Role of long chain omega-3 polyunsaturated fatty acids on weight management

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B Ed MES

Thesis submitted in fulfilment of the requirement to obtain the degree of Doctor of Philosophy in Human Physiology

School of Biomedical Sciences and Pharmacy
University of Newcastle, Australia

November 2012
Statement of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give my consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provision of the Copyright Act 1968.

..........................

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I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of my thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

............................

Irene Munro
Acknowledgements

A PhD thesis, like any body of work, has more than one contributor. To all those who have contributed to this research and thesis, including the participants who took part in the research, a very special thank you for your help. I wish to acknowledge NuMega Ingredients Pty Australia who supplied the fish oil and sunola oil capsules for all of the clinical trials. Also Nestle Nutrition, Australia who supplied the Optifast® bars and shakes for the meal replacements.

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Research publications central to this thesis

1. Chapter 3

   **Statement of contribution:** Major contribution to: the conception and design of the study; the recruitment of participants, collection and recording of data; drafting of the manuscript; revision of the manuscript.

2. Chapter 4

   **Statement of contribution:** Recruited participants; conducted the intervention and collected the clinical data; sample analysis; entered, analysed and interpreted the data; wrote the manuscript.

3. Chapter 5

   **Statement of contribution:** Recruited participants; conducted the intervention and collected the clinical data; sample analysis; entered, analysed and interpreted the data; wrote the manuscript.

4. Chapter 6

   **Statement of contribution:** Recruited participants; conducted the intervention and collected the clinical data; all sample analysis; entered, analysed and interpreted the data; wrote the manuscript.
Oral presentations with published abstracts


Poster presentation with published abstracts


Additional publications relevant to this thesis


Additional oral presentations with published abstracts relevant to this thesis


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<td>AF</td>
<td>adipose fraction</td>
</tr>
<tr>
<td>AGHE</td>
<td>Australian Guide to Healthy Eating</td>
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<tr>
<td>ALA</td>
<td>alpha linolenic acid</td>
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<td>AMPK</td>
<td>activated protein kinase</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>apoE</td>
<td>apolipoprotein E</td>
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<td>BIA</td>
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<td>BMI</td>
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<td>chemokine receptor 2</td>
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<td>dual-energy X-ray absorptiometry</td>
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</tr>
<tr>
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<td>ELISA</td>
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<td>fat free mass</td>
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<td>GC</td>
<td>gas chromatography</td>
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HDL-C  high density lipoprotein-cholesterol
HEWLD  healthy eating weight loss diet
hs    high sensitivity
IL-6   interleukin-6
kg     kilogram
kJ     kilojoule
L      litre
LA     linoleic acid
LCω -3 long chain omega-3
LCn -3 long chain omega-3
LCω -3PUFA long chain omega-3 polyunsaturated fatty acids
LDL-C  low density lipoprotein-cholesterol
LOX    lipoxygenase
LPL    lipoprotein lipase
LPS    lipopolysaccharide
LT     leukotriienes
LX     lipoxins
m      metre
mg     milligram
mL     millilitre
MM     muscle mass
mmol   millimol
MR     meal replacement
mRNA   messenger ribonucleic acid
MUFA   monounsaturated fatty acids
NEFA   non-esterified fatty acid
NFκB   nuclear factor kappa-B cells
ng     nanogram
PB     placebo
PG     prostaglandins
pg     picogram
PGI    prostacyclins
PI     post intervention
PPAR   peroxisome proliferator-activated receptor
PUFA   polyunsaturated fatty acids
RQ     respiratory quotient
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<td>subcutaneous adipose tissue</td>
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<td>SFA</td>
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<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<td>SR</td>
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<td>very low density lipoprotein</td>
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<td>µg</td>
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Synopsis of thesis

Despite an ever-growing body of research on obesity, investigating causes and possible solutions to address the problem, the prevalence of obesity continues to escalate. A major cause of obesity is attributed to poor eating behaviours driven by food advertising, lack of nutrition knowledge, lack of physical activity, lack of time and lack of will power to control food intake, and there is a plethora of research with a focus on changing dietary behaviour for weight management.

In part, this research also addressed dietary change, employing a reduced energy intake for weight loss supported with nutrition education and counselling to enable maintenance of the weight lost. However, consideration was also given to the internal interactions and changes that occur in the body when energy intake exceeds energy output resulting in weight gain and obesity, and whether these mechanisms could be manipulated to reduce weight gain through the inclusion of long chain omega-3 polyunsaturated fatty acids (LCω-3PUFA) in the diet.

Prospective studies in humans have reported that high levels of LCω-3PUFA were associated with low levels of obesity in males while higher intakes of LCω-3PUFA were associated with higher rates of obesity in females. The data on LCω-3PUFA concentrations in males and females had been sourced from dietary records with questionable reliability. Thus the first aim of this research was to investigate whether there was a relationship between plasma LCω-3PUFA and weight status in humans. The first research chapter (Chapter 3) reports on the relationship observed between plasma LCω-3PUFA composition and weight status in free living adults. Obese individuals, both males and females, had significantly lower levels of LCω-3PUFA compared to healthy-weight individuals.

Thus the aim of the first clinical trial (Chapter 4) was to investigate whether LCω-3PUFA supplementation, combined with a healthful diet with portion control and energy restriction would facilitate weight loss, improve blood lipids and inflammatory mediators. This was a double-blinded randomised controlled trial with two parallel groups. Both groups were instructed to follow the same diet for 12 weeks, one group consumed fish oil capsules and the other group consumed placebo capsules (monounsaturated oil). Despite a two-fold increase of EPA and
DHA in the treatment group, there were no significant differences in outcome measures between the two groups. Both the placebo and the fish oil supplemented groups lost similar amounts of weight, fat mass and fat free mass. Overall dietary compliance was poor representing a possible confounding factor on the outcomes.

The aim of the next clinical trial (Chapter 5) was to investigate whether LCω-3PUFA supplementation would facilitate weight loss, but this time in combination with a very-low-energy-diet (VLED), using meal replacements (MRs), to improve dietary compliance. The protocol for this trial was the same as the previous one apart from the change to the diet and with a shortened intervention of 4 weeks. Also, because of the anticipated rapid weight loss, a 10 week weight maintenance phase with continued supplementation was included. Although there was a greater than two-fold increase of EPA and DHA in the treatment group, there were no significant differences in outcome measures between the two groups after 4 weeks of weight loss. However, after a further 10 weeks of supplementation during weight maintenance, there was a significant reduction in anthropometric measurements, apart from fat free mass, in the treatment group but not the placebo group. The differences between the two groups were not significant.

The final clinical trial (Chapter 6) investigated potential benefits of loading the body cells/membranes with LCω-3PUFA prior to following a weight loss program. The protocol for the trial was similar to the previous two, but commenced with 4 weeks of prior-supplementation with fish oil or placebo in the treatment and placebo groups, respectively, while consuming their usual diet. This was followed by 4 weeks of dietary intervention where both groups again consumed a VLED with MRs plus continued supplementation. The same measurements were taken as for the previous trials. After 4 weeks of prior-supplementation there were no significant differences in outcome measures for either group. However, at 8 weeks a significant 3-way interaction between time, group and gender was observed for percentage reduction in weight and BMI, suggesting a significant effect of LCω-3PUFA for the fish oil group. There was also a significant reduction in percentage weight loss for females in the fish oil group. These results suggest that prior-supplementation with LCω-3PUFA, followed by supplementation with LCω-3PUFA and a VLED regimen may assist weight loss.
Chapter 1:

General Introduction
1.1 Obesity

1.1.1 Classifications of obesity and risk to health

For more than 50 years obesity has been officially classed as a disease (1). More recently it has been suggested that it is possible to be obese and healthy (2) however, research in this area remains sparse. Obesity is defined as a chronic condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that the health of individuals may be impaired (3; 4) and it is associated with significant morbidity and mortality (5). Obesity is classified by a body mass index (BMI) of ≥30 which is calculated by dividing weight in kilograms by the square of height in metres (kg/m$^2$). Overweight, which is a BMI of 25 – 29.9 kg/m$^2$, carries an increased risk to health; obesity with a BMI of 30 – 34.9 kg/m$^2$, carries a moderate risk to health; a BMI of 35 – 39.9 kg/m$^2$, carries a severe risk to health; morbid obesity with a BMI of ≥40 kg/m$^2$, is a very severe risk to health (6).

The obesity related health problems of moderate risk include respiratory difficulties, chronic musculoskeletal problems, skin problems and infertility. Higher risk health problems include cardiovascular problems such as hypertension, stroke and coronary heart disease (CHD), conditions associated with insulin resistance such as Type 2 Diabetes (T2D), and certain types of cancers, particularly hormone related and large-bowel cancers (3; 7). With morbid obesity the blood supply to the adipose tissue can reach 15-30% of cardiac output which can lead to high blood pressure and congestive heart failure (8).

1.1.2 Prevalence and cost of obesity

The prevalence of obesity has been rising exponentially worldwide, and in 2008 it was estimated that 1.5 billion people, 20 years and over, were overweight and approximately 500 million were obese (9). In Australia the prevalence of obesity has more than doubled over the past 20 years (10). Between 1980 – 1999/2000, in adults aged 25 - 64 years, it increased from 9 to 17% in males and from 8 to 20% in females and, by 2001, 2.4 million Australians aged ≥18 years were classified as obese (11). By 2008 it was estimated that obesity had increased to 3.8 million Australians with a cost to the Australian economy of $58 billion, including $8.3 billion in financial costs (12). By 2009 Australia had the 5th highest rate of obesity compared to all countries belonging to the Organisation for Economic Co-operation
and Development (OECD) \(^{10}\). Based on past trends it is projected that, by 2025, 6.9 million Australians are likely to be obese \(^{12}\). Such a rapid rise in the number of obese who might require treatment for obesity related health problems will place a significant burden on the health services of the country unless effective interventions are put in place.

1.1.3 Recording body measurements

An increase of approximately 20% of body weight above normal produces a 100% increase in body fat. \(^{13}\). It is important to accurately record such changes as body fat measurements can be used to assess risk for disease and to determine success of weight loss interventions \(^{14}\), however determining the amount and location of adipose tissue in the body is not easy. While BMI can be used to compare the prevalence of obesity within and between populations \(^{15};^{16}\), there is some difference of opinion on the use of BMI as a guide to determining the degree of risk to health. Although BMI has a high correlation with adiposity it does not identify the distribution of fat in the body \(^{17}\) or differentiate between fat mass (FM) and muscle mass (MM), for example the increased muscle mass of athletes giving them a high BMI.

**Waist circumference:** It has been suggested that a measure of waist circumference more accurately reflects intra-abdominal FM and total body fat, with a larger waist circumference indicating a greater burden of ill health than BMI \(^{18};^{19};^{5};^{7}\). However, as the location and size of fat deposits are associated with specific health risks \(^{14}\) waist circumference measurements should be used in combination with other methods of measurement.

**Skinfold thickness:** As 40-60% of total body fat is located/dispersed just beneath the skin it can be directly measured with calibrated callipers \(^{20}\). Though portable and inexpensive to use, reproducibility of results taken by different people could be a problem. If multiple sites on the partially clothed body are measured, it could be considered invasive \(^{20}\).

**Bioelectrical impedance:** Bioelectrical impedance analysis (BIA), an indirect measure of body composition, estimates total body water (TBW) by using an electric current to measure resistance and reactance of the body tissue.
Resistance (impedance) is low in lean tissue (which contains intracellular fluid and electrolytes that act as conductors of electricity) but high in fat tissue and is proportional to TBW. The bioelectrical impedance machine then calculates FM and fat free mass (FFM) using mathematical formulae based on measurements of height, weight, resistance and reactance \(^{(21; 22; 23)}\). Bioelectrical impedance is quick and simple to use, non-invasive, free from discomfort and it improves the accuracy of predicting TBW and FFM compared to other traditional anthropometric techniques \(^{(24)}\).

**Other methods:** Some methods for measuring body composition require large, expensive specialist equipment and qualified people to operate the machines, such as for Magnetic Resonance Imaging, Computer Tomography, Dual-Energy X-Ray Absorptiometry (DEXA), and Body Density – underwater weighing. DEXA cannot be used with people who have BMI > 35 because of technical limitations of DEXA with obese persons \(^{(25)}\).

### 1.2 Reducing body weight/fat mass

#### 1.2.1 Weight loss

It has been suggested that the health risks that are associated with overweight and obesity can be significantly reduced with a weight loss of between 5 – 10\% \(^{(26; 27)}\). Weight loss studies have shown that loss of adipose tissue (fat mass) through intentional weight loss results in decreased metabolic abnormalities \(^{(28; 29)}\), a decrease in circulating levels of proinflammatory biomarkers \(^{(30; 31)}\) and an increased expression of anti-inflammatory molecules \(^{(32)}\). According to the Dietitians Association of Australia, Australian women are spending $400 million per annum trying to lose weight \(^{(33)}\). They also report that 83\% of individuals who successfully lose weight regain that weight, plus more, within 2 years \(^{(33)}\). It would appear that the current strategies for weight management are not working.

#### 1.2.2 Reducing energy intake

While there is a plethora of popular weight reducing diets ranging in energy density and composition, with varying levels of immediate and long-term effectiveness and sustainability, there appear to be few effective strategies for losing weight intentionally and to reliably sustain the weight lost \(^{(34)}\). The traditionally preferred
dietary intervention for bringing about changes in weight and encouraging long term healthful eating is the modification of eating behaviours to healthful practices which include recommended portion control and energy restriction. Some popular diets are based on these guidelines, eg. Weight Watchers. However, with many popular diets energy intake is reduced by limiting consumption of one macronutrient - carbohydrate (CHO), protein or fat - which results in a greater intake of another. Thus, a low CHO diet may also be a high protein diet, eg. The Atkins Diet or The Zone Diet, or a low fat diet may increase the consumption of carbohydrate with a low glycaemic index (GI) foods, eg. The Low GI Diet. With these popular diets there is an increased risk of an inadequate intake of micronutrients which could result in nutritional deficiency. An extensive body of research has investigated the effectiveness of different weight loss diets and strategies, too numerous to discuss here in detail. However, to understand the complexities of dietary weight loss, the strengths and limitations of some of the most frequently used options will be briefly addressed.

### 1.2.3 Popular weight loss diets

A meta-analysis comparing the effectiveness of low-CHO diets versus low-fat diets investigated 5 trials involving 447 individuals. The authors reported that at 6 months there was a greater weight loss in individuals in the low-CHO group compared to the low-fat group but, by 12 months, the difference in weight loss between the two groups was no longer obvious. Despite the similarities in weight loss, other benefits that differed between the two groups were significant differences in blood lipids. Triglyceride (TG) and high density lipoprotein-cholesterol (HDL-C) values changed more favourably in the low-CHO diets, while total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) values changed more favourably in the low-fat diets. Similar findings for increased HDL-C and reduced TG were reported in a meta-analysis of 17 clinical trials on low-CHO diets.

A comparison of 4 popular, commercial weight loss programs (Dr Atkins New Diet Revolution, The Slim-Fast Plan, Weight Watchers Pure Points and ‘Eat yourself Slim’ Diet) involving 300 obese females in 5 different locations, showed that the reduction in weight over 6 months was not significantly different between the programs. Comparison of another 4 popular diets (Atkins, Ornish, Weight Watchers and Zone diets), initially involving 160 males and females, found that
reductions in body weight over 12 months were statistically significant but with no significant differences between the groups (36).

1.2.4 Very low energy diets with meal replacements

It would appear that differences in weight loss are influenced by energy restriction rather than by a particular nutrient or food group (44), with energy restriction achieved through a reduction in food intake. Dietary compliance when food intake is reduced can be difficult. To successfully lose weight a person has to persistently resist the temptation to eat more food or to consume indulgence foods. To manage such dietary change, strong self control is required. Carels et al. (2003) found that unsatisfactory weight loss and attrition during weight loss programs is associated with diminished self control (45). The satiating effects of a high protein diet can also be found with meal replacements (MRs) (46) in very low energy diets (VLED). MRs also facilitate weight loss because food choices are restricted. In Australia there has been a surge in the use of MRs for weight loss, with sales of $175 million in 2006 peaking at $259 million in 2008, and then declining to $208 million in 2011 (47).

Not only do MRs provide recommended daily intakes of micronutrients, they can result in rapid weight loss, eg. >15 kg over 8 weeks (48). Using MRs, studies have reported a weight loss of between 8.8% and 20% in 8 – 12 weeks (49; 50; 51; 52). A study comparing a VLED using MRs over 4 weeks with a slower LED of healthy eating over 12 weeks for weight loss in obese individuals observed a 7% weight reduction with the VLED and a 3.7% weight reduction with the LED (53) (Appendix 17). The possibility of weight rebound after following a VLED is discussed in 1.2.7 (below).

1.2.5 Pharmacotherapy

When weight loss through diet is unsuccessful, pharmacotherapies offer an alternative solution, with orlistat and sibutramine the favoured options (54). Sibutramine is a reputable inhibitor of noradrenalin, serotonin and, to a lesser extent, dopamine in the brain. It reduces food intake by producing feelings of satiety (55) and stimulates thermogenesis (54). A meta analysis of 29 randomised placebo-controlled trials on the effectiveness of sibutramine, reported a pooled mean weight loss of -4.45 kg at 12 months (56). Orlistat inhibits gastrointestinal
lipases which are needed to absorb dietary fat. Thus, by reducing fat absorption, energy intake is decreased\(^{(55)}\). A meta analysis of 22 randomised placebo-controlled trials on the effectiveness of orlistat and with 12 month outcomes, reported a pooled mean weight loss of -2.89 kg. Both treatments are associated with side effects. Sibutramine increases heart rate and blood pressure, and Orlistat is associated with flatulence and other negative gastrointestinal side effects\(^{(57,54)}\). Neither one is suitable for the long-term management of obesity.

### 1.2.6 Comparing weight management strategies

Franz et al. (2007) published a systematic review and meta-analysis of clinical trials comparing the effectiveness of the different weight management strategies on weight loss and management which included a follow-up at 1-year after the weight loss\(^{(58)}\). Results for diet alone (51 studies) or using MRs (7 studies), orlistat (13 studies) or sibutramine (7 studies) to assist weight loss and management were reported at 6 and 12 months. For diet alone, mean weight reduction at 6 months was -4.9 kg (-5%) which was maintained at 12 months. Weight loss for the other 3 strategies was much higher, -8.6 kg (-9.6%), -8.2 kg (-8.4%), and -8.3 kg (-8%) for MRs, sibutramine and orlistat respectively. At 12 months there was no change in mean weight in the sibutramine and orlistat studies, but there was weight regain of 2 kg in the MR studies\(^{(58)}\). While this review does give an indication of weight loss achieved and maintained, it should be noted that there was a difference in the duration of the treatment, both within and between the different strategies, which would have influenced the outcomes.

### 1.2.7 Weight maintenance

There is a commonly held belief that weight lost slowly is better maintained than rapid weight loss. However, it has been reported in Astrup et al. (2000) that a greater initial weight loss, usually achieved in the first 2 - 4 weeks, can improve supported long-term weight maintenance\(^{(59)}\). A meta analysis of 29 weight loss studies with a 5-year follow up showed that successful VLEDs led to significantly greater weight loss maintenance than hypoenergetic balanced diets\(^{(60)}\).

Successful long-term weight maintenance is defined as intentional weight loss of at least 10% of body weight and maintaining that weight for at least one year\(^{(61)}\). The majority of people who seek treatment for obesity can lose the weight but only a
small proportion are able to maintain their new dietary intake behaviour and prevent weight regain \(^{(62;33)}\).

Thus, it would appear that no current dietary weight loss treatment for obesity results in reliably sustained weight loss. This is possibly the result of compensatory metabolic processes which resist the maintenance of altered body weight \(^{(63)}\). Leibel et al (1995) observed a persistent reduction in total energy expenditure in individuals who had reduced their body weight by 10% or more, as if attempting to return the body to its previous weight \(^{(63)}\). It has been suggested that low metabolic rate and low ratio of fat to CHO oxidation predict body weight gain \(^{(64)}\). Compared to a control group, individuals who had lost weight and maintained it had a higher respiratory quotient (RQ) suggesting low rates of fat oxidation with a possible propensity to regain weight. After discontinuation of a low-energy diet, an elevated post-absorptive RQ shows that the endogenous lipid oxidation is low, a condition favouring weight regain \(^{(65)}\). Data supports the hypothesis that a reduced ability to oxidise fat is an important factor predisposing individuals to obesity \(^{(66)}\).

Thus, the search for improved weight management strategies remains, requiring an understanding of the complexity of obesity and the adipose organ.

### 1.3 Adipose tissue

Obesity is characterised by an increase in adipose tissue. The adipose organ is composed of two functionally different tissues, brown and white adipose tissue. Brown adipose tissue is involved in adaptive thermogenesis \(^{(67)}\) or energy dissipation, a function that is the opposite of white adipose tissue which is involved in the storage of energy \(^{(68)}\). This thesis will focus on white adipose tissue.

#### 1.3.1 What is adipose tissue?

Adipose tissue is a connective tissue made up of different types of cells with specific functions which are largely divided into two fractions, the adipocyte fraction (AF) and the stroma vascular fraction (SVF). The AF is composed of mature adipocyte cells with the main function of lipid storage. The SVF is composed of preadipocytes, mesenchymal stem cells, macrophages, endothelial cells and fibroblast which can be involved in the regulation of adipose tissue inflammation \(^{(69;70)}\). Both AF and SVF secrete a multiplicity of protein signals and factors, termed
adipokines or adipocytokines, which influence such biological functions as insulin sensitivity, inflammation, blood pressure, lipid metabolism, energy homeostasis and appetite (71; 72).

An organ with great plasticity, adipose tissue provides an almost unlimited storage site for energy consumed in excess of requirements (73; 74), with its size determined by the storage and release of fatty acids (FA)s in the adipocytes (75; 76). The excess energy is assimilated by fat cells and is stored as TG which makes up over 85% of adipose tissue (68) and, as the excess continues to grow, the adipocytes are able to change their diameter 20-fold and their capacity by up to several thousand-fold.

Adipose tissue provides not only for storage but it is involved in sensing the nutritional and metabolic status of individuals through several different signalling pathways, and influences the metabolism of carbohydrates, proteins and lipids (77). It is able to regulate energy metabolism as it is not only able to respond locally to metabolic stimuli by altering the expression of genes important in the mobilisation or storage of energy, but it can also activate signals to other tissues and systemically regulate energy balance (78).

1.3.2 Adipocytes

Fully differentiated adipocytes are formed from fibroblastic precursor cells (preadipocytes), or possibly from immature myoblast precursor cells (75). They are a critical component of metabolic control and endocrine organs (79); they are also a major site for the secretion of FA as well as cholesterol, retinol, prostanoids and steroid hormones (80). The adipocytes release the enzyme lipoprotein lipase (LPL) which breaks down circulating TGs to FA which are then stored in the adipocytes following uptake and re-esterification (80). Both adipocytes and muscle cells are known as the main source of synthesised and secreted LPL (81).

1.3.3 Adipocyte size and adipocyte number

Adipocytes represent between one- and two-thirds of the total number of cells in adipose tissue with the remainder composed of blood cells, endothelial cells, macrophages and adipose precursor cells of varying degrees of differentiation (82). Thus, adipose tissue mass is influenced by the number of adipocytes and their volume (83; 84; 8) and expansion can occur either by increasing the volume of pre-
existing adipocytes (hypertrophy) or by the generation of new small adipocytes (hyperplasia) \(^{(68, 85, 86)}\). The acquisition of fat cells occurs by replication and differentiation of preadipocytes \(^{(85)}\). Death occurs through preadipocyte or adipocyte necrosis or apoptosis \(^{(85, 87)}\).

There are differences of opinion on whether fat cell numbers increase with weight gain and subsequent variation in weight is determined by adipocyte volume. Studies have reported a decrease in adipocyte volume with significant weight loss but no reduction in adipocyte number \(^{(88)}\), and a significant increase in adipocyte volume with significant weight gain but no changes in adipocyte number \(^{(86)}\).

1.3.4 Adipose tissue development in childhood

The development of adipose tissue occurs at an early age, commencing in utero in the second trimester of pregnancy \(^{(89)}\) increasing in the third trimester and then extensively in early postnatal life \(^{(90, 91)}\). Thus, it is suggested that the pathway to obesity in many individuals begins before birth \(^{(92)}\) and that adipose tissue development in these early months is predictive of subsequent overweight and obesity \(^{(90)}\). For example, in a study of 70 Caucasian children it was reported that children’s BMI at 8 years could be positively predicted by their BMI at 2 years of age and age of adiposity rebound \(^{(93)}\). A cross-sectional study conducted in 1979 by Knittle et al., examined the increase in adipocyte size and number in children in different age groups. They reported that from 6 months to 1 year of age, increments in fat depots occur primarily because of increases in cell size with only minor contributions by cell number until 2 years of age. Between the ages of 2–10 years there was no significant change in cell number in non-obese children but they observed significant increases after that. However, in obese children there were significant increases in cell number in all age groups \(^{(94)}\). It has been suggested that disturbances to the nutrient supply to the foetus can affect adipocyte development and alter the way that it functions which can lead to excess body fat accumulation after birth \(^{(91)}\). It has also been suggested that interventions to inhibit this early increase in fat mass have the potential to interrupt this path to obesity \(^{(91)}\).
1.3.5 Adipose tissue development in adulthood

In adulthood there is a very low turnover rate of adipose cells supporting the finding that foetal and early postnatal periods are crucial windows in the development of adipose tissue depots (91). Studies have shown that adipocyte number is largely set towards the end of childhood and that the total number of adipose cells cannot be reduced through weight loss by dieting (95; 96) so that changes to adipose mass in adulthood can be attributed to changes to adipocyte volume. However, the use of $^{14}$C level in DNA to date the birth of cells has revealed that new adipocytes are constantly formed and these replace the adipocytes that have died with a turnover of 50% of adipocytes in subcutaneous fat mass every 8 years (88). It has been suggested by Arner et al. (2010) that when there is extended periods of weight gain in adults this might result in an increase in adipocyte number. While this has been observed in animal studies it has not been confirmed in humans. However, the number of adipocytes in adults does not decrease, even after prolonged weight loss (86).

The size of adipocytes within the adipose tissue is important because increased adipocyte size correlates with serum insulin concentrations, insulin resistance, and increased risk of developing T2D (97; 98). Also, adipocyte hypertrophy may impair adipose tissue function by inducing local inflammation, mechanical stress and altered metabolism (99; 100).

1.3.6 Visceral and subcutaneous adipose tissue

Adipose tissue can accumulate in different places in the body, e.g., visceral (internal) and subcutaneous (peripheral) depots (91), and there are distinct metabolic variations between visceral and subcutaneous deposits (68). Subcutaneous adipose tissue (SAT) is characterised by an accumulation of fat in the hypodermis, largely in the thighs and buttocks (101). Visceral adipose tissue (VAT), known as intra-abdominal fat, is composed largely of omental, mesenteric and retroperitoneal fat masses (102). Thus, visceral obesity is characterised by a definite accumulation of fat in the abdominal cavity. Visceral adiposity is measured as waist circumference, with a waist circumference > 102 cm in males and > 88 cm in females indicating increased risk of disease (103; 104; 105; 106; 107).
VAT and SAT are quite different in their sensitivity to lipolytic or antilipolytic hormones. It has been suggested that VAT, which is drained by the portal venous system directly connecting to the liver, is more sensitive to weight reduction than SAT because the main components of visceral abdominal fat, the omental and mesenteric adipocytes, are reported to be more metabolically active and have a higher lipolytic rate \(^{(108;102)}\). It has been shown that excess VAT is accompanied by elevated TGs, glucose and blood pressure, and reduced HDL-C \(^{(109)}\), and is linked to increased mortality, insulin resistance and T2D, hyperlipidemia, hypertension and atherosclerosis \(^{(110;111;103;106)}\).

VAT is also linked to the secretion of pro-inflammatory cytokines \(^{(109)}\). A study by Harman-Boehm et al. (2007) reported that the infiltration of macrophages into adipose tissue, triggering the inflammatory molecules and pathways, was enhanced in omental VAT versus SAT \(^{(112)}\). There is an obvious link between adipose tissue and non-infectious inflammation which will now be addressed.

### 1.4 Inflammation

Inflammation is the body’s natural response to injury or irritants. Known as acute inflammation, this occurs over very short periods of time, from seconds to days. However, when irritants continue to build up, for example, in response to smoking or consuming an unhealthy diet, the immune system can lose control and inflammation becomes chronic and can lead to damage of the heart, joints, brain cells, promote resistance to insulin and lead to T2D, cancer or stroke \(^{(113)}\). Thus, chronic low-grade inflammation, of the type associated with obesity, grows slowly and can lead to serious metabolic breakdown with implications for long-term health.

#### 1.4.1 Obesity and inflammation

The contribution by adipose tissue to inflammation was originally observed during acute infections and raised the possibility that there was a similar response in obesity \(^{(30)}\). Subsequently, it was observed that the induction of inflammatory mRNA transcripts in adipose tissue originated either in adipocytes or the resident macrophages that surround them. These adipocytes and resident macrophages contribute to local adipose inflammatory output independently, as well as together for a synergistic effect \(^{(30)}\).
1.4.2 Macrophages

Macrophages are cells that are produced by differentiation of monocytes in tissues. Macrophage infiltration of adipose tissue is implicated in the metabolic complications of obesity and macrophages are part of the inflammatory cascade within the expanded tissue mass \(^{(114)}\). Macrophage numbers and/or macrophage inflammatory gene expression in adipose tissue is positively correlated with adipocyte size and BMI in mice, and it is negatively correlated with weight loss in obese humans \(^{(115)}\).

1.4.3 Adipose tissue inflammation

It is now well established that increased adipose tissue mass contributes directly to an increase in systemic inflammation \(^{(30)}\). A possible suggestion on how this occurs is that there is a blood capillary close to each adipocyte providing a small blood flow to the adipose tissue; the greater the adipose tissue mass, the greater the blood supply \(^{(8)}\). As body weight continues to grow an increasing adipose mass outgrows its vascular supply and, with insufficient blood and oxygen, the frequency of adipocyte cell death increases dramatically \(^{(80)}\). This appears to stimulate the recruitment and activation of macrophages, derived from circulating monocytes \(^{(115)}\). Chemokine ligand 2 (CCL2) and chemokine receptor 2 (CCR2) recruit the monocytes \(^{(116; 117)}\) which attach to activated endothelial cells and then transmigrate through vessel walls to differentiate into macrophages \(^{(116; 119; 120; 121)}\). More than 90% of the macrophages gather around the dead adipocyte where they fuse to form syncytia that sequester and scavenge the residual free adipocyte lipid droplets and form multinucleate giant cells, a hallmark of chronic inflammation \(^{(115)}\). The accumulation of adipose tissue macrophages around dead adipocytes appears to make a significant contribution to obesity-induced inflammation involving extensive cell signalling with mature adipocytes and preadipocytes \(^{(72; 119; 120)}\). This process is illustrated in Figure 1.1 and 1.2 (adapted from Neels and Olefsky 2006) \(^{(119)}\). Another suggestion is that because the adipocyte is programmed to store a very tightly regulated amount of fat, adipocytes might become stressed when they need to store additional fat during weight gain and this could activate the secretion of chemokine attracting monocytes and macrophages, adding to adipokine production \(^{(122)}\).
Figure 1.1: Monocytes attach to activated endothelial cells, transmigrate through the cell wall, differentiating into macrophages which accumulate around adipocytes.

(Adapted from Neels and Olefsky, 2006). Permission to copy and communicate this work has been granted by the copyright owner, Journal of Clinical Investigation (Appendix 1).

Figure 1.2: The macrophages accumulate around the dead adipocytes and clear the necrotic debris.

(Adapted from Neels and Olefsky, 2006). Permission to copy and communicate this work has been granted by the copyright owner, Journal of Clinical Investigation (Appendix 1).
1.4.4 Chronic Inflammation

When chronic inflammation occurs in metabolically important organs such as the liver and adipose tissue it has a crucial role in the development of many chronic metabolic diseases such as T2D and CVD \(^{(123; 79; 87)}\). The increasing number of macrophages in obese adipose tissue are responsible for most of the adipocytokine production in that tissue \(^{(124)}\).

Adipocytes, like macrophages, are sensitive to cytokine-mediated inflammatory signals. By expressing a host of receptors, adipocytes can sense the presence of pathogens and inflammation and the stimulation of the receptors activate multiple inflammatory signal transduction cascades which induces and secretes inflammatory cytokines and acute phase reactants \(^{(30)}\). For example, when the toll-like lipopolysaccharide (LPS) receptor, TLR4, is stimulated with endotoxin, it activates certain NFκB signal transduction pathways \(^{(125)}\). These pathways then induce the expression of inflammatory mediators such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) \(^{(30)}\).

1.4.5 Inflammatory adipocytokines

Adipose tissue responds to metabolic signals through the release of a large number of proteins termed adipokines such as leptin and adiponectin and cytokines such as TNF-α and IL-6 \(^{(114; 79)}\), now known collectively as adipocytokines. Adipose tissue macrophages are a predominant source of TNF-α and a significant source of IL-6 \(^{(124; 126; 127; 79)}\), with IL-6 also being obtained from adipocytes and non-macrophage stromal vascular cells. Adipose tissue also secretes regulators of lipoprotein metabolism, such as LPL, apolipoprotein E (apoE) and cholesteryl ester transfer protein (CETP) \(^{(71)}\).
1.4.5.1 Tumour necrosis factor-α

TNF-α is a signalling molecule which is produced almost entirely by the adipose tissue macrophages \(^{(124)}\) (Figure 1.3), and has numerous effects on the metabolism of adipose tissue and other peripheral organs, e.g. it can alter glucose homeostasis and lipid metabolism \(^{(78)}\).

Serum or plasma concentrations of TNF-α in healthy adults are typically 0.01 – 2.0 pmol/L \(^{(128)}\). Studies in humans have reported that levels of TNF-α mRNA are increased in the abdominal SAT of the obese with the expression of TNF-α directly related to the degree of obesity \(^{(129}; 130; 131; 132)}\). Levels of TNF-α are also increased in plasma \(^{(133)}\) and in serum \(^{(134)}\). With weight loss in the obese, reductions in elevated levels of TNF-α have been reported in adipose tissue as well as in plasma \(^{(129}; 133)}\) or serum \(^{(135)}\).

It has been suggested that an obesity-related increase in circulating and adipose tissue TNF-α levels has the potential to limit a further increase in adipocyte volume and number through a variety of mechanisms, such as a reduction in lipogenesis, induction of insulin resistance, impairment of preadipocyte differentiation, and
increased apoptosis of preadipocytes and adipocytes \(^{(136)}\), and the induction and increase of adipocyte lipolysis \(^{(79)}\).

### 1.4.5.2 Interleukin-6

IL-6 is an inflammatory mediator \(^{(137)}\) that is abundantly expressed in adipose tissue \(^{(135}; 138)}\) (Figure 1.3). Over 90% of IL-6 expressed in adipose tissue is produced by cells other than adipocytes, such as macrophages and non-macrophage stromal vascular cells \(^{(124}; 79)}\). Serum or plasma concentrations of IL-6 which are typically 0.01 – 2.0 pmol/L in healthy adults \(^{(128)}\) are increased in obesity with production greater in the omental VAT than SAT depot \(^{(137}; 139}; 79)}\) and they decrease with weight loss \(^{(139)}\), IL-6 is believed to be involved in the regulation of glucose and lipid metabolism \(^{(137}; 140)}\). IL-6 reduces lipoprotein lipase (LPL) activity in adipose tissue \(^{(137}; 139)}\) and this results in increased circulating lipid levels \(^{(141)}\).

IL-6 is also a primary determinant of hepatic production of C-reactive protein (CRP) \(^{(142}; 143}; 144}; 80)}\). Elevated concentrations of IL-6 and CRP have been shown to predict the incidence of CHD in healthy individuals \(^{(145}; 146)}\). A study involving 14,916 healthy males reported that baseline levels of IL-6 were significantly elevated in apparently healthy men at risk for future myocardial infarction \(^{(147)}\).

### 1.4.5.3 C-reactive protein

CRP is an acute-phase protein found in the blood in response to inflammation. CRP is produced largely in the liver under the regulation of IL-6 \(^{(142}; 148)}\) (Figure 1.3). It has also been reported that plasma CRP levels are considerably higher in obese and overweight people compared to those of normal weight and that they decrease with weight loss suggesting a link between CRP and obesity associated risks for CVD and diabetes \(^{(149}; 150}; 151}; 138)}\), also that a state of low-grade systemic inflammation exists in overweight and obese individuals \(^{(152)}\). In healthy individuals, levels of CRP are normally <10 mg/L, with the mean level increasing slightly with age \(^{(153)}\). Acute inflammatory conditions are associated with high sensitivity (hs) CRP concentrations of >10 mg/L \(^{(154)}\).
CRP has become the primary independent marker of inflammation used to improve the detection of cardiovascular risk \((155; 30)\). In a number of clinical settings CRP has been shown to predict myocardial infarction, stroke and cardiovascular death \((156; 146)\) in healthy males \((157)\) and healthy females \((158)\). Not only has CRP been shown to independently predict future cardiovascular disease (CVD) events in individuals without known CVD (as reviewed by Clearfield, 2005) \((159)\), it has also been reported that determining the future risk of myocardial infarction is increased when baseline CRP levels are added to the predictive values of total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) \((160)\).

