerobic exercise to ameliorate the effects of sarcopenia has not received much scientific enquiry. The results of recent studies in previously sedentary older (>70 y) women and men reveal ~6-12% increases in quadriceps femoris volume concomitant with 13-30% improvements in aerobic capacity after 12 wk of aerobic training (Harber et al., 2009, 2012). In their investigation of male participants, almost a 2-fold greater quadriceps volume was observed at baseline (Harber et al., 2012) compared to the investigation of female participants (Harber et al., 2009). However, the aerobic training stimulus increased quadriceps volume of ~6% in males compared to a ~12% increase in females. As the increased muscle volume did not translate to increased muscle mass (Harber et al., 2009, 2012), the authors attributed the improved aerobic capacity (13 and 30%, for males and females, respectively) to the baseline quadriceps volume. Therefore, it is likely that the addition of other training modes is necessary to maximise retention and/or promote enhancement of muscle mass over the long-term to help prevent or mitigate the effects of sarcopenia.

Resistance-based exercise generates acute and sustained (i.e. up to 48 h) increases in rates of myofibrillar muscle protein synthesis. When protein feeding occurs in the 30 min – 1

h period after resistance exercise, this acute synthetic response is enhanced over non-feeding and promotes positive net protein balance in skeletal muscle (Moore et al., 2009). With chronic resistance training there is protein accretion and reduced protein breakdown (Koopman et al., 2004) leading to increases in muscle mass in young healthy populations (Moore et al., 2007). Older adults retain the capacity to enhance muscle mass but the anabolic response to both exercise and protein feeding is blunted (Drummond et al., 2008). As a consequence, the magnitude of the muscle protein synthetic response along with increases in muscle cross sectional area are generally less in older adults compared to their younger counterparts (Breen & Phillips, 2011). Studies that have focused on resistance exercise in the elderly have reported improvements in muscle cross-sectional area and strength (Ballor et al., 1988; Frontera, Meredith, O'Reilly, Knuttgen, & Evans, 1988; Peterson, Rhea, Sen, & Gordon, 2010). Therefore, resistance-training remains an essential exercise modality for the elderly in order to maintain (or increase) muscle mass and strength and conserve high levels of functional capacity. For the purpose of this thesis, unless defined otherwise, resistance exercise refers to the movement of free or machine based weights, for 8-15 repetitions, 2-4 sets at 40-70% of one repetition maximum (1RM).

Although resistance training retains or improves the relative quantity of lean tissue to total body mass, the low energetic requirements associated with this type of exercise typically result in only modest effects on fat loss. Willis and colleagues (2012) recently reported increased lean mass with resistance training (3 d/wk) compared to aerobic training (equivalent to ~19 km at 65-80% VO_2peak) over 8 months (1.1 kg vs. -0.1 kg, respectively). However, resistance training failed to promote a substantial loss of fat mass (-0.3 kg) compared to aerobic training (-1.7 kg). Consequently the resistance-training group gained body mass (Willis et al., 2012). Of note, their combined intervention provided the best improvements in body composition through similar lean mass gains to resistance only, similar fat mass losses to aerobic only, and therefore the greatest decrease in body fat percentage (Willis et al., 2012).

Ismail and colleagues (2012) also showed resistance exercise to be less effective at reducing visceral fat when compared with aerobic exercise. Consequently, the primary consideration with resistance exercise prescription in isolation is that the effects on fat metabolism are modest but that its role in retaining and/or increasing muscle mass is robust (Rolland et al., 2008). However, when exercise is included in a weight loss intervention enhanced weight loss is not related to exercise type (i.e. whether it is aerobic or resistance based), rather whether the exercise is supervised compared to unsupervised elicits the greatest losses of body mass (Caudwell, Gibbons, Finlayson, Näslund, & Blundell, 2014).

In summary, aerobic exercise promotes beneficial changes in muscle metabolism and reduces fat mass while resistance exercise preserves muscle mass. Accordingly, it seems intuitive that the optimal exercise prescription for healthy weight loss and muscle maintenance for individuals with sarcopenic obesity is the incorporation of both exercise modalities in a balanced supervised training program. Using such an approach, fat loss may be effectively achieved while maintaining sufficient quality and quantity of muscle mass to counteract sarcopenia, and extend a healthy lifespan (Wannamethee et al., 2007). Importantly, regular exercise without appropriate dietary intervention is a sub-optimal approach for healthy weight loss and it is imperative that exercise-diet interventions are employed to most effectively ameliorate the debilitating effects of sarcopenic obesity.

2.2.5 Dietary and exercise interventions

Many studies have manipulated combinations of diet and physical activity in weight loss protocols and have shown that energy restriction (in the absence of exercise) results in a loss of lean tissue (Ballor et al., 1988; Hoie et al., 1993; Layman et al., 2005; Villareal et al., 2011; Foster-Schubert et al., 2012) (**Figure 2.5A**). This becomes an essential consideration when prescribing nutritional modifications in the absence of any exercise intervention as a treatment for sarcopenic obesity. Studies that have incorporated dietary restriction together with an exercise program have generally resulted in better outcomes with regard to promoting body mass loss and improving body composition compared to those with diet or exercise modification alone (i.e. **Figure 2.5C** vs. **Figure 2.5A** and **B**, respectively).

Ballor and colleagues (1988) conducted the seminal study to investigate the effects of resistance exercise alone as well in combination with an energy-restricted diet, compared to energy restriction alone in obese middle-aged (~33 y) women. Prior to this study, resistance exercise had not been utilised to elicit increases or the maintenance of lean mass during body mass loss induced through dietary means. Whilst the diet-only and combined interventions both induced a ~4 kg body mass loss across the 8-wk intervention period, lean mass retention was superior in the combined intervention compared to the diet-only group (Ballor et al., 1988). Thus, resistance exercise was shown to generate favourable body composition changes in the face of mild energy restriction.

In an obese and older (~70 y) population of both men and women Frimel and colleagues (2008) combined a resistance and aerobic exercise (3 × 90 min/wk combined sessions) stimulus with a low energy diet (an energy deficit of ~750 kcal/d) for 6 months. The lack of gain of lean mass observed could be expected when considering the older age of the participant group compared to that of Ballor et al., (1988). However, Frimel and coworkers (2008) did find that the combined exercise and dietary stimulus was successful for

maintaining lean mass concomitant with a significant loss in fat mass compared to the energy-restricted diet alone, thus improving body composition (Frimel et al., 2008).

In an older-aged (~68 y) group of overweight women, Campbell and colleagues (2009) measured protein kinetics as well as total changes in protein-mineral and lean masses over a 16-wk intervention. The study compared resistance exercise (3 d/wk) with dietary induced energy restriction (-500 kcal/d) to the energy-restricted diet intervention alone. Whilst the loss of fat mass was not different between groups, the loss of lean mass was greater in the non-exercising dieting group (-1.6 kg) compared to the resistance training plus diet group (-0.3 kg). In this study, protein-mineral mass did not change, neither did the protein kinetics measured via leucine and phenylalanine tracers (Campbell et al., 2009). Therefore, the authors attributed the changes in lean mass in the non-exercising group to total body water (Campbell et al., 2009). In the energy intake of both groups, protein intakes were kept consistent and above the RDA at 1.0 g/kg BM/d which may have contributed to the lack of difference in protein-mineral mass.

Nicklas and colleagues (2009) utilised a 20 wk intervention of aerobic exercise training (3 d/wk) with a 400 kcal/d energy restricted diet. Two groups completed this combined intervention in which the aerobic exercise intensity differed (moderate: 45-50% VO_2peak vs. vigorous: 70-75% VO_2peak). For this group of overweight post-menopausal (~58 y old) women, neither moderate or vigorous aerobic exercise stimulus was able to mitigate lean mass losses induced by the energy restriction (Nicklas et al., 2009). These findings further highlight the need for a resistance exercise stimuli to maintain lean mass. Therefore, when implementing weight loss strategies, the only intervention with the capacity to mitigate the loss of lean mass during a period of energy deficit is resistance-based exercise.

Villareal and colleagues (2011) determined changes in body composition in response to either a diet (-500–750 kcal/d), exercise (3 d/wk aerobic and resistance) or a combined intervention for 52 wk in older (+65 y) obese men and women. They found no difference in

total body mass between the diet and combined groups; however the combination of the exercise intervention with dietary restriction resulted in 50% attenuation in the loss of lean mass that occurred in the diet-only group (Villareal et al., 2011). However, a loss of ~1.8 kg lean mass was still observed in the combined group, which may be expected due to the large energy deficit and the intermittent nature of exercise sessions. Further, greater improvements in physical function were observed in the combined group over the diet-only group.

Foster-Schubert and colleagues (2012) conducted a 1 y intervention with the aim of reducing body mass of their older (~58 y), obese postmenopausal women by 10% in the first six months, then maintain if for a further six months, until 12 months. To try to achieve this modest change in body mass, participants were assigned to an energy-restricted diet (~1300 kcal/d), an exercise program (45 min moderate intensity 5 d/wk where 3 d/wk was supervised), or a combined intervention of both diet and exercise. Whilst all three groups lost significant body mass from baseline, the combined exercise and diet group lost greater amounts of fat mass than the diet-only intervention, although lean mass maintenance was similar, and therefore had an improved body composition (Foster-Schubert et al., 2012).

In summary, energy restriction plus exercise stimulus compared to energy restriction alone results in a superior body composition outcome (**Figure 2.5C**). Specifically, when the exercise stimulus is resistance-based exercise, there is lean mass maintenance or gain of lean mass that is superior to aerobic exercise. Thus, to induce the most desirable changes in body composition, an energy-restricted diet should be concomitant with resistance exercise for lean mass maintenance as well as aerobic exercise for fat mass loss.
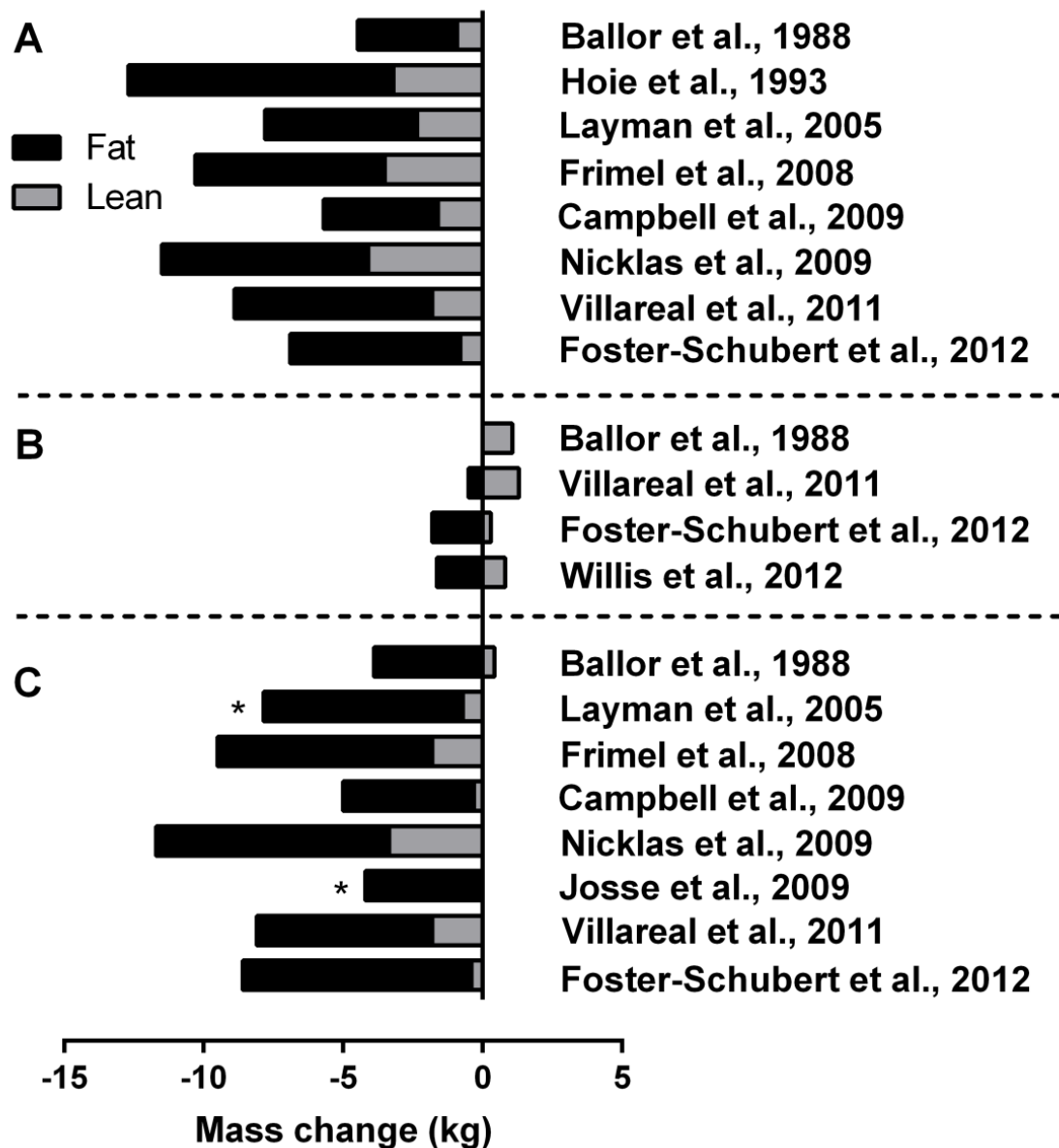
Figure 2.5. Data from body mass loss studies showing the effects of A) energy restriction alone, B) exercise alone, and C) energy restriction and exercise, on changes in fat mass and lean (fat-free) mass; *study of high protein diet.

In terms of dietary macronutrient composition, high protein diets are beneficial for minimising the loss of lean mass associated with energy-restricted diets in the absence of exercise (Leidy, Carnell, Mattes, & Campbell, 2007). However, there is no evidence to support the contention that high protein intake can prevent loss of skeletal muscle mass during prolonged periods of energy deficit. Of note, the study by Frimel and colleagues (2008) demonstrated that diets providing >20% of energy intake from protein may not be necessary with the inclusion of resistance exercise in a weight loss intervention (Frimel et al., 2008),

possibly due to lower leucine turnover and increased nitrogen retention with chronic resistance training (Moore et al., 2007).

In the controlled environment of a nursing home, increased protein intake in older women (~73 y) combined with progressive resistance exercise training has been shown to increase total and leg lean mass, as well as increase muscle strength compared with resistance training alone (Daly et al., 2014). However, increasing the protein intake above the RDA (rather than as a percentage of EI) with a resistance training program has not been shown to increase lean mass in two separate studies of older (~60 y) men and women (Andrews, MacLean, & Riechman, 2006; Iglay, Thyfault, Apolzan, & Campbell, 2007). Therefore, total protein intake above RDA levels during energy restriction in combination with chronic resistance-based training has not consistently demonstrated an additive effect of increasing lean mass in older (>60 y) adults. However, emerging evidence supports the notion that increased protein ingestion in the early post-exercise period following a single bout of exercise is beneficial in older individuals (Pennings et al., 2012; Yang, Breen, et al., 2012). Indeed, the interplay between the quantity and timing of protein ingestion after resistance exercise is a major factor regulating the capacity of the protein synthetic machinery for skeletal muscle maintenance and will be discussed subsequently (in Section 2.3.1).

Layman and colleagues (2005) have demonstrated an additive effect of resistance exercise with a high protein diet. In the female-only (40-56 y) group, increased protein intakes (~1.2 g/kg BM/d vs. 0.7 g/kg BM/d) over 4 months induced greater losses of fat mass. However, the higher protein intakes were only superior for retaining lean mass when an exercise stimulus (30 min walking on 5 d/wk plus 2 d/wk of ~30 min resistance exercise) was present. In order for protein intake to be increased CHO intake was reduced (i.e. similar fat intake between diets), with a total energy reduction of ~700 kcal/d. As such, the main effect on total body mass loss was through energy restriction, with no added benefit to body mass loss from the exercise stimulus. However, the effects of diet and exercise on the loss of body

fat were independent and additive. These differences between body fat and body mass losses highlight the importance of measuring body composition (Caudwell et al., 2014) rather than merely changes in total body mass.

Although many weight loss interventions only recruit female participants, a systematic review of both males and female studies found no effect of sex on weight loss, with any small differences in weight being largely attributable to exercise energy expenditure (Caudwell et al., 2014). Therefore, the recruitment of both male and female participants in weight-loss studies is important to ensure data can be extrapolated to the general population. A meta-analysis of 52 intervention studies showed the greatest weight losses were achieved through energy-restricted diets with a regular (daily) exercise stimulus (Weinheimer, Sands, & Campbell, 2010). Of importance, the authors found the resistance exercise component to be integral in reducing loss of lean mass that occurs with energy-restricted diets (Weinheimer et al., 2010).

In summary, the results of the majority of studies support the contention that a higher protein intake within an energy-restricted diet is necessary to facilitate maximal losses of fat mass in an overweight/obese population. An increased contribution to total energy requirements from protein is often at the expense of CHO intake (i.e. protein displaces energy from CHO). Whether the same reductions in fat mass can be attained with a high protein diet where the energy restriction is due to decreased contribution from fat and not CHO is currently unclear.

2.3 Role of dairy products/ foods in weight loss diets

2.3.1 Source, timing and distribution of protein in energy-restricted diets

While the evidence for increasing protein intake to decrease fat mass and maintain lean mass during periods of energy restriction is compelling, the source of dietary protein has received little study and has the potential to impact further on changes in body composition. In older men (~74 y), the ingestion of 20 g whey protein at rest acutely increased muscle protein synthesis (MPS) when compared to equal quantities of proteins casein and casein hydrolysate (Pennings et al., 2011). The authors attributed the increased MPS to the higher peak plasma leucine values and faster digestion rates of the whey protein (Pennings et al., 2011). In the 4-h recovery period from acute resistance exercise, 20 or 40 g of whey protein ingestion resulted in superior MPS over the equivalent amount of soy protein consumption in older (~71 y) men (Yang, Churchward-Venne, et al., 2012). Although both studies have fed proteins in supplement forms, both demonstrated the superiority of whey protein as the optimal source to maintaining MPS. When chronic in nature, whey protein intake may lead to the maintenance of lean mass in this older population.

The timing of protein intake across the day is also important to maintain MPS and to maintain lean mass. A skewed distribution of protein intake has been observed across elderly (65-94 y) community-dwelling, frail (i.e. required healthcare and physically inactive), and nursing home patients, where large portions of protein are typically consumed at dinner (i.e. the last meal; see **Figure 2.6**) (Tieland, Borgonjen-Van den Berg, van Loon, & de Groot, 2012). This skewed protein intake across the day may contribute to the decline in lean tissue mass, and when paired with an inactive population, is likely to ultimately impair functional outcomes. As such, it has been suggested that 25-30 g of high quality protein be consumed with each meal to prevent or slow down muscle loss with ageing (Paddon-Jones & Rasmussen, 2009).
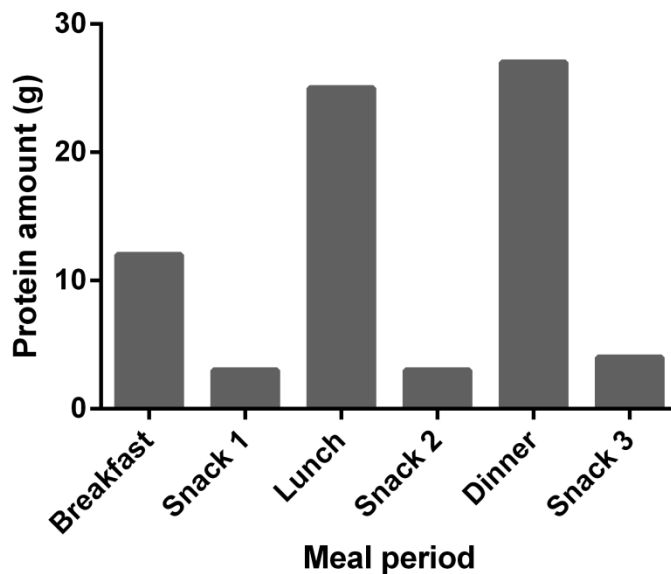
Figure 2.6. Daily protein intake in grams as distributed per mealtime from an average across data from community dwelling, frail and nursing home elderly people. Redrawn from Figure 71 of Tieland et al., Eur J Nutr, 2012:51:173-179.

The distribution of protein intake has been demonstrated to be important in promoting the maintenance of lean mass. Areta and colleagues (2013) compared different patterns of protein ingestion (a total of 80 g) over a 12 h period of recovery from resistance exercise in young (~25 y), male resistance-trained athletes. They measured maximal rates of MPS using a phenylalanine tracer technique when protein was ingested in 10, 20 or 40 g feedings (Areta et al., 2013). The ingestion rate of 20 g in four parts throughout the 12 h period was superior for stimulating MPS, however, the energy intake in this study was not energy-restricted.

Murphy and colleagues (2015) have recently shown that protein distribution across meals is integral to maintaining MPS in a short term (~4 wk) period of energy restriction compared to energy balance in older (~66 y), overweight men (Murphy et al., 2015). They investigated the rates of MPS in response to either Balanced (25% of total protein intake) or Skewed (7:17:72:4% of total protein intake) intakes across four meals (breakfast, lunch, dinner and pre-bed snack), where total energy was restricted by 300 kcal/d. In order to reach these protein intakes (1.3 g/kg BM/d), a whey protein beverage was added to the diets of the Balanced group, whereas the skewed group consumed protein from food sources only. When

energy-restricted, MPS was higher when the protein intake was Balanced (i.e. spread evenly throughout the day) compared to Skewed (Murphy et al., 2015). Therefore, MPS can be maintained when in energy restriction as long as the intake of protein is sufficiently greater than the RDA and is distributed evenly throughout meal times across a day.

Typically, many studies investigating the effects of protein intake on the maintenance of lean mass have used supplement protein rather than whole foods. The consumption of real foods within an energy-restricted eating plan should be encouraged for long-term adherence, but is harder to facilitate in research studies. In a controlled, nursing home environment, Daly and colleagues (2014) combined increased protein intakes through increased red meat consumption paired with resistance exercise in 60-90 y old women to increase lean mass compared with a typical CHO-based meal with resistance exercise over a four month period. Although the diet was not energy-restricted, the combination of real food through increased red meat consumption at lunch and dinner had a positive effect on improving body composition.

2.3.2 Dairy foods and calcium in energy-restricted diets

The contribution of dairy-based foods to total daily energy intake, as well as the type of dairy foods consumed (i.e. low vs high fat content) has altered dramatically over the past quarter of a century. Hu and colleagues (2000) showed the change in dairy food consumption through high and low-fat dairy servings from 1980-1990 as a part of a larger study (**Figure 2.7**). The trend of dairy serving intake consisted of a reduction in the consumption of high fat dairy products and increased consumption of low fat dairy products (Hu et al., 2000). Indeed up until recently, fat is been seen as the "enemy" in weight loss diets thus it might be expected that the trend in **Figure 2.7** would continue through to the present day, especially given recent media attention to popular "fad" diets. Unfortunately, no updated data have been reported, nor have similar statistics been produced specific to Australian consumers.

Results from the 1995 Australian National Nutrition Survey compared with the 2011-2012 Nutrition First Results of the Australian Health Survey show that increases in protein intake have occurred concurrently with a decreased intake in the amount of energy from milk products and dishes (**Table 2.1**). Although the intake of dairy foods in Australia appears to have declined slightly (**Table 2.1**), the consumption of dairy-based foods has been associated with many positive effects on fasting glucose, cholesterol, blood pressure and waist circumference, as examples (Rice, Cifelli, Pikosky, & Miller, 2011). With their high protein content dairy-based foods serve as practical options for increasing protein intakes at all meals and improving various health measures.
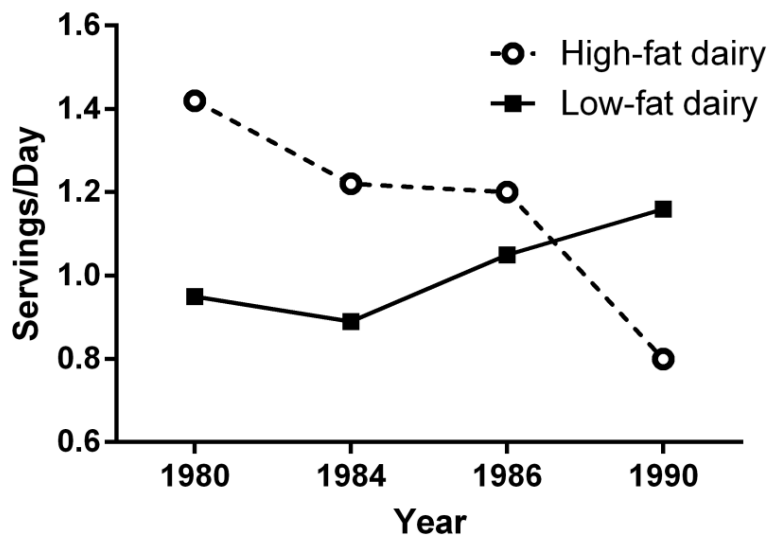
Figure 2.7. Age-adjusted trends in intake of dairy foods, where high-fat dairy included whole milk, hard cheese or cream cheese, ice cream and butter, low-fat dairy included skim or low-fat milk, yogurt, and cottage cheese. Redrawn from Figure 3, Hu et al., The New England Journal of Medicine (2000), 343(8): 530-537.

Table 2.1. Extracted information from the 2011-12 Australian Health Survey: Nutrition First Results and the 1995 National Nutrition Survey: Selected Highlights Australia for the average energy intake (kJ), the approximate energy contribution of Milk products and dishes (kJ), and the average macronutrient distribution (% of energy intake)

	Year	1995	2011	Δ	1995	2011	Δ
	Age group	**31-50 y**			**51-70 y**		
Energy, kJ							
	Men	11500	10200	-1300	9770	9340	-430
	Women	7830	7540	-290	7020	7270	+250
	All	9640	8870	-770	8380	8290	-90
Milk products and dishes, kJ	Men	1265[*]	906	-359	1026[*]	873	-153
	Women	940[*]	740	-200	856[*]	705	-151
Macronutrients, % of energy intake[‡]	CHO	44.9	43.4	-1.5	44.7	41.9	-2.8
	Fat	31.9	31.0	-0.9	31.0	30.7	-0.3
	Protein	16.7	18.4	+1.7	17.4	18.6	+1.2

CHO, carbohydrate; [*]Manually calculated from percentage contributions provided in the 1995 National Nutrition Survey in relation to total energy intake, where age brackets were 25-44 and 45-64 y; [‡]Total energy intake will not add to 100% due to excluding energy from fibre and other components.

Recently, two groups have undertaken meta-analyses of randomised control trials (RCTs) that have measured the effect of dairy food consumption on changes in body composition (Abargouei, Janghorbani, Salehi-Marzijarani, & Esmaillzadeh, 2012; M. Chen, Pan, Malik, & Hu, 2012). Chen et al., (2012) analysed 29 RCTs that included a range of dairy servings (1 to 6.5 servings/d) within energy-restricted or non-energy-restricted diets. The analysis was further subdivided by the duration of diet intervention: short (<1 y) or long (≥1 y) term. Short-term energy-restricted diets with increased dairy food intake resulted in a significant beneficial effect on the reduction in body fat, but when dietary intake was not energy-restricted there was no effect of the addition of dairy foods on changes in body composition (M. Chen et al., 2012).

Abargouei and colleagues (2012) utilised a different inclusion criteria of RCTs to their studies including 10 studies that met the criteria of including ≥3 − 6 dairy servings/d and a 500 kcal/d energy restriction. In agreement with Chen and colleagues (2012), Abargouei et al., (2012) also found greater reductions in body mass, fat mass and waist circumference in

individuals who consumed ≥3 dairy servings/d (containing >1000 mg calcium) compared to the control diets (containing <800 mg calcium). Therefore, it has been suggested that dairy foods should be included as a part of an energy-restricted diet as they reduce hunger and therefore induce appetite-lowering effects (Abargouei et al., 2012). However, it is worth noting that of the studies included in both meta-analyses (Abargouei et al., 2012; M. Chen et al., 2012) only one study has utilised energy restriction in combination with exercise (Josse, Atkinson, Tarnopolsky, & Phillips, 2011).

In a young (~19-45 y) group of premenopausal women, Josse and colleagues (2011) increased protein intake through the consumption of dairy foods. The aim of their study was to maximise fat mass losses with the retention of lean mass within an energy-restricted diet combined with resistance (2 d/wk) and aerobic (5 d/wk) exercise. Increasing the number of dairy serves from 1 serving/d (low dairy, protein: 0.7 g/kg BM/d) to 5 servings/d (moderate dairy, protein: 0.8 g/kg BM/d) or 7 servings/d (high dairy, protein: 1.3 g/kg BM/d) led to an increased total dietary calcium intake that was associated with larger gains of lean mass. However, no difference in the loss of fat mass was observed between the groups consuming three different levels of protein (Josse et al., 2011). In their study, increased protein intakes through high dairy food consumption made it necessary to reduce the CHO intake to match EI (Josse et al., 2011). Whether the same effect of high dairy protein intakes on increasing fat mass losses would be observed if fat intake was reduced and CHO intake remained similar to typical intakes (~55% CHO) is not known. Whether the same effect of increased lean mass occurs in an older age group consuming increased dairy products, prior to sarcopenic-inducing muscle losses (~30-60 y) has yet to be investigated.

It is plausible that increased intakes of dairy foods exert a positive effect on body composition due to the increased amount and type of calcium consumed. Zemel et al., (2009) compared the effect of increased calcium through dairy food sources to the same intake through calcium carbonate supplementation, and a low calcium control group. The young

(~25 y) male and female participant groups were placed on a 500 kcal/d energy-restricted diet for 12 wk. In that study, increased calcium intake achieved via dairy intake increased the loss of fat mass with an energy-restricted diet (Zemel et al., 2009). In contrast, Wagner and colleagues (2007) found no difference in the magnitude of weight loss (~4.1-5.4 kg) using the same energy deficit and intervention period (~500 kcal/d for 12 wk) between calcium supplementation (via calcium lactate or calcium phosphate) and low fat milk consumption. In that study, where each supplementation regimen resulted in a daily calcium intake of ~1500-1600 mg compared to placebo condition where intakes were ~800 mg, all conditions achieved a similar body mass loss (-5.8 kg) regardless of dietary macronutrient composition (Wagner et al., 2007). An explanation for the disparity between the results of these studies (Wagner et al., 2007; Zemel et al., 2009; Josse et al., 2011) may be the low baseline calcium intakes of the predominantly female participant cohorts. In the studies of Josse et al., (2011) and Zemel et al., (2009), participants had calcium intakes of ~500 mg at baseline (50% RDA), whereas Wagner and colleagues (2007) did not measure pre-study calcium intake. It appears that having a low calcium intake at baseline is an important factor influencing the magnitude of body weight loss independent of the change in the increase in daily calcium intake.

In this regard, Boon and colleagues (2005) have suggested a calcium intake "threshold" above which additional intake confers no further benefit in inducing changes in body composition. The calcium intake "threshold" concept was achieved using linear regression modelling to analyse the effects of dietary and body composition variables in a 23-year study of ~600 Dutch citizens. The study found no additional benefit of a high (>1200 mg) calcium intake over a moderate (800-1200 mg) intake on body composition (Boon et al., 2005). Shahar and colleagues (2010) found that an increase in dairy calcium intake rather than total calcium, promoted greater diet-induced weight loss. However, the total calcium intakes were >800 mg and were not modelled against weight change; furthermore only dairy calcium (~34-44% total calcium intake) was correlated to weight change. Therefore, the calcium

content of dairy foods may play an important role in enhancing weight loss. The high protein content of dairy foods make dairy an appropriate food to modify dietary composition in order to maximise positive outcomes associated with body composition, during periods of energy restriction.

2.4 Circulating microRNA analysis for individual responses

2.4.1 Reporting of individual responses

Studies assessing the effects of diet and exercise interventions on weight loss commonly report the magnitude of weight loss for the cohort under investigation as a mean, standard deviation, or standard error of measurement. In general, although it is possible to get an estimate of the "spread" or range of responses, it is not common practice for the individual variability in responses to be explored.

Some studies, however, present the variation of individual results providing a range of responses in the study cohort to a given intervention and the identification of "low" and "high" responders. For example, Davidsen et al., (2011) reported that among the 56 young men who undertook a standardised programme of resistance training with stringent dietary control for 12 wk, there were substantial differences in the amount of lean mass accretion (as determined by DXA) (Davidsen et al., 2011). Across the group, some participants achieved only small (~1 kg) lean mass gains compared to large (~4.5 kg) gains of lean mass in others (Davidsen et al., 2011). Using an aerobic, rather than resistance-based, exercise training stimulus aimed to induce a moderate energy deficit, King and co-workers (2009) measured weight loss in 58 overweight/obese men and women (King et al., 2009). They found that 45% of participants (**Figure 2.8**) failed to achieve the weight loss, predicted from previous research on energy expenditure (Leibel, Rosenbaum, & Hirsch, 1995), despite experiencing significant exercise-related health improvements, with the remaining 55% losing ≥3.3 kg (King et al., 2009). Herrmann and colleagues (2015) added aerobic exercise to the daily activities of young

(~23 y) men and women for 5 d/wk over a 10-month period in order to increase energy expenditure by 400 or 600 kcal/session. They reported 46% of participants to be 'non-responders' to the intervention as assessed by a weight loss of <5% body mass (Herrmann et al., 2015). The results of these recent studies (Davidsen et al., 2011; Herrmann et al., 2015; King et al., 2009) highlight the inherent differences in individual responses to given exercise interventions and emphasise a need to determine potential physiological mechanisms that may underpin such variation between individuals. With such information, the treatment for obesity can be applied to a much wider range of participants.
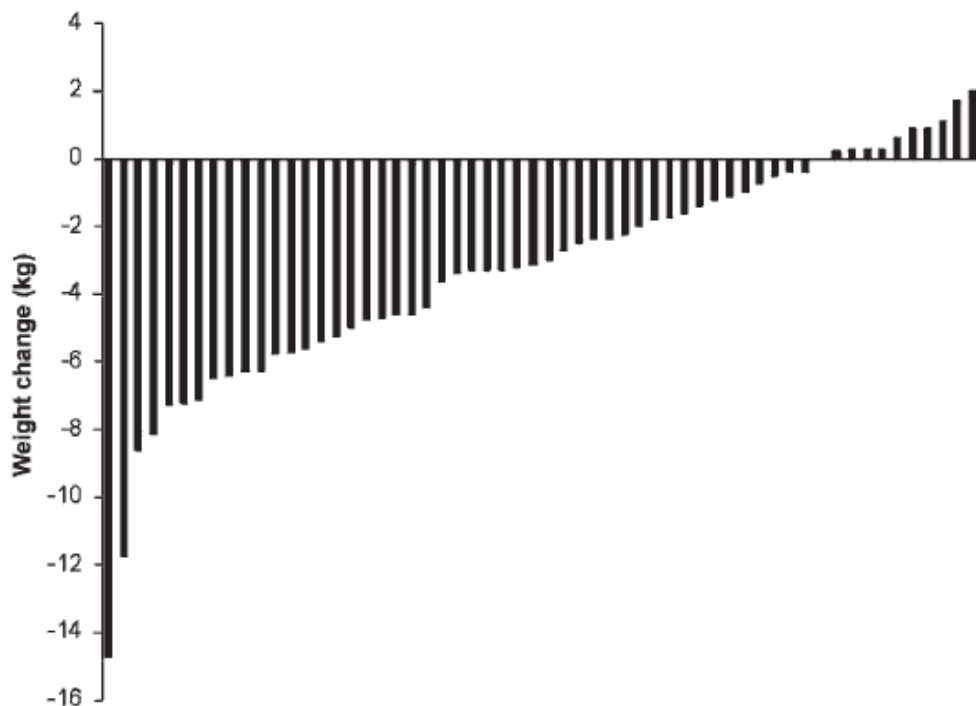
Figure 2.8. Individual responses of sedentary, overweight/obese men and women to an exercise-based weight loss intervention of 12 wk duration. Figure taken from Figure 1, King et al., *Br J Sp Med*, 2009; 43:924-927.

2.4.2 Genetic vs epigenetic factors

Intrinsic differences in responses between individuals weight loss in interventional studies underline the need to recruit large cohorts of participants. Larger cohorts allow the researchers to determine the efficacy of particular interventions under investigation. Intrinsic differences between individuals have been linked to genetic (i.e. heritability) and epigenetic

factors. Indeed, a study using identical twins demonstrated variation in weight loss responses between adult twins pairs to be almost 13 times greater than the variation within twin pairs to the same VLCD in an in-patient metabolic unit (Hainer et al., 2000).

Many of the metabolic adaptations to weight loss interventions are influenced by the transcriptional and translational regulation of genes encoding proteins with regulatory roles in metabolic responses and subsequent adaptations (Dela et al., 1994; Short et al., 2003). Epigenetics is the study of modifications in gene expression influenced by behaviour and environmental changes (i.e. dietary components, toxins and drugs, inflammation and physical activity) that occur without an alteration to the DNA sequence (Z. Chen & Riggs, 2005). Epigenetic alterations occur via modifications, such as conformational changes to DNA to influence changes in tissue-specific gene expression (Bannister & Kouzarides, 2011; Varley et al., 2013).

An emerging source of epigenetic modification are microRNAs (miRNAs), small (~22 nucleotides) non-coding strands of RNA (i.e. RNA sequences that do not produce a protein) that can promote messenger RNA (mRNA) degradation and/or supress protein translation (He & Hannon, 2004). A single miRNA can regulate the expression of over 100 different mRNAs and proteins while individual mRNAs can also be subject to regulation by multiple miRNAs (Ambros, 2004). The advancement of computational approaches such as TargetScan (Friedman, Farh, Burge, & Bartel, 2009), based on bioinformatic algorithms, allows for the prediction of mRNA targets by assessing the formation and stability of mRNA:miRNA duplexes (Dweep, Sticht, Pandey, & Gretz, 2011). The numerous targets of each miRNA make it difficult to determine how changes are influenced by physiological and pathological conditions (Aoi, 2014), however, the modulation of specific miRNA(s) may provide promising therapeutic targets in the management of obesity and metabolic diseases (Xie, Sun, & Lodish, 2009).

2.4.3 *MicroRNAs as biomarkers to explain individual differences to weight loss interventions*

MicroRNAs are expressed in multiple tissues throughout the body including muscle, liver and adipose tissue as well as in the blood, depending on their physiological function (Liang, Ridzon, Wong, & Chen, 2007). Although the mechanisms underlying the cellular secretion of miRNAs from specific tissues into the circulatory system (c-miRNAs) are not fully understood (Gupta, Bang, & Thum, 2010), they can be detected in plasma and serum (Lawrie et al., 2008; Mitchell et al., 2008). While c-miRNAs (c-miRs) appear to be robust in plasma from fresh or frozen and then re-thawed samples (Gilad et al., 2008), several confounding issues exist in the accurate quantification of c-miRs. For example, c-miRs can have low copy numbers (Aoi et al., 2013; Nielsen et al., 2014), samples need to be free of haemolysis (Kirschner et al., 2011), and there is no current consensus for the use of a house-keeping standard for data normalisation (Aoi & Sakuma, 2014). Moreover, studies measuring changes in c-miRs may provide more detailed information of the expression of predicted targets if samples from potential tissue of origin or destination (i.e. muscle, adipose tissue, etc.) are simultaneously measured. Further developments in methods of miRNA detection and target prediction will help this evolving research area progress towards their use within individualised treatments.