### 1.4.5.4 Leptin

Leptin is an adipocyte-secreted hormone with mature adipocytes secreting most of the leptin \((139; 124)\). Leptin expression and secretion is correlated with fat mass in humans \((161; 162; 139; 163)\), in mice and in rodents \((164; 165)\). Levels are higher in obese females compared to males, even after adjusting for body mass, which is partly due to the higher production of leptin by SAT in females and stimulation by estrogens \((166; 167)\). Not only does the concentration of leptin in adipose tissue and plasma correlate positively with adipose tissue mass, but also with adipocyte size, and TG concentrations \((167)\). Within individuals, larger adipocytes contain more leptin than smaller adipocytes \((168)\).

Leptin influences the modulation of glucose and lipid metabolism \((169)\) and, as humans become more obese, leptin’s role in regulating body weight becomes more complex. Leptin transmits a satiety signal through the bloodstream to the brain \((170)\) which was thought to signal the brain to inhibit food intake which would then decrease weight \((171)\). However, leptin levels do not rise in response to individual meals \((172)\), thus leptin is unlikely to serve as a meal-related satiety signal (Ahima 2000), and changes in leptin expression in response to fasting and feeding are out of proportion to corresponding changes in body weight or body fat \((173; 174)\).

### 1.4.5.5 Adiponectin

Adiponectin is an adipocyte-derived peptide that is synthesized and secreted exclusively by the adipose tissue \((175)\) (Figure 1.3) and has anti-inflammatory and insulin-sensitising properties. Adiponectin concentration is decreased with increasing obesity \((176; 177; 178)\), with concentrations in males lower than in females.
It has been suggested that this response of adiponectin to increased total body FM is due to its downregulation by the increased secretion of other adipocytokines, namely TNFα and IL-6 through the NFκB pathway. However, while studies have reported an increase in plasma adiponectin with weight reduction, others have shown a decrease in adiponectin with a reduction in weight.

Adiponectin regulates both lipid and glucose metabolism. It can promote hepatic FA oxidation thereby reducing lipid accumulation in the liver. It has also been shown to be independently associated with a reduced risk of T2D in healthy individuals providing protection against obesity-associated diseases.

1.5 Lipids

1.5.1 Early observations on influences of LCω-3PUFA and CVD

Several decades ago it was reported that, despite a high fat intake, the Inuit of Greenland and Alaska experienced a low incidence of cardiovascular mortality. The source of fat in the traditional Inuit diet was from seal and whale which is rich in long chain omega-3 polyunsaturated FA (LCω-3PUFA). The Inuit were also reported to have decreased serum concentrations of TC, TG, low density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein (VLDL) cholesterol and increased HDL-C levels. While recent studies have cast doubt on the original findings of the low incidence of ischemic heart disease among the Inuit, an interest in investigating the effects of ω3 on health had been initiated.

A review of studies by Calder (2004) now provides substantial evidence that consumption of fish or LCω-3PUFA does reduce the risk of cardiovascular mortality in Western populations.

1.5.2 Composition of lipids

Lipids comprise fats, sterols, phospholipids and waxes. Fats, a sub-group of lipids, are known as TGs and are made up of three FAs attached to a glycerol backbone. TGs make up approximately 90-95% of dietary fat with other main components being TC and phospholipids.
1.5.3 Fatty acids

Fatty acids are hydrocarbon chains with a carboxyl group at one end and a methyl group at the other end \(^\text{[194]}\). These can be saturated fatty acids (SFA), containing no carbon-to-carbon double bonds, monounsaturated fatty acids (MUFA), containing one double bond, or polyunsaturated fatty acids (PUFA), containing more than one double bond in the carbon-to-carbon (acyl) chain. In the diet there is an adequate supply of both SFA and MUFA, and they can also be synthesised by the human body to form non-fat precursors, such as glucose and amino acids, if there is a need \(^\text{[195]}\). PUFA cannot be synthesised by the human body.

1.5.4 Polyunsaturated fatty acids

1.5.4.1 Structure of PUFA

PUFA are identified by the number of double bonds in the acyl chain and the position of the first double bond, counted from the methyl end. For example, 18-carbon fatty acids:

- \(18:2\omega-6\) (Linoleic acid)
  \[
  \omega-12-9-\text{COOH}
  \]
  \(18:3\omega-3\) (\(\alpha\)-linolenic acid)
  \[
  \omega-15-12-9-\text{COOH}
  \]

1.5.4.2 Sources of PUFA

Mammals are unable to synthesise linoleic (LA) and \(\alpha\)-linolenic acids (ALA) because they lack the desaturase enzymes required. Mammals cannot convert oleic acid \((18:1\omega-9)\) into LA \((18:2\omega-6)\) because they would need the enzyme 12-desaturase which is found only in plants. Similarly, LA cannot be converted to ALA \((18:3n3)\) by mammals as they lack the necessary enzyme 15-desaturase, also found only in plants \(^\text{[195]}\). Unable to synthesise these long chain PUFAs (LCPUFA), humans must consume foods containing ALA (\(\omega-3\)) and LA (\(\omega-6\)), obtained largely from seeds and nuts. Linseed and rapeseed are sources of \(\omega-3\), and seeds from
sunflower, safflower, soybean and nuts such as Brazil nut and peanut are sources of ω-6. LA and ALA are then metabolised by desaturation and elongation to make the longer C20 and C22 PUFAs, precursors for the chemical messengers known as eicosanoids. This pathway of desaturation and elongation occurs predominantly in the liver of mammals (196; 194).

1.5.4.3 Metabolism of PUFA

As shown in Table 1.1, dietary LA (18:2ω-6) is converted to γ-linolenic acid (19:3ω-6), then to dihomo γ-linolenic acid (20:3ω-6), and then to arachadonic acid (AA) (20:4ω-6), which is the main precursor for the synthesis of eicosanoids.

20:4ω-6 (Arachadonic acid)

Competing with LA for the same metabolic enzymes, dietary ALA is converted to stearidonic acid (18:4ω-3), then to eicosatetraenoic acid (20:4ω-3), and then to eicosapentaenoic acid (EPA) (20:5ω-3 (Table 1.1) (196; 197). Thence there is a pathway for the further conversion of EPA to docosahexaenoic acid (DHA) (22:6ω-3) through elongation to docosapentaenoic acid (DPA) (22:5ω-3) and then 24:5ω-3 where desaturation then forms 24:6ω-3 which can be translocated from the endoplasmic reticulum to the peroxisomes where limited β-oxidation removes 2 carbons to give DHA (198). EPA and DPA can also be synthesised from DHA by retro-conversion because of the limited peroxisomal β-oxidation (199).
Table 1.1 Metabolism of ω-3 and ω-6 PUFAs\(^{(196;200;201)}\).

<table>
<thead>
<tr>
<th>Linoleic series:</th>
<th>Alpha-linolenic series:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-6</td>
<td>ω-3</td>
</tr>
<tr>
<td>18:2 ω-6 (Source: plants in the diet)</td>
<td>18:3 ω-3 α-linolenic acid</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>Δ6 desaturase</td>
</tr>
<tr>
<td>18:3 ω-6</td>
<td>18:4 ω-3</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>18:3 ω-6</td>
<td>C18-20 elongase</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>20:3 ω-6</td>
<td>20:4 ω-3</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
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<tr>
<td>20:4 ω-6</td>
<td>Δ5 desaturase</td>
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<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>20:5 ω-3 Eicosapentaenoic acid</td>
</tr>
<tr>
<td>↓</td>
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<tr>
<td>22:4 ω-6</td>
<td>C20-22 elongase</td>
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<td>↓</td>
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<tr>
<td>22:4 ω-6</td>
<td>22:5 ω-3</td>
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<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>24:4 ω-6</td>
<td>C22-24 elongase</td>
</tr>
<tr>
<td>↓</td>
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<tr>
<td>24:4 ω-6</td>
<td>24:5 ω-3</td>
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<tr>
<td>↓</td>
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<tr>
<td>24:5 ω-6</td>
<td>Δ6 desaturase</td>
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<tr>
<td>24:5 ω-6</td>
<td>24:6 ω-3</td>
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</tr>
<tr>
<td>22:5 ω-6</td>
<td>β-oxidation</td>
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<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>22:5 ω-6</td>
<td>22:6 ω-3 Docosahexaenoic acid</td>
</tr>
</tbody>
</table>

### 1.5.5 Dietary intake of ω-6 and ω-3

It has been suggested that over 150 years ago the diet of humans contained a balance of ω-6 and ω-3, with a ratio of 1:1, but the Western diet of today is deficient in ω-3 with a suggested average ω-6 to ω-3 ratio of between 10:1\(^{(194)}\) and 20:1\(^{(202;203)}\). In Australia, the average daily consumption of ω-6 and ω-3 by adults is 10.8 g/d and 1.17 g/d respectively, a ratio of over 9:1\(^{(204)}\). The enzyme Δ6-desaturase that catalyses the first step in the conversion of linoleic acid (LA) and α-linolenic acid (ALA), has a greater affinity for the former so that under the same concentrations of LA and ALA, more AA is formed than EPA\(^{(197)}\). With higher concentrations of LA in the diet, the metabolism of ω-6 places even greater demands on the desaturase and elongase enzymes\(^{(205)}\). To compensate for this dietary imbalance, increasing amounts of LCω-3PUFA
through the consumption of oily seafood such as salmon, tuna, sardines, sea bream, and mackerel, or fish oils partially replaces AA in cell membranes with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This, in turn, leads to decreased production of AA derived mediators which is one of the potentially beneficial anti-inflammatory effects of ω-3 FAs (196).

Where oily seafood is not available, supplementation with capsules of LCω-3PUFA from fish oil is a possible option. After consumption, the subsequent appearance of EPA and DHA in plasma lipids, platelets, erythrocyte, leukocytes, colonic tissue and cardiac tissue occurs at different rates and to different extents, possibly depending on gender and age. Near maximal incorporation of EPA and DHA into serum cholesteryl esters occurs within 30 days and into erythrocytes within 56-182 days (199).

1.5.6 Lipid storage and metabolism

Oxidation of fat is not determined by fat intake but rather by the deficit of total energy intake relative to energy expenditure (206). This suggests that fat consumed in excess of daily energy requirements is stored, contributing to increased adipose tissue.

The liver is central to whole-body lipid metabolism (207). In the small intestine, dietary fats are emulsified to form micelles by bile salts from the gall bladder, and are then degraded into FAs and glycerol by pancreatic lipase. They are then re-esterified in TGs and secreted into the lymphatic vessels in the form of chylomicrons which move across the blood vessel membrane and into the blood stream (208; 209). LPL hydrolyses the TGs of the chylomicrons and the FAs produced are taken up by adipocytes for storage or a small fraction is made available as fuel for energy (210).

1.5.7 Function of LCω-3PUFA

It has been suggested that dietary PUFAs function as a partitioner of energy sources away from storage and towards oxidation, thus directing FAs away from TG synthesis and assimilation and towards FA oxidation. LCω-3PUFA also appear to have the unique ability to enhance thermogenesis resulting in a reduction in FA deposition in the body (211).
It has also been reported that ω-3 PUFA can modify appetite as PUFA can interact with neuroendocrine factors that participate in brain-intestinal loop signals that are related to energy metabolism, for example, ghrelin, and leptin. LCω-3PUFA also play an important role in the synthesis of inflammatory mediators (discussed in section 1.6 below).

1.5.8 Gender differences

Recent studies have revealed that conversion of ALA into its longer chain derivatives is not at all efficient in adult humans especially males. Studies have reported that young women convert a greater proportion of ALA to EPA and DHA compared with young men of a similar age. It was also observed in three population-based studies that, compared to males, females had a higher percentage of total FAs as DHA in plasma and adipose tissue FAs. It has been suggested that these sex differences could be attributed to differences in FA metabolism.

1.6 Inflammatory mediators

In humans, inflamed cells contain high proportions of the ω-6PUFA, AA, and low proportions of ω-3PUFA. AA is the precursor of 2-seried prostaglandins and 4-series leukotrienes which are highly active mediators of inflammation. Consumption of fish oil reduces levels of AA and replaces AA in inflammatory cell membranes with EPA, decreasing production of AA derived mediators, with beneficial anti-inflammatory effect of ω-3.

1.6.1 Eicosanoids

The link between FAs and inflammation occurs through the inflammatory mediators known as eicosanoids which are hormones that are derived from oxygenated 20-carbon PUFAs liberated from cell-membrane phospholipids. They include prostaglandins, thromboxanes and leukotrienes, formed from precursor FAs when oxygen atoms are incorporated into the FA chains. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses.
1.6.2 Phospholipids

Before eicosanoid synthesis can occur, the precursor FAs, EPA (20:5ω-3), AA (20:4ω-6) and dihomo-gamma-linolenic acid (DGLA) (20:3ω-6) must be mobilised from membrane phospholipids by an enzyme, usually phospholipase A₂ (228). As there are large amounts of AA in most cell membranes, compared to EPA and DGLA, AA is the principal precursor for eicosanoid synthesis. Prostanoids, a subclass of eicosanoids, consists of prostaglandins (PG) which are mediators of inflammation, thromboxanes (TX) which are mediators of vasoconstriction, and prostacyclins (PGI₂) which are active in the resolution phase of inflammation. Prostanoids are effectors as well as regulators of inflammation (226).

1.6.3 Prostaglandins

Prostaglandins have roles in a wide variety of homeostatic biological functions and inflammation (229). They are lipid mediators that are synthesised in the cell from the essential FAs. Eicosanoids that originate from ω-3 and ω-6 FAs compete for a common enzyme, prostaglandin endoperoxide synthase, better known as cyclooxygenase (COX), which is a key enzyme required for the conversion of AA to prostaglandins (230) prostacyclins and thromboxanes.

1.6.4 Cyclooxygenase and lipoxygenase

It would appear that the consumption of fish oil results in reduced levels of AA with EPA replacing AA in inflammatory cell membranes. This occurs by EPA inhibiting the release of AA from phospholipids by phospholipase A₂ and competitively inhibiting the oxygenation of AA by COX (231), thereby decreasing the capacity of inflammatory cells to synthesise eicosanoids from AA. Thus EPA serves as a substrate for COX and lipoxygenase (LOX), the enzymes that initiate the synthesis of PGs, TXs, PGI₂ and leukotrienes (LTs).

Mast cells contain two types of COX, COX 1 which is expressed constitutively in most tissues, and COX-2 which has a major role in different inflammatory conditions (232). The free AA can be metabolised by COX enzymes to produce the 2-series PGs and TXs, or by LOX enzymes to form LTs and lipoxins (LXs) (233; 232; 195; 196), all of which have key regulatory roles in inflammation immune function and cell proliferation (193).
1.7 Genes

In addition to being a source of energy, dietary fat functions as a regulator of gene expression, impacting on lipid, carbohydrate and protein metabolism (234). Nutrient regulation of gene expression is fundamental in the development of nutritionally related pathophysiologies such as diabetes and heart disease (235).

FAs regulate the activity of several transcription factors such as peroxisome proliferator-activated receptors (PPARs), sterol regulatory element-binding protein (SREBP), and NFκB (236). FAs can also act on gene expression through their effects on enzyme-mediated pathways such as COX and LOX (234).

Ingestion of ω-3 or ω-6 PUFA leads to rapid inhibition of the activation of many proteins/enzymes involved in both CHO metabolism and lipogenesis. Post absorption, dietary FAs are partitioned toward oxidation and away from esterification. This has been clearly shown in animals fed very long chain (VLC) ω-3PUFA leading to enhanced hepatic FA oxidation (a PPARα-stimulated process), reduced TG synthesis and reduced VLDL-TGs (237).

Eicosanoids originating from ω-3 and ω-6 FAs compete for a common enzyme (COX) during their metabolic conversion to the physiologically active PGs, PGI2s and TXs. It follows that the consumption of large quantities of one type of FA will saturate this enzyme and thus restricts the metabolic production of other types of eicosanoids.

1.8 Blood lipids and glucose

1.8.1 Blood glucose

Glucose is the primary source of energy for the cells in the body. The mean normal fasting level of blood glucose in humans is 4 mmol/L. Blood glucose homeostasis is maintained primarily by the hormone insulin which transfers glucose from the blood into the cells and, an inadequate secretion of insulin to maintain normal blood glucose levels, contributes to the development of T2D (238).

It has been suggested that the location of adipose tissue, eg. accumulated visceral fat and not subcutaneous fat, might cause the reduction of glucose tolerance and
lipid metabolism\textsuperscript{(101)}. Intra-abdominal visceral fat, largely composed of omental and mesenteric adipocytes, is closely linked to metabolism in the liver. As VAT increases, FFAs are directed to the liver for enhanced production of glucose, TGs and VLDL\textsuperscript{(239)}. An excessive increase in FFAs to the liver causes overproduction of VLDL which could result in hypertriglyceridemia\textsuperscript{(101)}, and might also result in a reduction in the insulin sensitivity of hepatocytes and disturbed glucose metabolism\textsuperscript{(240)}. This could be the first step in the development of T2D\textsuperscript{(101)}.

The size of the adipocyte within the adipose tissue is also important\textsuperscript{(241)}. A large adipocyte size correlates with serum insulin concentrations, insulin resistance, and increased risk of developing T2D\textsuperscript{(97, 98)}. Obese persons with a few large adipocytes are more glucose intolerant and hyperinsulinemic than persons that have the same degree of obesity but have many small adipocytes\textsuperscript{(97, 98)}.

\textbf{1.8.2 Triglycerides}

High levels of TGs in humans have been shown to be an independent risk factor for CHD\textsuperscript{(242)}. A major cause of overproduction of TGs is an increase in the availability of FA in the liver, with non-esterified fatty acids (NEFAs) contributing the largest fraction to VLDL-TG production\textsuperscript{(243)}. It well established that \(\omega-3\) FAs have a lowering effect on TGs. According to Harris (2006), six studies investigating the effects of \(\omega-3\) FAs on TG metabolism in humans, reported that \(\omega-3\) FA consumption reduced the production rate of VLDL-C, thus establishing that part of the mechanism by which \(\omega-3\) FAs reduce serum TG concentrations is by inhibiting hepatic VLDL-C secretion rates\textsuperscript{(244)}. The mechanisms by which TG synthesis could be reduced by \(\omega-3\) FAs has been explored in animal studies and three possible options have been suggested. These include reduced availability of FAs, increased phospholipid synthesis, and decreased activity of TG synthesising enzymes\textsuperscript{(244)}.

It is recognised that elevated fasting and postprandial plasma TG concentrations increase the risk of CVD. LC\(\omega-3\)PUFA is able to lower both of them\textsuperscript{(245, 246)}. 
1.8.3 Lipoprotein lipase

The enzyme lipoprotein lipase (LPL) is released from adipocytes to elicit the breakdown of circulating TGs to FAs which are subsequently stored within fat cells following uptake and reesterification (80). Both adipocytes and muscle cells are known as the main source of synthesized and secreted LPL (81). TG storage in the adipocyte is determined by the activity of the enzyme LPL.

1.8.4 Cholesterol

Cholesterol is not synthesised by adipose tissue but it is taken up and stored within the tissue (80) and travels in the blood as lipoproteins. Lipoproteins can be categorised as low density lipoproteins, high density lipoproteins and very low density lipoproteins. Chylomicrons are also a category of TG-rich lipoproteins which are formed in the intestine from dietary fat and appear in the blood after a fat-containing meal (247).

1.8.5 Low density lipoprotein cholesterol

LDL-C is identified as a depositor of cholesterol and other lipids into peripheral cells which have an LDL receptor, including the cells of the vascular endothelium. Thus LDL-C has been identified as an independent risk factor for CHD (248; 249).

LDL-C particles are of two sizes and density. A majority of small dense LDL-C particles is associated with increased plasma TG and apoB and decreased HDL-C and apoA1 (250; 251). A predominance of these small LDL-C particles is associated with an increased risk of myocardial infarction, insulin resistance and T2D (250).

Viscerally obese individuals have increased plasma concentrations of small, dense LDL-C particles which are associated with higher risk of CVD (109).

1.8.6 High density lipoprotein cholesterol

HDL-C is an independent, inverse coronary risk factor with recommended plasma concentrations of 1 mmol/L (40 mg/dL) in males and 1.3 mmol/L (50 mg/dL) in females (249). It has been suggested that every 1 mg/dL increase in HDL-C reduces CVD risk by 3% (252). Abdominal obesity is associated with reduced levels of HDL-C (253).
The flow of cholesterol is significantly mediated by HDL-C metabolism, ApoA1 and reverse cholesterol transport which enables the removal of cholesterol from macrophages and its transport to the liver where it is excreted in the form of bile acids and cholesterol (254). Other protective properties of HDL-C are the reduction of oxidation, vascular inflammation and thrombosis, also the improvement of endothelial function (249).

Abdominal obesity is associated with reduced levels of HDL-C (253). However, studies which investigated the effect of intentional weight reduction on HDL-C reported no significant relationship between weight reduction and changes to levels of HDL-C. But further analysis showed that HDL-C levels decreased during the weight loss phase and then increased significantly when weight stabilised at the reduced level (255).

### 1.9 Effects of LCω-3PUFA supplementation

Dietary LCω-3PUFA from fish oils have been reported to have profound effects on lipid metabolism (256). Benefits of LCω-3PUFA include decreased blood TG concentrations, lower blood pressure, decreased thrombosis and cardiac arrhythmias, decreased production of chemoattractants, growth factors, adhesion molecules, inflammatory eicosanoids and inflammatory cytokines; increased nitric oxide production, endothelial relaxation, increase heart rate variability (193).

#### 1.9.1 Effects of LCω-3PUFA on body weight and fat mass

**1.9.1.1 Cross-sectional studies: humans**

There are few studies that have considered the effects of LCω-3PUFA on body weight and FM in humans. However, two long-term prospective studies which were investigating the effect of LCω-3PUFA on the risk of stroke also reported on the effect of LCω-3PUFA on weight. In the Health Professional Follow-Up Study involving 43,671 males, it was observed that the percentage of overweight participants was inversely related to LCω-3PUFA intake through fish consumption (257). In the Nurses’ Health Study involving 79,839 females, higher intakes of fish and LCω-3PUFA were associated with higher rates of obesity (258). It has been suggested that it is possible that the differences in the two studies could be
attributed to gender differences in response to the effects of LCω-3PUFA on weight and FM or limitations with the dietary records used (259). When the FA composition of abdominal adipose tissue of 84 obese individuals was analysed, there was an inverse association between the distribution of visceral abdominal fat and the ω-3 content of adipose tissue (260), while the ω-3 content of the SAT was inversely associated with adipocyte diameter (261), suggesting that higher levels of ω-3 are associated with reduced abdominal obesity (259).

Before conducting intervention trials on the potential benefits of supplementation with LCω-3PUFA on human health, it was important to explore and confirm the efficacy and safety of such supplementation using animal models.

1.9.1.2 Animal studies

The studies that have been conducted in animals have used different fats and oils as well as different concentrations of LCω-3PUFA.

A study compared the influence of dietary ω-3PUFA compared to ω-6PUFA on lipid composition and metabolism in rats over 1 week. Despite a comparable food intake, body weight increments and adipocyte size were significantly smaller in the rats fed ω-3PUFA compared to rats fed ω-6PUFA (262). As the rats fed ω-3PUFA also had reduced concentrations of serum TGs, this could have contributed to the results.

Six groups of mice were fed with perilla oil, soybean oil or lard, or a combination of each of these fats with controlled amounts of EPA and DHA, all of similar energy levels. The study showed that the weight of the epididymal fat pads were significantly reduced in the mice fed EPA and DHA mixed with perilla oil or soybean oil, and narrower areas of visceral fat were observed in all three groups fed EPA and DHA (263).

A study involving mice used a high fat diet to induce weight gain in the animals. Weight gain was lower in mice consuming a diet enriched with EPA/DHA and, in mice consuming diets with the content of EPA/DHA increased from 1 – 12% of dietary lipids, the animals lost weight (264).
In another study, no difference in total body mass was observed when rats were fed fat supplemented diets of either lard or fish oil, despite similar quantities of food being consumed. While there was a significant increase in fat pad mass in both groups, the fat pad mass of the fish oil fed rats was considerably less \(^{(265)}\). In another study involving rats, they were fed diets containing fish oil, olive oil or beef tallow. The fish oil group gained more lean body mass but less fat mass \(^{(266)}\).

As these studies differ considerably in the way they were conducted, particularly with the type and number of animals used, the diets given, the different fats and oils used as controls, and the duration of the feeding, it is not possible to draw firm conclusions about the specific effects of LCω-3PUFA, but it would appear that potentially there are positive outcomes with respect to body weight and fat mass. However, results obtained in animals can be very different to the outcomes with humans. For example, a study comparing the effects of LCω-3PUFAs on serum lipid and lipoprotein concentrations in humans and in different animals, including dogs, mice, rats, rabbits, pigs, hamsters and monkeys, reported that LCω-3PUFAs consistently lowered serum TG concentrations in humans but not in most of the animals \(^{(267)}\).

### 1.9.1.3 Randomised controlled trials: humans

In humans a few studies have investigated the effect of LCω-3PUFA alone, and in combination with an energy-restricted diet, on body weight and FM. After following a control diet for 3 weeks, 6 non-obese individuals subsequently consumed a diet in which 6 g/day of visible fat was replaced with 6 g/day of fish oil. There was a significant reduction in FM of -0.88 kg with the fish oil diet compared to -0.3 kg in the control diet but weight reduction was similar in both diets \(^{(268)}\). Not only could the very small sample size have influenced the results but the one-way order of treatment, whereby all participants firstly consumed a control diet followed by a diet where visible fat was replaced with the same amount of fish oil, is also a possible confounding factor.

A study involving 27 obese, post menopausal females with T2D consumed a diet with either 3 g/d LCω-3PUFA or 3 g/d paraffin oil over 2 months. Despite no reduction in body weight with either group, there was a significant reduction in total FM and subcutaneous adipocyte diameter in the fish oil group \(^{(269)}\). A larger study
with 93 overweight, insulin resistant females consumed either an energy restricted diet supplemented with LCω-3PUFA or a placebo oil, or a placebo oil with no weight loss diet (control), for 12 weeks. A significant reduction in weight was observed in both of the weight loss and oil supplemented groups compared to the control group with no significant difference between the former (31). Thus, it would appear that changes in weight could be attributed to the reduced energy diets and not the LCω-3PUFA. In another study oily fish, white fish or capsules with fish oil or sunflower oil (control) were consumed in combination with an energy-restricted diet involving 278 overweight/obese males and females over 8 weeks. Weight loss and a reduction in waist circumference was significantly greater for males only in the three groups consuming fish or fish oil compared to the control (270). Outcomes from the studies by He, Song et al. (2004) (257) and Iso, Rexrode et al. (2001) (258), discussed in the cross-sectional studies above, suggested that males but not females responded to the effects of LCω-3PUFA. In the study by Thorsdottir, Tomasson et al. (2007) (270) significant differences were also observed in weight reduction in males but not females even when the diet contained white fish and no LCω-3PUFA. The authors suggested that the reduction in weight observed in the white fish group could be attributed to fish protein but this would not explain why males responded to this intervention and not females.

1.9.2 Effects of LCω-3PUFA on inflammatory markers

The beneficial effect of supplementation with ω-3, raising levels of circulating adiponectin has been reported in human studies (31; 271) and in rodent models of obesity (272; 273; 271). However, the effect of ω-3 on leptin levels does vary. Supplementation with ω-3 has been reported to reduce plasma leptin levels in association with reduced adiposity in mice (263; 274; 264) while studies in rats have reported that ω-3 increases plasma leptin levels despite a reduction in adiposity (275; 276; 272). It has also been suggested that ω-3 could increase plasma leptin levels independent of adiposity (277). In a study involving males, where ω-3 was consumed as a supplement for 6 weeks, it had no effect on plasma leptin concentrations (278). Another study, which combined an energy-restricted diet with the consumption of white fish, oily fish, or fish oil capsules over 8 weeks, reported that changes to leptin levels were almost the same for the fish groups and control, but a greater, though not significant, increase in the leptin levels was observed in the capsule group (279).
A cross-sectional study of 727 females reported lower levels of CRP and IL-6 in participants with the higher levels of EPA and DHA: CRP levels were 29% lower and IL-6 levels were 23% lower in the highest quintile of total ω-3 FA intake \(^{(280)}\). Another study involving males reported no change to circulating levels of CRP after 45 days of supplementation with DHA but, a significant 15% reduction in CRP was observed when DHA supplementation was continued to 91 days \(^{(281)}\). There was also a reduction of almost 20% in concentrations of IL-6 by day 91 but there was no change to TNF-α \(^{(281)}\). Other studies have reported a significant reduction in CRP with the consumption of fish oil \(^{(282)}\) and with fish and sardine oil \(^{(283)}\). However, other studies have reported that CRP concentrations were unaffected by ω-3 intake \(^{(284,285)}\), while another reported no significant changes to concentrations of CRP, IL-6 and TNF-α after supplementation with EPA or DHA \(^{(286)}\).

A study involving 138 sedentary, overweight males and females reported a reduction of 10% and 12% in levels of IL-6, and a change of 0.2% and -2.3% in levels of TNF-α with an intake of 1.25 g/d or 2.5 g/d ω-3 PUFAs, respectively. There were no changes in weight as the subjects did not follow an energy restricted diet \(^{(287)}\). In another study involving obese males who did not follow an energy restricted diet, no change in weight or inflammatory markers was observed when ω-3 capsules were consumed over 6 weeks. However, when the men subsequently followed a VLED for 8 weeks, they did reduce their weight accompanied by a small reduction in TNF-α \(^{(29)}\).

The outcomes for the different inflammatory biomarkers are complicated by the diversity in the studies. Concentrations and composition of ω-3 consumed, duration of the intake, whether or not an energy restricted diet was part of the intervention, the number of participants, age, gender and health status would all have had an effect.

**1.9.3 Effects of LCω-3PUFA on blood lipids**

It is now well established that LCω-3PUFAs have a TG lowering effect in humans \(^{(288)}\). Harris conducted a meta-analysis of 65 studies which showed that ω-3 FAs lowered serum TGs in a dose-dependent manner \(^{(289)}\).

Jacobson et al. (2012) conducted a review of 22 studies on the differential effects of EPA and DHA on LDL-C, HDL-C and TGs \(^{(290)}\). Because of the different
combinations and concentrations of EPA and DHA, the different lengths of the interventions, and the different numbers of participants involved in the studies and, in some studies males or females only, a comparison of the results show some conflicting outcomes. The majority of the studies show a decrease in TG levels with DHA supplementation, alone or in combination with EPA although there was a considerable range in the reduction levels of TG. For example, TG levels reduced by 8.9% - 30.3% in the 12 studies with DHA only supplementation (290). Overall, there was an increase in HDL-C in response to DHA treatment, with levels increased from 2.9% - 18.3%, but changes in response to EPA were much smaller and a few reported a small decrease in HDL levels (290). On average, LDL-C levels increased in response to treatment with DHA while treatment with EPA showed no definite trend to HDL-C levels in either direction (290).

An earlier review of 41 studies on the effect of omega-3 FAs on plasma lipids, which involved normolipidemic subjects, reported similar results (291). All of the 41 studies reported a reduction in TG levels in response to ω-3 FA treatment, ranging from 1.1% - 65% with a total mean reduction of 25%. There was an overall increase of 3% in levels of HDL-C with an increase in 27 of the studies, a decrease in 11 and no change in 2 studies. Results reported for LDL-C were more ambivalent, with levels reduced in 23 studies, increased in 15, and no change in 2 studies resulting in no change overall (291). (There was no data for HDL-C and LDL-C available for one of the studies). As with the previous review, the number of participants in the different studies varied, concentrations and sources of ω-3 FAs also varied as did the length of the treatment. Differences between the effects of DHA and EPA were not explored.

1.10 Conclusion

There are conflicting reports on the relationship between LCω-3PUFA and weight status. It would appear that LCω-3PUFAs have the potential to facilitate weight loss and inhibit weight gain. Studies with animals are more consistent in their reports of reduced weight and fat mass in response to supplementation with EPA and DHA but results in animals are very different to the outcomes that can be achieved in humans. Not only is dietary compliance more difficult to control in humans, but the concentrations of LCω-3PUFAs administered in animal studies are generally higher than is deemed to be safe for humans. Also, relatively few
randomised controlled trials investigating the effectiveness of LCω-3PUFA on weight loss in humans have been conducted. Thus, this thesis investigates the efficacy of supplementation with LCω-3PUFA on weight management in humans.

1.11 Aims

1. To investigate the relationship between plasma ω-3 PUFA concentration and various anthropometric measures in healthy-weight, overweight and obese adults.

2. To investigate whether LCω-3PUFA supplementation, combined with a structured weight loss program, will facilitate weight loss and improvements in blood lipids and inflammatory mediators.

3. To investigate whether LCω-3PUFA supplementation, combined with a VLED, will facilitate weight loss and weight maintenance, improvements in blood lipids and inflammatory mediators.

4. To examine whether prior supplementation with LCω-3PUFA, followed by a concomitant administration of a VLED, will facilitate weight loss, improve blood lipids and inflammatory mediators.

1.12 Hypotheses

1. Plasma LCω-3PUFA is associated with weight status in adults.

2. LCω-3PUFA of marine origin will facilitate weight loss in obese adults when taken in combination with a weight loss programme using a VLED.

3. After weight loss, continued supplementation with LCω-3PUFA will inhibit weight regain during a period of weight maintenance.

4. Increasing concentrations of LCω-3PUFA in body cells and membranes of obese individuals prior to commencing a reduced energy weight loss diet will assist weight loss.
Chapter 2:

General Methods
2.1 Ethical Approval

Ethical approval was obtained from the University of Newcastle Human Research Ethics Committee for all of the studies described in this thesis. Informed, written consent was obtained from all participants prior to their commencement in a study. Apart from the first study, the other studies described in this thesis were registered with the Australian New Zealand Clinical Trials Registry.

Ethical approval was conditional upon a risk assessment of hazardous tasks and activities being made and approval to conduct these tasks and activities was obtained from the University of Newcastle Safety Committee.

2.2 Clinical Assessment

2.2.1 Anthropometric measurements

Anthropometric measurements were taken with participants dressed in light clothing and without shoes. Standing height was measured to the nearest 0.1 cm using a Holtain Crymych stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated balance beam scale (PCS Measurement, NSW, Australia). BMI was calculated in kilograms (kg) per metre squared (weight/height$^2$). Waist circumference (WC) was measured at the mid-point between the lowest rib and the top of the hipbone; the hip measurement was taken at the fullest point of the hip, as viewed from the side. The waist:hip ratio was calculated as waist girth in centimetres (CM) divided by the hip girth.

2.2.2 Bioelectrical impedance measurements

Fat mass (FM), fat free mass (FFM) and muscle mass (MM) were measured using bioelectrical impedance. Two machines were used:

(i) BIOSCAN 916S (Maltron International, Rayleigh, Essex, UK) uses single-frequency bioelectrical impedance. It is calibrated for all age groups and different nationalities. A gel-electrode measured impedance, a total of four electrodes are used, one to the back of each hand and one to the top of each foot. Measurements are taken with the person in the supine position,
arms not in contact with the sides of the body and legs not in contact with each other.

(ii) InBody 230 (Biospace Co., Ltd. Seoul, Korea) uses direct segmental multi-frequency bioelectrical impedance analysis enabling separate measurement of the trunk, arms and legs for body fat. The person is measured in the standing position and there is a total of eight contact points used, two on the soles of each foot as the person stands on the base of the machine, and two on the inside of each hand which grip the handles.

Bioelectrical impedance analysis (BIA), an indirect measure of body composition, estimates total body water (TBW) by using an electric current to measure resistance and reactance of the body tissue. Resistance (impedance) is low in lean tissue (which contain intracellular fluid and electrolytes that act as conductors of electricity) but high in fat tissue and is proportional to TBW. The bioelectrical impedance machine calculates FM and FFM using mathematical formulae based on measurements of height, weight, resistance and reactance. BIA improves the accuracy of predicting TBW and FFM compared to other traditional anthropometric techniques.

Dual-energy x-ray absorptiometry (DEXA) is often used as a criterion method for adult body measurements. A study compared the validity and reliability of measurements obtained using InBody to results obtained with DEXA. All body composition devices were correlated to DEXA ($r = 0.54 - 0.97; P \leq 0.01$). A high correlation between contact measured impedance and gel-electrode measured impedance has also been reported.

2.2.3 Physical activity

Level of exercise was assessed by questionnaire at the commencement and completion of the intervention to determine whether change had occurred. Participants were not encouraged to increase activity for the duration of the study as this could influence change to weight and some biomarkers.
2.2.4 Dietary analysis

Dietary intake was assessed with a 3-day food diary over three consecutive days (two week days and one weekend day) to determine everyday nutrient and energy intake. All food and liquid consumed each day was recorded with quantities determined by cup and spoon measures, weights on food products, and whole foods or parts thereof. Self report activity levels were also recorded. A dietary software program, Foodworks® Professional 2009, version 6 (Xyris Software (Australia) Private Ltd) was used to analyse the food records for each participant for an average of daily macro and micro nutrients consumed as well as for fatty acids. With individual information on the levels of activity, gender, age, height and weight, individual energy requirements were calculated.

2.3 Clinical Trials

2.3.1 Intervention

The treatment group received 6 x 1 g/d fish oil capsules, each capsule containing 700 mg LCω-3PUFA with 270 mg DHA and 70 mg EPA (ratio 1:3.9). The control group received a placebo of Sunola oil. Both oils contained the antioxidant Coviox®T-70, 3000ppm in the tuna oil and 600ppm in Sunola oil. Two capsules were taken with each meal. The capsules were visually identical and both were flavoured with peppermint to disguise the flavour of the oil inside. Duration of supplementation differed with each trial, see figures 2.2, 2.3 and 2.4 below.
2.3.2 Procedure

The overall recruitment and baseline procedure flow diagram for all of the clinical trials is presented in Figure 2.1, below.

Enquiries received by telephone. Telephone interview to assess for eligibility

**Inclusion criteria:**
- BMI = 30–40 kg/m²
- Age = 18–55 years
- Can swallow capsules

**Exclusion criteria:**
- Has inflammatory condition
- Is diabetic
- Allergic to fish
- Already taking fish oil capsules
- > 2 oily fish meals/week
- Already following an energy restricted diet
- Pregnant
- Lactating

**Accept:** randomly assign to control or treatment group

**Treatment:** Fish Oil (FO)
6x1 g/d capsules ω3 = 1.62 g DHA, 0.42 g EPA

**Control:** Placebo (PB)
6x1g/d capsules
Sunola oil

**Baseline processing – first visit:**
After >10 h overnight fast:
- Collect anthropometric measurements: height, weight, hip, waist
- Measure fat mass (FM), fat free mass (FFM), muscle mass (MM) using bioelectrical impedance assessment (BIA)
- Collect 25ml fasting blood sample
- Collect 3-day food diary
- Complete medical questionnaire

**Figure 2.1** Flow diagram: Initial procedure for all three clinical trials
EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid
Procedures for clinical trials 2, 3, and 4 are presented as flow diagrams in Figures 2.2, 2.3 and 2.4 respectively (below).

**Weight loss diet – Weeks 1-12**
Energy reduced (5000 kJ females, 6000 kJ males), healthy eating diet

**Weeks 1, 2, 3, 4**
- Energy density of foods
- Food groups and portions to be consumed/day
- Portion sizes in each food group
- Understanding and using food labels in the selection of food
- Develop healthy eating plans

**Weeks 1, 2, 3, 4, 8, 12**
- Weight, waist, hip measurements recorded
- Supplements distributed
- Individual counselling for diet issues

**End of week 12:**
After >10 h overnight fast
- Measure FM, FFM, MM using bioelectrical impedance
- Collect anthropometric measurements: weight, hip, waist
- Collect 25ml fasting blood sample
- Collect medical questionnaire
- Collect 3-day food diary

Further 12 weeks of support provided for weight maintenance

**Figure 2.2** Flow diagram: Procedure for Clinical Trial 1 over 12 weeks
FO – fish oil; PB – placebo; FM – fat mass; FFM – fat free mass; MM – muscle mass
Weight loss diet with meal replacements (MR) – 4 weeks
3,000 kJ/day

Weeks 1 & 2:
Breakfast: MR shake;
Lunch: MR bar
1 cup salad
Dinner: MR shake
2 cups steamed veg
>2 L water/day

Week 3:
Breakfast: MR shake;
Lunch: Light meal
1 cup salad
Dinner: MR shake
2 cups steamed veg
>2 L water/day

Week 4:
Breakfast: MR shake;
Lunch: Light meal
1 cup salad
Dinner: MR shake
2 cups steamed veg
>2 L water/day

Weeks 1, 2, 3, 4, 6, 8, 10, 12
Weight, waist, hip measurements recorded. Individual counselling given

End of weight loss, week 4:
After >10 h overnight fast
- Measure FM, FFM, MM using bioelectrical impedance
- Collect 25ml fasting blood sample

Weight maintenance + continued supplementation: 10 weeks

Treatment – FO (n 17)
(1 no show for final measurement)

Control – PB (n 12)
(2 no show for final measurement)

End of maintenance, week 14:
After >10 h overnight fast
- Measure FM, FFM, MM using bioelectrical impedance machine
- Collect anthropometric measurements: weight, hip, waist
- Collect 25ml fasting blood sample
- Collect medical questionnaire
- Collect 3-day food diary

Figure 2.3  Flow diagram: Procedure for Clinical Trial 2 over 14 weeks
FO – fish oil; PB – placebo; FM – fat mass; FFM – fat free mass;
MM – muscle mass
End of preparation phase, 4 wks
After >10 h overnight fast
- Measure FM, FFM,MM using bioelectrical impedance
- Collect anthropometric measurements: weight, hip, waist
- Collect 25ml fasting blood sample
- Collect 3-day food diary

Preparation phase - supplementation – Weeks 1-4
Usual healthy diet, without fast foods PLUS 6x1g capsules supplement / day

Treatment = FO (n 20)
6x1 g/d capsules FO

Control = PB (n 19)
6x1g/d capsules PB

Baseline processing as in Figure 2.1

Weight loss diet with meal replacements (MR) – Weeks 5-8
3,000 kJ/day

Weeks 1 & 2:
Breakfast: MR shake;
Lunch: MR bar
1 cup salad
Dinner: MR shake
2 cups steamed veg
>2 L water/day

Week 3:
Breakfast: MR shake;
Lunch: Light meal
1 cup salad
Dinner: MR shake
2 cups steamed veg
>2 L water/day

Week 4:
Breakfast: MR shake;
Lunch: Light meal
1 cup salad
Dinner: Light meal
2 cups steamed veg
>2 L water/day

Weeks 5, 6, 7, 8
Weight, waist, hip measurements recorded. Individual counselling given

End of weight loss, week 8:
- Measure FM, FFM,MM using bioelectrical impedance
- Collect anthropometric measurements: weight, hip, waist
- Collect 25ml fasting blood sample
- Collect medical questionnaire
- Collect 3-day food diary

Figure 2.4 Flow diagram: Procedure for Clinical Trial 3 over 8 weeks
FO – fish oil; PB – placebo; FM – fat mass; FFM – fat free mass; MM – muscle mass
2.4 Biochemical analysis

2.4.1 Blood collection

After a ≥10 h overnight fast, blood samples were collected by qualified phlebotomists following the Venipuncture and Patent Identification procedure used by the Hunter Area Pathology Service (HAPS), the ‘Pathology Training Guide’ by Macri International for routine blood collection (CPCS-JHH-SOP0070) and with reference to the University of Newcastle Policy AcS 03:18 ‘Policy on minimum standards for blood collection from students, staff or others during teaching and research activities’. 20th January, 2003. An overall collection and processing flow diagram is presented in Figure 2.5.

Blood samples were collected into vacutainers (Becton Dickinson, Australia/New Zealand, Sydney, Australia):

- For analysis of blood lipids, including TC, HDL-C, LDL-C and TGs, vacutainers coated with lithium heparin, an anti-coagulant. The plasma and red blood cells are separated in the tube with a barrier of PST gel during centrifuge.

- For glucose analysis, vacutainers coated with sodium fluoride and potassium oxalate which are antiglycolytic agents and preserve glucose for up to 5 days.

- For analysis of inflammatory markers and fatty acids, vacutainers coated with ethylenediaminetetraacetic acid (EDTA) to prevent clotting and platelet clumping.

2.4.2 Blood processing

The vacutainers were centrifuged (Heraeus Biofuge Stratos) at 30000 x g, at 4°C for 10 minutes. The plasma fraction of the blood in the EDTA tubes was then removed and aliquots of plasma were stored in 1.5 mL microfuge tubes at -80°C until required for further analysis. The lithium heparin and sodium fluoride coated
tubes (containing plasma) were then taken to Hunter Area Pathology Services (Newcastle) for plasma analysis of blood lipids and blood glucose, respectively.

**Figure 2.5** Flow diagram: Procedure for blood processing after each collection

HAPS – Hunter Area Pathology Service; HDL – high density lipoprotein; LDL – low density lipoprotein; EDTA – Ethylenediaminetetraacetic acid

### 2.4.3 Fatty acid analysis (plasma)

**Fatty acid determination**: Total fatty acids were determined using the method established by Lepage and Roy (1986) (294). 2 mL of methanol:toluene mixture (4:1 v/v), containing C19:0 (4 µg/mL) internal standard, was added to 100 µL plasma. Fatty acids were methylated by slowly adding 200 µL acetyl chloride drop-wise...
while vortexing and then heating for 1 hour at 100°C. After rapid cooling, 5 mL K₂CO₃ (potassium carbonate) was added to halt the reaction. The sample was centrifuged at 3000 x g at 4°C for 10 minutes to separate the layers. 100 µL was drawn from the top toluene layer and used for gas chromatography (GC) analysis of the fatty acid methyl esters. A Hewlett Packard HP6890 Gas Chromatograph System (Hewlett Packard, Palo Alto CA, USA), with 30 m x 0.25 mm (DB-255) fused carbon-silica column, coated with cyanopropylphenyl (J & W Scientific, Folsom, CA) was used. Both the injector and detector port temperatures were set at 250°C; the oven temperature began at 270°C for 2 minutes and then rose by 10°C/minute to a temperature of 190°C and held for 1 minute before increasing 3°C/minute to 220°C and maintained to give a full run time of 28 minutes. A split ratio of 10:1 and an injection volume of 5 mL were used. Fatty acid methyl ester peaks were ascertained by comparing their retention times with the retention times of synthetic standards of known fatty acid composition (Nu Check Prep Elysian, MN, USA) and were quantified using Chemstations Version A.04.02 for GC analysis.

2.4.4 Assays

2.4.4.1 Cytokine analysis

High sensitivity (hs) enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) were used to determine plasma levels of hs-TNF-α and hs-IL-6. Minimal detectable concentration of the kits was 0.106 pg/ml and 0.039 pg/ml respectively with and intra- and inter-assay coefficients of variation (CV) of <9%.

Analysis of hs-CRP was performed using an immunoturbidimetric method (Hunter Area Pathology Services, Newcastle, Australia) with a minimal detection level of 0.15 mg/L.

2.4.4.2 Adipokine analysis

ELISA kits (R&D Systems) were used to determine plasma levels of leptin and adiponectin, employing a quantitative sandwich enzyme immunoassay technique with a detection limit of 15.6 pg/mL and 3.9 ng/mL respectively, and inter- and
intra-assay CV of less than 5.5% and 7% respectively. Alternatively, plasma leptin levels were quantified with a commercial double-antibody enzyme immunometric assay (EIA) (Cayman Chemical Company, Ann Arbor, MI, USA) with a detection limit of 1.0 ng/mL and inter-and intra-assay CVs of less than 9%. Adiponectin levels were also determined using an alternative ELISA kit (SPI-bio, Montigney, Le Bretonneux, France). Intra- and inter-assay CVs were 6.4% and 7.3% respectively with a detection range of 0.1 – 10.0 µg/mL.

2.5 Statistical analysis

Results are presented as mean ± standard deviation (sd) or mean ± standard error of mean (SEM) with P ≤0.05 indicating significance. One-way ANOVA was used to test group mean differences. Within group changes from baseline were determined using paired samples t-tests. Pearson product-normal correlations (r) were used to show relationships.

Mixed design 3-way ANOVA was also used to measure 3-way interactions between group, time and gender, and 2-way ANOVA to measure 2-way interactions between time and group.

All statistical analyses were carried out using SPSS software: Study 1 (version 15.0, SPSS. Inc); Studies 2 and 3 (version 17.0, SPSS. Inc); Study 4 (version 19.0 SPSS. Inc).
Chapter 3:

Plasma n-3 polyunsaturated fatty acids are negatively associated with obesity

Prospective studies in humans have previously reported that high levels of LCω-3PUFA were associated with low levels of obesity in males and higher intakes of LCω-3PUFA were associated with higher rates of obesity in females. Thus this research commenced with an investigation into whether there was a relationship between plasma LCω-3PUFA and weight status in humans.

3.1 Abstract

The objective of this study was to investigate the relationship between plasma n-3 PUFA composition and weight status. A total of 124 adults, stratified by weight status: healthy weight (n=21), overweight (n=40) and obese (n=63) were recruited. Fasting blood samples, anthropometric measures and body composition were collected. Plasma fatty acid composition was determined by GC. BMI, waist and hip circumference were inversely correlated with n-3 PUFA, EPA and DHA (P<0.05 for all) in the obese group. Obese individuals had significantly lower plasma concentrations of total n-3 PUFA, compared with healthy weight individuals (4.53±1.11 vs. 5.25±1.43 %). When subjects were pooled and stratified into quartiles of total n-3 PUFA, a significant inverse trend was found for BMI (P=0.002), waist and hip circumference (P=0.01 and P<0.001 respectively). Higher plasma levels of total n-3 PUFA are associated with a healthier BMI and waist and hip circumference. Our findings suggest that n-3 PUFA may play an important role in weight status and abdominal adiposity.
3.2 Introduction

Obesity is a consequence of the excessive accumulation of fat in adipose tissue which can result in significant morbidity and mortality. Health problems associated with obesity include cardiovascular disorders such as hypertension, stroke and coronary heart disease, conditions associated with insulin resistance such as type 2 diabetes, and certain types of cancers (15; 7). A weight loss of between 5 - 10% can substantially reduce these risks (26; 27), however successfully maintaining weight loss, in the long-term, is difficult (295). Hence, effective strategies to improve adherence to weight loss and weight maintenance are needed (36).

The consumption of n-3 PUFA, namely EPA and DHA, have been linked to reduced CVD risk (296; 31; 297), and to reduced fasting glucose levels, providing a protective effect against the development of type 2 diabetes (298). There is also continuing debate as to whether or not n-3 PUFA contribute to weight loss.

Dietary fatty acids are an important source of adipose tissue fatty acids and play a significant role in adipose tissue metabolism (206). Intake of n-3 PUFAs has been shown to influence the fatty acid composition of membrane phospholipids, thus modulating several metabolic processes that take place in the adipocyte (280; 299; 300; 301). Lipid management at the cellular level influences the degree of the development of disease and co-morbidities in obesity (302). Indeed, abnormal n-3 PUFA metabolism in studies of obese children has been suggested (303; 304; 305; 306), therefore, not only the amount of dietary fat, but also the composition of dietary fat, plays an important role in adipose tissue metabolism and thus on body fat accumulation.

In the present study, we investigate the relationship between plasma n-3 PUFA concentrations and various anthropometric measures in healthy weight, overweight and obese adults. We hypothesize that plasma n-3 PUFA is associated with weight status, more specifically obesity. Perhaps n-3 PUFA could assist weight loss by complementing existing weight loss approaches through their influence on biomarkers of obesity (307; 280; 308). We also examine whether the contribution of n-3 PUFA concentration to covariates of body composition is independent of weight status.
3.3 Experimental Methods

3.3.1 Participants

A total of 124 male and female free-living participants, aged between 18 - 70 years were recruited from the university campus and the general community of Newcastle, Australia. Exclusion criteria for participation were: diagnosed diabetes mellitus; liver or other endocrine dysfunction; evidence of CVD, including angina or hypolipidemic medication; chronic inflammatory disease; consumption of fish oil supplements; consumption of more than two fatty fish meals per week; on a restricted diet; BMI < 20 or > 40 kg/m$^2$; tobacco smoking. Further biochemical exclusion criteria included fasting glucose >6.8 mmol/l (122.5 mg/dl).

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki; all procedures involving human subjects were approved by the Human Research Ethics Committee of the University of Newcastle, Australia. Written informed consent was obtained from all subjects.

3.3.2 Anthropometry assessment

All anthropometric measurements were made with participants wearing light clothing and no shoes. BMI was calculated as body mass in kilograms (kg) divided by the square of height in meters (m) to the nearest 0.1 (kg/m$^2$) using a calibrated balance beam scale (PCS Measurement, NSW, Australia). Waist circumference was measured at the mid-point between the lowest rib and the top of the hip bone; the hip measurement was taken at the fullest point of the hip, as viewed from the side. The waist:hip ratio was calculated as waist girth in centimetres (cm) divided by hip girth (cm). Single frequency bioelectrical impedance was used to determine fat mass (FM) and fat-free mass (FFM) (Maltron International, Rayleigh Essex, UK). Measurements were taken in the supine position following a >10h fast with no physical activity or alcohol consumption 24h before testing. Calculations determined percentage FM $[(FM/body weight) \times 100]^{(309)}$. 
3.3.3 Plasma fatty acid analyses

Fasting (>10 h) blood samples were collected into tubes pre-coated with EDTA by venipuncture. Samples were prepared by centrifuging for 10 min x 3000 g at 4°C. Plasma samples were collected and stored at -80°C until further analysis. The fatty acid composition of plasma lipids was determined according to a modification in the method of Lepage and Roy (294), using an acetyl chloride methylation procedure. Fatty acid methyl esters were quantified using GC (Hewlett Packard 6890; Hewlett Packard, Palo Alto, CA, USA). The identity of each fatty acid peak was ascertained by comparison of the peak’s retention time with the retention times of synthetic standards of known fatty acid composition (Nu Check Prep, Elysian, MN, USA). The relative amount of each fatty acid was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids. Concentrations of n-3 PUFA were calculated by summing the respective 18–22 carbon atom fatty acids (linolenic acid, EPA, docosapentaenoic acid and DHA). Fatty acid results are reported as percentage of total fatty acids.

3.3.4 Statistical analysis

Data are presented as mean values and standard deviations. Preliminary assumption testing was conducted to check for normality, linearity, outliers and homogeneity of variance, with no serious violations noted for anthropometric and body composition measurements. Variables that were not normally distributed were log-transformed prior to analysis. Comparisons between the different groups were made with one-way ANOVA and post hoc testing. P<0.05 was considered significant. Data were further explored with all weight status groups pooled and stratified into quartiles of n-3 PUFA. All statistical analyses were carried out with SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA).

3.4 Results

Among the 124 adults, the average age was 49.5 (SD 10.7) years with 37% being male. Participants were stratified into weight status according to BMI; healthy weight 20–24.9 kg/m² (n 21), overweight 25–29.9 kg/m² (n 40), and obese 30–40 kg/m² (n 63). Anthropometric characteristics of the three groups are presented in Table 3.1. The healthy weight group had a significantly lower body weight, BMI,
waist circumference, hip circumference and FM ($P<0.001$ for all) compared to the obese group. The overweight group had a significantly lower body weight, BMI, waist circumference, hip circumference ($P<0.001$ for all) and FM ($P=0.03$) compared to the obese group.

Table 3.1. Subject characteristics
(Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Healthy weight (n=21)</th>
<th>Overweight (n=40)</th>
<th>Obese (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.28$^a$ 8.56</td>
<td>49.87$^a$ 11.46</td>
<td>43.79$^b$ 12.22</td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>66.34$^a$ 9.23</td>
<td>80.54$^b$ 8.08</td>
<td>95.53$^c$ 14.50</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>23.12$^a$ 1.61</td>
<td>27.74$^b$ 1.56</td>
<td>33.59$^c$ 2.72</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>83.07$^a$ 9.37</td>
<td>94.05$^b$ 7.84</td>
<td>104.49$^c$ 8.86</td>
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<tr>
<td>Hip (cm)</td>
<td>91.36$^a$ 6.45</td>
<td>104.31$^b$ 8.18</td>
<td>118.63$^c$ 8.94</td>
</tr>
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<td>Waist-to hip ratio</td>
<td>0.91 0.09</td>
<td>0.90 0.08</td>
<td>0.98 0.08</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>33.81$^a$ 7.37</td>
<td>37.36$^a$ 7.38</td>
<td>40.90$^b$ 6.70$^b$</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>66.57$^a$ 8.07</td>
<td>62.89$^a$ 7.90</td>
<td>59.10$^b$ 6.70$^b$</td>
</tr>
</tbody>
</table>

$^a$, $^b$, $^c$ Mean values within a row with unlike superscript letters were significantly different ($P<0.05$)

The correlation between plasma $n$-3 PUFA concentration and features of anthropometry were explored separately for each weight status. No significant correlation was observed for the healthy weight and overweight groups (data not shown). Correlations between plasma $n$-3 PUFA concentration and BMI, waist circumference, hip circumference, waist:hip ratio and FM in the obese group were analysed (Table 3.2). Total $n$-3 PUFA, EPA and DHA were inversely correlated with BMI ($P=0.004$, $P=0.009$, $P=0.004$, respectively), waist circumference ($P=0.03$, $P=0.05$, $P=0.02$, respectively) and hip circumference ($P<0.001$, $P=0.009$, $P=0.002$, respectively).
Table 3.2 Associations between plasma n-3 PUFA (% of total fatty acids) and measures of anthropometry in obese subjects  
(Pearson's correlations)

<table>
<thead>
<tr>
<th></th>
<th>Total n-3 PUFA</th>
<th>LNA</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.40</td>
<td>-0.11</td>
<td>-0.32</td>
<td>-0.36</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>-0.27*</td>
<td>-0.01</td>
<td>-0.24*</td>
<td>-0.28*</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>-0.41*</td>
<td>-0.07</td>
<td>-0.32*</td>
<td>-0.38*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.17</td>
<td>0.12</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>-0.03</td>
<td>-0.12</td>
<td>0.14</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

* P<0.05

When participants were stratified into quartiles according to total n-3 PUFA composition (thirty-one subjects per quartile; quartile 1: 3.4 (SD 0.06); quartile 2: 4.1 (SD 0.02); quartile 3: 4.9 (SD 0.06); quartile 4: 6.7 (SD 0.1)% total fatty acids), a highly significant inverse trend was found for BMI (P=0.002), waist circumference (P=0.01) and hip circumference (P<0.001) (Figure 3A-C). No trends were found for waist-to-hip ratio (P=0.50) and FM (P=0.16). Post hoc analysis shows a significant difference between the lowest and highest quartiles for BMI (P=0.004), waist circumference (P=0.03) and hip circumference (P=0.001).
Fig. 3.1 Quartiles of plasma n-3 PUFA concentration for (A) BMI (kg/m\(^2\)), (B) waist circumference (cm) and (C) hip circumference (cm). Values are means (n 31 per quartile), with standard deviations represented by vertical bars. \(^{a,b}\) Mean values with unlike letters were significantly different (P<0.05). For BMI, P for trend=0.002; for waist circumference, P for trend=0.01; for hip circumference, P for trend<0.001.

### 3.5 Discussion

In the present cross sectional study, we observed significantly lower plasma concentrations of n-3 PUFA in obese men and women compared to healthy weight individuals. The finding of the present study is that measures of weight status
were correlated with plasma n-3 PUFA composition when participants were stratified into quartiles of total n-3 PUFA concentration.

Similar findings were reported in a study comparing serum phospholipid fatty acids with adipose tissue in 25 normal-weight and obese adolescents, which found that concentrations of n-3 PUFA were significantly lower in obese vs lean, age matched, females\(^{(308)}\). Another study involving 120 normal-weight and overweight adolescents found that overweight adolescents had lower total n-3 PUFA and lower DHA concentrations compared to normal-weight adolescents, independent of body fat and fat distribution\(^{(310)}\). When dietary intake was also considered in a study of 134 age- and sex-matched normal-weight and overweight children, the BMI z-score of the obese children was negatively associated with plasma n-3 PUFA and DHA, despite obese children having higher intakes of the main fatty acid families, including PUFA\(^{(311)}\).

Previous observational studies which also considered food intake indicate a negative association of fish consumption with central obesity measures\(^{(312)}\). In rodents, feeding fish oil enriched diets have been shown to prevent abdominal fat accumulation compared with other types of dietary oils\(^{(256, 313, 314)}\). In human subjects, replacement of 6 g of visible fat/d with 6 g of fish oil/d for three weeks resulted in reduced fat mass and increased basal lipid oxidation\(^{(268)}\). A recent study has shown that the inclusion of lean fish, fatty fish or fish oil to a nutritionally balanced diet resulted in a greater weight loss within four weeks compared with diets devoid of seafood or marine supplements\(^{(270)}\). Another study which investigated the dietary intake of 132 children aged 4-years, reported that a low n-3 PUFA intake was associated with higher body weight\(^{(315)}\). These studies, along with our observations, suggest that n-3 PUFA supplementation may play an important role in preventing weight gain and improving weight loss when n-3 PUFA are supplemented concomitantly with a structured weight loss programme. Furthermore, inclusion of n-3 PUFA in a weight loss programme may provide additional health benefits\(^{(31)}\).

The results presented are biologically plausible because several mechanisms underlying the association between n-3 PUFA and obesity have been shown. One possibility is that n-3 PUFA could increase basal fat oxidation which may in turn reduce fat mass\(^{(268, 297)}\). Animal studies have shown that n-3 PUFA
supplementation may be associated with increased expression of mitochondrial uncoupling protein \(^{316}\), a system of thermogenesis that can provide a defence against obesity. Furthermore, a recent study has shown that n-3 PUFA intake increases postprandial satiety in overweight and obese individuals during weight loss \(^{317}\). Fatty acids may interact with neuroendocrine factors including insulin \(^{318} ;^{319}\), ghrelin \(^{213} ;^{214}\) and leptin \(^{215} ;^{216} ;^{320}\) to modulate brain-intestinal loop signals for energy metabolism and appetite control. A recent study found that ghrelin is negatively correlated with body weight and total n-3 PUFA in normal-weight subjects \(^{321}\), suggesting that n-3 PUFA can modulate appetite. Thus, the idea that fish oil can regulate weight status via improved appetite control along with a subsequent reduction in calorie intake is plausible and worthy of further investigation.

A limitation of our study is that it does not explain why plasma n-3 PUFA concentration was lower in obese individuals. A possible reason could be that the diets of obese adults are such that their intake of n-3 PUFA (marine foods) is lower than in normal weight individuals. Alternatively, lower plasma n-3 PUFA levels in obese individuals may be a reflection of increased utilisation or oxidative damage to these highly unsaturated fatty acids. Indeed obesity has been associated with increased oxidative stress \(^{322}\). Future studies should also examine long-term biomarkers of n-3 PUFA status, such as n-3 index, to further explore the relationship with obesity. We also acknowledge the small sample size of our study, and recognise that a large-scale multi centre trial would be appropriate; certainly future studies should account for ethnicity and family history of disease and obesity.

Regardless of the mechanisms by which n-3 PUFA may assist in the maintenance of weight status \(^{302} ;^{317}\), a significant inverse trend for BMI, waist circumference and hip circumference was observed when participants were stratified into quartiles of plasma n-3 PUFA concentration. Whether improvements in plasma concentration of n-3 PUFA by dietary supplementation with marine oils may reduce abdominal adiposity, or obesity in general, merits investigation. Interestingly, anthropometric measures correlated with the major n-3 PUFA (EPA and DHA) but not the parent n-3 PUFA (linolenic acid).
In summary, we have reported, an inverse relationship between plasma concentrations of n-3 PUFA and anthropometric measures of obesity including BMI, waist and hip circumference. Previous studies involving children and adolescents have shown a negative correlation between adiposity and plasma n-3 PUFA and DHA concentrations, but there appears to be a paucity of research in adults. These studies make the basis for conducting more intervention trials in adults examining the influence of dietary supplementation with n-3 PUFA-rich fats/oils in assisting weight loss and weight maintenance.
Chapter 4:

Dietary supplementation with long chain omega-3 polyunsaturated fatty acids and weight loss in obese adults

In Chapter 3 it was observed that obese males and females had significantly lower levels of LCω-3PUFA compared to healthy-weight individuals. Thus the aim of this first clinical trial was to investigate whether supplementation with LCω-3PUFA, combined with a healthful diet with portion control and energy restriction would facilitate weight loss, improve blood lipids and inflammatory mediators.

4.1 Abstract

Background: Obesity is associated with elevated levels of inflammation and metabolic abnormalities, with increased risk of developing insulin resistance, type 2 diabetes, stroke and CVD. Nutrients that can assist in weight loss may also reduce the risk of obesity related co-morbidities.

Aim: The aim of this study was to investigate whether LCn-3PUFA, combined with a reduced energy diet, facilitated weight loss and improvements in blood lipids and inflammatory mediators.

Design: A double blind randomised controlled trial with two parallel groups. Both groups followed a low energy diet for 12 weeks, one group consumed 6x1g capsules/d monounsaturated oil (Placebo) (n=18), the other 6x1g capsules/d LCn-3PUFA (Fish Oil) (n=17). Fasting blood samples, anthropometric measurements and 3-day food diaries were collected at baseline and post intervention.

Results: There was a two-fold increase in plasma levels of EPA and DHA in the Fish Oil group (p<0.001). There were no significant difference within and between the Placebo and the Fish Oil groups for weight reduction (3.37% and 4.35% respectively), fat mass reduction (8.95% and 9.76% respectively), or changes in inflammatory biomarkers and blood lipids apart from TGs, reduced by 27% in FO (p<0.05). For Fish Oil group there were significant correlations between leptin and weight loss (p=0.01) and leptin and EPA and DHA (p<0.05 for both).

Conclusion: Dietary LCn-3PUFA supplementation during a weight loss program does not appear to assist weight loss. Poor dietary compliance may be a contributing factor in accurate assessment of the role of these fatty acids in weight loss.
4.2 Introduction

The original concept of obesity was as an adipose tissue storage depot for energy in excess of requirements. It is now well established that adipose tissue is also an active endocrine organ associated with low-grade systemic inflammation through the expression of proinflammatory mediators such as tumour necrosis factor-α (TNFα), Interleukin-6 (IL-6) (124), C-reactive protein (CRP) (155) and leptin (323) all of which have been linked to increased risk of CVD (144). Adipose tissue also secretes the anti-inflammatory mediator, adiponectin, which has direct positive effects on vascular function (324).

In addition to body size, the location of body fat can be a critical indicator of disease risk. Visceral adiposity, measured by waist circumference (WC) >102cm (males) and >88cm (females), is one of a group of established risk factors for several metabolic diseases (103) which, in addition to CVD, include insulin resistance, type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia (106). The other contributing risk factors in the group are impaired fasting plasma glucose (324), raised levels of TGs and of LDL and reduced levels of high-density lipoprotein cholesterol (HDL-C) (247). Waist-to-hip ratio (WHR) is also used to measure the distribution of body fat and cardio-metabolic risks, and a high reading is considered to be a better predictor of CVD than a large waist circumference alone (325).

Loss of adipose tissue through weight loss results in a decrease in circulating levels of proinflammatory biomarkers (30) and decreased metabolic abnormalities (29). There is no shortage of dietary interventions for weight loss but dietary adherence can be difficult (36) and weight loss is difficult to achieve and maintain (295). It has been suggested that supplementation with long chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) will reduce metabolic risk factors and reduce inflammation (225; 195). It has also been shown that supplementing the diet with LCn-3PUFA of marine origin reduces body weight in mice (326; 264).

The purpose of this study was to investigate the effect of LCn-3PUFAs on weight loss in obese adults by comparing two groups who were following the same energy controlled weight loss diet, with one group consuming fish oil and the other placebo capsules.
4.3 Method

4.3.1 Participants

Both male and female participants, aged 18–60 years and with a BMI of between 30–40 kg/m², were recruited from the university campus and the general public in Newcastle, Australia to take part in a weight loss study. People with diagnosed diabetes mellitus, a chronic inflammatory condition, already following an energy restricted diet, allergic to fish, taking fish oil capsules or consuming two or more oily fish meals per week were excluded from the study, as were women who were pregnant or lactating. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the Human Research Ethics Committee of the University of Newcastle, Australia. Written, informed consent was obtained from participants prior to commencement. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000659000).

4.3.2 Study design

This was a double blind randomised controlled trial. Simple randomisation using computer generated random numbers was used to allocate participants to one of two parallel groups. For 12 weeks one group consumed 6 x 1g capsules of placebo per day (placebo group), containing Sunola Oil (monounsaturated oil), and the other group consumed 6 x 1g capsules of fish oil per day (fish oil group). Each fish oil capsule contained tuna oil comprising 360mg LCn-3PUFA with 270 mg Docosahexaenoic Acid (DHA) and 70 mg Eicosapentaenoic Acid (EPA) (HiDHA® NuMega Ingredients Pty Ltd, Australia). All capsules contained peppermint to mask the flavour. Two capsules were consumed with each meal, that is breakfast, lunch and dinner providing 1.62 g DHA and 0.42 g EPA per day. Both groups of participants followed an energy reduced, portion controlled healthy eating weight loss diet (HEWLD) comprising a daily intake of 5000 kJ for females to 6000 kJ for males. For the first four weeks participants attended weekly so that they could receive nutrition education and counselling, with the sessions focusing on the energy density of foods, understanding and using food labels, appropriate portion sizes and the number of portions to be consumed daily from the different food groups. This information was used to build a healthy diet using the guidelines from
the Australian Guide to Healthy Eating (AGHE) for the participants to follow. At these meetings, participants were weighed so that progress could be monitored and their supplements replenished. Thereafter participants attended monthly to weigh in and collect their supplements.

4.3.3 Dietary assessment

Prior to commencing the weight loss program, dietary intake was assessed with a 3-day food diary to determine every-day nutrient and energy intake. Participants used the weights given on food products and handy measures of cups and spoons to record food intake which was analysed using the dietary software program, Foodworks Professional 2009, version 6 (Xyris Software (Australia) Pty Ltd) and the mean values were calculated. In the final two weeks of the weight loss program participants again completed a 3-day food diary to measure changes in dietary intake.

4.3.4 Anthropometric assessment

On the first day of the weight loss trial, anthropometric measurements were taken in the morning after a 10-hour overnight fast and no alcohol consumption, with participants dressed in light clothing and without shoes. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated balance beam scale (PCS Measurement, NSW, Australia). BMI was calculated in kilograms (kg) per meter squared from weight and height. WC was measured at the mid-point between the lowest rib and the top of the hipbone; the hip measurement was taken at the fullest point of the hip, as viewed from the side. The waist: hip ratio was calculated as waist girth in centimetres (cm) divided by the hip girth (cm). Fat mass (FM), fat-free mass (FFM) and muscle mass (MM) were measured using single-frequency bioelectrical impedance (Maltron International, Rayleigh, Essex, UK). Under identical conditions, these anthropometric measurements were repeated at the end of the weight loss phase.
4.3.5 Biochemical analyses

4.3.5.1 Blood sample collection

Fasting (≥ 10 h) blood samples were collected into tubes, EDTA, Lithium Heparin and Sodium Fluoride, by venipuncture at baseline and again at the end of the weight loss phase. The samples were prepared by centrifuging (Heraeus Biofuge Stratos) for 10 min at 3000 g at 4°C. Plasma samples from the EDTA tubes were collected and stored at -80°C awaiting further analysis. The Lithium Heparin and Sodium Fluoride tubes were taken to Hunter Area Pathology Services (Newcastle) for plasma analysis for blood lipids and blood glucose, respectively.

4.3.5.2 Plasma fatty acid analysis

The plasma lipids were analysed for fatty acid composition using an acetyl chloride methylation procedure, a modification of the method of Lepage and Roy \(^{(294)}\). Fatty acid methyl esters were quantified using a GC (Hewlett Packard 6890, Hewlett Packard, Palo Alto, CA, USA) and identified by comparing peak retention times with the retention times of synthetic standards of known fatty acid composition (Nu Check Prep, Elysian, MN, USA).

4.3.5.3 Analysis of inflammatory markers

High sensitivity (hs) enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) were used to determine levels of hs-TNF-α and hs-IL-6. Minimal detectable concentration of the kits was 0.106 pg/ml and 0.039 pg/ml respectively with an intra- and inter-assay coefficients of variation (CV) of <9%. Analysis of hs-CRP was conducted using an immunoturbidimetric method (Hunter Area Pathology Service, Newcastle, NSW, Australia) with a minimal detection level of 0.15 mg/l. Plasma leptin levels were quantified with a commercial double-antibody enzyme immunometric assay (EIA) (Cayman Chemical Company, Ann Arbor, MI, USA) with a detection limit of 1.0 ng/mL and inter- and intra-assay CVs of less than 9%. Adiponectin levels were determined using an ELISA kit (SPI-bio, Montigney le Bretonneux, France). Intra- and inter-assay CV were 6.4% and 7.3% respectively with a detection range of 0.1–10.0 µg/mL.
4.4 **Statistical analyses**

Data are presented as mean values and standard deviations, with $p \leq 0.05$ indicating significance. ANOVA was used to test group mean differences within groups and changes from baseline were determined using paired samples t-test. Pearson product-normal correlations ($r$) were used to show relationships, with $p \leq 0.05$ indicating significance. All statistical analyses were carried out with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA).