The expressions of particular c-miRs have emerged as promising biomarkers of disease. As such, the dysregulation of c-miRs have been associated with a number of different pathologies such as cancer and cardiovascular disease (Carè et al., 2007; Thum, Catalucci, & Bauersachs, 2008). This has raised the possibility of whether the expression of certain c-miRs may also be implicated in development and/ or treatment of obesity. Indeed, altered expression of several c-miRs (c-miR-223 and -143) have been measured in whole blood samples across a range of clinical cohorts including morbidly obese (BMI >40 kg/m^2), obese (BMI 30-39.9 kg/m^2) and overweight/control (BMI <29.9 kg/m^2)) (Kilic et al., 2015). Both of

these c-miRs were expressed higher in the morbidly obese and obese groups compared to the overweight/normal BMI group, potentiating a possible the role for these c-miRs in the pathogenesis of obesity. Whether other c-miRs are associated in the development of obesity requires further investigation.

The expression of particular c-miRs prior to and following acute and chronic exercise has received recent scientific enquiry. Several recent reviews (Aoi, 2014; Flowers, Won, & Fukuoka, 2015; Kirby & McCarthy, 2013; Zacharewicz, Lamon, & Russell, 2013) have summarised the range of exercise protocols (aerobic and resistance exercise, both chronic and acute) across different subject populations (healthy adolescent or adults). Together the information from these reviews, as well as additional studies of obese or diabetic populations, have started to form an integrated picture of which c-miRs expression are altered by different exercise stimuli in different participant groups. As an example, in the aforementioned study of Davidsen and colleagues (2011) of "responders" and "non-responders" to 12 wk of resistance training (as per Section 2.4.1), the change in lean mass was strongly correlated with the change in miR-378 (**Figure 2.9**). However, few studies have investigated the effect that changing dietary intake has on c-miRs in weight loss interventions. Indeed, Flowers and co-workers (2015) identified the need for further studies with both diet and exercise components to elucidate the likely existence of clinically predictive biomarkers associated with weight loss.
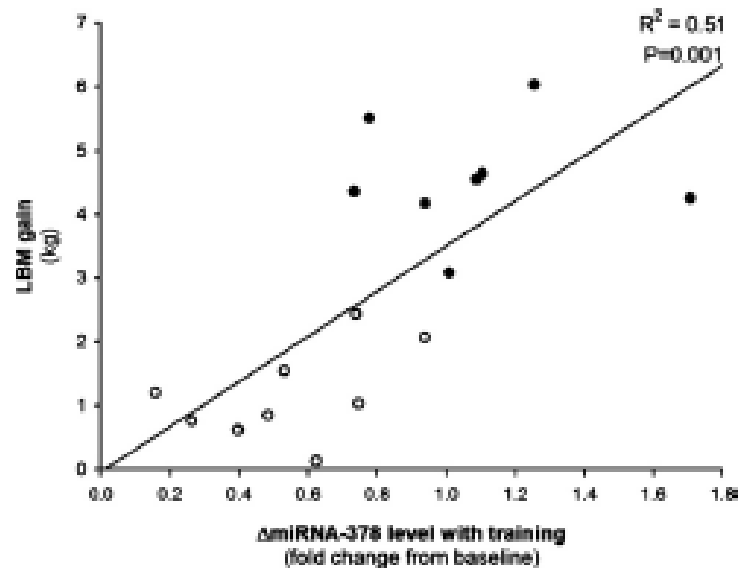
Figure 2.9. Correlation between the change in lean mass and the change in a single miRNA measured before and after 12 wk of resistance training in healthy, young men. Figure taken from Figure 3, Davidsen et al., *J Appl Physiol*, 2011:110:309-317.

One of the first groups to investigate the expression of c-miRs following weight loss was Ortega and colleagues (2013). These workers induced weight loss through surgery (gastric bypass resulting in 33% decrease in body mass) or energy restriction (500–1,000 kcal/d deficient diet for 14 wk resulting in 17% decrease in body mass). They reported significant changes in the expression of ten c-miRs when weight loss was induced through surgery, compared with no change in any c-miR following the dietary intervention (**Table 2.2**). Furthermore, at baseline, 18 c-miRs were differentially expressed across BMI ranges, between non-obese (BMI <30 kg/m^2) and morbidly obese (BMI ≥40 kg/m^2) participant groups. While the authors did not examine the expression targets of these altered c-miRs, they concluded that the specific regulation of c-miRs may constitute novel biomarkers for risk estimation and classification of morbidly obese patients (Ortega et al., 2013).

Table 2.2. Summary of c-miR analysis studies relating to weight loss through surgery, diet and diet-exercise interventions

First author, year	Sample	Intervention details	Main outcome	c-miRNAs differentially expressed
Ortega, 2013	n=6 patients (men and women) pre BMI = 42.9 kg/m^2	Pre and post (14 wk) bariatric surgery	-37 kg weight loss (~-33%); ~16 kg fat loss; BMI -14 kg/m^2	↑ miR-21, miR-130b, miR-221, miR-423-5p, ↓ miR-122, miR-140-5p, miR142-3p, miR-193a-5p, miR-222, miR-483-5p
	n=9 subjects (men and women) pre BMI = 34.4 kg/m^2	Pre and post (14 wk) low calorie (500-1000 kcal/d deficit) dietary intervention	-17% weight loss BMI -5.7 kg/m^2	NS
Milagro, 2013	n=10 subjects (women) pre BMI ~ 35 kg/m^2	Pre and post (8 wk) low calorie (800-880 kcal/d intake) dietary intervention	Comparison of "non-responders" (NR) -3.1 ± 0.7 kg body mass loss vs. "responders" (R) -10.8 ± 0.6 kg body mass loss	Baseline: ↑ miR-223, miR-935, and miR-4772 in NR; ↑ miR-224 and miR-376b in R <u>Correlated to weight loss:</u> +ve: miR-935, miR-4772, miR-199b, miR-874; -ve: miR-589, miR-148b
Wen, 2015	n=121 subjects (men and women) n=40 overweight BMI=25.7 kg/m^2; n=40 obese BMI=30.2 kg/m^2; n=41 normal weight BMI=21.7 kg/m^2.	Pre and post (3 mo) "alimentary control and taking exercise" i.e. uncontrolled calorie-reduced low fat diet (1200-2000 kcal/d) and outdoor aerobic sports for 30 min/d, 5 d/wk.	Overweight: -1.7 kg body mass loss Obese: -2.4 kg body mass loss	<u>Baseline:</u> ↓ miR-223 in obese < overweight < normal Post intervention: ↑ miR-223 expression in overweight and obese

Key: BMI, body mass index; c-miRNA, circulation microRNA; NS, non-significant changes

The expression pattern of c-miRs following a weight loss intervention has also been investigated (Milagro et al., 2013). Specifically, a subset of 'responders' ($n=5$, >5% BM loss) and 'non-responders' ($n=5$, <5% BM loss) from a previous 8 wk energy-restricted diet (800 – 1,000 kcal/d) intervention were compared for baseline and post-intervention c-miR expression using high throughput sequencing of c-miRs (**Table 2.2**). Strong correlations were reported between six of the c-miRs measured (c-miR-148b, -199b, -589, -874, -935 and -4772) and magnitude of weight loss. Furthermore, an alternative set of five c-miRs were differentially expressed at baseline between groups (c-miR-223, -224, -376b, -935, and -4772). While these findings suggest c-miRs may be used as prognostic biomarkers for weight loss, neither of these weight loss interventional studies included exercise-induced energy expenditure, which may alter the regulation of c-miRs as exercise differentially changes c-miR expression compared to energy restriction.
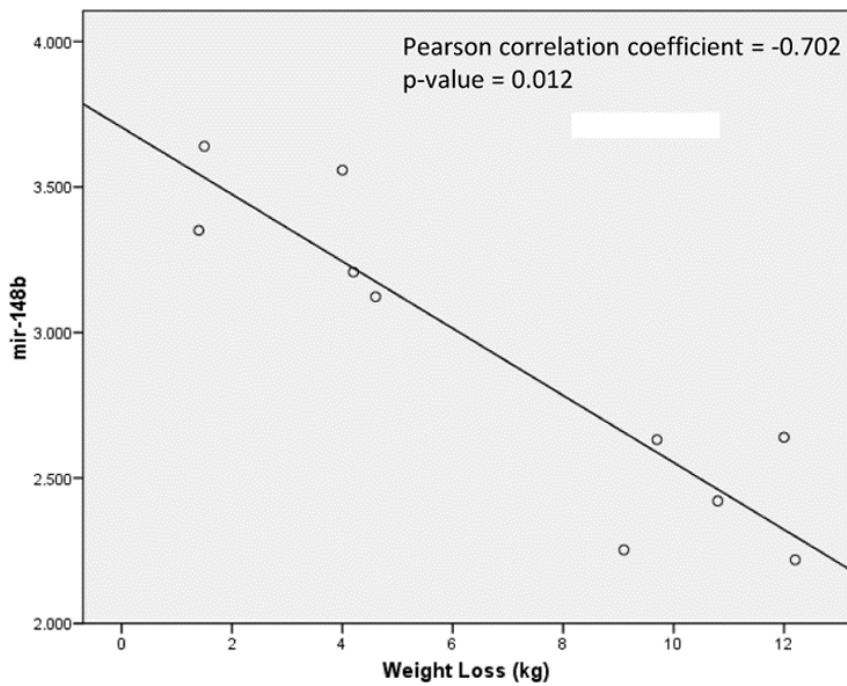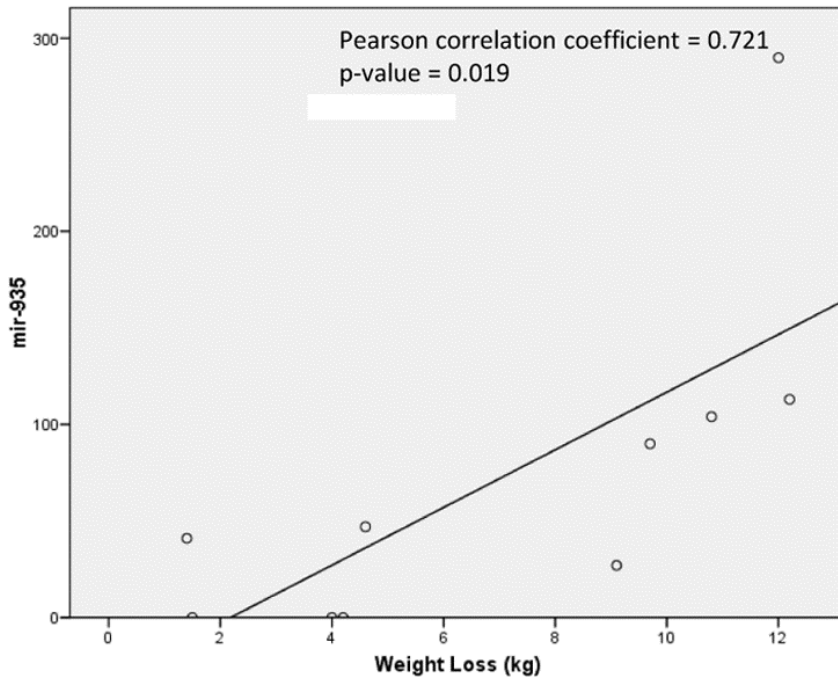
Figure 2.10. Correlation of the expression of c-miR-935 (top) and -148b (bottom) with the magnitude of weight loss from an 8 wk dietary intervention. Figure taken from figure 3, Milagro et al., PLoS One, 2013:8(1):e54319.

To date, only one study has quantified differential expression in a single c-miR in response to a combined diet and exercise intervention. Wen and colleagues (2015) did not monitor but provided advice to participants to complete an energy-restricted diet (~-300-500 kcal/d) combined with exercise on 5 d/wk (**Table 2.2**). Due to the lack of scientific control employed by the authors, a minimal (~1.7 kg) but significant body mass loss was achieved by the participant group (Wen et al., 2015). The results showed a lower baseline expression of c-miR-223 in overweight and obese participants compared to overweight controls. There was an increased expression of c-miR-223 as participants lost more weight across the intervention period (Wen et al., 2015), with the lowest quartile of c-miR-223 expression having the highest prevalence of obesity (Wen et al., 2015). These results support the findings of Kilic and colleagues (2015) and suggest that c-miR-223 may have a potential role as an important biomarker for obesity. However, in this study neither the diet or exercise interventions were quantified thus the results of this study may have limited applicability to the results of other interventions.

At present, the studies investigating the expression of c-miRs in responses to chronic weight loss interventions incorporating structured dietary and exercise programs are lacking. Moreover, little is known regarding the expression pattern of c-miRs from 'high' responders and 'low' responders following combined diet and exercise weight loss interventions. Such information could provide novel insights into potential mechanisms that can influence the success (or failure) of weight loss with exercise and diet interventions. Having the tools to be able to identify individuals that can "successfully lose" weight prior to dietary and exercise interventions has the potential to revolutionise disease management (Mutch et al., 2007).

2.5   Changes in body composition in the follow-up period

As previously discussed, intervention studies are a popular method for evaluating the efficacy of diet and/or weight loss treatment programs (Santarpia, Contaldo, & Pasanisi, 2013). However, the long-term effect of these interventions on clinical outcomes (such as body composition) once the intervention controls (i.e. dietitian assistance, recording food intake, supervised and monitored exercise training) are removed is not as well characterised. Such information is imperative, not only for preventing weight cycling and declines in body composition post-intervention, but also to determine whether these interventions are effective in the long-term. For example, weight cycling (whereby an individual loses then regains some or all of the lost weight) may enhance the risk of sarcopenia (Lee et al., 2010). Evidence from follow-up studies reveal this negative effect of weight cycling and highlight that lean mass typically lost during the weight loss phases (**Figure 2.5**) is not gained back in the regain phase, leading to an increased body fat percentages (K. M. Beavers et al., 2011; Byrne et al., 2003).

Byrne and colleagues (2003) compared periods of weight loss (~20-24 wk, until reaching >10 kg weight loss) and regain (1 y thereafter) in overweight females (~36 y). Of the total weight lost during the intervention period 10% was lean mass. However, the amount of lean mass regained in the follow-up period was less than the amount lost within the intervention, negatively affecting body composition. A later study investigated whether the ratio of fat to lean mass gained during the regain phase was similar to that lost during the energy restriction intervention phase (5 mo) in overweight females (~58 y) (K. M. Beavers et al., 2011). The authors found considerable variability in the participant responses in the weight regain phase (**Figure 2.11**). Beavers and colleagues (2011) found that a 1 kg loss of fat mass during the weight-loss intervention period concurrently occurred with 0.26 kg loss of lean mass. However, following the removal of intervention controls only 0.1 kg of lean mass was regained for every 1 kg of fat mass regained in the 1 y follow-up period. Therefore, the

intervention had a detrimental effect on the body composition of these women in the post-intervention period. Although ~76% of participants were re-gainers, on average the ~3 kg regain meant that they were still 8 kg lighter than baseline measures (K. M. Beavers et al., 2011).
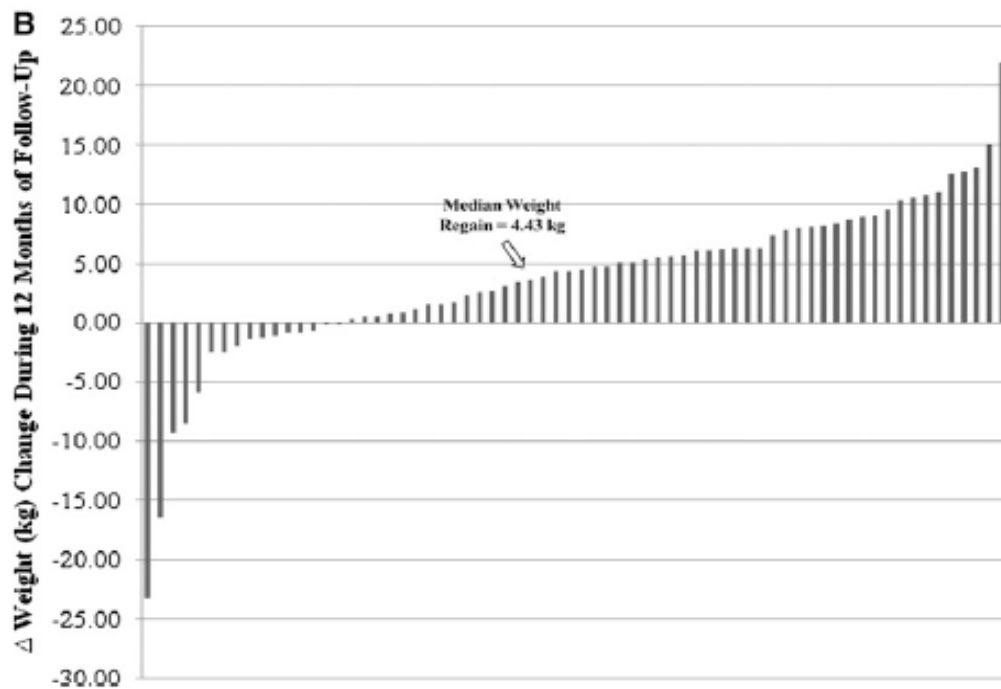
Figure 2.11. Post-intervention change in body mass through a 12-month follow-up period after a dietary intervention (5 mo) in postmenopausal, overweight women. Figure taken from Figure 1B of K. M. Beavers et al., Am J Clin Nutr, 2011:94:767-74.

Similarly, Lee and colleagues (2010) measured changes in body composition patterns for both the men and women (~73 y) considered as weight-cyclers (defined as individuals with 3% body mass loss followed by regain of ±3% baseline body mass). Their results demonstrated that proportionally more lean mass was lost in the weight loss phase than amount of lean mass gained in the regain phase (Lee et al., 2010). However, the relative total lean mass changes were greater (i.e. worse) in the regain phase for the male participants compared to the females, which could not be explained by higher initial lean mass or a lower health status in the men (Lee et al., 2010). This inconsistent pattern of lean mass changes between sexes has been previously observed in a study comparing body composition changes of males and females from age 70-79 y (Ding et al., 2007). A linear decline in lean mass was

observed with age in women, but this decline was further accelerated in the male participants (Ding et al., 2007). As yet, there has been no explanation for why the loss of total lean mass is accelerated in males.

Not all studies of weight regain have found the same disparity in body composition changes between the intervention and weight regain phases. Bosy-Westphal and colleagues (2013) also investigated weight loss (~13 wk intervention) and regain (after 6 months follow-up) in overweight and obese women (~33 y). Their primary objective was to investigate weight loss and regain from a perspective of the distribution of adipose tissue and secondarily, the composition of lean mass. Unlike the results of previous studies who have found less lean mass in the weight regain phase, they did not find any differences in lean mass changes in those who regained back their weight (Bosy-Westphal et al., 2013).

Not only do these phases of weight cycling (loss then regain) affect long-term body composition, and therefore the predisposition to develop sarcopenia, but there is also an increased cardiometabolic risk with weight regain (D. P. Beavers, Beavers, Lyles, & Nicklas, 2013). Measures such as systolic and diastolic blood pressure, as well as fasting glucose, in "regainers" (i.e. those who regained weight in the time following the intervention) had returned to baseline 1 y post-intervention (D. P. Beavers et al., 2013). Such outcomes suggest the importance of the degree of energy deficit when designing diet and exercise interventions to achieve other beneficial effects as well as aiming to reduce obesity (D. P. Beavers et al., 2013).

The reason for the difference in the type of tissue regained after weight loss is currently unknown, but it may be linked to protein intakes reducing to baseline after high intakes during intervention periods. It has been suggested that weight regain is dependent on the tissue type in which it occurs and this will have further implications on long-term body composition changes (Lee et al., 2010). The studies of weight regain/follow-up highlight the need for follow-up studies to enhance our understanding of the efficacy of an intervention

protocol and whether such interventions can be translated into long-term changes that are beneficial for health outcomes. In terms of individual responses to an intervention, there is a need to identify those who may be prone to weight regain as to provide individualised and intensive weight management strategies following the intervention. Finally, studies examining weight cycling (i.e. weight loss and weight regain) highlight how minimising the loss of lean mass during weight loss interventions is vital for long-term health outcomes and the prevention of sarcopenia.

Chapter THREE

3   *Study 1*: Standardised DXA measurements

The following chapter outlines the formative work completed to investigate the reliability for using dual energy x-ray absorptiometry (DXA) to estimate body composition in *Studies 2* and *4*.

This chapter will not be submitted for publication and is an extended methodology of the specific procedures conducted and used in the prospective studies.

3.1　　　　Introduction

The four-compartment model for determining body composition – comprising of body density from air-displacement plethysmography or hydrodensitometry, total body water from isotope dilution and bone mineral density from dual energy x-ray absorptiometry (DXA) (Fuller et al., 1992) – is widely accepted as the criterion measure for quantifying fat mass (FM) and lean mass (LM). However, conducting the testing for the four-compartment model technique is costly and often impractical due to the number of laboratory visits required and pre-test standardisation procedures. Consequently, dual energy x-ray absorptiometry (DXA) has been used as an alternative technique to estimate body composition, with DXA scans becoming a routine tool for estimating FM and LM in the majority of research studies during the past 20 years. DXA has several advantages over other methods, such as bioelectrical impedance, subcutaneous skinfold measures and underwater weighing, when quantifying body composition using a single analytical technique due to the fewer limitations (i.e. time and participant discomfort) and its greater accuracy (Andreoli, Scalzo, Masala, Tarantino, & Guglielmi, 2009). However, methodological details of DXA protocols are often under-reported with regard to pre-test control (i.e. standardisation of diet and physical activity), subject positioning on the scanning bed, reliability and technical error, and scan data analysis (Nana, Slater, Hopkins, & Burke, 2012a).

Early studies utilising DXA scans to determine body composition routinely reported the technical error of measurement with which to interpret the meaningfulness of the magnitude of effect for various interventions (Mazess, Barden, Bisek, & Hanson, 1990; Calbet, Moysi, Dorado, & Rodríguez, 1998; Ryan, Nicklas, & Dennis, 1998). Technical errors arise from differences in calibration, human error in position, and errors within the instrumentation, whereas biological errors are due to the inherent variability in a homeostatic individual (Coggan & Costill, 1984). When obtaining repeated measurements, as in randomised clinical trials, the measurement error should include both the technical error as

well as the biological daily variation. As such, the coefficient of variation (CV) including both technical and biological errors is an important measure of reliability that should be included in the interpretation of the results of DXA estimates of body composition. Unfortunately, many studies of DXA-determined changes in body composition in response to diet and exercise interventions have failed to report key information related to the errors of measurement, the CV and the method for calculating the CV (Nicklas et al., 2009; Josse et al., 2011; Foster-Schubert et al., 2012). Measurement error in DXA-derived estimates of body composition has been identified as a challenge in determining the efficacy of weight loss strategies (Caudwell et al., 2014) and failure to report measurement error is contrary to established principals for best practice in the use of DXA technologies (Plank, 2005).

The available literature on the reliability of DXA-derived measures of body composition report typical CVs across studies from 1-2% for total body mass (BM) (Frimel et al., 2008; Layman et al., 2005; Nana et al., 2012a), with 0.7-1.8% for LM and 1.4-8.2% for FM (Calbet et al., 1998; De Lorenzo, Andreoli, & Candeloro, 1997; Mazess et al., 1990; Nana et al., 2012a; Ryan et al., 1998; Williams et al., 2006). However, these previous studies have also found a wide variation in the measurement of CVs, potentially due to differences in scanners and technicians, so it becomes important to conduct within laboratory measures. Therefore, the purpose of the current study was to determine test-retest reliability when quantifying two-compartment body composition in normal to obese participants. Based on previous reports, CVs of less than 2% for a single operator, single machine study were expected.

3.2 Methods

3.2.1 Participants

Thirteen participants were recruited to participate from staff and post-graduate students employed and/or studying at RMIT University Bundoora at the time of the study. All participants were asked to read an information sheet (Appendix A, Section 9.1.2) regarding the risks associated with DXA measurements, and given the option of asking questions of the technician, prior to providing their consent to take part in this study. The study was approved by the RMIT Human Ethics Committee within the application for *Study 2*.

3.2.2 Procedure

Each participant completed a series of four separate measures of body composition within a 4-7 day period. On the morning of a scan, participants reported to the laboratory in an overnight fasted (10 h) and rested (no exercise) state (Nana et al., 2012a; Nana, Slater, Hopkins, & Burke, 2013). Participants were asked to achieve euhydration by regular intake of fluid and avoidance of heavy exercise on the day prior to each scan. Participants' voided their bladder immediately prior to each scan and wore the same light, loose fitting clothing. Participants BM was determined at each visit using Wedderburn scales (Tanita BWB-620, Wedderburn, VIC). Height was recorded on the first visit using a stadiometer.

A single whole-body or two half-body scans were performed according to the investigator's visual judgement of whether the participant fitted the scanning area (i.e. 197.5 cm × 60.0 cm) (Tataranni & Ravussin, 1995). Participants were positioned for each single scan using a standardised protocol on the scanner bed of the GE Lunar Prodigy Pro (GE Healthcare, United Kingdom). Specifically, participants' hands were placed in custom-made "paddles" so each hand rested in the sagittal plane through internal rotation (90°) of the distal forearm, with 3 cm of foam on the inside of the paddle to ensure separation of the arms from the leg/gynoid (hip) segments. Participants' legs were positioned using a foam block that both

separated the feet by 15 cm and kept the feet in a dorsiflexed position (see **Figure 3.1**). Where participants did not fit within the range/area of the x-ray scanner, two scans were undertaken. For double scans participants were positioned to ensure that the entire right side scan was captured, which for most of the larger individuals meant the left arm and sometimes hip was not included. Once the initial scan was complete the participant was re-positioned for scanning of the other (left) side of the body, with the right arm/hip being excluded from the scan (Nana, Slater, Hopkins, & Burke, 2012b). Manufacture settings were used to determine scan thickness (standard or thick) settings, which are based on the BMI of the individual.

Data analysis

Scans were analysed using software (Encore 2009, version 12.20.033) pre-set limits of interest for whole-body and regional (trunk, arm and leg) bone mineral-free lean and fat tissue. With the use of specific anatomic landmarks, the legs and arms were individually isolated on each scan through the glenohumeral joint of the shoulder, at the top of the iliac crests and through the femoral neck of the acetabulofemoral joint (**Figure 3.2**) (Byrne et al., 2003). The calculation of soft tissue mass of the arms and legs from the trunk was at predetermined positions, as described by Byrne and co-workers (2003). Total limb fat and lean tissue was calculated from summed arm and leg fat and bone mineral-free lean tissues, respectively. BM from DXA was calculated as the sum of non-bone fat-free tissue, fat tissue and bone mineral, as per the software.

Figure 3.1. Example of the hand paddle and foot placement block utilised when scanning a participant to ensure standardised placement on the scanner.
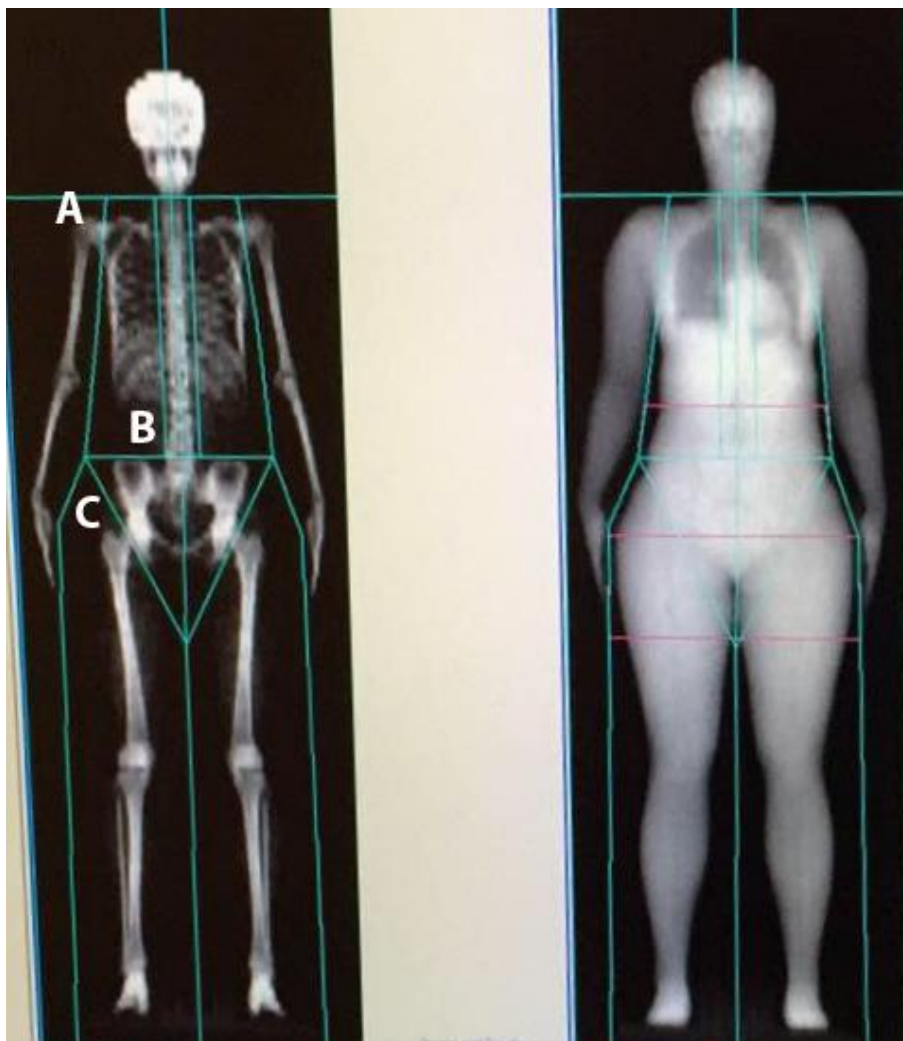
Figure 3.2. Example of scan positioning using the pre-set limits for region of interest **A**: glenohumeral joint of the shoulder; **B**: top of the iliac crests; and **C**: femoral neck of the acetabulofemoral joint.

Where partial scans were combined, the sums of the left and right sides were manually calculated using Microsoft Excel spreadsheets. Microsoft Excel was also used to collate data and calculate coefficient of variation using the following equation (CV = SD/mean). Intraclass correlation coefficients, as a measure of agreement of measures between scan days, were calculated using a reliability spreadsheet (www.sportsci.org; Hopkins, 2015).

Data were analysed using SPSS (version 20.0). Two factor ANOVA was used to determine differences in the four repeated scans (time) and BM measurement (scales vs DXA). One factor ANOVA was used to determine differences in FM and LM, and body fat percentage with repeated scans. Linear regression analysis was performed to assess the relationship between BMI and individual CV measures of total, fat and lean mass and body fat percentage. Data are presented as mean ± standard deviation (SD) and significant differences were established when $P<0.05$.

3.3 Results

Descriptive measures of the participants are listed in **Table 3.2**. Of the thirteen participants, two required two scans (one of each side, 1M/1F).

Table 3.1. Descriptive measures of participants completing the series of four reliability scans. Data are mean ± SD (range).

	All (*n=13*)	Females (*n=7*)	Males (*n=6*)
Age (y)	41.4 ± 13.8 (22.8 – 66.4)	41.4 ± 12.3 (28.1 – 57.0)	41.8 ± 16.6 (22.8 – 66.4)
Body mass (kg)	82.7 ± 22.1 (60.4 – 128.8)	74.8 ± 24.1 (60.4 – 128.8)	91.8 ± 17.0 (78.4 – 125.0)
Height (m)	1.70 ± 0.10 (1.53 – 1.85)	1.63 ± 0.07 (1.53 – 1.76)	1.78 ± 0.05 (1.71 – 1.85)
BMI (kg/m^2)	28.5 ± 5.1 (23.6 – 41.8)	28.1 ± 6.3 (23.6 – 41.8)	29.1 ± 3.9 (26.6 – 36.8)

Key: BMI, body mass index.
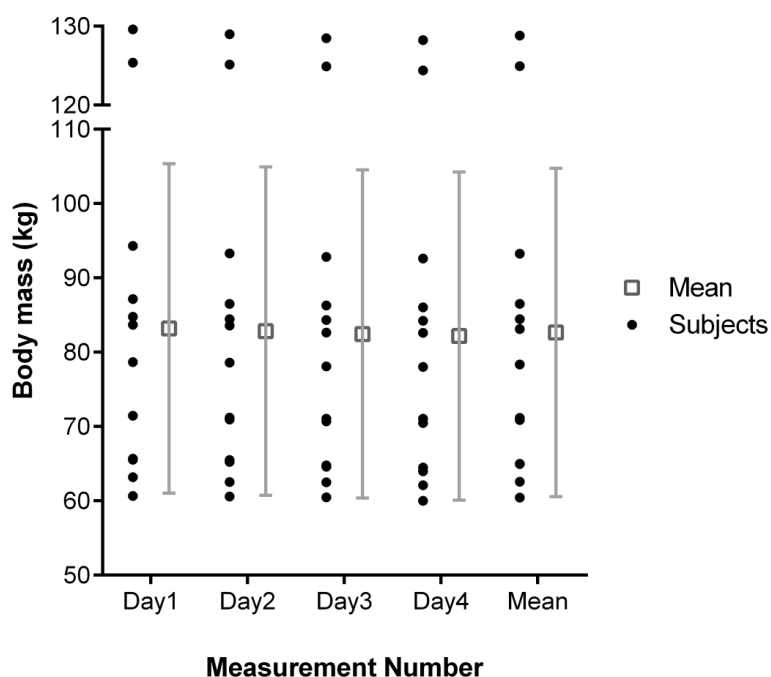
Figure 3.3. Individual measures (•, *n*=13) of body mass measured using scales over the four measurement days, the mean of all subjects on each occasion, and the group mean ± SD (□) across the four measures.

There were no differences in BM determined from the DXA and scales (0.114 ± 0.339 kg, 95%CI: -0.091 – 0.320 kg; **Table 3.2**). However, there was a main effect of time (P=0.001) as well as an interaction effect of measurement type (scale vs. DXA) × day of measurement (P=0.043). The greatest mean difference between the four scan measures was 0.99 ± 0.32 kg for BM (DXA; range: 0.50 – 1.50 kg), 0.73 ± 0.35 kg for FM (range: 0.07 – 1.22 kg), 1.15 ± 0.68 kg for LM (range: 0.42 – 3.19 kg) and 1.0 ± 0.3% for body fat percentage (range: 0.5 – 1.5%). The smallest mean difference between the four scan measures was 0.09 ± 0.10 kg for BM, 0.15 ± 0.15% for body fat percentage, 0.08 ± 0.09 kg for FM and 0.15 ± 0.24 kg for LM.

Despite differences in body composition measures across four scans, the intraclass correlation coefficient between each scan ranged from 1.000 for BM measured by DXA and scales, and between 0.999 – 1.000 for FM, 0.997 – 0.999 for FFM, and 0.995 – 0.998 for body fat percentage. The CV across the four measurements for each component of body composition was small and the highest CV (1.3%) was calculated for body fat percent (**Table**

3.2). There were no significant relationships between the CVs measured for total mass, lean mass or body fat percentage and BMI. However, a moderate negative linear relationship was measured between fat mass and BMI (P=0.011, R^2= 0.46) whereby a larger BMI was associated with a smaller CV for fat mass [data not shown].

Table 3.2. Grouped results for total body measures from (*n*=13) participants participating in four scans over seven days.

Variable	Value	SD	CV (%)
Body mass, scale (kg)	82.66 ± 22.10	0.46 ± 0.18	0.6
Body mass, DXA (kg)	82.78 ± 22.13	0.46 ± 0.15	0.6
Fat mass (kg)	28.42 ± 12.60	0.32 ± 0.15	1.1
Lean mass (kg)	51.25 ± 12.17	0.52 ± 0.30	1.0
Body fat percentage (%)	35.02 ± 7.65	0.43 ± 0.14	1.3

Key: CV, coefficient of variation; DXA, dual energy x-ray absorptiometry; SD, standard deviation.
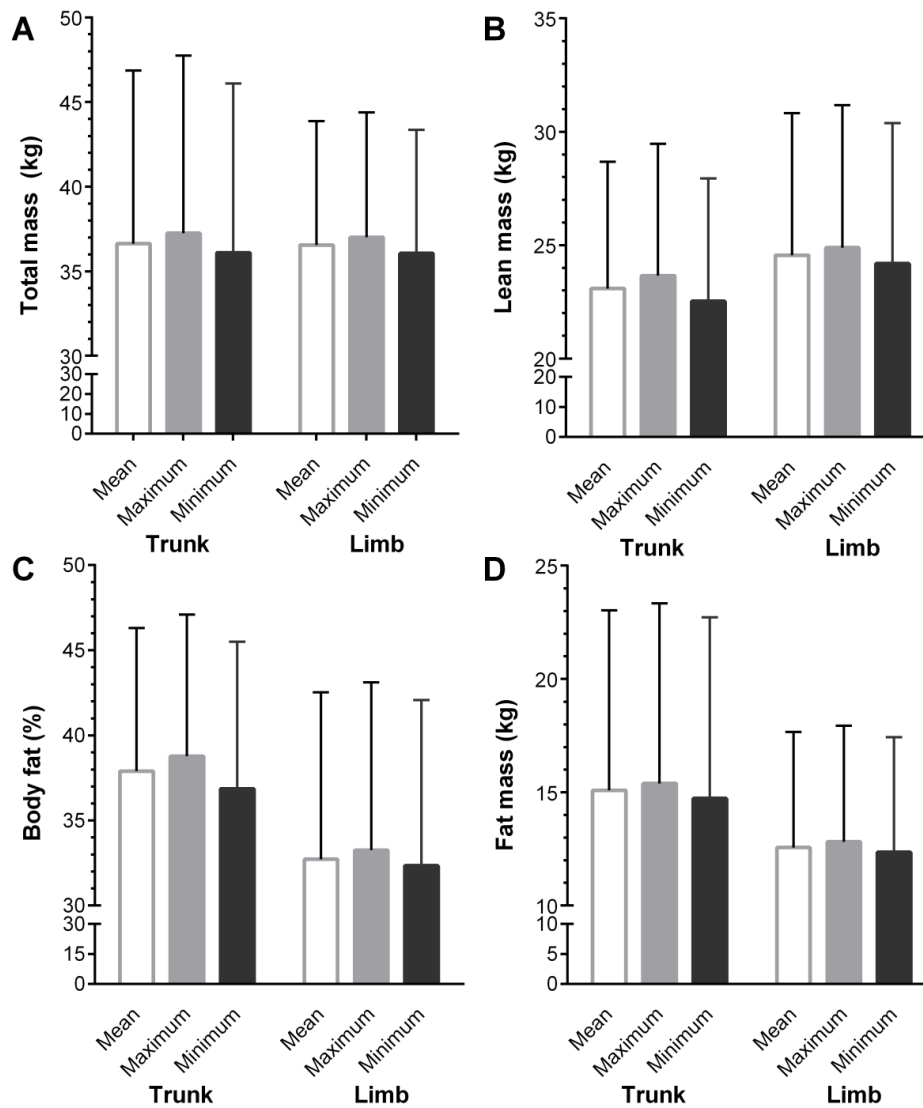
Figure 3.4. Mean (±SD) and range of **A**: total mass, **B**: lean mass, **C:** fat percent and **D**: fat mass for trunk and limb anatomical regions across the four DXA scans (*n*=13).