4.5 **Results**

Forty three participants commenced the study, seven withdrew and one was excluded at commencement of study when initial blood results identified him as being diabetic. At the end of the study two participants from the Fish Oil group were excluded. One did not comply with the consumption of the fish oil capsules as evidenced by an 8% return of unused capsules and the very modest increase in plasma LCn-3PUFA. The other participant did not comply with the energy reduced diet as evidenced by a weight gain of 2 kg and a 3-day food diary at the end of the study showing a consumption of 9,000 kJ/d. Of the 33 participants who completed the study, 18 were in the Placebo group (6 males, 12 females) and 15 were in the Fish Oil group (FO) (5 males, 10 females). Two extreme outliers were excluded from the inflammatory biomarker adiponectin (1 from placebo and 1 from FO group) and two from hs-CRP (both placebo).

4.5.1 **Diet and supplementation**

Dietary intake was calculated from 3-day food diaries completed prior to commencement of the study and again at 12 weeks. There were no significant differences in daily dietary intake between the two groups at either time-point. Table 4.1 shows mean daily energy and nutrient intake at baseline and the mean daily intake difference from baseline for both groups. There were slightly greater reductions in protein, CHO and fat intake by FO than placebo and the mean reduction in energy intake by FO was a greater (-10.9%) compared to placebo (-6.7%) but the differences were not significant. Baseline EPA and DHA levels were very similar for FO and placebo. After 12 weeks of supplementation with fish oil capsules, there was a >2-fold increase in plasma levels of both EPA and DHA in FO. This difference from baseline was highly significant ($p<0.001$) as was the
difference in EPA and DHA levels between the two groups (p<0.001) post intervention (Table 4.1).

4.5.2 Anthropometric measurements

Baseline anthropometric characteristics and changes from baseline are shown in Table 4.2. Over the 12 weeks body weight reduced by 3.37% (-3.17 kg) and 4.35% (-4.19 kg) for placebo and FO respectively. There was a reduction in FM of 8.95% (-3.43 kg) and increase in FFM of 0.67% (0.29 kg) for placebo. For FO there was a reduction in both FM of 9.76% (-3.8 kg) and FFM of 0.51% (-0.36 kg). The differences from baseline within groups were significant for weight, FM, BMI (p<0.001 for all in both groups). Differences between groups were not significant. The baseline waist circumference was above the acceptable range <102 cm for males and <88 cm for females for almost all participants, with one male (FO) and two females (placebo) below the recommended cut-off point. After weight loss eight participants had reduced their waist circumference to below the cut-off point, 4 placebo and 4 FO. Within groups the reduction in waist circumference was significant (p<0.01 and p<0.05 for FO and placebo respectively) but the differences between the two groups was not.

4.5.3 Blood biomarkers

The changes in blood lipids from baseline to the end of the intervention are summarised in Table 4.3. Apart from a significant 27% (-0.53 mmol/l) reduction in TGs (p<0.05) in FO, which was significantly greater than the 1.09% (-0.25 mmol/l) reduction in placebo (p<0.05), there were no other significant differences for blood lipids from baseline within and between groups. While changes from baseline were modest, some were positive for health, for example, HDL-C in FO and TC in placebo, while others were negative, for example, TC and LDL-C in FO and HDL-C and LDL-C in placebo.
Table 4.1 Baseline values and changes or percent changes from baseline for daily energy and nutrient intake and LCn-3PUFA

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=18)</th>
<th></th>
<th>Fish Oil (n=15)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PI</td>
<td>Δ</td>
<td>BL</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>8170 ± 2457</td>
<td>7069 ± 1819</td>
<td>-1123 ± 2044</td>
<td>7928 ± 2832</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>94.73 ± 25.71</td>
<td>92.26 ± 20.20</td>
<td>-0.50 ± 23.39</td>
<td>100.73 ± 34.99</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>197.35 ± 67.73</td>
<td>158.33 ± 47.38</td>
<td>-47.94 ± 63.32</td>
<td>220.93 ± 94.86</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>73.69 ± 31.00</td>
<td>55.87 ± 25.13</td>
<td>-7.00 ± 20.83</td>
<td>60.58 ± 23.65</td>
</tr>
<tr>
<td>LCn3-PUFA (%)</td>
<td>4.21 ± 0.89</td>
<td>4.82 ± 1.21</td>
<td>0.61 ± 0.75a</td>
<td>4.33 ± 0.94b</td>
</tr>
<tr>
<td>(20:5n-3) EPA (%)</td>
<td>0.92 ± 0.30</td>
<td>1.09 ± 0.40</td>
<td>0.17 ± 0.33a</td>
<td>1.06 ± 0.40b</td>
</tr>
<tr>
<td>(22:6n-3) DHA (%)</td>
<td>2.00 ± 0.62</td>
<td>2.37 ± 0.64</td>
<td>0.36 ± 0.43a</td>
<td>2.03 ± 0.67b</td>
</tr>
</tbody>
</table>

Mean values ± standard deviations
In each row superscripts with a common letter differ, p<0.001
Table 4.2 Baseline values and changes from baseline for anthropometric measurements.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=18)</th>
<th></th>
<th>Fish Oil (n=15)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PI</td>
<td>Δ</td>
<td>BL</td>
</tr>
<tr>
<td>Age</td>
<td>41.11 ± 11.27</td>
<td></td>
<td>39.94 ± 11.70</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.95 ± 16.39</td>
<td>90.78 ± 16.12</td>
<td>-3.17 ± 3.09***</td>
<td>94.92 ± 11.53</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.53 ± 3.58</td>
<td>31.42 ± 3.70</td>
<td>-1.11 ± 0.99***</td>
<td>32.55 ± 2.07</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>38.98 ± 7.49</td>
<td>35.55 ± 8.06</td>
<td>-3.43 ± 3.51***</td>
<td>39.67 ± 6.88</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>54.40 ± 10.43</td>
<td>54.69 ± 10.02</td>
<td>0.29 ± 1.77</td>
<td>55.27 ± 9.51</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>103.11 ± 12.11</td>
<td>100.86 ± 11.65</td>
<td>-2.25 ± 3.93*</td>
<td>101.73 ± 6.89</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>119.67 ± 9.78</td>
<td>117.14 ± 10.43</td>
<td>-2.53 ± 2.46***</td>
<td>119.33 ± 6.93</td>
</tr>
<tr>
<td>Waist:hip</td>
<td>0.86 ± 0.08</td>
<td>0.86 ± 0.08</td>
<td>0.01 ± 0.02</td>
<td>0.86 ± 0.08</td>
</tr>
</tbody>
</table>

Mean values ± standard deviations
*p<0.05; **p<0.01; ***p<0.001
Table 4.3  Baseline values and changes from baseline for blood biomarkers.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=18)</th>
<th></th>
<th></th>
<th>Fish Oil (n=15)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PI</td>
<td>Δ</td>
<td>BL</td>
<td>PI</td>
<td>Δ</td>
</tr>
<tr>
<td>Trigs (mmol/l)</td>
<td>1.33 ± 0.71</td>
<td>1.07 ± 0.40</td>
<td>-0.25 ± 0.59</td>
<td>1.32 ± 1.00</td>
<td>0.80 ± 0.46</td>
<td>-0.53 ± 0.83*</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.84 ± 0.93</td>
<td>4.74 ± 0.73</td>
<td>-0.10 ± 0.56</td>
<td>4.91 ± 0.91</td>
<td>5.02 ± 0.84</td>
<td>0.11 ± 0.78</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.16 ± 0.24</td>
<td>1.12 ± 0.27</td>
<td>-0.03 ± 0.18</td>
<td>1.21 ± 0.27</td>
<td>1.25 ± 0.25</td>
<td>0.04 ± 0.22</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.07 ± 0.81</td>
<td>3.13 ± 0.66</td>
<td>0.06 ± 0.47</td>
<td>3.12 ± 0.79</td>
<td>3.25 ± 0.88</td>
<td>0.13 ± 0.49</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.71 ± 0.51</td>
<td>5.02 ± 0.57</td>
<td>0.31 ± 0.50</td>
<td>4.81 ± 0.39</td>
<td>4.95 ± 0.58</td>
<td>0.14 ± 0.54</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>34.32 ± 13.47</td>
<td>35.82 ± 19.38</td>
<td>1.50 ± 12.00</td>
<td>36.27 ± 19.92</td>
<td>35.36 ± 20.60</td>
<td>-0.91 ± 10.57</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)*</td>
<td>12.18 ± 6.41</td>
<td>13.36 ± 7.33</td>
<td>1.17 ± 5.86</td>
<td>13.27 ± 4.93</td>
<td>13.04 ± 6.37</td>
<td>-0.23 ± 4.62</td>
</tr>
<tr>
<td>hs-TNF-α (pg/ml)</td>
<td>1.34 ± 1.13</td>
<td>1.01 ± 1.11</td>
<td>-0.33 ± 0.80</td>
<td>1.72 ± 1.62</td>
<td>0.96 ± 1.28</td>
<td>-0.76 ± 0.50***</td>
</tr>
<tr>
<td>hs-IL-6 (pg/ml)</td>
<td>2.92 ± 2.34</td>
<td>2.98 ± 3.89</td>
<td>0.07 ± 1.82</td>
<td>1.96 ± 1.19</td>
<td>1.73 ± 0.67</td>
<td>-0.23 ± 1.20</td>
</tr>
<tr>
<td>hs-CRP (µg/ml)b</td>
<td>4.34 ± 3.59</td>
<td>4.15 ± 4.21</td>
<td>-0.18 ± 2.53</td>
<td>3.57 ± 4.36</td>
<td>3.68 ± 4.22</td>
<td>0.11 ± 1.71</td>
</tr>
</tbody>
</table>

Mean values ± standard deviations

* Data available n=31 (placebo: n=17, fish oil: n=14)

b Data available n=31 (placebo: n=16; fish oil: n=15)

*p<0.05  ***p<0.001
Changes to inflammatory biomarkers, both within groups and between FO and placebo, were also modest and non-significant due to the large variance in the results. The results are shown in Table 4.3.

Correlations between changes in anthropometric measurements, blood biomarkers and LCn-3PUFA were computed. For both groups there were positive and significant correlations between weight loss and changes to BMI and FM (p<0.001 for both), also changes to waist (p<0.001 for FO and p<0.01 for placebo). For FO there were also positive and significant correlations between leptin and changes to weight, FM, EPA and DHA (p<0.05 for all), suggesting a possible interaction with LCn-3UFA weight loss and leptin. However, there was no correlation with LCn-3PUFA and weight loss.

4.6 Discussion

Although studies with animals have shown that dietary intake of LCn-3PUFA may reduce body weight in obese animals, and may reduce body fat accumulation, particularly visceral fat in animals fed a high fat diet \(^{(328)}\), the effectiveness of supplementation with LCn-3PUFA as an adjunct to weight loss in humans is less certain. It has been suggested that LCn-3PUFA may reduce body fat in humans by suppressing the appetite and increasing fat oxidation and energy expenditure \(^{(328)}\) but studies with humans have provided conflicting results. The hypothesis of this study was that supplementing an energy restricted diet with LCn-3PUFA may have a complementary effect on weight loss.

In this study we observed a significant increase in EPA and DHA from baseline in FO and a significantly greater level of EPA and DHA in FO compared to placebo. Despite the increase in LCn-3PUFA in FO, over the 12 weeks the differences in loss of weight and FM between the two groups were marginal and not significant, suggesting that LCn-3PUFA may not contribute to loss of weight or FM in humans. Similar results have been reported in comparable studies involving supplementation with LCn-3PUFA on weight loss. One study involving overweight, insulin-resistant females, used a very low energy diet for weight loss with a treatment group receiving supplementation of 2.9g DHA + 1.3 g EPA each day. No significant difference in
loss of weight or FM was observed between the treatment and control groups \(^{(31)}\). In another two similar studies individuals followed energy controlled, but not restricted diets. In both studies, supplementation with Lcn-3PUFA in the proportion of 1 g EPA + 0.7 g DHA/day for the treatment group, influenced a significantly greater decrease in FM in the treatment group compared to the control group but no significant differences in weight loss between the two groups \(^{(268; 269)}\). Using oily fish to provide 3.65 g Lcn-3PUFA/d, a study reported no significant difference in weight loss between participants who followed an energy restricted diet alone and an energy restricted diet + fish. There was also no change in weight in a diet of usual energy intake + fish \(^{(329)}\). Another study with an energy restricted diet plus lean fish or fatty fish or Lcn-3PUFA capsules, reported that the diets did not differ in their effect on weight loss for females, but weight loss in males was significantly greater in all three treatment groups when compared with the control group \(^{(270)}\). It is proposed that this difference between males and females could be attributed to a different response to marine foods by gender or to a different effect of the intervention diet on weight loss for males and females \(^{(270)}\).

A possible reason for the different outcomes from these studies could be the different amounts of Lcn-3PUFA supplementation and the relative proportions of EPA to DHA. It is noteworthy that the controversy about the weight loss potential of omega-3 fatty acids is limited to the human studies while animal studies have consistently demonstrated body weight reduction following administration of omega-3 fatty acids in the diet. These differences could be attributed to dietary compliance in humans versus animals. It is easier to manage dietary compliance when working with animals compared to working with humans, particularly when faced with feelings of hunger on an energy restricted diet. Self-reporting of dietary compliance can be misleading both through under-reporting and over-reporting dietary intake \(^{(330; 331)}\). Moreover, the effect of Lcn-3PUFA on weight reduction, if any, is likely to be small, gradual changes over time and not occur abruptly. Therefore, it is likely that variable compliance (both diet and physical activity), coupled with short duration of the intervention period may negate any small and gradual beneficial effects of dietary supplementation with Lcn-3PUFA on weight loss.
Not only have studies with mice found that dietary supplementation with LCn-3PUFA reduces body weight and fat mass but it has been shown to prevent weight gain (326; 264). Also, feeding fish oil-enriched diets to rodents, compared to other types of dietary oils, has been shown to prevent abdominal fat accumulation (256; 313; 314). Perhaps LCn-3PUFAs are more effective in preventing weight gain rather than assisting weight loss. In this context, studies with humans have shown that when compared to healthy-weight individuals, significantly lower concentrations of n-3 PUFA have been observed in obese females and/or males (310; 306; 332).

It is well established that weight loss has a positive influence on blood lipids. LCn-3PUFAs are also known to be effective in lowering TG levels (333; 244) which were the findings in our study with a significant decrease in TGs from baseline for FO. It has been suggested that TC is not affected by LCn-3PUFA (291; 333). Harris (1989) also contends that LDL-C are not affected by LCn-3PUFA (291) while Balk et al. (2006) suggests that LCn-3PUFA increases LDL-C (333). Our findings for TC and LDL-C agree with those reported by Harris (1989) with no significant changes in FO or between FO and placebo. It is possible that the modest increase in TC observed in FO could reflect the amount of saturated fat in the diet of individuals. Obesity is associated with decreased plasma levels of HDL-C. A modest increase of HDL-C is associated with weight loss (334; 335) and an increase of 5-10% is associated with consumption of LCn-3PUFA (291). While our results showed such an increase in HDL-C concentrations, that is 5% (0.04 mmol/) for FO, the variance in the results could have been influenced by individual differences in weight lost.

Weight loss is known to influence a decrease in circulating inflammatory markers (336) and LCn-3PUFA have been shown to have anti-inflammatory properties (337; 205; 338). In keeping with the modest weight loss in this study, plasma leptin levels reduced by a small amount in FO but, despite a similar weight loss in placebo, leptin levels in that group increased. Although the differences were not significant, there was a significant, positive correlation between leptin and weight loss (p = 0.01) and leptin and EPA and DHA (p<0.05 for both) in FO. This would appear to indicate that the reduction of leptin in FO could also be attributed to LCn-3PUFA. In a study involving overweight men and women serum leptin levels were significantly reduced in a weight loss plus fish meal diet but not in a weight loss diet or usual energy plus fish
diet, showing that this reduction occurred from an interaction between n-3 fatty acids and weight loss \(^{(327)}\). In our study there was a modest increase in adiponectin levels in placebo but a reduction in FO, which suggests that weight loss and not LCn-3PUFA influenced the increase. These results appear to be in keeping with a study investigating the effect of LCn-3PUFA consumption on adiponectin in overweight and obese men and women and found that weight loss influenced an increase on adiponectin concentrations not LCn-3PUFA \(^{(339)}\).

In our study there was an overall mean increase of hs-CRP and an overall mean decrease of TNF-\(\alpha\) and IL-6 by FO, but changes were very small and not significantly different to placebo. Findings for the effect of LCn-3PUFA on CRP are not consistent. A study involving healthy postmenopausal women found that there was a reduction in serum hs-CRP with supplementation of 7g/d LCn-3PUFA but not with 14 g/d \(^{(340)}\). Another study involving healthy males and females, found that there was no significant reduction in serum hs-CRP with daily supplementation of 2 g or 6.6 g LCn-3PUFA \(^{(284)}\).

4.9 Conclusion

While studies with animals have consistently shown that LCn-3PUFA influences weight loss, studies in humans have produced conflicting results. Reasons for this might include the different amounts of LCn-3PUFA consumed by humans in the various studies and the relative proportion of EPA to DHA. A major factor could be the uncertainty of dietary compliance which can be controlled in animals but not as easily in humans. Future studies on LCn-3PUFA and weight loss should consider more effective management strategies to encourage dietary compliance.
Chapter 5:

Dietary supplementation with omega-3 polyunsaturated fatty acids does not promote weight loss when combined with a very low energy diet

In the previous study both the placebo and fish oil supplemented groups lost similar amounts of weight, fat mass and fat free mass. A possible reason for this was attributed to poor dietary compliance. Thus, the aim of this next clinical trial was to again investigate whether LCω-3PUFA supplementation would facilitate weight loss but this time in combination with a VLED using meal replacements to improve dietary compliance.

5.1 Abstract

Obesity is associated with elevated levels of inflammation and metabolic abnormalities which are linked to cardiovascular disease. The aim of this study was to investigate whether LCn-3PUFA, combined with a very low energy diet (VLED), facilitated weight loss and weight maintenance, improvements in blood lipids and inflammatory mediators. This was a double blind randomised controlled trial with two parallel groups. For 14 weeks one group consumed 6x1g capsules/d monounsaturated oil (Placebo), the other group consumed 6x1g capsules/d LCn-3PUFA (Fish Oil) each comprising 70 mg EPA and 270 mg DHA. Both groups followed a VLED for 4 weeks (n=14 Placebo, n=18 Fish Oil), then 10 weeks of weight maintenance (n=12 Placebo, n=17 Fish Oil). Fasting blood samples, anthropometric measurements and 3-day food diaries were collected at baseline, at 4 weeks and 14 weeks. A greater than two-fold increase occurred in plasma levels of EPA and DHA in the Fish Oil group (P<0.001). At 4 weeks the mean weight loss was -6.54 (SD 2.08) kg (-6.9%) for Placebo and -6.87 (SD 1.83) kg (-7.7%) for Fish Oil. At week 14, after the maintenance phase, there was a further mean decrease in weight, -1.57 (SD 3.7) kg (1.85%) for Placebo and -1.69 (SD 2.32) kg (-1.9%) for Fish Oil. Both groups experienced improved metabolic profiles and there was a significant reduction in fat mass for the FO group at week 14 but not for PB. However, it would appear that supplementation with LCn-3PUFA had no significant effect on weight loss or weight maintenance over the 14 weeks.
5.2 Introduction

Obesity is a progressive and relapsing chronic disorder that is associated with increased risk of morbidity and mortality including the development of insulin resistance, type 2 diabetes, hypertension, dyslipidemia\(^{(106)}\) and cholesterol abnormalities\(^{(247)}\). It is also associated with low-grade systemic inflammation through the expression of proinflammatory mediators such as tumour necrosis factor-α (TNFα), Interleukin-6 (IL-6)\(^{(124)}\), C-reactive protein (CRP)\(^{(155)}\), and leptin\(^{(323)}\), all of which have been linked to increased risk of cardiovascular disease (CVD)\(^{(144)}\). Adipose tissue also secretes the anti-inflammatory mediator, adiponectin which has direct positive effects on vascular function\(^{(324)}\).

Intentional weight loss with a reduction of adipose tissue improves many of these medical complications with a decrease in circulating levels of proinflammatory biomarkers\(^{(29; 31)}\) and a reduction of metabolic abnormalities\(^{(28)}\). Dietary supplementation with long chain omega-3 polyunsaturated fatty acids (LC n-3PUFA) is also known to suppress production of proinflammatory cytokines\(^{(225)}\) and increase levels of HDL-C\(^{(29; 341)}\). LC n-3PUFA also lowers TG levels\(^{(342; 341)}\) and to a considerably lower level than weight loss does\(^{(245)}\). Animal studies have shown that supplementing the diet with LC n-3PUFA can reduce body weight and fat mass in mice\(^{(326; 264)}\) and it is possible that, in humans, LC n-3PUFA may also reduce body fat\(^{(328)}\) and it could have a similar effect on body weight.

There is a dose-dependent relationship with weight lost and improvements to health and it has been suggested that a modest weight loss of 5-10% of initial body weight can make a difference\(^{(27; 343)}\). There is a possibility that LC n-3PUFA could facilitate this by increasing weight loss when taken in combination with a weight loss program. The aim of this study was to investigate the effect of LC n-3PUFA on weight loss and short-term weight loss maintenance in obese adults by comparing two groups who followed the same very low energy diet (VLED) using meal replacements (MRs), with one group consuming fish oil capsules. We hypothesised that (i) LC n-3PUFA of marine origin may facilitate weight loss in combination with a weight loss program using a VLED, and that (ii) continued supplementation with LC n-3PUFA may inhibit weight regain during a period of weight maintenance. Furthermore, the combination
of weight loss and LC n-3PUFA supplementation may have a concomitant positive effect on cardiovascular health determinants.

5.3 Methods

This was a randomised, double blind placebo controlled intervention with two parallel groups. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Human Research Ethics Committee of the University of Newcastle, Australia. Written, informed consent was obtained from all participants prior to commencement. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12609000204246).

5.3.1 Participants

Both male and female participants, aged 18 – 60 years and with a BMI of between 30 – 40 kg/m², were recruited from the university campus and the general public in Newcastle, Australia to take part in a weight loss study. People with diagnosed diabetes mellitus, a chronic inflammatory condition, already following an energy restricted diet, allergic to fish, taking fish oil capsules or consuming two or more oily fish meals per week were excluded from the study, as were women who were pregnant or lactating.

A power calculation estimated that 16 participants per group would allow a detection of approximately 2% difference in weight loss between the two diet groups at the 0.05 level of significance, with a statistical power of 80% and assuming a standard deviation of 2%. Allowing for a 20% drop-out rate, 20 participants per group would be required.

5.3.2 Study design

Simple randomisation using computer generated random numbers allocated participants to one of two parallel groups. Supplement containers were labelled with a code by an independent researcher before the trial commenced so that neither the
principal researcher nor the participants could identify the contents of the supplements, and supplements contained peppermint to disguise identifiable flavours. For 4 weeks one group consumed 6 x 1g capsules of placebo per day (placebo group), containing Sunola Oil (monounsaturated oil), and the other group consumed 6 x 1g capsules of fish oil per day (fish oil group). Each fish oil capsule contained tuna oil comprising 360mg LC n-3 PUFA with 70 mg Eicosapentaenoic Acid (EPA) and 270 mg Docosahexaenoic Acid (DHA) (ratio 1:3.9). Both the tuna oil and the Sunola Oil contained the antioxidant Coviox®T-70, 3000ppm in the tuna oil and 600ppm in the Sunola Oil. Two capsules were consumed with each meal, that is breakfast, lunch and dinner, providing 1.62 g DHA and 0.42 g EPA per day.

For the four weeks participants followed a VLED of 3000 kJ/day. To encourage dietary compliance and to reduce differences between participants in the foods that they consumed, for the first two weeks participants received Optifast® bars and shakes to replace meals according to the Intensive Phase of the Optifast Very Low Calorie Diet Program (Novartis, Australia), supplemented daily with raw and cooked vegetables and 2 litres of drinking water. This 3000 kJ/day diet is composed of 40% protein, 16% fat and 40% carbohydrate plus vitamins and minerals. At the same time, participants were given individual nutrition education and counselling, with the sessions focusing on the energy density of foods, understanding and using food labels, appropriate portion sizes and the number of portions to be consumed daily from the different food groups. This information was used to build a healthy diet using the guidelines from the Australian Guide to Healthy Eating (AGHE) (327), and applied during weeks three and four, when the meal replacements were gradually phased out and healthy kilojoule controlled meals were phased in. All participants attended weekly so that weight loss progress could be monitored and to receive the next supply of meal replacements and capsules.

To address the potential for weight loss rebound after the weight loss phase, participants progressed to a weight stabilisation/maintenance phase. For 10 weeks they continued to take the fish oil or placebo supplements as allocated in the weight loss phase, and continued to consume healthy meals with food choices and portion sizes as recommended by the AGHE. Participants now attended fortnightly to obtain
their capsules, monitor their weight and discuss diet related issues. An accredited practicing dietitian was part of the research team to advise on health care.

5.3.3 Dietary assessment

Prior to commencing the weight loss program, dietary intake was assessed with a 3-day food diary (2 week-days and 1 weekend day) to determine every-day nutrient and energy intake. Participants used the weights given on food products and handy measures of cups and spoons to record food intake which was analysed using the dietary software program, Foodworks® Professional 2009, version 6 (Xyris Software (Australia) Pty Ltd) and the mean values were calculated. In the final week of weight maintenance participants again completed a 3-day food diary to measure changes in dietary intake.

5.3.4 Anthropometric assessment

On the first day of the weight loss trial, anthropometric measurements were taken in the morning after a 10-hour overnight fast and no alcohol consumption, with participants dressed in light clothing and without shoes. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated balance beam scale (PCS Measurement, NSW, Australia). BMI was calculated in kilograms (kg) per meter squared from weight and height. Waist circumference (WC) was measured at the mid-point between the lowest rib and the top of the hipbone; the hip measurement was taken at the fullest point of the hip, as viewed from the side. The waist: hip ratio was calculated as waist girth in centimetres (cm) divided by the hip girth (cm). Fat mass (FM), fat-free mass (FFM) and muscle mass (MM) were measured using single-frequency bioelectrical impedance (Maltron International, Rayleigh, Essex, UK) and (InBody 230, Biospace Co., Ltd. Seoul, Korea). Under identical conditions, these anthropometric measurements were repeated at the end of the weight loss and weight maintenance phases.
5.3.5 Biochemical analyses

5.3.5.1 Blood sample collection

Fasting (≥ 10 h) blood samples were collected into tubes, EDTA, Lithium Heparin and Sodium Fluoride, by venipuncture at baseline, at the end of the weight loss phase and again at the end of weight maintenance. The samples were prepared by centrifuging (Heraeus Biofuge Stratos) for 10 min at 3000 g at 4°C. Plasma samples from the EDTA tubes were collected and stored at -80°C awaiting further analysis. Plasma from the Lithium Heparin and Sodium Fluoride tubes was analysed for blood lipids and blood glucose, respectively, by Hunter Area Pathology Services (Newcastle).

5.3.5.2 Plasma fatty acid analysis

The plasma lipids were analysed for fatty acid composition using an acetyl chloride methylation procedure, a modification of the method of Lepage and Roy \(^{344}\). Fatty acid methyl esters were quantified using a GC (Hewlett Packard 6890, Hewlett Packard, Palo Alto, CA, USA) and identified by comparing peak retention times with the retention times of synthetic standards of known fatty acid composition (Nu Check Prep, Elysian, MN, USA).

5.3.5.3 Analysis of inflammatory markers

Enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) were used to analyse inflammatory markers. The assays used to determine plasma leptin and adiponectin levels employ the quantitative sandwich enzyme immunoassay technique with a detection limit of 15.6 pg/mL and 3.9 ng/mL respectively and inter- and intra-assay coefficients of variance (CVs) of less than 5.5% and 7% respectively. High sensitivity ELISA kits were used to determine levels of tumour necrosis factor-α (hs-TNF-α) and interleukin-6 (hs-IL-6). Minimal detectable concentration of these kits was 0.106 pg/ml and 0.039 pg/ml respectively with an intra- and inter-assay CVs of <9%. Analysis of high-sensitivity C-reactive protein (hs-CRP) was conducted using an immunoturbidimetric method (Hunter Area...
Pathology Service, Newcastle, NSW, Australia) with a minimal detection level of 0.15 mg/l.

5.4 **Statistical analyses**

ANOVA was used to test group mean differences, and within group changes from baseline were determined using paired samples t-tests. Pearson product-normal correlations (r) were used to show relationships. Data are presented as mean values and standard deviations, with \( P \leq 0.05 \) indicating significance. All statistical analyses were carried out with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA).

5.5 **Results**

Forty participants commenced the study (20 per group) but eight did not complete; two reacted to the supplements, one participant relocated to another city, one withdrew for personal reasons, one withdrew because of a broken ankle and three participants did not keep appointments for final measurements and blood collection. Thirty two participants completed the weight loss phase, 18 in the Fish Oil group (FO) (15 females and 3 males) and 14 in the Placebo group (PB) (11 females and 3 males). Extreme outliers were identified for hs-CRP (\( n=2 \) FO and \( n=2 \) PB) and were excluded from the analysis of this biomarker. The 32 participants continued to weight stabilisation/maintenance but only 29 completed this phase. Three participants did not keep their appointments for final measurements and blood collection, one female FO, and one male and one female PB. In the weight maintenance phase other extreme outliers were identified and excluded, (\( n=1 \) PB for glucose and \( n=2 \) FO for adiponectin).

5.5.1 **Diet**

Mean energy intake of participants prior to commencing the study was 8273 (SD 1669) kJ/d for FO and 7846 (SD 2399) kJ/d for PB; the differences were not significant. During weight loss all participants followed a very similar 3000 kJ/d MR diet for 4 weeks. The 3-day food diaries completed towards the end of the
maintenance phase showed a mean energy intake of 5080 (SD 1294) kJ/d for FO and 6007 (SD 1758) kJ/day for PB. Again, the differences between the two groups were not significant.

The percentage fatty acid composition of plasma lipids at baseline showed no significant difference between the two groups. Supplementation with LC n-3 PUFA in FO significantly increased eicosapentaenoic acid (EPA; C20:5n-3) (p<0.001) and docosahexaenoic acid (DHA; C22:6n-3) (P<0.001) (Table 5.1) and these increased levels were significantly higher than those levels found in PB at the end of weight loss (P<0.001 for both). Levels of EPA and DHA remained significantly higher for FO than for PB at the end of weight maintenance (P<0.001 for both).

### Table 5.1 Baseline values and changes for LCn-3PUFA.

<table>
<thead>
<tr>
<th>LCN3-PUFA</th>
<th>Baseline (%)</th>
<th>4 weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>(20:5n-3) EPA (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FO*</td>
<td>1.02</td>
<td>0.30</td>
</tr>
<tr>
<td>PB†</td>
<td>0.88</td>
<td>0.26</td>
</tr>
<tr>
<td>(22:6n-3) DHA (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FO*</td>
<td>2.05</td>
<td>0.54</td>
</tr>
<tr>
<td>PB†</td>
<td>2.07</td>
<td>0.57</td>
</tr>
<tr>
<td>Ratio n-6:n-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FO*</td>
<td>8.75</td>
<td>2.00</td>
</tr>
<tr>
<td>PB†</td>
<td>8.90</td>
<td>1.59</td>
</tr>
<tr>
<td>Ratio 18:1/18:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FO*</td>
<td>2.90</td>
<td>0.51</td>
</tr>
<tr>
<td>PB†</td>
<td>2.89</td>
<td>0.35</td>
</tr>
<tr>
<td>Δ</td>
<td>0.01</td>
<td>0.99</td>
</tr>
</tbody>
</table>

FO, fish oil group; PB, placebo group
* n 18; † n 14

### 5.5.2 Anthropometric measurements

#### 5.5.2.1 Weight loss phase

At commencement of the study the baseline measurements for weight, BMI, FM, FFM, waist and hip were not significantly different between PB and FO (Table 5.2).
At the end of four weeks of weight loss there was a significant reduction in weight -7.7% (-6.87 kg), FM -15% (-5.47 kg) and FFM -2.6% (-1.36 kg) in FO, (P<0.001 for all). In PB there were also significant reductions in, weight -6.9% (-6.54 kg) and FM -13.9% (-5.53 kg) (P<0.001 for both), and in FFM -1.8% (-1.0 kg) (P<0.02). For both FO and PB there was a significant reduction in WC -6.4% (-6.53 cm) and -4.8% (-4.96 cm) respectively, also in hip measurement -3.7% (-4.44 cm) and -3.5% (-4.23 cm) for FO and PB respectively, (P<0.001 for all) (Table 2). There was no significant difference in the changes between the two groups. Controlling for BMI using 2-stage multiple regression confirmed this result. Correlations between changes in anthropometric measurements, weight loss and LC n-3PUFA were computed. There were positive, significant correlations between weight loss and reductions to BMI and FM (P<0.001 for FO and PB), FFM (P<0.05 FO and P<0.001 PB); also between EPA and a reduction in FM (P≤0.02) in FO.

5.5.2.2 Weight maintenance phase

The number of participants completing this phase reduced by two in PB to n=12, and by one in FO to n=17. Table 5.2 shows the reductions in anthropometric measurements during the weight maintenance phase (week 14). For FO there were significant reductions in weight -1.9% (-1.69 kg), BMI -1.8% (-0.6 kg/m²) (p=0.01 for both), FM -5% (-1.62 kg, P=0.03), WC -2.4% (-2.29 cm, P=0.003), and hip circumference -2.1% (-2.38 cm, P≤0.001). For PB the changes from weight loss to weight maintenance were not significant. A comparison of changes between FO and PB showed no significant differences. For FO there was a positive, significant correlation between DHA and weight loss (P=0.02) and loss of FM (P=0.025).

5.5.3 Blood biomarkers

5.5.3.1 Weight loss phase

The changes in blood lipids from baseline to the end of weight loss (week 4) are summarised in Table 5.3. For both FO and PB, there were significant reductions in TC (P<0.001 and P≤0.002 respectively), and LDL-C (P<0.05 for both), with small, non-significant differences between the two groups. There were positive, significant
correlations between weight loss and TC for FO (P<0.05) and PB (P≤0.001), also between weight loss and LDL-C for FO (P<0.05) and PB (P≤0.001). For FO there were significant reductions in TG (P<0.001) but not for PB. For both FO and PB there were significant reductions in HDL-C (P<0.01 for both). There was a significant reduction in glucose for FO (P<0.05) but not for PB. The only significant reduction observed in the inflammatory biomarkers was for leptin (P<0.001 for FO and PB) and hs-IL-6 (P<0.05) for PB (Table 5.3).

5.5.3.2 Weight maintenance phase

At the end of ten weeks of weight maintenance (week 14 in the study) there were more changes in blood lipids for both FO and PB, as shown in Table 5.3. An increase in TGs and TC was significant for FO and PB (P<0.05 for both) and in HDL-C for PB only (P<0.01) (Table 5.3). HDL-C did increase for FO but the difference was not significant. There was a significant decrease in levels of TNF-α and a significant increase in adiponectin levels for FO (P≤0.05 for both) (Table 5.3). Other changes in inflammatory biomarkers were non-significant apart from leptin which increased in PB (P<0.01) (Table 5.3). Differences between the two groups were not significant.
### Table 5.2  Baseline values and changes for anthropometric measurements

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th></th>
<th></th>
<th></th>
<th>Fish Oil</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL (n=14)</td>
<td>4 weeks (n=14)</td>
<td>Δ (n=14)</td>
<td>14 weeks (n=12)</td>
<td>Δ (n=12)</td>
<td>BL (n=18)</td>
<td>4 weeks (n=18)</td>
<td>Δ (n=18)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Mean: 42.3</td>
<td>SD: 9.10</td>
<td>Mean: 40.5</td>
<td>SD: 10.9</td>
<td>Mean: 40.5</td>
<td>SD: 10.9</td>
<td>Mean: 48.9</td>
<td>SD: 11.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean: 94.2</td>
<td>SD: 16.60</td>
<td>Mean: 87.6</td>
<td>SD: 15.4</td>
<td>Mean: 88.9</td>
<td>SD: 10.8</td>
<td>Mean: 82.0</td>
<td>SD: 10.3</td>
</tr>
<tr>
<td></td>
<td>-6.54</td>
<td>2.08</td>
<td>1.57</td>
<td>3.70</td>
<td>-6.87</td>
<td>1.83</td>
<td>81.2</td>
<td>8.74</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Mean: 178.3</td>
<td>SD: 9.1</td>
<td>Mean: 176.5</td>
<td>SD: 9.7</td>
<td>Mean: 176.5</td>
<td>SD: 9.7</td>
<td>Mean: 176.5</td>
<td>SD: 9.7</td>
</tr>
<tr>
<td></td>
<td>-2.27</td>
<td>0.59***</td>
<td>-0.61</td>
<td>1.40</td>
<td>-2.46</td>
<td>0.77***</td>
<td>29.9</td>
<td>2.62</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>Mean: 40.7</td>
<td>SD: 7.65</td>
<td>Mean: 35.2</td>
<td>SD: 7.28</td>
<td>Mean: 38.0</td>
<td>SD: 9.03</td>
<td>Mean: 32.5</td>
<td>SD: 8.85</td>
</tr>
<tr>
<td></td>
<td>-5.53</td>
<td>1.34***</td>
<td>-0.99</td>
<td>3.45</td>
<td>-5.47</td>
<td>1.39***</td>
<td>31.3</td>
<td>8.74</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>Mean: 53.5</td>
<td>SD: 10.8</td>
<td>Mean: 52.5</td>
<td>SD: 10.5</td>
<td>Mean: 50.9</td>
<td>SD: 6.25</td>
<td>Mean: 49.5</td>
<td>SD: 6.02</td>
</tr>
<tr>
<td></td>
<td>-1.00</td>
<td>1.29*</td>
<td>-0.46</td>
<td>0.77</td>
<td>-1.36</td>
<td>0.83***</td>
<td>50.0</td>
<td>6.10</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>Mean: 103.1</td>
<td>SD: 11.2</td>
<td>Mean: 98.1</td>
<td>SD: 11.1</td>
<td>Mean: 100.8</td>
<td>SD: 6.88</td>
<td>Mean: 94.3</td>
<td>SD: 5.99</td>
</tr>
<tr>
<td></td>
<td>-4.96</td>
<td>3.54***</td>
<td>-2.29</td>
<td>4.34</td>
<td>-6.53</td>
<td>4.40***</td>
<td>92.2</td>
<td>6.34</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>Mean: 118.0</td>
<td>SD: 7.49</td>
<td>Mean: 113.8</td>
<td>SD: 6.47</td>
<td>Mean: 117.1</td>
<td>SD: 9.15</td>
<td>Mean: 112.6</td>
<td>SD: 7.80</td>
</tr>
<tr>
<td></td>
<td>-4.23</td>
<td>2.38***</td>
<td>-1.71</td>
<td>3.70</td>
<td>-4.44</td>
<td>2.81***</td>
<td>110.7</td>
<td>7.30</td>
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<tr>
<td>Waist:hip</td>
<td>Mean: 0.87</td>
<td>SD: 0.08</td>
<td>Mean: 0.86</td>
<td>SD: 0.08</td>
<td>Mean: 0.86</td>
<td>SD: 0.07</td>
<td>Mean: 0.84</td>
<td>SD: 0.06</td>
</tr>
<tr>
<td></td>
<td>-0.01</td>
<td>0.03</td>
<td>-0.01</td>
<td>0.02</td>
<td>-0.02</td>
<td>0.04**</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

BL, baseline; FM, fat mass; FFM, fat free mass; WC, waist circumference.