The CVs for the limb and trunk regions were greater when compared to the total body measures. The CV for FM for four independent scans was 3.5% and 2.0% for limb and trunk regions, respectively, while FM was not different between regions (-2.52 ± 4.90 kg; 95%CI: -5.48 – 0.44 kg, P=0.088, **Figure 3.4D**). Body fat percentage was lower in the trunk compared with the limbs (-5.17 ± 7.92 kg; 95%CI: -9.95 – -0.39 kg, P=0.036, **Figure 3.4C**). There was a greater proportion of LM in the limb compared with the trunk (1.48 ± 2.42 kg; 95%CI: 0.02 – 2.94 kg, P=0.048, **Figure 3.4B**). Consequentially, the CV for LM was 1.8% for limb and 2.2% for trunk regions. Total mass for the trunk (36.63 ± 10.24 kg) and limb (36.55 ± 7.33

kg) regions were similar (95%CI: -2.95 – 2.79 kg, **Figure 3.4A**) with CVs of 1.4% and 1.3%, respectively.

3.4   Discussion

The present study was conducted to establish the reliability of DXA estimates of body composition under the specific conditions of the current laboratory (i.e. DXA scanner model, technician expertise, participant presentation and the positioning protocol). Such information is important in determining the precision of these measurements and the ability to detect changes in body composition in subsequent activities.

The participants used in this study represented those likely to be recruited for the subsequent clinical intervention trial (Chapter 4) and included both 'normal' weight and overweight/obese individuals (BMI range: 27-40 kg/m^2). These results show daily test-retest reliability in this population of normal weight and overweight/obese individuals. The largest variance of a single compartment across four scans undertaken within a 7 d period was total LM (1.15 kg) and is similar to the error reported by Müller and colleagues (2012). Müller and colleagues (2012) reported the minimum detectable change of FM for a DXA scan as 1.0 kg while Williams and colleagues (2006) have shown a bias towards overestimating FM by up to 3 kg in obese subjects when using DXA. Nonetheless, reliability measured in the current investigation was good for each compartment comprising body composition with low CVs (<1.3%).

Short-term changes in BM of non-weight stable individuals violate some of the underlying assumptions when using DXA to estimate body composition (Müller et al., 2012). For example, several acute changes that occur as a result of energy-restriction (i.e. fluid-electrolyte changes, or changes in stores of muscle substrates) create non-steady state conditions and directly affect the measure of LM (Heymsfield et al., 2011). Understanding the limitations of the application of DXA technology to estimate body composition and the

minimum detectable changes in FM and LM is important for the utilization of these protocols in studies determining short-term (<6 month) changes in body composition.

In the current study, all scans were completed within a 7 d period to ensure that participants were relatively weight-stable and that any variability reflected biological and technical error, rather than true change. The quantification of the background 'noise' in technical and biological variability allows the calculation of the real world changes in body composition seen during a weight loss intervention. Factors such as hydration status (fluid shifts and sodium status), food consumption and bowel contents are likely to have contributed to the variation in DXA and scale measurements of BM from day to day. The CV values calculated in the present study are similar or lower than those reported for whole body measures in several previously conducted weight loss intervention studies (Frimel et al., 2008; Layman et al., 2005; Mazess et al., 1990; Ryan et al., 1998). The CV of measures of limb and trunk (regional) mass were much greater than the CV for total body mass. Such a difference is likely to be related to the application of a similar measurement error to the smaller mass of the trunk and limb regions. However, the contribution of the technician's reliability in undertaking the demarcation of regions must also be taken into account. The large CV of regional mass is comparable to the results reported in similar studies (Mazess et al., 1990; Ryan et al., 1998).

The use of DXA for quantifying regional changes in body composition has recently been questioned and it has been suggested DXA is not sensitive enough to determine changes in regional LM and FM with adequate precision (Pourhassan et al., 2013). The appendicular (limb) and trunk data also show more variability than the total body composition data. The standard deviation across scans showed 0.5 kg variation in LM while at the individual level one participant exhibited ~3.2 kg variation in LM between scans. Consequently, the large inter-individual variation that may not be apparent from group means is an important consideration for interpreting individual data following interventions with the aim of inducing

changes in LM and FM. DXA has been used as a primary method to compare LM between dominant and non-dominant arms of professional tennis players (Calbet et al., 1998) as well as for trunk compared to limb weight regain after a period of weight loss (Byrne et al., 2003). Magnetic resonance imaging (MRI) has emerged as the criterion measure to accurately estimate regional changes in body composition (Pourhassan et al., 2013). Where MRI is not available and/or practical, the use of DXA for regional changes should be accompanied by coefficients of variation for both the limb and trunk regions with which the physiological meaningfulness of changes in compartments can be appropriately interpreted.

For regional DXA estimates to be as reliable, a clear standardised positioning protocol should be utilised. Recently, a series of independent but related studies was undertaken to determine the effect of different methods of positioning individuals on the scanning bed in a variety of somatotypes that typically require special consideration due to their larger than average size (i.e. height and BM) (Nana et al., 2012b). In addition, the effect of daily activities (Nana et al., 2012a) and specific exercise sessions (Nana et al., 2013) were also quantified to establish their effect on the accuracy and reliability of DXA derived estimates of body composition. The results of these studies indicate that accurate summation of partial DXA scans to assess body composition of athletes who do not fit within the limits of the scan bed can be achieved by strategies that determine and minimise the biological and technical errors of measurement. These strategies include limiting the 'noise' generated by exercise/activity and/or eating/drinking prior to scanning, as well as employing specific positioning and analytical protocols. However, whether the same principles apply when participants are not undertaking high levels of physical activity and typically present with lower muscle mass and higher FM has not been determined.

Nana and colleagues (2012b) determined the effect of summating two scans (the left and right side) on body composition. The dual scans were undertaken on individuals who were all able to be positioned for left/right scans with all body parts fully supported within the

confines of the scanner bed but not the scan area (Nana et al., 2012b). Similarly, Rothney et al., (2009) undertook an analysis of left/right scans on obese individuals but all individuals could fit within the width of the scanner bed. It is important to note that when quantifying body composition of larger individuals (either total mass or hip width), such that a scan of the left and right side is required, the accuracy of the DXA has not been established using clear positioning guidelines. Tataranni and Ravussin (1995) measured 21 subjects with dual scans because they did not fit within the scanner width but provided no details regarding standardised positioning of their participants. Despite this, they found no difference between sides for body fat percentage or LM, although FM differed significantly between left and right sides by 0.7 kg. In the case of standardising the appropriate position of larger (BMI >30 kg/m^2 or width >60 cm) individuals on the scanner bed for a single side scan it is not uncommon for the contralateral arm (and sometimes a portion of the torso) to be unsupported by the scanner bed. In the present study the two larger (BMI 37 and 42 kg/m^2) participants were positioned for the right scans with some of their left side off the scanner bed (and vice versa). Thus, it is unclear how the positioning in such cases may alter repeated estimates of body composition measured by DXA. Considering a 0.7 kg difference in FM has been reported between dual scans, the analysis of individuals' body composition whom do not fit within the scanner bed width is an area in need of further investigation.

Pertinent to the use of DXA-derived estimates of body composition in weight loss interventions, Heymsfield and colleagues (2011) have conducted a systematic review of the early (1-6 wk) changes in body composition during voluntary weight loss. Early body composition changes are primarily attributed to losses of LM (bone-free, protein, glycogen and fluid-electrolytes) (Heymsfield et al., 2011). However, only a single study was reviewed that included exercise during the intervention period (Redman et al., 2007) and suggested that adding exercise to a dietary intervention appears to influence LM loss kinetics in the early phase of weight loss interventions. Indeed, the findings of Müller et al. (2012) indicate the 3

wk period when commencing a weight loss intervention is insufficient for DXA to accurately quantify changes in body composition due to dynamic changes in tissues in this early period (4-6 wk), sometimes termed the 'adaptive' phase (Heymsfield et al., 2011). Therefore, LM changes early in an intervention may be more difficult to detect with the requisite precision but thereafter DXA should be accurate when standardised procedures (for hydration, food, and exercise, as well as positioning) are employed.

In summary, the use of DXA scans for quantifying fat and lean mass and in determining the efficacy of specific interventions to estimate changes in body composition can be considered reliable. Further, estimates of limb and trunk regions seems to be more problematic and larger relative changes may be necessary to permit assessment by DXA given that the reliability of these measures is lower compared with total body analysis. Despite recent trends to omit reliability and/or technical error of measurement of DXA, future studies should include coefficients of variation and intraclass correlation coefficients to better define the physiological effects and ecological validity of weight loss studies.

Preface to Chapter FOUR

The following chapter utilises DXA to determine the most effective diet composition during energy restriction to maximise the loss of fat mass while retaining lean mass. Loss of skeletal muscle mass can occur at a rate of up to 1% per year after attainment of peak muscle mass (Baumgartner et al., 1998) and the obesity epidemic poses added complications to sarcopenia where excess body fat exacerbates the deteriorating body composition.

Middle-age (i.e. 35-60 y) represents a group where the loss of lean mass is progressive, but where such losses have not yet accelerated (Robinson et al., 2012). Combining appropriate nutrition with resistance and aerobic exercise likely represents the best chance to retain lean mass whilst losing fat mass. Therefore, the effect of higher protein intakes through increased dairy food consumption in a dietary and exercise intervention to improve body composition during weight loss will be assessed in the subsequent chapter.

Chapter FOUR

4   *Study 2*: A randomised trial of high dairy protein, variable carbohydrate diets and exercise on body composition in adults with obesity

This chapter is comprised of the following manuscript that has been submitted (25th September 2015) and is currently under review in *Obesity (Silver Spring)*:

Parr, E.B., Coffey, V.G., Cato, L.E., Phillips, S.M., Burke, L.M., Hawley, J.A. A randomised trial of high dairy protein, variable carbohydrate diets and exercise on body composition in adults with obesity

Due to the limited word count (3 500) allowed by *Obesity (Silver Spring)*, several sections from the methods were removed and placed into a Supplemental Information file, and will be available online only when published. For this thesis the additional information has been added to the chapter.

4.1 Abstract

Objective: This study determined the effects of 16 wk high-dairy protein, variable carbohydrate (CHO) diets and exercise training (EXT) on body composition in men and women with overweight/obesity.

Methods: 111 participants (age 47 $\pm$ 6 y, body mass 90.9 $\pm$ 11.7 kg, BMI 33 $\pm$ 4 kg/m^2 values mean $\pm$ SD) were randomly stratified to either: high dairy protein, moderate-CHO (HDPMC; 40% CHO: 30% Protein: 30% Fat; ~4 dairy servings); high dairy protein, high-CHO (HDPHC: 55%: 30%: 15%; ~4 dairy servings); or control (CON; 55%: 15%: 30%; ~1 dairy serving) diets. Energy restriction (500 kcal/d) was achieved through diet (~250 kcal/d) and EXT (~250 kcal/d). Body composition was measured using dual-energy x-ray absorptiometry (DXA) before, mid-way and upon completion of the intervention.

Results: Eighty-nine (25 M/64 F) of 111 participants completed the 16-wk intervention, losing 7.7 $\pm$ 3.2 kg FM (P<0.001) and gaining 0.50 $\pm$ 1.75 kg LM (P<0.01). There was no difference in changes in body composition (FM or LM) between the groups.

Conclusions: Compared to a healthy control diet, energy-restricted high-protein diets containing different proportions of fat and carbohydrate confer no advantage to weight loss or change in body composition in the presence of an appropriate exercise stimulus.

4.2   Introduction

The coexistence of diminished muscle mass and increased fat mass (FM) is referred to as 'sarcopenic obesity' and as the proportion of older inactive adults increases, the incidence of this condition will escalate and have a dramatic impact on the lives of an increasing number of the population. Accordingly, a critical health issue facing many adults is how to preserve muscle mass while reducing FM in the face of reduced physical activity and increased longevity (Parr et al., 2013).

Many investigations targeting weight loss have manipulated combinations of diet and exercise training (EXT) and reported that energy restriction in the absence of an exercise stimulus results in a loss of both fat and lean tissue (Ballor et al., 1988; Foster-Schubert et al., 2012; Layman, 2003). However, energy-restricted higher-protein, reduced-carbohydrate (CHO) diets partially offset the deleterious loss of LM observed in the face of greater (~55% of energy intake) CHO intakes (Abete et al., 2010; Krieger et al., 2006; Layman, 2004). Accordingly, during periods of reduced energy intake, protein consumption in excess of the recommended daily intake (RDI; 0.8 g/kg) may be necessary to preserve LM (Churchward-Venne, Murphy, Longland, & Phillips, 2013). However, the optimal macronutrient ratio (i.e., fat to CHO) needed to promote the greatest rates of FM loss when protein intakes are high remains highly controversial. Indeed, whether it is the lowering of dietary CHO (Krieger et al., 2006) or fat (Wycherley et al., 2012) during energy-restricted, high-protein diets that favourably predisposes an individual to an increased FM loss is unknown. In previous studies (Krieger et al., 2006; Wycherley et al., 2012) comparisons have only been made between high versus adequate protein intake with little consideration to the macronutrient ratio of CHO, fat and protein.

With regard to high quality protein sources, the incorporation of dairy foods into weight-loss diets has been employed in some (Josse et al., 2011; Major et al., 2008; Shahar et al., 2010; Zemel et al., 2005, 2009; Zemel & Miller, 2004), but not all studies (Bowen,

Noakes, & Clifton, 2005), with greater FM loss than non-dairy diets or diets with calcium supplementation. Indeed, combining higher protein diets with EXT, especially resistance exercise (REX), works synergistically to maintain LM during weight loss in women (Layman et al., 2005; Meckling & Sherfey, 2007). However, to date no studies have determined the effect of altering the ratio of CHO to fat, within energy-restricted diets using dairy products, in middle-aged adults with overweight/obesity, where maintenance of LM during weight loss was the primary goal. Hence, the objective of this study was to determine the effect of 16 wk of energy-restricted high dairy protein (> RDI) diets with variable CHO and fat content to a control diet (meeting RDI for protein), in combination with EXT, on changes in body composition and selected risk factors for obesity in men and women with overweight/obesity. We hypothesized that a lower CHO intake, coupled with high protein consumption through full fat dairy products, when combined with EXT, would induce greater loss of FM and maintain LM compared to a control or higher CHO diet.

4.3   Methods

4.3.1 Design

The study was a randomised trial of three energy-restricted diets, two with high protein (~30% energy intake (EI)) and one control (~15% EI protein), during a 16 wk intervention. Primary outcome measures (body composition comprising fat, lean and total mass) were assessed before (wk 0), mid-way (wk 8) and post (wk 16) intervention using dual-energy x-ray absorptiometry (DXA). Secondary outcome measures were anthropometry, blood analytes, aerobic fitness, strength, estimates of daily activity and energy expenditure and dietary analyses. The study was undertaken at the Royal Melbourne Institute of Technology (RMIT) University Bundoora (Victoria, Australia) campus from March 2011 to October 2013 and approved by the RMIT University Human Research Ethics committee (Project number 76/11). All participants were provided with written informed consent prior to

participation. This trial was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612000021875). No changes to the methodology or trial outcomes, as described on the registry and below, occurred after the trial commenced.

4.3.2 Participants

Four hundred and fifty individuals registered their interest in the study via phone or email of which 115 men and women commenced pre-study screening (**Figure 4.1**). Participants (males and females aged 35-59 y, overweight or obese [BMI 27-40 kg/m^2] and sedentary) were eligible to participate based on their responses to a validated medical screening questionnaire (Pre-exercise screening system, (Sports Medicine Australia, 2005) and completing medical clearance as required (67%, aged >45 y for males, >55 y for females, or >2 cardiac risk factors). Four participants were excluded through the pre-screening process (see **Figure 4.1**). Use of prescription medication for elevated BP and/or cholesterol, depression, anxiety and arthritis was permitted if participants had been taking them for ≥3 months prior to the study. Participants discontinued nutritional supplements prior to starting the trial. Participants were instructed verbally and in writing on how to complete a 7-d food diary prior to an initial consultation with the study dietitian and were provided with measuring cups and spoons to use throughout the study to assist with both their adherence to dietary plans and recording of portion sizes of their food and drink intake.

Participants commenced the study in April-2012 with the final group beginning in June-2013. If participants dropped out in the first 8 wk they were removed from the random allocation such that they were replaced when possible. Recruitment continued until a minimum of 30 participants had been allocated to each group.

4.3.3 Dietary allocation

Participants (*n*=111) were assigned to one of three diets through stratified randomisation. The stratification was by sex (M/F), age (35-39, 40-44, 45-49, 50-54 and 55-

59 y brackets), and BMI (27-29, 30-34 and 35-40 kg/m^2) to ensure equal numbers of subjects in each group. The stratification scheme was successful as there were no between-group differences in baseline age or body composition characteristics. Participant randomisation for group assignment was completed by the same investigator who informed the study dietitian. From then on, apart from the study dietitian, all investigators were blinded to the assigned dietary group by using participant codes unrelated to their dietary assignment.

4.3.4 Dietary intervention

Dietary interventions were implemented as a free-living energy-restricted eating plan where energy intake was based on a mild restriction (-250 kcal/d) from estimated maintenance energy requirements (Frankenfield, Roth-Yousey, & Compher, 2005). Dietary macronutrient content was manipulated to achieve the following targets (**Table 4.1**):

• High dairy protein, high carbohydrate (HDPHC; ~30% protein, 55% CHO, 15% fat; 4+ dairy servings/d focusing on sweetened, low fat choices)

• High dairy protein, moderate carbohydrate (HDPMC; ~30% protein, 40% CHO, 30% fat; 4+ dairy servings/d focusing on unsweetened/artificially sweetened, full-fat choices)

• Low dairy protein, high carbohydrate (CON; ~15% protein, 55% CHO, 30% fat: 1-2 dairy servings/d)

4.3.5 Dietary implementation and assessment

Dietary intervention followed a transition from a prescribed menu meeting the desired energy restriction and macronutrient composition (wk 0-8) to a flexible self-chosen plan (wk 9-16) based on a points system. Participants met fortnightly with a dietitian and were provided with education resources. Menus for each diet provided for three meals/day and a "Dairy/Snack Basket" (food choices that achieved most of the nutrient manipulation for each diet; **Table 4.1**). For the higher protein diets, the Baskets contained foods equivalent to 4-5 dairy servings (defined by the Australian Guide to Healthy Eating, (National Health and

Medical Research Council, 2011)) where two servings were to be consumed as soon as practical post-exercise. In the moderate protein CON diet, "Baskets" provided CHO-rich choices (e.g. non-dairy) for post-exercise recovery snacks and meal additions.

For the higher protein diets, two dairy servings were to be consumed as soon as practical post-exercise to provide an optimal high-quality protein according to sports nutrition guidelines (Phillips & Van Loon, 2011). Baseline dietary intake was assessed via a 7-day food diary (7DD) using household measures to quantify food intake (FoodWorks® Professional Edition Food Composition database (Version 7.0, Xyris Software Pty Ltd, QLD, Australia). Compliance to each dietary intervention was assessed by the study dietitian via daily checklists (all wk; foods through to points) and the completion of 7DD's (wk 1, 4, 8, 12 and 16). The 7DD's were analysed using serving sizes derived from the Australian Dietary Guidelines (National Health and Medical Research Council, 2011), as per the baseline records.

Table 4.1. Sample of a 1-day meal plan for each of the diets (1600 kcal/d version)

Diet		Control (low dairy protein, high carbohydrate)	High dairy protein, moderate carbohydrate	High dairy protein, high carbohydrate
Goal macronutrient ratio (CHO:Protein:Fat)		55%:15%:30%	40%:30%:30%	55%:30%:15%
Breakfast		1 wholemeal crumpet 1 medium tomato 20 g slice reduced fat cheese	1 wholemeal crumpet 1 medium tomato 1 poached egg **250 ml full fat milk***	1 wholemeal crumpet 1 medium tomato 1 poached egg **250 ml low fat milk** 1 piece fruit
Lunch		2 cups chicken noodle soup 1 slice wholegrain bread 1 tsp margarine **1 fruit and nut bar** **1 piece fruit**	2 cups chicken noodle soup 2 crispbreads 2 slices deli turkey 1 piece fruit	2 cups chicken noodle soup **200 g tub low fat unsweetened yoghurt** 1 piece fruit
Dinner		2/3 cup Italian mince 1 cup pasta 1 cup salad vegetables **10 cashew nuts**	1 cup Italian mince ½ cup pasta Chicken salad (50 g chicken, **25 g reduced fat cheese,** 1 cup salad)	1 cup Italian mince 1 cup pasta Chicken salad (50 g chicken, 1 cup salad)
Recovery snack		**1 piece fruit** **50 g yoghurt covered sultanas**	**175 g tub low fat natural yoghurt** **250 ml full fat milk**	**250 ml yoghurt smoothie drink** **250 ml low fat milk**
Nutrient analysis	Meals	1053 kcal, 125 g CHO, 57 g protein, 31 g fat, 497 mg calcium	1001 kcal, 112 g CHO, 80 g protein, 27 g fat, 310 mg calcium	1194 kcal, 147 g CHO, 85 g protein, 23 g fat, 385 mg calcium
	"Basket"	495 kcal, 85 g CHO, 8 g protein, 13 g fat, 85 mg calcium	568 kcal, 46 g CHO, 36 g protein, 20 g fat, 1110 mg calcium	447 kcal, 67 g CHO, 36 g protein, 3 g fat, 1560 mg calcium
	Total	1548 kcal. 210 g CHO, 65 g protein, 44 g fat, 582 mg calcium	1569 kcal, 158 g CHO, 116 g protein, 47 g fat, 1420 mg calcium	1569 kcal, 214 g CHO, 121 g protein, 26 g fat, 1945 mg calcium

***Bolded items** correspond to a "Basket" of foods that could be consumed as a post-exercise recovery snack or added to the meal structure. For the first 8 weeks, participants consumed a prescribed meal plan consisting of a meal structure + one "Basket" per day. From weeks 8-16, participants were encouraged to develop their own meal structure (using a points system to achieve a desired energy and macronutrient intake) and add one of 5-7 "Basket" combinations to the day's intake

4.3.6 Exercise training

All participants undertook supervised REX training three d/wk, completing 48 sessions in 16 wk. Individualised training programs were based on one repetition maximum (1RM) tests for each exercise. REX training loads were increased every 4 wk after further 1RM training-specific testing, in order to provide a progressive overload stimulus. A range of exercises were employed to train the same muscle groups (chest, back, legs and core) for 3-4 sets of 8-15 reps at 40-70% of 1RM. Exercise diaries kept by the study trainers were used to ensure the appropriate weight and number of sets was completed. On the other four days, participants completed aerobic exercise equating to 250 kcal/d energy expenditure (e.g. 4 km walk, 16 km cycle or 1 km swimming, or equivalent combinations).

4.3.7 Intervention compliance

Dietary non-compliance was defined as exceeding prescribed energy restriction by >200 kcal/d or demonstrating an inability or unwillingness to adhere to the "Dairy/Snack Baskets" and the desired macronutrient manipulation. Being non-compliant to the REX was defined as missing >2 consecutive training sessions.

4.3.8 Body composition and anthropometry

Whole-body DXA scans (GE Lunar Prodigy Pro, GE Healthcare; software: Encore 2009, version 12.20.033) were undertaken ~10 days prior to study commencement, upon completion of wk 8, and after the completion of wk 16. Pre-study scan reliability (*Study 2*, n=13) was assessed and resulted in a variance of $\pm$ 320 g FM (1.1% CV) and $\pm$ 520 g LM (1.0% CV) per scan. Participants were scanned before 0930 h after an overnight fast. Forty-six of the 111 participants were scanned using half scans, where one whole side was scanned, followed by the other side (Nana et al., 2012b). Regions of interest were pre-set from the Encore software and were adjusted accordingly for each individual subject such that the arms were separated at the shoulder joint, the legs were separated at the neck of the femur, the mid

line at the top of the pelvis bones, and the tissue was encapsulated by the lines. Scans were assessed to the closest mass that the scanner gave in comparison to the scale mass. Upon arrival to the lab, subjects voided their bladder and collected a urine sample that was later measured for urine specific gravity (USG; Atago, Japan) to ensure euhydration. After height and BM were measured, subjects were positioned on the scanner bed using foam hand and foot placers to ensure consistency in scan positioning. Following a scan, waist (minimal circumference) and hip (maximal gluteal protuberance) circumferences were measured in duplicate and the waist-hip ratio calculated.

4.3.9 Strength and aerobic fitness testing

Each participant's 1RM was determined on three exercise machines: chest press, lat pulldown and 45° leg press, prior to, at the midpoint, and after the 16 wk intervention, independent of the 1RMs measured for training purposes. Aerobic fitness was assessed using an incremental cycle test to volitional fatigue (Hawley & Noakes, 1992) using indirect calorimetry (True One 2400, Parvo Medics, USA). Exercise intensity was increased by 15 W (females) and 25 W (males) every 150 s until volitional exhaustion. Peak oxygen consumption (VO_2peak) was taken as the highest O_2 uptake recorded for 30 s.

4.3.10 SenseWear™ energy expenditure monitoring

SenseWear Armbands (SWA, Bodymedia, Pittsburgh, PA, USA) were worn for four consecutive day periods to estimate daily energy expenditure pre, mid (wk 8-9) and post (wk 16) the intervention. Participants wore the SWA on their dominant arm (determined by handedness) over the triceps muscle for four 24-h periods (midnight to midnight) within a 7 d period, inclusive of one weekend day. Estimates of daily energy expenditure were measured prior to the intervention, at wk 8-9 and in wk 16 of the intervention through the recorded data, as well as manually inputted data of sex, BM, height and handedness, based on the SWA proprietary algorithm, as previously validated (St-Onge, Mignault, Allison, & Rabasa-Lhoret,

2007). Second-by-second values are reported as either kcal or metabolic equivalents (METs). Data were downloaded to a computer using SenseWear Professional 7.0 software (Bodymedia Inc, Pittsburgh, PA, USA) and exported for analysis in Microsoft Excel.

4.3.11 Blood samples and laboratory analyses

After a DXA scan, a fasted blood sample (9 mL) was taken from an antecubital vein and subsequently analysed for cholesterol, insulin, glucose, and triglycerides concentrations. A second tube (4 mL EDTA) was taken for fasted blood glucose analyses for the OGTT using an YSI 2900 analyser (YSI Life Sciences, Yellow Springs, OH, USA). OGTT's were conducted pre and post intervention using a 75 g 300 mL glucose solution (Point of Care Diagnostics, NSW, Australia). Sequential 4 mL blood samples were timed from ingestion of the drink over 30, 60, 90 and 120 min, spun at 3000 g, 4 °C, for 10 min and the plasma was stored at -80 °C for later analysis. The homeostasis model assessment (HOMA) score was subsequently calculated (Matthews et al., 1985). LDL cholesterol was calculated using the following formula, which is only valid for triglyceride values up to and including 4.5 mmol/L.

LDL-Cholesterol = [Total Cholesterol] – [HDL-Cholesterol] – ([Triglyceride]/2.18)

Plasma leptin and adiponectin, pre and post intervention were subsequently analysed using enzyme-linked immunosorbent assay (ELISA) kits (SPI Bio, Montigny le Bretonneux, France).

4.3.12 Statistical analysis

Statistical power sample size calculations (0.8, P<0.05) were undertaken using G*Power 3.1.2. software using the raw data from the recently reported changes in body composition following the only other chronic (16 wk) weight loss study that manipulated both nutrition and exercise (Josse et al., 2011). Estimated sample size for detecting changes in fat mass following a 16 wk intervention was calculated at n=30 for control diet and n=25 for high dairy diet.

Further statistical analyses were all performed using SPSS (version 20.0). Prior to hypothesis testing, primary endpoint data were examined for normality and outliers (>3 standard deviations (SD)). Analyses were conducted on all participants who completed the intervention via per protocol (all variables) and those who completed but did not adhere to the intervention protocols (intent to treat; performed only on the primary outcome variables of body composition). Participants who started but did not complete were included only in baseline analyses of primary outcome variables (body composition) to determine recruitment bias.

Linear mixed models were used to analyse all data which had more than one time point (i.e. all changes across time), using intervention allocation or sex as the grouping variable and baseline data as covariates. Where time effects were measured, post-hoc t-tests were used to determine differences between time points within groups. Effect sizes were calculated, using the linear mixed model analysis as the estimated differences between intervention groups, with 95% confidence intervals. Baseline data were analysed between groups using one-factor ANOVA with appropriate post hoc tests (LSD). ANCOVA was used for analysis with covariates. Linear regression analysis was performed to assess the relationship between baseline and changes pre-post intervention in body composition. Significance was set at $P<0.05$ and data are presented as mean $\pm$ SD.

4.4   Results

4.4.1 Randomisation

Ninety individuals ($n=25$ M, $n=65$ F) completed the intervention as per study protocol. One female was excluded from primary outcome measures as her values were > 3 SD from the mean for total body and FM loss (**Figure 4.1**). Participants who began but did not complete the intervention ($n=21$), as detailed in Figure 1, had a greater baseline trunk fat (24.4 $\pm$ 7.7 vs. 21.1 $\pm$ 4.0 kg, $P=0.007$) compared with all those who completed ($n=90$). Age, BM,

and BMI of unsuccessful participants did not differ between groups. There were no differences among groups for age, physical characteristics, blood analytes and exercise variables before study commencement (Error! Reference source not found.).
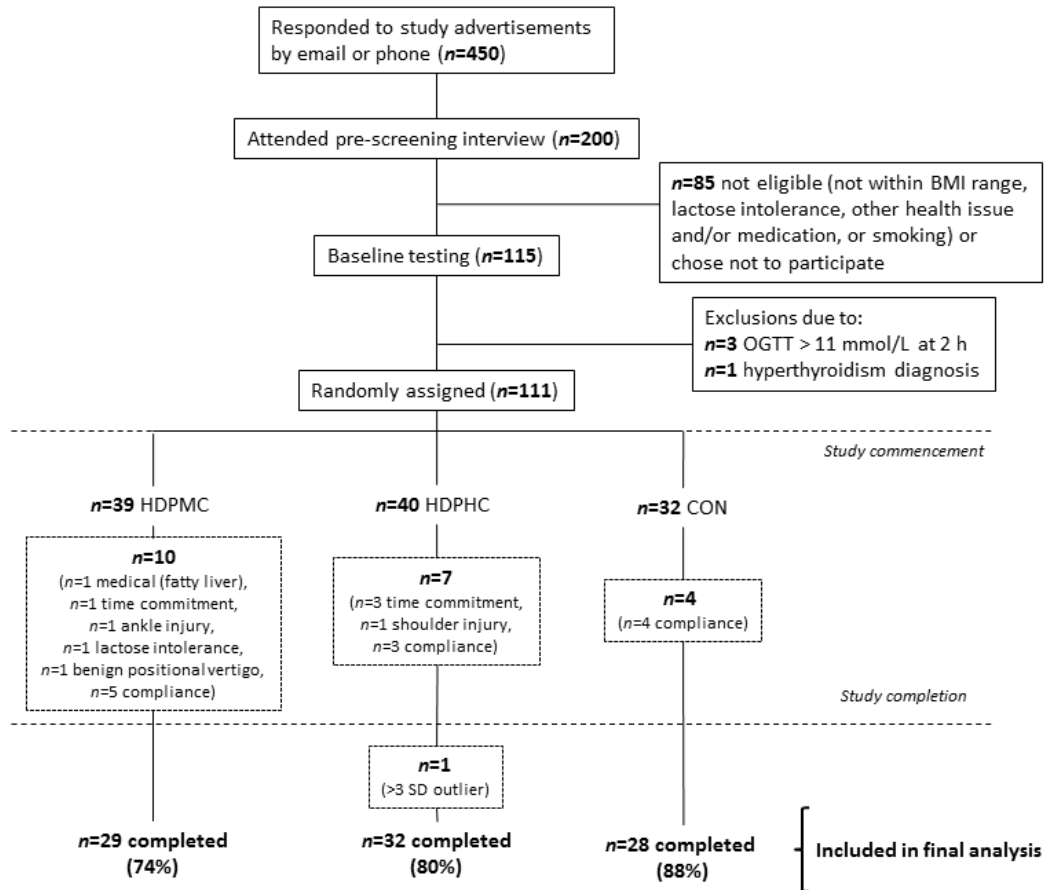
Figure 4.1. Flowchart of participants throughout the intervention. HDPHC, high dairy protein moderate fat; HDPMC, high dairy protein moderate fat; CON, control. Compliance was to the diet and/or the exercise training

Table 4.2. Summary of baseline participant characteristics, body composition, blood, and exercise variables of the 89 participants who completed the 16 week exercise-diet intervention within the protocol design

Variable	Diet condition		
	HDPMC (*n*=29)	**HDPHC** (*n*=32)	**CON** (*n*=28)
Age (y)	47.0 ± 5.9	47.1 ± 5.7	47.2 ± 6.5
Height (cm)	168.0 ± 8.0	168.0 ± 6.5	165.5 ± 11.0
Sex (M/F)	8/21	10/22	7/21
Body composition			
Body mass, DXA (kg)	91.7 ± 12.6 (73.9 – 121.8)	90.3 ± 9.9 (73.4 – 114.2)	90.5 ± 13.6 (66.9 – 116.8)
BMI (kg/m^2)	32.6 ± 3.9 (27.4 – 41.4)	32.1 ± 3.6 (26.5 – 41.6)	33.0 ± 3.7 (27.9 – 40.6)
Fat mass (kg)	40.8 ± 8.3 (25.2 – 60.5)	38.1 ± 8.3 (22.2 – 55.4)	39.4 ± 8.5 (23.9 – 59.9)
Fat mass (%BM)	46.2 ± 7.5 (27.3 – 56.3)	43.6 ± 7.3 (27.8 – 55.0)	45.2 ± 7.6 (28.9 – 55.2)
Lean mass (kg)	47.8 ± 10.6 (36.5 – 73.2)	49.1 ± 7.8 (37.8 – 68.2)	48.0 ± 10.8 (30.4 – 72.3)
Waist circumference (cm)	105.6 ± 10.0	105.0 ± 10.6	104.1 ± 9.0
Waist:hip ratio	0.91 ± 0.08	0.91 ± 0.09	0.91 ± 0.07
Trunk fat, DXA (kg)	21.9 ± 3.7	20.5 ± 4.5	21.1 ± 3.6
Blood variables			
Glucose (mmol/L)	5.4 ± 0.6	5.4 ± 0.5	5.5 ± 0.6
Insulin (mIU/mL)	7.7 ± 6.0	6.1 ± 3.2	7.6 ± 5.8
Fasted HOMA index	1.9 ± 1.6	1.5 ± 0.9	2.0 ± 1.7
Total Cholesterol (mmol/L)	5.1 ± 1.0	5.2 ± 1.1	5.5 ± 0.8
LDL Cholesterol (mmol/L)	3.1 ± 0.7	3.3 ± 0.9	3.4 ± 0.7
HDL Cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.4	1.3 ± 0.3
Triglycerides (mmol/L)	1.7 ± 1.9	1.4 ± 0.8	1.5 + 0.5
Adiponectin (ng/mL)	7.2 ± 2.0	8.8 ± 3.7	8.2 ± 3.0
Leptin (ng/mL)	24.5 ± 17.6	25.2 ± 15.9	25.1 ± 19.4
OGTT AUC (AU)	234 ± 104	238 ± 121	275 ± 145
Exercise variables			
VO$_2$ peak, absolute (L/min)	2.03 ± 0.61	2.11 ± 0.50	2.15 ± 0.70
1RM Chest press (kg)	42 ± 15	43 ± 17	45 ± 16
1RM Leg press (kg)	180 ± 55	176 ± 54	184 ± 53
1RM Lat pulldown (kg)	56 ± 15	55 ± 17	57 ± 17

All values are means ± SDs, (Range: min-max). 1RM, one repetition maximum; AUC, area under the curve; AU, arbitrary units; BM, body mass; CON, control (low dairy protein, high carbohydrate); DEXA, dual energy x-ray absorptiometry; HDL, high density lipoprotein; HOMA index, homeostasis model assessment; HDPMC, High dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; LDL, low density lipoprotein; OGTT, oral glucose tolerance test; VO$_2$ peak, peak oxygen consumption.
No significant differences were observed among groups at baseline for any variable ($P>0.05$).

4.4.2 Body composition

BM decreased across time with no differences between groups (HDPMC: -7.2 ± 3.3 kg, -7.4 ± 4.1 %Δ; HDPHC: -7.0 ± 3.3 kg, -7.4 ± 4.0 %Δ; CON: -7.7 ± 3.6 kg, -7.4 ± 3.9 %Δ; P=0.42). The loss of body fat in all groups was significant in both absolute and relative-to-baseline changes across time (P<0.001, **Table 4.3** and **Figure 4.2A**). A greater change occurred in the first 8 wk such that all groups' BM, trunk fat loss, and decrease in BMI (**Table 4.3**), and body FM loss (**Figure 4.2A**) was greater than the second 8 wk. Similarly, body fat percentage decreased significantly across time in all groups, in absolute and relative-to-baseline changes (P<0.001, **Table 4.3** and **Figure 4.2B**). Post-hoc analysis showed no effect of time for the change in body fat percentage for HDPHC participants, due to the increase in LM in the second 8 wk of the intervention (**Figure 4.2C**). No relationship between starting BM and change in BM over the intervention was observed (P=0.21, R^2=0.02).

The change in LM was not different between groups (HDPMC: 0.8 ± 1.8 kg; HDPHC: 0.7 ± 2.0 kg; CON: 0.1 ± 1.6 kg), although the magnitude of change across time differed such that a group × time interaction was observed (P=0.05, **Table 4.3** and **Figure 4.2C**). Specifically, the HDPMC group accrued more LM in the first 8 wk compared with HDPHC (P=0.016) and CON (P=0.021), and consequently HDPHC and CON participants' gained LM at a faster rate in the second 8 wk (**Figure 4.2C**). Regional LM analysis showed the HDPMC participants gained more trunk LM than the HDPHC (P=0.025) and CON participants (P=0.008) in the first 8 wk of the intervention, with no differences in limb LM (**Figure 4.2C**). However, the change in both limb and trunk LM was not different in the second 8 wk between groups as this trunk LM was not maintained for the HDPMC participants. When comparing data from males and females the changes in FM, BM and body fat percentage did not differ across time [data not shown], but the rate of change in LM was different. There was no difference in LM change after 8 wk (M: -0.27 ± 1.51 kg vs F: 0.12 ± 1.68 kg) but during the

subsequent 8 wk period there was a greater accretion of LM in males (M: 0.96 ± 1.11 kg vs F: 0.30 ± 1.05 kg, P=0.014). Waist circumference, waist to hip ratio and trunk fat decreased across the 16 wk intervention (**Table 4.3**). BMI decreased across the intervention (-2.6 ± 1.2 km/m^2 to 29.9 ± 3.7 kg/m^2) but was not different between groups.
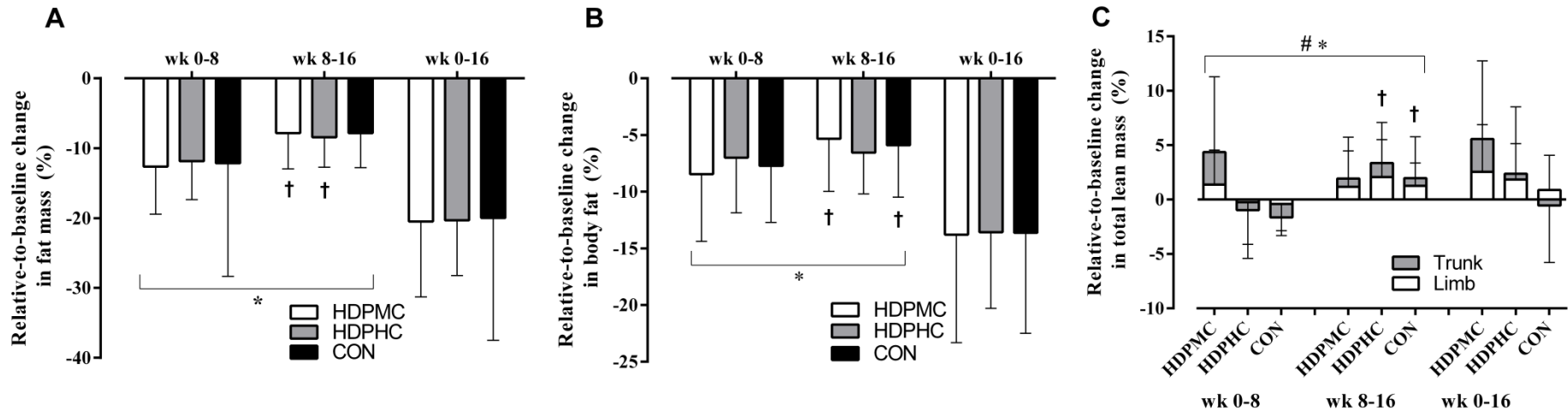
Figure 4.2. Effects of a 16 wk diet and exercise intervention on the percentage change relative to baseline in **A**: fat mass, **B**: body fat percentage and **C**: lean mass for three different diets (HDPMC: high dairy protein moderate carbohydrate, (n=29); HDPHC: high dairy protein moderate fat (n=32); CON: control; low dairy protein, high carbohydrate (n=28)). Data are mean (± SD) (total n=89); Linear mixed model analysis, significantly different by: * time (P<0.05); # group × time interaction (P<0.05); post-hoc within-group ANOVA analysis, significantly different from: † Week 0-8 vs 8-16 (P<0.05).

Table 4.3. Absolute changes in body composition variables of participants across intervention groups for the first 8 weeks of the intervention (Δ 0-8) and the second 8 weeks of the intervention (Δ 8-16)

Variable	Diet	Time point		P		
		Δ 0-8	Δ 8-16	Group	Time	Interaction
Body mass, kg				NS	$P<0.01$	NS
	HDPMC	-4.3 ± 2.3	-2.9 ± 1.9[*]			
	HDPHC	-4.7 ± 2.4	-2.3 ± 1.8[*]			
	CON	-5.3 ± 2.3	-2.5 ± 1.7[*]			
BMI, kg/m^2				NS	$P<0.01$	NS
	HDPMC	-1.5 ± 0.7	-1.1 ± 0.7[*]			
	HDPHC	-1.7 ± 0.8	-0.9 ± 0.7[*]			
	CON	-2.0 ± 0.8	-0.9 ± 0.7[*]			
Body fat, kg				NS	$P<0.01$	NS
	HDPMC	-4.9 ± 2.3	-3.0 ± 1.8[*]			
	HDPHC	-4.3 ± 1.7	-3.1 ± 1.6[*]			
	CON	-4.9 ± 5.2	-2.9 ± 1.8[*]			
Body fat, %				NS	$P<0.01$	NS
	HDPMC	-3.8 ± 2.5	-2.3 ± 1.9[*]			
	HDPHC	-2.9 ± 1.8	-2.8 ± 1.5			
	CON	-3.3 ± 1.8	-2.5 ± 1.8[*]			
Lean mass, kg				NS	$P<0.01$	$P=0.05$
	HDPMC	0.7 ± 1.7	0.1 ± 1.2			
	HDPHC	-0.4 ± 1.7	0.8 ± 1.2[*]			
	CON	-0.3 ± 1.2	0.5 ± 1.0[*]			
Trunk fat, kg				NS	$P<0.01$	NS
	HDPMC	-3.2 ± 1.5	-1.9 ± 1.2[*]			
	HDPHC	-2.7 ± 1.4	-2.0 ± 1.1[*]			
	CON	-3.2 ± 1.3	-1.7 ± 1.2[*]			
Waist circumference, cm				NS	$P<0.01$	NS
	HDPMC	-5.5 ± 3.3	-3.4 ± 1.6[*]			
	HDPHC	-5.2 ± 3.0	-2.5 ± 3.0[*]			
	CON	-5.1 ± 2.5	-2.9 ± 2.8[*]			
Waist:hip ratio				NS	$P=0.02$	NS
	HDPMC	-0.02 ± 0.03	-0.01 ± 0.02			
	HDPHC	-0.01 ± 0.03	0.00 ± 0.03[*]			
	CON	-0.01 ± 0.02	-0.01 ± 0.03			

All values are means ± SDs. NS, $P>0.05$. All body composition variables were measured with DXA. DXA, dual energy x-ray absorptiometry; HDPMC, High dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate. Significant differences observed between groups were measured via one-factor ANOVA where: *DXA* * significantly different from 0-8 wk ($P<0.05$).

4.4.3 Diet

At baseline, self-reported energy intake was greater for participants randomly assigned to the CON diet compared to either the HDPMC (P=0.04) or the HDPHC diet (P=0.003) (**Table 4.4**). When included as a covariate these differences had no influence on FM loss, LM maintenance, body fat percentage loss and total BM loss. Baseline protein intakes were above the Australian RDI for protein for adults, whereas baseline calcium intakes were below the daily RDI for calcium (1000 mg) for men and women. There were no differences in estimated energy requirements between groups (HDPMC: 2110 ± 275 kcal/d; HDPHC: 2106 ± 209 kcal/d; CON: 2078 ± 324 kcal/d) or the energy band assigned for dietary intake through the intervention (i.e. estimated energy requirements minus 250 kcal/d; HDPMC: 1834 ± 268 kcal/d; HDPHC: 1844 ± 224 kcal/d; CON: 1796 ± 319 kcal/d). Participants were prescribed energy intakes amounting to 273 ± 65 kcal/d less than the estimated energy requirements, but reported energy intakes of 489 ± 171 kcal/d less than requirements, with no differences between groups for either prescribed or reported intakes.

As intended, protein intake was similar between HDPHC and HDPMC (in absolute amounts or as a percentage of total energy intake). Protein intake in the high protein groups was greater than CON (P<0.001, for g/d and % total energy). Dairy servings were not different between groups at baseline but higher in HDPMC (P<0.001) and HDPHC (P<0.001) than CON. Calcium intakes were higher for the two dairy groups (HDPMC and HDPHC), greater than baseline (P<0.05) with no change in calcium intake for the CON group.

Table 4.4. Dietary analysis of overweight or obese, sedentary, but otherwise healthy, 35-59 y old participants prior to beginning the intervention (baseline), an average of consumption during the 16 wk intervention, and change from baseline for each intervention dietary group

	Baseline			Week 1-16 Average			Change from baseline		
	Diet			Diet			Diet		
Variable	**HDPMC**	**HDPHC**	**CON**	**HDPMC**	**HDPHC**	**CON**	**HDPMC**	**HDPHC**	**CON**
n	**27**	**30**	**25**	**27**	**27**	**27**	**27**	**26**	**24**
Energy (kJ/d)	8162 ± 1859	7739 ± 1573	9114 ± 1957[ab]	6689 ± 942	6791 ± 783	6733 ± 977	-1473 ± 1271	-848 ± 1619	-2391 ± 1813[ab]
Energy (kcal/d)	1950 ± 443	1849 ± 376	2177 ± 468[ab]	1597 ± 224	1622 ± 187	1608 ± 234	-353 ± 303	-203 ± 387	-571 ± 433[ab]
Energy (kJ/kg BM/d)	89 ± 19	87 ± 19	101 ± 22[ab]	73 ± 9	75 ± 7	75 ± 9	-16 ± 14	-10 ± 19	-27 ± 20[ab]
CHO (%TE)	43 ± 5	43 ± 7	43 ± 7	39 ± 2	47 ± 3[a]	54 ± 3[ab]	-4 ± 6	3 ± 7[a]	11 ± 7[ab]
Protein (g/d)	95 ± 16	99 ± 20	107 ± 22[ab]	113 ± 17	119 ± 15	79 ± 12[ab]	18 ± 13	22 ± 21	-29 ± 19[ab]
Protein (g/kg BM/d)	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.2[ab]	1.2 ± 0.1	1.3 ± 0.2[a]	0.9 ± 0.1[ab]	0.2 ± 0.1	0.2 ± 0.2	-0.3 ± 0.2[ab]
Protein (g/kg lean BM/d)	2.0 ± 0.4	2.0 ± 0.4	2.3 ± 0.6[ab]	2.4 ± 0.3	2.4 ± 0.3	1.7 ± 0.3[ab]	0.4 ± 0.3	0.4 ± 0.4	-0.6 ± 0.4[ab]
Protein (%TE)	20 ± 3	22 ± 3	21 ± 4	29 ± 1	31 ± 2[a]	21 ± 1[ab]	9 ± 3	9 ± 4	0 ± 4[ab]
Fat (g/d)	76 ± 16	70 ± 22	84 ± 25[b]	56 ± 9	39 ± 6[a]	45 ± 9[ab]	-20 ± 13	-29 ± 20	-40 ± 25[ab]
Fat (%TE)	35 ± 5	33 ± 6	34 ± 5	32 ± 3	22 ± 3[a]	25 ± 2[ab]	-4 ± 5	-11 ± 5[a]	-9 ± 5[a]
Calcium, mg/d	793 ± 363	909 ± 272	795 ± 151	1398 ± 183	1576 ± 226[a]	721 ± 88[ab]	605 ± 308	673 ± 344	-81 ± 187[ab]
Dairy servings (#)	1.3 ± 0.8	1.3 ± 0.6	1.2 ± 0.5	3.6 ± 0.6	3.9 ± 0.7	1.0 ± 0.3[ab]	2.4 ± 0.9	2.6 ± 0.8	-0.1 ± 0.5[ab]
Dietary Fibre (g/d)	20 ± 5	22 ± 7	23 ± 6[a]	22 ± 5	24 ± 4	31 ± 6[ab]	3 ± 5	3 ± 9	8 ± 7[ab]
Alcohol (g/d)	4 ± 6	4 ± 6	5 ± 7	1.1 ± 1.6	1.4 ± 2.4	1.3 ± 1.5	-3.2 ± 5.2	-2.5 ± 4.6	-3.8 ± 6.6
Alcohol (%TE)	1 ± 2	1 ± 2	2 ± 2	1 ± 1	1 ± 1	1 ± 1	-1 ± 2	-1 ± 2	-1 ± 2

All values are means ± SD. Week 1-16 Average were analysed using the 7DD records from weeks 1, 4, 8, 12 and 16. BM, body mass; CHO, carbohydrate; HDPMC, high dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate); TE, total energy.

Significant differences observed between groups were measured via one-factor ANOVA where: [a] significantly different to HDPMC ($P<0.05$); [b] significantly different to HDPHC ($P<0.05$).

4.4.4 Blood glucose and lipids

Blood glucose and total cholesterol concentrations, as well as triglyceride, insulin, HDL and LDL cholesterol concentrations remained unchanged across time with no interaction (**Table 4.5**). HDL cholesterol was different among groups (P=0.002) but no time or interaction effect was observed. HOMA index and area under the curve (AUC) for the OGTT test both decreased across time (P<0.05) but were not different between groups. Both leptin and adiponectin concentrations, as well as AUC for OGTT tests, all decreased pre to post intervention (P<0.01) with no group effect (**Table 4.5**).

4.4.5 Aerobic capacity and strength

Aerobic fitness increased over the 16 wk (P<0.001), with no differences between groups (HDPMC: 0.26 ± 0.21 L/min; HDPHC: 0.21 ± 0.18 L/min; CON: 0.25 ± 0.21 L/min; **Table 4.6**). Maximal strength for chest, leg and back muscle groups all increased with time (P<0.001), but with no differences between groups (total 1RM of three exercises; HDPMC: 66 ± 28 kg; HDPHC: 76 ± 24 kg; CON: 74 ± 29 kg).

4.4.6 Energy expenditure

There were no differences at baseline for estimated energy expenditure (2754 ± 521 kcal/d) between groups. Estimates of energy expenditure from SWA monitors did not change across the intervention, although a trend for decreased energy expenditure occurred across time (Δwk 8, group mean: 64 ± 1255 kcal/d, Δwk 16 group mean: -207 ± 1443 kcal/d, P=0.05), with no group × time differences (**Figure 4.3**).
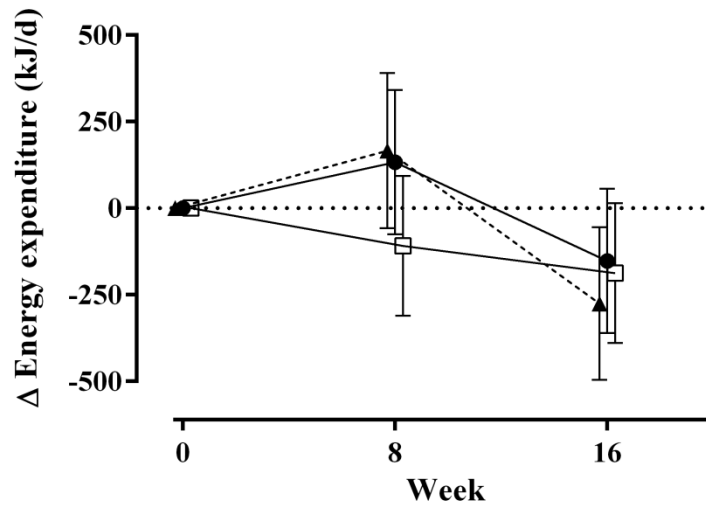
Figure 4.3. Effects of a 16 wk diet and exercise intervention on the changes in energy expenditure (kJ/day) when consuming a high dairy protein, moderate carbohydrate (HDPMC) diet (●), a high dairy protein, moderate fat (HDPHC) diet (□), or a control (CON) diet (▲).

Table 4.5. Blood profiles of overweight or obese, sedentary, but otherwise healthy, 35-59 y old participants across intervention groups (HDPMC, $n=29$; HDPHC, $n=32$; CON, $n=28$) for the first 8 wk of the intervention (Δ 0-8) and the second 8 wk of the intervention (Δ 8-16)

Variable	Diet	Time point		P		
		Δ 0-8	Δ 8-16	Group	Time	Interaction
Glucose, mmol/L				NS	$P<0.01$	NS
	HDPMC	-0.1 ± 0.5	-0.0 ± 0.3			
	HDPHC	-0.1 ± 0.4	-0.1 ± 0.5			
	CON	-0.1 ± 0.5	-0.2 ± 0.3			
Insulin, mIU/mL				NS	NS	NS
	HDPMC	-2.0 ± 3.4	-0.4 ± 2.3			
	HDPHC	-0.2 ± 3.5	-0.7 ± 2.9			
	CON	-1.6 ± 4.3	-0.5 ± 2.9			
Fasted HOMA index				NS	$P=0.01$	NS
	HDPMC	-0.5 ± 0.9	-0.1 ± 0.6			
	HDPHC	-0.1 ± 0.9	-0.2 ± 0.8			
	CON	-0.5 ± 1.4	-0.2 ± 0.7			
Total Cholesterol, mmol/L				NS	NS	NS
	HDPMC	-0.4 ± 0.6	0.0 ± 0.4			
	HDPHC	-0.4 ± 0.6	0.0 ± 0.5			
	CON	-0.5 ± 0.7	0.0 ± 0.5			
LDL Cholesterol, mmol/L				NS	NS	NS
	HDPMC	-0.2 ± 0.5	0.1 ± 0.3			
	HDPHC	-0.1 ± 0.6	0.0 ± 0.4			
	CON	-0.3 ± 0.5	0.0 ± 0.2			
HDL Cholesterol, mmol/L				$P<0.01$	NS	NS
	HDPMC	-0.1 ± 0.2	0.1 ± 0.2			
	HDPHC	-0.1 ± 0.2	0.0 ± 0.2			
	CON	0.0 ± 0.1[a,b]	0.0 ± 0.2[a,b]			
Triglycerides, mmol/L				NS	NS	NS
	HDPMC	-0.5 ± 1.3	-0.1 ± 0.3			
	HDPHC	-0.3 ± 0.5	0.0 ± 0.3			
	CON	0.0 ± 0.3	0.0 ± 0.3			
Adiponectin, ng/mL				NS	P<0.01	NS
	HDPMC	-0.7 ± 4.3				
	HDPHC	-1.6 ± 4.4				
	CON	-1.0 ± 4.6				
Leptin, ng/mL				NS	P<0.01	NS
	HDPMC	-10.6 ± 10.7[*]				
	HDPHC	-10.7 ± 10.2[*]				
	CON	-7.7 ± 11.4[*]				
OGTT AUC, AU				NS	P<0.01	NS
	HDPMC	-57 ± 100[*]				
	HDPHC	-87 ± 100[*]				
	CON	-107 ± 107[*]				

Table 4.5. Continued

All values are means ± SD. Analyses are from whole blood unless specified. AUC, area under the curve; AU, arbitrary units; HOMA index, homeostasis model assessment; HDL, high density lipoprotein; HDPMC, High dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate); LDL, low density lipoprotein; OGTT, oral glucose tolerance test.
Significant differences observed between groups were measured via one-factor ANOVA where:

[a] significantly different to HDPMC (P<0.05), [b] significantly different to HDPHC (P<0.05).* Main effect of time within group (*P*<0.05).

Adiponectin and Leptin were analysed from plasma; samples were not available for all participants such that HDPMC, *n*=29; HDPHC, *n*=30; and CON, *n*=25.

For OGTT analyses, samples were not available for all participants such that HDPMC, *n*=28; HDPHC, *n*=30; and CON, *n*=26.

Table 4.6. Exercise variables (aerobic and strength) measured in overweight or obese, sedentary, but otherwise healthy, 35-59 y old participants across dietary intervention groups (HDPMC, $n=29$; HDPHC, $n=32$; CON, $n=28$) prior to beginning the intervention (wk 0), at the intervention mid-point (wk 8) at the conclusion of the intervention (wk 16)

| Variable | Diet condition | | | | | | | | |
| | HDPMC | | | HDPHC | | | CON | | |
	Wk 0	Wk 8	Wk 16	Wk 0	Wk 8	Wk 16	Wk 0	Wk 8	Wk 16
VO_2 peak, absolute (L/min)	2.03 ± 0.61	2.25 ± 0.61	2.32 ± 0.68	2.11 ± 0.50	2.24 ± 0.52	2.30 ± 0.50	2.15 ± 0.70	2.32 ± 0.76	2.31 ± 0.89
VO_2 peak, relative (mL/kg/min)^2	21.9 ± 5.2	25.6 ± 5.4	27.0 ± 6.4	23.2 ± 5.4	26.1 ± 6.0	27.7 ± 6.1	23.2 ± 6.2	26.7 ± 7.5	28.4 ± 8.2
Peak power output (W)	148 ± 51	165 ± 48	175 ± 51	149 ± 39	165 ± 41	173 ± 42	152 ± 56	171 ± 63	182 ± 65
1RM tests									
Chest press (kg)	42 ± 15	45 ± 16	49 ± 15	43 ± 17	48 ± 18	52 ± 19	45 ± 16	48 ± 16	52 ± 17
Leg press (kg)	180 ± 55	209 ± 50	231 ± 53	176 ± 54	216 ± 61	234 ± 57	184 ± 53	213 ± 64	238 ± 55
Lat pulldown (kg)	56 ± 15	60 ± 17	64 ± 18	55 ± 17	60 ± 16	64 ± 17	57 ± 17	62 ± 19	66 ± 20

All values are means ± SDs. No significant differences were observed between groups as measured using linear mixed model analysis for time, group, or interaction effects, where baseline values were included as covariates ($P \geq 0.05$). Relative VO_2 peak was calculated using the measured body weight at the corresponding time point.

1RM, one repetition maximum; HDPMC, high dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate); VO_2 peak, peak oxygen consumption.

4.5   Discussion

This study has determined the effect of high-protein, dairy-based energy-restricted diets with variable CHO and fat content on changes in body composition and selected health parameters after 16 wk of energy restriction and daily EXT in overweight and obese men and women. A novel aspect of the current study was that the diets used normal fat versus low-fat carbohydrate-sweetened dairy foods to manipulate the macronutrient content of the diets. The major findings were: 1) a significant loss of FM coupled with the preservation of LM and improvements in health-related parameters occurred for all dietary intervention groups; 2) variable CHO and fat contribution with high-dairy protein intakes neither enhanced the rate of fat loss nor promoted greater gains in LM compared to a control diet; 3) when protein intake meets or exceed RDIs, and this intake is spread across the day, favourable changes in body composition can be attained with mild energy restriction and an appropriate exercise stimulus, and 4) moderate CHO restriction *per se* was not necessary to achieve meaningful changes in body composition or improvements in health parameters and risk factors for obesity.

In previous studies that have examined the role of macronutrient composition on weight loss/maintenance, higher protein intakes (i.e., >25% of energy) are most often incorporated into daily meal plans at the expense of CHO-based foods (Krieger et al., 2006; Layman et al., 2005; Meckling & Sherfey, 2007; Wycherley et al., 2012). Through the use of dairy foods as the primary tool to modify protein source, and with energy intakes equally restricted, no differences were observed in changes in body composition, health markers, or functional outcomes whether dietary treatments were based on high-CHO low-fat dairy choices (HDPHC) or full-fat unsweetened dairy (HDPMC). The results of the current study suggest that provided total energy intake is mildly restricted (i.e. ~-500 kcal/d) and there is an appropriate exercise stimulus, higher protein intake from dairy products can be included in a weight loss diet regardless of their macronutrient composition. Furthermore, the results

support the contention that modest CHO restriction *per se* is not necessary to achieve optimal body composition changes or improvements in health parameters and risk factors for obesity.

Two recent meta-analyses on the efficacy of dairy-based weight loss diets have been published (Abargouei et al., 2012; M. Chen et al., 2012) and in ten studies which reported dairy intakes of greater than three servings/day in combination with a mild energy restriction, reductions in BM, FM and waist circumference, along with greater retention of LM, were greater than in the control diets (Abargouei et al., 2012). However, the current study did not find an improvement in body composition when dairy food consumption was increased, possibly because participants' calcium intakes were already above the "calcium threshold" to improve body composition (Boon et al., 2005).

Although no differences in LM between groups were observed post-intervention, there were subtle differences in the time course of change in LM loss. However, such variability in the rate of change in LM may not accurately reflect significant changes in "functional" lean tissue but rather other components quantified by DXA including, but not limited to, lean tissue in the trunk region, intestinal contents and internal organs. Thus, the variation in trunk LM measures accounts for the temporal differences between treatments, a notion also supported by comparable limb LM and functional strength changes between groups.

This study has shown that the inclusion of REX three times a week within a weight loss program for middle-aged adults was associated with the retention of LM. The results of the current study are in support of Willis and co-workers (2012) who have shown that concomitant aerobic and REX in overweight or obese adults is superior for healthy body composition changes compared to either training mode alone. Due to the regular structured (resistance) and unstructured (aerobic) activity of all groups, the effects on body composition and other parameters that may have been observed in a diet-only study may have been overridden by the potent exercise stimulus. Previous evidence (Bryner et al., 1999) would suggest that the large FM losses and maintenance of LM that has been observed would not

have been evident with either a diet- or exercise-only intervention (Weinheimer et al., 2010). The results of the current study suggest that adequate protein consumed across the day is sufficient to maintain LM and that protein intake above the RDI confers no additional benefit for the maintenance of LM in this age group, providing there is a REX stimulus.

The use of real foods within energy-restricted, high protein diets provides a more holistic approach to long-term dietary change compared to the use of liquid meals (Bryner et al., 1999), whey, soy or other protein isolates (Ballor et al., 1988) or single brands/supplements/foods (Farnsworth et al., 2003; Josse et al., 2011; Zemel et al., 2005). Indeed, the dairy consumption in the current study provided a realistic ~30% of daily energy intake (~4 servings) in both of the higher protein intervention groups, compared to 6-7 servings of standardised low fat dairy products that provided up to 50% of total energy in a previous investigation (Josse et al., 2011). The concept of "dairy baskets", consisting of combinations of commonly available dairy foods (milk, flavoured milk, cheese, yogurt, drinking yogurt etc.,) provided a simple, practical method to systematically increase protein and calcium intake while differentiating the intake of dairy fat and CHO.

Recruiting apparently healthy individuals for the current study may have limited the potential for the current intervention to induce large improvements in markers of cardiometabolic health (i.e. fasting glucose, insulin, blood lipid and cholesterol concentrations). However, the overweight cohort of this study presented with elevated fasting leptin, adiponectin and OGTT concentrations and all these parameters were significantly improved post intervention. A potential limitation of this study was that participants prepared and recorded their own food intake from dietary plans that evolved from tight prescriptions to self-determined choices. This offers less control and relies on self-report compared with methods such as provision of pre-prepared meals or "live-in" metabolic ward research designs. However, the dietary methodology utilised, which encouraged education and guided

preparation of dietary plans in both males and females, has an important "real world" application and, the overall free-living experimental design adds ecological validity.

In conclusion, the results of this study demonstrate robust benefits of 16 wk of mild energy-restriction in combination with exercise training on changes in body composition, health and functional parameters in men and women with overweight/obesity. A stimulus comprising resistance- and aerobic-based exercise is sufficient to maintain lean mass when overweight individuals are in mild energy restriction, at least when daily protein intake meets or exceeds the recommended daily intakes. Significant differences in energy intake and dietary macronutrient content were achieved using novel and innovative education and professional support, and through the use of whole dairy foods to manipulate the macronutrient content of the diets. This allowed individuals the freedom to select high quality protein foods according to personal preference within a lifestyle involving appropriate energy intake and regular exercise during healthy weight loss or weight maintenance. Such an approach has substantial merit for long-term weight maintenance and the associated improvements in the health profile of middle-aged individuals.

4.6   Supplementary Results

Table 4.7. Effect sizes and 95% confidence intervals for body composition variables measured in overweight or obese, sedentary, but otherwise healthy, 35-59 y old participants between dietary intervention groups (HDPMC, n=29; HDPHC, n=32; CON, n=28) across the first 8 weeks and the entire duration (0-16) weeks of the diet and exercise intervention.

| | Absolute effect sizes (95% CI's) | | | | | |
| | Δ wk 0-8 | | | Δ wk 0-16 | | |
	HDPMC vs HDPHC	**HDPMC vs CON**	**HDPHC vs CON**	**HDPMC vs HDPHC**	**HDPMC vs CON**	**HDPHC vs CON**
Body mass, kg	0.5 (-1.0 , 2.0)	1.1 (-0.5 , 2.6)	0.6 (-0.9 , 2.0)	-0.1 (-1.6 , 1.4)	0.6 (-0.9 , 2.1)	0.7 (-0.8 , 2.2)
BMI, kg/m^2	0.1 (-0.4 , 0.6)	0.4 (-0.1 , 0.9)	0.3 (-0.2 , 0.8)	-0.1 (-0.6 , 0.5)	0.3 (-0.3 , 0.8)	0.3 (-0.2 , 0.9)
Fat mass, kg	-0.7 (-2.1 , 0.7)	0.0 (-1.5 , 1.4)	0.7 (-0.7 , 2.1)	-0.6 (-2.0 , 0.8)	-0.2 (-1.6 , 1.3)	0.4 (-1.0 , 1.8)
Fat mass, %BM	-1.2 (-2.5 , 0.2)	-0.6 (-2.0 , 0.8)	0.6 (-0.8 , 1.9)	-0.7 (-2.0 , 0.6)	-0.4 (-1.8 , 0.9)	0.2 (-1.1 , 1.6)
Lean mass, kg	1.0 (0.2 , 1.9)	1.0 (0.2 , 1.9)	0.0 (-0.9 , 0.8)	0.3 (-0.5 , 1.2)	0.7 (-0.2 , 1.6)	0.4 (-0.5 , 1.2)
Waist circumference, cm	-0.3 (-2.0 , 1.5)	-0.2 (-2.1 , 1.6)	0.0 (-1.8 , 1.8)	-1.1 (-2.9 , 0.6)	-0.8 (-2.6 , 1.0)	0.4 (-1.4 , 2.1)
Waist:hip ratio	-0.01 (-0.02 , 0.01)	-0.01 (-0.02 , 0.01)	0.00 (-0.02 , 0.01)	-0.01 (-0.03 , 0.00)	-0.01 (-0.02 , 0.01)	0.01 (0.00 , 0.03)
Trunk fat, DEXA, kg	-0.6 (-1.5 , 0.4)	0.0 (-1.0 , 0.9)	0.5 (-0.4 , 1.4)	-0.4 (-1.3 , 0.5)	-0.2 (-1.2 , 0.7)	0.1 (-0.8 , 1.1)
Trunk lean, DEXA, kg	0.7 (0.1 , 1.3)	0.8 (0.1 , 1.4)	0.1 (-0.5 , 0.7)	0.4 (-0.2 , 1.1)	0.6 (0.0 , 1.3)	0.2 (-0.4 , 0.8)
Limb fat, DEXA, kg	-0.1 (-0.7 , 0.4)	0.1 (-0.5 , 0.7)	0.3 (-0.3 , 0.8)	-0.2 (-0.7 , 0.4)	0.0 (-0.6 , 0.6)	0.2 (-0.4 , 0.8)
Limb lean, DEXA, kg	0.3 (-0.1 , 0.7)	0.4 (0.0 , 0.8)	0.1 (-0.3 , 0.5)	0.0 (-0.2 , 0.5)	0.0 (0.0 , 0.8)	0.0 (-0.1 , 0.6)

Effect sizes were calculated, using the linear mixed model analysis, as the estimated differences between intervention groups with 95% confidence intervals; no significant differences were measured ($P \geq 0.05$). DEXA, dual energy x-ray absorptiometry; HDPMC, high dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate).

Table 4.8. Effect sizes and 95% confidence intervals for blood profile variables measured in overweight or obese, sedentary, but otherwise healthy, 35-59 y old participants between dietary intervention groups (HDPMC, n=29; HDPHC, n=32; CON, n=28) across the first 8 weeks and the entire duration (0-16) weeks of the diet and exercise intervention

| | Absolute effect sizes (95% CI's) | | | | | |
| | Δ wk 0-8 | | | Δ wk 0-16 | | |
	HDPMC vs HDPHC	HDPMC vs CON	HDPHC vs CON	HDPMC vs HDPHC	HDPMC vs CON	HDPHC vs CON
Glucose, mmol/L	-0.98 (-2.41 , 0.14)	-0.40 (-1.87 , 0.12)	0.58 (-0.86 , 0.08)	-0.68 (-2.12 , 0.14)	-0.22 (-1.69 , 0.11)	0.46 (-0.98 , 0.06)
Insulin, mIU/mL	-0.02 (-0.22 , 0.17)	-0.01 (-0.22 , 0.19)	0.01 (-0.19 , 0.21)	0.07 (-0.12 , 0.27)	0.14 (-0.07 , 0.34)	0.07 (-0.13 , 0.27)
Fasted HOMA index	-0.2 (-0.6 , 0.1)	-0.1 (-0.4 , 0.3)	0.2 (-0.2 , 0.5)	-0.1 (-0.5 , 0.2)	0.0 (-0.4 , 0.4)	0.1 (-0.2 , 0.5)
Total Cholesterol, mmol/L	-0.02 (-0.28 , 0.24)	-0.01 (-0.28 , 0.26)	0.00 (-0.26 , 0.27)	-0.02 (-0.28 , 0.24)	0.01 (-0.26 , 0.28)	0.03 (-0.23 , 0.30)
LDL Cholesterol, mmol/L	0.1 (0.0 , 0.1)	-0.1 (-0.1 , 0.0)	-0.1 (-0.2 , 0.0)	0.1 (0.0 , 0.1)	0.0 (-0.1 , 0.1)	-0.1 (-0.2 , 0.0)
HDL Cholesterol, mmol/L	-0.1 (-0.4 , 0.1)	0.0 (-0.3 , 0.2)	0.1 (-0.1 , 0.3)	-0.1 (-0.3 , 0.2)	0.0 (-0.2 , 0.3)	0.1 (-0.1 , 0.3)
Trigylerides, mmol/L	0.0 (-0.2 , 0.2)	0.0 (-0.2 , 0.2)	0.0 (-0.2 , 0.2)	-0.1 (-0.3 , 0.1)	-0.2 (-0.3 , 0.0)	-0.1 (-0.2 , 0.1)
Adiponectin, ng/mL	--	--	--	0.8 (-1.0, 2.6)	0.2 (-1.7, 2.1)	-0.6 (-2.5, 1.3)
Leptin, ng/mL	--	--	--	0.6 (-7.5, 7.4)	-2.9 (-10.7, 4.9)	-3.0 (-10.8, 4.8)
OGTT AUC, AU	--	--	--	28 (-7 , 62)	24 (-13 , 60)	-4 (-40 , 31)

Effect sizes were calculated, using the linear mixed model analysis, as the estimated differences between intervention groups with 95% confidence intervals; no significant differences were measured (P≥0.05). AUC, area under the curve; AU, arbitrary units; HOMA index, homeostasis model assessment; HDL, high density lipoprotein; HDPMC, high dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate); OGTT, oral glucose tolerance test.

Table 4.9. Effect sizes and 95% confidence intervals for exercise (aerobic and strength) variables measured in overweight or obese, sedentary, but otherwise healthy, 35-59 y old participants between dietary intervention groups (HDPMC, n=29; HDPHC, n=32; CON, n=28) across the first 8 weeks and the entire duration (0-16) weeks of the diet and exercise intervention

	Absolute effect sizes (95% CI's)					
	Δ wk 0-8				Δ wk 0-16	
	HDPMC vs HDPHC	**HDPMC vs CON**	**HDPMC vs HDPHC**	**HDPMC vs CON**	**HDPMC vs HDPHC**	**HDPMC vs CON**
VO$_2$ peak, L/min	0.04 (-0.05 , 0.14)	0.02 (-0.08 , 0.12)	-0.02 (-0.12 , 0.08)	0.05 (-0.05 , 0.14)	0.01 (-0.09 , 0.11)	-0.03 (-0.13 , 0.06)
VO$_2$ peak, mL/kg · min^{-1}	0.6 (-0.6 , 1.8)	0.0 (-1.3 , 1.2)	-0.6 (-1.8 , 0.6)	0.4 (-0.8 , 1.6)	-0.3 (-1.6 , 0.9)	-0.7 (-1.9 , 0.5)
Peak power output, W	-1.4 (-8.4 , 5.6)	-2.9 (-10.1 , 4.4)	-1.5 (-8.6 , 5.7)	1.0 (-6.0 , 8.0)	-3.0 (-10.3 , 4.2)	-4.1 (-11.2 , 3.1)
1RM tests						
Chest press, kg	-2 (-4 , 1)	0 (-3 , 2)	1 (-1 , 4)	-2 (-4 , 0)	0 (-3 , 2)	2 (-1 , 4)
Leg press, kg	-11 (-22 , 1)	-6 (-18 , 6)	5 (-7 , 16)	-6 (-18 , 5)	-7 (-19 , 5)	-1 (-12 , 11)
Lat pulldown, kg	-1 (-4 , 1)	-1 (-4 , 1)	0 (-2 , 3)	-2 (-4 , 1)	-1 (-4 , 1)	0 (-2 , 3)

Effect sizes were calculated, using the linear mixed model analysis, as the estimated differences between intervention groups with 95% confidence intervals; No significant differences were measured ($P \geq 0.05$); Relative VO$_2$ peak was calculated using the measured body weight at the corresponding time point.

1RM, one repetition maximum; HDPMC, high dairy protein moderate carbohydrate; HDPHC, high dairy protein high carbohydrate; CON, control (low dairy protein, high carbohydrate); VO$_2$ peak, peak oxygen consumption.

Preface to Chapter FIVE

The randomised trial (Chapter 4) showed a wide distribution for the change in body mass across all participants (**Figure 5.0**). Interaction of genes and environment may define an individual's response and epigenetic factors may also have the capacity to explain the differences between those who lost large (high responders) versus small (low responders) amounts of body mass. Plasma samples from the OGTT testing were used to quantify circulating microRNA concentrations and characterise changes in high and low responders. Two studies have undertaken c-miRNA analysis associated with loss of body weight with dietary intervention only (Milagro et al., 2013; Ortega et al., 2013). Several studies have also investigated the c-miRNA response to various exercise interventions (single session and chronic training) (Aoi & Sakuma, 2014). Currently, no studies have utilised c-miRNA analysis to examine differences in the magnitude of weight loss between individuals.
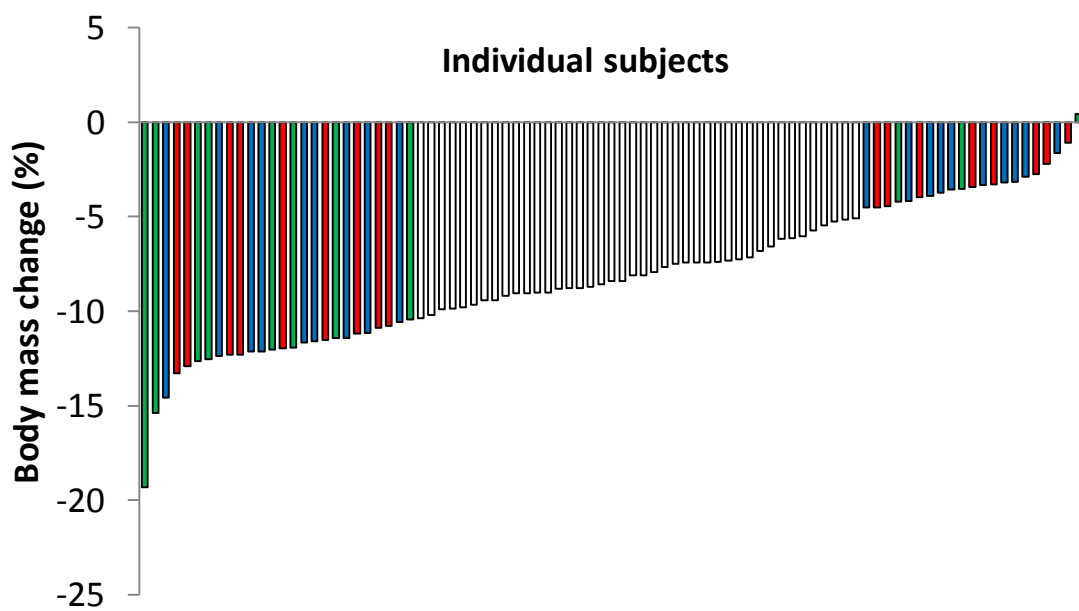
Figure 5.0. Change in body mass relative to baseline by all participants who completed the 16 wk diet and exercise intervention. Blue: denotes high dairy protein, high carbohydrate (HDPHC) diet participants; Red: denotes high dairy protein, moderate carbohydrate (HDPMC) diet participants; Green: denotes moderate protein, moderate carbohydrate (CON) diet participants.

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Chapter FIVE

5 *Study 3*: Modulation of circulating microRNAs between 'high' and 'low' responders following a 16-wk diet and exercise weight loss intervention

This chapter is comprised of the following manuscript in preparation to be submitted to *PLoS One:*

Parr, E.B., Camera, D.M., Coffey, V.G., Phillips, S.M., Burke, L.M., Hawley, J.A. Modulation of circulating microRNAs between 'high' and 'low' responders following a 16-wk diet and exercise weight loss intervention

5.1 Abstract

Background: Interactions between diet and physical activity and genetics contribute to the variance in body mass (BM) between individuals, and subsequently the loss of body mass in response to a given intervention. The modulation of circulating concentrations of microRNAs (c-miRNAs) may play a role in regulating the biological variation in response to energy restriction.