Mean values were significantly different within groups: *p<0.05; **p<0.01; ***p<0.001
Table 5.3 Baseline values and changes for blood biomarkers (mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th></th>
<th></th>
<th></th>
<th>Fish Oil</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>4 weeks</td>
<td>Δ</td>
<td>14 weeks</td>
<td>Δ</td>
<td>BL</td>
<td>4 weeks</td>
<td>Δ</td>
</tr>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=14)</td>
<td>(n=12)</td>
<td>(n=12)</td>
<td>(n=18)</td>
<td>(n=18)</td>
<td>(n=18)</td>
<td>(n=17)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Trigs (mmol/l)</td>
<td>1.28</td>
<td>0.51</td>
<td>1.02 0.48</td>
<td>-0.26 0.49</td>
<td>1.20</td>
<td>0.52</td>
<td>0.14 0.21*</td>
<td>1.15 0.59</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.40</td>
<td>0.77</td>
<td>4.54 1.17</td>
<td>-0.86 0.84**</td>
<td>5.27</td>
<td>1.15</td>
<td>0.55 0.76*</td>
<td>4.93 0.79</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.37</td>
<td>0.28</td>
<td>1.19 0.20</td>
<td>-0.17 0.17**</td>
<td>1.44</td>
<td>0.28</td>
<td>0.21 0.21**</td>
<td>1.36 0.28</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.43</td>
<td>0.79</td>
<td>2.90 0.96</td>
<td>-0.54 0.72*</td>
<td>3.29</td>
<td>1.05</td>
<td>0.26 0.71</td>
<td>3.05 0.68</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.01</td>
<td>0.47</td>
<td>4.96 0.56</td>
<td>-0.05 0.33</td>
<td>5.14</td>
<td>0.64</td>
<td>0.21 0.33†</td>
<td>5.14 0.68</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>32.20</td>
<td>14.40</td>
<td>12.50 8.77</td>
<td>-19.70 11.80***</td>
<td>20.90</td>
<td>9.84</td>
<td>8.70 7.69**</td>
<td>32.10 20.20</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>10.20</td>
<td>5.76</td>
<td>10.40 5.11</td>
<td>0.20 2.73</td>
<td>12.00</td>
<td>8.27</td>
<td>1.02 4.24</td>
<td>9.97 5.99</td>
</tr>
<tr>
<td>hs-CRP (µg/ml)</td>
<td>4.50</td>
<td>3.97</td>
<td>3.52 3.78</td>
<td>-0.97 2.83§</td>
<td>4.14</td>
<td>3.00</td>
<td>0.50 2.81</td>
<td>4.78 4.47</td>
</tr>
<tr>
<td>hs-IL-6 (pg/ml)</td>
<td>2.07</td>
<td>0.95</td>
<td>1.60 0.77</td>
<td>-0.47 0.78*</td>
<td>1.45</td>
<td>0.63</td>
<td>-0.27 0.69</td>
<td>2.37 1.73</td>
</tr>
<tr>
<td>hs-TNF-α (pg/ml)</td>
<td>1.13</td>
<td>0.98</td>
<td>0.93 0.89</td>
<td>-0.20 1.18</td>
<td>0.32</td>
<td>0.42</td>
<td>-0.42 0.70</td>
<td>1.08 0.78</td>
</tr>
</tbody>
</table>

BL, baseline; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; hs-CRP, high-sensitivity C-reactive protein; hs-IL-6, high-sensitivity IL-6; hs-TNF-α, high-sensitivity TNF-α.

Mean values were significantly different within groups: *p<0.05; **p<0.01; ***p<0.001
† n 11.
‡ n 15.
§ n 12.
|| n 16.
5.6 Discussion

The main purpose of this study was to investigate whether LC n-3PUFA would assist weight loss when administered as a supplement during a weight loss diet. To this end it was essential that the diet was controlled and participant energy intake was as similar as possible to enable the measurement of the effect of the LC n-3PUFA. By using MRs participants were able to follow a VLED of 3000kJ/d for 4 weeks. Dietary compliance is more favourable with MRs than with a conventional reduced energy diet because the prescribed meal content is set, that is portion sizes are predetermined, food choice is largely removed and so too are the temptations presented when preparing food. MRs are also satiating with the effect lasting for between 3 and 5 hours after consumption \(^{(46)}\) thus further facilitating dietary compliance.

Significant reductions in weight, FM, FFM, WC and hip measurements for both groups in this study highlight the efficacy of the VLED but fail to show a significant weight loss difference from supplementation with LC n-3PUFA, despite the significant increase in plasma concentrations of EPA and DHA. There is conflicting evidence on the effect of LC n-3PUFA on weight loss. Some animal studies have shown that supplementation with LC n-3PUFA is effective in reducing weight and fat mass in mice \(^{(264)}\) while others have shown no significant effect \(^{(345; 346)}\). Studies involving humans have also reported conflicting results. A small number of severely obese women following a VLED reported a significant weight loss in the group who also consumed LC n-3PUFA (2.8g/d n3; EPA:DHA = 2:1) compared to the control \(^{(297)}\). A significantly greater weight loss was observed in males but not in females consuming an energy restricted diet including lean fish (0.27g/d n3; 0.05g/d EPA + 0.21g/d DHA, in the ratio 1:4), or fatty fish (3.0g/d n3; 0.77g/d EPA + 1.37g/d DHA, in the ratio 1:1.8), or LC n-3PUFA (1.42g/d n3; 0.63g/d EPA + 0.43g/d DHA, in the ratio 1.5:1), compared to the control \(^{(270; 342)}\). However, no significant differences were observed when participants were given LC n-3 PUFA supplements (1.3g/d EPA and 2.9g/d DHA, in the ratio 1:2.2) in combination with a VLED \(^{(31)}\), or with an energy controlled diet plus exercise (3g/d EPA + DHA, in the ratio 5:1) \(^{(347)}\), or with energy controlled but not restricted diets (1.1g/d EPA and 0.7g/d DHA, in the ratio 1.6:1) \(^{(268)}\), (1.8g/d n3; 1.08g/d EPA and 0.72g/d DHA, in the ratio 1.5:1) \(^{(269)}\), although
there was a significantly greater decrease in FM in the treatment groups compared to the control for these last two studies. A significant weight gain has also been observed in some studies when the diet of cancer patients was supplemented with LC n-3PUFA\(^{(348)}\).

To explain the reason for these conflicting results is difficult. It is possible that the energy level of the diet that accompanies supplementation could be a factor. However, in two studies that used a VLED with LC n-3 PUFA supplementation, one study reported a significant weight loss compared to the control \(^{(297)}\), while the other study reported no significant differences \(^{(31)}\). It is also possible that the amount of LC n-3PUFA consumed, or the proportion of EPA to DHA could be a contributing factor. Studies with mice have reported that body weight and fat mass decrease relative to the amount of LC n-3PUFA in the diet \(^{(326)}\), and it has also been shown that LC n-3PUFA prevents weight gain \(^{(326; 264)}\). Similar findings have been reported in humans with significantly lower levels of LC n-3PUFA found in obese females and/or males compared to healthy weight individuals \(^{(310; 306; 332)}\). It would appear that the accumulation of LC n-3PUFA in the body could be an important factor for weight management so we investigated this possibility in the 10-week weight maintenance phase.

It is a commonly held belief that slow weight loss is better preserved long term \(^{(59)}\) and that large amounts of initial weight loss lead to more weight regain \(^{(349)}\). However, it has also been reported that a greater initial weight loss is associated with a more significantly sustained weight loss maintenance \(^{(59; 60)}\). The potential is there for a weight loss rebound after a VLED unless new manageable and effective dietary habits are established. In this study the VLED was supported with a 10 week transition period of weight maintenance using the AGHE to establish healthy eating habits while participants continued to consume the supplements they had been allocated during the weight loss phase. The significant decreases in weight, BMI, FM, WC and hip measurements for FO but not for PB during this phase would appear to support the suggestion that the accumulation of LC n-3PUFA in the body might contribute to weight management. This was further supported by the significant correlations between the level of DHA and reduction in weight, BMI and FM \((p= 0.021, p=0.024\) and \(p=0.025\) respectively) for FO during this phase.
As anticipated, weight loss had a positive influence on blood lipids which is highlighted by the significant relationships with weight loss and reductions in TC and LDL-C in both the FO and the PB groups. The reduction in HDL-C levels was similar for both groups. Reductions in levels of TC and LDL-C are associated with clinical benefits but a reduction in HDL-C is undesirable because of its recognised cardioprotective effects (350). There are conflicting reports on the effect of weight loss on HDL-C with studies either showing small increases or small decreases or no change at all (335). Overall, it would appear that HDL-C levels decrease with active weight loss and then increase when a stabilised reduced weight is reached (255; 351). It has been suggested that the reduction in HDL-C occurs because lipoprotein lipase is reduced during active weight loss subsequently reducing TG rich lipoprotein synthesis which impairs VLDL-C catabolism and the transfer of lipids to HDL-C, thereby reducing HDL-C concentrations. With the stabilisation of weight at the reduced level, lipoprotein lipase increases as does hydrolysis of VLDL-C and the transfer of lipids to HDL-C resumes (255). After the 10 weeks of weight maintenance in our study, HDL-C levels did increase for both groups, but significantly only for the PB group, reaching a level higher than that at baseline. Levels of TG, TC and LDL-C also increased, probably as a consequence of resuming the consumption of normal meals with a different fat content. However, they remained lower than the baseline levels.

It has been reported that LC n-3PUFA has no effect on TC levels (291; 333), although there are differences of opinion on whether LC n-3PUFA increases levels of LDL-C (333) or has no effect (291). Recent studies (352; 353) have shown that LCn-3PUFA supplementation results in a small increase in LDL-C, however it is accompanied by an increase in the size of these particles which are less atherogenic (354). It is well known that LC n-3PUFA is effective in lowering TG levels (244), which supports our findings of a significant reduction in TGs in FO only. During weight loss there were no significant correlations between blood lipids and EPA or DHA, though there was for glucose which reduced significantly and was positively and significantly correlated with EPA (p≤0.05).
It would appear that weight loss and LC n-3PUFA, either independently or combined, influenced positive changes in the inflammatory markers. While the changes were modest, some of the changes within the groups were significant but, between the groups, they were not. Overall, the study was not adequately powered to determine significance between the groups with such small changes.

Leptin is important for the regulation of food intake, energy expenditure and adiposity and decreasing leptin levels are associated with dietary energy restriction and weight loss, while a return to a regular food intake is associated with increased leptin levels. Accordingly, in our study there was a significant decrease in plasma leptin within both groups with dietary energy restriction and weight loss. A return to regular food intake during maintenance saw a significant increase in leptin for PB only, with a very modest increase in leptin for FO, the difference between the two groups almost reaching significance (p=0.052), suggesting a possible influence of LC n-3PUFA on leptin levels in the FO group. A study by Mori et al reported that a significant reduction in serum leptin was influenced by an interaction between LC n-3PUFA and energy restrictions but there were no significant effects from LC n-3PUFA or weight loss alone. However, the evidence on the effect of fish oil on plasma leptin levels in association with changes in adiposity or independent of adipose tissue mass, shows very conflicting results.

Reduced levels of CRP, a surrogate marker of CVD risk, are directly related to a reduction in weight loss. However, in our study, the results show that although there was a greater decrease in CRP levels for PB during the weight loss phase compared to FO, CRP continued to decrease during the maintenance phase for FO while it increased for PB. This would suggest that the greater decrease in CRP for FO could be attributed to LC n-3PUFA. Studies investigating the effect of LC n-3PUFA on CRP have reported conflicting results, with one study finding that LC n-3PUFA but not weight loss was associated with a significant reduction in CRP, and the other reporting that LC n-3PUFA had no effect on serum concentrations of CRP. Differences in the size and age of the two groups studied and the length of the intervention could account for the conflicting results. It has also been
suggested that such contradictory findings could be attributed to other components in the diet which might positively affect plasma CRP (357).

Weight loss but not the composition of the diet is reported to be the influence in the reduction of IL-6 and TNF-α (29). However, a study involving 28 healthy subjects reported that dietary supplementation with fish oil can suppress the production of TNF-α (358). Our study did not show any positive additional effects of LC n-3PUFA on changes to IL-6 or TNF-α which agrees with the findings of a study involving healthy, moderately hypertriglyceridemic subjects where supplementation with 0.85g/d and 3.4g/d of EPA and DHA over 8 weeks had no effect on levels of IL-6 and TNF-α (359). It has been suggested that, as IL-6 is secreted by adipose tissue, loss of FM with weight loss could contribute to the reduction of IL-6 (29). Adiponectin has been reported to increase in response to diet induced weight loss (180), and LC n-3PUFA (283), although the change in the latter was not significant, possibly because of the small sample size. In our study, weight loss resulted in an increase in adiponectin for PB but decreased for FO, both not significant. There was, however, a significant increase in adiponectin levels for FO during maintenance indicating a possible delayed influence from LC n-3PUFA. It has been suggested that a weight loss >10% is needed for a significant increase in adiponectin levels to occur (180).

The ratio of plasma 18:1/18:0 is used as a measure of stearoyl-coenzyme A desaturase (SCD) activity in humans (360) that has been in turn implicated in weight gain and TG synthesis. In the current study, the change in 18:1/18:0 ratio was significantly lower in FO compared to PB group (P<0.05) (Table 1). The reduction in the 18:1/18:0 ratio in the FO group correlated negatively with change in plasma TG levels but not with change in BMI or body weight. Considering that TG levels in plasma correlate with adipose tissue (360), future studies may examine longer-term effects of fish oil supplementation on body weight and fat mass.

In summary, it appears that short-term supplementation with LC n-3PUFA did improve the metabolic profile of the participants for the duration of the study, but it did not have any influence on weight loss. There was a significant decrease in FM for the FO group during the maintenance phase but, compared to the PB group, the difference was modest. While it is possible that this difference in FM could be
attributed to supplementation with LC n-3PUFA, a larger cohort of subjects would be required to determine significance with such modest changes. Also, this study does not rule out the possibility that a deficiency of omega-3 fatty acids may cause rapid weight gain. The role of omega-3 fatty acids in weight gain has not been previously examined and is worthy of further examination. The potential benefits of loading the body cells/membranes with omega-3 fatty acids prior to implementation of the weight loss program merits further examination.

5.7 Limitations

A limitation of this study is the small sample size from the reduced completion rate. More studies with a larger sample size and possibly a longer weight stabilisation/maintenance phase are needed to further investigate these findings.
Chapter 6:

Prior supplementation with long chain omega-3 polyunsaturated fatty acids for weight loss in obese adults: results of a double-blinded randomised controlled trial.

In the previous study, the observation that there was a significant reduction in weight and fat mass in the treatment group but not the placebo group with 10 weeks of weight maintenance suggests a possible influence of LCω-3PUFA on weight over time. Thus, this final clinical trial investigated the potential benefits of loading body cells/membranes with LCω-3PUFA prior to following a weight loss program.

6.1 Abstract

Obesity has been linked with low levels of ω-3 fatty acids. However, intervention studies have generally failed to establish benefits of dietary supplementation with ω-3PUFA in reducing body weight or fat mass in humans. The aim of this study was to investigate whether supplementation with LCω-3PUFA alone, then continued supplementation consumed concomitantly with a very low energy diet (VLED), facilitated weight loss, improvements in blood lipids and positive changes to inflammatory mediators. This was a double blind randomised controlled trial with two parallel groups. For 4 weeks of prior supplementation, one group consumed 6x1g capsules/d monounsaturated oil (Placebo), the other group consumed 6x1g capsules/d LCω-3PUFA (Fish Oil) each comprising 70 mg EPA and 270 mg DHA, while consuming their usual diet. Each group continued with their supplements for another 4 weeks while both groups followed a VLED regimen (n=19 Placebo, n=20 Fish Oil). Fasting blood samples, anthropometric measurements and 3-day food diaries were collected at baseline, at 4 weeks and at 8 weeks. At 4 weeks levels of EPA and DHA increased two-fold in the Fish Oil group (P<0.001), with no significant changes to anthropometric measurements for either group. At 8 weeks a significant 3-way interaction between time, group and gender was observed for percentage weight loss, F(1,35)=5.55, P=0.024, and BMI, F(1,35)=5.3, P=0.027. There was also a significant reduction in percentage weight loss in females in the FO group. It would appear that prior supplementation with LCω-3PUFA had a positive influence on weight loss over the 8 weeks.
6.2 Introduction

Obesity, classified by a body mass index (BMI) of ≥30kg/m$^2$, is defined as the abnormal or excessive accumulation of fat that may impair health (9). It is associated with cholesterol abnormalities (247) and with increased levels of systemic inflammation (114) that contribute to cardiovascular risk (CVD) (30).

There is substantial evidence that the consumption of fish or LCω-3PUFA reduces the risk of cardiovascular mortality in Western populations (296; 193; 31). However, there is no consensus on the beneficial effects of supplementation with LCω-3PUFA on reducing adiposity. Studies on animals, investigating the effects of LCω-3PUFA on weight, have reported conflicting results. Some studies with rodents have reported beneficial effects of supplementation with LCω-3PUFA on reducing weight and/or fat mass (264; 361) or the reduction/prevention of weight gain (362; 363; 364), while other studies have shown no significant effects (345; 346). Similarly, there is conflicting evidence on the effects of supplementation with LCω-3PUFA on adiposity in humans. In some studies LCω-3PUFA has been associated with weight loss (297; 270), in others with a decrease in adipose tissue mass (268; 269), while other studies have reported no effect on adiposity (31; 347).

It has also been reported that concentrations of LCω-3PUFA are significantly lower in obese compared to normal weight adults (332), adolescents (310; 306), and children (311) suggesting that high concentrations of LCω-3PUFA in the body inhibit the development of adiposity or have possibly reduced weight previously gained. Whether increasing the levels of LCω-3PUFA in obese individuals, prior to commencing a reduced energy weight loss diet, will result in a greater reduction in weight is yet to be established. Therefore the aim of this study was to examine whether prior supplementation with LCω-3PUFA without dietary change, immediately followed with continued supplementation of LCω-3PUFA, consumed concomitantly with a very low energy diet (VLED), may assist weight loss, reduce fat mass and improve blood biomarkers associated with possible beneficial consequences for the determinants of cardiovascular health.
6.3 Methods

This was a randomised, double blind placebo controlled intervention with two parallel groups.

6.3.1 Participants

Obese men and women (BMI 30–40 kg/m\(^2\)), aged 18 – 60 years were recruited from the university campus and the general public in Newcastle, Australia to participate in a weight loss study. Excluded from the study were men and women with diagnosed type 2 diabetes, a chronic inflammatory condition, already following an energy restricted diet, allergic to fish, taking fish oil capsules or consuming two or more oily fish meals per week, also women who were pregnant or lactating.

A power calculation estimated that 16 participants per group were needed to detect a difference of approximately 2% in weight loss between the two weight loss groups at the 0.05 level of significance, with a statistical power of 80% and assuming a standard deviation of 2%. Allowing for a 20% drop-out rate, 20 participants per group would be needed. When 42 participants had been recruited (21 in each group), recruitment was discontinued.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki: ethical approval was obtained from the Human Research Ethics Committee of the University of Newcastle, Australia and written, informed consent was obtained from participants prior to commencement. The trial was registered with the Australia New Zealand Clinical Trials Registry (ACTRN12610000654055).

6.3.2 Study design

Participants were allocated to one of two parallel groups using computer generated numbers. Supplement containers were labelled with a different code for each group by an independent researcher before the trial commenced so that neither the principle researcher nor the participants could identify the contents of the
supplements, and supplements were flavoured with peppermint to disguise identifiable tastes. For 8 weeks one group consumed 6 x 1g capsules of placebo/day (placebo group), containing sunola oil (monounsaturated oil), and the other group consumed 6 x 1g capsules of fish oil/day (fish oil group). Each fish oil capsule contained tuna oil comprising 360mg LCω-3PUFA with 70 mg Eicosapentaenoic Acid (EPA) and 270 mg Docosahexaenoic Acid (DHA) (ratio 1:3.9). Two capsules were consumed with each meal, breakfast, lunch and dinner, providing 1.62 g DHA and 0.42 g EPA per day. Both the tuna oil and the Sunola oil contained the antioxidant Coviox®T-70, 3000ppm in the tuna oil and 600ppm in the Sunola oil.

The intervention was divided into two phases. For the first phase of 4 weeks, during prior supplementation (the preparation phase), participants in the fish oil group consumed 6 x 1g capsules/day fish oil and, in the placebo group 6 x 1g capsules/day placebo, with both groups also following a healthy diet, that is their usual diet without the inclusion of fast foods. A 4 week period of supplementation to increase levels of EPA and DHA in the body was deemed appropriate because, in a previous study by Munro et al. (2012), an almost 2-fold increase in EPA and 2.5-fold increase in DHA had been observed after 4 weeks of supplementation with LCω-3PUFA (365). A study reported similar changes after 7 weeks of supplementation with krill oil or fish oil (366), indicating that there was little apparent benefit in extending the length of initial supplementation. For the second phase of 4 weeks, the weight loss phase, participants continued consuming the supplements allocated at the commencement of the study while following a very low energy diet (VLED) of 3000 kJ/day using meal replacements (MRs). This phase was limited to 4 weeks because of the very restricted energy intake. For the first two weeks (weeks 5 and 6) participants received Optifast® bars and shakes to replace meals according to the Intensive Phase of the Optifast Very Low Calorie Diet Program (Novartis, Australia), supplemented daily with raw and cooked vegetables and 2 litres of drinking water. This diet provides ≥3000 kJ per day composed of 40% protein, 16% fat and 40% carbohydrate plus vitamins and minerals. At the same time, participants were given individual nutrition education and counselling, with the sessions focusing on the energy density of foods, understanding and using food labels, appropriate portion sizes and the number of portions to be consumed daily from the different food
groups. This information was used to build a healthy diet using the guidelines from the Australian Guide to Healthy Eating (AGHE)\textsuperscript{(327)}, and applied during the final two weeks of the weight loss phases (weeks 7 and 8) when the MRs were gradually phased out and healthy kilojoule controlled meals were phased in. All participants attended weekly so that weight loss progress could be monitored and to receive the next supply of meal replacements and capsules. An accredited practicing dietitian was part of the research team to advise on health care.

6.3.3 Dietary assessment

Prior to commencing the weight loss program, dietary intake was assessed with a 3-day food diary to determine every-day nutrient and energy intake. Participants used the weights given on food products and handy measures of cups and spoons to record food intake which was analysed for nutrient intakes using the dietary software program, Foodworks® Professional 2009, version 6 (Xyris Software (Australia) Pty Ltd) and the mean values calculated.

6.3.4 Anthropometric assessment

On the first day of the preparation phase, anthropometric measurements were taken in the morning after a 10-hour overnight fast and no alcohol consumption, with participants dressed in light clothing and without shoes. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated balance beam scale (PCS Measurement, NSW, Australia). BMI was calculated in kilograms (kg) per metre squared from weight and height. Waist circumference (WC) was measured at the mid-point between the lowest rib and the top of the hipbone; the hip measurement was taken at the fullest point of the hip, as viewed from the side. The waist: hip ratio was calculated as waist girth in centimetres (cm) divided by the hip girth (cm). Fat mass (FM), fat-free mass (FFM) and muscle mass (MM) were measured using direct segmental multifrequency bioelectrical impedance (InBody 230, Biospace Co., Ltd. Seoul, Korea). Under identical conditions, these anthropometric measurements were repeated at the beginning and the end of the weight loss phase.
6.3.5 Biochemical analyses

6.3.5.1 Blood sample collection

Fasting (≥ 10 h) blood samples were collected into vacutainers, EDTA, Lithium Heparin and Sodium Fluoride, by a phlebotomist at commencement of the preparation phase and at baseline and the end of the weight loss phase. The samples were prepared by centrifuging (Heraeus Biofuge Stratos) for 10 min at 3000 g at 4°C. Plasma samples from the EDTA tubes were collected and stored at -80°C awaiting further analysis. The Lithium Heparin and Sodium Fluoride vacutainers were taken to Hunter Area Pathology Services (Newcastle) for plasma analysis for C-reactive protein (CRP) and blood lipids, and for blood glucose respectively.

6.3.5.2 Plasma fatty acid analysis

The plasma samples were analysed for fatty acid composition using an acetyl chloride methylation procedure, a modification of the method of Lepage and Roy (344). Fatty acid methyl esters were quantified using a GC (Hewlett Packard 6890, Hewlett Packard, Palo Alto, CA, USA) and identified by comparing peak retention times with the retention times of synthetic standards of known fatty acid composition (Nu Check Prep, Elysian, MN, USA).

6.3.5.3 Analysis of inflammatory mediators

Enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) were used to analyse inflammatory markers. The assays used to determine plasma leptin and adiponectin levels employ the quantitative sandwich enzyme immunoassay technique with a detection limit of 15.6 pg/mL and 3.9 ng/mL respectively and inter- and intra-assay coefficients of variance (CVs) of less than 5.5% and 7% respectively. High sensitivity ELISA kits were used to determine levels of tumour necrosis factor-α (hs-TNF-α) and interleukin-6 (hs-IL-6). Minimal detectable concentration of these kits was 0.106 pg/ml and 0.039 pg/ml respectively.
with an intra- and inter-assay CVs of <9%. Analysis of high-sensitivity C-reactive protein (hs-CRP) was conducted using an immunoturbidimetric method (Hunter Area Pathology Service, Newcastle, NSW, Australia) with a minimal detection level of 0.15 mg/l.

6.3.6 Statistical analyses

For anthropometric measurements, mixed design 3-way ANOVA was used to measure 3-way interactions between group, time and gender. Within group changes from baseline were determined using paired samples t-test and ANOVA was used to test group mean differences. Pearson product-normal correlations (r) were used to show relationships. Data are presented as mean values and standard error of mean (SEM), with P<0.05 indicating significance. All statistical analyses were carried out with SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA).

6.4 Results

Of the 42 participants who commenced the study, 39 completed the 8-week intervention, 20 in the Fish Oil group (FO) (15 females, 5 males) and 19 in the Placebo group (PB) (15 females, 4 males). Of the 3 who did not complete; 2 withdrew in week 2 for family reasons and one did not keep appointments for measurements and blood collection. Five extreme outliers were identified for hs-CRP (n = 2PB and 3FO) and were excluded from the analysis of this biomarker.

6.4.1 Diet

The mean daily energy intake of participants was similar for the two groups with 8293 ± 2597 kJ/d for FO and 8727 ± 2469 kJ/d for PB. At commencement of the study the percentage fatty acid composition of plasma lipids showed no significant difference between the two groups. After supplementation during the preparation phase with LCω-3PUFA in the FO group, both eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3) had increased almost 2-fold from week 0 to week 4 (P<0.001 for both), and they were significantly higher than the
levels in the PB group (P<0.001 for both) (Figure 6.1). There were very modest changes to levels of EPA and DHA after the weight loss dietary regimen (week 8) and they remained significantly higher in the FO group compared to the PB group (P<0.001 for both).

![Graph showing changes in EPA and DHA levels](image)

**Fig. 6.1** Baseline values and percent changes for LCn-3PUFA at prior-supplementation and weight loss phases (0, 4 & 8 weeks) (Mean values ± SEM)

*** p<0.001

### 6.4.2 Anthropometric measurements

At commencement of the study the baseline measurements for weight, BMI, FM, FFM, MM, WC and hip were not significantly different between the two groups, PB and FO and at the end of the 4 weeks preparation phase anthropometric measurements were relatively unchanged (Table 6.1).

After 4 weeks of weight loss with continued supplementation with LCω-3PUFA in the FO group there was a significant reduction in body weight -7.01% (-6.12 kg), FM -13.6% (-4.36 kg), FFM -3.2% (-1.68 kg), WC -5.8% (-5.69 cm), and hip
measurement -3.3% (-3.78 cm) (P<0.001 for all). However, after continued supplementation with placebo in the PB group there were also significant reductions in, body weight -6.2% (-5.79 kg), FM -11.9% (-4.46 kg), WC -5.3% (-5.68 cm), and hip measurement -3.3% (-3.82 cm) (P<0.001 for all), and in FFM -2.3% (-1.33 kg) (P<0.01) (Table 1). Mixed design 3-way ANOVA was used to investigate percentage change to anthropometric measurements between weeks 4 and 8, and the interaction between the FO and the PB groups, and gender. A significant 3-way interaction between time, group and gender was observed for percentage weight loss, F(1,35)=5.55, P=0.024, and for percentage decrease to BMI, F(1,35)=5.3, P=0.027. When mixed design 2-way ANOVA was then used to investigate percentage decrease in weight for males and females, a significant 2-way interaction between time and group for percentage weight loss in females was observed, F(1,28)=8.86, P=0.046 for a 1-tailed test.

Correlations between changes in anthropometric measurements, weight loss and LCω-3PUFA were computed. For both FO and PB there were positive, significant correlations between weight loss and reductions to BMI (P<0.001 for both), FFM (p<0.01 for both) and FM (P<0.001 for PB and P<0.05 for FO). For FO only there was a positive, significant correlation between a reduction in FM and a reduction in WC (P<0.01), BMI and hip measurement (P<0.05 for both). There was a negative and non significant correlation between percentage weight change and EPA, r = -0.316 (P=0.175), and DHA, r = -0.283 (P=0.227).
Table 6.1 Baseline values and changes for anthropometric measurements at preparation and weight loss phases (4 and 8 wks)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=19)</th>
<th>Fish Oil (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 weeks</td>
<td>4 weeks Δ</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>47.11 ± 2.05</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.55 ± 2.67</td>
<td>92.86 ± 2.71 0.32 ± 0.25</td>
</tr>
<tr>
<td>Weight (%)</td>
<td>0.34 ± 0.27</td>
<td>-6.61 ± 0.28</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.57 ± 0.82</td>
<td>33.70 ± 0.83 0.13 ± 0.09</td>
</tr>
<tr>
<td>BMI (%)</td>
<td>0.38 ± 0.27</td>
<td>-6.29 ± 0.44</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>38.35 ± 1.58</td>
<td>38.59 ± 1.64 0.19 ± 0.25</td>
</tr>
<tr>
<td>FM (%)</td>
<td>0.81 ± 1.26</td>
<td>-11.85 ± 1.10</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>54.20 ± 2.15</td>
<td>54.27 ± 2.20 0.07 ± 0.39</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>106.34 ± 2.29</td>
<td>106.66 ± 2.24 0.32 ± 0.45</td>
</tr>
<tr>
<td>WC (%)</td>
<td>0.34 ± 0.44</td>
<td>-5.31 ± 0.51</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>118.08 ± 1.46</td>
<td>117.92 ± 1.48 -0.16 ± 0.24</td>
</tr>
<tr>
<td>Hip (%)</td>
<td>-0.13 ± 0.21</td>
<td>-3.26 ± 0.34</td>
</tr>
<tr>
<td>Waist:hip</td>
<td>0.90 ± 0.02</td>
<td>0.91 ± 0.02 0.00 ± 0.00</td>
</tr>
</tbody>
</table>

(Mean value ± SEM)

Significant changes within groups: *p<0.05; **p<0.01; ***p<0.001
Table 6.2 Baseline values and changes for blood biomarkers at preparation and weight loss phases (4 and 8 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=19)</th>
<th></th>
<th>Fish Oil (n=20)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 weeks</td>
<td>4 weeks</td>
<td>Δ</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Trigs (mmol/l)</td>
<td>1.76 ± 0.30</td>
<td>1.57 ± 0.30</td>
<td>-0.19 ± 0.09</td>
<td>1.31 ± 0.28</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.24 ± 0.23</td>
<td>5.29 ± 0.23</td>
<td>0.05 ± 0.09</td>
<td>4.60 ± 0.20</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.36 ± 0.08</td>
<td>1.37 ± 0.08</td>
<td>0.01 ± 0.03</td>
<td>1.22 ± 0.07</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.07 ± 0.24</td>
<td>3.21 ± 0.25</td>
<td>0.14 ± 0.09</td>
<td>2.78 ± 0.19</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.10 ± 0.14</td>
<td>5.06 ± 0.15</td>
<td>-0.04 ± 0.11</td>
<td>4.94 ± 0.16</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>42.06 ± 4.45</td>
<td>40.44 ± 4.57</td>
<td>-1.62 ± 1.95</td>
<td>17.45 ± 2.64</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>9.31 ± 1.72</td>
<td>8.96 ± 1.40</td>
<td>-0.36 ± 0.54</td>
<td>8.63 ± 1.58</td>
</tr>
<tr>
<td>hs-CRP (μg/ml)</td>
<td>4.59 ± 0.56</td>
<td>4.40 ± 0.62</td>
<td>-0.18 ± 0.85†</td>
<td>3.82 ± 0.74</td>
</tr>
<tr>
<td>hs-IL-6 (pg/ml)</td>
<td>2.49 ± 0.51</td>
<td>2.45 ± 0.50</td>
<td>-0.05 ± 0.34</td>
<td>2.85 ± 0.63</td>
</tr>
<tr>
<td>hs-TNF-α (pg/ml)</td>
<td>1.49 ± 0.32</td>
<td>1.51 ± 0.30</td>
<td>0.03 ± 0.06</td>
<td>1.45 ± 0.27</td>
</tr>
</tbody>
</table>

Data available: † n=17; ‡ n=17
(Mean value ± SEM)

Significant changes within groups: *p<0.05; **p<0.01; ***p<0.001
6.4.3 Blood biomarkers

The changes in blood biomarkers from baseline to the end of the 4 week preparation phase were very modest and non significant for both groups (summarised in Table 6.2) apart from TGs which showed a significant reduction in the FO group (P<0.05) (Table 6.2).

At week 8, after 4 weeks of a VLED and continued supplementation with LCω-3 PUFA, there were significant changes to blood biomarkers within each group. For both FO and PB there was a significant reduction in total cholesterol (TC) (P<0.001 for both). In FO there were significant reductions in high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) (P<0.001 for both) and in triglycerides (TG)s and glucose (P<0.01 for both). For PB there were significant reduction in HDL-C, LDL-C and TGs (P<0.01 for all) but not glucose (Table 6.2). The only significant reduction observed in the inflammatory mediators was for leptin (P<0.001 for FO and PB). Differences between the two groups were not significant.

6.5 Discussion

Despite compliance with supplement consumption, as evidenced by the significant increase in levels of EPA and DHA in the FO group, anthropometric measurements were relatively unchanged in both FO and PB at the end of the 4 week preparation phase, with no significant differences between the two groups, suggesting that, within this short time-frame, LCω-3PUFA consumed without dietary energy restriction had no effect on body weight or FM. To compare the effect of LCω-3PUFA during the 4-week weight loss phase, dietary compliance was facilitated with the use of MRs which have a satiating effect that lasts for between 3 and 5 hours after consumption (46). While there were significant reductions in all anthropometric measurements within both groups at the end of the weight loss phase, it appeared that the differences in the reductions between the two groups were not significant. Also, there was no apparent significant association between changes to EPA or DHA and changes to weight or fat mass during the preparation phase or the weight loss phase in either group. A possible suggestion for this is that during the preparation phase, when levels of EPA and DHA increased
significantly, there was no change to anthropometric measurements; during the weight loss phase, when there was a significant change to anthropometric measurements, changes to EPA and DHA were extremely modest.