Objective: To quantify selected c-miRNAs with putative roles in energy metabolism and exercise adaptations following a 16 wk energy-restricted diet and exercise intervention in individuals with large (high responders; HiRes) versus small (low responders; LoRes) losses in BM.

Methods: From a cohort of 89 male and female overweight/obese participants who completed the intervention (500 kcal/d energy restriction from diet [250 kcal/d] and exercise [250 kcal/d]), a subgroup of HiRes (>10% BM loss, *n*=22) and LoRes (<5% BM loss, *n*=18) were identified. RNA was extracted, quantified and reverse transcribed from fasted plasma samples collected pre and post intervention. Quantification of thirteen c-miRNA selected *a priori* was performed on a customised 96-well miScript miRNA PCR Array.

Results: Loss of BM (-11.0 ± 2.3 kg vs -3.0 ± 1.3 kg; P<0.01) and fat mass (FM; -11.1 ± 2.6 kg vs. -3.9 ± 1.6 kg; P<0.01) was greater for HiRes than LoRes (P<0.001). Expression of c-miR-935 was higher in LoRes compared to HiRes pre- (~47%; P=0.025) and post- (~100%; P<0.01) intervention and was the only c-miRNA differentially expressed at baseline between groups. The abundance of c-miR-221-3p and -223-3p increased pre- to post-intervention in both groups (~57-69% and ~25-90%, P<0.05). There was a post-intervention increase in c-miR-140 expression only in LoRes compared to HiRes (P=0.016).

Conclusion: The differential expression of selected c-miRNAs between high and low responders before and after a chronic exercise and diet weight loss intervention indicate c-miRNAs may contribute to the magnitude of weight loss during energy deficit.

5.2   Introduction

Obesity is the excessive accumulation of fat mass resulting from a chronic imbalance between energy intake and energy expenditure. Common behavioural strategies for weight management and reducing fat mass include dietary energy restriction and increased energy expenditure through physical activity (Wing & Hill, 2001). However, the efficacy of each strategy alone or in combination is associated with considerable inter-individual variability (Hainer et al., 2008). While compliance to prescribed diet and physical activity interventions contributes to the variance in fat loss between individuals, differences in genotype that may predispose a given individual to be "resistant" to the loss of fat mass are also likely to be important (Bouchard et al., 2010).

Previous studies have reported "high" and "low" responders to weight loss interventions, defined as individuals who either lost significant or little/no body mass, respectively (Bouchard et al., 2010; Milagro et al., 2013; Moleres et al., 2013). Such variability in weight loss responses may, in part, be attributed to epigenetic factors modulated by behavioural/environmental influences (Bouchard et al., 2010; Campión, Milagro, & Martínez, 2009; Milagro et al., 2011; Petronis, 2001).

MicroRNAs, small non-coding strands of RNA, are an epigenetic mechanism and regulatory step of gene expression. MicroRNAs (miRNAs) are posttranscriptional regulators of messenger RNA (mRNA) that promote mRNA degradation and suppress or inhibit protein translation (Lai, 2002). While particular miRNAs are highly enriched in various tissues throughout the body, a subset of miRNAs are detectable in circulation (c-miRNAs) and can be transported to other tissues with potential to act in an endocrine manner on targeted cells (Aoi, 2014). To date, only one study has reported differential abundance of selected c-miRNAs associated with 'high' and 'low' responders following an 8-wk energy-restricted diet (Milagro et al., 2013) while others have shown no significant differences before and after a 14-wk weight loss diet despite a 17% loss in body mass (Ortega et al., 2013). Of note, these studies

did not incorporate exercise prescription during the weight loss interventions. Exercise is a well-established modulator of gene expression and can also alter c-miRNA abundance (Aoi et al., 2013; Baggish et al., 2011).

The study of c-miRNAs is a relatively new field of research and although many miRNAs have been discovered and quantified, and their roles vary considerably depending on tissue origin and what conditions (i.e. cancer, exercise, diet) they are measured under. The aim of the current study was to investigate whether the expression of specific c-miRNAs previously shown to be modulated by energy restriction or exercise are 'predictive' of the magnitude of weight loss between high' and 'low' responders following a 16 wk diet and exercise intervention. A second aim was to determine whether changes in these c-miRNAs as a result of the weight loss intervention are associated with loss of body mass and changes in glucose tolerance. It was hypothesised there would be a differential abundance of c-miRNAs implicated in the regulation of fat metabolism and exercise adaptation between high and low weight loss responders both before and after the intervention.

5.3   Methods

5.3.1 Participants

Participant characteristics have been previously described (*Study 2*). Briefly, 111 males and females aged 35-59 years began a 16-wk weight loss intervention incorporating diet and exercise prescription. Prior to commencing the intervention, participants had a BMI of 27-40 kg/m^2 (body mass 67-122 kg) and were sedentary but otherwise apparently healthy. All participants gave written consent to participate in the study that was approved by the Royal Melbourne Institute of Technology (RMIT) University Bundoora (Victoria, Australia) Human Research Ethics Committee. The trial was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612000021875). Participants were recruited and participated in the trial between March 2012 and October 2013. Of the initial participant

cohort, 89 individuals successfully completed the intervention and were subsequently ranked according to the magnitude of percentage body mass (BM) loss pre- to post-intervention from highest to lowest, irrespective of dietary group (as described below). From this ranking, participants were divided into quartiles and the criteria for high and low responders was formulated based on the 1st vs 4th quartiles: the upper quartile were individuals decreased ≥~10% (high responders) and the lower quartile individuals decreased ≤5% (low responders) of initial BM. There were a total of 49 individuals that were categorised as reducing BM ≤5% or ≥10% after the 16 wk intervention.

5.3.2 Experimental Design

The experimental design has been described elsewhere (*Study 2*). Briefly, participants undertook a 16 wk intervention during which an energy deficit of 500 kcal/d was induced using an energy-restricted diet (~250 kcal/d) and daily exercise (~250 kcal/d). Participants were stratified according to sex, age, and BMI and then randomly assigned to one of three dietary groups of different macronutrient intake (high dairy protein, high carbohydrate (HDPHC) ~30% protein, 55% CHO, 15% fat; High dairy protein, moderate carbohydrate (HDPMC) ~30% protein, 40% CHO, 30% fat; Low dairy protein, high carbohydrate (control, CON) ~15% protein, 55% CHO, 30% fat). For all groups, energy intake was restricted by 250 kcal/d based on estimated energy balance requirements (Frankenfield, Roth-Yousey, & Compher, 2005). Body composition was measured via whole-body dual energy absorptiometry (DXA) scans (GE Lunar Prodigy Pro, GE Healthcare; software: Encore 2009, version 12.20.033) before and after the 16 wk intervention. Dietary intake was assessed through analysing 7 day food records in Foodworks® Professional Edition Food Composition database (Version 7.0, Xyris Software Pty Ltd, QLD, Australia) collected in weeks 1, 4, 8, 12 and 16 and averaging intake across the study intervention period.

After an overnight fast participants reported to the laboratory for a DXA scan and fasted blood samples were taken from an antecubital vein. Blood was collected using a 9 mL

tube and was immediately spun at 3000 g at 4 °C for 10 min before analysis for insulin concentration. Subsequently, a 4 mL EDTA tube was taken for baseline blood glucose analyses as part of the oral glucose tolerance test (OGTT) using a YSI 2900 analyser (YSI Life Sciences, Yellow Springs, OH, USA). OGTT's were conducted before and after the intervention period using a 75 g (300 mL) commercially produced glucose solution (Point of Care Diagnostics, NSW, Australia) consumed within 5 min. Sequential 4 mL blood samples were collected 30, 60, 90 and 120 min post-ingestion. The remaining sample was spun at 3000 g at 4 °C for 10 min and stored at -80 °C for later analysis. The homeostasis model assessment (HOMA) score was calculated from glucose and insulin concentrations, as previously described (Matthews et al., 1985).

5.3.3 *Plasma RNA Extraction and Quantification*

Plasma samples stored at -80 °C were thawed on ice and subsequently centrifuged at 3000 g for 10 min at 4 °C to pellet cell debris. Thereafter, 1.5 µL of plasma was loaded onto a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA) to determine levels of free haemoglobin as previously described (Kirschner et al., 2011). Samples measuring above 0.20 at 414 nm were indicative of elevated free haemoglobin and were excluded from further analysis (*n*=6). 200 µL of the plasma was then transferred to a 1.5 mL tube and mixed with 1 mL Qiazol lysis reagent (Cat No. 217184; Qiagen, VIC, Australia) and 3.5 µL of miRNeasy Spike-In Control *Caenorhabditis elegans* miR-39 (cel-miR-39; Qiagen, VIC, Australia). 200 µL of chloroform was subsequently added to the samples and vortexed for 15 s before being centrifuged for 15 min at 12 000 g and 4 °C. The upper aqueous phase (~600 µL) was then transferred to a 2 mL tube and mixed thoroughly with 100% ethanol. Samples were transferred to RNeasy mini spin columns (Qiagen, VIC, Australia) in a 2 mL collection tube and centrifuged at 8 000 g for 15 s at room temperature. After washing and centrifuging with RWT and RPE buffers (Qiagen, VIC, Australia), columns were washed a final time with 80% ethanol. Columns were then centrifuged with lids open to dry any residual ethanol as per the

manufactures directions. For RNA elution, columns were placed in a new 1.5 mL collection tube and 14 μL of RNase-free water was added directly to the spin column membrane and centrifuged for 1 min at full speed. Extracted RNA was quantified using the NanoDrop 1000 spectrophotometer and measuring absorbance at 260 nm and 280 nm and equilibrated with RNase-free water for subsequent cDNA synthesis.

5.3.4 Reverse Transcription (RT) and Pre-Amplification

50 ng RNA was reverse transcribed using a miScript II RT Kit (Cat. No. 218160, Qiagen, Melbourne, Australia) in a BioRad thermal cycler (BioRad, Australia) according to the manufacturer's instructions. The resulting cDNA was then diluted in RNase-free water and pre-amplified using a miScript PreAMP PCR Kit (Cat No. 331452; Qiagen, Australia) according to the manufacturer's specifications. Pre-amplified cDNA was then diluted in RNase-free water and stored at -20 °C.

5.3.5 Real-Time PCR

Quantification of miRNAs was performed on a Qiagen customised 96-well miScript miRNA PCR Array (Custom Catalogue Number: CMIHS02269). The array contained positive and reverse transcription controls and 13 miRNAs selected *a priori* from previous studies showing changes following exercise, dietary intervention and/or weight loss including: hsa-miR-21-5p, hsa-miR-126-3p, hsa-miR-140-5p, hsa-miR-142-3p, hsa-miR-148a-5p, hsa-miR-148b-5p, hsa-miR-199a-3p, hsa-miR-221-3p, hsa-miR-223-3p, hsa-miR-423-5p, hsa-miR-589-3p, hsa-miR-874-5p and hsa-miR-935. PCR arrays were run using a miScript SYBR Green PCR Kit (218073; Qiagen, VIC Australia) with microRNA abundance normalised to cel-miR-39-3p abundance, and expression was not different at either time point or between groups (P=0.77). The 2ΔΔCT method of relative quantification was used to calculate the relative abundance of miRNAs in plasma (Livak and Schmittgen '01). Where the

relative abundance of miRNAs in plasma were >3 SD from the mean, measures were excluded from analysis at both time points (i.e. pre and post).

5.3.6 *Selection of Predicted miRNA Targets*

The TargetScan bioinformatics algorithm (Version 6.2, www.targetscan.org) was used for target prediction of miRNAs altered between responders and non-responders or pre to post intervention for those that were determined to be statistically significant.

5.3.7 *Statistical analysis*

To estimate whether differences between time points were statistically significant, a linear mixed model with an AR1 covariance matrix was used for each outcome measure. The interaction between time × group was included to allow the groups to change differently over time and to obtain least significant difference (LSD) post hoc comparisons of group within time. As this was an exploratory study, no adjustments were made for multiple comparisons in order to avoid type II errors [19]. Independent sample t-tests were used to assess baseline participant characteristics pre intervention and baseline differences in c-miRNA expression where main effects were observed. Linear regression analysis was performed to assess the relationship between baseline participant characteristics and baseline c-miRNA abundance, and between changes in participant characteristics and c-miRNA abundance pre- and post-intervention. Data are mean ± standard deviation (SD) and were analysed using SPSS (Version 20.0) with significance set at P<0.05.

5.4 Results

5.4.1 Participant characteristics

The mean age of participants was 47 ± 6 y. Forty-nine individuals lost $\leq 5\%$ or $\geq 10\%$ of their initial body mass after the 16 wk intervention. Participant characteristics pre and post intervention are listed in **Table 5.1**.

Table 5.1. Participant characteristics measured pre and post intervention of High (HiRes) and low (LoRes) responders to the 16 week diet and exercise intervention.

	Low responders (*n*=18)		High responders (*n*=22)		P values		
	Pre	Post	Pre	Post	Group	Time	Interaction
Age (years)	46.3 ± 5.7	-	48.2 ± 6.2	-	-	-	-
Body mass (kg)	94.4 ± 11.7	92.3 ± 12.0[#]	89.9 ± 14.2	82.8 ± 12.1[#*]	P=0.04	P<0.01	P<0.01
Body Mass Index (kg/m^2)	33.6 ± 4.7	32.5 ± 4.5[#]	31.9 ± 3.7	27.9 ± 3.3[#*]	P=0.02	P<0.001	P<0.001
Fat mass (kg)	41.2 ± 11.2	37.4 ± 12.0[#]	37.5 ± 8.7	26.4 ± 8.3[#*]	P=0.03	P<0.01	P<0.001
Lean mass (kg)	50.2 ± 8.2	51.1 ± 8.1[#]	49.3 ± 9.5	49.5 ± 9.7	NS	P=0.05	NS
Fasting blood glucose concentration (mmol/L)	5.3 ± 0.5	5.3 ± 0.5	5.8 ± 0.7[*]	5.4 ± 0.6[#]	NS	P=0.02	P<0.01
Oral glucose tolerance test AUC (AU)	210 ± 104	159 ± 78	316 ± 153[*]	186 ± 88[#]	P=0.04	P<0.001	P=0.05

Data are mean ± SD; P<0.05: [#] difference between time points within condition, [*] difference between groups within time points.

Nine participants were excluded from analysis: six (*n*=4 HiRes/*n*=2 LoRes) through blood sample haemolysis and three with missing samples (*n*=2 HiRes/*n*=1 LoRes) (**Figure 5.1**). Therefore, 18 LoRes and 22 HiRes participants were included in the final analysis and each diet intervention was represented in the HiRes (*n*=8 HPMC, *n*=5 HPHC, *n*=9 CON), and LoRes (*n*=6 HPMC, *n*=9 HPHC, *n*=3 CON) groups.
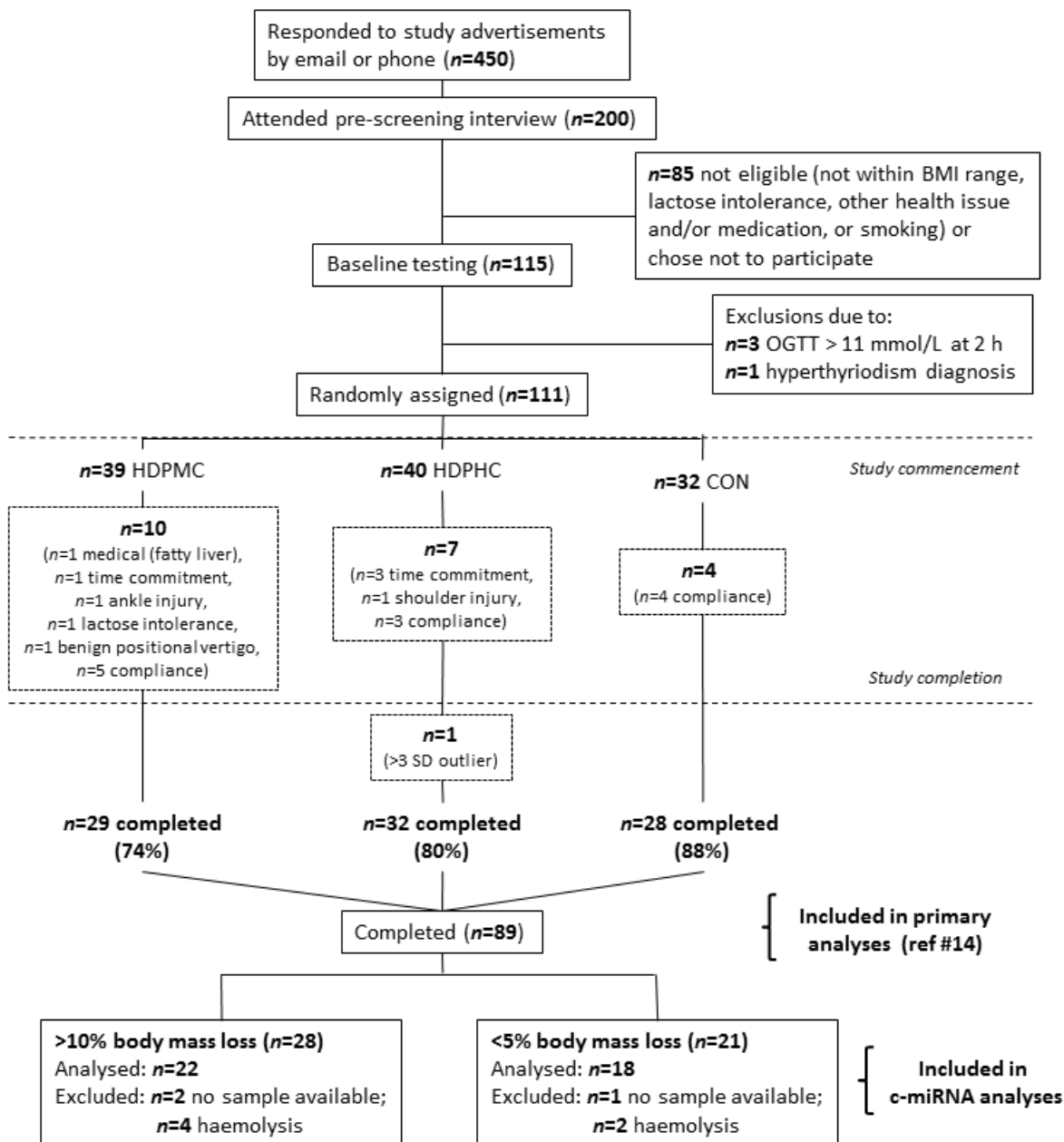
Figure 5.1. Flowchart of participants throughout the 16 wk diet and exercise intervention and in the subsequent analysis of high (HiRes) and low (LoRes) responders to the intervention. HDPHC, high dairy protein moderate fat; HDPMC, high dairy protein moderate fat; CON, control. Compliance was to the diet and/or the exercise training.

5.4.2 Plasma c-miRNA

The c-miR-221-3p and -223-3p abundance increased across time from pre- to post-intervention in both groups (57-69% and 25-90%, P=0.04 and P=0.05, respectively; **Figure 5.2B** and **C**). There was a significant effect for time for c-miR-223 (P=0.05) as a result of the increase in expression above baseline in HiRes after the intervention (**Figure 5.2C** P=0.038). A group difference was observed for c-miR-935 (P=0.001), where c-miR-935 was the only microRNA differentially expressed at baseline being higher in LoRes than HiRes (47%; P=0.046, **Figure 5.2D**). Such a difference in c-miR-935 was still evident at the conclusion of the 16 wk intervention period (100%; P<0.001). A group difference for c-miR-140 was evident (P=0.016) and post hoc tests showed no difference between responders at baseline for c-miR-140 but an increase in expression in LoRes (23%) compared to HiRes post-intervention (8%, P=0.014). There were modest effects of the 16 wk weight loss intervention on the abundance of other c-miRNA targets (**Table 5.2**).

Table 5.2. Plasma c-miRNA abundance at baseline (pre-intervention) and the relative fold change in abundance after a 16 wk diet and exercise intervention in low and high responders for weight loss.

c-miRNA	Pre-intervention			Relative fold change		
	Low responders (n=18)	High responders (n=22)	P value	Low responders (n=18)	High responders (n=22)	P value
miR-21-5p	2.47 ± 2.44	1.94 ± 2.30	0.96	0.28 ± 0.88	-0.05 ± 1.11	0.30
miR-126-3p	1.41 ± 1.10	1.16 ± 0.85	0.41	0.27 ± 1.16	0.25 ± 1.24	0.84
miR-140-5p	1.34 ± 0.91	0.92 ± 0.72	0.12	0.23 ± 0.66	0.07 ± 1.04	0.44
miR-142-3p	1.42 ± 1.03	0.98 ± 0.75	0.12	-0.02 ± 0.95	0.22 ± 1.08	0.54
miR-148a-5p	1.15 ± 0.76	0.92 ± 0.67	0.35	-0.10 ± 1.11	-0.02 ± 0.67	0.75
miR-148b-5p	1.39 ± 1.26	0.82 ± 0.63	0.08	-0.03 ± 1.08	0.97 ± 2.76	0.34
miR-199a-3p	2.50 ± 2.45	1.39 ± 1.42	0.08	0.19 ± 1.41	0.72 ± 1.87	0.60
miR-221-3p	1.28 ± 1.22	1.09 ± 1.06	0.62	0.51 ± 1.43	0.63 ± 1.97	0.97
miR-223-3p	1.75 ± 1.50	1.36 ± 1.38	0.42	0.25 ± 1.48	0.82 ± 1.77	0.34
miR-423-5p	1.11 ± 0.58	1.18 ± 0.94	0.80	0.14 ± 0.76	0.65 ± 1.40	0.68
miR-589-3p	1.37 ± 1.03	0.88 ± 1.22	0.20	0.04 ± 1.42	0.22 ± 1.22	0.78
miR-874-5p	1.12 ± 0.79	0.81 ± 0.54	0.17	0.21 ± 1.10	0.18 ± 1.33	0.84
miR-935	1.06 ± 0.52	0.72 ± 0.35	0.03	0.17 ± 0.74	-0.12 ± 0.59	0.18
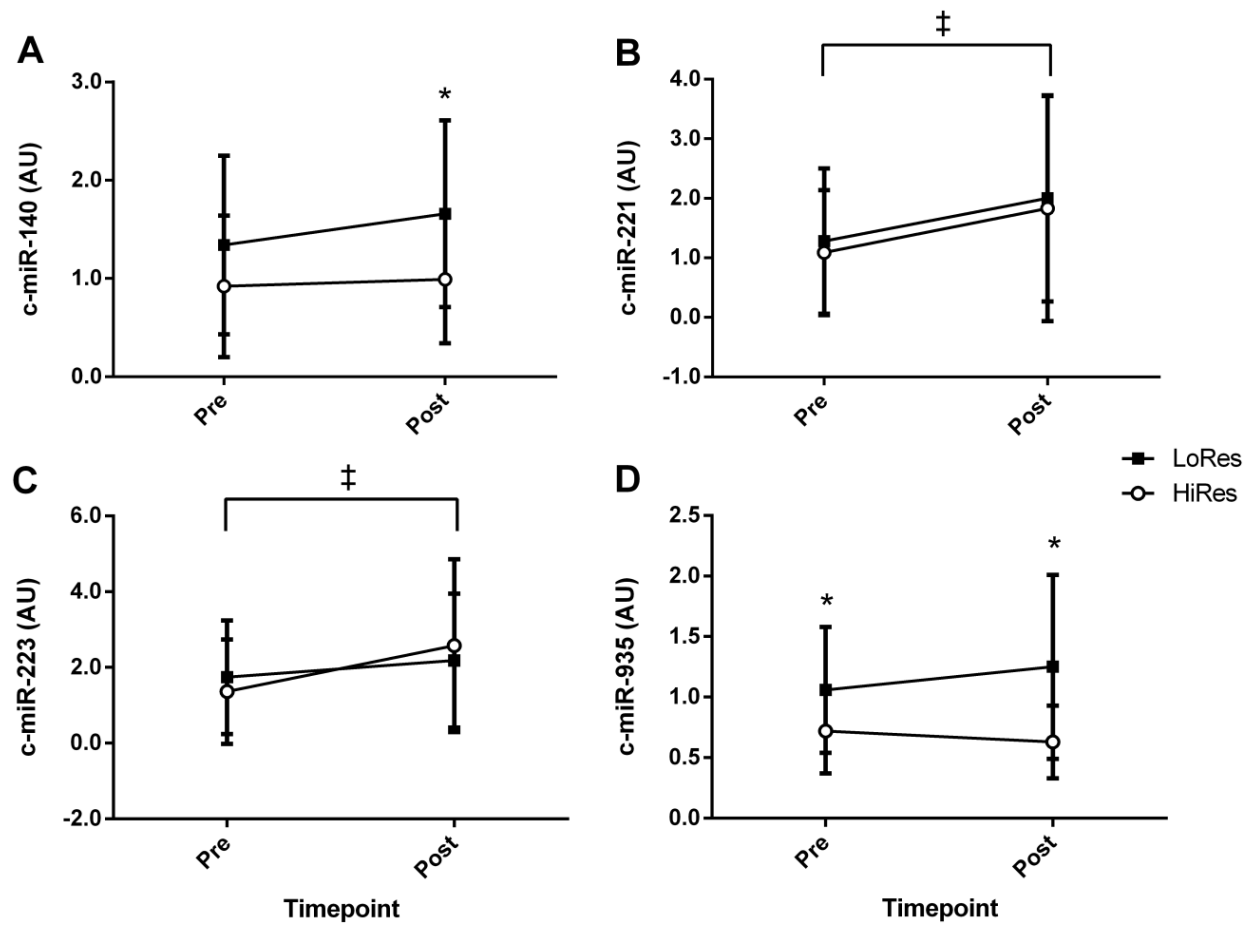
Figure 5.2. Plasma c-miR abundance prior to and after a 16 week diet and exercise weight loss intervention for high responders (HiRes, *n*=22) and low responders (LoRes, *n*=18) **A**: c-miR-140, **B**: c-miR-221; **C**: c-miR-223, and **D**: c-miR-935; data are mean ± SD; P<0.05: ‡ difference pre vs post, *difference HiRes vs. LoRes.

5.4.3 Target Prediction of altered c-miRNAs

TargetScan identified 345 predicted targets for c-miR-140, 448 for c-miR-221, 310 for c-miR-223 and 263 for c-miR-935. Of these, the predicted targets that are related to obesity, diabetes, substrate metabolism and physiological adaptation responses to exercise (e.g. mitochondrial biogenesis, muscle hypertrophy) were further classified **Table 5.3**).

Table 5.3. Selected target mRNAs of microRNAs showing altered expression patterns (P<0.05) between high and low responders, pre and post energy-restricted diet and exercise intervention. Target mRNAs have been previously implicated in substrate (fat) metabolism, obesity, diabetes and exercise adaptation responses.

c-miRNA	Sequence (source: mirbase.org)	TargetScan algorithm	Description (source: www.ncbi.nlm.nih.gov)
hsa-mir-140	UGUGUCUCUCUCUGUGUCCUGCCAGU GGUUUUACCCUAUGGUAGGUUACGUC AUGCUGUUCUACCACAGGGUAGAACC ACGGACAGGAUACCGGGGCACC	ARL15	Expressed in insulin-responsive tissues including adipose tissue and skeletal muscle; implicated in insulin signaling and insulin-stimulated glucose transport (Li et al., 2014)
		CROT	Involved in lipid metabolism and fatty acid beta-oxidation
		CRTC3	May induce mitochondrial biogenesis and attenuate catecholamine signaling in adipose tissue
		FNDC5	Gene encodes a secreted protein released from muscle cells during exercise implicated in the development of brown fat
		SOCS7	Proposed to regulating glucose homeostasis (Capuano et al., 2013)
		STRADB	Component of a kinase protein complex regulating energy-generating metabolism
hsa-mir-221	UGAACAUCCAGGUCUGGGGCAUGAAC CUGGCAUACAAUGUAGAUUUCUGUGU UCGUUAGGCAACAGCUACAUUGUCUG CUGGGUUUCAGGCUACCUGGAAACAU GUUCUC	CYP7A1	Gene encodes a member of the cytochrome P450 superfamily of enzymes catalysing the synthesis of cholesterol and other lipids
		PIK3R1	Involved in the metabolic actions of insulin
		SOCS7	Proposed to regulate glucose homeostasis (Capuano et al., 2013)
hsa-mir-223	CCUGGCCUCCUGCAGUGCCACGCUCC GUGUAUUUGACAAGCUGAGUUGGACA CUCCAUGUGGUAGAGUGUCAGUUUGU CAAAUACCCCAAGUGCGGCACAUGCU UACCAG	HDAC4	Involved in histone acetylation and chromatin remodelling with a potential role in GLUT4 transcription (Richter & Hargreaves, 2013)
		IGF1R	Receptor binding insulin-like growth factor responsible for the activation of the IGF-signalling cascade
		PLA2G6	Catalyses the release of fatty acids from phospholipids
		SORBS1	Involved in the signaling and stimulation of insulin

Table 5.3 continued.

hsa-mir-935	GGCGGGGGCGCGGGCGGCAGUG GCGGGACGGCCCCUCGGCCAUC CUCCGUCUGCCCAGUUACCGCU UCCGUACCGCCGCCGCUCCCGCU	HMGB1	Putative involvement in the maintenance of chronic low-grade inflammation associated with increased adiposity (Guzmán-Ruiz et al., 2014)
		MEF2D	Involved in the control of muscle cell differentiation and development
		PHIP	Modulates insulin signaling
		PPARGC1B	Stimulates the activity of several transcription factors and nuclear receptors involved in fat oxidation, non-oxidative glucose metabolism, and the regulation of energy expenditure.

Key: ARL15, ADP-ribosylation factor-like 15; CROT, carnitine O-octanoyltransferase; CRTC3, CREB regulated transcription coactivator 3; CYP7A1, cytochrome P450, family 7, subfamily A, polypeptide 1; FNDC5, fibronectin type III domain containing 5; HDAC4, histone deacetylase 4; HMGB1, high mobility group box 1; IGF1R, insulin-like growth factor 1 receptor; MEF2D, myocyte enhancer factor 2D; PIK3R1, phosphoinositide-3-kinase, regulatory subunit 1 (alpha); PHIP, pleckstrin homology domain interacting protein; PLA2G6, phospholipase A2, group VI (cytosolic, calcium-independent); PPARGC1B, peroxisome proliferator-activated receptor gamma, coactivator 1 beta; SOCS7, suppressor of cytokine signaling 7; SORBS1, sorbin and SH3 domain containing 1; STRADB, STE20-related kinase adaptor beta.

5.4.4 Anthropometric, diet and changes in aerobic fitness

There were no differences in body mass, BMI and fat mass between groups at baseline (**Table 5.1**). The HiRes participants lost 8 kg more body mass (P<0.01) and 7.2 kg more fat mass (P=0.03), than LoRes respectively, with significant group × time interactions (both P<0.01). A corresponding decrease in BMI was also evident for HiRes compared to LoRes following the 16 wk intervention period (P=0.02) where an interaction effect was also observed (P<0.001).

Using the Mifflin St Jeor equation (Frankenfield et al., 2005) for sedentary individuals, the energy requirements of HiRes and LoRes were 2081 ± 352 kcal/d and 2174 ± 202 kcal/d, respectively. Dietary analysis of individual food diaries obtained during the diet intervention showed the reported energy intake was not different between groups (HiRes: 1622 ± 262 kcal/d (n=18) vs. LoRes: 1606 ± 136 kcal/d (n=15), P=0.84). Pre-intervention peak aerobic power (VO$_2$peak) did not differ between groups (HiRes: 2.21 ± 0.68 L/min; LoRes: 2.20 ± 0.56 L/min, P=0.97), and was significantly increased (P<0.001) by the same magnitude in response to exercise training (HiRes: 0.27 ± 0.19 L/min, LoRes: 0.25 ± 0.18 L/min, P=0.70).

5.4.5 Blood parameters

At baseline, fasting blood glucose was higher in HiRes than LoRes (**Table 5.1**, P=0.008), with a significantly greater decrease in blood glucose in HiRes over the 16 wk intervention than LoRes (P<0.001). The area under the curve (AUC) from the OGTT was also greater in HiRes compared with LoRes pre-intervention (**Table 5.1**, P=0.006). The 16 wk intervention resulted in a reduction in glucose AUC (-91 ± 117; P<0.01) across both groups that was significant for HiRes (P<0.001), but not the LoRes group (P=0.07). A group × time interaction effect was observed for insulin concentration (P=0.05). Insulin concentrations

were not different between groups at baseline (HiRes: 8.1 ± 5.4 mIU/mL vs. LoRes: 7.3 ± 6.0 mIU/mL, P=0.60) but decreased significantly over the 16 wk intervention in the HiRes group only (HiRes: -2.9 ± 4.1 mIU/mL, P<0.001; LoRes: -1.2 ± 3.9 mIU/mL; P=0.51). No differences in HOMA index were observed between groups despite a decrease across time (HiRes, Pre: 2.15 ± 1.69, Post: 1.21 ± 0.75; LoRes, Pre: 1.80 ± 1.63, Post: 1.61 ± 1.27 P=0.003) with a significant group × time interaction (P=0.045).

5.4.6 Relationships between c-miRNAs and anthropometric measures

Regression equations were used to show the abundance of several baseline c-miRNA's were weakly associated with pre-intervention body mass including c-miR-221 (P=0.02, R^2=0.15), c-miR-21 (P=0.01, R^2=0.16) and c-miR-126 (P=0.05, R^2=0.10). These weak associations explained less than 20% of the variance between baseline c-miRNA abundance and pre-intervention body mass. There were no relationships between baseline c-miRNA abundance and pre-intervention fat mass, lean mass or BMI, while a weak relationship was found between baseline c-miR-140 and age (P=0.04, R^2=0.11). There were no significant relationships between the change in c-miRNA abundance and the changes in total, fat or lean masses as a result of the 16 wk diet and exercise intervention.

5.5 Discussion

This is the first study to characterise changes in targeted c-miRNAs in a cohort of high and low responders with a 16 wk intervention involving dietary energy restriction and exercise in overweight/obese individuals. The results demonstrate altered expression of c-miRNAs -140, -221, -223 and -935 in human plasma that were associated with divergence in the magnitude of change in body mass. These findings provide a novel profile of c-miRNA that may be related to the beneficial effects of exercise- and/or diet-induced energy restriction for weight loss.

Diet and exercise interventions generate large variability in weight loss responses between individuals that is often under-reported (King et al., 2012). The expression profile of particular c-miRNAs have emerged as promising biomarkers for certain disease states and this raises the possibility their regulation may affect body composition changes and contribute to differences between individual responses. Utilising previous studies that demonstrated robust changes in c-miRNAs following exercise, energy restriction or surgical interventions with putative roles in weight loss, 13 c-miRNAs were identified for analysis (Milagro et al., 2011; Mooren, Viereck, Krüger, & Thum, 2014; Ortega et al., 2013; Sawada et al., 2013). The current study shows increased levels of c-miR-935 in the LoRes compared to HiRes at baseline and increases in c-miR-140, -221 and -223 abundance following a 16 wk weight-loss intervention. The increased expression of c-miR-935 in LoRes compared to the HiRes group (**Figure 5.2D**) supports previous findings of higher c-miR-935 abundance at baseline in a group of non-responders (<5% body weight) to a low energy (800-880 kcal/d) diet-only weight-loss intervention (Milagro et al., 2013). Using the Target Scan algorithm, several predicted targets of c-miR-935 were identified with purported roles in various exercise-mediated adaptations and energy metabolism (

Table 5.3). It is currently unknown if this disparity in baseline c-miR-935 abundance between the HiRes and LoRes groups up- or down-regulates the expression of these targets in a tissue-specific manner (i.e. adipose tissue, skeletal muscle, etc.). Regardless, the findings of the current study support those of Milagro and colleagues (2013) and provide further evidence for c-miR-935 as a biomarker with potential to predict individual weight loss responses.

There was an increase in c-miR-140 abundance in LoRes compared to HiRes after the weight-loss intervention. Increased basal c-miR-140 abundance has been reported in obese/morbidly obese groups and is markedly reduced after bariatric surgery (Ortega et al., 2013) although its expression was unchanged following a diet-only weight loss intervention (Ortega et al., 2013). Previous research has also showed higher c-miR-140 abundance in individuals with a high (BMI $\geq$ 40 kg/m^2) compared to normal (BMI$\leq$ 30 kg/m^2) body mass (Ortega et al., 2013). In contrast, the results of the current study show increased c-miR-140 expression in individuals "resistant" to losing fat mass with an exercise and diet intervention (i.e. low responders). A putative target of c-miR-140 is the muscle integral membrane protein fibronectin type III domain containing 5 (FNDC5), which may play a role in this purported resistance to fat mass loss. The hormone irisin is the proteolytically processed product of the FNDC5 gene secreted by skeletal muscle cells in response to exercise where it can circulate to white adipose tissue and stimulate 'beige' fat development (Boström et al., 2012). Considering beige adipocytes are more metabolically active and induce greater energy expenditure than white adipocytes, it is possible the higher c-miR-140 expression post-intervention in LoRes down regulates FNDC5 activity and subsequently reduces cell metabolic activity and attenuates weight loss.

Recent evidence from overweight and obese humans implicates c-miR-223 as a potential biomarker for obesity (Kilic et al., 2015; Wen, Qiao, & Wang, 2015). Indeed, reduced c-miR-223 levels have been observed in morbidly obese (BMI >40 kg/m^2) and obese (BMI 30-39.9 kg/m^2) individuals compared to normal-overweight individuals (BMI 20-29.9

kg/m^2) (Kilic et al., 2015). Moreover, c-miR-223 abundance has been shown to increase after a 12 wk unstructured dietary and exercise intervention in obese and overweight Chinese participants (Wen et al., 2015). These results indicate that increases in c-miR-223 are associated with diet and exercise interventions that result in loss of body mass. The increases in c-miR-223 with body mass losses corresponds with the current study where increases in c-miR-223 and -221 abundance post-intervention were evident in both HiRes and LoRes groups. Previous reports of c-miR-221 responses to dietary or exercise interventions are equivocal, with one study showing a decrease in c-miR-221 3 d after a bout of resistance exercise (Sawada et al., 2013) while another reported increased c-miR-221 abundance after bariatric surgery weight loss but not dietary-induced weight loss (Ortega et al., 2013). Thus, the increase in c-miR-221 abundance across the 16 wk intervention in LoRes and HiRes (**Figure 5.2B**) indicates these changes may be attributed to the exercise program rather than changes in body mass per se.

Although participants were grouped according to body mass loss, glucose tolerance was also different between groups at baseline, with higher fasting glucose concentration and a greater area under the curve for OGTT in HiRes. It seems plausible the impaired glucose sensitivity at baseline permitted greater metabolic adaptation and weight loss, and that enhanced glucose disposal/glycaemic stability in response to an exercise and diet intervention is a hallmark of HiRes. The c-miR-126 has been implicated as a biomarker for impaired fasting glucose and glucose tolerance (Liu et al., 2014). However, despite the differential OGTT responses at baseline, no differences in c-miR-126 abundance were observed between HiRes and LoRes groups suggesting any potential for c-miR-126 modulation with impaired glucose metabolism may be limited to a pre-diabetic population (Liu et al., 2014). Nonetheless, glucose tolerance may be a determinant of weight loss responder status.