Analysis of changes to anthropometric measurements using mixed design 3-way ANOVA did, however, reveal a significant 3-way interaction between time, group and gender for both percentage weight loss and percentage decrease for BMI suggesting a significant effect of LCω-3PUFA for the FO group. To further explore this result, a mixed design 2-way ANOVA revealed a significant 2-way interaction between time and group for percentage weight loss in females, suggesting a significant effect of LCω-3PUFA for FO group. It has previously been reported that females are more responsive to the metabolism of LCω-3PUFA compared to males (220; 224) possibly due to hormonal factors (367). However, this study was not designed to detect sex differences, and the result for a significant difference in percentage weight loss for females should be treated with caution, though it does suggest that further research exploring gender differences to weight loss supplemented with LCω-3PUFA is warranted.

A limited number of previous studies have reported on the effects of combined supplementation with LCω-3PUFA and dietary energy restriction for weight loss over a period of 8 weeks or longer. Energy restricted or controlled diets combined with supplementation with LCω-3PUFA (1.3g/d EPA and 2.9g/d DHA, ratio 1:2.2) over 12 weeks (31), (1.08g/d EPA and 0.72g/d DHA, ratio 1.5:1) over 8 weeks (269), and (3g/d EPA + DHA, ratio 5:1) over 24 weeks in combination with exercise (347), all reported no significant difference in weight reduction compared to the control, although Kabir et al., (2007) did report a significantly greater decrease in FM in the treatment group compared to the control. Another study, but with only 6 participants, also reported a reduction in FM after a 3-week intake of 6g/d of fish oil (1.1g/d EPA and 0.7g/d DHA, ratio 1.6:1) and with a control diet ad libitum (266). A significant reduction in BMI was reported in a group of severely obese females following a VLED over 3 weeks with an intake of 2.8g/d LCω-3PUFA (EPA:DHA ratio 2:1) (297). Clearly the published literature varies greatly with respect to dosage of LCω-3PUFA, the EPA:DHA ratio, duration of supplementation, healthy status of the study participants and gender and it is, therefore, difficult to draw any firm conclusions from human studies,
However, studies with rodents have reported that LCω-3PUFA in the diet prevents or limits weight gain (262; 326; 264; 368), modulates fat deposition (256; 277), or results in weight loss (362; 361). It has been suggested that body weight and FM decrease relative to the amount of LCω-3PUFA in the diet (326). However, caution should be applied when comparing results from animals and humans because the doses in animal studies vary considerably and are generally higher than those considered safe in humans, for example 85.2 g EPA + DHA/ kg/ day (369), to 1 g EPA/ kg/ d (277), and could influence differences in pharmacokinetics of EPA and DHA supplementation (328).

In humans it is possible that an extended period of LCω-3PUFA intake during the growth years rather than dietary supplementation with large doses over a short duration in adults influences body weight. In this context, several studies have reported a significantly higher concentration of LCω-3PUFA in normal weight compared to obese individuals (310; 306; 311; 332) but timing and duration of intake of LCω-3PUFA in the individuals is unknown. It is possible that incorporation of LCω-3PUFA into the adipose tissue is needed for any effects on weight loss and, despite long-term supplementation in adults, increase in the levels of EPA and DHA in adipose tissue is reportedly modest (31). As late foetal and early postnatal life is a highly sensitive period when adipose tissue expands rapidly (94; 305; 91) this could be a critical window of opportunity to improve the balance of fatty acids involved in adipogenesis and lipogenesis. Studies in animals have reported that DHA inhibits adipogenesis (370; 371). A study in humans, where mothers received DHA supplementation from 21 weeks gestation until the end of the third month of lactation, reported a significant time-dependent effect of DHA on weight and BMI reduction in their infants at 21 months (372). Thus, it would appear that when increasing levels of LCω-3PUFA for weight management, gender, age, duration of supplementation, concentration and ratio of EPA:DHA all require further consideration.

Weight loss is associated with reductions in blood lipids which is beneficial to health (255). Accordingly, after weight loss this study showed significant reductions in levels of TGs, TC and LDL-C within both FO and PB, with no significant difference between the two groups. There was, however, also a significant reduction in TG in the FO group, but not in the PB group, after the preparation phase Supplementation with LCω-3PUFA is firmly associated with a reduction in TG levels (291; 244; 373; 374) which supports our finding. The subsequent significant reduction in TG in both groups after the weight loss phase
could be as a result of the considerable weight reduction experienced by both groups over the 4 weeks, though continued supplementation LCω-3PUFA did influence a greater reduction in TG in the FO group. Reductions in TC and LDL-C were also greater in FO than PB but, again, the differences were modest and not significant. It has been suggested that LCω-3PUFA has no effect on TC levels (291; 333) and opinions on the effect of LCω-3PUFA on LDL-C are inconclusive. Studies have reported that supplementation with LCω-3PUFA resulted in small increases in LDL-C levels (352; 353). However, it has been suggested that an increase in LDL-C is accompanied by an increase in the size of the LDL particles which are thought to be less atherogenic (354).

A reduction in HDL-C also occurred in both FO and PB which is not desirable because it is known to have cardioprotective effects (350). Reports on the effect of weight loss on levels of HDL-C are conflicting; some studies have shown small increases (375; 376; 377), some have reported small decreases (378; 379), and others have shown no changes at all (380; 335). It would appear that HDL-C levels reduce during the period when weight is decreasing, as occurred in this study. It has been suggested that during a decrease in weight, a reduction in triglyceride rich lipoprotein synthesis impairs VLDL-C catabolism with a subsequent reduction in the transfer of lipids to HDL-C. When weight stabilises, lipoprotein lipase increases and with it the hydrolysis of VLDL-C and a resumption in the transfer of lipids to HDL-C (255). From a review of studies, Dattilo et al. (1992) reported a mean decrease in HDL-C during actual weight loss which then increased when a stable weight was achieved (255). A study by Munro et al. (2012) with an identical weight loss diet conducted over the same length of time as the current study, also reported a decrease in HDL-C levels at the end of the weight loss phase. Then, during the 10 weeks of maintenance that followed, HDL-C levels increased, reaching levels higher than those recorded at baseline (365). Studies have also shown that LCω-3PUFA, particularly DHA, increases HDL-C concentrations (381) but, despite the greater concentration of DHA in the supplementation with LCω-3PUFA in this study, HDL-C levels did not increase. It is possible that this occurred as a consequence of the considerable weight loss, as discussed above.

Apart from leptin, the changes to inflammatory mediators in this study were extremely modest. After 4 weeks of prior supplementation with LCω-3PUFA and with virtually no weight change, there was an extremely modest increase in leptin for FO and reduction
for PB. However, after the 4 week weight loss phase there was a significant reduction in leptin for both FO and PB (64% and 57% respectively) but no significant difference between the two groups, suggesting that weight loss and not LCω-3PUFA influenced the changes. The magnitude of these reductions is similar to the findings in other studies with similar energy restricted diets but no supplementation with LCω-3PUFA \(^{(382; 383; 53)}\). There is conflicting evidence on the effect of LCω-3PUFA on plasma leptin levels \(^{(169)}\), with one study reporting that EPA increases leptin levels \(^{(384)}\), another suggesting that LCω-3PUFA reduces leptin levels \(^{(385)}\). The changes to adiponectin levels were very modest with no distinct trend for weight loss and supplementation with LCω-3PUFA. Madsen et al. (2008) reported an increase in levels of adiponectin in response to diet induced weight loss but suggest that a reduction in weight of at least 10% is required to show a significant increase in levels of adiponectin \(^{(180)}\).

After the 4 weeks of prior supplementation CRP levels increased for FO and decreased for PB. However, after 4 weeks of weight loss there was an almost identical decrease in CRP for both groups. Conflicting findings have been reported from studies investigating the effect of diet and/or weight on CRP levels. One study reported that LCω-3PUFA and not weight loss was associated with a significant reduction in CRP \(^{(283)}\), while another study reported an inverse association with LCω-3PUFA and serum CRP in men \(^{(386)}\). Observational studies have reported that LCω-3PUFA is associated with lower levels of CRP \(^{(280; 387)}\), while randomised control trials have reported no effect of LCω-3PUFA on CRP levels in healthy people \(^{(285; 359)}\). It has also been suggested that a reduction in the level of CRP is directly related to a reduction in weight \(^{(356)}\). However, it would appear that a significant reduction in the long-term requires weight loss to be greater than 10% \(^{(180)}\) and it is possible that not only differences in the length of the intervention, but differences in ages of subjects within the groups and components of the different diets could influence levels of plasma CRP \(^{(169)}\).

After the preparation phase and after the weight loss phase there were no significant differences in changes to IL-6 and TNF-α either within groups or between groups, suggesting that LCω-3PUFA had no effect. It has been reported that dietary supplementation with LCω-3PUFA can suppress the production of TNF-α \(^{(358)}\), however no significant changes in IL-6 and TNFα were observed in a study when subjects
received either 2 g, 4 g or 6 g/d of Hi DHA (26% DHA and 6% EPA) over 12 weeks \(^{(388)}\). Similar findings were reported in a study that did not observe any effect on levels of IL-6 concentrations after supplementation with 3.4 g/d of LCω-3PUFA for 6 weeks \(^{(389)}\). Another study reported that weight loss and not LCω-3PUFA influenced a significant reduction in IL-6 and a non-significant reduction in TNF-α \(^{(29)}\). These authors suggest that as IL-6 is secreted by adipose tissue, a reduction in FM could contribute to a reduction of IL-6 levels.

### 6.6 Conclusion

It has been suggested that compared to males, females are more responsive to the metabolism of LCω-3PUFA, possibly because of the hormone oestrogen and they therefore benefit from enhanced thermogenesis from LCω-3PUFA. However, because of the small numbers involved in this study, the greater percentage reduction in weight and BMI observed in females in the FO group should be treated with caution. The findings suggest that further research is needed to investigate gender differences in the potential link between LCω-3PUFA, including DHA and EPA on adiposity and weight.

### 6.7 Limitations

Although sufficient participants completed the study to detect significant differences in anthropometric measurements, the sample size was small. Overall, changes to blood biomarkers were greater in the fish oil group and, with a larger sample size, these differences could have been significant.

**Acknowledgements**

NuMega Ingredients Pty Ltd, Australia, supplied the fish oil and sunola oil capsules. Nestle Nutrition, Australia, supplied the Optifast® bars and shakes for the meal replacements.
Chapter 7:

General discussion
7.1 Discussion

This thesis comprises four individual studies of original research, three of which are clinical trials. The studies have been presented as four individual chapters.

7.2 The influence of supplementation with LCω-3PUFA and dietary energy restriction on adiposity

This research was driven by a curiosity to explore possible ways of addressing weight loss and weight management with a particular focus on improvements in health. Obesity and increased adiposity are associated with chronic, low-grade systemic inflammation which has been linked to CVD. With weight loss, a reduction in adipose tissue is associated with a decrease in these markers of inflammation. It has also been suggested that LCω-3PUFA is associated with a reduction in inflammation (225) and a reduction in CVD risk (390; 31; 297). The consumption of LCω-3PUFA has reduced body weight and FM in mice (326; 264) and it is possible that supplementing the diet with LCω-3PUFA in humans might also reduce body fat (328). The possibility of reducing adipose tissue inflammation without weight loss remains an emerging area of research.

The first step in this research was to confirm a link between LCω-3PUFA and adiposity. Previous research using data from two long term prospective studies (258; 257) involving large cohorts of adults had reported conflicting associations between LCω-3PUFA and adiposity. Thus, the study reported in Chapter 3 investigated a potential association between weight status, more specifically obesity, and plasma LCω-3PUFA concentrations in adults. Plasma was deemed a more reliable source of LCω-3PUFA data compared to the self-report data in the prospective studies which had been obtained from food consumed and itemised in food frequency questionnaires. We observed that total ω-3 PUFA, EPA and DHA were inversely correlated with BMI, WC and hip circumference in obese individuals. This appears to be the first time that this relationship has been reported in adults though our findings are similar to those obtained from studies conducted with adolescents (310; 306) and children (311) which reported lower levels of LCω-3PUFA in those who were overweight and obese.
The hypothesis that LCω-3PUFA is associated with weight status in adults is supported. Thus, the potential for dietary supplementation with ω-3 PUFA to reduce adiposity warranted further investigation.

The first clinical trial, which is reported in Chapter 4, investigated the potential of consuming LCω-3PUFA as an adjunct to weight loss when combined with an energy reduced diet. The approach taken for dietary change was to encourage the development of healthful eating behaviour through portion control, nutrition education, and counselling. Both groups experienced a reduction in weight but the difference between the two groups was not significant suggesting that LCω-3PUFA had no effect on weight change. Also, the weight loss over 12 weeks was modest suggesting poor dietary compliance which was confirmed with the 3-day food diaries completed at the end of the intervention. Animal studies have consistently shown a reduction in body weight in response to dietary supplementation with LCω-3PUFA. This could be influenced by dietary compliance which can be enforced in caged animals but not in free-living humans. A more effective weight loss strategy was needed that would address the feelings of hunger that accompany a reduction in food intake, to provide for a more consistent compliance across all participants.

The issue of dietary compliance was addressed through the use of MRs for a VLED consumed concomitantly with LCω-3PUFA for weight loss in the second clinical trial, reported in Chapter 5. Munro et al. (2011) had reported significantly greater reductions in weight and fat mass with a VLED using MRs for rapid weight loss over a short period of 4 weeks compared to a slow, longer-term, healthy eating diet over a longer period of 12 weeks (53) (Appendix 17). The weight loss phase in the second clinical trial followed that same time frame of 4 weeks because of the very low energy intake with MRs. The weight loss phase was then followed with a 10-week supervised weight maintenance phase of balanced meals with healthy food choices, to support participants in the maintenance of their changed dietary behaviour as they continued to consume their supplements. Significant reductions in anthropometric measurements within each group, but with no significant differences between the two groups after 4 weeks of weight loss, suggested that the changes were influenced by the VLED and not by LCω-3PUFA. It is possible that the significant weight reduction in both groups masked any
potential effect of LCω-3PUFA. The expected weight reduction from the consumption of LCω-3PUFA is yet to be elucidated, and quantification could be difficult as it could depend on a number of influences such as concentration and ratio of EPA to DHA, duration of consumption, age, gender, location of the fat mass, and the level of adiposity.

The question of duration of intake of LCω-3PUFA required until it potentially influences adiposity is an interesting one. Although weight stabilisation rather than weight reduction was the aim of the maintenance phase of 10 weeks in the second clinical trial, there was a continued reduction in weight in both the fish oil and placebo groups. While the reduction in weight, BMI, FM, WC and hip was not significantly different between the two groups or within the PB group over the 10 weeks, they were significantly different within the FO group, suggesting that this might be attributed to supplementation with LCω-3PUFA.

It has been suggested that for the full biological effect of DHA to be reached in humans, it may take 4-6 months after DHA supplementation commences (31). Krebs (2006) reported on changes to plasma and adipose tissue with daily supplementation of 1.3 g EPA and 2.9 g DHA in the ratio of 1:2.2 in overweight women. After 12 weeks plasma levels of DHA had increased by 0.55% and by week 24 a further 1% increase was observed with no indication that levels had reached a plateau. Initially plasma levels of EPA increased more rapidly and by 12 weeks an increase of 1.79% was observed which then levelled out to a further increase in EPA of only 0.11% by week 24 (31). Although total plasma levels of EPA were greater than those for DHA at 24 weeks, it appears possible that DHA might continue to increase with continued supplementation. Changes to levels of EPA and DHA in adipose tissue were much more modest. Adipose tissue levels of EPA barely changed during supplementation, increasing by 0.02% at 12 weeks and then a further 0.01% at 24 weeks, while adipose tissue levels of DHA followed a similar trend to plasma levels with an increase of 0.08% at 12 weeks and a further increase of 0.14% at 24 weeks, and potentially still increasing slowly (31).

Age of intake also appears to make a difference to the rate of absorption of fatty acids. A study on elderly men reported that it took more than 3 years for the linoleic acid (LA)
content of their body fat to reflect the increased levels of LA in their diet \(^{(391)}\) while a study conducted with infants no more than 5 days old, reported that the LA content of their subcutaneous fat became very similar to their levels of LA intake from formula milk 7 weeks after commencing the formula \(^{(392)}\). As late fetal and early postnatal life is a highly sensitive period when adipose tissue expands rapidly \(^{(94; 305; 91)}\) this could be a critical window of opportunity to improve the balance of fatty acids involved in adipogenesis and lipogenesis. Studies in animals have reported that DHA inhibits adipogenesis \(^{(370; 371)}\). In humans, a study where mothers received DHA supplementation from 21 weeks gestation until the end of the third month of lactation reported a significant time-dependent effect of DHA on weight and BMI reduction in their infants at 21 months \(^{(372)}\).

Thus, it would appear that when increasing intake of LC\(\omega-3\)PUFA for weight management, age of commencement, concentration and ratio of EPA to DHA all require further consideration. The hypothesis that LC\(\omega-3\)PUFA of marine origin will facilitate weight loss in obese adults when taken in combination with a weight loss programme using a VLED is not supported as there was no significant difference between the FO and PB groups. However, the hypothesis that after weight loss, continued supplementation with LC\(\omega-3\)PUFA will inhibit weight regain during a period of weight maintenance is supported. There was a significant reduction in weight and adiposity in the FO group over the 10 weeks of maintenance but not in the PB group. Although there was no significant difference between the two groups this might be a consequence of the small number of participants remaining in the PB group at the end of the maintenance phase.

The observation in the second clinical trial that a significant reduction in weight and FM continued in the FO group after 4 weeks of supplementation with LC\(\omega-3\)PUFA during the weight loss phase was deemed worthy of further investigation. The third clinical trial, reported in Chapter 6, examined whether prior supplementation with LC\(\omega-3\)PUFA followed by the concomitant administration of a VLED with MRs would facilitate weight loss. In the previous study, the second clinical trial, levels of EPA had increased almost 2-fold and DHA had increased 2.5 fold over the 4 weeks of weight loss. Similar changes had been reported in another study after 7 weeks of supplementation with krill.
oil or fish oil (366) and there did not appear to be any immediate benefit to increasing the 4-week duration of prior supplementation. A 4-week VLED with MRs was again used for weight loss with participants continuing to consume the supplements that they had been allocated at the commencement of the study. After 4 weeks of prior supplementation with LCω-3PUFA, levels of EPA and DHA had increased almost 2-fold in the FO group. Despite continued supplementation at the same concentration of EPA and DHA and with the same frequency over the same time frame, changes over the 4-week weight loss phase in FO group were extremely small. While there were no changes in measures of adiposity at the end of prior supplementation (4 weeks) in either the PB or FO groups, there were significant reductions in weight, FM WC and hip circumference within each group at the end of the weight loss phase (8 weeks), suggesting a positive effect of the VLED. However, significant differences were observed between the groups, with a significantly greater reduction in percentage weight and BMI in the FO group suggesting a positive effect from supplementation with LCω-3PUFA. The hypothesis, increasing concentrations of LCω-3PUFA in body cells and membranes of obese individuals prior to commencing a reduced energy weight loss diet will assist weight loss, is supported.

There was also a significantly greater reduction in percentage weight loss in females in the FO group. While this study was not designed to detect sex differences on the effect of LCω-3PUFA on adiposity, it has been suggested that there are gender differences in the metabolism of LCω-3PUFA. These differences could include gender differences in the rates of β-oxidation, the composition and mobilisation of adipose tissue, and possible influences of sex hormones on the desaturase and elongase enzymes involved in the synthesis of LCω-3PUFA (393). Gender differences have been observed in the composition of adipose tissue in human studies with women found to have more DPA and DHA in adipose tissue compared to men (194). It has been reported that females are more responsive to the metabolism of DHA compared to males and have a higher percentage of DHA in plasma and adipose lipids (224) possibly due to hormonal factors (367). It is possible that gender differences in the composition of adipose tissue may directly affect circulating concentrations of LCω-3PUFA to increase the availability of ALA for synthesis of LCω-3PUFA in women compared with men (194). Thus, it is possible that the higher concentration of DHA in the supplements used in the current
study, EPA:DHA ratio of almost 1:4, may have been favourable for weight reduction in females.

7.3 The effect of LCω-3PUFA supplementation and dietary energy restriction on plasma lipids

It is well established that weight loss is associated with reductions in blood lipids apart from HDL-C, with different studies reporting small increases \(^{(375;376;377)}\), small decreases \(^{(378;379)}\), or no changes in HDL-C at all \(^{(380;335)}\). In all three of our clinical trials, levels of HDL-C reduced with weight loss apart from in the FO group in Trial 1 where there was an extremely modest increase. These reductions are undesirable as HDL-C is known to have cardioprotective effects. However, it has been suggested that reductions are short term \(^{(255)}\). HDL-C levels reduce because lipoprotein lipase is reduced during actual weight loss resulting in a reduction in TG-rich lipoprotein synthesis which impairs VLDL-C catabolism and a subsequent reduction in the transfer of lipids to HDL-C. When weight stabilises at the reduced level, lipoprotein lipase increases and with it the hydrolysis of VLDL-C, and the transfer of lipids to HDL-C resumes \(^{(255)}\). This was observed in our second clinical trial (Chapter 5) where there was a reduction in HDL-C with weight loss and then an increase during the 10 weeks of weight maintenance.

TG levels decrease in response to a reduction in weight. However, it is also well established that LCω-3PUFA have a lowering effect on TGs \(^{(333;244)}\). These influences were observed in all three Clinical Trials with a significant reduction in TGs in the LCω-3PUFA supplemented (FO) groups only during the weight loss phases in Trials 1 and 2, and the prior supplementation phase, which did not include weight loss, in Trial 3, suggesting that the reductions could be attributed to the effect of LCω-3PUFA. A non-significant reduction in TG was also observed in the PB groups during the same time frames and most likely occurred in response to a reduction in weight.

In Trial 2, the maintenance phase provided an opportunity to observe changes that occurred to plasma lipids when the VLED ceased and regular foods were consumed but supplementation was continued. By the end of this 10-week maintenance phase in Trial 2, there was a small but significant increase in TG levels in both the PB and FO
groups. It is possible that this increase could be the result of a return to regular food consumption with a different fat content. However, it has been suggested that LCω-3PUFA lowers TG levels by reducing the synthesis and secretion of VLDL particles and, through the up-regulation of enzymes such as lipoprotein lipase, increases the removal of VLDL and chylomicron particles. Thus, the lowering influence of LCω-3PUFA on TGs should have applied in the FO group with continued supplementation of LCω-3PUFA in the 10-week maintenance phase. A possible explanation for the significant increase could also be the small sample size.

A change, similar to that observed with TG, occurred with TC and LDL-C. While there had been a significant reduction in TC and LDL-C within both PB and FO during the 4-week weight loss phase, levels increased during the 10-week maintenance phase in both groups, significantly only for TC in both groups. With the resumed consumption of regular food and changes to dietary fat during the maintenance phase, an increase in TC and LDL-C levels might be expected, particularly as it has been reported that LCω-3PUFA have no effect on TC levels although there are differences of opinion on whether LCω-3PUFA has any effect on LDL-C. Thus it does appear that resumption of a normal diet with weight maintained at the reduced level can influence a return of lipids to pre intervention levels.

### 7.4 The influence of LCω-3PUFA supplementation and dietary energy restriction on inflammatory mediators

The consumption of LCω-3PUFA has been linked to reductions in inflammation in both animals and humans, and a reduction in weight also decreases circulating inflammatory mediators. However, in all three of our Clinical Trials there were no significant differences in the changes to the inflammatory mediators between PB and FO groups. It is possible that this has occurred because the trials were not powered to detect significant changes in inflammatory mediators between the groups, but were powered to detect significant differences in anthropometric measurements. Nonetheless, significant changes were observed with some of the biomarkers within each group.
7.4.1 Leptin

There was a significant decrease in leptin levels at the end of the weight loss phase for the PB and FO groups in both of the studies using VLEDs for weight loss (Trial 2 and Trial 3), suggesting that weight loss and not LCω-3PUFA affected these changes. Similar observations have been reported in studies with comparable energy restricted diets but without supplementation with LCω-3PUFA (382; 383; 53). While decreasing leptin levels are associated with dietary energy restriction (173) and weight loss (165), there is conflicting evidence on the effect of LCω-3PUFA on plasma leptin levels (169), with one study suggesting that EPA increases leptin (384) and other suggesting that LCω-3PUFA reduces leptin levels (385). In Trial 1 there was a very modest change in plasma leptin levels, probably reflecting the moderate weight loss while, in Trials 2 and 3, the magnitude of the reduction in leptin was considerable, in response to the greater weight loss. However, it has been suggested that changes in leptin expression in response to dietary restriction and food consumption are out of proportion compared to associated changes in body weight or body fat (173; 395).

7.4.2 Adiponectin

The findings from studies investigating the effects of weight reduction on levels of adiponectin are inconsistent with studies reporting an increase in plasma adiponectin with weight reduction (181; 151) while others have reported a decrease in adiponectin with weight loss (182; 183). However, studies investigating the effect of supplementation with LCω-3PUFA have reported more consistent results with weight reduction associated with an increase in adiponectin levels observed in human studies (31) although it can also vary. These conflicting results are clearly observed in our Clinical Trials with adiponectin levels increased in PB after weight loss but decreased in FO after weight loss and supplementation with LCω-3PUFA in Clinical Trials 1 (slow weight loss) and 2 (VLED). A significant increase in adiponectin levels was subsequently observed in FO group after the weight maintenance phase in Trial 2, with no change in PB group. Adiponectin levels decreased after prior supplementation in both PB and FO groups in Trial 3, and continued to decrease with weight loss in PB group but increased with weight loss in FO group. It has been suggested that a weight reduction of at least 10%
is required for a significant increase in adiponectin to occur \(^{(180)}\) and we did not achieve such a reduction in any of our Clinical Trials.

### 7.4.3 C-reactive protein

Levels of CRP, which are higher in those who are overweight and obese, decrease with weight loss \(^{(356)}\). In a review of four intervention studies, Balk et al. (2006) reported that there was no significant change in hs-CRP levels with consumption of LCω-3PUFA \(^{(333)}\). While we observed a similar trend in our Clinical Trials, with a reduction in hs-CRP after weight loss in PB and FO groups in Clinical Trials 2 and 3, and in PB group in Clinical Trial 1, the differences were not significant. However, a significant effect of LCω-3PUFA intake on a reduction in CRP has been reported in one study \(^{(283)}\).

### 7.4.4 Tumour necrosis factor-α and Interleukin-6

There is more consistency with our results for TNF-α, with a reduction observed after the weight loss phase in all 3 Clinical Trials for both PB and FO groups. There was a further reduction in TNF-α in both PB and FO groups during the maintenance phase of Trial 2. However the differences between the groups were not significant suggesting that reductions in TNF-α were a consequence of weight loss and that supplementation with LCω-3PUFA had no effect. Results for IL-6 were not consistent across the three Clinical Trials. There was a reduction in IL-6 for both PB and FO groups after weight loss in Clinical Trial 2 (VLED) while, for both PB and FO groups, levels of IL-6 decreased after prior supplementation but then increased after weight loss in Clinical Trial 3 (also VLED). Published reports on changes to these inflammatory mediators are conflicting. It has been reported that weight loss and not an intake of LCω-3PUFA influenced a reduction in IL-6 and TNF-α \(^{(29)}\), while another study reported that LCω-3PUFA can suppress the production of TNF-α \(^{(358)}\). However another study reported findings similar to those observed in our Clinical Trials, that an intake of LCω-3PUFA had no significant effect on levels of IL-6 and TNF-α.
7.5 *Mechanisms of action*

Although the potential amount of weight that might be reduced with the consumption of LCω-3PUFA is yet to be quantified, a number of possible mechanisms for the process have been proposed. It has been suggested that dietary PUFA partitions energy sources, directing glucose towards glycogen storage and directing fatty acids away from the synthesis and assimilation of triglycerides and towards fatty acid oxidation. LCω-3PUFA also appear to enhance thermogenesis resulting in a reduction in fatty acid deposition in the body.\(^{(211)}\)

Mechanistically, most evidence suggests that LCω-3PUFAs reduce the synthesis and secretion of VLDL particles while also raising TG removal from VLDL and chylomicron particles through the up-regulation of enzymes such as lipoprotein lipase\(^{(394)}\).

The main effects of LCω-3PUFA on modulating lipid metabolism are to promote lipolysis and enhance hepatic fatty acid oxidation and to inhibit fatty acid synthesis and VLDL secretion\(^{(396}; 397}\). Both of these processes shift the balance of fatty acid metabolism towards oxidation rather than storage.

It has been shown that the intake of LCω-3PUFA up-regulates the expression of genes involved in fatty acid oxidation in the liver and down regulates the expression of genes involved in adipose tissue. Through these processes, the balance of fatty acid metabolism is shifted towards oxidation and reduced storage of fat\(^{(328}; 398}\). It is suggested that AMP-activated protein kinase (AMPK) in adipose tissue is activated by DHA and EPA which could be a mechanism for their effect on fatty acid oxidation\(^{(398)}\).

Functions of LCω-3PUFA can be mediated by increasing the oxidation-related enzyme activities including carnitine palmitoyl transferase-1 (CPT-1) and acyl CoA oxidase\(^{(399}; 400}\). CPT regulates the balance between fat and CHO oxidation by promoting an increased transport of fatty acids into the mitochondria and their subsequent oxidation\(^{(259)}\). The mitochondrial expression of CPT-1 is regulated upstream by peroxisome proliferator-activated receptors (PPARs) and by 5'-AMP-activated protein kinase (AMPK).
The mechanisms by which LCω-3PUFA reduce body fat are still being explored and it is possible that a combination of influences will apply.

7.6 Conclusion

This thesis has investigated the influence of LCω-3PUFA on adiposity in humans. Observations from the clinical trials suggest that LCω-3PUFA can contribute to small reductions in body weight but these modest changes can be easily obscured by differences in dietary energy intake and possibly by energy expenditure. Other possible confounders include gender, age, duration and concentration of supplementation, and ratio of EPA:DHA. However, there does appear to be a significant relationship between higher levels of LCω-3PUFA and lower levels of adiposity in adults, as observed in our first study, which is supported by similar studies in adolescents and children. This suggests that the benefit of LCω-3PUFA is in preventing the development of adiposity. Thus, the timing of intake is extremely important and would appear to be of maximum benefit at the age when adipose tissue is developing, in utero, continuing through childhood as a maintenance strategy. This suggests an important direction for future research.

7.7 Limitations

The small sample size in each of the clinical trials limited the significance of the findings. Also, a longer period for the interventions might have made a difference to the effect of LCω-3PUFA supplementation on weight.


183. Abbasi F, Chang SA, Chu JW, et al. (2006) Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or
adiponectin multimeric complexes. Am J Physiol Regul Integr Comp Physiol 290, R139-144.


Appendices
Appendix 1

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With many thanks for your help

Irene Munro
Nutraceuticals Research Group
School of Biomedical Sciences and Pharmacy
The University of Newcastle
Callaghan NSW 2308

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Appendix 3

Statements from 3 co-authors relating to a paper published with Irene Munro (1)

Statement from co-authors relating to a paper published with Irene Munro

I. Manohar L. Garg
(co-author’s name in full),

attest that Research Higher Degree candidate Irene Munro contributed to the following paper:


- To the conception and design of the study
- To the recruitment of participants
- Collection and recording of data
- To the drafting of the manuscript
- Revision of the manuscript

(Signature of Co-Author)

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Irene A. Munro
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- to the drafting of the manuscript - 40%
- revision of the manuscript - 40%

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to the paper/publication entitled:

Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity.


(Signature of Co-Author) ____________________________ Date: __________

(Signature of Assistant Dean Research Training (ADRT))

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• to the drafting of the manuscript – 40%
• revision of the manuscript – 50%

... to the paper/publication entitled:

Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity.


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Appendix 4

Statements from co-author relating to a paper published with Irene Munro (2)

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- To the conception and design of the study
- To the recruitment of participants
- Conducted the intervention and collected the clinical data
- Analysed inflammatory mediators
- Entered, analysed and interpreted the data
- To the drafting of the manuscript

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Appendix 5

Statements from co-author relating to a paper published with Irene Munro (3)

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- To the recruitment of participants
- Conducted the intervention and collected the clinical data
- Analysed inflammatory mediators
- Entered, analysed and interpreted the data
- To the drafting of the manuscript
- To the revision of the manuscript

(Signature of Co-Author)
Manohar L. Garg

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Appendix 6

Statements from co-author relating to a paper published with Irene Munro (4)

Statement from co-authors relating to a paper published with Irene Munro

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- To the conception and design of the study
- To the recruitment of participants
- Conducted the intervention and collected the clinical data
- Analysed inflammatory mediators
- Entered, analysed and interpreted the data
- To the drafting of the manuscript

(Signature of Co-Author)
Manohar L. Garg  
Date:

(Signature of Candidate)
Irene A. Munro  
Date:

(Signature of Assistant Dean Research Training (ADRT))
Professor John Rostas  
(Full Name of ADRT)
Prof. John Rostas  
Deputy Head of Faculty (Research)  
Faculty of Health  
Date:

Office of Graduate Studies, East Wing, The Chancellery  
Telephone: (02) 4921 6537  Fax: (02) 4921 6908  Email: research@newcastle.edu.au
Participant information statement: Clinical Trial 1
Information Statement
Omega-3 fats and weight loss

Investigators: A/Professor Manohar Garg and Irene Munro

You are invited to take part in a study investigating the role of omega-3 polyunsaturated fats on weight loss. This study is being conducted by Irene Munro, a PhD student with A/Professor Manohar Garg at the University of Newcastle.

Fish oil fatty acids (omega-3) are known to reduce inflammation caused by weight gain. Weight loss is assisted with reduced inflammation. Thus, in reducing inflammation, omega-3 fatty acids may prove to be an effective means of assisting in weight loss. This project will investigate the extent to which the consumption of omega-3 fat assists in weight loss.

Who can take part?
To take part in this study, you must be obese (BMI>30) and interested in embarking on a weight loss program. You cannot take part if you are currently taking anti-inflammatory drugs, you have been consuming fish oil supplements or more than two oily fish meals per week for the past 6 weeks, if you have an allergy to fish products, and/or you are unable to swallow capsules.

Participation in the study is voluntary and only those people who give their informed consent will be included in the project. You can withdraw from the project at any time without giving a reason for doing so.

What will you be asked to do?
If you agree to participate, you will be asked to attend up to six one-hour meetings over a period of three months with a dietitian at the university. The dietitian will provide information and support to help you lose weight. Parking vouchers will be provided for your visits. On your first visit, and again on your last visit, your height, weight and body composition will be measured, and a qualified nurse will collect 25mL (approximately 1 tablespoon) of your blood after an overnight fast. This will take an additional 15 minutes on these two occasions.

- At home you will be asked to complete a short quality of life questionnaire and complete a food record in which you write down everything you eat and drink for 3 days. Reply paid envelopes will be provided for you to post these back to the university.
During the study you will be asked to consume 6 x 1g capsules/day of fish oil (the taste is masked) or 6 x 1g capsules/day of Sunola oil (a monounsaturated oil).

**How long does it take?**
You will be asked to take the capsules until you reach your target weight or for three months, whichever comes first.

**What are the risks and benefits of participating?**
There are some risks from having blood collected, including bruising or bleeding from the collection site, feeling faint or dizzy. However, these risks are minimal and a very experienced and qualified person will take your blood in order to minimise these risks. The benefits are that you can obtain information about your health from the results of your blood analysis. There are no known risks associated with the consumption of fish oil with the dose used in this trial unless you are allergic to fish products, or Sunola oil (a monounsaturated oil).

**How will the information collected be used?**
The overall results of this research will be published in scientific journals and will be available to participants. The data collected will also contribute to Irene Munro’s PhD thesis. Information about individuals will not be released and participants will not be identified in any reports arising from the project.

**How will your privacy be protected?**
The information you provide will be treated with the same respect for privacy and confidentiality that is given for all medical information collected about you when you visit your local doctor.

Initially, information collected about you for this project will be stored on a computer database at the University of Newcastle, only identifiable by a participant identification number and with no other identifying details. The database will be password protected on a computer that is kept in a locked room. When all of the information for the project has been collected, all information will be de-identified and your personal details will be unknown. A master copy of participants and their code numbers will be held by the chief investigator so that participants can be re-identified if necessary. Only the researchers at the University of Newcastle conducting this research will have access to this information which will be stored at the University for 15 years.

Participant blood samples will only be accessible to the researchers involved in the study. The blood samples will be stored in a secured laboratory at the University until successful analysis has been completed and they will then be destroyed, following standard procedures.