The responses observed in the present study do not appear to be related to alternate adaptive responses to exercise as no differences were observed between the two groups in

aerobic fitness (VO$_2$max) after the 16 wk intervention. Further, only weak correlations were found between baseline body mass, BMI, or fat mass measures and selected c-miRNA abundance in the current study and no significant correlations between changes in anthropometric measures and changes in c-miRNA abundance were observed. These results contrast those of Milagro and colleagues (2013) who showed correlations between several miRNA's measured in peripheral blood mononuclear cells and the magnitude of weight loss (n=10). However, a strength of the current study was the larger participant cohort (n=40) to provide a greater representation of the overweight/obese population. The inclusion of exercise in addition to dietary energy restriction to generate the overall energy deficit may also have contributed to disparity in results between studies.

A limitation of the present work is the lack of concomitant sampling of tissues (i.e. skeletal muscle and adipose tissue) in which c-miRNAs may originate and/or target in order to regulate post-transcriptional processes. The time-course of appearance and disappearance of individual c-miRNAs following an exercise and diet intervention remains to be established and may vary according to the length of intervention and the time at which samples were obtained. Moreover, whether the change in abundance of specific c-miRNA is a cause or consequence of changes in body mass during energy restriction requires further study.

In conclusion, this is the first study to characterise changes of selected c-miRNAs following a combined and structured exercise and diet weight loss intervention. This study provides new information showing the abundance of c-miRNA -221 and -223 are modulated with exercise and diet, and that c-miRNA -935 and 140 are differentially expressed between high and low responders before and after a chronic weight loss intervention, respectively. The results indicate c-miRNAs may have potential as biomarkers of the magnitude of weight loss to an exercise and diet intervention between different individuals. Progression within this field will require *in vitro* mechanistic studies in cell culture and animal knockout models to

validate the cellular targets of c-miRNAs and the conditions (i.e. exercise,diet) under which c-miRNAs regulate cellular mechanisms controlling weight loss.

Preface to Chapter SIX

Although many studies have completed interventions with diet and exercise, few have followed participants after intervention controls have been removed. Whilst this aspect of the study was not funded, it was of interest to potentially try and encourage participants to continue their healthy lifestyle that had required so much work to implement. Thus offering the participants of the clinical trial intervention (*Study 2*) follow-up DXA scans was initially as an incentive to the participants to continue their healthy habits with some form of accountability.

As reviewed in Section 2.5, the literature regarding following up participants after the controlled phase of an intervention provides a check for these individuals as well as efficacy of the education from studies incorporating changing to healthier eating patterns through diet and/or increased physical activity and movement. The ability for participants to maintain a healthier lifestyle post-intervention (dependent on the initial intervention success) can then inform future interventions and form platforms for public health promotion.

Chapter SIX

6 *Study 4*: Maintenance of body composition in the 12 months following a 16 wk diet and exercise intervention in overweight/obese middle-aged adults.

This chapter is comprised of the following manuscript in preparation to be submitted to *Obesity (Silver Spring):*

Parr, E.B., Coffey, V.G., Phillips, S.M., Burke, L.M., Hawley, J.A. Maintenance of body composition in the 12 months following a 16 wk diet and exercise intervention in overweight/obese middle-aged adults.

6.1 Abstract

Objective: To monitor body composition during the 12-month follow-up period after a 16 wk diet and exercise weight loss intervention.

Method: All overweight/obese participants (*n*=89) who completed the intervention (500 kcal/d energy-restriction from diet and exercise) were invited back, after intervention support had been withdrawn, at 3, 6, 9 and 12 months post-intervention for serial measures of body composition (total body, fat and lean masses) using dual-energy x-ray absorptiometry (DXA).

Results: Of the original cohort (*n*=89), 60 participants presented for at least one measurement, with attendance rates 42-68% across the follow-up scans. Twenty-five participants (28%) attended all four scans such that body mass (BM) measured 3 months (3MPo; -1.1 ± 2.0 kg, 95%CI: -3.3 – 1.1 kg) and 12 months post-intervention (12MPo; 1.0 ± 3.5 kg, 95% CI: -3.3 – 1.2 kg) was not different to the end of the intervention (wk 16). The BM change during the first 3-month post-intervention period (wk 16-3MPo) was significantly correlated to that after 12 months (wk 16-12MPo, r^2=0.68, P<0.001).

Conclusion: The modest increase in body and fat mass in the 12 months of free-living conditions following a 16-wk diet and exercise weight loss intervention is a positive finding regarding the interventions effectiveness.

6.2   Introduction

Recent estimates from American data indicate that approximately 50% of individuals who undertake a period of intentional weight loss remain weight stable within the first 12-months post-intervention, but that 75% of these individuals are unable to maintain this weight for five years or longer (McGuire, Wing, & Hill, 1999). This propensity for an initial weight loss followed by the failure to maintain weight losses (typically resulting in weight regain) has been termed "weight cycling" and has been shown to subsequently increase the risk of developing type-2 diabetes (Delahanty et al., 2014) and the incidence of cardiometabolic diseases (Montani, Schutz, & Dulloo, 2015). Many weight loss interventions fail to address the impact that the initial weight loss induced by study participation has on long-term weight status. Accordingly, a better understanding of the factors that enable some individuals to maintain weight loss over the long-term, versus those that are predisposed to weight cycling, is important to prevent the negative effects on changes in body composition on health outcomes (Pourhassan et al., 2013; Santarpia et al., 2013).

To date, in the majority of studies, the weight loss achieved during an experimental intervention is typically the single outcome measure for demonstrating the efficacy of that intervention. However, a critical question is whether this change of weight loss should only be considered meaningful if it can be maintained once participants return to free-living conditions. In this regard, several studies have monitored participants for periods of 1-3 y following clinical weight loss trials lasting 12-16 wk in duration of energy-restricted diets to determine patterns of weight regain (K. M. Beavers et al., 2011; Byrne et al., 2003; Cleanthous, Noakes, Keogh, Mohr, & Clifton, 2007). While the proportion of weight regainers varied across the interventions (from 56 to 68% of participants), and the magnitude of weight change over the follow-up period from baseline also varied substantially (from as low as 4% and up to 50% of participants), in all studies average overall body composition and total mass of the participants were still improved compared to pre-intervention values.

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In a 3-y follow-up after a 12 wk dietitian counselled, energy-restricted diet in 33-68 y old women, 44% had a clinically important ($\geq$5%) loss of body mass (BM) from baseline (Cleanthous et al., 2007). The authors attributed the success in the long-term maintenance of BM losses to the dietary monitoring strategy that was employed. Indeed, it has been demonstrated that individuals who maintain weight loss typically sustain these changes through the continued strategies utilised during the original intervention along with regular weight checks, whilst both maintainers and regainers desired more support during their maintenance (2 y) phase (Reyes et al., 2012). The role of combined diet and exercise education on the capacity to maintain BM after weight loss (i.e. body composition) when participants move from a research environment to free-living conditions in weight loss studies is unclear. Therefore, the primary aim of the present study was to assess the changes in body composition after a 16 wk diet and exercise weight loss intervention during the subsequent 12-month follow-up period without any intervention support. Based on previous research, it was hypothesised that there would be a range of BM changes across participants, with some regressing towards pre-intervention BM (i.e. increasing BM) and others maintaining the large losses (i.e. no change in BM), during the subsequent 12-month follow-up period.

6.3 Methods

6.3.1 Participants

Eighty-nine participants (n=25 M, n=64 F) completed a 16 wk diet and exercise intervention, as described in *Study 2*. Participants included in the study had a BMI range of 27-40 kg/m^2 and were sedentary but otherwise healthy. Briefly, participants were randomly stratified by age, sex and BMI to one of three dietary interventions: high dairy protein, moderate carbohydrate (HDPMC); high dairy protein; high carbohydrate (HDPHC) or low dairy protein, high carbohydrate (control, CON) for the duration of the intervention. The diets were designed to induce an energy reduction of 250 kcal/d below energy balance, based on energy requirements estimated using the Mifflen St Jeor equation (Frankenfield et al., 2005). The dietary intervention was implemented through a progression from daily food checklists (wk 1-6) through to self-selected food choices (wk 7-16) from a points-based system based on the total daily energy intake. Fortnightly meetings with the study dietitian ensured participants' intake and planning was monitored and recorded through the use of weekly food records. Initially, recipes were provided through the use of the Australian Institute of Sport's Survival for the Active Family Cookbook (Text Pacific, Sydney, 2009) where all recipes were converted to the aforementioned points-based system for self-selected planning in the later weeks.

In addition to the dietary energy restriction, participants also undertook daily exercise to induce a further 250 kcal/d energy deficit through 3 d/wk of supervised one-on-one resistance training. On the other 4 d/wk participants completed unsupervised aerobic exercise (e.g. 4 km walk, 16 km cycle or 1 km swimming, or equivalent exercise to induce the desired energy deficit). Primary outcome measures of the intervention were body composition (total, fat and lean masses, and body fat percentage) determined pre (wk 0), mid (wk 8) and post (wk 16) intervention.

Upon completion of the 16-wk intervention, and after the initial post-study measurements had been taken, participants had a final consultation with the study dietitian to discuss their ongoing dietary plan. If requested, participants were given extra copies of the resources used during the study to assist them in continuing their weight loss and/or maintenance. After this final consultation, participants had no further contact with the study dietitian. With regards to exercise training, participants were provided with a free 6-month membership at the RMIT Bundoora gym and were offered a 6-month training program to complete unsupervised. Dietary intakes and exercise training sessions were not recorded and any training through the 12-month follow-up period was performed without supervision.

Participants who completed *Study 2* (*n*=89) were all invited to participate in the collection of follow-up body composition data via DXA scans at 3, 6, 9 and 12 months after completion of the initial 16 wk intervention (**Figure 6.1**). Participants were contacted ~3 wk prior to each of the scheduled 3-month scan dates and volunteers who accepted the invitation reported to RMIT University's Bundoora campus to complete scans. At the 12-month follow-up scan, participants were asked to provide verbal information regarding their exercise pattern during this period, which was noted by the investigator.

6.3.2 *Experimental Procedure and Data Analysis*

The scan protocol, using dual-energy x-ray absorptiometry (DXA), was conducted and analysed as described in Chapter 3. Briefly, participants reported to the laboratory in the morning, after a ≥10 h fast and having refrained from exercise (Nana et al., 2012a, 2013). Participants were asked to void their bladder prior to each scan and wearing the same light, loose fitting clothing. Body mass (BM) was determined at each visit using Wedderburn scales (Tanita BWB-620, Wedderburn, Victoria) with mass recorded to the nearest 0.05 kg. Height was recorded at the first visit (wk 0) using a stadiometer.

Participants were positioned using a standardised protocol on the scanner bed of the GE Lunar Prodigy Pro (GE Healthcare, United Kingdom), as described in *Section 3.2.2*, to

measure total BM, fat mass (FM) and lean mass (LM). Where participants in *Study 2* were larger than the usable area of the scanning bed, two scans of the left and right side were undertaken using the same procedure (described in *Section 3.2.2*). Scan results from *Study 2* time-points of pre-intervention (wk 0) and post-intervention (wk 16) were compared to those measured in the follow-up phase (3, 6, 9 and 12 months post-intervention).

6.3.3 Statistical Analyses

Data were analysed using SPSS version 20.0. Data were pooled across the three intervention dietary groups for analysis. Linear mixed models were used to determine changes across time, accounting for the missing data points, with post-hoc analysis (LSD). Two separate sets of analyses were completed. Firstly, for all participants who attended any scan measurement and secondly, an analysis was conducted for the cohort (n=25) that completed all four measurement points. Linear regression analysis was conducted to determine relationships between changes in BM, FM and LM across different time points. Data are mean ± standard deviation (SD) and significant differences were established when P<0.05.
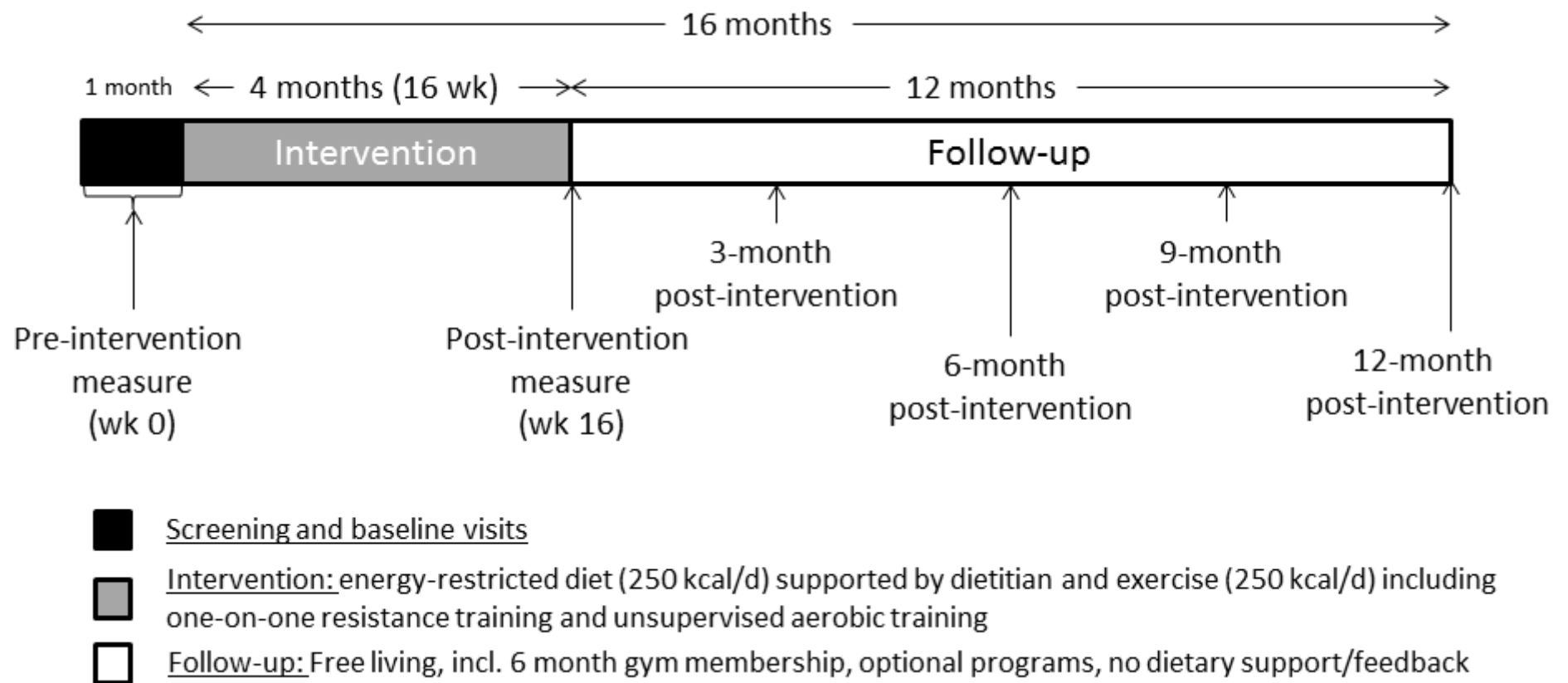
Figure 6.1. Overview of the study design and measurement times for participants in the 12 months following the 16 wk supervised diet and exercise intervention.

6.4 Results

6.4.1 Participant attendance

Of the 89 participants who completed the 16 wk diet and exercise intervention in *Study 2*, 60 participants (67%) reported for a minimum of one scan and 25 participants (28%) undertook all four scans during the 12-month follow-up period (**Table 6.1**).

Table 6.1. Participant attendance matrix at scans during the 12-month study period.

Number of scans	*n*	Subtotal	3MPo	6MPo	9MPo	12MPo
			Time point			
4	25	25	x	x	x	x
3	5		x	x		x
	3		x	x	x	
	5		x		x	x
	1	14		x	x	x
2	2		x			x
	1			x		x
	3		x			x
	3		x	x		
	2	11	x		x	
1	9		x			
	1	10				x

Key: x = attendance; 3MPo, 3 month post-intervention; 6MPo, 6 month post-intervention; 9MPo, 9 month post-intervention; 12MPo, 12 month post-intervention.

Sixty-four percent of *Study 2* participants reported to the laboratory for the 3-month post-intervention (3MPo) scans, 42% reported at 6 months (6MPo), 43% at 9 months (9MPo) and 48% at 12 months post-intervention (12MPo). There were comparable numbers of participants from the three diet allocations represented at each scan (**Table 6.2**).

Table 6.2. Participant attendance (*n*) at scans by dietary allocation.

Diet allocation	wk 0 / wk 16	3MPo	6MPo	9MPo	12MPo
			n		
HDPMC	29	18	14	14	16
HDPHC	32	23	12	11	16
CON	28	16	11	11	11
Total	**89**	**57**	**37**	**36**	**43**

Key: 3MPo, 3 month post-intervention; 6MPo, 6 month post-intervention; 9MPo, 9 month post-intervention; 12MPo, 12 month post-intervention; CON, control diet; HDPHC, high dairy protein high carbohydrate; HDPMC, high dairy protein moderate carbohydrate; wk 0, pre-intervention measure; wk 16, post-intervention measure.

6.4.2 Body mass

During the initial intervention period (wk 0 to wk 16), 88 participants decreased BM (-7.4 ± 3.3 kg) while a single participant increased BM (0.5 kg; **Figure 6.2A**). Across the entire group, BM significantly decreased (-7.3 ± 3.4; P<0.001) and LM was maintained (0.5 ± 1.8 kg, P=0.01), such that FM was significantly decreased (-7.7 ± 3.2 kg; P<0.001).

BM at 3MPo (*n*=57) was significantly lower than at wk 0 (-8.0 ± 3.9 kg; P<0.001; **Figure 6.2B**), but was not different from wk 16 (-0.6 ± 2.1 kg; **Figure 6.3A**). LM was maintained from wk 0 (0.5 ± 2.1 kg; P=0.04) such that no changes in LM were observed between wk 16 and 3MPo. Of these attendees, 89% of participants (*n*=51) maintained or decreased BM (-0.8 ± 1.9 kg) while the other six participants increased BM compared with the wk 16 scan (2.9 ± 0.5 kg).

At the 6MPo and 9MPo time-points, BM was significantly lower than at wk 0 (-8.0 ± 4.4 kg and -6.7 ± 4.7 kg, respectively; P<0.001; **Figure 6.2C** and **D**), represented by significant changes in FM (-8.4 ± 4.5 and -6.8 ± 4.3 kg, respectively) with no change in LM. At each of these time-points, one participant had regained BM such that they were heavier than at wk 0. Compared with wk 16, the 6MPo and 9MPo scans showed 26 and 23 participants (70 and 64%), respectively, had maintained or decreased BM (-1.3 ± 2.3 kg and -

1.1 ± 2.2 kg). Furthermore, 11 and 13 participants increased their BM (6MPo: 3.3 ± 1.0 kg and 9MPo: 5.0 ± 2.4 kg) (**Figure 6.3B** and **C**).

At 12MPo (*n*=43), BM and FM remained significantly different from wk 0 (-5.8 ± 4.9 kg (**Figure 6.2E**) and -5.8 ± 5.0 kg, respectively; P<0.001) with no change in lean mass. The 12MPo scan revealed that 49% of participants had regained >2 kg BM from wk 16, with the mean BM and FM gains among these 21 participants being 5.4 ± 2.2 kg (**Figure 6.3D**) and 5.4 ± 2.6 kg, respectively. Five participants regained mass such that BM at 12MPo exceeded their mass prior to beginning the diet and exercise intervention (1.9 ± 1.1 kg; **Figure 6.2E**). Overall, an increase of ~2 kg BM occurred between scans during the 3 to 12 months study period. At the 12MPo scan, 24 of the 43 participants (56%) reported to be continuing some form of structured exercise training on a regular (≥3 d/wk) basis.
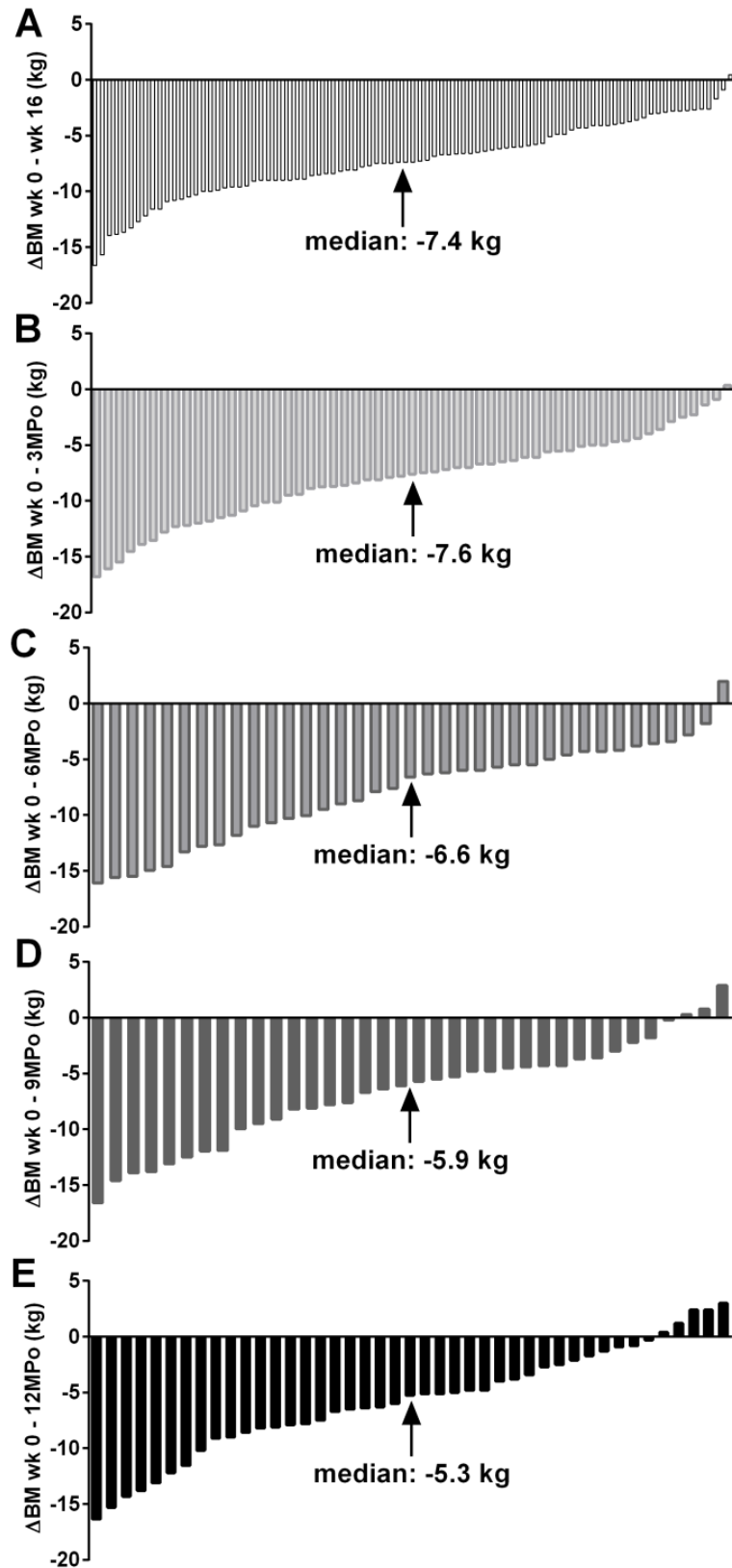
Figure 6.2. Individual change in body mass from baseline to **A**: the end of 16 wk diet and exercise weight loss intervention (wk 16; *n*=89); **B**: 3-months post-intervention (3MPo, *n*=57); **C**: 6-months post-intervention (6MPo, *n*=37); **D**: 9-months post-intervention (9MPo, *n*=36); **E**: 12-months post-intervention (12MPo, *n*=43).
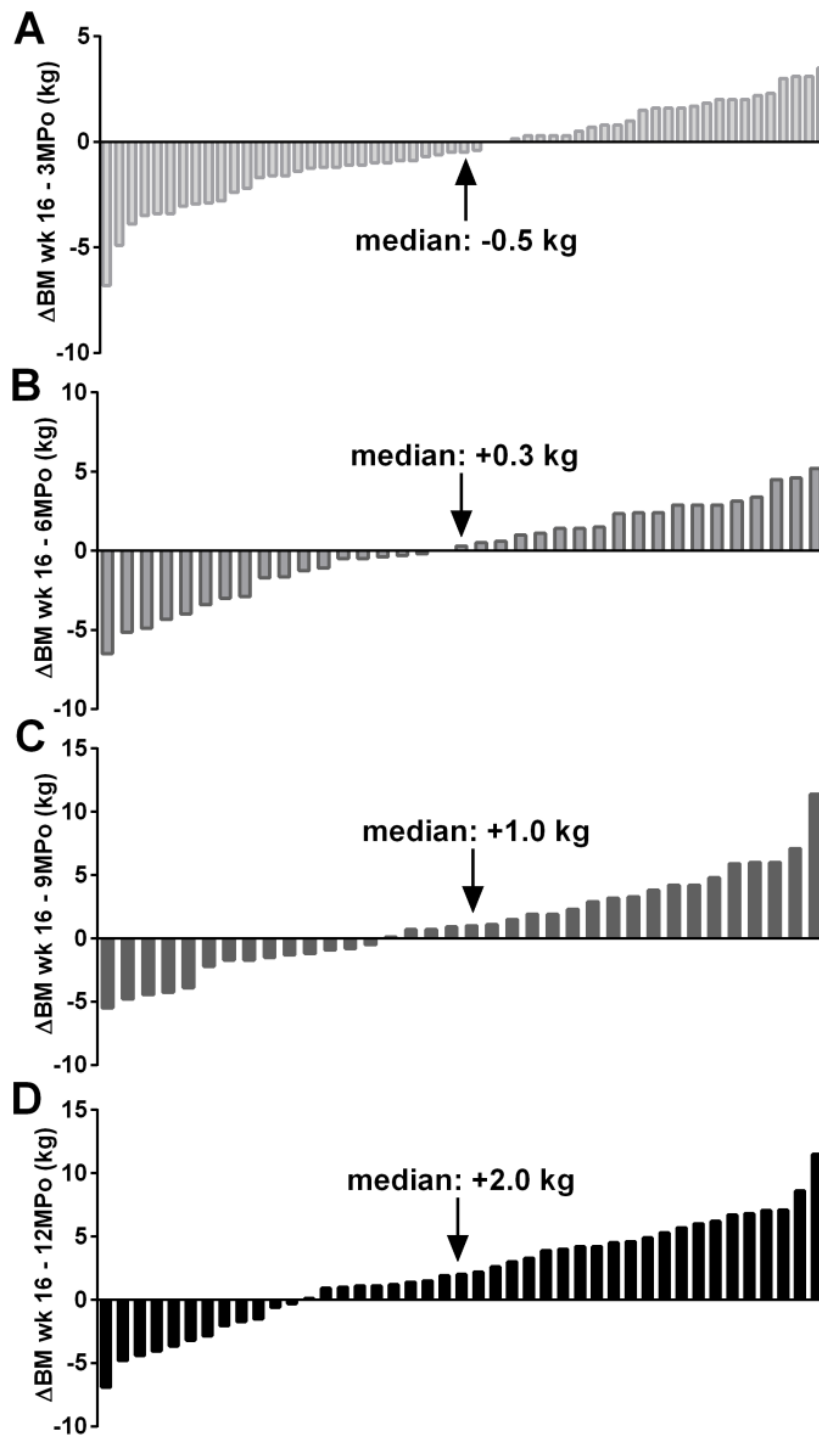
Figure 6.3. Individual change in body mass from the end of the 16 wk diet and exercise intervention (wk 16) to **A**: 3-months post-intervention (3MPo, *n*=57); **C**: 6-months post-intervention (6MPo, *n*=37); **D**: 16 9-months post-intervention (9MPo, *n*=36); **E**: 12-months post-intervention (12MPo, *n*=43).

6.4.3 Subgroup analysis

The BM and composition measures from pre-intervention through to 12MPo were similar for all participants compared to the subgroup of 25 participants who completed all DXA scans (**Figure 6.4**).
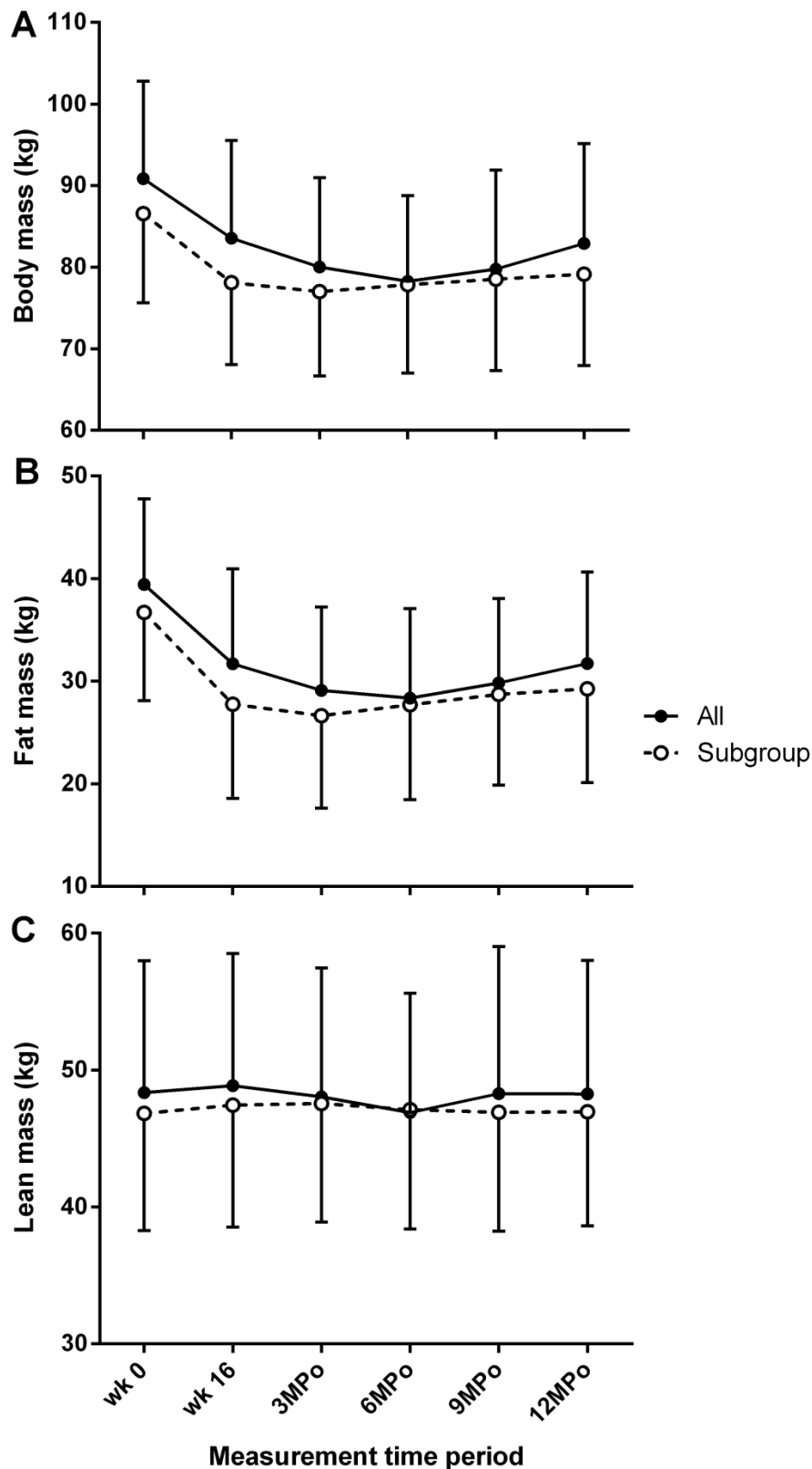
Figure 6.4. Mean (± SD) **A**: total body, **B**: lean and **C**: fat masses during the 16-month total measurement period. Data are for subgroup that completed all scan measurements (dash lines/white symbols; *n*=25) versus all participants that attended (solid lines/symbols;) before and after a 16 wk diet and exercise weight loss intervention (wk 0 and wk 16, *n*=89) and 3 months (3MPo, *n*=58), 6 months (6MPo, *n*=37), 9 months (9MPo, *n*=36) and 12 months (12MPo, *n*=43) post-intervention.

There was a significant effect of time (P<0.001) for the subgroup that completed all scans such that BMI, BM and FM were higher at wk 0 than at any other time point (P<0.01; **Table 6.3**). For this subgroup, BM decreased by -8.5 ± 2.6 kg, (range: -13.3 to -0.9 kg; 95%CI: -14.5 – -2.4 kg, P=0.006) during the 16 wk intervention. There were no differences at any time point in LM (**Table 6.3**). Accordingly, as the changes in total BM directly reflect changes in FM, only changes in total BM mass will be presented as they are more easily compared with the outcomes of other literature in this area.

There was a small but non-significant decrease in BM for the sub-group of n=25 from 16 wk to 3MPo -1.1 ± 2.0 kg (range: -4.90 to +3.5 kg, 95%CI: -3.3 – 1.1 kg, P=0.33; **Table 6.3**). At 6MPo, BM was comparable to 3MPo with large variation between participants (range: -6.5 to +5.2 kg; 95%CI: -2.5 – 1.9 kg, P=0.80; **Table 6.3**). The 9MPo time point showed a small increase of 0.4 kg in BM (range: -5.5 to +6.0 kg; 95%CI: -1.8 – 2.6 kg, P=0.72; **Table 6.3**). At 12MPo, mean BM was not different to 16 wk (+1.0 ± 3.5 kg, 95% CI: -3.3 – 1.2 kg, P=0.36; **Table 6.3**). However, the distribution of BM change across participants was large (range: -6.9 to +7.1 kg) and the greater increase in BM from 16 wk-12MPo compared with 16 wk-3MPo approached significance (95%CI: -0.1 – 4.4 kg, P=0.059; **Table 6.3**). At the 12MPo scan, 19 of the 25 participants (76%) reported to be continuing some form of structured exercise training on a regular (≥3 d/wk) basis.

Correlation analysis showed a significant association between the change in BM in the initial weight loss intervention (wk 0 to wk 16) and BM at the completion of the present study (wk 0 to 12MPo) (r^2=0.40, P<0.01; **Figure 6.5A**). There was also a significant correlation between the change in BM during the first 3 month post-intervention period (wk 16-3MPo) to that after 12 months (wk 16-12MPo, r^2=0.68, P<0.001; **Figure 6.5C**).

Table 6.3. Change (Δ) in BMI and body composition during the 12-month period after a 16 wk diet and exercise weight loss intervention for participants who completed all four measurement points (n=25).

Variable	Pre (wk 0)	Post (wk 16)	12MPo	Δwk 16-0	Δ3MPo – wk 16	Δ6MPo – wk 16	Δ9MPo – wk 16	Δ12MPo – wk 16	Δ12MPo – wk 0
BMI (kg/m^2)	31.5 ± 3.8	28.4 ± 3.4	28.7 ± 3.5	-3.1 ± 1.0[‡]	-0.4 ± 0.7	-0.1 ± 1.2	0.1 ± 1.3	0.3 ± 1.4	-2.8 ± 1.9[‡]
Total mass (kg)	86.8 ± 10.9	78.1 ± 10.1	79.2 ± 11.2	-8.5 ± 2.6[‡]	-1.1 ± 2.0	-0.3 ± 3.1	0.4 ± 3.4	1.0 ± 3.5	-7.4 ± 4.5[‡]
Fat mass (kg)	36.7 ± 8.6	27.8 ± 9.2	29.3 ± 9.2	-9.0 ± 2.5[‡]	-1.1 ± 2.4	-0.1 ± 3.3	1.0 ± 3.4	1.5 ± 3.9	-7.4 ± 4.6[‡]
Lean mass (kg)	46.8 ± 8.5	47.4 ± 8.9	47.0 ± 8.3	0.6 ± 2.0	0.1 ± 1.5	-0.3 ± 1.5	-0.5 ± 1.4	-0.5 ± 1.8	0.1 ± 2.6

BMI, body mass index; 3MPo, 3 month post-intervention; 6MPo, 6 month post-intervention; 9MPo, 9 month post-intervention; 12MPo, 12 month post-intervention; wk 0, baseline pre-intervention measures; wk 16, post-intervention measures.
[‡] Significantly different change, P<0.01. *P=0.059 for Δ3MPo-wk16 vs. Δ12MPo-wk16.
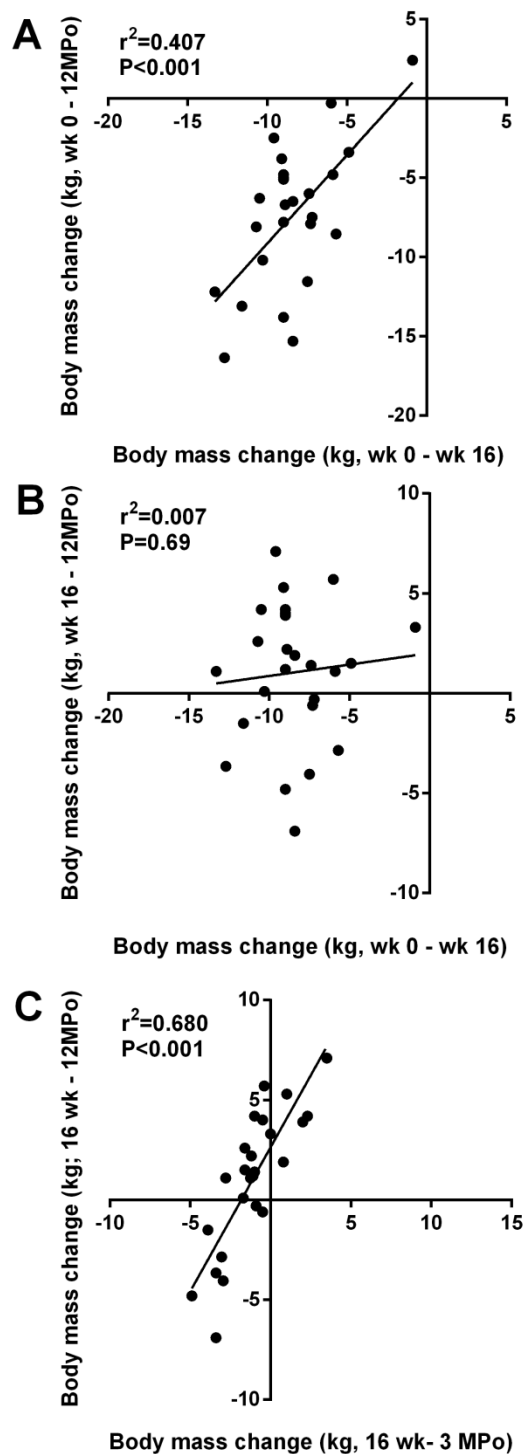
Figure 6.5. Linear correlations of participants attending follow-up scans until 12 months post-intervention (*n*=25) for **A**: the body mass change from baseline to post intervention (x-axis) against the body mass change from baseline to 1 y follow-up, inclusive (y-axis); **B**: the body mass change from baseline to post intervention (x-axis) against the body pass change in the follow-up period (wk 16 – 12MPo; y-axis); and **C**: the follow-up changes from post-intervention (wk 16) to the 3 month time point (x-axis) against the body mass change in the follow-up period (wk 16 – 12MPo; y-axis).

6.5   Discussion

The main finding of the present study, based on observations of a population representing half of the subjects enrolled in a previously conducted clinical trial, was that increases in BM and FM occur when individuals return to free-living conditions after a 16 wk supervised diet and exercise intervention. Results show that in the 12-month follow-up period, there was a small increase in FM, together with the maintenance of LM, which resulted in favourable improvements in body composition from baseline. However the average results across the participant group hides the individual responses, where some attendees were tracking towards their pre-intervention BM and FM and a select group (*n*=5) had increased BM greater than their pre-intervention measure, both by 12-months post-intervention (**Figure 6.2E**). In addition, the BM changes (i.e. increase) during the initial 3-month period of free-living conditions may have some predictive utility of longer-term body composition outcomes following a weight loss intervention. Although these results are only indicative of the participant group who returned for measurements, there is the possibility that they could be exacerbated if the rest of the cohort attended and presented with similar or greater regressions of BM towards baseline.

The body composition maintenance found in these follow-up measures is in contrast to the results of previous studies that have shown a greater magnitude of FM accrual with modest changes in LM during periods of weight regain (Byrne et al., 2003; K. M. Beavers et al., 2011). Beavers et al., (2011) examined body composition 1-y after a 5-month diet-only weight loss intervention in postmenopausal women. In that study (K. M. Beavers et al., 2011), 72.5% of participants were defined as weight regainers (>2 kg increase in BM), whereas 27.5% were identified as maintainers (D. P. Beavers et al., 2013). Significant inter-individual variability in theregain of BM was observed across participants with the largest proportion of weight regained being fat tissue (K. M. Beavers et al., 2011). While Byrne and colleagues (2003) did not investigate individual differences in their 1-y follow-up of women after a ~20

wk diet-induced weight loss intervention, they did report the magnitude of weight regain to be 44-50% of the original weight lost (~6 kg) and similarly the majority of the mass regain was FM. In the current study, ~50% of participants regained >2 kg of BM with a large variation observed between individuals (**Figure 6.3E**).

The mean BM regained by participants in the current study was the consequence of alterations in FM, whereas in the studies that have employed diet-only interventions some LM was also regained (K. M. Beavers et al., 2011; Byrne et al., 2003). The disparity in the type of tissue regained between the current and previous investigations is likely due to the change in LM observed within the intervention periods. In the present study, LM was maintained across the entire intervention period, whereas between 10 to 30% of weight loss was LM in the aforementioned studies (K. M. Beavers et al., 2011; Byrne et al., 2003). Consequently, due to the large FM losses, body composition was improved in all interventions at the follow-up periods. However, D Beavers et al. (2013) reported that when weight loss was not maintained there was a significant increase in cardiometabolic risk, as determined by increased total cholesterol, triglyceride, glucose and insulin concentrations that had previously improved during the intervention period. The current study shows LM can be maintained in a pre-sarcopenic cohort in the 12 months after a short-term (16 wk) exercise and diet intervention.

The addition of resistance exercise training to an energy-restricted weight loss programme prevents the loss of LM but also improves the quality of skeletal muscle and metabolic health (Hunter et al., 2008). The possibility exists that the improved morphology and metabolic status of some individuals' skeletal muscle promoted the maintenance of BM during the follow-up period. Despite an increase in FM from 3- to 12-months follow-up, body composition after 12 months free-living was improved (i.e. decreased FM and maintenance of LM) compared to before the initial intervention. It seems plausible to suggest that the general maintenance of an improved body composition may be attributed to the education and

improved behaviours (i.e. exercise and dietary habits) gained throughout the intervention period. Indeed, in the subgroup of participants who completed all four scan measures compared to all of those who attended the 12 month scan, a greater proportion (76% vs. 56%) reported completing regular ($\geq$3 d/wk) structured exercise.

In line with the results of previous studies (K. M. Beavers et al., 2011; King et al., 2009), there was significant variation across the participant group for the changes in BM during both the 16 wk intervention and 12 month follow-up period in the present investigation. Therefore, the body composition outcome throughout the follow-up period is likely to be related to an individual's choice to continue with some or all of the lifestyle changes required during the study intervention (i.e. regular exercise, dietary intake awareness of energy and macronutrient intake) despite no supervision or ongoing professional support during this period.

In the present study, a strong association was found between the BM change in the first 3-months post-intervention and the 12-month follow-up period. Those individuals who had lost BM between the post-intervention (16 wk) and the 3MPo follow-up scan were more likely to maintain that loss or at least have a small ($\leq$2 kg) mass regain. Conversely, individuals who had a greater increase of BM and FM during the early post-intervention period were less likely to maintain BM long-term. In this regard, it may be possible to predict an individual's capacity for maintenance of BM or likelihood for weight regain in the longer-term during the initial months and could provide a "window of opportunity" for lifestyle correction through reinstating professional support. Regardless, maintenance of BM or composition in the follow-up period for the majority of participants (*n*=24) indicates a sustained effect of the 16 wk diet and exercise intervention, especially when the BM changes (i.e. losses) during the intervention were substantial (>5% BM). A limitation of the present study was an inability to quantify any change in dietary/exercise knowledge and an

understanding of energy requirements at the conclusion of the intervention or during the 12-month post-intervention period.

As the sub-group of participants (n=25) in the current study were willing to complete all scans, it could be argued that they are more likely to comply with healthy lifestyle practices after study completion as they were prepared (high intrinsic motivation) to maintain their changes in lifestyle (Teixeira et al., 2006). Therefore, it is plausible to have a greater proportion of weight losers or maintainers in the follow-up phase than in a randomly selected cohort and these participants may be more likely to adhere to a change in lifestyle. The likelihood of increased adherence to changes towards a healthier lifestyle is also supported by the high proportion of participants in this cohort who continued to undertake structured exercise. Consequently, extensive monitoring and education during the first 3-months post-intervention period may provide greater insight on the predictive utility of long-term weight stability when returning to free-living conditions.

The apparent lack of relationship between the loss of BM within the intervention and the change in BM during the follow-up period (wk 16 to 12MPo) is in contrast with the results of Bosy-Westphal and colleagues (2013) who reported greater regain of BM post-intervention with increasing loss of BM during the intervention. Over half of the participants who attended the 12-month scan in the current study either lost or maintained (±2 kg of post-intervention) BM throughout the 12-month follow-up period. This proportion of 'losers' or 'maintainers' in the present study is greater than has been previously reported (McGuire et al., 1999). However, both the small sample size (n=25) and the nature of the data collection (post-intervention) are limited compared with the larger subject cohort (n=500) and the retrospective interview nature of the data collection reported by McGuire et al., (1999).

In summary, there were small increases in body and fat mass in the 12-months following a 16-wk diet and exercise weight loss intervention. Whilst participants received extensive exercise and dietary education and support during the 16-wk intervention, the small

change in mass when participants returned to free-living conditions is a positive finding with regard to the interventions effectiveness. Although body composition status at 3-months follow-up was moderately predictive of changes in body mass at 12 months, there was also substantial variation in body composition changes between individuals. Therefore, it seems prudent to recommend extensive monitoring and continuing education in the initial 3-months after a supervised intervention to assist in long-term weight stability as well as future studies incorporating the presentation of individual variation throughout periods of follow-up. A variety of variables measured in the follow-up period, in addition to body composition, would advance the understanding of the efficacy of scientific interventions when participants return to free-living conditions.

Chapter SEVEN

7 Summary and future directions

The primary aim of this thesis was to investigate the effect of practical diet and exercise programs on acute and long-term changes in body composition in overweight/obese adults, including variability in responses to different macronutrient combinations within the energy restricted diets as well as the evidence for individual responses. This body of work focussed on identifying appropriate strategies to improve the body composition and health status of overweight/obese individuals, particularly by reducing body fat while maintaining muscle (lean) mass. Such findings could play an important role in reducing the burden of obesity and obesity-related disease treatment, which in Australia is estimated to cost $21 billion, annually.

The first step of this program of research was to establish the precision of a DXA protocol for measuring body composition under the specific conditions of the study. Therefore, *Study 1* was performed on a group of individuals with characteristics similar to the study population at baseline (obese/overweight) and as a result of the intervention (normal to overweight body composition). A standardised protocol of participant positioning was implemented which, together with the involvement of a single machine (GE Lunar Prodigy) and a sole technician, served to minimise the expected technical error of measurement. The biological error (i.e. day-to-day variability caused by changes within the participant) was minimised by having participants present according to a protocol that standardised factors known to influence gut contents and body water. This protocol has previously been shown to optimise the precision of DXA estimates of body composition in exercising populations. Although the completion of the reliability study added extra work to this body of research, it was considered important to establish the specific precision in measuring body composition and to provide confidence that meaningful changes could be detected in subsequent work. It is noted that many other intervention studies have failed to undertake such a preparation and

although it requires additional measures to be added to already large studies, it is imperative that reliability investigations are included in any clinical trial outcome to understand the minimum real change measured.

Following the establishment of the reliability of the specific DXA protocol for body composition measurement, a program for weight loss and body composition change was designed (*Study 2*, Chapter 4). Despite the high global prevalence of overweight and obesity, many studies typically place emphasis on losing weight per se and fail to recognise the separate but inter-related problems associated with the accelerated loss of lean (muscle) in ageing (i.e. greater than 60 years of age). The current program aimed to improve the quality and quantity of lean mass concomitantly with reductions in fat mass; thus improving the health of middle-aged adults and preserving function and mobility as they enter the later stages of life.

Meta-analyses have found there are increased losses of body and fat masses when dairy foods are included in energy-restricted diets. Therefore, based on the scientific evidence that increased protein intake maintains lean mass and accelerates fat mass losses when a diet is energy-restricted, *Study 2* determined the effectiveness of dairy foods to increase protein intake within altered macronutrient (fat/carbohydrate) ratios on changes in body composition and selected health parameters. The first hypothesis of this study was that increased protein intake through dairy foods within a lower carbohydrate, energy-restricted diet when combined with an appropriate exercise stimulus would maintain lean mass and accentuate fat mass losses. The second hypothesis of the study was that the higher protein intake through dairy foods within energy-restricted diets would maintain lean mass compared with the recommended daily intake of protein.

Both of the original research hypotheses were refuted. Against a background of mild energy restriction and an exercise program (3 d/wk resistance and 4 d/wk aerobic training), similar fat mass losses and retention of lean mass were observed regardless of the increased

intake of (dairy) protein and its pairing with either a reduction in fat or carbohydrate intake. Additionally, independent of dietary protein manipulation, the previously sedentary, overweight/obese middle-aged adults successfully improved their aerobic capacity, increased maximal strength, improved glucose sensitivity, reduced insulin concentrations, as well as improved body composition through the energy deficit from the combined energy-restricted diet and exercise intervention in all protocols tested.

The design of the clinical intervention made it incapable of distinguishing how the chosen diets would have acted independently of an exercise stimulus. However, the results of this study suggest that the dietary energy deficit acts independent of macronutrient composition to induce losses in body fat. The macronutrient composition between diets was significantly different (40% carbohydrate and 30% fat vs. 50% carbohydrate and 20% fat) and such dietary intakes represent "real world" intakes and use of commercially available foods. For example, participants did not need to rely on specific supplements and could make substitutions and meal plans, under the guidance of the study dietitian. Thus, the innovative dietary protocol ensured that individuals could fit within familial and social situations that involve food choices. While the participants were able to fit their menu plans and meal choices within their normal living situations, not all participants enjoyed the changes to their diets. Furthermore, with regard to the daily exercise requirement, many commented that their adherence was underpinned by their knowledge that they would only continue to receive support while their exercise participation was maintained.

The innovative nutrition and training program adds a novel contribution to the treatment of obesity and supports the recommendation that individuals trying to induce weight loss through dietary means should include an appropriate exercise stimulus. Specifically, large losses of fat mass were induced with mild energy restriction (-250 kcal/d) and daily exercise training. Additionally, resistance-based exercise and aerobic training assisted in the maintenance of daily energy expenditure and the retention of lean mass. The finding of lean

mass retention is atypical in the literature of energy restriction through diet-only or diet and aerobic exercise studies. The combination of resistance training of 30-45 min on 3 d/wk and aerobic exercise (mainly walking) for ~45 min on 4 d/wk synergistically acted to maintain lean mass and create an energy deficit to induce losses of fat mass. Therefore, such an approach is integral in the preservation of age-related losses of skeletal muscle mass. The majority of the participants did not understand the significance of the resistance-based exercise prior to study entry, and those who understood the basis for its inclusion based on skeletal muscle and metabolic health seemed to be those that continued this type of exercise. However, for many, the removal of the one-on-one support meant the end of their resistance-training regime. Thus, while this study confirms the recommendation for overweight and obese adults to consume healthy proportions of macronutrients (i.e. especially protein) within a mildly energy-restricted diet, the proviso of maintaining sufficient exercise including resistance exercise appears to be less well appreciated, since it seemed that many individuals would rather try to manipulate their diet than continue to exercise.

Although lacking in evidence-base, popular media currently promote unrealistic energy-restricted diets intakes that are highly skewed in macronutrient composition (i.e. very low carbohydrate, ketogenic diets). These diets are marketed with the appeal of producing rapid, positive results in terms of total weight loss rather than body composition changes. In part, such "sensationalism" can be traced to some well-known scientists although there are few valid scientific data to support the testimonials of large, sustainable weight losses attributed to such diets. Furthermore, whether these extreme dietary conditions would further interfere with the compliance to a beneficial resistance and aerobic stimulus is unknown and warrants scientific investigation.

It could be argued that energy restriction for periods longer than 16 weeks may be hard to maintain affecting adherence and therefore body composition outcomes. Developing and testing intermittent energy restricted dietary programs with periods of energy balance

compared with a block of dietary restriction, with continuing exercise training, would be of interest through quantifying the magnitude of fat losses and retention of body composition changes. Furthermore, a quantification of resting metabolic rate before and after such an intermittent energy restriction period would be a key measure to take to either support or refute the use of such technique towards a long-term strategy for body composition maintenance. There is the potential that intermittent energy restriction would provide increased perturbations to energy metabolism to increase fat losses whilst maintaining resting metabolic rate and therefore may provide a more long-term solution to encourage the retention of body composition changes.

Overall, the intervention in *Study 2* was successful in improving body composition and other health parameters across the participant group. However, the quantity of body mass lost varied substantially within the cohort, as represented by different changes in fat rather than lean masses. An epigenetic approach was considered to explain some of the differences across participants, as those who lost the greatest amounts of body and fat masses were not those who started with the largest total mass, nor were they individuals who exercised more or induced greater energy-restriction through their diet. Thus, in an attempt to determine some of the mechanistic factors contributing to this variation, the expression of circulating microRNAs (c-miRs) were examined in the plasma samples of participants who lost the most body mass (deemed high responders) compared to those who lost the least body mass through the 16-wk intervention (deemed low responders). Therefore, the third study (Chapter 5) determined the differential responses of individuals to a diet and exercise body mass loss intervention through epigenetic (c-miR) analyses.

The select c-miRs investigated were chosen based on previous findings from studies of diet or exercise interventions. It was hypothesised that an altered expression of c-miRs between high and low responders would be identified. Indeed, the altered expression of four of 13 targeted c-miRs was measured; two were differentially expressed between high and low

responders and two were differentially expressed pre- to post-intervention. The algorithm program (TargetScan) predicted targets of the differentially expressed-miRs that have purported roles in insulin signalling, glucose regulation as well as lipid and fatty acid oxidation. As muscle and adipose tissue were not sampled, future research projects should sample skeletal muscle and adipose tissue concomitantly with plasma to investigate these predictive targets whilst simultaneously measuring circulating miRNAs in the plasma.

The field of epigenetic analysis using c-miRs is relatively new and holds exciting promise to advance the management of disease through individual sample testing. Specifically, epigenetic analyses may have the ability to predict how an individual will respond to an intervention and pave the way for personalised obesity "treatment". For example, obese individuals may be screened prior to starting a diet and exercise intervention based on their c-miR profile. This screening process may be able to be completed using pre and post intervention samples post-hoc across many studies to further flesh out the larger scale effects that c-miRs may be able to indicate. However, this innovation brings with it many negative connotations that will not be handled by the scientists who discover them but by the health professionals on the ground *per se*. Until the linking literature is proficient in illustrating the cause and effect of these epigenetic measures from measures of the effected bodily tissues, the application of these results in a wider context should be used with caution.

In the fourth study (Chapter 6), the same large cohort of participants in the 16 wk diet and exercise weight loss intervention were followed for an additional 12-month period to test the maintenance of improved body composition. The impetus for these measurements was to provide a method of support to the *Study 2* participants that was in the form of measureable feedback during this follow-up period and could be used as a method of encouragement whilst being largely "hands-off". The follow-up period, during which participants are free-living, can also be used to provide insight into the long-term efficacy of the sustainability of the outcomes of an intervention. As weight cycling (where body mass is cycled through periods

of regain and losses) has been shown to increase the incidence of type-2 diabetes and cardiovascular disease, preventing periods of weight cycling has the potential to enhance the long-term health of many individuals. Indeed, weight cycling may be worse than the obese state of the individual due to the additional stress of the fluctuations of cardiovascular risk measures. Weight cycling also has the potential to worsen body composition through lean mass losses followed by an inability to regain back sufficient quantities of the metabolically important lean tissue, especially with aging. Therefore, examining the efficacy of interventions that would minimise fat mass regain and preserve lean mass are integral for long-term preventative health care.

In *Study 4* it was hypothesised that there would be a range of body mass responses across participants, with some regressing towards pre-intervention body mass and others maintaining the large losses, during the periodic (3-monthly) measures throughout the subsequent 12-month follow-up period to the 16-wk intervention. Throughout the 16-wk intervention, participants received intensive dietary advice and support, along with supervised exercise training. Although, a 6-month gym membership with optional programs was supplied to participants post-intervention, no further support was provided by the research team during the 12-month follow-up period.

Of all participants who returned 12-months post-intervention (*n*=43), an insignificant amount of body mass (+2 kg) was regained, although 49% of those who attended (*n*=21) gained >2 kg. The regain in body mass was due to the regain of fat mass with little change in lean mass over the follow-up period. However, due to the limited retention of participants it is unknown whether this is representative of the entire trial population. A quarter of the participants from *Study 2* (*n*=25, 28%) returned for all four follow-up measures, at 3, 6, 9 and 12-months post-intervention period. The results from this subgroup analysis showed an insignificant amount of fat mass (+1 kg) was regained throughout this period compared with the large fat mass loss (-8.5 kg) throughout the 16-wk intervention. Again, in this subgroup,

these changes were indicative of fat mass changes as lean mass did not change throughout the follow-up period. Further, the 3-month measurement point was largely predictive (~60%) of the 12-month post-intervention body mass outcome. Collectively, these results have shown the 16 week rigorous dietary and exercise intervention was effective for preventing significant weight regain in some but not all of participants through the 12-month period post-intervention. As highlighted by *Study 3*, individual differences are often hidden within group means and this was certainly the case across the results from *Study 4,* whereby some individuals improved by increasing fat mass losses over that already achieved during the intervention and some regressed to body masses measuring greater than at study entry.

The results of *Study 4* raise several points of discussion. Although participants did not weight cycle during the 12-month follow-up, it is unknown whether weight cycling (i.e. further loss then subsequent regain of body mass) occurred beyond the follow-up period. Further, extensive monitoring and continuing education in the initial 3-months after a supervised intervention may be required to assist with long-term stability of body composition changes. How this extra monitoring would occur in the real world poses a practical conundrum. Collectively, the result of these studies suggests having a support network consisting of personal trainers and dietitians may be required, which is simply unrealistic for the everyday person. Future studies integrating continual education and support are required to determine the long-term effectiveness of interventions on maintaining body composition changes as well as to ascertain the level of support that may be required, and over what length of time, in order to establish the best-case outcomes for the majority of the population. Specifically, if the proposed intermittent energy-restriction with continual exercise (as suggested under *Study 2*) were to proceed, a key factor to be considered would be the long-term efficacy of the changes observed to body composition. Practically, the long term follow up of participants after an intervention is difficult, and it can be argued that the collection of

that information could influence the participant's outcomes. However, the information gathered from a long term follow up would be invaluable in terms of health outcomes.

The data collected in *Study 4* only related to body composition, as participants' dietary records and exercise habits were not measured during the follow-up period. Further, the retention rate of participants in the follow-up period was lower than in other studies, which may have biased the results towards the more successful participants. Thus, it is likely that the less successful participants, who opted not to present for follow-up scans, are those who lacked successful weight management or who require more support after the removal of the intervention controls. A better design of follow-up measurements to target these less successful individuals, through extensive monitoring and continuing education in the initial 3-months after a supervised intervention, is a starting point for future research in this area.

Principally, it seems that two different groups of participants volunteered for this study. The first group were those individuals who came into the study willing to make long-term changes, learn from the information provided by the world-renowned team of dietitians and be able to take the skills from the program forward. These individuals make up the positive results from the follow-up measures (*Study 4*), and are the minority of the participant group. The second group were the majority of the participant cohort who required the support and encouragement provided by the research team to attend and complete the necessary requirements and fell by the wayside when the study ended. These "unsuccessful" individuals were not necessarily those who did not lose the most weight, although undeniably some were, but those that did not have the willingness to make the positive changes regarding reducing energy intake and adding in regular, daily exercise with a resistance component. Overlaying the varying nature of participant willingness to such a large-scale clinical trial increases the applicability of the data to the real world, but makes studies like this incredibly hard to run. Furthermore, with the genetic inferences of the resultant c-miR analysis, and therefore the potential for the promotion of individualised treatments, the likelihood of such large-scale

real-world labour-intensive studies, such as that conducted for this thesis, occurring again is limited.

Whilst the overwhelming take-home message from this body of work is that supervision is required to enhance the positive responses to the interventions conducted, this is a dire recommendation for the health care system that is already struggling to treat the larger individuals that are now more prevalent in today's society. The consequential increased reliance on health professionals is going to create a society dependent on the distribution, frequency and appropriateness of advice. The specificity that individuals may soon require in terms of treatment variations, due to genetic and epigenetic factors continually being discovered, is exciting but is going to further exacerbate stresses on the health care system if individuals do not take responsibility for their food and movement patterns.

It may be considered that the work of this thesis has simply reinforced the notions of diet and exercise being integral for improving health outcomes. However, the improved body composition with large intakes of dairy foods while concomitantly undertaking exercise training, the epigenetic quantification of different responses by groups of individuals to the same intervention and the positive maintenance of body composition changes in the period post intervention have all shown that people can make positive changes provided adequate support systems are in place. The challenge for future researchers is to find the appropriate amount of support that needs to be given to an individual in order for them to reach and maintain a healthy body composition and lead a healthier life in the face of negative (i.e. marketing and mixed messages) and societal (i.e. work-family balance) pressures.

In conclusion, the current body of work investigated the effects of a diet and exercise intervention on changes in body composition, and selected health parameters, from four different perspectives:

1. Established the reliability of a standardised protocol encompassing biological and technical variability of DXA for the analysis of body composition. Such work should be

encouraged to be completed within all settings where different operators, machines or procedures occur.

2. Demonstrating the effectiveness of a combined diet and exercise intervention to facilitate healthy weight loss within a short-term (16 wk) intervention period. These results strengthen the evidence base for the inclusion of both aerobic and resistance training stimuli to induce robust losses of fat mass whilst maintaining lean mass when dietary intakes are energy-restricted.

3. Epigenetic responses of individuals to such interventions were found to differ between individuals; some to the intervention itself and some related to the different magnitude of weight loss success. Such responses may be used in the future to predict individual outcomes in weight loss studies and interventions.

4. The efficacy of a diet and exercise intervention through follow-up for a 12-month post-intervention period found that only a small amount of weight regain was measured. This demonstrates that, at least in the individuals willing to be re-scanned, the exercise and dietary intervention had the capacity to be effective in making sustainable, improvements to body composition.

Collectively, the findings from these studies provide insight into the measurement, improvement, individual diversity and maintenance of body composition through diet and exercise strategies that can improve health and fitness outcomes for obese individuals.

Chapter EIGHT

8   List of References

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Chapter NINE

9   Research Portfolio Appendix

a)   Publications and Statement of Contribution of others

1.   **Parr, E.B**., Coffey, V.G., Hawley, J.A. 'Sarcobesity': a metabolic conundrum, *Maturitas*, 2013, 74(2):109-113.

Contribution statement: EBP was primarily responsible for the literature search and summary, and the first draft of the review. VGC was involved in summarising, drafting and writing the manuscript. JAH was involved in the initial concept, manuscript preparation and final proofing.

Approximate percentage contributions: E. B. Parr 65%; V.G. Coffey 20%; J. A. Hawley 15%.

I acknowledge that my contribution to the above paper is 65%.

E.B. Parr: Date: 27th October 2015

As principal supervisor of this project, I certify that the above contributions are true and correct:

J.A. Hawley: Date: 27th October 2015

2. **Parr, E.B**., Coffey, V.G., Cato, L.E., Phillips, S.M., Burke, L.M., Hawley, J.A. A randomised trial of high dairy protein, variable carbohydrate diets and exercise training on changes in body composition

Submitted to *Obesity (Silver Spring)* on 25th of September 2015.

Contribution statement: EBP was primarily responsible for obtaining ethical approval, data collection, data and statistical analyses, writing and submitting the manuscript. VGC was involved in grant submission, data collection, data analysis and reviewing the manuscript. LEC was involved in dietary design and implementation. SMP was involved in grant submission and reviewing the manuscript. LMB was involved in grant submission, dietary design and revision of the manuscript. JAH was involved in grant submission, data analysis and reviewing the manuscript.

Approximate percentage contributions: E. B. Parr 65%; V.G. Coffey 10%; L.E. Cato 3%; S.M. Phillips 2%; L.M. Burke 10%; J. A. Hawley 10%.

I acknowledge that my contribution to the above paper is 65%.

E.B. Parr: Date: 27th October 2015

As principal supervisor of this project, I certify that the above contributions are true and correct:

J.A. Hawley: Date: 27th October 2015

3. **Parr, E.B**., Camera, D.M., Coffey, V.G., Phillips, S.M., Burke, L.M., Hawley, J.A.

   Modulation of circulating microRNAs between 'high' and 'low' responders following a 16-wk diet and exercise weight loss intervention

   In preparation, to be submitted to *PLoS One*.

Contribution statement: EBP was primarily responsible for study design, sample collection, analysis and writing the manuscript. DMC was involved in study design, sample analysis, statistical analysis and writing the manuscript. VGC was involved in the initial grant submission, data and statistical analysis and final edits of the manuscript. SMP and LMB were involved in the initial grant submission and final edits of the manuscript. JAH was involved in the initial grant submission, study design, data analysis and writing the manuscript.

Approximate percentage contributions: E. B. Parr 65%; D.M. Camera 10%; V.G. Coffey 10%; S.M. Phillips 2%; L.M. Burke 3%; J. A. Hawley 10%.

I acknowledge that my contribution to the above paper is 65%.

E.B. Parr: Date: 27th October 2015

As principal supervisor of this project, I certify that the above contributions are true and correct:

J.A. Hawley: Date: 27th October 2015

b) Additional Publications

4. **Parr, E.B**., Camera, D.M., Areta, J.L., Burke, L.M., Phillips, S.M., Hawley, J.A., Coffey, V.G. Alcohol ingestion impairs maximal post-exercise rates of myofibrillar protein synthesis following a single bout of concurrent training, *PLoS One*, 2014, 9(2):e88384. DOI: 10.1371/journal.pone.0088384

Accepted 6 January 2014; Published 12 February 2014;

Contribution statement: EBP was primarily responsible for gaining obtaining approval, data collection, data and statistical analyses, writing and submitting the manuscript. DMC was involved in data collection, analysis of the muscle tissue samples, data and statistical analyses, and writing the manuscript. JLA assisted with trial completion and the analysis of the muscle tissue samples. LMB was involved in study design and writing the manuscript. SMP was involved in the study design, the analysis of muscle tissue for FSR and amino acid concentrations and final edits of the manuscript. JAH was involved in the study design, data analysis and writing the manuscript. VGC was involved in study design, data collection, data and statistical analysis, and writing the manuscript.

Approximate percentage contributions: E. B. Parr 65%; D.M Camera 5%, J.L. Areta 5%, L.M. Burke 5%, S.M. Phillips 5%, J. A. Hawley 5%, V.G. Coffey 10%.

I acknowledge that my contribution to the above paper is 60%.

E.B. Parr: Date: 27th October 2015

As principal supervisor of this project, I certify that the above contributions are true and correct:

J.A. Hawley: Date: 27th October 2015

c) Conference Presentations

1. **Parr, E.B.,** Camera, D.M., Areta, J.L., Burke, L.M., Phillips, S.M., Hawley, J.A., Coffey, V.G. Alcohol consumption after a bout of concurrent training impairs rates of muscle protein synthesis, *Sports Dietitians Australia National Conference*, Melbourne, October 18th, 2013. [Oral presentation]

Contribution Statement: This presentation was based on the work from Publication #4 (see above for author contributions). The presentation was designed and delivered by EBP. DMC, JLA, JAH and VGC reviewed the presentation and provided feedback.

2. **Parr, E.B.,** Camera, D.M., Areta, J.L., Burke, L.M., Phillips, S.M., Hawley, J.A., Coffey, V.G. Alcohol consumption after a bout of concurrent training impairs rates of muscle protein synthesis, *Sports and Exercise Science New Zealand Conference*, Christchurch, November 29th, 2013. [Oral presentation; **Awarded best student presentation**]

Contribution Statement: This presentation was based on the work from Publication #4 (see above for author contributions). The presentation was designed and delivered by EBP. DMC, JLA, JAH and VGC reviewed the presentation and provided feedback.

3. **Parr, E.B.,** Coffey, V.G., Burke, L.M., Phillips, S.M., Hawley, J.A. Effect of increased consumption of dairy protein on weight loss during a diet and exercise intervention, *International Sports and Exercise Nutrition Conference*, Newcastle upon Tyne, December 17th, 2014. [Oral presentation]

Contribution Statement: This presentation was based on the work from Publication #2 (see above for author contributions). The presentation was designed and delivered by EBP. JAH and VGC reviewed the presentation and provided feedback.

4. **Parr, E.B**., Camera, D.M., Burke, L.M., Coffey, V.G., Hawley, J.A. Changes in circulating microRNAs between high and low responders to a 16-wk diet and exercise weight loss intervention, *European College of Sports Sciences (ECSS),* Malmo, Sweden, June 24-27, 2015. [Poster presentation]

Contribution Statement: This presentation was based on the work from Publication #3 (see above for author contributions). The presentation was designed and delivered by EBP. DMC, VGC and JAH reviewed the poster and provided feedback.

d) Individual papers

9.1   Published paper which forms the basis of Chapter 2

Parr, E.B., Coffey, V.G., Hawley, J.A. 'Sarcobesity': a metabolic conundrum, *Maturitas*, 2013, 74(2):109-113.

Due to copyright restrictions, the published version of this journal article is not available here. Please view the published version online at: http://doi.org/10.1016/j.maturitas.2012.10.014

e) Ethics approvals, letters to participants and consent forms

The following approval notices cover all three studies for the work completed during this thesis. For *Study 4*, an amendment request was made to the original ethics application, which is also attached (pp 193-195), to allow the follow-up measurements to be conducted.

9.1.1 Study 2: Effect of dairy-based high-protein, variable carbohydrate diets and exercise on muscle maintenance and movement

A. Notice of Approval:

RMIT
UNIVERSITY

Human Research Ethics Committee (HREC)
Research and Innovation office

Notice of Approval

Date: **16 January 2012**

Project number: **76/11**

Project title: **Effect of dairy-based high-protein, variable carbohydrate diets and exercise on muscle maintenance and movement**

Risk classification: **More than low risk**

Investigator: **Evelyn Parr**

Approved: **From: 20 January 2012   To: 31 December 2013**

Terms of approval:
1. **Responsibilities of investigator**
   It is the responsibility of the above investigator to ensure that all other investigators and staff on a project are aware of the terms of approval and to ensure that the project is conducted as approved by HREC. Approval is only valid whilst investigator holds a position at RMIT University.
2. **Amendments**
   Approval must be sought from HREC to amend any aspect of a project including approved documents. To apply for an amendment use the request for amendment form, which is available on the HREC website and submitted to the HREC secretary. Amendments must not be implemented without first gaining approval from HREC.
3. **Adverse events**
   You should notify HREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
4. **Plain Language Statement (PLS)**
   The PLS and any other material used to recruit and inform participants of the project must include the RMIT university logo. The PLS must contain a complaints clause including the above project number.
5. **Annual reports**
   Continued approval of this project is dependent on the submission of an annual report.
6. **Final report**
   A final report must be provided at the conclusion of the project. HREC must be notified if the project is discontinued before the expected date of completion.
7. **Monitoring**
   Projects may be subject to an audit or any other form of monitoring by HREC at any time.
8. **Retention and storage of data**
   The investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

In any future correspondence please quote the project number and project title above.

A/Prof Barbara Polus
Chairperson
RMIT HREC

cc: Dr Peter Burke (Ethics Officer/HREC secretary), Prof John Hawley (supervisor).

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RMIT UNIVERSITY

INVITATION TO PARTICIPATE IN A RESEARCH PROJECT
PROJECT INFORMATION STATEMENT

Project Title:

Effect of dairy-based high-protein, variable-carbohydrate diets and exercise on muscle maintenance and movement

Investigators:

Ms Evelyn Parr (Associate Lecturer/ PhD Student, Exercise Science, RMIT University
evelyn.parr@rmit.edu.au, 0413 477 697)

Professor John Hawley (Research Director, Exercise Science, RMIT University
john.hawley@rmit.edu.au, 9925 7353)

Dr Vernon Coffey (Research Officer / Project Supervisor, Exercise Science, RMIT University
vernon.coffey@rmit.edu.au, 9925 7356)

Collaborators:
Professor Louise Burke (Principal Dietician, Australian Institute of Sport, Canberra)
Professor Stuart Phillips (Head of Dept of Kinesiology, McMaster University, Canada)

Dear ...

You are invited to participate in a research project being conducted by RMIT University. This information sheet describes the project in straightforward language, or 'plain English'. Please read this sheet carefully and be confident that you understand its contents before deciding whether to participate. If you have any questions about the project, please ask one of the investigators.

Who is involved in this research project? Why is it being conducted?

Ms. Evelyn Parr, Dr. Vernon Coffey and Professor John Hawley are researchers from the Exercise Metabolism Group (Discipline of Exercise Sciences) at RMIT University. Professor Hawley is the Head of this group and directs and supervises all the applied exercise research within the Exercise Metabolism Laboratory. Ms Parr and Dr Coffey are the principal researchers for this study and are responsible for the co-ordination and administration of the experimental trials involved in this project. Professor Louise Burke is the Head of Sports Nutrition at the Australian Institute of Sport (Canberra based) and is the principal dietician for this study. Professor Phillips is the Head of the Research group at McMaster University and will collaborate on analyses for this project.
This project is being conducted to determine the potential for increased consumption high-protein, dairy-based diets during diet- and exercise-induced weight loss to promote fat mass loss and lean mass gain. The project has been approved by the RMIT Human Research Ethics Committee (Ref 76/11).

1

Why have you been approached?

You have been approached because you have registered your interest with regard to participating in this study by responding to the advertisement calling for volunteers or requesting information on future studies as a former participant in exercise research with the Exercise Metabolism Group at RMIT University.

What is the project about? What are the questions being addressed?

The willingness of the majority of Australians to adopt a sedentary lifestyle accompanied by an excess energy intake underpins the current epidemic of lifestyle diseases. In an effort to prevent the rise of inactivity-related conditions, medical and health professionals prescribe energy-restricted weight-loss programmes combined with increased physical activity as a means to reduce body mass (weight) and improve health outcomes. However, typical diet-induced weight loss leads to an imbalance in **body composition** that reduces an individuals' functional capacity (i.e., movement and activity patterns) and increases their susceptibility to metabolic disorders. Specifically, the complex interactions between elevated levels of body fat and a reduced muscle size result in a high fat: muscle ratio, leading to a cascade of events that accelerate pre-existing health conditions (i.e., sarcopenia) and simultaneously predispose an individual to further (new) health risks.

At present, the optimal diet to achieve weight loss and promote favourable body composition changes in overweight adults remains highly controversial. More information is needed to determine the effects of different diets on energy metabolism, energy balance and body composition. Indeed, the biggest concern for the majority of Australian adults is how to lose fat mass while preserving muscle mass and the health benefits that accrue from a nutrient-rich high-quality protein diet and physical activity. Accordingly, the aim of this project is to discover and characterise the best composition of mildly energy restricted high-protein (dairy-based), variable-CHO diets consumed in association with resistance training to improve health.

If I agree to participate, what will I be required to do?

The study is based at RMIT University, Bundoora, and your current health and medical status will initially be assessed in order to be deemed eligible to participate. Participants will complete 16 weeks of a specific diet and exercise program designed to increase or maintain muscle mass and simultaneously reduce fat mass to promote health. This includes regular dietary counselling with a qualified dietitian and fitness centre/gym membership for 16 weeks of supervised exercise sessions.

You will be required to provide your written consent prior to your participation in the project. Prior to participating in the study you will also be required to complete a validated medical screening checklist. You will be excluded from the study if you have any the contradictory condition to exercising or dietary restriction that may be a risk to your health. Prior to the study, your height and weight will be measured and you will also complete a Food Frequency Questionnaire validated for dairy foods to verify your habitual dairy consumption. At this time you will also be instructed on how to accurately complete a seven-day food record and will be given your own set of measuring cups and spoons to use throughout the study.

Volunteers will be compensated for travel ($250.00) to the Exercise Metabolism Laboratory at the RMIT University Bundoora West campus (Plenty Rd, Bundoora). Each session's duration will vary according to the specific requirements of that day. Specifically, you will be required to attend approximately three (3-5) initial interview/testing sessions prior to beginning the study and during week 8 and week 16 of the study. Preliminary visits will vary from 30-120 minutes in duration and exercise training sessions will be approximately 45 minutes duration. Overall, we anticipate you will complete 80 training sessions over 16 weeks.

Experimental Diets
All participants will have initial dietary and clinical assessment performed prior to study entry. This involves completing a seven-day food record i.e. all food and fluid you consume over a seven day period will need to be measured and recorded. At the same time, a measure of energy expenditure will be collected using a Sensewear Pro energy expenditure device (BodyMedia). The device is similar to a matchbox size monitor that you wear on your upper arm with an armband. Once we have this data it will be used as a starting point from which subsequent diets and dietary advice will be based. All dietary assessment and counselling will be undertaken by the study dietician.

2

All eligible participants will then be randomly assigned to one of three experimental groups. The three groups differ in the amount and type of protein that you will consume throughout the intervention period, and the dietary macronutrient distribution.

1. High protein (via high dairy) and moderate carbohydrate
2. High protein (via high dairy) and high carbohydrate
3. Moderate protein and high carbohydrate

Prior to beginning the dietary and exercise regime, participants will complete the following preliminary tests, which will be re-tested at half way (8 weeks) and at the completion (16 weeks) of the study.

Preliminary Tests

- **VO_2peak test**

The VO_2peak test is an incremental test to exhaustion on a stationary cycle ergometer. The workload increases every 2½ minutes until you can no longer maintain a pedalling rate of 60-70 rpm. This test is expected to last between 10-15 minutes. During the test expired air will be sampled as you breathe into a mouthpiece attached to an automated gas analyser. The results from this test will provide you with a peak oxygen consumption reading and peak power output (PPO) and physiological data regarding your cardiovascular health and fitness.

- **Strength test**

Muscular strength will be determined during a series of one repetition maximum (1RM) for a series of weight training exercises. Prior to commencing the maximal test participants will warm-up by performing exercises at sub-maximal intensities separated by 60 seconds rest. Following the warm-up each single exercise repetition will be performed with 3 min recovery periods between each attempt, as participants undertake exercise of increasing weight until the repetition load that can be completed once but not a second time is determined. The determined loads will be used to set the training intensity.