**What do you need to do if you want to participate?**
Ensure that you understand the contents of this Information Statement before you consent to participate. If there is anything that you do not understand, or you have questions about the study, please contact Irene Munro, telephone 4921 5638.
If you would like to participate, please phone Irene Munro on 4921 5638 to arrange a time convenient for your first visit. You must sign the Consent Form which you can bring it to your first visit or you can post it to the University of Newcastle (address provided at the bottom of the page).

Thank you for considering this invitation to participate in our study

Yours sincerely,

Dr Manohar Garg
Associate Professor, Discipline of Pharmacy

Irene Munro
PhD Student

Note: This project has been approved by the University’s Human Research Ethics Committee, Approval H-159-1205. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher. If an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308. Telephone: 02 4921 6333, email: Human-Ethics@newcastle.edu.au
Participant information statement: Clinical Trial 2
Information Statement
Omega-3 fats and weight loss
Investigators: A/Professor Manohar Garg and Irene Munro

You are invited to take part in a study investigating the role of omega-3 polyunsaturated fats on weight loss. This study is being conducted by Irene Munro, a PhD student with A/Professor Manohar Garg at the University of Newcastle.

Fish oil fatty acids (omega-3) are known to reduce inflammation caused by weight gain. Weight loss is assisted with reduced inflammation. Thus, in reducing inflammation, omega-3 fatty acids may prove to be an effective means of assisting in weight loss, and maintaining that loss. This project will investigate the extent to which the consumption of omega-3 fat assists in weight loss management.

Who can take part?
To take part in this study, you must be obese (BMI>30) and interested in embarking on a weight loss program. You cannot take part if you are currently taking anti-inflammation drugs, you have been consuming fish oil supplements or more than two oily fish meals per week for the past 6 weeks, if you have an allergy to fish products, and/or you are unable to swallow capsules.

Participation in the study is voluntary and only those people who give their informed consent will be included in the project. You can withdraw from the project at any time without giving a reason for doing so.

What will you be asked to do?

1. Weight loss phase
If you agree to participate, you will be asked to attend up to four one-hour meetings over a period of one month at the university. These meetings will provide nutrition information and support to guide you through weight loss and maintenance. For these four weeks you will be asked to follow a very low calorie diet to help you with your weight loss. The very low calorie diet will provide a total of 3,000 kilojoules (715 Calories) per day. Participants will have the option of choosing meal replacements, nutritionally balanced meals or a combination of both to achieve this. Parking vouchers will be provided for your visits.

On your first visit, and at the end of the month, your height, weight and body composition will be measured, and a qualified nurse will collect 25mL (approximately.../2
1 tablespoon) of your blood after an overnight fast. This will take an additional 15 minutes on these two occasions.

- At home, prior to your first visit, you will be asked to complete a short quality of life questionnaire, a behaviour questionnaire, and complete a food record in which you write down everything you eat and drink for 3 days. You will be asked to bring these with you to your first visit.
- During the study you will be asked to consume 6 x 1g capsules/day of fish oil (the taste is masked) or 6 x 1g capsules/day of Sunola oil (a monounsaturated oil).

2. Weight maintenance phase
For the three months that follow we will provide you with support to help you to put into practice the nutrition information that you have been given to maintain the weight that you have lost.

- You will be asked to continue consuming 6 x 1g capsules/day of fish oil or 6 x 1g capsules/day of Sunola oil (a monounsaturated oil) for the duration of this phase.
- At the end of the maintenance phase you will be asked to complete another short quality of life questionnaire and to complete a food record in which you write down everything you eat and drink for 3 days.
- At the end of these three months, your weight and body composition will again be measured, and a qualified nurse will collect 25mL (approximately 1 tablespoon) of your blood after an overnight fast.

How long does it take?
The weight loss phase lasts for four weeks and the maintenance phase lasts for 10 weeks

What are the risks and benefits of participating?
There are some risks from having blood collected, including bruising or bleeding from the collection site, feeling faint or dizzy. However, these risks are minimal and a very experienced and qualified person will take your blood in order to minimise these risks. The benefits are that you can obtain information about your health from the results of your blood analysis. There are no known risks associated with the consumption of fish oil with the dose used in this trial unless you are allergic to fish products, or Sunola oil (a monounsaturated oil).

How will the information collected be used?
The overall results of this research will be published in scientific journals and will be available to participants. The data collected will also contribute to Irene Munro’s PhD thesis. Information about individuals will not be released and participants will not be identified in any reports arising from the project.

How will your privacy be protected?
The information you provide will be treated with the same respect for privacy and confidentiality that is given for all medical information collected about you when you visit your local doctor. Initially, information collected about you for this project will be stored on a computer database at the University of Newcastle, only identifiable by a participant...
identification number and with no other identifying details. The database will be password protected on a computer that is kept in a locked room. When all of the information for the project has been collected, all information will be de-identified and your personal details will be unknown. A master copy of participants and their code numbers will be held by the chief investigator so that participants can be re-identified if necessary. Only the researchers at the University of Newcastle conducting this research will have access to this information which will be stored at the University for 15 years.

Participant blood samples will only be accessible to the researchers involved in the study. The blood samples will be stored in a secured laboratory at the University until successful analysis has been completed and they will then be destroyed, following standard procedures.

**What do you need to do if you want to participate?**

Ensure that you understand the contents of this Information Statement before you consent to participate. If there is anything that you do not understand, or you have questions about the study, please contact Irene Munro, telephone 4921 5638.

If you would like to participate, please phone Irene Munro on 4921 5638 to arrange a time convenient for your first visit. You must sign the Consent Form which you can bring it to your first visit or you can post it to the University of Newcastle (address provided at the bottom of the page).

Thank you for considering this invitation to participate in our study

Yours sincerely,

____________________________
Dr Manohar Garg
Associate Professor, Discipline of Pharmacy

____________________________
Irene Munro
PhD Student

**Note:** This project has been approved by the University's Human Research Ethics Committee, Approval H-159-1205. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher. If an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308.

Telephone: 02 4921 6333, email: Human-Ethics@newcastle.edu.au
Participant information statement: Clinical Trial 3
Information Statement
Omega-3 fats and weight loss

Investigators: Professor Manohar Garg and Irene Munro

You are invited to take part in a study investigating the role of omega-3 polyunsaturated fats on weight loss. This study is being conducted by Irene Munro, a PhD student with Professor Manohar Garg at the University of Newcastle.

Fish oil fatty acids (omega-3) are known to reduce inflammation caused by weight gain. Weight loss is assisted with reduced inflammation. Thus, in reducing inflammation, omega-3 fatty acids may prove to be an effective means of assisting in weight loss. This project will investigate the extent to which the consumption of omega-3 fat assists in weight loss management.

Who can take part?
To take part in this study, you must be obese (BMI > 30) and interested in embarking on a weight loss program. You cannot take part if you are currently taking anti-inflammatory drugs, if you have been consuming fish oil supplements or more than two oily fish meals per week for the past 6 weeks, if you have an allergy to fish products, and/or you are unable to swallow capsules.

Participation in the study is voluntary and only those people who give their informed consent will be included in the project. You can withdraw from the project at any time without giving a reason for doing so.

What will you be asked to do?
If you agree to participate, you will be asked to attend six meetings at the university, two during the Preparation phase and four during the Weight Loss phase. Parking vouchers will be provided for your visits.

1. Preparation phase – 4 weeks

- On your first visit, and at the end of the month, your height, weight and body composition will be measured, and a qualified nurse will collect 25mL (just over 1 tablespoon) of your blood after an overnight fast.
- At home, prior to your first visit, you will be asked to complete a short quality of life questionnaire, a behaviour questionnaire, and complete a food record in which you write down everything you eat and drink for three days. You will be asked to bring these with you to your first visit.
During the 4 weeks of the preparation phase you will be asked to consume either 6 x 1g capsules/day of fish oil (the taste is masked) or 6 x 1g capsules/day of Sunola oil (a monounsaturated oil) while continuing to consume your regular diet.

2. Weight loss phase – 4 weeks

During this phase, you will be asked to attend up to four one-hour meetings over a period of one month at the university. These meetings will provide nutrition information and support to guide you through the first weeks of weight loss. For these four weeks you will be asked to follow a very low calorie diet to help you with your weight loss. The very low calorie diet will provide a total of 3,000 kilojoules (715 Calories) per day. Participants will have the option of choosing meal replacements, nutritionally balanced meals, or a combination of both to achieve this.

- You will be asked to continue consuming 6 x 1g capsules/day of fish oil or 6 x 1g capsules/day of Sunola oil (a monounsaturated oil) for the duration of this phase.
- At the end of the weight loss phase you will be asked to complete another short quality of life questionnaire, your weight and body composition will again be measured, and a qualified nurse will collect 25mL (just over 1 tablespoon) of your blood after an overnight fast.

How long does it take?
The preparation phase lasts for four weeks and the weight loss phase lasts for four weeks.

What are the risks and benefits of participating?
There are some risks from having blood collected, including bruising or bleeding from the collection site, feeling faint or dizzy. However, these risks are minimal and a very experienced and qualified person will take your blood in order to minimise these risks. The benefits are that you can obtain information about your health from the results of your blood analysis. There are no known risks associated with the consumption of fish oil with the dose used in this trial unless you are allergic to fish products, or Sunola oil (a monounsaturated oil).

How will the information collected be used?
The overall results of this research will be published in scientific journals and will be available to participants. The data collected will also contribute to Irene Munro’s PhD thesis. Information about individuals will not be released and participants will not be identified in any reports arising from the project.

How will your privacy be protected?
The information you provide will be treated with the same respect for privacy and confidentiality that is given for all medical information collected about you when you visit your local doctor.
Initially, information collected about you for this project will be stored on a computer database at the University of Newcastle, only identifiable by a participant identification number and with no other identifying details. The database will be password protected on a computer that is kept in a locked room. When all of the information for the project has been collected, all information will be de-identified and your personal details will be unknown. A master copy of participants and their code numbers will be held by the chief investigator so that participants can be re-identified if necessary. Only the researchers at the University of Newcastle conducting this research will have access to this information which will be stored at the University for 15 years.

Participant blood samples will only be accessible to the researchers involved in the study. The blood samples will be stored in a secured laboratory at the University until successful analysis has been completed and they will then be destroyed, following standard procedures.

What do you need to do if you want to participate?
Ensure that you understand the contents of this Information Statement before you consent to participate. If there is anything that you do not understand, or you have questions about the study, please contact Irene Munro, telephone 4921 5638.

If you would like to participate, please phone Irene Munro, on the number above, to arrange a time convenient for your first visit. You will be asked to bring the signed Consent Form with you to your first visit.

Thank you for considering this invitation to participate in our study.

Yours sincerely,

________________________________
Dr Manohar Garg
Professor, School of Biomedical Sciences

____________________________________
Irene Munro
PhD Student

Note: This project has been approved by the University's Human Research Ethics Committee, Approval H-159-1205. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher. If an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308.
Telephone: 02 4921 6333, email: Human-Ethics@newcastle.edu.au
Participant letter of information for first visit
Preparation for Your First Appointment

Thank you for your interest in our study “Omega-3 polyunsaturated fatty acids and weight loss”. We are very pleased that you are joining our study and provide the following information for your first appointment.

This letter confirms your appointment on _________________ at ________________.

In preparation for your appointment:

- **Please do not** engage in exercise or have anything to eat or drink (apart from drinking water) after 9pm on the night before your appointment until after your appointment. This is very important as it could influence the results of the study.

- You should continue to take your usual medications.

- **Please do not** consume alcohol during the 24 hours prior to your appointment.

- At your first appointment your fasting blood will be collected and your body composition measures taken. You will be asked to remove your shoes and socks/stockings so that these measurements can be taken.

- Please complete the following questionnaires and bring them with you to your first appointment.
  - Consent form
  - Pre-trial medical questionnaire
  - 3-day food diary

- If you have any questions when completing these questionnaires, please call me on 4921 5638.

A map and parking permit are enclosed. Please use the parking areas indicated on the map, and display the permit on the dashboard of your car.

I look forward to seeing you,

Irene Munro
Nutraceuticals Research Group
Appendix 11

Participant consent form: Clinical Trial 1
Consent Form for Research Project

The role of long chain omega-3 polyunsaturated fatty acids on weight loss and weight maintenance

Investigators: A/Professor Manohar Garg and Irene Munro
Telephone: 4921 5638

I agree to participate in the above research project and give my consent freely. I understand that the study will be carried out as described in the Information Statement, a copy of which I have retained.

- I understand that my participation is voluntary and entirely my choice.
- I understand I can withdraw my consent for participation at any time and do not have to give any reason for withdrawing.
- I understand that my personal information will remain confidential to the researchers.
- I have the opportunity to have questions answered to my satisfaction.
- The final outcome of my results from this research will be freely available to me and will be posted to me at the completion of the study. Results of this research conducted at the University of Newcastle will be published in scientific journals with no identifying details of participants included.

I consent to the following:

- Having my height, weight and body composition recorded
- Completing a 3-day food diary at the beginning and end of the study
- Completing a short health survey at the beginning and end of the study
- Providing a 25mL blood sample at beginning and end of the weight loss phase
- Consuming 6 x 1g capsules/day of fish oil or 6 x 1g capsules/day of placebo during the study

Participant name ____________________ Person asking for consent ____________________

Signature: ___________________________ Signature: ________________________________

Date: _______________________________
Participant consent form: Clinical Trial 2
Consent Form for Research Project

The role of long chain omega-3 polyunsaturated fatty acids on weight loss and weight maintenance

Investigators: A/Professor Manohar Garg and Irene Munro
Telephone: 4921 5638

I agree to participate in the above research project and give my consent freely. I understand that the study will be carried out as described in the Information Statement, a copy of which I have retained.

- I understand that my participation is voluntary and entirely my choice.

- I understand I can withdraw my consent for participation at any time and do not have to give any reason for withdrawing.

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- The final outcome of my results from this research will be freely available to me and will be posted to me at the completion of the study. Results of this research conducted at the University of Newcastle will be published in scientific journals with no identifying details of participants included.

I consent to the following:

- Having my height, weight and body composition recorded
- Completing a 3-day food diary at the beginning and end of the study
- Completing a short health survey at the beginning and end of the study
- Providing a 25mL blood sample at the beginning and end of the weight loss phase and again at the end of the weight maintenance phase of the study
- Consuming 6 x 1g capsules/day of fish oil or 6 x 1g capsules/day of placebo during the study

Participant name ___________________ Person asking for consent ___________________

Signature: __________________________ Signature: __________________________

Date: __________________________
Participant consent form: Clinical Trial 3
Consent Form for Research Project

The role of long chain omega-3 polyunsaturated fatty acids on weight loss

Investigators: Professor Manohar Garg and Irene Munro
Telephone: 4921 5638

I agree to participate in the above research project and give my consent freely. I understand that the study will be carried out as described in the Information Statement, a copy of which I have retained.

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- The final outcome of my results from this research will be freely available to me and will be posted to me at the completion of the study. Results of this research conducted at the University of Newcastle will be published in scientific journals with no identifying details of participants included.

I consent to the following:

- Having my height, weight and body composition recorded
- Completing a 3-day food diary at the beginning of the study
- Completing a short health survey at the beginning and end of the study
- Providing a 25mL blood sample at the beginning and end of the preparation phase and again at the end of the weight loss phase
- Consuming 6 x 1g capsules/day of fish oil or 6 x 1g capsules/day of placebo during the study

Participant name ___________________ Person asking for consent ___________________

Signature: __________________________ Signature: __________________________

Date: __________________________
Pre-trial medical questionnaire
Pre-trial Medical Questionnaire

The role of long chain omega-3 fatty acids in weight loss and maintenance

Participant code: _______________

Date of birth: ____/____/____  Sex: ____  Height: ______cm  Weight: ______kg

Please enter information or tick boxes to answer, as appropriate

1. Please list all current medical conditions for which you are receiving treatment:

Condition: _______________  Condition: _______________
Year diagnosed: ___________  Year diagnosed: ___________

Condition: _______________  Condition: _______________
Year diagnosed: ___________  Year diagnosed: ___________

2. Have you ever had:

A stroke?  Yes [ ]  No [ ]  Angioplasty?  Yes [ ]  No [ ]
A heart attack?  Yes [ ]  No [ ]  By-pass surgery?  Yes [ ]  No [ ]
Angina?  Yes [ ]  No [ ]
Other heart/vascular surgery  Yes [ ]  No [ ]
Other heart-related problems?  Specify ________________________

3. Please list all medications that you take, as prescribed by a doctor:

Brand name: ___________  Brand name: ___________  Brand name: ___________
Dose: _________________  Dose: _________________  Dose: _________________
Frequency: ____________  Frequency: ____________  Frequency: ____________

4. Please list all over-the-counter medications you take regularly

Brand name: ___________  Brand name: ___________  Brand name: ___________
Dose: _________________  Dose: _________________  Dose: _________________
Frequency: ____________  Frequency: ____________  Frequency: ____________
5. Please list all vitamin, mineral, and/or herbal supplements you take regularly

<table>
<thead>
<tr>
<th>Brand name:</th>
<th>Dose:</th>
<th>Frequency:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand name:</td>
<td>Dose:</td>
<td>Frequency:</td>
</tr>
<tr>
<td>Brand name:</td>
<td>Dose:</td>
<td>Frequency:</td>
</tr>
</tbody>
</table>

6. How many alcoholic beverages do you normally consume per week?

- 0-3 □
- 4-7 □
- 8-10 □
- More than 10 □

7. Do you smoke?
   - Yes □
   - No □

8. Do you exercise regularly
   - Yes □
   - No □

9. If yes, please enter type of exercises and frequency per day, week or month

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Hours/day</th>
<th>Hours/week</th>
<th>Hours/month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking, medium pace</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking, briskly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running/jogging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bike riding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming (laps)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gym (specify activity):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sport (please specify):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other physical activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(please specify):</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 15

Post-trial medical questionnaire
Post-trial Medical Questionnaire

The role of long chain omega-3 fatty acids in weight loss and maintenance

Participant code: ________________________

Date of birth: ___/___/___   Sex: ____   Height: ______cm   Weight: _____kg

Please enter information or tick boxes to answer, as appropriate

1. Please list all medications that you took regularly during the trial, as prescribed by a doctor:

   Brand name:_____________   Brand name:_____________   Brand name:_____________
   Dose:____________________   Dose:____________________   Dose:____________________
   Frequency:_______________   Frequency:_______________   Frequency:_______________

2. Please list all over-the-counter medications you took regularly during the trial

   Brand name:_____________   Brand name:_____________   Brand name:_____________
   Dose:____________________   Dose:____________________   Dose:____________________
   Frequency:_______________   Frequency:_______________   Frequency:_______________

3. Please list all vitamin, mineral, and/or herbal supplements you took regularly during the trial

   Brand name:_____________   Brand name:_____________   Brand name:_____________
   Dose:____________________   Dose:____________________   Dose:____________________
   Frequency:_______________   Frequency:_______________   Frequency:_______________

6. On average, how many alcoholic beverages did you consume per week during the trial?

   0-3 □   4-7 □   8-10 □   More than 10 □
7. Did you smoke?  Yes ☐  No ☐

8. Did you exercise regularly  Yes ☐  No ☐

9. If yes, please enter type of exercises and frequency per day, week or month

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Hours/day</th>
<th>Hours/week</th>
<th>Hours/month</th>
</tr>
</thead>
<tbody>
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<td>Running/jogging</td>
<td></td>
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</tr>
<tr>
<td>Bike riding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming (laps)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gym (specify activity):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sport (please specify):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other physical activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(please specify):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Record for a 3-day food diary
Instructions for recording food and drink consumption

You are being asked to record everything that you eat and drink over a period of 3 consecutive days, made up of 2 week days and 1 weekend day. The information you record is very important to the success of this study. Instructions (below) will help you to fill in your food and drink consumption in the most accurate manner.

If you have any questions, please contact Irene Munro, telephone 4921 5638.

Instructions:

- Write down everything that you eat and drink (including water) for three consecutive days (2 week days and one weekend day).
- Complete this in the time frame you are given by Irene.
- Start a new page each day (pages are provided):
  - List the food and give a description of method of cooking (e.g. grilled rump steak or fried rump steak). An example of how to record the information is given below.
  - State the weight of the food (from labels on packaged foods) or use standard household measure, such as a cup, a tablespoon, a teaspoon.
  - Specify brand names of products for processed foods (e.g. Nestle diet yoghurt)
  - Include supplements, and any condiments such as sauce or salad dressing
- Try to record each item when you eat or drink, or at the end of the meal, so that nothing is forgotten.

Example:

<table>
<thead>
<tr>
<th>Time</th>
<th>Food/Drink</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 am</td>
<td>Corn flakes</td>
<td>Kellogg’s</td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Dairy Farmer’s litre</td>
<td>100 mL</td>
</tr>
<tr>
<td></td>
<td>Egg</td>
<td>Boiled</td>
<td>2 medium</td>
</tr>
<tr>
<td></td>
<td>Black coffee</td>
<td>Nescafe instant</td>
<td>1 cup</td>
</tr>
<tr>
<td>12 noon</td>
<td>Salad sandwich</td>
<td>Wholegrain bread</td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td>Baby spinach leaves</td>
<td></td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>Tomato slices</td>
<td></td>
<td>3 slices</td>
</tr>
<tr>
<td></td>
<td>Beetroot</td>
<td>Edgell’s tinned</td>
<td>30 grams</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>Grated</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>Red delicious</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td>Tropical fruit yoghurt</td>
<td>Nestle diet</td>
<td>125 mL</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
<td>500 mL</td>
</tr>
<tr>
<td>3 pm</td>
<td>Coke</td>
<td>Diet</td>
<td>500 mL</td>
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<td>Etc....</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Food/Drink</td>
<td>Description</td>
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</tbody>
</table>
Publication related to this research

This work was conducted to compare two different weight loss strategies for improved dietary compliance during weight loss, a weakness observed in Clinical Trial 1.

Weight loss and metabolic profiles in obese individuals using two different approaches

Irene A. Munro and Manohar L. Garg*

Received 12th July 2011, Accepted 22nd August 2011
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Intentional weight loss with a reduction in adipose tissue is associated with an improvement in medical complications linked to obesity. The aim of this study was to compare the effect of two different weight loss diets on obese individuals (BMI 30–40 kg m⁻²) for improvements in anthropometric measurements and blood biomarkers. Study 1 comprised a low energy diet (LED) of 5000–6000 kJ d⁻¹ for a slow but steady weight loss over 12 weeks. Study 2 comprised a very low energy diet (VLED) of 3000 kJ d⁻¹ using meal replacements for rapid weight loss over a shorter period of 4 weeks followed by 10 weeks of weight stabilisation to prevent rebound after rapid weight loss. Nutrition information sessions were given to both groups. Fasting blood samples, anthropometric measurements and 3-day food diaries were collected at baseline and again at completion of weight loss, at 12 weeks for LED group and 4 weeks for VLED group. Mean weight loss in the LED group (n = 18) was −3.17 kg (−3.7%) compared to a −6.54 kg (−7%) loss in the VLED group (n = 14) (p < 0.001). The VLED group experienced significantly greater reductions in fat mass, −13.9% compared to −8.9% for the LED group (p < 0.05). Significantly greater reductions in blood glucose (p < 0.05), cholesterol and LDL-C (p < 0.01 for both), and waist circumference (p ≤ 0.05) were noted in VLED compared to those for LED diet. Short-term, rapid weight loss produced the desired ≥5% weight loss suggested to substantially reduce metabolic abnormalities associated with obesity, and to reduce health risks.

1. Introduction

Obesity is a chronic metabolic disorder linked to the development of cardiovascular disease (CVD), and associated with increased risk of morbidity and mortality, including the development of insulin resistance and type 2 diabetes mellitus (T2DM), disturbances in blood lipids and dyslipidemia, and increased levels of inflammatory biomarkers.

Intentional weight loss with a reduction in adipose tissue, particularly central or visceral adipose tissue, improves many of these medical complications by decreasing levels of inflammatory biomarkers and reducing metabolic abnormalities. It has been suggested that a weight loss of between 5 and 10% is needed to substantially reduce these risks, and a number of strategies are available to assist with weight loss. Popular weight loss programs focus on reducing energy intake, either by decreasing total fat intake (e.g. the Ornish diet), reducing fat intake and increasing consumption of low GI foods (e.g. the Low GI Diet), extreme carbohydrate restriction (e.g. the Atkins diet) or reducing carbohydrate and increasing protein intake (e.g. the Zone diet). With the exception of low fat diets, the resulting weight loss with these diets is less than the energy level of the diets would indicate, most likely as a consequence of poor dietary compliance, but it is unrealistic to expect that dieters can restrict their food intake in the face of hunger. The use of commercial food replacements (MRs) for a very low energy diet (VLED) reduces feelings of hunger and improves compliance resulting in significantly greater weight loss than with low energy diets (LEDs).

The aim of this study was to compare two different dietary weight loss strategies, a rapid, short-term VLED using MRs and a slow, longer-term healthy eating LED, for weight and fat loss, improvements in blood lipid levels and improvements in levels of inflammatory biomarkers, namely tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), C-reactive protein (CRP), leptin and adiponectin.

2. Method

2.1 Participants

Both male and female participants, aged 18–55 years and with a BMI of between 30–40 kg m⁻², were recruited from the university campus and the general public in Newcastle, Australia. Exclusion criteria were diagnosed diabetes mellitus, a chronic inflammatory condition, already following a kilojoule-restricted diet, or women who were pregnant or lactating. Forty
healthy, obese individuals met the criteria to participate in one of two studies which were conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the Human Research Ethics Committee of the University of Newcastle, Australia. Written, informed consent was obtained from participants prior to commencement.

2.2 Study design

Two weight loss studies were designed to target a weight loss of 5% of initial body weight using two very different dietary weight loss strategies. This was a non-randomised intervention. Initially participants were recruited to take part in Study 1 and this was then followed using the same process to recruit participants for Study 2. Study 1 comprised of a slow and steady weight loss program of 5000 kJ d\(^{-1}\) for females and 6000 kJ d\(^{-1}\) for males. To accommodate the slow weight loss, the study took place over 12 weeks. This healthy eating LED was based on the Australian Guide to Healthy Eating (AGHE)\(^{29}\) and enabled participants to eat a variety of healthy foods, albeit a reduced amount. Study 2 comprised a fast VLED of 3000 kJ d\(^{-1}\) using MRs. Because of the very low energy intake and rapid weight loss, the study was conducted over a shorter time frame of 4 weeks. For the first 2 weeks, participants received Optifast\textsuperscript{®} bars and shakes to replace meals according to the Intensive Phase of the Optifast Very Low Calorie Diet Program (Novartis, Australia), supplemented daily with raw and cooked vegetables and 2 L of drinking water. In weeks three and four MRs were gradually phased out and healthy low energy meals, based on the AGHE, were phased in so that by the end of week 4 participants no longer consumed MRs. After rapid weight loss, the potential is there for weight rebound unless new manageable and effective dietary habits are established. Thus, at the end of the weight loss phase, the VLED participants were observed for a further 10 weeks while they established healthy eating behaviours which stabilised their weight.

Both of the studies commenced with individual nutrition education sessions, all of which were conducted by the same educator to ensure uniformity of information presented. The topics covered included the energy density of foods, understanding and using food labels, appropriate portion sizes and the number of portions to be consumed daily from the different food groups. Both studies were conducted during the summer season to ensure a similar availability of food for both groups.

Prior to commencing the weight loss program, participants recorded their food intake over three days (2 weekdays and 1 day at the weekend). Participants were shown how to use weights given on food labels and handy measures to record their dietary intake which was then analysed using the dietary software program, Foodworks\textsuperscript{®} Professional 2009, version 6 (Xyris Software (Australia) Pty Ltd). Participants in LED group again completed a 3-day food diary in the final week of weight loss; the VLED group again completed a 3-day food diary after 10 weeks of dietary stabilisation to measure dietary compliance.

2.3 Anthropometric measurements

On the first day of the weight loss study, anthropometric measurements were taken in the morning following a 10 h overnight fast with no alcohol consumption, with all participants dressed in light clothing and without shoes. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated balance beam scale (PCS Measurement, NSW, Australia). Waist circumference (WC) was measured at the mid-point between the lowest rib and the top of the hipbone; the hip measurement was taken at the fullest point of the hip, as viewed from the side. Fat mass (FM) and fat-free mass (FFM) were measured using single-frequency bioelectrical impedance (Maltron International, Rayleigh, Essex, UK). Under identical conditions, the anthropometric measurements were repeated at the end of the study.

2.4 Biochemical analyses

Fasting (≥10 h) blood samples were collected into tubes, EDTA, Lithium Heparin and Sodium Fluoride, by venipuncture at baseline and again at the end of the weight loss phase. The samples were prepared by centrifuging (Heraeus Biofuge Stratos) for 10 min at 3000 g at 4 °C. Plasma samples from the EDTA tubes were collected and stored at −80 °C awaiting further analysis. The Lithium Heparin and Sodium Fluoride tubes were taken to Hunter Area Pathology Services (Newcastle) for plasma analysis for blood lipids and blood glucose, respectively.

High sensitivity enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) were used to determine levels of tumor necrosis factor-\(\alpha\) (hs-TNF-\(\alpha\)) and interleukin-6 (hs-IL-6). Minimal detectable concentration of the kits was 0.106 pg mL\(^{-1}\) and 0.039 pg mL\(^{-1}\) respectively with an intra- and inter-assay coefficients of variation (CV) of <9%. Analysis of high-sensitivity C-reactive protein (hs-CRP) was conducted using an immunoturbidimetric method (Hunter Area Pathology Service, Newcastle, NSW, Australia) with a minimal detection level of 0.15 mg L\(^{-1}\). Plasma leptin levels were quantified with a commercial double-antibody enzyme immunometric assay (EIA) (Cayman Chemical Company, Ann Arbor, MI, USA) with a detection limit of 1.0 ng mL\(^{-1}\) and inter- and intra-assay CVs <9%. Adiponectin levels were determined using an ELISA kit (SPI-bio, Montigney le Bretonneux, France). Intra- and inter-assay CV were 6.4% and 7.3% respectively with a detection range of 0.1–10.0 μg mL\(^{-1}\).

2.5 Statistical analyses

Data are presented as mean values and standard deviations, with a significance level of 0.05 indicating significance. A power calculation for a 5% reduction for the primary outcome, weight loss, at the 0.05 level of significance at 80% power indicates that a sample of 13 subjects per group would be required to complete the study. Allowing for a 20% drop-out rate, recruitment of 16 subjects per group is needed. One-way ANOVA was used to test group mean differences, and within group changes from baseline were determined using paired samples t-tests. Pearson product-normal correlations (\(r\)) were used to show relationships. All statistical analyses were carried out with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA).

3. Results

Forty healthy, obese participants commenced the study, with 20 participants in each of the two groups. Thirty two of these
participants completed one of the two weight loss diets, 18 participants (6 males and 12 females) in the LED group and 14 participants (3 males and 11 females) in the VLED group. Although the completion rates for each group were not the same, this difference in the number of participants in each group was not significant \( (p = 0.85) \). At the commencement of the two studies, daily energy intake for each group was calculated and was found to be similar, 8022 kJ d\(^{-1}\) (8127 kJ d\(^{-1}\) males and 7966 kJ d\(^{-1}\) females) for LED group and 7846 kJ d\(^{-1}\) (9684 kJ d\(^{-1}\) males and 7345 kJ d\(^{-1}\) females) for VLED group \( (p = 0.76) \). At baseline there were no significant differences between the two groups for age \( (p = 0.64) \), gender \( (p = 0.47) \), or blood biomarkers, apart from HDL levels which were higher for VLED group \( 1.37 \pm 0.28 \text{ mmol L}^{-1} \), compared to LED group \( 1.16 \pm 0.24 \text{ mmol L}^{-1} \) \( (p = 0.03) \). There were also no significant differences in baseline anthropometric measurements, weight \( (p = 0.97) \), height \( (p = 0.74) \), BMI \( (p = 0.68) \), FM \( (p = 0.53) \), FFM \( (p = 0.81) \), WC \( (p = 0.99) \) or hip \( (p = 0.60) \).

At the end of the weight loss period, that is after 12 weeks for the LED group and after 4 weeks for the VLED group, there was a significant reduction in weight, BMI, FM and hip circumference within both groups \( (p < 0.001) \), and WC \( (p < 0.001) \) and \( (p < 0.05) \) for VLED group and LED group, respectively (Table 1). By the end of 4 weeks the VLED group reduced weight by 0.05 kg \( \pm 0.72 \text{ mmol L}^{-1} \) compared to LED group, \( p < 0.05 \). Individually, only two (out of 14) VLED participants reduced their weight by less than 5%, while 15 (out of 18) LED participants reduced their weight by less than 5%, with 15 (out of 18) LED participants had a weight reduction of less than 5%. The reduction in FM was significantly greater for VLED group, \( 13.9\% \) \( (-5.54 \text{ kg}) \) compared to LED group, \( -8.9\% \) \( (-3.43 \text{ kg}) \) \( (p < 0.05) \), which corresponded with the significantly greater reduction in WC for VLED group \( (p = 0.05) \) (Table 1).

With the greater weight reduction there was a significantly greater reduction in TC for the VLED group than for the LED group, \(-0.86 \pm 0.84 \text{ mmol L}^{-1} \) compared to \(-0.10 \pm 0.56 \text{ mmol L}^{-1} \), respectively \( (p = 0.005) \); also a greater reduction in LDL-C for the VLED group than for the LED group, \(-0.54 \pm 0.72 \text{ mmol L}^{-1} \) compared to \(-0.06 \pm 0.47 \text{ mmol L}^{-1} \), respectively \( (p = 0.008) \). The reduction in leptin levels was significantly greater for the VLED group compared to the LED group, \(-19.67 \pm 11.82 \text{ mmol L}^{-1} \) and \(-0.26 \pm 13.82 \text{ mmol L}^{-1} \), respectively \( (p < 0.001) \). There was also a significant difference in the change to glucose levels with a decrease of \(-0.05 \pm 0.33 \text{ mmol L}^{-1} \) for the VLED group and an increase of \( 0.31 \pm 0.50 \text{ mmol L}^{-1} \) for the LED group \( (p = 0.03) \) (Table 2). Changes to inflammatory markers, which are shown in Table 2, were not significantly different between the two groups.

Following weight loss, 12 of the 14 participants in the VLED group continued for 10 weeks of weight stabilisation using whole foods after relying on MRs to assist their weight loss. During this time, they lost a small amount of weight \(-1.57 \pm 3.70 \), which was accompanied by small, non-significant reductions in FM, FFM, WC and hip circumference, which are shown in Table 3. There was a significant rebound in levels of triglycerides, TC \( (p < 0.05 \) for both) and leptin \( (p < 0.01) \). There was also an increase in levels of LDL-C and hs-CRP, but the differences were not significant. The level of HDL-C, which had decreased with weight loss, increased significantly \( (p < 0.01) \) and was now higher than the baseline level had been. Levels of adiponectin increased and IL-6 and TNF-\( \alpha \) decreased but the changes were not significant.

### 4. Discussion

There are differences of opinion on the optimal diet for weight loss and weight management. Registered dietitians typically recommend portion control to reduce energy intake as a weight loss strategy. All of the participants in this study were instructed on portion control for weight management, and it was also the primary strategy used for weight loss with the LED group. Commencing the study with a mean baseline energy intake of 8022 kJ d\(^{-1}\) (7966 kJ d\(^{-1}\) females and 8127 kJ d\(^{-1}\) males), the target energy intake for the LED group was 5000 kJ d\(^{-1}\) for females and 6000 kJ d\(^{-1}\) for males. Reducing energy intake by 2000 kJ d\(^{-1}\) should have resulted in a weight loss of 0.5 kg week\(^{-1}\) with an anticipated loss of 6 kg over the 12 weeks, but this did not happen. The mean weight loss was 3.17 kg, with some participants achieving a much better weight loss than others; for example, a reduction of 11.5 kg (10%) and 9.5 kg (11.6%) for two participants while two other participants did not lose any weight at all. The participants were responsible for choosing their food and limiting the amount that they consumed, which would not have been easy when they had to manage feelings of hunger. Dietary compliance was challenging, as evidenced by the mean daily intake of 7552 kJ d\(^{-1}\) for males

### Table 1 Baseline values and changes from baseline for anthropometric measurements

<table>
<thead>
<tr>
<th></th>
<th>LED (( n = 18 ))</th>
<th>VLED (( n = 14 ))</th>
<th>( \Delta )</th>
<th>( \Delta )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age/yr</strong></td>
<td>41 \pm 3.0</td>
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<tr>
<td><strong>Weight/kg</strong></td>
<td>93.95 \pm 16.40</td>
<td>90.78 \pm 16.12</td>
<td>-3.17 \pm 3.09***</td>
<td>94.16 \pm 16.64</td>
<td>87.63 \pm 15.41</td>
</tr>
<tr>
<td><strong>BMI/kg m(^{-2})</strong></td>
<td>32.53 \pm 3.58</td>
<td>31.42 \pm 3.70</td>
<td>-1.11 \pm 0.99***</td>
<td>33.04 \pm 3.17</td>
<td>30.77 \pm 3.16</td>
</tr>
<tr>
<td><strong>FM/kg</strong></td>
<td>38.90 \pm 7.49</td>
<td>35.55 \pm 8.06</td>
<td>-3.43 \pm 3.51***</td>
<td>40.69 \pm 7.65</td>
<td>35.15 \pm 7.28</td>
</tr>
<tr>
<td><strong>FFM/kg</strong></td>
<td>54.40 \pm 10.43</td>
<td>54.69 \pm 10.02</td>
<td>0.29 \pm 1.77</td>
<td>53.50 \pm 10.82</td>
<td>52.50 \pm 10.53</td>
</tr>
<tr>
<td><strong>WC/cm</strong></td>
<td>103.11 \pm 12.11</td>
<td>100.86 \pm 11.65</td>
<td>-2.25 \pm 3.93*</td>
<td>103.11 \pm 11.99</td>
<td>98.14 \pm 11.10</td>
</tr>
<tr>
<td><strong>Hip/cm</strong></td>
<td>119.67 \pm 9.78</td>
<td>117.14 \pm 10.43</td>
<td>-2.53 \pm 2.46***</td>
<td>117.99 \pm 7.49</td>
<td>113.75 \pm 6.47</td>
</tr>
<tr>
<td><strong>Waist : hip</strong></td>
<td>0.86 \pm 0.08</td>
<td>0.86 \pm 0.08</td>
<td>0.00 \pm 0.03</td>
<td>0.87 \pm 0.08</td>
<td>0.86 \pm 0.08</td>
</tr>
</tbody>
</table>

*Mean value \pm standard deviation. Significant changes within groups: \* \( p < 0.05 \); \*\* \( p < 0.01 \); \*\*\* \( p < 0.001 \). \( P \) shows the significant differences between LED and VLED.
and 6701 kJ d⁻¹ for females, recorded in the 3-day food diaries completed at the end of weight loss.