- **Body composition analysis**

A dual energy X-ray absorptiometry scan (DXA) is a specialised X-ray technique to provide a measure of your body composition. This is just like a normal X-ray, with no pain involved and it will measure total body mass, fat mass and lean mass. You will be scanned wearing light clothing at the same time of day (early morning) and will be instructed to follow standardised diet and exercise control before each scan. The scan will last for 3-4 minutes, and will be conducted in weeks 0, 8 and 16 of the study.

- **Resting metabolic rate**

Resting metabolic rate will be measured using a mouth piece to measure your oxygen intake at rest for a 30 min period whilst lying down. This will be measured on three separate occasions during the same visit as the whole-body DXA scans (i.e., in the week prior to study commencement (week 0), at the half-way point of the intervention period (week 8) and upon study completion (week 16).

- **Anthropometry**

Waist and hip circumference will be measured on three separate occasions during the same visit as you undertake your whole-body DXA scans. The measurement will be taken twice, and the result averaged for the most accurate measurement.

- **Blood and Urine Sampling**

Resting blood samples (three 6 mL tubes) will be obtained on three occasions, in the week prior to study commencement, at the half-way point of the intervention (week 8) and at the end of the study (week 16) in the morning after a 10-12 hr overnight fast. Blood will be taken through a venepuncture to a vein in the elbow/lower arm area, by a phlebotomist (i.e. someone qualified and experienced in taking blood). You will also be asked to provide a small urine sample when arriving at the laboratory.

- **Glucose Monitor**

The glucose sensor is a device routinely used by diabetics to provide information on changes in glucose levels in the body and will be inserted when you complete the seven day food record and wear the Sensewear armband. The sensor will be removed up to six days later. A small fibre/wire will be inserted just under your skin on your side adjacent to your stomach and is essentially painless to insert. This wire will then be attached to a data recorder approximately the size of a 50 cent piece that sits on skin surface beside it. The recorder is taped to your body and will be barely detectable throughout the day. The glucose sensor measures the glucose concentration and stores an average value every 5 minutes (Medtronic, iPro2 professional model) and is viable for six days with no further intervention required until it is removed. The monitor will be used twice more, for weeks 8 and 16 of the training intervention.

3

Dietary Monitoring

All participants will receive individual diet counselling by the study dietitian on a biweekly basis. Each subject will be provided with an individualised food plan detailing their personal food intakes (in grams) to achieve the energy restriction. You will also be required, on days on which exercise is completed, to consume a recovery drink during the 30-minute period following the session.

You will be supplied with recipes and protein/fat/carbohydrate "counters" to allow you to plan and track meals to achieve the dietary prescription. Every two weeks thereafter, you will be asked to provide a three-day food record to track compliance with the nutritional intervention protocol, as well as a daily checklist in which dairy serves and post-exercise drinks are noted. Biweekly counselling sessions will be used to provide feedback regarding compliance to the test diet. You will also be instructed not to take any supplements throughout the intervention period. At the middle and the end of the study periods, subjects will be instructed to keep a 7-day food diary to allow a more comprehensive analysis of self-reported intake on the dietary treatments.

Study Exercise Program

Study participants will undertake 16 weeks of a supervised exercise-training programme in association with their allocated diet (described previously). The core part of the exercise prescription will be progressive resistance-training performed 3 days/week (e.g. Monday, Wednesday and Friday on the RMIT campus) supervised by trained personnel (personal trainer). Based on the results of the preliminary exercise testing, you will perform three sets of multiple exercises (e.g. seated leg extension, hamstring curls, seated row and chest-press) with 60 seconds recovery between each set, in the form of circuit training. The weight lifted by each subject will be recorded every session and only increased once you can complete three sets of 10 repetitions or more at any given weight. Resistance-training exercise logs will be given to you and completed at the time of training. These logs will be checked frequently by study personnel to ensure compliance.

In addition to the resistance exercise programme, you will be encouraged to engage in various modes of aerobic exercise on the days you do not perform resistance training (e.g. Tuesday, Thursday and at the weekend). During the week, you will have the option of group indoor cycling or aerobics dance classes held at RMIT, or exercising on your own at the gym. At the weekend, you will perform one bout of walking per day. The goal of every exercise session is to expend 250 kcal (i.e. create an exercise-induced energy deficit that is equal to the diet-induced deficit). As the energy cost of walking 1 mile (1.6 km) is approximately 100 calories participants, on average, will be required to walk 4 km on Saturdays and Sundays. You can periodically track your exercise energy expenditure and will be given access to a Sensewear Pro energy expenditure device (BodyMedia) to wear during selected sessions. The Sensewear Pro will be pre-programmed so that you exercise for the prescribed duration over the weekend training sessions (walking).

Control of exercise testing sessions
In order to standardise the testing sessions, the following standardisation procedures will need to be followed, and these will be discussed with you prior to each session:

Caffeine and Alcohol Consumption

Caffeine and alcohol will affect test results. Accordingly, you will be required to abstain from caffeine for 24 hours and alcohol 48 hours prior to week 0, 8 and 16 testing sessions.

Physical Activity

For the most reliable and valid test results it is important for you to be physically rested. You will be required to perform no structured exercise for the 48 hours prior a laboratory-based testing session.

What are the risks or disadvantages associated with participation?

Venipuncture (blood sampling) of participants is slightly discomforting and can lead to the possibility of bruising and infection. The use of sterile, disposable needles, syringes, swabs, etc. will markedly reduce the possibility of infection caused by the catheterisation procedure. The use of qualified and experienced staff will reduce the likelihood of bruising as this is primarily caused by poor venipuncture techniques. Although the possibility of infection and bruising is small, if by chance it does eventuate, consult your doctor immediately and inform the researcher.

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The radiation dose of each DXA is very small and participants will have a maximum of three scans and this is approximately equal to ~2.0 µSv per year. This will ensure that the total radiation exposure is kept to the minimum and is well under the exposure limits for volunteer by research set by the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) of 5 mSv per year (>100 times less).

Finally, while all physical exertion involves some possible risk of injury or complication the exercise sessions in this study will be supervised and mainly involve lower intensity, resistance exercise and will be progressively increased corresponding with your improvements in fitness and strength.

What are the benefits associated with participation?

Individuals who take part in the research study will benefit in being provided with information about their starting health and fitness as well as the opportunity to improve their health by regular exercise and having intensive dietary counselling. You will have access to personal trainers and a dietitian throughout the study period and these professionals will educate you to enable you to continue to change lifestyle patterns for beneficial effects on your health. While of no direct benefit to you the findings from this study will provide new information to health professionals regarding the best practice for lifestyle intervention in the community to promote health and well being in Australian society.

What will happen to the information I provide?

Information for each volunteer will be identified by a code so that your name does not appear on any data sheets. Individual information will be kept for 5 years in a locked cabinet at RMIT University and only the principal investigators (Ms Evelyn Parr, Dr. Vernon Coffey, Profs. John Hawley, Louise Burke and Stuart Phillips) will have access to this information. At the conclusion of this five-year period all material containing confidential information will be destroyed. If you wish to gain access to your data contact the principal researcher and it will be provided to you. Please note that no material that could personally identify you will be used in any reports or presentations of this project.

Any information that you provide can be disclosed only if (1) it is to protect you or others from harm, (2) a court order is produced, or (3) you provide the researchers with written permission.
The results from this study will be presented at scientific conferences and published in peer-reviewed scientific journals.

What are my rights as a participant?

Participation in this project is voluntary, that is, it is your choice to participate. You do not have to take part in this study. If you choose to participate you may withdraw (stop taking part) at any time without having to give a reason. Should you use the right to withdraw this will not affect your relationship with RMIT University, you may withdraw without prejudice. You are also encouraged to ask questions if you require clarification about any part of this research project or have any queries as you have the right to have any question answered at any time.

Whom should I contact if I have any questions?

Please contact Ms. Evelyn Parr (evelyn.parr@rmit.edu.au ph: 0413 477 697), Dr. Vernon Coffey (vernon.coffey@rmit.edu.au ph: 9925 7356), Professor John Hawley (john.hawley@rmit.edu.au ph: 9925 7353) if you have any question relating to participation in this study.
Yours sincerely,

Ms. Evelyn Parr
Dr. Vernon Coffey Ph.D
Professor John Hawley Ph.D
Professor Louise Burke Ph.D
Professor Stuart Phillips Ph.D

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C. Informed Consent Form for Participants

RMIT HUMAN RESEARCH ETHICS COMMITTEE

Prescribed Consent Form for Persons Participating In Research Projects Involving Tests and/or Medical Procedures

COLLEGE OF	**Science, Engineering and Health (SEH)**
SCHOOL OF	**Medical Sciences (Discipline of Exercise Sciences)**
Name of participant:	
Project Title:	Effect of dairy-based high-protein, variable-carbohydrate diets and exercise on muscle maintenance and movement

Name(s) of investigators:				
	(1)	**Ms Evelyn Parr**	Phone:	0413477697
	(2)	**Prof John Hawley**	Phone:	9925 7353
	(3)	**Dr Vernon Coffey**	Phone:	9925 7356
	(4)	**Prof Louise Burke**	Phone:	02 6214 1351
	(5)	**Prof Stuart Phillips**	Email:	phillis@mcmaster.ca

1. I have received a statement explaining the tests/procedures involved in this project.

2. I consent to participate in the above project, the particulars of which - including details of tests or procedures - have been explained to me.

3. I agree to follow the dietician's advice, adhere to the dietary condition I will be randomised to and will inform the researchers should there be any unavoidable situation where I am unable to adhere to the prescribed procedure.

4. I authorise the investigator or his/her assistant to use with me the tests or procedures referred to in 1 above.

5. I acknowledge that:
   (a) The possible effects of the tests or procedures have been explained to me to my satisfaction.
   (b) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied (unless follow-up is needed for safety).
   (c) The project is for the purpose of research and/or teaching. It may not be of direct benefit to me.
   (d) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (e) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to RMIT. Any information which will identify me will not be used.
   (f) I have read the plain language statement including details of the dietary conditions, exercise training, and measurements that will be taken and have completed the medical screening questionnaire.

Participant's Consent

Name: _____   **Date:** _____
       (Participant)

Name: _____   **Date:** _____
       (Witness to signature)

Participants should be given a photocopy of this consent form after it has been signed.

Any complaints about your participation in this project may be directed to the Executive Officer, RMIT Human Research Ethics Committee, Research & Innovation, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 2251.
Details of the complaints procedure are available from the above address

D. Request for Project Amendment

RMIT
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Request for Amendment/Extension of Human Research Ethics project

Note: This form is intended to be completed as an electronic document and is set up as a series of tables. The table will enlarge to the size you require as you type or press the Enter key. For check boxes, double click on the left mouse button and a 'check boxes form field' dialog box will appear: choose 'Checked' and 'OK'. If you want to uncheck it, double click on left mouse button and a 'check boxes form field' dialog box will appear: choose 'Not checked' and 'OK'.

All changes to a project must be approved before they are implemented. If data collection continues beyond the date for which the project was approved then the project is considered to be not approved and data collected will un-useable.

Project No:	Project title
76-11	Effect of a dairy-based high-protein, variable carbohydrate diets and exercise on muscle maintenance and movement

Project approved until:	1 February 2015
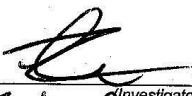
Project being undertaken for award of degree:	PhD ☒

Principal Investigator	Ms Evelyn Parr
Email	evelyn.parr@rmit.edu.au
Address:	Building 223.02.21, Medical Sciences, Bundoora West
Name of Supervisor (If applicable):	Prof. John Hawley

Project summary	Summarise the original project. Assume when preparing your summary that the reader does not have a copy of the original application so this summary needs to 'stand alone'.

Energy restriction promotes weight loss but it is indiscriminate as to the tissue loss that occurs: both fat and muscle are lost simultaneously. Thus, typical diet-induced weight loss leads to an imbalance in body composition that reduces an individuals' functional capacity and increases their susceptibility to sarcopenia and metabolic disorders. This project comprises a chronic intervention (i.e., clinical trial) in previously sedentary, overweight (i.e. over-fat) humans 35-55 years old to determine the potential for higher-protein (dairy-based), lower-carbohydrate diets and exercise to preserve (or increase) muscle mass, while promoting weight loss by decreasing whole-body fat mass/visceral fat mass and concomitantly enhancing resting metabolic rate. Comprehensive body composition analyses coupled with functional outcome measures of muscle size, strength, cardiovascular fitness, activities of daily living and blood profiling will advance and expand the science to support strategies relating to the role of protein consumption for muscle maintenance and functional capacity with weight loss. The information derived from this project will provide practical, evidence-based recommendations for diet prescription that can be rapidly incorporated into daily living and will form an integral component in the development of future combinational therapies for the prevention and treatment of sarcopenia. This is a critical first step for improving the standard of living for a large portion of Australian society.

SECTION 1 – Amendments

1.	List those aspects of the project that you would like to amend. Make sure to include a rationale and background for each of the amendments. Cite any references where appropriate.

Our original application and subsequent amendments outlined primary and secondary outcome measures for our weight loss study across 16 weeks.

We intend that the results of this trial can be disseminated to stakeholders in the community such as dieticians, GPs and other allied health professionals. However, we currently have no provision to evaluate whether the tools provided to participants allows them to effectively continue the diet and exercise programme outside of the strictly controlled 16-week clinical trial. During the evaluation we are seeking to gain an indication of the study effectiveness in the 'real world' and allow suggestions and feedback from completed study participants.

On this basis, we request approval to contact participants at 3, 6, 9 and 12 months after the conclusion of the 16-week dietary and exercise intervention.

1) Initial contact for each of these time points would happen by the preferred contact method the participants had indicated in pre-screening. All current participants have indicated either by phone or by letter. The follow-up would consist of an informal discussion of 15-20 min asking about the continued compliance with diet and exercise tools, such as dietary records sheets and personal training programmes provided to participants throughout the 16-week programme. This will permit the investigators to evaluate the utility of study resources and allow for amendments before dissemination to allied health professionals.

2) In return, participants would be offered the chance to undertake a repeat testing which may include any of the following approved tests including 1) peak oxygen uptake (VO2peak) test 2) muscular strength testing, 3) resting blood analyses (glucose/insulin/lipid profile), 4) body composition analysis using DXA (dual-energy x-ray absorptiometry) scans, 5) Waist and hip circumferences, 6) urine analyses and 7) seven day diet record with energy expenditure measures (Sensewear) and continuous blood glucose monitors. It may also include, as outlined in the previous amendment 8) Oral Glucose Tolerance Test (OGTT) or 9) muscle biopsies. All tests will be offered to participants and they may choose which ones they would like to repeat to gain feedback on their continued progress. This would also provide further motivation for past participants to maintain healthy diet and exercise habits.

2.	Explain how the requested amendments will alter the original approved project.
	Remember to revise any documents associated with the approved application (e.g. plain language statement, questionnaire) that will be altered if the amendment is approved • If appropriate attach copies of documents that will be changed by the amendment (use 'track changes') • If appropriate attach a table comparing the amendment details with what is approved in the current application.

The Plain Language Statement that participants will view at pre-screening has been altered and changes are highlighted in red font (see attached).

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Signed: _____ Date: 16/7/12
(Investigator)
Evelyn Fong

Student No: S3327293
(if applicable)

Signed: _____ Date: 16/7/12
(Supervisor – if applicable)

RMIT HREC or College Human Ethics Advisory Network:	Approved On: Signed:	Issued: Date:

RMIT UNIVERSITY

INVITATION TO PARTICIPATE IN A RESEARCH PROJECT
PROJECT INFORMATION STATEMENT

Project Title:

Effect of dairy-based high-protein, variable-carbohydrate diets and exercise on muscle maintenance and movement

Investigators:

Ms Evelyn Parr (Associate Lecturer/PhD Student, Exercise Science, RMIT University evelyn.parr@rmit.edu.au, 0413 477 697)

Professor John Hawley (Research Director, Exercise Science, RMIT University john.hawley@rmit.edu.au, 9925 7353)

Dr Vernon Coffey (Research Officer / Project Supervisor, Exercise Science, RMIT University vernon.coffey@rmit.edu.au, 9925 7356)

Ms Daniela Manche (Project Dietician, Exercise Science, RMIT University, daniela.manche@rmit.edu.au, 0408 177 815)

Collaborators:
Professor Louise Burke (Principal Dietician, Australian Institute of Sport, Canberra)
Professor Stuart Phillips (Head of Dept of Kinesiology, McMaster University, Canada)

Dear ...

You are invited to participate in a research project being conducted by RMIT University. This information sheet describes the project in straightforward language, or 'plain English'. Please read this sheet carefully and be confident that you understand its contents before deciding whether to participate. If you have any questions about the project, please ask one of the investigators.

Who is involved in this research project? Why is it being conducted?

Ms. Evelyn Parr, Dr. Vernon Coffey and Professor John Hawley are researchers from the Exercise Metabolism Group (Discipline of Exercise Sciences) at RMIT University. Professor Hawley is the Head of this group and directs and supervises all the applied exercise research within the Exercise Metabolism Laboratory. Ms Parr and Dr Coffey are the principal researchers for this study and are responsible for the co-ordination and administration of the experimental trials involved in this project. Professor Louise Burke is the Head of Sports Nutrition at the Australian Institute of Sport (Canberra based) and is the principal dietician for this study. Professor Phillips is the Head of the Research group at McMaster University and will collaborate on analyses for this project.

This project is being conducted to determine the potential for increased consumption high-protein, dairy-based diets during diet- and exercise-induced weight loss to promote fat mass loss and lean mass gain. The project has been approved by the RMIT Human Research Ethics Committee (Ref 76/11).

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Why have you been approached?

You have been approached because you have registered your interest with regard to participating in this study by responding to the advertisement calling for volunteers or requesting information on future studies as a former participant in exercise research with the Exercise Metabolism Group at RMIT University.

What is the project about? What are the questions being addressed?

The willingness of the majority of Australians to adopt a sedentary lifestyle accompanied by an excess energy intake underpins the current epidemic of lifestyle diseases. In an effort to prevent the rise of inactivity-related conditions, medical and health professionals prescribe energy-restricted weight-loss programmes combined with increased physical activity as a means to reduce body mass (weight) and improve health outcomes. However, typical diet-induced weight loss leads to an imbalance in **body composition** that reduces an individuals' functional capacity (i.e., movement and activity patterns) and increases their susceptibility to metabolic disorders. Specifically, the complex interactions between elevated levels of body fat and a reduced muscle size result in a high fat: muscle ratio, leading to a cascade of events that accelerate pre-existing health conditions (i.e., sarcopenia) and simultaneously predispose an individual to further (new) health risks.

At present, the optimal diet to achieve weight loss and promote favourable body composition changes in overweight adults remains highly controversial. More information is needed to determine the effects of different diets on energy metabolism, energy balance and body composition. Indeed, the biggest concern for the majority of Australian adults is how to lose fat mass while preserving muscle mass and the health benefits that accrue from a nutrient-rich high-quality protein diet and physical activity. Accordingly, the aim of this project is to discover and characterise the best composition of mildly energy restricted high-protein (dairy-based), variable-CHO diets consumed in association with resistance training to improve health.

If I agree to participate, what will I be required to do?

The study is based at RMIT University, Bundoora, and your current health and medical status will initially be assessed in order to be deemed eligible to participate. Participants will complete 16 weeks of a specific diet and exercise program designed to increase or maintain muscle mass and simultaneously reduce fat mass to promote health. This includes regular dietary counselling with a qualified dietitian and fitness centre/gym membership for 16 weeks of supervised exercise sessions.

You will be required to provide your written consent prior to your participation in the project. Prior to participating in the study you will also be required to complete a validated medical screening checklist. You will be excluded from the study if you have any the contradictory condition to exercising or dietary restriction that may be a risk to your health. Prior to the study, your height and weight will be measured and you will also complete a Food Frequency Questionnaire validated for dairy foods to verify your habitual dairy consumption. At this time you will also be instructed on how to accurately complete a seven-day food record and will be given your own set of measuring cups and spoons to use throughout the study.

Volunteers will be compensated for travel ($250.00) to the Exercise Metabolism Laboratory at the RMIT University Bundoora West campus (Plenty Rd, Bundoora). Each session's duration will vary according to the specific requirements of that day. Specifically, you will be required to attend approximately four (4-5) initial interview/testing sessions prior to beginning the study and during week 8 and week 16 of the study. Preliminary visits will vary from 30-120 minutes in duration and exercise training sessions will be approximately 45 minutes duration. Overall, we anticipate you will complete 80 training sessions over 16 weeks.

Experimental Diets
All participants will have initial dietary and clinical assessment performed prior to study entry. This involves completing a seven-day food record i.e. all food and fluid you consume over a seven day period will need to be measured and recorded. At the same time, a measure of energy expenditure will be collected using a Sensewear Pro energy expenditure device (BodyMedia). The device is similar to a matchbox size monitor that you wear on your upper arm with an armband. Once we have this data it will be used as a starting point from which subsequent diets and dietary advice will be based. All dietary assessment and counselling will be undertaken by the study dietician.

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All eligible participants will then be randomly assigned to one of three experimental groups. The three groups differ in the amount and type of protein that you will consume throughout the intervention period, and the dietary macronutrient distribution.
1. High protein (via high dairy) and moderate carbohydrate
2. High protein (via high dairy) and high carbohydrate
3. Moderate protein and high carbohydrate

Prior to beginning the dietary and exercise regime, participants will complete the following preliminary tests, which will be re-tested at half way (8 weeks) and at the completion (16 weeks) of the study (unless stated otherwise).

Preliminary Tests

- **VO$_2$peak test**

The VO$_2$peak test is an incremental test to exhaustion on a stationary cycle ergometer. The workload increases every 2½ minutes until you can no longer maintain a pedalling rate of 60-70 rpm. This test is expected to last between 10-15 minutes. During the test expired air will be sampled as you breathe into a mouthpiece attached to an automated gas analyser. The results from this test will provide you with a peak oxygen consumption reading and peak power output (PPO) and physiological data regarding your cardiovascular health and fitness.

- **Strength test**

Muscular strength will be determined during a series of one repetition maximum (1RM) for a series of weight training exercises. Prior to commencing the maximal test participants will warm-up by performing exercises at sub-maximal intensities separated by 60 seconds rest. Following the warm-up each single exercise repetition will be performed with 3 min recovery periods between each attempt, as participants undertake exercise of increasing weight until the repetition load that can be completed once but not a second time is determined. The determined loads will be used to set the training intensity.

- **Body composition analysis**

A dual energy X-ray absorptiometry scan (DXA) is a specialised X-ray technique to provide a measure of your body composition. This is just like a normal X-ray, with no pain involved and it will measure total body mass, fat mass and lean mass. You will be scanned wearing light clothing at the same time of day (early morning) and will be instructed to follow standardised diet and exercise control before each scan. The scan will last for 3-4 minutes, and will be conducted in weeks 0, 8 and 16 of the study.

- **Resting metabolic rate**

Resting metabolic rate will be measured using a mouth piece to measure your oxygen intake at rest for a 30 min period whilst lying down. This will be measured on three separate occasions during the same visit as the whole-body DXA scans (i.e., in the week prior to study commencement (week 0), at the half-way point of the intervention period (week 8) and upon study completion (week 16).

- **Anthropometry**

Waist and hip circumference will be measured on three separate occasions during the same visit as you undertake your whole-body DXA scans. The measurement will be taken twice, and the result averaged for the most accurate measurement.

- **Blood and Urine Sampling**

Resting blood samples (three 6 mL tubes) will be obtained on three occasions, in the week prior to study commencement, at the half-way point of the intervention (week 8) and at the end of the study (week 16) in the morning after a 10-12 hr overnight fast. Blood will be taken through a venepuncture to a vein in the elbow/lower arm area, by a phlebotomist (i.e. someone qualified and experienced in taking blood). You will also be asked to provide a small urine sample when arriving at the laboratory.

- **Oral Glucose Tolerance Test (known as OGTT)**

An OGTT will be conducted at two time points, prior to entering the study and at the end of the study (week 16). The test involves consuming a bolus of glucose (75 g solution) within 5 minutes and then subsequent blood samples to see your reaction (change in blood glucose levels) in the blood over a 2 hour period. For the blood sampling a qualified phlebotomist will insert an indwelling catheter (a needle) into a forearm vein. The catheter consists of a needle and tubing. The tubing is fed over the top of the needle (which has punctured a vein) and into the vein. The needle is then withdrawn, leaving only the plastic sterile tubing in your vein for the remainder of the experiment. A tap (stopcock) is placed into the

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tubing so the flow of blood along the tubing can be controlled. This catheter procedure allows the extraction of multiple blood samples without the need for multiple venipunctures/catheterisations (puncturing of the vein). The test will run for 2 hours and over this period five 4 ml samples will be taken.

- **Glucose Monitor**

The glucose sensor is a device routinely used by diabetics to provide information on changes in glucose levels in the body and will be inserted when you complete the seven day food record and wear the Sensewear armband. The sensor will be removed up to six days later. A small fibre/wire will be inserted just under your skin on your side adjacent to your stomach and is essentially painless to insert. This wire will then be attached to a data recorder approximately the size of a 50 cent piece that sits on skin surface beside it. The recorder is taped to your body and will be barely detectable throughout the day. The glucose sensor measures the glucose concentration and stores an average value every 5 minutes (Medtronic, iPro2 professional model) and is viable for six days with no further intervention required until it is removed. The monitor will be used twice more, for weeks 8 and 16 of the training intervention.

- **Muscle and fat biopsies (OPTIONAL)**

The biopsy technique is a rapid procedure (~5-10 seconds) that is performed to obtain small samples of skeletal muscle and adipose (fat) tissue for metabolic analysis. The biopsies enable the measurement of changes at the cellular level that are important to understanding how dairy can help achieve optimal body composition.

Each muscle biopsy will be taken from the quadriceps (thigh) muscle by a registered physician experienced in this procedure. After the administration of local anaesthetic a small incision is made in the skin overlying the muscle. The biopsy needle will be inserted into the muscle via the incision and a small amount of tissue, around the same size as a small kernel of corn, will be removed. During this part of the procedure you will experience some pressure and a tendency for the muscle to cramp, however this will only persist for a few seconds. At the conclusion of each experimental trial, incisions will be closed with surgical tape and dressed with a pressure bandage to prevent infection. Ice packs will also be applied to reduce any local swelling or pain associated with the procedure. To assist you with post-trial dressing changes, dressings will be supplied and information given on wound cleaning. You will also be contacted in the days following the muscle biopsy procedure to verify your recovery.

The fat biopsy is taken from a small incision in the abdomen. The fat biopsy technique is performed similar to the muscle biopsy with the exception that following administration of local anaesthetic this procedure is essentially painless because there is little nerve supply to fat tissue. Care of the incision is also the same as for the muscle biopsy.

Biopsy samples will be undertaken before commencing the diet and training modification (week 0) and during week 8 and week 16.

Dietary Monitoring

All participants will receive individual diet counselling by the study dietitian on a biweekly basis. Each subject will be provided with an individualised food plan detailing their personal food intakes (in grams) to achieve the energy restriction. You will also be required, on days on which exercise is completed, to consume a recovery drink during the 30-minute period following the session.

You will be supplied with recipes and protein/fat/carbohydrate "counters" to allow you to plan and track meals to achieve the dietary prescription. Every two weeks thereafter, you will be asked to provide a three-day food record to track compliance with the nutritional intervention protocol, as well as a daily checklist in which dairy serves and post-exercise drinks are noted. Biweekly counselling sessions will be used to provide feedback regarding compliance to the test diet. You will also be instructed not to take any supplements throughout the intervention period. At the middle and the end of the study periods, subjects will be instructed to keep a 7-day food diary to allow a more comprehensive analysis of self-reported intake on the dietary treatments.

Study Exercise Program

Study participants will undertake 16 weeks of a supervised exercise-training programme in association with their allocated diet (described previously). The core part of the exercise prescription will be progressive resistance-training performed 3 days/week (e.g. Monday, Wednesday and Friday on the RMIT campus) supervised by trained personnel (personal trainer). Based on the results of the preliminary exercise testing, you will perform three sets of multiple exercises (e.g. seated leg extension, hamstring curls, seated row and chest-press) with 60 seconds recovery between each set, in the form of circuit training. The weight lifted by each subject will be recorded every session and only increased once you

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can complete three sets of 10 repetitions or more at any given weight. Resistance-training exercise logs will be given to you and completed at the time of training. These logs will be checked frequently by study personnel to ensure compliance.

In addition to the resistance exercise programme, you will be encouraged to engage in various modes of aerobic exercise on the days you do not perform resistance training (e.g. Tuesday, Thursday and at the weekend). During the week, you will have the option of group indoor cycling or aerobics dance classes held at RMIT, or exercising on your own at the gym. At the weekend, you will perform one bout of walking per day. The goal of every exercise session is to expend 250 kcal (i.e. create an exercise-induced energy deficit that is equal to the diet-induced deficit). As the energy cost of walking 1 mile (1.6 km) is approximately 100 calories participants, on average, will be required to walk 4 km on Saturdays and Sundays. You can periodically track your exercise energy expenditure and will be given access to a Sensewear Pro energy expenditure device (BodyMedia) to wear during selected sessions. The Sensewear Pro will be pre-programmed so that you exercise for the prescribed duration over the weekend training sessions (walking).

Control of exercise testing sessions
In order to standardise the testing sessions, the following standardisation procedures will need to be followed, and these will be discussed with you prior to each session:

Caffeine and Alcohol Consumption

Caffeine and alcohol will affect test results. Accordingly, you will be required to abstain from caffeine for 24 hours and alcohol 48 hours prior to week 0, 8 and 16 testing sessions.

Physical Activity

For the most reliable and valid test results it is important for you to be physically rested. You will be required to perform no structured exercise for the 48 hours prior a laboratory-based testing session.

Follow-up Testing

After your participation in the formal 16-week diet and exercise program has ended, we intend to have provided you with the necessary tools that allow the continuation of a self-directed healthy diet and exercise program. In order to evaluate the effectiveness of these tools, such as dietary resources, food plans and exercise programs, some participants may be contacted post-study. We will ask your permission to continue to contact you when you are nearing the end of the study itself. At 3, 6, 9 and 12 months post-study, investigators will phone you to discuss your current diet and exercise habits and how you have used the tools provided to you in the study. You will also be given the opportunity to make suggestions on how the resources provided might be improved. During this discussion, you may be asked to complete a seven-day food record. You will also be offered some repeat testing, such as those outlined in preliminary testing above, to allow you to track your own progress in terms of body composition post-study. These testing sessions will be subject to the same standardisation controls as outlined above and results may be retained by study investigators.

What are the risks or disadvantages associated with participation?

Venipuncture/cannulation (blood sampling) of participants is slightly discomforting and can lead to the possibility of bruising and infection. The use of sterile, disposable needles, syringes, swabs, etc. will markedly reduce the possibility of infection caused by the catheterisation procedure. The use of qualified and experienced staff will reduce the likelihood of bruising as this is primarily caused by poor venipuncture techniques. Although the possibility of infection and bruising is small, if by chance it does eventuate, consult your doctor immediately and inform the researcher.
The radiation dose of each DXA is very small and participants will have a maximum of three scans and this is approximately equal to ~2.0 µSv per year. This will ensure that the total radiation exposure is kept to the minimum and is well under the exposure limits for volunteer by research set by the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) of 5 mSv per year (>100 times less).

Finally, while all physical exertion involves some possible risk of injury or complication the exercise sessions in this study will be supervised and mainly involve lower intensity, resistance exercise and will be progressively increased corresponding with your improvements in fitness and strength.

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What are the benefits associated with participation?

Individuals who take part in the research study will benefit in being provided with information about their starting health and fitness as well as the opportunity to improve their health by regular exercise and having intensive dietary counselling. You will have access to personal trainers and a dietitian throughout the study period and these professionals will educate you to enable you to continue to change lifestyle patterns for beneficial effects on your health. While of no direct benefit to you the findings from this study will provide new information to health professionals regarding the best practice for lifestyle intervention in the community to promote health and well being in Australian society.

What will happen to the information I provide?

Information for each volunteer will be identified by a code so that your name does not appear on any data sheets. Individual information will be kept for 5 years in a locked cabinet at RMIT University and only the principal investigators (Ms Evelyn Parr, Ms Daniela Manche, Dr. Vernon Coffey, Profs. John Hawley, Louise Burke and Stuart Phillips) will have access to this information. At the conclusion of this five-year period all material containing confidential information will be destroyed. If you wish to gain access to your data contact the principal researcher and it will be provided to you. Please note that no material that could personally identify you will be used in any reports or presentations of this project.

Any information that you provide can be disclosed only if (1) it is to protect you or others from harm, (2) a court order is produced, or (3) you provide the researchers with written permission.
The results from this study will be presented at scientific conferences and published in peer-reviewed scientific journals.

What are my rights as a participant?

Participation in this project is voluntary, that is, it is your choice to participate. You do not have to take part in this study. If you choose to participate you may withdraw (stop taking part) at any time without having to give a reason. Should you use the right to withdraw this will not affect your relationship with RMIT University, you may withdraw without prejudice. You are also encouraged to ask questions if you require clarification about any part of this research project or have any queries as you have the right to have any question answered at any time.

Whom should I contact if I have any questions?

Please contact Ms. Evelyn Parr (evelyn.parr@rmit.edu.au ph: 0413 477 697), Dr. Vernon Coffey (vernon.coffey@rmit.edu.au ph: 9925 7356), Professor John Hawley (john.hawley@rmit.edu.au ph: 9925 7353), or Ms. Daniela Manche (Daniela.manche@rmit.edu.au ph: 0408 177 815) if you have any question relating to participation in this study.

Yours sincerely,

Ms. Evelyn Parr
Dr. Vernon Coffey Ph.D
Professor John Hawley Ph.D
Ms Daniela Manche APD
Professor Louise Burke Ph.D
Professor Stuart Phillips Ph.D

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A. <u>DXA guidelines</u>

DAIRY-FIT Clinical Trial

AUSTRALIAN INSTITUTE OF SPORT — RMIT UNIVERSITY

DXA assessment protocol

Dual energy X-ray absorptiometry (DXA)

Dual energy X-ray absorptiometry or DXA is the main technique used to measure bone mineral density to diagnose osteoporosis. Nowadays, it is also a popular tool to assess body composition as it is non-invasive, time efficient, and can generate detailed body composition information, that is, it will provide information on whole body and regional (arms, legs, trunk) bone mineral content, lean and fat mass. This information is pertinent for our study. One whole body scan will take approximately 5-15 minutes to complete.

DXA works by exposing the body to a very small dose of radiation, for example, one whole body DXA scan will produce a radiation exposure that is only 1/100th amount received from a seven-hour aeroplane flight.

Previous research at the AIS has found DXA results to be affected by the amount of food and fluid consumed before the scan, especially for lean mass estimates (Nana et al., 2012). Your hydration level also affects the results so your hydration status will be tested using urine specific gravity (USG), and will be used to help interpret your body composition results. Also, all subjects are required to be overnight-fasted and scanned first thing in the morning to ensure that all the "noise" or error associated with food and fluid ingestion is minimised.

One limitation of the DXA machine is the small size of the scanning bed. The DXA machine was not originally designed for whole body measurements (it was designed for spine scans only); therefore, the size of the scanning bed can be too narrow for some people. To accommodate this, multiple scans may be needed.

Preparation regimen and tips for a DXA scan
- Participants need to be fasted and rested (no exercise before the scan)
- Participants should be hydrated on the morning of testing, try to stay well hydrated the day before by consuming fluids regularly (i.e. tea, milk, smoothies, water etc.).
- A urine sample will be collected on the morning of testing for better interpretation of lean mass changes so please try to avoid going to the toilet before you arrive.
- Some people will not fit the scanning bed. In this instance, whole body composition will be estimated by the addition of 2-3 partial scans.
- Appropriate clothing should be worn for the scan, preferably a loose t-shirt and shorts, however gowns are available to change into and be worn throughout the scan. For females – bras with wires are not allowed to be worn, so please wear a support singlet or similar.
- All metal objects and jewelleries should be removed as these can interfere with the scanner
- To enhance the consistency between the scans, we will attempt to place you on the scanning bed in a standardised position every time
- Ideally, there will be one trained technician carrying out all the procedures (i.e. positioning and analysing the scans), especially for longitudinal monitoring of body composition

If you have any questions regarding this DXA scanning protocol, please do not hesitate to contact Evelyn Parr at 03 9925 7073 or evelyn.parr@rmit.edu.au.
Last Updated: March 2012

B. Training Guidelines

DAIRY-FIT Clinical Trial

AUSTRALIAN INSTITUTE OF SPORT

RMIT UNIVERSITY

Training guidelines

Your participation in the DairyFit study includes completion of three resistance training sessions per week as suited to your individual time schedules at RMIT's fitness centre (BNASC).

The fitness program also includes 250 calories of exercise _per day on your_ alternate (non-resistance exercise) days. We request that these exercise sessions be recorded in your dietary booklet (under the appropriate section) and we will also periodically monitor the activities using the Sensewear armbands.

For your information and to assist you in selecting the appropriate exercise mode and volume/duration here are some specific examples to use 250 calories of energy:

- 4 km Walk/jog/run
- 1 km Swim
- 16 km Cycle
- Use the machines in the gym with the "Calorie goal" program (under other programs) – treadmill, bike, cross-trainer all available
- Any combination of above (e.g. swim 500 m and walk 2 km)

The exercise does not need to be completed in a single session. In this case, please split your recovery snack between sessions if appropriate.
Remember your recovery snack should be consumed within half an hour of all your exercise sessions!!

Thank you,

The DairyFit Team

C. Introduction to the Diet

Australian Government
Australian Sports Commission

fuelling your success
AIS Sports Nutrition

**AUSTRALIAN
INSTITUTE OF SPORT**

Dairy Fit Project

Welcome to this collaboration between RMIT University and the Australian Institute of Sport. We hope that together we will learn habits to achieve lifetime goals of healthy eating and fitness.

Over the next 16 weeks you will undergo a number of stages to gradually take ownership of new eating and exercise patterns that will help you maintain lower body fat levels and increased muscle mass and function.

Stage 1: Retraining your eating: **Weeks 1-2**

During the initial period you will be provided with a set eating plan, based on your personal characteristics and designed to start loss of body fat while preserving/increasing muscle mass.

Eating to this prescription will take all the guess work out of your days meals and snacks, and help you to start a new routine.

You will be provided with a weekly meal plan, including.

- A 7 day grid, summarising your daily pattern of 3 meals and snacks, as well as a recovery snack to follow your training session.
- An individual page for each day, providing more detail of the days menu, and space for you to make notes on how well you went, any concerns and queries, and any tips that may help you or your colleagues to follow the plan.
- Recipes for main meals (dinners and some lunches), many coming from your copy of the AIS Survival for the Active Family Cookbook .

Over the 16 weeks, resources will be gradually provided to help you understand and add flexibility to this new eating pattern. The first resources are summarised below.

Resource	Description
Dairy Baskets	Your daily meal plans provide a basket of 4 - 5 dairy products. Each basket is interchangeable with another (i.e. 4 – 5 points from one day can be swapped for the 4 – 5 points on another). This way, you can choose the dairy foods that you prefer.
Free Kick Points	These foods provide extra bulk and flavour to your meal plan. You can add up to 4 or 6 units from the Limited Free Kick Food lists each day, as well as free choice from the Unlimited Free Kick Foods.
Time Out Points	Sometimes everyone needs a little 'time out' from their diet. You can add 4 Time Out points per week to your meal plan.
Tips and strategies to make dietary changes	These are some ideas to help you stick to your eating plan by making it enjoyable and practical to follow. We will continually update these tips from the ideas that you bring back to us.