The MRs for the VLED group initially removed the responsibility of managing food intake from a variety of foods, and this appears to have facilitated dietary compliance. A study comparing two weight loss groups, LED and VLED, found that increasing the variety of food choice was associated with greater food consumption and that a less varied diet resulted in the greatest decrease in food cravings, possibly mediated by reduced hunger. The study found that the LED group (n = 19) reduced weight by 6.51 kg over 12 weeks while consuming 5024 kJ d⁻¹ and the VLED (n = 22) reduced weight by 11.97 kg over 6 weeks while consuming 3349 kJ d⁻¹ with MRs²² showing greater weight reduction in a shorter period for VLED, which is similar to the findings in our study. Other studies using MRs for VLED of between 2520–3570 kJ d⁻¹ for weight loss have also reported considerable weight loss over short periods of time, for example Haugaard (2007) reported a mean weight reduction of 9.3 kg (8.8%) over 8 weeks,²³ Harder (2004) reported a mean weight loss of 10.9 kg (11%) over 8 weeks,²⁴ while Clement (2004) reported a mean weight loss of 6 kg (6.4%) over 4 weeks.²⁵ Using MRs has been criticized because they obviate the need to choose from a variety of foods and to control portions when replacing meals and do not encourage the development of improved eating habits.²⁶ Concerns have also been raised about fast and/or large decreases in weight and the potential for weight regain. However, a study by Nackers et al. (2010) found that losing weight slowly does not lead to greater long term weight loss and smaller regain,²⁷ although a large decrease in weight, considered to be >30%, has been associated with a greater weight regain.²⁸ The weight reduction in the VLED group in this study was 7% (−6.54 kg) over 4 weeks which was twice the amount lost by the LED group over 12 weeks, but to address these concerns, the VLED group did not continue with their weight loss unchecked. To minimize the potential for weight rebound and to fully implement the development of healthy eating behaviours

Table 2 Baseline values and changes from baseline for blood biomarkers²⁴

<table>
<thead>
<tr>
<th></th>
<th>LED (n = 18)</th>
<th>VLED (n = 14)</th>
<th>Δ</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PI (week 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trigs/mmol L⁻¹</td>
<td>1.33 ± 0.71</td>
<td>1.07 ± 0.40</td>
<td>−0.25 ± 0.59</td>
<td></td>
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<tr>
<td>TC/mmol L⁻¹</td>
<td>4.84 ± 0.93</td>
<td>4.74 ± 0.73</td>
<td>−0.10 ± 0.56</td>
<td></td>
</tr>
<tr>
<td>HDL-C/mmol L⁻¹</td>
<td>1.16 ± 0.24</td>
<td>1.12 ± 0.27</td>
<td>−0.04 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>LDL-C/mmol L⁻¹</td>
<td>3.07 ± 0.81</td>
<td>3.13 ± 0.66</td>
<td>0.06 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>Glucose/mmol L⁻¹</td>
<td>4.71 ± 0.51</td>
<td>5.02 ± 0.57</td>
<td>0.31 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Leptin/ng mL⁻¹</td>
<td>35.21 ± 13.60</td>
<td>34.95 ± 19.16</td>
<td>−0.26 ± 13.82</td>
<td></td>
</tr>
<tr>
<td>Adiponectin/mg mL⁻¹</td>
<td>11.92 ± 6.32</td>
<td>13.83 ± 7.38</td>
<td>1.91 ± 6.48</td>
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<tr>
<td>hs-CRP/mg mL⁻¹</td>
<td>5.95 ± 6.46</td>
<td>4.79 ± 5.43</td>
<td>−1.16 ± 3.72</td>
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<tr>
<td>hs-IL-6/pg mL⁻¹</td>
<td>2.75 ± 2.39</td>
<td>2.95 ± 3.69</td>
<td>0.19 ± 1.58</td>
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<tr>
<td>hs-TNF-α/pg mL⁻¹</td>
<td>1.34 ± 1.13</td>
<td>1.01 ± 1.11</td>
<td>−0.33 ± 0.80</td>
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Table 3 Changes from the end of weight loss to the end of weight stabilisation for VLED²⁴

<table>
<thead>
<tr>
<th></th>
<th>VLED (n = 12)</th>
<th>Δ</th>
<th>P value</th>
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<tr>
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<td>End weight loss</td>
<td>End weight stabilisation</td>
<td></td>
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<tr>
<td>Weight/kg</td>
<td>83.55 ± 10.01</td>
<td>81.98 ± 10.28</td>
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<td>BMI/kg m⁻²</td>
<td>30.46 ± 3.11</td>
<td>29.85 ± 3.37</td>
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<td>FM/kg</td>
<td>33.45 ± 5.90</td>
<td>32.46 ± 5.68</td>
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<td>FFM/kg</td>
<td>50.13 ± 8.00</td>
<td>49.67 ± 7.95</td>
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<td>WC/cm</td>
<td>95.83 ± 9.11</td>
<td>93.54 ± 10.69</td>
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<td>Hip/cm</td>
<td>112.42 ± 5.95</td>
<td>110.70 ± 6.78</td>
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<td>Trigs/mmol L⁻¹</td>
<td>1.06 ± 0.52</td>
<td>1.20 ± 0.52</td>
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<td>TC/mmol L⁻¹</td>
<td>4.73 ± 1.17</td>
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<td>HDL-C/mmol L⁻¹</td>
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<td>LDL-C/mmol L⁻¹</td>
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<td>Glucose/mmol L⁻¹</td>
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<td>Leptin/ng mL⁻¹</td>
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<tr>
<td>Adiponectin/mg mL⁻¹</td>
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<td>1.02 ± 4.24</td>
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<tr>
<td>hs-CRP/mg mL⁻¹</td>
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<td>5.01 ± 4.14</td>
<td>0.50 ± 2.81</td>
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<tr>
<td>hs-IL-6/pg mL⁻¹</td>
<td>1.48 ± 0.73</td>
<td>1.46 ± 0.63</td>
<td>−0.03 ± 0.69</td>
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<tr>
<td>hs-TNF-α/pg mL⁻¹</td>
<td>0.74 ± 0.56</td>
<td>0.31 ± 0.42</td>
<td>−0.42 ± 0.70</td>
</tr>
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</table>

Mean values ± standard deviation.
after weight loss, the participants followed a healthy balanced diet for 10 weeks to stabilise their weight and to not intentionally continue to reduce weight. However, while they were trying to establish the optimal energy intake for maintenance, there was a further mean weight reduction of 1.85% (~1.57 kg).

The greater weight loss with the VLED group was unsurprising since energy intake of that group was 60% of the energy intake for the LED group. However the reduced energy intake for the LED group did occur over a longer time frame, that is 12 weeks compared to 4 weeks for the VLED group, so they had a longer time in which to lose weight. Despite the significant differences between the two groups in the amount of weight lost, the reduction in triglyceride levels was very similar for both groups. With weight loss, levels of LDL-C and glucose increased for the LED group while, for the VLED group, levels of LDL-C and glucose reduced. There was a greater reduction in TC for the VLED group relative to weight lost (0.13 mmol L⁻¹ per kg of weight lost) compared to the LED group (0.03 mmol L⁻¹ per kg of weight lost) but the difference was not significant.

HDL-C also decreased and again the reduction was greater for the VLED group (0.03 mmol L⁻¹ per kg of weight lost) compared to the LED group (0.01 mmol L⁻¹ per kg of weight lost) but the difference between the two groups was not significant. While there are clinical benefits of a reduction in levels of TC and LDL-C, it is well recognised that HDL-C has a cardioprotective effect and a reduction is undesirable. Previous studies have reported different effects of weight loss on HDL-C, some showing small increases, others a small decrease and some no change at all.38 It would appear that HDL-C levels are reduced during active weight loss and levels subsequently increase when a stabilized, reduced weight is attained.19,18 Dattilo (1999) suggests that this occurs because lipoprotein lipase decreases during active weight loss, reducing triglyceride rich lipoprotein synthesis which impairs VLDL-C catabolism and the transfer of lipids to HDL-C, thereby reducing HDL-C concentrations. When weight then stabilises at a reduced level, lipoprotein lipase increases as does hydrolysis of VLDL-C and the transfer of lipids to HDL-C resumes.35 With the 10-week weight stabilisation/ maintenance phase in the VLED group in our study, there was a significant increase in HDL-C to above baseline levels. Triglyceride, TC and LDL-C levels also increased during the 10-week weight stabilisation phase but they remained lower than baseline levels. The reason for the increases could be attributed to the change from MRs to resuming meal consumption which was not significant.

There was a significant 60.6% decrease in leptin after 4 weeks of weight loss in the VLED group (p < 0.001). The magnitude of this reduction is similar to those reported in a number of studies. Wisse et al., observed a 61% reduction in fasting leptin after 14 days of energy restriction of 1900 kJ d⁻¹, a 52% reduction after 14 days of a low energy balanced-deficit diet of 5500 kJ d⁻¹ and a 76% reduction in leptin after 14 days of fasting.13 Mars et al., observed a 39.4% reduction in fasting leptin after a 4-day, 65% energy restricted diet.34 Dubuc et al., reported a 35–65% reduction in fasting leptin after 7 days of an energy restricted MR diet of either 2646 kJ d⁻¹ for females or 3528 kJ d⁻¹ for males.35 These authors contend that the decrease in leptin does not correspond to the changes in body fat.33,35 It has been suggested that decreasing leptin levels are associated with self-perceived increases in hunger,14,15 a mechanism in place to regulate food intake to prevent starvation. Controlling that hunger response can be difficult and one of the reasons for the high failure rate with weight loss diets. However, after the 10 weeks of stabilisation, leptin levels had significantly increased (p < 0.01) although they did not return to baseline levels.

In summary, the very low energy, rapid weight loss diet produced the desired ≥5% weight loss that had been suggested for a reduction of metabolic abnormalities, and for the 4-week period an improvement in blood biomarkers was observed. The concerns for weight rebound following rapid weight loss have been addressed in this study with a short rapid weight loss phase followed by a recovery/stabilisation phase to reinforce balanced eating behaviours. This very low energy diet, followed by a recovery cycle to stabilise weight, can be followed until the desired weight is achieved, at which point the weight stabilisation diet can be followed for weight maintenance.

5. Limitations

A limitation of this study is the small sample size with its low completion rate. More studies with a larger sample size, and which extend the dietary weight loss periods, are needed to further investigate these findings.

6. Acknowledgements

Nestle Nutrition, Australia, supplied the Optifast® bars and shakes for meal replacements.

References

Statement from co-author relating to a paper published with Irene Munro (5)

Statement from co-authors relating to a paper published with Irene Munro

I. Manohar L. Garg
(co-author's name in full),

attest that Research Higher Degree candidate Irene Munro contributed to the following paper:


- To the conception and design of the study
- To the recruitment of participants
- Conducted the intervention and collected the clinical data
- Analysed inflammatory mediators
- Entered, analysed and interpreted the data
- To the drafting of the manuscript
- To the revision of the manuscript

(Signature of Co-Author)

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Date:

(Signature of Candidate)

Irene A. Munro

Date:

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Publication related to this research

Dietary compliance is a weakness when conducting trials for weight loss, as observed in Clinical Trial 1. This work was conducted to investigate whether other methods of dietary compliance for improved weight loss could be found to assist with weight loss during clinical investigations.

Using personality as a predictor of diet induced weight loss and weight management

Irene A Munro1, Miles R Bore2, Don Munro2 and Manohar L Garg1*

Abstract

Background: A major challenge for successful weight management is tailoring weight loss programs to individual needs. The aim of this study was to investigate whether personality traits could be used to match individuals to a compatible weight loss program that would maximize weight loss.

Method: Two different weight loss trials were conducted, both with a weight loss greater than 5% the measure of success. Fifty-four individuals, BMI 30-40 kg/m2, either followed a slow, healthy eating weight loss diet (HEWLD) of 5000-6000 kJ/day for 12 weeks (n = 22), or a fast, very low energy diet (VLED) of 3000 kJ/day for 4 weeks (n = 32). Anthropometric measurements were recorded at baseline, at the end of the weight loss period and, for VLED, at the end of 10 weeks of weight maintenance. Personality traits were measured at baseline using the Tangney Self Control Scale plus 3 of the scales from the Five Factor Model - Neuroticism, Conscientiousness and Extraversion.

Results: The percentage weight loss was significantly greater in VLED (-7.38%) compared to HEWLD (-4.11%), (p < 0.001). Weight loss in HEWLD was positively correlated with Anxiety, a facet of Neuroticism. Weight loss in VLED was positively correlated with Neuroticism (r = 0.5, p < 0.01), and negatively correlated with Dutifulness and Discipline, facets of Conscientiousness, (p < 0.05 for both). No link was observed between weight loss and the personality trait, Self Control, in either HEWLD or VLED.

Conclusion: The personality factor, Neuroticism, was linked to successful weight loss (that is ≥ 5%) with a particular weight loss treatment, suggesting that there is a potential to use measures of personality to identify appropriate weight loss/management strategies for individuals.

Trial registration: Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12611000716965

Background

Obesity is a risk factor for several chronic diseases that are largely preventable, such as insulin resistance, type 2 diabetes mellitus, hypertension, dyslipidemia [1] and cardiovascular disease [2]. In addition to the significant morbidity and mortality that arises from these diseases, there is considerable social stigmatization associated with obesity. It has been suggested that a weight loss of 5-10% can significantly reduce the health risks [3,4] and there is no shortage of strategies available to assist with weight loss. Popular weight loss programs focus on weight reduction by restricting energy intake, either by reducing kilojoules (e.g. Weight Watchers), restricting carbohydrate and increasing protein intake (e.g. the Atkins diet) [5]. Another strategy is a very low energy diet (VLED) using commercial meal replacements [6]. However, weight loss can be difficult to achieve or maintain and long term dietary compliance rates, are low, overall [7,8]. Restricting the intake of food in the face of hunger and temptation requires strong self control. Carels (2003) found that unsatisfactory weight loss and attrition during weight loss programs is associated with diminished self control [9]. When dietary control fails, other weight loss strategies may be sought including appetite suppressants such as Sibutramine, fat blockers such as Xenical or Orlistat [10], laxatives and diuretics, but these are not sustainable in the long term. More effective strategies to improve compliance rates for weight loss and weight maintenance are needed [5].

A major challenge for successful weight management is tailoring programs to meet individual needs, that is,
matching personal attributes and behaviors to a particular weight loss program, such as whether a person with a particular personality will achieve a better weight loss outcome while following a time-convenient web-based weight reducing dietary program, for example, Collins 2010 [11], or in sessions that provide face-to-face personal support, for example, Cognitive Behavior Therapy [12]. Personality traits are measurable attributes of people and can be used to explain behavior. It is possible, therefore, that the profiling of personality traits could be matched with the various weight loss programs to identify those that could result in improved weight loss outcomes for the individuals concerned. Indeed, a number of studies have investigated the link between personality traits and weight loss with varying degrees of success [13-16]. However, their use of different tests to measure different traits makes it difficult to compare the findings.

Since about 1990, the Five Factor Model (FFM) [17] has become dominant in the study of personality, with a large proportion of studies of personality explicitly using measures based on it, or referring to it, as the standard approach. The 5 variables it proposes and their descriptive characteristics are Neuroticism (low self esteem, anxious, irritable and worrying), Conscientiousness (efficient, thorough, organized and hard working), Extraversion (socially stimulated, energetic, enthusiastic and pleasure seeking), Openness (imaginative, adventurous and spontaneous), and Agreeableness (sympathetic, gentle, trusting and warm) [18].

Associations between some of these personality traits and body weight and/or BMI have been reported. In different studies, Neuroticism has been found to be both positively and negatively correlated with obesity [18-20]. Conscientiousness is consistently associated with adiposity [19], with high Conscientiousness related to low BMI [18-20]. It has been suggested that there is less evidence for the association between BMI and the 3 remaining traits [19]; however, in different studies, high Extraversion has been correlated both positively and negatively with obesity [18,19]. Thus, there is evidence that 3 traits, Neuroticism, Conscientiousness and Extraversion, could provide a basis to identify personality attributes for successful weight loss. Another more specific trait likely to be predictive is Self Control. The Tangney Self Control Scale (SCS) [21] has been used in a number of studies to measure self control in relation to eating behaviors and weight [22,23] and was therefore included in the study.

The purpose of the present study was to determine whether the personality traits described above could be used to match individuals to a compatible weight loss program to maximize weight loss. For example, would a particular personality type respond better to a slow weight loss program using self-control to manage dietary compliance with a range of food choices, while another personality type might respond better to a more restrictive but faster weight loss? This was investigated with 2 groups of people using 2 different weight loss programs. We hypothesized that there would be a significant positive correlation between the personality trait, Self Control, and weight loss on a program that required participants to follow a healthy eating weight loss diet (HEWLD) and restrict their food intake without the aid of satiety enhancing supplementation. We were, however, uncertain which personality trait(s) would correlate with weight loss on a program that removed food choices and reduced feelings of hunger with satiety enhancing meal replacements. Therefore we explored the relationship between weight loss and the 3 FFM dimensions that have been found to correlate with weight control, Conscientiousness, Neuroticism and Extraversion, and the 6 facets for each (as listed Table 1).

Methods
Participants
Both male and female participants were recruited from the university campus and the general community in Newcastle, Australia, to take part in one of two different weight loss/management trials. For both trials participants were required to have a BMI of between 30 - 40 kg/m² and be aged 18 - 60 years. People with diagnosed diabetes mellitus, a chronic inflammatory condition, or who were already following an energy restricted diet, were excluded from the study, as were women who were pregnant or lactating. This study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and approved by the Human Research Ethics Committee of the University of Newcastle, Australia. Written, informed consent was obtained from participants prior to commencement. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12611000716965)

Study design
Two weight loss/management trials were conducted with participants having no prior knowledge that the 2 different trials were to be offered. Initially participants were recruited to take part in a weight loss study that provided for a slow and steady loss of weight over 12 weeks. Later, following the same process, participants were recruited to take part in the second study that provided for a quick weight loss over a short period of time, 4 weeks, and was based on meal replacements. A weight loss greater than 5% was considered as the measure of success. To ensure uniformity of information presented all information given to the participants was provided by one person, and included nutrition education and counseling sessions which were conducted over the first 4 weeks of the trials. The nutrition education
sessions focused on the energy density of foods, understanding and using food labels, appropriate portion sizes as well as the number of portions to be consumed daily from the different food groups. This information was used to help participants build a healthy diet using the guidelines from the Australian Guide to Healthy Eating (AGHE) [24].

In the first trial participants followed a portion controlled, reduced energy, HEWLD comprising 5000 kJ for females to 6000 kJ for males daily for 12 weeks. The diet was based on the AGHE [24] which allowed participants to eat a variety of healthy foods, albeit a reduced amount. The changed eating patterns, enabling a steady weight loss, are considered to be a sound strategy for the development of life-long healthy eating habits. However, if the stomach is accustomed to holding and digesting larger volumes, managing hunger could be a problem and dietary compliance difficult. With the second trial participants followed a very low energy diet (VLED) of 3000 kJ/day for 4 weeks. For the first 2 weeks participants received Optifast® bars and shakes to replace meals according to the Intensive Phase of the Optifast® Very Low Calorie Diet Program (Novartis, Australia) [25], supplemented daily with raw and cooked vegetables and 2 liters of drinking water. This is a rigid diet and the shakes and bars are satiety enhancing because of the high polydextrose content and/or high protein content, which helps to reduce hunger and facilitate compliance. The lack of variety of food choice is very restricting but enables a faster weight loss over a shorter period. During weeks 3 and 4, the meal replacements were gradually phased out and healthy kilojoule controlled meals were phased in so that participants would learn to choose healthy foods and correct portion sizes, based on the AGHE. Immediately following the 4 weeks of weight loss, and still observing the principles of the AGHE, participants progressed to 10 weeks of weight maintenance to reinforce healthy eating behaviors. This meant that the weight loss/management period was then similar for both groups. An accredited practicing dietitian was part of the research team to advise on health care.

**Instruments**

Prior to commencement, individual diets were assessed with a 3-day food diary to determine every-day nutrient and energy intake. The diaries were analyzed using the program FoodWorks Professional 2009, version 6 (Xyris

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### Table 1 Correlations of personality with percentage weight loss and BMI loss for HEWLD and VLED, and weight maintenance for VLED

<table>
<thead>
<tr>
<th></th>
<th>HEWLD r (n = 22)</th>
<th>HEWLD BMI change r (n = 22)</th>
<th>VLED r (n = 32)</th>
<th>VLED BMI change r (n = 32)</th>
<th>Maintenance r (n = 29)</th>
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<td>128</td>
<td>436*</td>
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<td>128</td>
<td>563**</td>
<td>553**</td>
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<td>089</td>
<td>375*</td>
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*p < 0.05, **p < 0.01 (2-tailed)
Software (Australia) Pty Ltd.) and the mean values calculated.

At the same time, 2 questionnaires were given to participants to measure personality. There are several self-report instruments based on the FFM; in this study, a public-domain test based on the work of L.R. Goldberg (2006) was used [26], namely the International Personality Item Pool http://ipip.ori.org 'Big Five' questionnaire. This instrument has shown to be highly reliable in many investigations using it and has been used locally for several years so local norms are available [27]. Three of the 5 scales were used, Neuroticism, Extraversion and Conscientiousness but not the other 2 as previous research has not found them to be clearly related to dietary weight loss behavior. There were 10 items for each of 6 facets (subscale), to a total of 180 items, each rated on a 5-point scale. Both facets and scale totals were used to test specific relationships with weight gain/loss behaviors. The FFM was used in conjunction with a second instrument, the Tangleyn Self Control Scale (SCS) which measures general self-control, such as the ability to refrain from acting on undesired behavioral tendencies [21]. This 36-item scale has been shown to be reliable (alpha coefficient = .85) and has been used previously in research on eating styles [22,28].

On the first day of the weight loss trial, anthropometric measurements were taken in the morning after ≥ 10 hour overnight fast, with participants dressed in light clothing and without shoes. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated balance beam scale (PCS Measurement, NSW, Australia). BMI was calculated in kilograms per meter squared from weight and height. Under identical conditions, these anthropometric measurements were repeated at the end of the weight loss phase as well as at the end of the maintenance phase in the second trial, the VLED.

Statistical analyses
ANOVA was used to test group mean differences, and Pearson product-normal correlations (r) were used to show relationships, with p ≤ 0.05 indicating statistical significance (critical value for r = 0.423 for n = 22, and r = 0.349 for n = 32, 2-tailed). Multiple regression analysis was used to test the possibility that weight loss was a function of the interaction of group (treatment) with personality.

Results
Twenty two participants (8 males) completed the HEWLD and 32 participants (6 males) completed the VLED trials. There was no significant difference in age, baseline body weight, BMI and daily energy intake between the 2 groups (Table 2).

A weight loss of at least 5% has been suggested as necessary to significantly reduce the health risks arising from obesity [3,4]. Only 5 of the 22 participants following the HEWLD had lost 5% or more of their body weight after 12 weeks with a mean weight loss and BMI reduction of 4% for both. The changes from baseline were not significantly different. After 4 weeks of the VLED, the mean body weight of participants had reduced by 7% (p = 0.02) and BMI had also reduced by 7% (p = 0.002). All but 2 of these participants had achieved a weight loss of 5% or above. The weight loss between the 2 groups was significantly different (p < 0.001); the individual differences in weight loss for both groups shown in Figures 1 and 2.

Three participants chose not to continue to the maintenance phase of the VLED. Twenty-nine participants completed the 10 weeks of weight maintenance, 2 of whom regained 2-3% (1.7-2.5 kg) and one person regained just under 6% weight (4.3 kg). Three participants did not change their weight at all, 6 lost a further 4-7% and one lost a further 11% weight. The others continued to lose or gain small amounts (Figure 3).

The mean personality scores for HEWLD and VLED are shown in Table 3; there were no significant differences between the 2 groups. In the current obese sample, scale reliabilities as measured by Cronbach’s alpha for the main personality traits were .86 for Neuroticism, .79 for Extraversion, .80 for Conscientiousness, and .85 for Self Control, all of which are satisfactory. The correlations between personality (both facet and whole scale scores) and percentage weight loss and percentage BMI change for both HEWLD and VLED, and between personality and percentage weight maintenance for VLED, were computed (Table 1). For the HEWLD there were no significant correlations between personality and weight loss or BMI change. However, for the VLED group, there was a significant, positive correlation between the Neuroticism personality trait and weight loss, BMI change and Maintenance change, in particular the facets Anxiety, Anger, Depression, Self consciousness and Vulnerability, with significance

<table>
<thead>
<tr>
<th>Table 2 Descriptive characteristics of participants at baseline</th>
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</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Daily kj intake</td>
</tr>
</tbody>
</table>

(Mean values and standard deviations)
ranging from < 0.05 to 0.001, but not the facet, Immoderation. Weight loss and BMI change were also negatively correlated with the Conscientiousness facet, Discipline, and weight loss with the facet, Dutifulness, (p < 0.05 for all). None of the facets of personality within the factor Conscientiousness were significantly correlated with weight maintenance. For both HEWLD and VLED there were no significant correlations between Extraversion (both facet and whole scale scores) and weight loss or BMI change, or between Self Control in the Tangney SCS and weight loss or BMI change. It should be noted that though the correlation between change in the weight loss phase and the maintenance phase for the VLED group was r = 0.48, indicating moderate independence between the phases, the patterns of correlations within personality were similar. Because of the small sample sizes it was not practicable to apply a Bonferroni or similar correction to compensate for the large number of comparisons, so the results should be interpreted with caution.

Multiple regression analysis was used to check that there were no significant interactions between groups (as treatments) and personality. None were found, partly due to the small sample sizes.
**Discussion**

The aim of this study was to investigate the possibility that different personality traits are differentially related to different weight loss diets so that individuals might in future be matched to treatments to maximize weight loss. In particular, we hypothesized that there would be a significant positive correlation between the personality trait, Self Control, and weight loss on a program that required participants to follow a healthy diet and restrict their food intake without the aid of satiety enhancing supplementation (HEWLD). No specific predictions were made about which personality trait(s) would correlate most strongly with weight loss on the other program that initially removed food choices but reduced feelings of hunger with satiety enhancing meal replacements (VLED).

Using 2 different weight loss strategies, we found there was a significant difference in weight change from baseline in the VLED group, but the change in weight loss from baseline was not significantly different for the HEWLD group. A lack of willpower or self-discipline is often blamed for the inability to lose and manage weight but controlling hunger is not easy. Participants in the HEWLD were required to reduce their food intake and to eat healthy foods. This reduction in food intake would have been associated with increased feelings of hunger requiring considerable self control to resist the immediate temptation to assuage the pangs of hunger for the delayed reward of weight loss. The mean scores for self control were similar for HEWLD and the VLED, 118.5 and 117.3 respectively. For both trials, the correlations between self control and weight change or BMI change are negative and not significant. In particular, the treatment outcome in the HEWLD, that is low weight loss, was not associated with our Self Control measure. A study by Carels (2003) investigating failure to lose weight during treatment with 44 obese, postmenopausal women found that one of the significant influences on poor treatment outcome was diminished self control [9], but they attributed the poor treatment outcomes to a number of other causes as well. A study which examined the control of eating (restraint, measured using the TFEQ [29]) and a possible association with general self control (measured using the SCS [21]) found that, among the obese participants and dieters, restraint was related to more successful weight control [22]. They found that overweight and obese participants had higher restraint scores compared to those of normal weight; however, normal weight participants had higher self control scores. Also, current and past dieters had higher restraint scores compared to never-dieters; however never-dieters had higher self control scores. We did

![Figure 3 Individual weight change during the 10 week weight maintenance phase of VLED](http://www.ijbnpa.org/content/8/1/129)

**Table 3 Personality scores for participants at baseline**

<table>
<thead>
<tr>
<th>Personality Traits</th>
<th>HEWLD (n = 22)</th>
<th>VLED (n = 32)</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Norm</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>134.6</td>
<td>23.9</td>
<td>-.45</td>
</tr>
<tr>
<td>Extraversion</td>
<td>162.3</td>
<td>20.4</td>
<td>-.32</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>181.2</td>
<td>18.6</td>
<td>+.03</td>
</tr>
<tr>
<td>Self Control</td>
<td>118.5</td>
<td>13.8</td>
<td>+.01</td>
</tr>
</tbody>
</table>

*With respect to undergraduate norms with n = 237 for IPIP scales, Tangney et al (2004) for Self Control*
not measure eating restraint in our study, but the findings of the study by Konttinen (2009) appear to indicate that self control is not associated with the control of food intake among overweight and obese individuals and current and past dieters. It has been suggested that, overall, self control is unrelated to decision making [30]. The study found that, in their daily lives, individuals who were high in self control made less frequent references to positive and negative affect and physiological states, for example feeling tired and hungry, and were seen as being less spontaneous compared to individuals with low self control [30]. They suggest that self control may inhibit affective experiences in general. Tangney (2004) suggests that individuals that are high in self control favor long-term goals in the guidance of their behavior [21], so it is possible that these individuals are not concerned with the immediate issues of their weight and are focused on other aspects of their lives. While the HEWLD was underpowered to determine significant correlations, there was no association between weight loss and self control in the VLED group either.

It is possible that an alternative measure for self control is the trait Conscientiousness. In the current study Conscientiousness and Tangney Self Control were highly correlated (r = .769 for HEWLD and r = .701 for VLED, p < .001 for both). Previous research using the FFM has reported significant negative correlations between Conscientiousness and adiposity in males and females; that is, high Conscientiousness was associated with a low BMI [18–20]. In particular, the facets “order” and “self discipline” were strongly associated with weight [20]. Sutin (2011) suggests that people with high scores in these facets are thinner because they are organized and follow their diet and meal plans [19]. However, it should be noted that these studies were about personality profiles and weight/BMI, not about weight loss [18–20]. In the current study the correlations between Conscientiousness and weight loss were negative and modest for both groups apart from the 2 facets “dutifulness” and “self discipline” that were significantly and negatively correlated with weight loss in the VLED group. If, as Sutin suggests, those that are high in Conscientiousness are thinner, then there is less need for them to follow a weight loss regime. Thus, in the current sample, high Conscientiousness could be comparatively lower in this group wanting to lose weight, and that may also explain the conflicting results compared to previous studies that did not focus on weight loss. It is also possible that people high in Conscientious do not respond well to the weight loss regimes followed in this study.

Despite prior research reporting that high Extraversion was associated with a high BMI [19] and a high BMI in males but not in females [18] our study found no significant relationship between Extraversion and weight loss or BMI in this group.

The VLED participants consumed satiety enhancing meal replacements and, with hunger initially controlled, the small bars and shakes enabled the stomach to reduce in size so that when healthy meals were phased in to the diet, participants were satisfied with less food. The satiating effect of the Optifast® bars and shakes appear to have facilitated dietary compliance in the VLED group, as evidenced by > 5% weight loss in all but 2 of the participants over 4 weeks. In this group there was a significant positive correlation between weight loss and Neuroticism and its facets “anxiety, anger, depression, self consciousness and vulnerability” but not “immoderation”.

Previous studies on personality and adiposity have reported that high Neuroticism is significantly associated with a high BMI in females [18] and a high BMI in both males and females [19]. Elfhag (2005) suggests that disinhibited eating is related to the factor Neuroticism [31]. It has also been suggested that unrestrained eaters, who eat as a natural response to hunger and appetite, appear to reduce their food intake more radically with enhanced satiety, resulting in greater weight loss [32]. A study by Elfhag (2008), using the NEO Personality Inventory-Revised which is based on the FFM of personality [33], reported that weight loss supplemented with Sibutramine, which enhances satiety, was significantly and positively correlated with the factor Neuroticism, and 2 of its facets, ‘anxiety’ and ‘depression’ [34].

There is also the suggestion that Sibutramine appears to be more effective with people who eat as a natural response to hunger and not as a response to cognition and conscious control [34]. Optifast® provides a similar satiating effect to Sibutramine and weight loss with both showing a significant correlation with Neuroticism. It is possible that the VLED group was also people who responded to hunger and appetite rather than control for weight loss but our study did not measure eating behavior so this cannot be confirmed.

The VLED continued to maintenance after the 4 weeks of weight loss to determine whether the participants could continue to manage their weight, and for this phase participants continued consuming a diet based on the AGHE. This extended their trial to match the time span of the HEWLD. Although weight loss for the 2 phases, that is weight loss and maintenance, was only moderately correlated, the correlations with personality are very similar.

Some studies have found no link between personality and weight loss. The Dutch Personality Questionnaire which includes 7 scales; Neuroticism, Social anxiety, Rigidity, Hostility, Egoism, Dominance and Self-esteem was used with gastric banding for weight loss and the findings showed that none of the personality variables was associated with weight outcomes [14]. The 10-item
Life Orientation Test was used to determine whether dispositional optimism had a predictive value for weight loss with no significant results [35]. A study by Poston [15] used the Karolinska Scales of Personality to predict weight loss and found that the personality traits identified by the Karolinska Scales of Personality did not predict weight loss. We hypothesized that self control would be associated with being able to limit food intake and restrict food choices so it was interesting to observe that, in our study, there was no link between weight loss and self control in the Tangney SCS. However there do not appear to be any studies which positively associate the personality attribute self control with weight loss.

Schwartz (1995) surveyed a group of obesity experts to determine whether or not they agreed about factors important for matching individuals to weight loss treatment [36]. She refers to previous research that suggests that people with different personality styles, levels of obesity and disturbed eating practices will respond differently to the various treatments. The position of the American Dietetic Association on weight management is that it is important to find ways to optimize individualized treatments appropriately [37]. Using measures of personality traits to identify appropriate weight loss and weight management strategies remains an intriguing possibility, but so far none of the relationships that have been found are reliable enough to base treatment regimes on them.

Conclusion
This was an exploratory study to determine whether there was a relationship between personality and different methods of weight loss. The results of this study have shown that there is a link between the personality factor, Neuroticism, and successful weight loss with a particular weight loss treatment that facilitates dietary compliance with enhanced satiety. These results were unexpected as Neuroticism normally has an inhibited effect on behavior [38]; those who are higher in Neuroticism are more likely to be anxious, depressed and have poor self esteem and so do not perform as well in many things they do. As noted above, Elfhag (2005) also found associations between neuroticism and eating behavior, but much remains to be clarified about these relationships. Surprisingly to us, there were no significant relationships with weight loss and Self-Control, as one might expect self control would play a part in behaviors related to weight control. It was predicted that the HEWLDD would show such an association, as compliance with that weight loss diet required self control. Unfortunately the sample size for this group was too small to show significant results. Overall, the number of participants involved in this study was relatively small so the results should be interpreted with caution. It is also clear that both theory and measurement methods require further development for the links between personality and weight management behavior to be understood.

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Authors’ contributions
All authors contributed to the concept and design of the research project. IM conducted the trials and collected the data, analyzed and interpreted the data and drafted the manuscript. DM analyzed and interpreted the data and drafted components of the manuscript. MB, DM, and MG provided essential materials and provided critical review. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References


Statement from co-authors relating to a paper published with Irene Munro (6)

Statement from co-authors relating to a paper published with Irene Munro

I attest that Research Higher Degree candidate Irene Munro contributed to the following paper:


- To the conception and design of the study
- To the recruitment of participants
- Conducted the intervention and collected the clinical data
- To entering, analysing and interpreting the data
- To the drafting of the manuscript
- To the revision of the manuscript

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